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Online identification of the fluorescent whitening agent 4,4-bis(2-sulfostyrol)biphenyl using a sweeping technique combined with capillary electrophoresis/77 K fluorescence spectroscopy

The feasibility of combining the techniques of online concentration and CE/low-temperature fluorescence spectroscopy in the detection and identification of *E,E*-4,4'-bis(2-sulfostyryl)biphenyl (DSBP) in synthetic detergents at 77 K is demonstrated. The technique involves the use of sweeping-MEKC, and was used for the initial online concentration and separation, after which a cryogenic molecular fluorescence experiment was performed at 77 K. The proposed method not only permits the separation and detection of *E,E*-DSBP in a synthetic detergent sample, but also ensures that the online spectrum is readily distinguishable and can be unambiguously assigned at 77 K. The photoconversion and isomer separation of DSBP are also described.

Keywords: 4,4'-Bis(2-sulfostyryl)biphenyl / 77 K Low-temperature fluorescence spectroscopy / Sweeping-MEKC DOI 10.1002/elps.200500325

1 Introduction

Online sample concentration techniques [1–3], including the so-called sweeping and stacking modes, have recently become popular and important. By applying these methods, sensitivity can be improved by several orders of magnitude. Several excellent descriptions of online sample concentration techniques, and their applications, can be found in the published literature [4–8]. However, even when these techniques are used in CE, the methods that are normally used for the identification of sample constituents are dependent on the migration time or the spiking method used. It should be noted that, if the migration time scale shifts (commonly known as drift) or the spiking-standards are difficult to obtain, problems would be encountered in identifying unknown constituents.

DSBP (4,4'-bis(2-sulfostyryl)biphenyl) is a stilbene-type fluorescent whitening agent (FWA) and, like other stilbene-type FWAs, degrades to variable extents on exposure to sunlight or hypochlorite. It is water-soluble (because of the sulfonate group) and has a high affinity for cellulose (because of its large structure), which makes it a suitable product for whitening applications [9–18], and is commonly used in cleaning detergents for laundry washing. The worldwide production of this compound was 3000 tons in 1992 [9]. DSBP absorbs UV light at ~360 nm and emits blue visible light at a maximum wavelength of ~430 nm with a fluorescence quantum yield of 0.8 [11]. The ability to absorb short-wavelength light also makes it susceptible to photochemical transformations. The exposure of *E,E*-DSBP to light causes reversible isomerization of the *E,Z*- and *Z,Z*-forms of the stilbene moiety (Fig. 1). DSBP is currently commercially available in the *E,E*-form. As a result, the identification of the *E,Z*- and *Z,Z*-form isomers is still a difficult task. Hence, the separation and identification for these isomers have become an important issue.

We previously reported on the determination of six stilbene-type FWAs [10], based on the CE and HPLC methods by means of UV-absorbance. However, the photoconversion properties of these agents have not yet been investigated. In this study, DSBP was selected as a model compound, and we now report on the use of an online sample concentration technique (sweeping-MEKC) to further improve the LOD by means of fluorescence detection. Furthermore, in an attempt to precisely identify the isomers by means of "fluorescence fingerprinting," the online 77 K fluorescence spectra of DSBP isomers were obtained. These low-temperature spectra are particularly useful for qualitative analysis, since this technique provides spectral-sharpening both in absorption and emission [19–25]. The current work also extends this method to the determination of DSBP in an actual sample (a synthetic detergent), and the results are reported herein.

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Abbreviations: DSBP, 4,4'-bis(2-sulfostyryl)biphenyl; **FWA**, fluorescent whitening agent

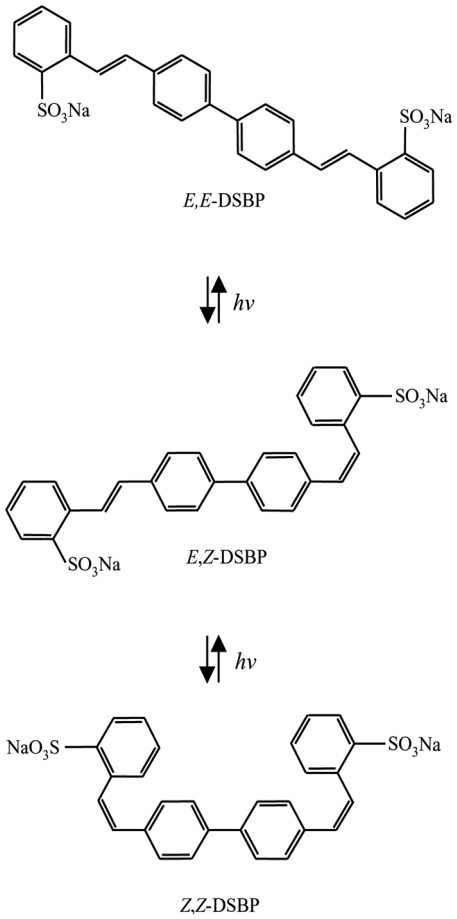

Figure 1. Molecular structures and photochemical reaction schemes for DSBP.

