Determination of Phloxine B and Uranine in Water by Capillary Zone Electrophoresis

Qing X. Li*, **Jocelyn P. Alcantara-Licudine**, and **Li-Ping Li**

Department of Environmental Biochemistry, University of Hawaii, Honolulu, HI 96822

Abstract

Phloxine B and uranine are color additives in drugs and cosmetics as well as potential photoactive insecticides. A capillary zone electrophoretic (CZE) method is developed to determine phloxine B and uranine in water. A fused-silica capillary (67cm, 75-μm i.d.) and borate buffer are used. Migration of phloxine B and uranine increases slightly as the pH of the running buffer increases between the range of 8–9. Although there are only slight effects of ionic strength on the analyte migration in the range of 0–20mM NaCl in the running buffer, the migration of phloxine B and uranine increases as the percentage of methanol in the samples increases. Methanol shows little effect on the quantitation of phloxine B and uranine. The CZE procedure is applied to determine phloxine B and uranine fortified in tap and stream water samples. Solid-phase extraction is employed to recover the analytes spiked in the water samples. Recoveries range from 87–112% for phloxine B spiked at 10–200 ppb in the tap and stream water. Uranine recoveries are 86–91% at fortification levels of 10–50 ppb in the water samples.

Introduction

Phloxine B and uranine (Figure 1) are used as color additives in drugs and cosmetics. They are also common dyes and stain reagents in research laboratories. These chemicals are characterized as having strong light absorption and fluorescence. Recent studies showed that phloxine B and uranine were toxic to fruit flies (1–4). The U.S. Environmental Protection Agency (EPA) has approved a field experiment permit to test phloxine B and uranine as a replacement for malathion in fruit fly control on coffee in Hawaii, oranges in California, and grapefruit in Texas (5). Field studies have been conducted in these states and are now going on in Guatemala on about 50,000 acres of coffee fields (J.R. Heitz, PhotoDye International, Inc., Linthicum, MD, personal communication, 1997). Methods of rapid analysis are needed to investigate the environmental effect and fate of these chemicals.

A method for the analysis of phloxine B, uranine, and related xanthene dyes in soil was recently reported using supercritical fluid extraction (SFE) and high-performance liquid chromatography (HPLC) (6). Capillary electrophoresis is a fast-growing analytical technique in a wide range of applications (7–10). The use of capillary zone electrophoresis (CZE) appears to be promising and rapidly increasing in environmental analysis and monitoring (11,12). CZE was reported for analyzing polar pesticides such as phenoxyalkyl acids (13), paraquat and diquat (14–17), glyphosate (18,19), and sulfonylureas (20,21). Phloxine B and uranine are polar aromatic compounds containing carboxylic and phenolic groups. They are water-soluble and exhibit fluorescence and strong absorption in the visible (VIS) region. Thus, they are suitable for direct VIS or fluorescent detection with CZE. In CZE, separation is attributed to differential electrophoretic migration of the analytes and electroosmotic flow of the bulk solution when an electric field is applied.

In this study, determination of phloxine B and uranine in water was studied using CZE. The effects of solvents that dissolve the analytes were investigated, as well as the effects of pH value and salt concentration of running buffer on the migration times of the analytes. Solid-phase extraction (SPE) was employed to concentrate the analytes for CZE determination.
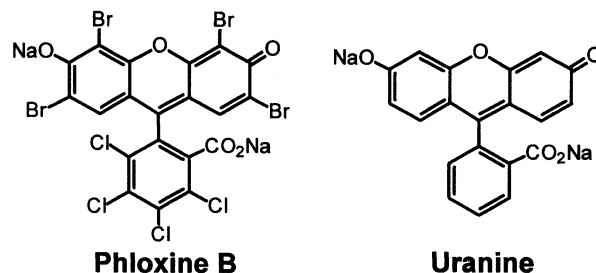

Figure 1. Structures of phloxine B and uranine.

---

* Author to whom correspondence should be addressed.

