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PHOTODIODE ARRAY DETECTION IN HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS

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SUMMARY

A photodiode array detection system was applied to high-performance capillary electrophoresis. Spectra were obtained for mixtures of aromatic compounds and water-soluble vitamins. Photodiode array detection enabled accurate characterization of separated components and was a powerful tool for the investigation of mixed zones.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) has become one of the most rapidly developing techniques for the separation of both charged and neutral compounds. The main modes of HPCE are capillary zone electrophoresis[1,2], micellar electrokinetic capillary chromatography[3], capillary gel electrophoresis[4,5] and isoelectric focusing[6]. These techniques offer fast and highly efficient separation of various compounds ranging from small molecules, such as amino acids, to biopolymers, such as proteins and DNAs. Successful separation by HPCE is dependent on the sampling and detection method, as the sample amounts are very small because of the use of small-diameter capillaries. Ewing *et al.*[7] reviewed the newest detection methods for HPCE and listed the detection limits for spectrophotometric, mass spectrometic, electrochemical and radiometric detectors. Recently[8] an attempt was made to utilize the sensitivity of photodiode array detection in the separation and analysis of minute quantities of complex substances, especially those of biological origin. However, the small diode array employed limited detection to less than 20 wavelengths. The present work covers the application of a full 512-element diode array to provide complete coverage of the 200–380-nm UV range with the concomitant advantage of contour-plot electropherograms enabling rapid and accurate characterization of complex samples.

EXPERIMENTAL

Reagents

Protein research-grade sodium dodecyl sulfate (SDS) was purchased from Na-

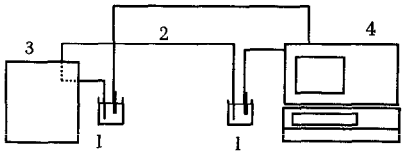

Fig. 1. Schematic of the HPCE system. 1 = Buffer reservoirs; 2 = capillary tubes; 3 = modified SPD-M6A; 4 = IP-3A control unit.

karai (Kyoto, Japan). Other reagents of analytical-reagent grade were obtained from Wako (Osaka, Japan) and were used without further purification. Deionized and distilled water was obtained with a Model WG-25 water purification system (Yamato, Tokyo, Japan) and was used for all buffer solutions. A mixture of resorcinol (3.7 m*M*), phenol (3.7 m*M*), *p*-nitroaniline (0.6 m*M*), nitrobenzene (0.3 m*M*), *o*-nitroaniline (0.6 m*M*) and 2-naphthol (0.6 m*M*) in buffer solution containing SDS was used as a sample of aromatics. A water-soluble vitamin standard solution containing 0.81 m*M* nicotinamide, 0.49 m*M* pyridoxine hydrochloride (B_6), 0.52 m*M* caffeine, 0.39 m*M* calcium pantothenate, 0.27 m*M* riboflavin (B_2), 0.074 m*M* vitamin B_{12} and 0.3 m*M* thiamine (B_1) was used to show the applicability of the detection system employed. A commercially available vitamin tablet, Popon S (Shionogi & Co., Osaka, Japan), was dissolved in 200 ml of distilled water to provide a realistic sample. All solutions were filtered through a membrane filter of 0.2-μm pore size prior to use.

Instrumentation

Fig. 1 is a block diagram of the apparatus used. The SPD-M6A UV–VIS photodiode array detector (Shimadzu, Kyoto, Japan) was modified for capillary electrophoresis, and the control unit of the IP-3A isotachophoresis analyzer (Shimadzu) was employed as high-voltage controller. As shown in Fig. 2, the standard detector cell normally used for high-performance liquid chromatography (HPLC) was removed and the electrophoresis capillary, a segment of its polyimide coating removed by pyrolysis, was positioned to within 1 mm of the slit of 65 μm width by 5 mm length. Light intensity was adjusted by aperture control. The fused-silica capillaries were obtained from Scientific Glass Engineering (Melbourne, Australia). Injection of sample into the capillary was performed by siphoning[9]. Routinely, 50 cm -long capillaries were used.

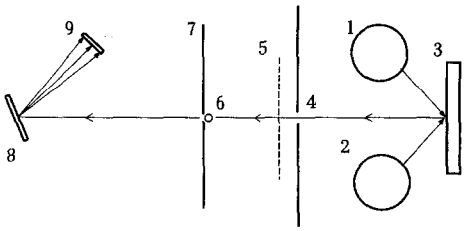

Fig. 2. Optical path for the modified photodiode array detection system. 1 = Deuterium lamp; 2 = tungsten lamp; 3 = mirror; 4 = aperture; 5 = shutter; 6 = fused-silica capillary tubes; 7 = slit; 8 = holographic grating; 9 = photodiode array.