2 Materials and methods

2.1 Chemicals

DSBP was obtained from Ciba-Geigy (Basel, Switzerland). SDS was obtained from Sigma (St. Louis, MO, USA). Phosphoric acid was obtained from J. T. Baker (Philipsburg, NJ, USA). ACN and MeOH (99.8%) were obtained from Acros (Geel, Belgium). The synthetic detergents were purchased from local supermarkets (Taiwan). A C-18 column (J.T Baker, NJ, USA) was used for the SPE. All other chemicals were of analytical grade and are commercially available.

2.2 CE apparatus

The CE setup and data acquisition system used were similar to that described previously [26], but the light source was changed to a high intensity discharge (HID) lamp (PHILIPS 85126 WX D2R), instead of a Xenon lamp, since the line at a wavelength of 365 nm is particularly well suited for pumping the analyte (DSBP). This sharp line source can be simply selected by a short-pass filter (254–400 nm pass) or by means of a monochromator. In the former case (for quantitative analysis), about 5 mW in luminous intensity can be obtained. A UV lens was used for focusing on the capillary (fused-silica capillary, 75 μm ID; J&W Scientific, CA, USA). The fluorescence emission was collected by means of a microscope eyepiece (10 ×), passed through a cut filter and a slit, focused by a second lens, and then detected by a photomultiplier tube. In the other case (for qualitative analysis), the excitation light was selected by a monochromator (Acton Research Corporation; Model SP-150). Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator (Acton Research Corporation; Model SP-300i), followed by detection using another photomultiplier tube. For acquiring online fluorescence spectra at 77 K, a locally designed capillary-Dewar was used [20, 26], which can be used for introducing liquid nitrogen.

2.3 Sample preparation

The *E,E*-DSBP standard was examined by ESI-MS (Thermo Finnigan LCQ Advantage) and NMR (Bruker AVANCE 400) to confirm its purity as a standard in further experiments. The sample pretreatments of the DSBP standard and commercially synthetic detergents were undertaken in a dark room to prevent photodegradation. The synthetic detergents were extracted using an octadecyl silica (ODS) extraction column at a rate below 5 mL/min. The ODS extraction column had been previously washed with 10 mL of methanol (MeOH) and 5 mL of distilled water. FWAs were eluted with 5 mL of MeOH. The eluent was centrifuged to dryness and redissolved in an appropriate volume (1–100 mL) of the matrix using the sweeping-MEKC.

3 Results and discussion

3.1 Online sample concentration

DSBP standard can be easily separated by either MEKC or sweeping-MEKC modes. In the case of MEKC, the optimal conditions for DSBP were achieved using a phosphate buffer (30 mM) containing SDS (75 mM) in an ACN–water solution (15:85 v/v). In the case of sweeping-MEKC, the same buffer system was used, but the sample was dissolved in the matrix (30 mM phosphate buffer without SDS); the sample solution injection length was 15 cm. When the sweeping-MEKC mode was applied, a

~300-fold improvement in detection sensitivity could be obtained. Using this approach, the lowest concentration of 10 ppb (S/N = 3, LOD ~ 1 ppb) can be easily obtained. This is better than our previous data using HPLC and UV-CE that provided an OD of DSBP in the range of 7.6 (HPLC) and 328 ppb, respectively [10]. Table 1 summarizes and compares the linearity, LOD values, RSD% of peak area and migration times, and plate numbers for MEKC and sweeping-MEKC, respectively. The linearity of these methods for DSBP was also fairly good. Using this approach, six commercially available synthetic detergents, that are routinely sold in Taipei supermarkets, were examined. Four synthetic detergents were found to contain DSBP and their concentrations were 480, 261, 2.6, and 122 ng/mg, respectively. In a river pollution case, the concentration of DSBP in sewage effluents (rivers near Tokyo Bay) was reported to be ~8 ppb [13]. The CE/sweeping-MEKC method provides sufficient sensitivity for monitoring DSBP in such an environment.

