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Separation of antibiotics by high-performance capillary electrophoresis with photodiode-array detection

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ABSTRACT

The separation of six antibiotics by high-performance capillary electrophoresis (HPCE) with UV photodiode-array detection is reported. The effect of pH on the separation was studied. Simultaneous detection at different wavelengths and characterization of separated components by spectral analysis were performed.

INTRODUCTION

Capillary zone electrophoresis (CZE) has developed into a high-efficiency separation technique for small samples of ionic species [1–6]. In CZE, ionic species separate under the influence of a high-voltage (15–40 kV) potential field based on their electrophoretic mobilities. Although high-performance liquid chromatography (HPLC) has been the conventional method used for the analysis of antibiotics, in recent years the use of capillary electrophoresis (CE) has assumed considerable importance [7,8]. However, the use of photodiode-array detection for the CE analysis of antibiotics has not been studied. Further, the study of antibiotics has usually involved only one family of the antibiotics, *e.g.*, the study of β-lactam antibiotics by Nishi *et al.* [7] and the separation and determination of aminoglycoside antibiotics by Gambardella *et al.* [8]. In this work, the separation of selected antibiotics of different types was investigated. The separation of these antibiotics has not previously been performed using either HPLC or HPCE. A photodiode-array detector was employed for detection at different wavelengths simultaneously.

EXPERIMENTAL

The experiments were performed on a laboratory-built HPCE system. A 15-kV laboratory-built power supply was used to deliver the necessary potential across the column. A fused-silica capillary tube (50 μm I.D. × 50 cm effective length) (Polymicro Technologies, Phoenix, AZ, USA) was used as the separation column. The peaks were detected with an SPD-M6A UV–VIS photodiode-array detector (Shimadzu, Kyoto, Japan) with accompanying software. The detector cell was modified as described by Kobayashi *et al.* [9].

Six antibiotics (Fig. 1) were purchased from Sigma (St. Louis, MO, USA) and sodium dihydrogenphosphate dihydrate and sodium tetrahydroborate from Fluka (Buchs, Switzerland). Buffer solution was prepared by dissolving sodium dihydrogenphosphate and sodium tetraborate in water purified with a Milli-Q system (Millipore, Bedford, MA, USA). The pH conditions studied were 6.07, 6.57, 7.07, 7.50, 8.09 and 8.50.

Samples were introduced by the gravity feed method. This was carried out by placing the tip of the separation column at the high-potential end in the sample solution at a level 8 cm higher than the buffer reservoir. The time for each injection was 8 s. The capillary end was then rinsed by dipping into