Experimental

Reagents and standards

Phloxine B and uranine were purchased from ICN Biochemicals (Cleveland, OH). Phloxine B was purified by silica gel column chromatography, as previously reported (6). Sodium hydroxide (NaOH), sodium chloride (NaCl), boric acid (H_3BO_3), and optima-grade MeOH were purchased from Fisher Scientific (Pittsburgh, PA). Sodium borate decahydrate ($Na_2B_4O_7$–$10H_2O$) and phenyl SPE cartridges were obtained from J.T. Baker, Inc. (Phillipsburg, NJ). Water was filtered through a Sybron/Barnstedt Nanopure II water system set at 17.8 mega ohm-cm resistance. Helium (99.95% purity), nitrogen (99.99% purity), and air were purchased from Gaspro (Honolulu, HI).

Standard stock solutions were 1 mg/mL of phloxine B or uranine in MeOH. Working standard solutions of phloxine B and uranine were diluted with deionized water at appropriate concentrations. Standard solutions were kept at 4°C and covered with aluminum (Al) foil.

CZE analysis

The CZE analysis was performed on a Dionex CES I (Dionex, Sunnyvale, CA) equipped with ultraviolet–visible (UV–vis) and fluorescence detectors. The capillary was fused silica (65 cm × 75-μm i.d.). The optimized running buffer was 10mM $Na_2B_4O_7$–50mM H_3BO_3 (pH 8.5), which was filtered through a 47-mm, 0.45-μm Whatman Nylon filter (Whatman International, Maldstone, England). Other CZE conditions were as follows: polarity on "+" position; gravity injection, 100 mm for 10 s; control mode, voltage; constant potential, 20,000 V; current, 150 μA; and run time, 10–15 min. The destination vial and capillary were flushed with a running buffer for 6 and 120 s, respectively. Single rinsing of the capillary was employed between runs for most determinations unless indicated otherwise. A Dionex 4400 integrator was set at a chart speed of 0.5 or 1.0 cm/min. Absorptions were monitored at 215 nm for both phloxine B and uranine or at 546 nm for phloxine B and 493 nm for uranine. Uranine was also monitored by fluorescence (excitation wavelength [λ_{ex}], 493 nm; emission wavelength [λ_{em}], 515 nm). Samples were in aqueous solutions unless indicated otherwise. The data were the average of three to seven replicates.

The CZE conditions for studying the effects of buffer ionic strength, buffer pH, and MeOH on solute migration were the same as the CZE conditions described above except for the following modification. Between injections, the destination vial and capillary were flushed with 0.1M NaOH aqueous solution for 6 and 120 s, respectively, followed by the running buffer for 6 and 360 s, respectively. Benzyl alcohol was used as a neutral marker to obtain the electroosmotic flow (EOF) and analyte mobilities.

Water samples and fortification

Water samples were from a tap in the laboratory and collected from Manoa stream in Honolulu, Hawaii. An appropriate amount of phloxine B or uranine stock solution was added in 1 L water in flasks covered with aluminum foil. Three replicates were prepared in each concentration. The samples were analyzed directly or after SPE concentration.

SPE of water samples

Water samples were acidified to obtain a pH value less than or equal to 2 using 1N HCl aqueous solution. The water samples (20 or 100 mL) were passed through the columns using a Baker-10 SPE system at a flow rate of about 4 mL/min after the cartridges were washed sequentially with MeOH (10-mL) and deionized water (10 mL). The analytes adsorbed on the cartridges were eluted with 10-mL of MeOH and the eluants were collected in 10-mL flasks. The solvent was evaporated to near dryness under nitrogen after the eluants were transferred into graduated test tubes (15 mL). Deionized water was added to dissolve the residues, and the volume (2 mL for most cases) was adjusted for quantitative CZE analysis.