RESULTS AND DISCUSSION

Basic considerations

It was necessary to determine the feasibility of using a capillary in place of the standard cell of the spectrometer. Two HPLC configurations were set up: one employing the standard 1-cm path length cell, and one with a 100-μm capillary in the cell position. Since the effective "cell" path length in the capillary was considerably smaller than in the 1-cm cell, a corresponding decrease in light intensity incident upon the capillary was necessary in order to prevent saturation of the diode array. Intensity control was achieved simply by controlling the aperture width at the light source (see Fig. 2). Even at a full-scale absorbance of 0.01 absorbance unit, both systems displayed equivalent response to a number of samples as illustrated by the superimposed spectra of a sample of pyrene in methanol (Fig. 3). (The offset in wavelength between the spectra is a software artifact as the data from one instrument were used with the other instrument without appropriate wavelength calibration.) The result showed that the SPD-M6A detector could adopt a capillary as cell. The system was subsequently used for micellar electrokinetic capillary chromatography.

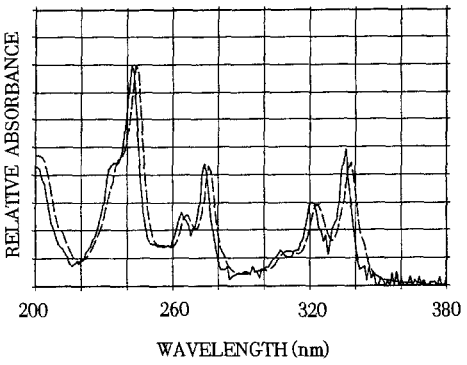

Fig. 3. Superimposed spectra of pyrene in methanol. The full-line spectrum was obtained by the modified photodiode array system with fused-silica capillary tubes (I.D. 100 μm). The broken-line spectrum was obtained by the original system with HPLC detection cell.

Micellar electrokinetic capillary chromatography

Separation of aromatic compounds. Fig. 4 shows the electropherogram of the mixture of aromatics performed in 100-μm and 75-μm capillaries. The volume injected by means of siphoning the sample was estimated by the time required for the sample to reach the detector and was calculated to be 6–7 nl for a 75-μm capillary. Although the spectra obtained using both capillary widths were comparable, some noise appeared in the spectra obtained with the 75-μm capillaries. These data indicate that a capillary diameter of *ca.* 75 μm represents the lower limit for obtaining spectra of a quality equivalent to or better than those obtained with the standard HPLC configuration of the detector.

Separation of water-soluble vitamins. Fig. 5 shows the electropherogram and spectra of the mixture of six water-soluble vitamins and caffeine obtained with the 75-μm and 100-μm capillaries. Determination of water-soluble vitamins in commer-