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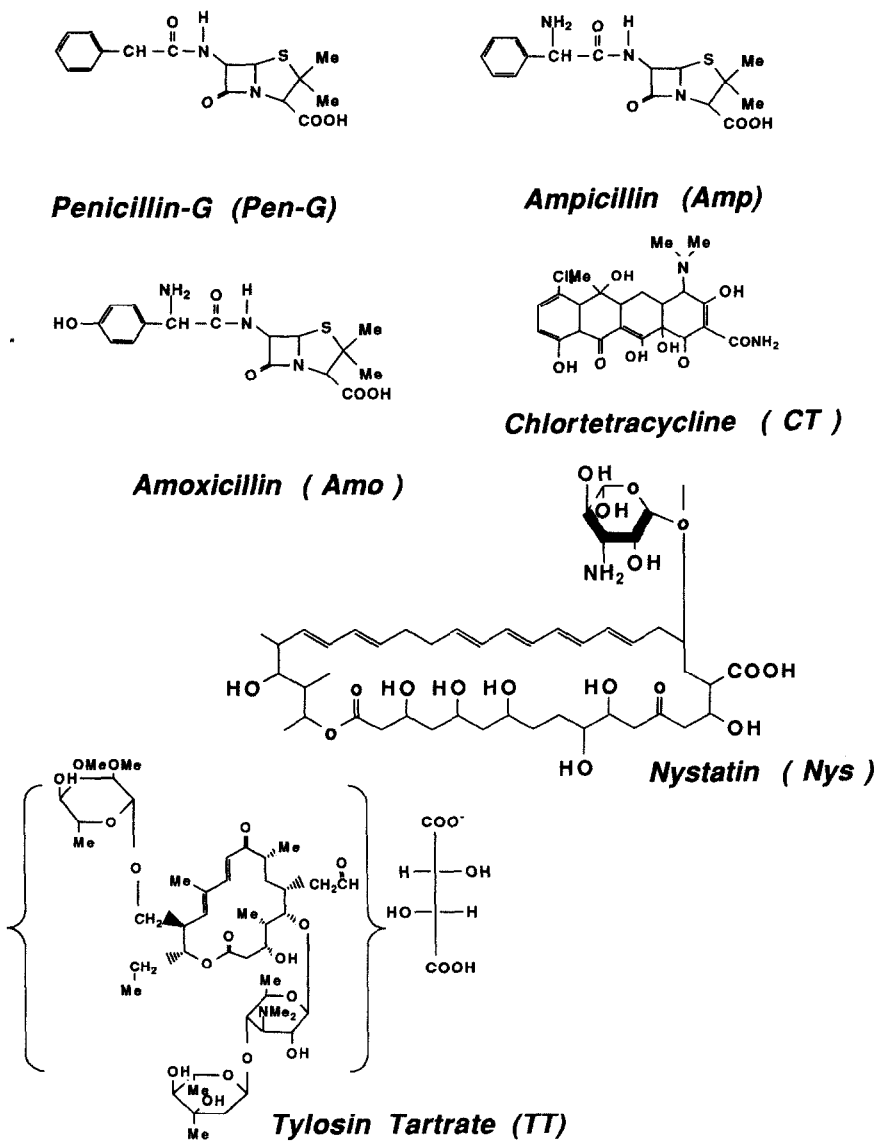

Fig. 1. Structures of the six antibiotics. Me = Methyl.

a rinsing solution of similar composition to the electrophoretic medium. The capillary end was then transferred back to the reservoir before the power supply was switched on. The amount injected by this method is estimated to be 3 nl per injection. The capillary column was flushed periodically with water. The relative standard deviation for the migration times was found to be 0.78% (n = 8). In all electrophoretic runs, methanol was used as the electroosmotic marker.

Solutions of the six antibiotics were prepared in methanol at the following concentrations: tylosin tartrate 2.5 mM, chlortetracycline 4.3 mM, amoxicillin 4.6 mM, pencillin G sodium 3.4 mM, ampicillin (sodium salt) 4.6 mM and nystatin 2.1 mM. As antibiotics degrade rapidly, all samples were kept in

a refrigerator when not in use and new solutions were prepared every few days.

A tablet obtained commercially, which was stated to contain 250 mg of ampicillin trihydrate, was analysed for the presence of the antibiotic. The tablet was pulverized and then 200 ml of methanol were added to extract the ampicillin present. After shaking the mixture for about 5 min, the suspension was filtered through a Whatman No. 1 filter-paper and the filtrate was evaporated by a rotatory evaporator (Eyela; Tokyo Rikakikai, Tokyo, Japan) at room temperature until white residue was observed. The white residue was dissolved in 100 ml of methanol, the solution obtained was filtered and 10 ml of methanol were used for rinsing. The resulting solution was analysed for ampicillin using the standard addition method.

RESULTS AND DISCUSSION

Results of the capillary electrophoretic experiments are shown in Fig. 2. The antibiotics migrated in the following order of increasing migration times: TT < NYS < AMO < AMP < CT < PEN-G. TT has the shortest migration time. The reason could be that it contains a tertiary amino group, which could be protonated under the pH conditions studied, resulting in it having a positive charge. As a result, both electrophoretic and electroosmotic flows would be in the same direction. Consequently, it migrated faster than methanol, which is neutral. The other five antibiotics migrated more slowly than methanol.

The above trend was observed at pH 7.07, 7.50 and 8.09. At pH 6.07, it was observed that CT migrated faster than AMO and AMP. This could be due to the fact that at lower pH, deprotonation of the OH groups is suppressed, resulting in CT being less negatively charged than AMO and AMP. At pH 8.50, CT migrated faster than AMP because the charge on CT can be delocalized over the four rings whereas the charge on AMP is not delocalized.