Results and Discussion

CZE detection of phloxine B and uranine

Phloxine B and uranine were analyzed by VIS absorption detection at 546 and 493 nm, respec-

Table I. Detection Limit of Phloxine B and Uranine by CZE–VIS and Fluorescence Detection

Chemical	Detector	Detection Limit* Concentration (μg/mL)	Detection Limit* Amount injected[†] (pg)
Phloxine B	VIS (546 nm)	0.13	1.7
Uranine	VIS (493 nm)	0.05	0.7
	fluorescence (λ_{ex}, 493 nm; λ_{em}, 515 nm)	0.03	0.4

* Detection limit is defined by the smallest amount of chemical injected that gives a peak in the linear range of the standard curve (i.e., background signal ratio ≈ 1:2).
[†] Gravity injection is described in the Experimental section. Injection volume was calculated by using the formula $V = (2.84 \times 10^{-8} \times H \times T \times D^4) / L$, where V is the injected volume (nL), H is the injection height, T is the injection time, D is the inner diameter of the capillary, and L is the length of the capillary. Amount = $V \times C$, where V and C are injection volume and analyte concentration, respectively.

Table II. Recoveries of Phloxine B and Uranine Spiked in Water by SPE

Compound	Concentration (ppb)	Recovery (%) Tap water	Recovery (%) Stream water
Phloxine B	10		98.4* ± 4.7
	100	99.1 ± 1.3	104.2 ± 2.1
	200	111.8 ± 2.5	87.0 ± 2.5
Uranine	10		90.8 ± 7.6
	26	87.1 ± 2.1	85.8 ± 1.8
	51	90.6 ± 2.9	88.5 ± 1.3

* Concentrations were determined by an HPLC method previously reported (6).

tively. Uranine was also measured by fluorescence detection (λ_{ex}, 493 nm; λ_{em}, 515 nm). Calibration curves were obtained to determine concentrations of phloxine B and uranine. The linear correlation coefficient (r) was 0.999 for a phloxine B curve (1.7–104.7 pg injected) by VIS absorption. The linear correlation coefficient was 1.000 for a uranine curve by VIS absorption (0.7–71.0 pg injected) or fluorescence (0.4–71.0 pg injected). Fluorescence measurement of uranine was more sensitive and selective than VIS absorption. The detection limit of uranine by fluorescence (0.4 pg) was almost half of that by VIS absorption (0.7 pg) and about four times lower than that of phloxine B by VIS absorption (1.7 pg) (Table I). Electropherograms of phloxine B and uranine are shown in Figure 2. Phloxine B and uranine in
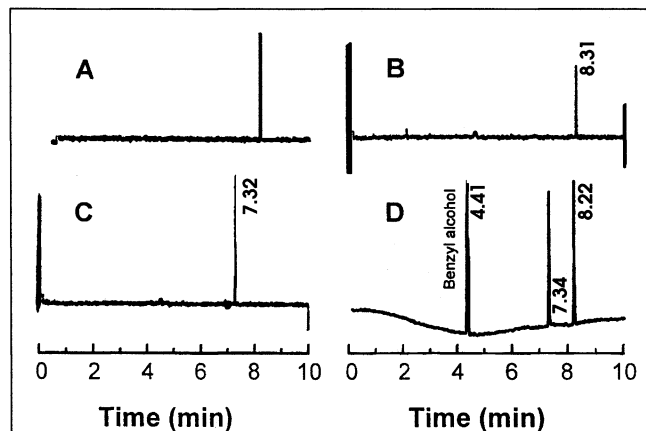
a mixture were well-separated and simultaneously determined using UV detection at 215 nm. The detection limit and background noise were relatively high. Therefore, VIS absorption and fluorescence were used to lower the detection limit of phloxine B and uranine.

Effect of pH and NaCl concentration of running buffer on analyte migration

Buffer pH effects on CZE runs were examined in the range of pH 8–9 where it was somewhat near the pK_a (i.e., phenolic proton) values of the analytes. The migration time of phloxine B and uranine decreased from 7.5 to 6.9 min and from 8.4 to 7.8 min, respectively, as the buffer pH value increased in the range of pH 8.1–9.1 (Figure 3). The electroosmotic flow was practically invariant (0.81–0.85 × 10^{-3} cm^2/Vs). There were no significant influences of buffer pH 8–9 on peak symmetry. The buffer pH 8.5 was optimum and was used in this study.

