

# Electrochemical Behavior and Differential Pulse Polarographic Determination of Piperacillin Sodium

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In an aqueous piperacillin sodium solution, a well-defined single wave or single peak was observed by direct current(DC) polarography or differential pulse polarography(DPP). The peak potential change per pH unit was -54 mV in the phosphate buffer at 18°C, which indicated that protons were involved in the electrochemical reduction of the 2,3-dioxopiperazine moiety of piperacillin sodium with a  $H^+/e^-$  ratio of one. Using a phosphate buffer of pH 4.3, the  $1.0 \times 10^{-7}$  M piperacillin sodium single peak could be determined by DPP with relative standard deviation of 1.6%(n=3). Piperacillin sodium could be analyzed without interference from penicillin G-potassium, which enabled the employment of DPP as a fast and simple technique for monitoring the synthetic process of the antibiotic.

**Key words:** Differential pulse polarography, Cyclic voltammetry, Piperacillin sodium

## INTRODUCTION

Antibiotics are low-molecular-weight microbial metabolites that inhibit the growth of other microorganism in low concentrations (Lancini *et al.*, 1995). Chemically, antibiotics possess many organic functional groups including hydroxyl, carboxyl, carbonyl and nitrogen functions, etc.. Antibiotics with electrochemically reducible functional groups in their structure such as chloramphenicol (Hahn and Jeon, 1992), tetracyclines (Hahn, 1982), bacitracin (Jacobsen and Pederstad, 1977) and norfloxacin(Jaber and Lounici, 1994) have been determined directly by differential pulse polarography (DPP). However, most of the penicillin antibiotics have been indirectly determined. This is because the penicillin backbone (a four-membered  $\beta$ -lactam ring fused to a five-membered sulfur-containing thiazolidine ring) is not electrochemically reducible. Nitrosation or alkaline cleavage of the  $\beta$ -lactam ring has thus been used prior to polarographic determinations of the penicillin antibiotics (Siegerman, 1979). Among the penicillin antibiotics, ampicillin and amoxycillin have been determined by forming their respective nickel complexes and measuring their electrochemical properties by DPP (Lyle and Yassin, 1993). Penicillin-V in a fermentation broth has been determined by flow-injection analysis based on measuring

the pH change caused by the enzymatic hydrolysis of penicillin to penicilloic acid(Meier and Tran-Minh, 1992 ; Carlsen, *et al.*, 1993). Reversed-phase high performance liquid chromatography (HPLC) with pulsed amperometric detection has been used to indirectly monitor several types of penicillin by carefully matching the detector waveform with the chromatographic mobile phase (Kirchmann and Welch, 1993).

Piperacillin sodium is semisynthetic penicillin derived from penicillin G, which has been used therapeutically against various bacterial infections including septicemia and bronchitis. Piperacillin sodium has been officially determined by a microbial assay using *pseudomonas aeruginosa* NCTC 10490 and a spectrophotometric method that measures the absorbance of the product at 490 nm produced by reacting the antibiotic with hydroxylamine HCl and ferric ammonium sulfate(Ministry of Health and Welfare, 1986). The official microbial assay requires at least one day to measure the potency of the antibiotic and the spectrophotometric method also needs some pretreatment. Therefore, in the production process, these methods cannot satisfy the time limits required differentiating piperacillin sodium from penicillin G. Recently capillary electrophoresis(CE) was adapted for the separation and quantification of piperacillin from tazobactam in the drug(Pajchel and Tyski, 1999) and was compared with the validated HPLC chemical method. Both methods used UV detection. The linearity of CE was confirmed for piperacillin concentrations within the range 0.08~2 mg/ml with a detection limit of 0.01 mg/ml, which is compara-

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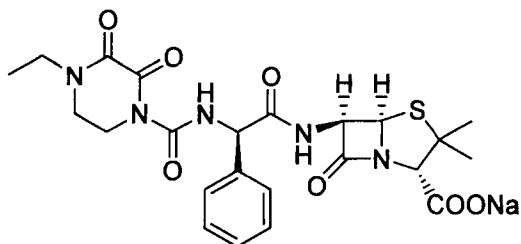


Fig. 1. Chemical structure of piperacillin sodium

tively high due to the low molar absorptivity. The chemical structure of piperacillin sodium contains the penicillin backbone with a (4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido side chain (Fig. 1). Since piperacillin sodium contains an electrochemically reducible dioxo group and has not been reported for its electrochemical property, the electrochemical behavior was investigated to develop a differential pulse polarographic analytical procedure for piperacillin sodium, which can be used to monitor the synthetic process.