Table 1. LOD values, RSD values, and plate numbers for the DSBP standard with the MEKC sweeping-MEKC modes

(A) MEKC	
Equation of the line	$y = 0.4416x + 0.0128$
CV	0.9998
LOD (S/N = 3)	0.7 ppm
Detection range	0.1–10 ppm
RSD	
(a) Migration time	
Intraday (n = 3)	1.0
Interday (n = 3)	3.9
(b) Peak area	
Intraday (n = 3)	5.7
Interday (n = 3)	8.9
Plate number	2.2×10^5
(B) Sweeping-MEKC (sample zone: 15 cm)	
Equation of the line	$y = 0.0582x + 0.1252$
CV	0.9997
LOD (S/N = 3)	1 ppb
Detection range	10–400 ppb
RSD	
(a) Migration time	
Intraday (n = 3)	1.9
Interday (n = 3)	2.1
(b) Peak area	
Intraday (n = 3)	6.0
Interday (n = 3)	8.6
Plate number	1.0×10^6

Total length/effective length = 86 cm/80 cm. Exciting source: 365 nm line of a high-intensity discharge (HID) lamp (power, ~5 mW).

3.2 Photoconversion and isomer separation of DSBP

As mentioned above, DSBP can be photoconverted in three isomeric forms, *i.e.*, the *E,E-, E,Z-*, and *Z,Z*-DSBP forms (Fig. 1). Thus, it is necessary to realize the photoconversion process in order to exactly estimate the amount of each component, if exposed to UV light. In this study, we found that no *E,Z-* and *Z,Z*-DSBP forms are present before the irradiation (data not shown). However, after an hour exposure to UV light, additional smaller peak appears near the DSBP peak. It is well known that *trans*-stilbene (*E*-type) is weakly fluorescent ($\Phi_F = 0.05$) and *cis*-stilbene (*Z*-type) is nonfluorescent at room temperature. This indicates that the fluorescence intensity of the DSBP isomer could be *E,E-* > *E,Z-* > *Z,Z*-DSBP. Thus, we assigned the smaller peak to *E,Z*-DSBP (data not shown). Meanwhile, *Z,Z*-DSBP could also be formed. We separated and detected this test sample again by means of the UV-absorbance method and the result showed that the third peak (*Z,Z*-DSBP) indeed appears. It should be noted that the measurement of an actual spectrum for a single isomer is extremely difficult if the analyte is undergoing photoconversion during the measurement. Therefore, online identification would be ideal for compounds such as this, when standards are not easily acquired. Although a CCD camera can be used to instantly record the fluorescence, nearly all of the detectors of CE systems currently in use obtain data at room temperature. These fluorescence spectra only provide a broadband of fluorescence spectrum and the identification of isomers is difficult, because the isomers have similar fluorescence behaviors at room temperature. Assuming that the isomerization proceeded extremely slowly at 77 K and no solid-phase photoconversion occurred, our system was capable of measuring the 77 K fluorescence spectra online for the *E,E-* and *E,Z*-DSBP isomers, as described in Section 3.3.

3.3 Online spectral measurement of *E,E-* and *E,Z*-DSBP at 77 K

Figure 2 shows the online 77 K spectra of the CE-separated peaks of the test *E,E*-DSBP after exposure to UV light for 1 h. Herein, a locally designed capillary-Dewar was custom-made and consisted of a double-walled quartz flask for introducing liquid nitrogen [19, 20]. The capillary was bent into the shape of a hoop, secured to a glass rod, and positioned in the central region of the capillary-Dewar. The separation was observed on a computer monitor. Once the CE-separated analytes appeared on the screen, the power supply was immediately turned off and liquid nitrogen poured directly into the capillary-

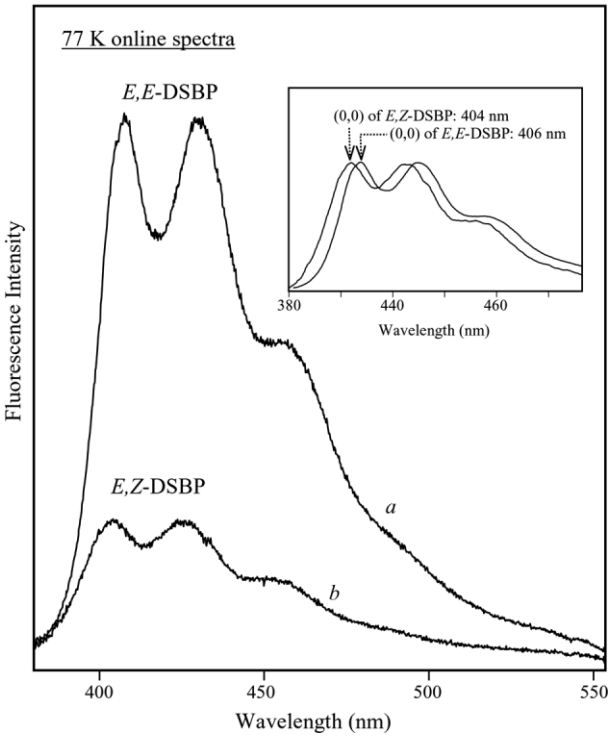

Figure 2. Online 77 K spectra of the CE-separated peaks. Spectra a and b assigned to corresponding *E,E*-DSBP and *E,Z*-DSBP, respectively. Inset, the scale-modified spectra. Observed wavelength of the (0,0) origin band of *E,E*- and *E,Z*-DSBP was 406 and 404 nm, respectively.