UV photodiode-array detection was found to be very useful in the identification of the antibiotic peaks. All six antibiotics absorb in the region 198–200 nm. Nystatin and tylosin tartrate were also found to absorb in the region 306–308 nm (peaks 1′ and 3′ in Fig. 3), whereas chlortetracyline absorbs in the region 388–390 nm (peak 6′ in Fig. 4). With the use of two different channels at the same time, the identification of these peaks would be unambiguous as they would appear in both electropherograms.

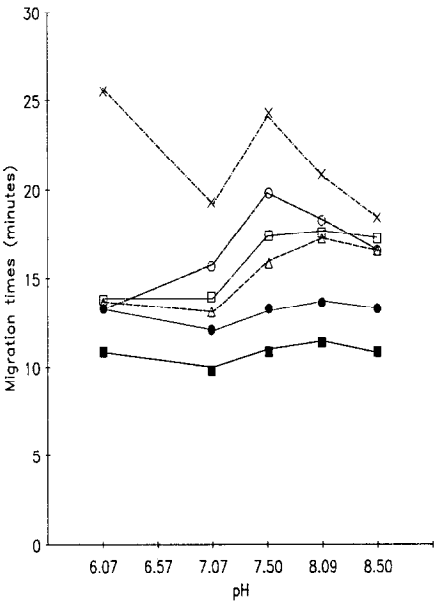

Fig. 2. Plot of migration time against pH conditions studied. ■ = TT; □ = AMP; △ = AMO; ○ = CT; ● = NYS; × = PEN-G.

To demonstrate the usefulness of the procedure,

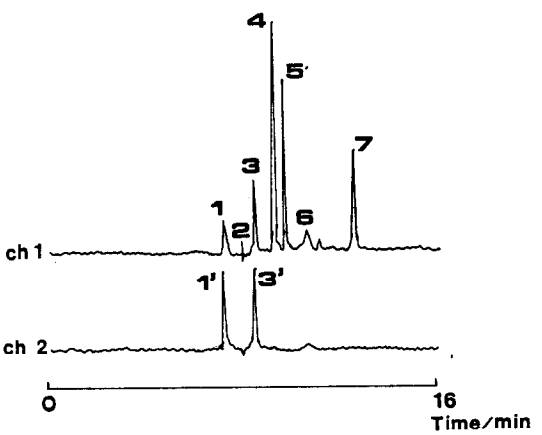

Fig. 3. Electropherogram of the six antibiotics. 1 = TT; 1′ = TT; 2 = methanol; 3 = NYS; 3′ = NYS; 4 = AMO; 5 = AMP; 6 = CT; 7 = PEN-G. Electrophoretic solution, 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.06); separations tube, 50 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV, 36 μA; detection wavelengths, channel 1 198–200 nm and channel 2 306–308 nm.

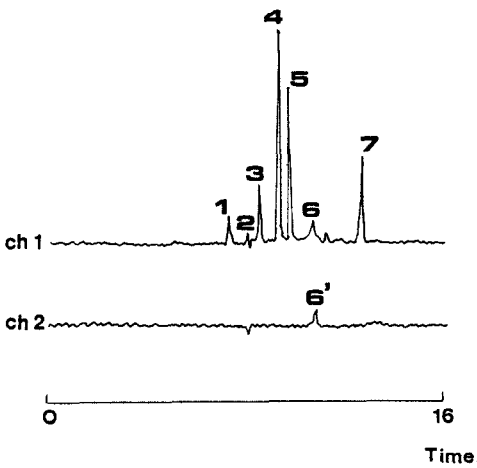

Fig. 4. Electropherogram of the six antibiotics. 1 = TT; 2 = methanol; 3 = NYS; 4 = AMO; 5 = AMP; 6 = CT; 6′ = CT; 7 = PEN-G. Electrophoretic solution, 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.06); separation tube, 50 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV, 36 μA; detection wavelengths, channel 1 198–200 nm and channel 2 380–390 nm.