## MATERIALS AND METHODS

### Instruments

Electrochemical measurements were done using a 303 Static Mercury Drop Electrode attached to a 174A Polarographic Analyzer (EG & G Princeton Applied Research Company) with a RE0074 Omnigraphic X-Y recorder.

### Chemicals and solutions

Piperacillin sodium and penicillin G potassium were supplied by the Cheiljedang Corporation and Chong Kun Dang Pharmaceutical Company. All other chemicals were extra pure grade. The phosphate buffer solutions were prepared by mixing 0.10 M sodium phosphate dibasic (Shinyo Pure Chem. Co., Ltd.) and 0.10 M potassium phosphate monobasic (Oriental Chem. Ind.) to adjust the appropriate pH values. The 0.10 M tartarate buffer solutions were prepared by dissolving tartaric acid (Junsei Chem. Co., LTD.) in distilled water and adjusting the pH value with ammonia (Junsei Chem. Co., LTD.). The 0.10 M acetate buffer solutions were prepared similarly using sodium acetate trihydrate (Shinyo Pure Chem. Co., Ltd.) and glacial acetic acid (Duksan Pharm. Co., LTD.).

### Procedure

For direct current (DC) and differential pulse (DP) polarographic analysis, approximately 10-ml of the test solutions were placed in a glass cell bottom and deoxygenated for 10 minutes using purified  $N_2$  gas. The usual experimental conditions were as follows; a medium mercury drop size (area;  $0.015 \text{ cm}^2$ ), a scan rate of  $-5 \text{ mV/s}$  (or  $-2 \text{ mV/}$

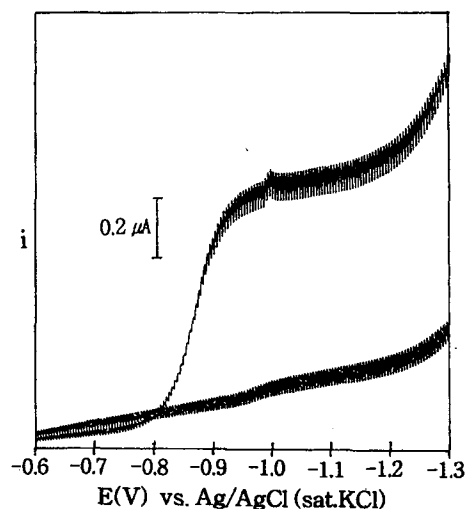


Fig. 2. A DC polarogram of  $0.88 \times 10^{-4} \text{ M}$  piperacillin sodium in a pH 3.4 phosphate buffer. drop size: medium ( $A=0.015 \text{ cm}^2$ ), drop time: 1 sec, scan rate:  $-5 \text{ mV/sec}$

s) and a modulation amplitude of 25 mV (or 50 mV) were used for DPP.