NaCl (0–20mM) added in the buffer (10mM Na$_2$B$_4$O$_7$–50mM H$_3$BO$_3$, pH 8.5) slightly affected the migration times of the target analytes. The migration times were 7.3, 7.5, and 7.6 min, respectively, for phloxine B and 8.4, 8.8, and 8.9 min, respectively, for uranine when the NaCl concentrations were 0, 10, and 20mM in the running buffer. The average electrophoretic mobilities of phloxine B and uranine were –0.276 × 10^{-3} and –0.337 × 10^{-3} cm^2/Vs, respectively, when no NaCl was added in the buffer. When the NaCl concentrations were 10 and 20mM in the buffer, the average electrophoretic mobilities were –0.329 × 10^{-3} and –0.351 × 10^{-3} cm^2/Vs, respectively, for phloxine B and –0.340 × 10^{-3} and –0.342 × 10^{-3} cm^2/Vs, respectively, for uranine. The electroosmotic flow was 0.772 × 10^{-3}, 0.810 × 10^{-3}, and 0.827 × 10^{-3} cm^2/Vs, respectively, at the corresponding NaCl concentrations. These variations were insignificant and probably due to instrumental measurement errors.

Figure 2. Electropherograms of uranine (3.6 pg [A] by fluorescence [λ_{ex}, 493 nm and λ_{em}, 515 nm] and [B] by VIS [493 nm]); phloxine B (17 pg [C] by VIS [546 nm]); and a mixture of phloxine B, uranine (34 pg each), and benzyl alcohol (1.7 ng [D] by UV [215 nm]). The running buffer was 10mM Na$_2$B$_4$O$_7$–50mM H$_3$BO$_3$ (pH 8.5).
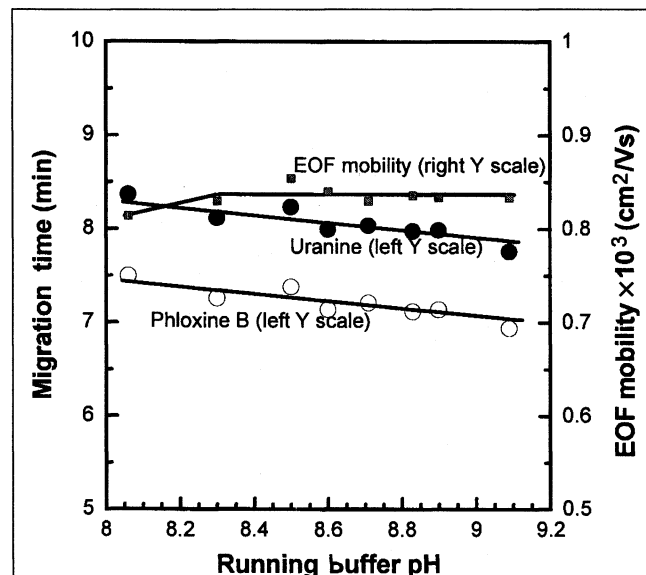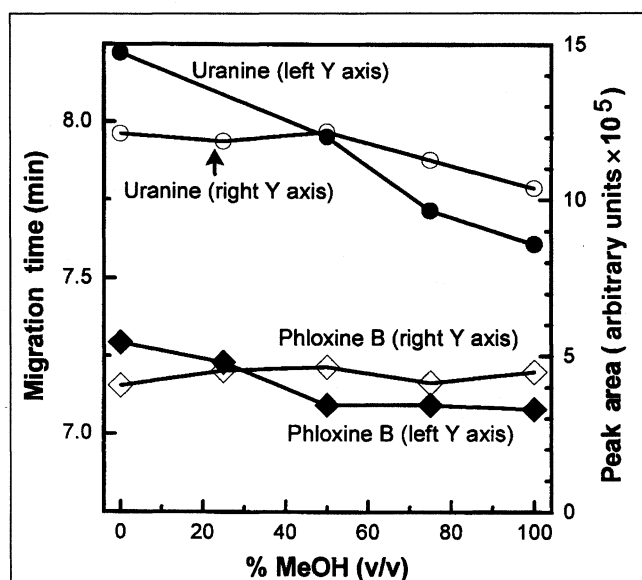

Figure 3. The effect of running buffer pH on the migration of phloxine B and uranine. The pH values of the running buffer were adjusted by adding various amounts of H$_3$BO$_3$ to 10mM Na$_2$B$_4$O$_7$. Data were the average of three to seven replicates. The standard deviations averaged 1.6% of the mean values for phloxine B, uranine, and benzyl alcohol as a neutral marker.