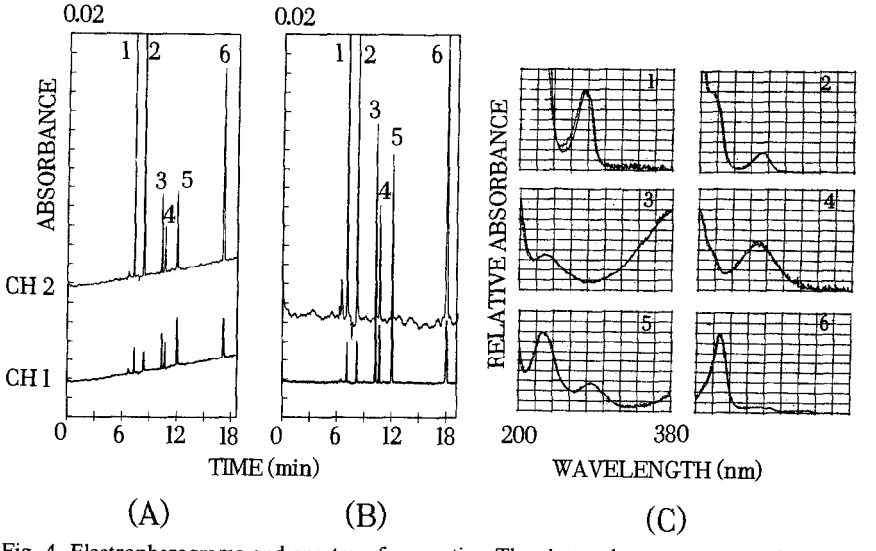

Fig. 4. Electropherograms and spectra of aromatics. The electropherograms were obtained by micellar electrokinetic capillary chromatography with 75 μm I.D. (A) and 100 μm I.D. (B) fused-silica capillary tubes. Buffer: 50 m*M* SDS in 10 m*M* Tris–phosphate, pH 6.9; sample injection: 10 s at 3.5 cm height difference; detection: channel 1 (CH1) at 250–253 nm; channel 2 (CH2) at 207–210 nm. (A) 15 kV, *ca.* 32 μA, detection at 0.02 a.u.f.s.; (B) 15 kV, *ca.* 38 μA, detection at 0.02 a.u.f.s. (C) Superimposed spectra of components separated: 1 = resorcinol; 2 = phenol; 3 = *p*-nitroaniline; 4 = nitrobenzene; 5 = *o*-nitroaniline; 6 = 2-naphtol. Broken line, spectra obtained with 75-μm capillaries; full line, spectra obtained with 100-μm capillaries.

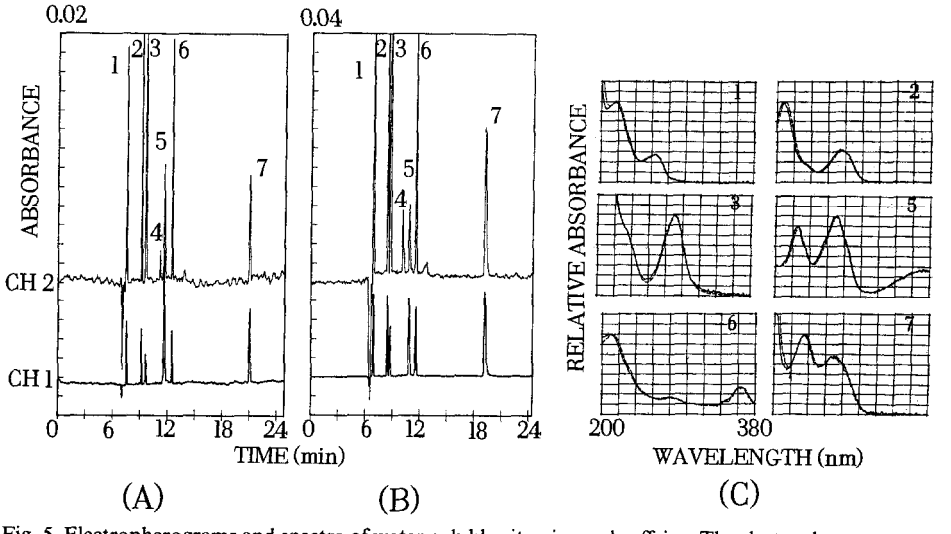

Fig. 5. Electropherograms and spectra of water-soluble vitamins and caffeine. The electropherograms were obtained by micellar electrokinetic capillary chromatography using 75 μm I.D. (A) and 100 μm I.D. (B) fused-silica capillary tubes. Buffer: 50 m*M* SDS in 50 m*M* Tris–borate, pH 8.4; sample injection: 10 s at 3.5 cm height difference; detection: CH1 at 250–253 nm, CH2 at 207–210 nm. (A) 15 kV, *ca.* 33 μA, detection at 0.02 a.u.f.s. (B) 15 kV, *ca.* 42 μA, detection at 0.04 a.u.f.s. (C) Superimposed spectra of the components separated: 1 = nicotinamide; 2 = pyridoxine hydrochloride (B_6); 3 = caffeine; 5 = riboflavin (B_2); 6 = vitamin B_{12}; 7 = thiamine (B_1). Full line, spectra obtained with 75-μm capillaries; broken line, spectra obtained with 100-μm capillaries. Since calcium pantothenate (peak 4) has a very weak UV absorbance, no effective absorption profile was observed.

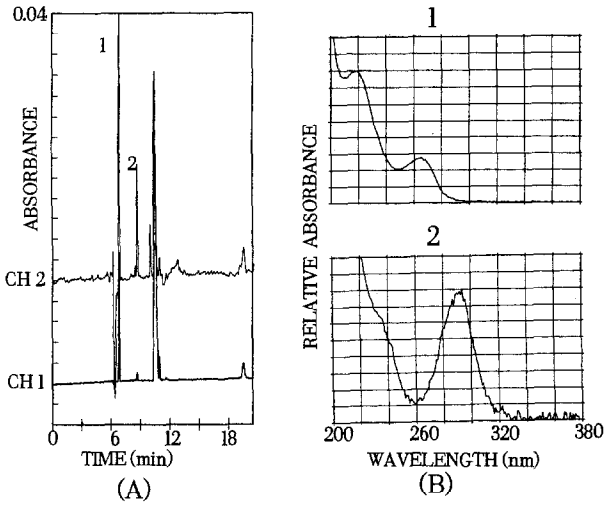

Fig. 6. Separation of vitamins and caffeine in tablets by micellar electrokinetic capillary chromatography. (A) Electropherograms obtained using 100 μm I.D. fused-silica capillary tubes. (B) Spectra of nicotinamide (1) and caffeine (2). Buffer: 50 m*M* SDS in 50 m*M* Tris–borate, pH 8.4, sample injection: 10 s at 3.5 cm height difference; detection: CH1 at 250–253 nm, CH2 at 207–210 nm, at 0.04 a.u.f.s.; voltage: 15 kV, *ca.* 42 μA.