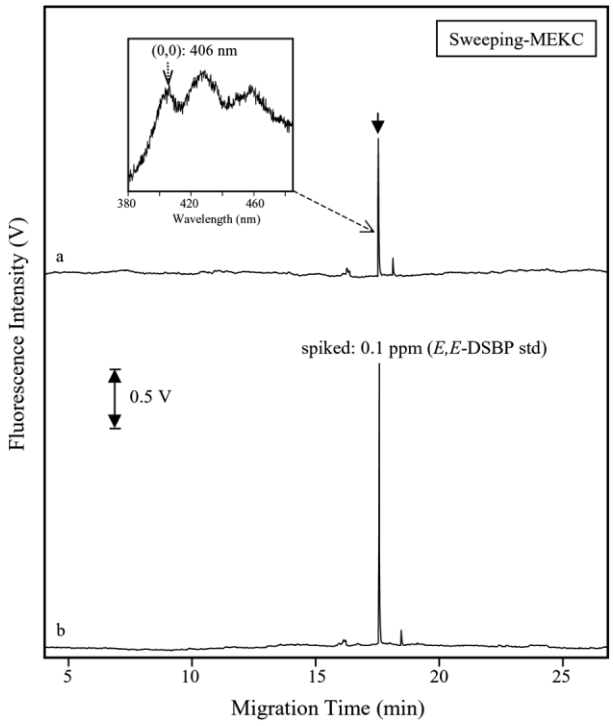

Figure 3. CE electropherograms a and b show the result obtained from a synthetic detergent extract; electropherogram a, before spiking with the standard; electropherogram b, after spiking with 0.1 ppm *E,E*-DSBP standard. Inset spectrum shows the online 77 K spectra of the CE-separated analyte (arrow) from the synthetic detergent extract.

Dewar. Once frozen, arbitrary detection times can be used to completely characterize the separated analytes by 77 K low-temperature spectroscopy. Spectra a and b in Fig. 2 are assigned to *E,E*- and *E,Z*-DSBP, respectively. The inset shows the scale-modified spectra. The observed wavelength of the (0,0) origin band of *E,E*- and *E,Z*-DSBP was 406 and 404 nm, respectively. These standard spectra are useful for achieving a fluorescence fingerprinting analysis.

3.4 Online spectral identification of *E,E*-DSBP in synthetic detergent at 77 K

Figure 3 shows typical CE electropherograms obtained from an extract of a synthetic detergent, by applying the sweeping-MEKC mode; electropherograms a and b show the result obtained before and after spiking with the *E,E*-DSBP standard (100 ppb), respectively. In comparison with the two electropherograms, we assigned the peak marked by an arrow as *E,E*-DSBP, and its concentration was calculated to be 115 ppb. The inset spectrum shows the online 77 K spectra of the CE-separated analyte (arrow) from the synthetic detergent extract. The 77 K fluorescence spectrum of the arrow marked peak was superimposable with the spectrum of the *E,E*-DSBP standard. Thus, we conclude that *E,E*-DSBP in synthetic detergent could be absolutely identified using this approach. This proposed method may solve problems encountered for analytes that have a weak fluorescence intensity, a broader fluorescence behavior at room temperature, or are present at low levels.

4 Concluding remarks

This work demonstrates that *E,E*-DSBP in a synthetic detergent can be readily distinguishable and unambiguously assigned through online sample concentration, thus improving the sensitivity and online 77 K fluorescence spectral identification (fluorescence fingerprinting at 77 K). The complete, optimal separation of DSBP could be achieved with phosphate buffer (30 mM) containing SDS (75 mM) in an ACN–water solution (15:85 v/v) and a ~300-fold improvement in detection sensitivity was obtained compared with the MEKC method. Although all of the separation conditions are discussed as well as the

results of excitation by an HID lamp, it is definitely clear that the use of a combination of a sweeping technique and a laser, especially a UV-Argon laser at 351 nm, can further improve the analysis of DSBPs in environmental samples.

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