Figure 4. The effect of MeOH percentage in the samples on the migration and detection of phloxine B and uranine. The running buffer was 10mM Na$_2$B$_4$O$_7$–50mM H$_3$BO$_3$ (pH 8.5). Little change (0.796–0.801 × 10^{-3} cm^2/Vs) in the EOF was observed when the sample was dissolved in various percentages of MeOH. Data were the average of three replicates. The standard deviations averaged 0.43% of the mean values for phloxine B and uranine.

Effect of MeOH in samples on analyte migration and detection

MeOH was used to elute the analytes adsorbed on the SPE cartridges. Thus, studies were to determine effects of MeOH in the samples on CZE performance. Phloxine B and uranine generally migrated faster as the MeOH concentration in the samples increased from 0 to 100% (Figure 4). The influence of MeOH on the migration time of uranine was greater than that of phloxine B. The migration times of uranine dissolved in 0, 50, 75, and 100% MeOH were 8.2, 8.0, 7.7, and 7.6 min, respectively. The electroosmotic flow had little change (0.796–0.801 × 10^{-3} cm^2/Vs) when the sample was dissolved in various percentages of MeOH. MeOH in the samples may alter the analyte charge. When a MeOH zone in front of the solute zone migrates along the capillary, MeOH may also decrease the effective wall charge and consequently improve the overall solute mobility. MeOH in the samples had little effect on the quantitation of phloxine B and uranine.

Phloxine B and uranine recoveries in water samples by SPE

Phloxine B and uranine are potential photoactive insecticides to control fruit flies (1–4). Baits containing these chemicals are used for spray applications in fields. These chemicals may be present in various environmental compartments if they are used in the environment. Stream and tap water samples were used as model matrices to develop CZE methods for environmental monitoring of these chemicals.

The recoveries of phloxine B in the tap and stream water ranged from 87 to 112% at the spiking levels of 10–200 ppb using the phenyl cartridges (Table II). The recoveries of uranine in the tap and stream water samples were observed from 86 to 91% at the spiking levels of 10–51 ppb. Phloxine B and uranine retained in the SPE cartridges can be quantitatively eluted with a small amount of MeOH (10 mL). This SPE method will allow fast and quantitative extraction of phloxine B and uranine in environmental water samples.

Measurement correlation

The CZE procedure was used to determine phloxine B and uranine fortified at levels of 0.01–5 ppm in tap and Manoa stream water samples. The samples were directly analyzed by CZE when the concentrations of phloxine B and uranine were high (e.g., ≥ 0.5 ppm). SPE was employed for these samples containing low concentrations (< 0.5 ppm) of the analytes. Results of CZE determinations correlated very well with the spiking concentrations. The correlation coefficients (r) were 0.996 with a slope of 1.13 and 0.999 with a slope of 1.28 for phloxine B in tap and stream water, respectively. The same correlation coefficient (r) of 0.999 with slopes of 1.15 and 1.09 was obtained for uranine in tap and stream water, respectively.

Conclusion

The determination of phloxine B and uranine by CZE was fast and simple. This CZE method will be useful for assessing environmental effects of the potential photoactive insecticides phloxine B and uranine. It will also be a valuable tool for basic laboratory studies in which these dyes are used. Because use of organic solvent is reduced considerably, as compared with classical methods such as HPLC, CZE is environmentally compatible.