cially available tablets was carried out by using the above spectra for identification. Fig. 6 shows the separation of the components of a vitamin tablet using a 100-μm capillary with the spectra matched to the spectra of the standard mixture. Since reproducibility and peak shape in capillary electrophoresis are sensitive to the state of the inner wall of the capillary, constitution of electrolyte solution, sample concentration and other factors[9], variations in retention times of the components were observed. Fig. 7 shows the profile obtained with the six water-soluble vitamin standards and caffeine. Six main peaks with one shouldering peak were observed. Investigation of the peaks by the contour plot method (Fig. 8A) showed that peak 1 consisted of pyridoxine (B_6) and nicotinamide in the reverse order compared to the data in Fig. 5.

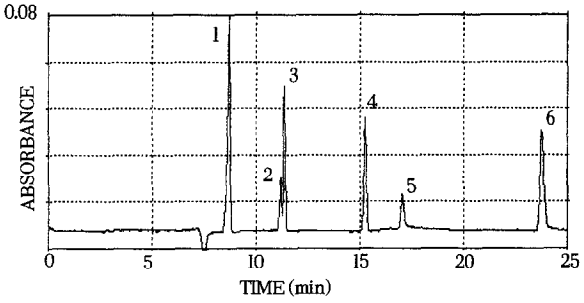

Fig. 7. Electropherogram of water-soluble vitamins and caffeine using 75 μm I.D. fused-silica capillary tube. Buffer: 50 m*M* SDS in 10m*M* Na_2HPO_4; sample injection: 10 s at 3.5 cm height difference; voltage: 15 kV, *ca.* 33 μA; sample: same as in Fig. 5. Peaks: 1 = pyridoxine hydrochloride and nicotinamide; 2 = caffeine; 3 = calcium panthothenate; 4 = riboflavin; 5 = vitamin B_{12}; 6 = thiamine.

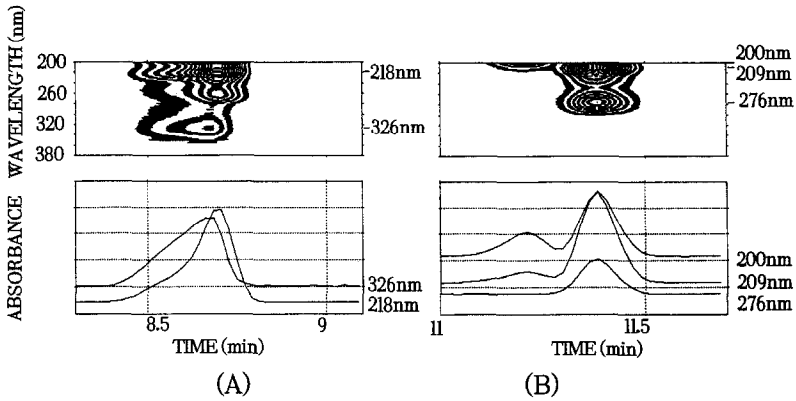

Fig. 8. Investigation of the peaks in Fig. 7 by the contour plot method. (A) Extended aborption curves at 218 and 326 nm and the contour plot for peak 1. (B) extended aborption curves at 200, 209, and 276 nm and the contour plot for peaks 2 and 3.

The time difference between the apices of the peaks of these two components was 1.8 s. Spectra of this double peak slightly changed depending on the retention time observed; however, detection at a single wavelength only suggested the posibility of a mixed zone by the shape of the leading edge of the peak. Fig. 8B shows the contour plots of two components, peaks 2 and 3, which were eluted closely together but with symmetrical peak shapes. In this case, however, the pH of the buffer solution was changed from 9.0 to 7.7 after 12 h operation time. At low salt concentration and a pH value different from the pK value of the buffer, reproducible separations cannot be expected. Therefore, to avoid tailing of the thiamine peak, a 50 mM Tris–borate buffer was used with low electric conductivity and a relatively high salt concentration for high pH. These results show the feasibility of using the SPD-M6A UV–VIS spectrophotometric detector in HPCE. This detection system takes advantage of full UV spectrum scanning with the concomitant benefits of contour plotting of spectra for rapid and accurate characterization of complex samples.

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