a commercially available tablet was analysed for the presence of ampicillin. Fig. 5 is an electropherogram of the extracted sample of an ampicillin tablet together with the UV spectrum obtained on-line for ampicillin. A peak purity check (purity index = 0.9951) and the contour plot (Fig. 6) confirmed that ampicillin was the only antibiotic present in the tablet. It was found that the amount of ampicillin present was 220 mg [relative standard deviation =

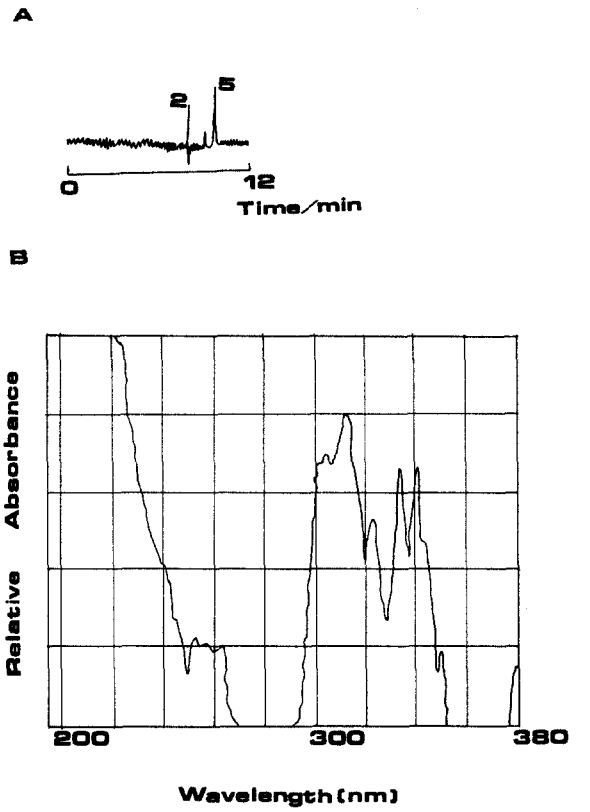

Fig. 5. (A) Electropherogram of ampicillin extract. (2) Methanol; (5) AMP. (B) Spectrum of ampicillin. Electrophoretic solution, 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.06); separation tube, 50 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV, 36 μA; detection wavelength, channel 1 198–200 nm.

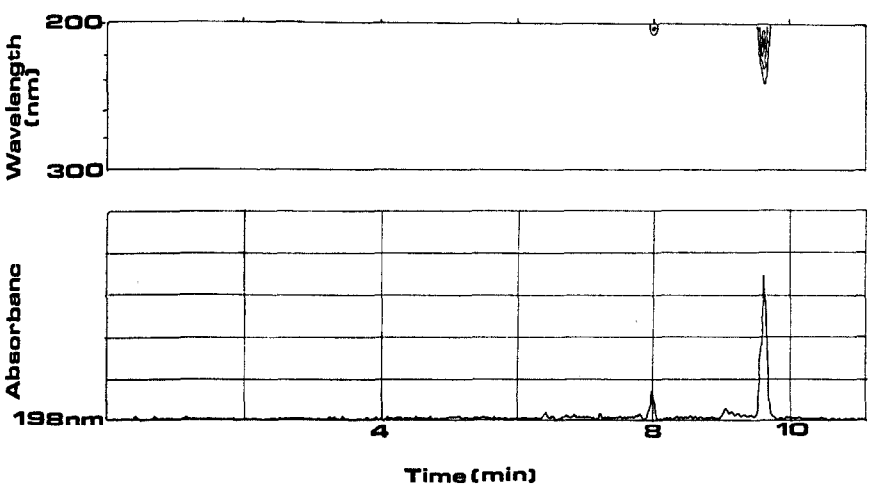

Fig. 6. Contour plot of ampicillin extract and its electropherogram at 198 nm.

9.6% ($n = 3$)]. Considering that for every 1.15 mg of trihydrate only 1 mg of ampicillin is present [10], the amount of ampicillin trihydrate present in the tablet was calculated to be 253 mg. This value agrees very well with the stated amount of 250 mg.

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