Acknowledgments

References

1. J.R. Heitz. Pesticidal applications of photoactivated molecules. *Light-Activated Pest Control.* J. R. Heitz and K.R. Downum, Eds. ACS symposium series 616. American Chemical Society, Washington DC, 1995, pp. 1–16.
2. N.J. Liquido, G.T. McQuate, and R.T. Cunningham. Light-activated toxicity of phloxine B and uranine to Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidate), adults. *Light-Activated Pest Control.* J.R. Heitz and K.R. Downum, Eds. ACS symposium series 616. American Chemical Society, Washington DC, 1995, pp. 82–106.
3. N.J. Liquido, G.T. McQuate, and R.T. Cunningham. Light-activated toxicity of phloxine B and fluorescein in methyleugenol to Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidate), males. *Light-Activated Pest Control.* J. R. Heitz and K.R. Downum, Eds. ACS symposium series 616. American Chemical Society, Washington DC, 1995, pp. 107–114.
4. R.L. Mangan and D.S. Moreno. Development of phloxine B and uranine bait for control of Mexican fruit fly. *Light-Activated Pest Control.* J.R. Heitz and K.R. Downum, Eds. ACS symposium series 616. American Chemical Society, Washington DC, 1995, pp. 115–126.
5. US EPA. Issuance of experimental use permit (EUP), 11312-EUP-100. *Fed. Register.* **60(193):** 52183–52184 (1995).
6. J.P. Alcantara-Licudine, M.K. Kawate, and Q.X. Li. Method for the analysis of phloxine B, uranine and related xanthene dyes in soil using supercritical fluid extraction and high-performance liquid chromatography. *J. Agric. Food Chem.* **45(3):** 766–73 (1997).
7. J.W. Jorgenson and K.D. Lukacs. Zone electrophoresis in open-tubular glass capillaries. *Anal. Chem.* **53:** 1298–1302 (1981).
8. J.P. Landers. Clinical capillary electrophoresis. *Clin. Chem.* **41:** 495–509 (1995).
9. P.F. Cancalon. Capillary electrophoresis: A new tool in food analysis. *J. AOAC International* **78:** 12–15 (1995).
10. R.L. St. Claire, III. Capillary electrophoresis. *Anal. Chem.* **68:** 569R–586R (1996).
11. G. Dineli, A. Vicari, and P. Catizone. Monitoring of herbicide pollution in water by capillary electrophoresis. *J. Chromatogr. A* **733:** 337–347 (1996).
12. W.C. Brumley. Techniques with potential for handling environmental samples in capillary electrophoresis. *J. Chromatogr. Sci.* **33:** 670–85 (1995).
13. M.W.F. Nielen. (Enantio-)separation of phenoxy acid herbicides using capillary zone electrophoresis. *J. Chromatogr.* **637:** 81–90 (1993).
14. Y.Y. Wigfield, K.A. McCormack, and R. Grant. Simultaneous determination of residues of paraquat and diquat in potatoes using high-

performance capillary electrophoresis with ultraviolet detection. *J. Agric. Food Chem.* **41:** 2315–18 (1993).

15. J. Cai and Z. El Rassi. Capillary zone electrophoresis of two cationic herbicides, paraquat and diquat. *J. Liq. Chromatogr.* **15(6&7):** 1193–1200 (1992).

16. D. Kaniansky, F. Ivanyl, and F.I. Onuska. On-line isotachophoretic sample pretreatment in ultratrace determination of paraquat and diquat in water by capillary zone electrophoresis. *Anal. Chem.* **66:** 1817–24 (1994).

17. M. Tomita, T. Okuyama, and Y. Nigo. Simultaneous determination of paraquat and diquat in serum using capillary electrophoresis. *Biomed. Chromtogr.* **6:** 91–94 (1992).

18. M. Tomita, T. Okuyama, Y. Nigo, B. Uno, and S. Kawai. Determination of glyphosate and its metabolite, (aminomethyl) phosphonic acid, in serum using capillary electrophoresis. *J. Chromatogr.* **571:** 324–30 (1991).

19. M.G. Cikalo, D.M. Goodall, and W. Matthews. Analysis of glyphosate using capillary electrophoresis with indirect detection. *J. Chromatogr. A* **745:** 189–200 (1996).

20. G. Dinelli, A. Vicari, and P. Catizone. Use of capillary electrophoresis for detection of metsulfuron and chlorsulfuron in tap water. *J. Agric. Food Chem.* **41:** 742–46 (1993).

21. A.J. Krynitsky and D.M. Swineford. Determination of sulfonylurea herbicides in grains by capillary electrophoresis. *J. AOAC International* **78:** 1091–96 (1995).