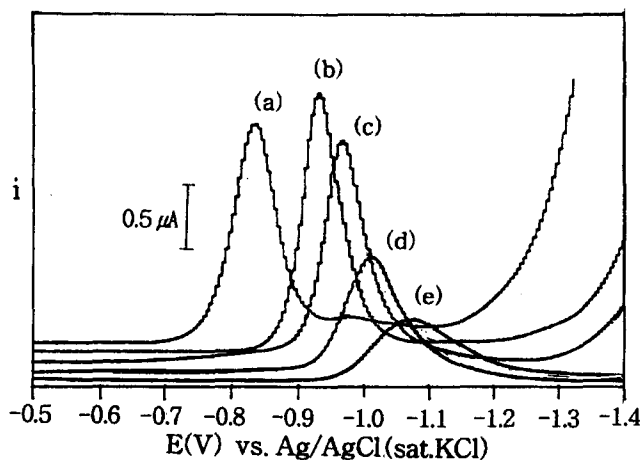
## RESULTS AND DISCUSSION

### DC polarography of piperacillin sodium

The  $0.88 \times 10^{-3} \text{ M}$  piperacillin sodium solutions, prepared with three different buffers (pH 3.4 phosphate buffer, pH 6.5 acetate buffer and pH 9.0 tartarate buffer), showed well defined single waves at a dropping mercury electrode (DME) in the potential range  $-0.3 \text{ V}$  to  $-1.8 \text{ V}$  (referenced to a Ag/AgCl in sat. KCl, electrode at room temperature,  $18^\circ\text{C}$ ). The half-wave potentials were shifted toward the negative directions as the pH values of the media were increased indicating the involvement of protons in the electrochemical reduction of the piperacillin sodium. The  $E_{1/2}$  change per pH unit was  $-40 \text{ mV}$ , which was smaller than the theoretical value ( $-58 \text{ mV}$  at  $18^\circ\text{C}$ ) considering a  $H^+/e^-$  ratio of one. The smaller  $E_{1/2}$  change per pH unit might be due to different interactions between the antibiotic and the different supporting electrolytes. The Fig. 2 shows the DC polarogram of  $0.88 \times 10^{-4} \text{ M}$  piperacillin sodium in a pH 3.4 phosphate buffer at  $24^\circ\text{C}$ .

### DPP of piperacillin sodium

The DP polarograms of  $1.0 \times 10^{-5} \text{ M}$  piperacillin sodium obtained using various pH phosphate buffers at room temperature ( $18^\circ\text{C}$ ) are shown in Fig. 3. The peak potential ( $E_p$ ) and peak current ( $I_p$ ) data are listed in Table I. As the pH increased, the  $E_p$  moved toward the negative directions. The  $E_p$  change per pH unit was  $-54 \text{ mV}$  between pH 3.02 and 7.00 with a correlation coefficient ( $r$ ) of 0.993. The experimental value was very close to the



**Fig. 3.** DP polarograms of  $1.00 \times 10^{-5}$  M piperacillin sodium at various phosphate buffer pH. pH: (a) 3.02 (b) 4.18 (c) 5.05 (d) 6.00 (e) 7.00 drop size: medium ( $A=0.015$  cm<sup>2</sup>), drop time: 1 sec, scan rate: -5 mV/sec, modulation amplitude: 25 mV

**Table I.** DPP of  $1.0 \times 10^{-5}$  M piperacillin sodium in phosphate buffers

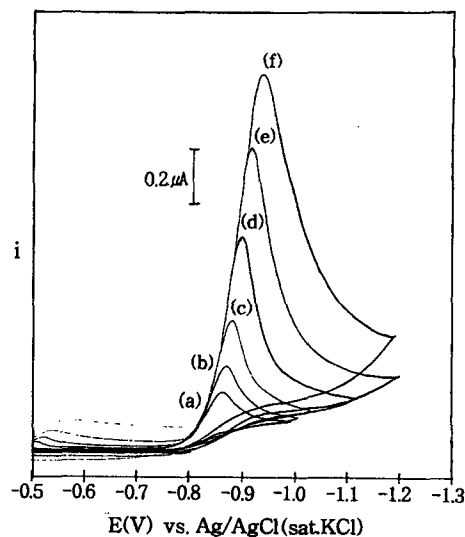
pH	$E_p$ (V)	$I_p$ ( $\mu$ A)
3.02	-0.840	1.675
4.18	-0.925	1.950
5.05	-0.970	1.700
6.00	-1.010	0.950
7.00	-1.060	0.450
8.00	-1.225	0.300

drop size: medium ( $0.015$  cm<sup>2</sup>), drop time: 1sec, scan rate: -5 mV/sec, modulation amplitude: 25 mV

theoretical  $\Delta E_p/pH$  of -58 mV at 18°C, confirming  $H^+/e^-$  ratio of one. The peak currents in the pH 3.02~5.05 buffers were greater than the  $I_p$  values in more alkaline solutions. This means that hydrogenation is involved in the electrochemical reduction step. The DP data obtained with acetate and tartarate buffer solutions showed similar patterns. However, the  $\Delta E_p/pH$  was -66 mV with the tartarate buffer (pH 3.0~7.0), and -49 mV with acetate buffer (pH 3.5~6.5). Although the buffer constituents appear to interact with the antibiotic, it still may be concluded that a proton is involved in the electrochemical reduction of piperacillin sodium with a  $H^+/e^-$  ratio of one.

#### Cyclic voltammetry(CV) of piperacillin sodium

The cyclic voltammograms of  $0.88 \times 10^{-4}$  M piperacillin sodium at a hanging mercury drop electrode(HMDE) are shown in Fig. 4. The cathodic peak potentials( $E_{pc}$ ) and peak currents( $I_{pc}$ ) at different scan rates ( $v$ ) are listed in Table II. The  $E_{pc}$  change and the linear plot of  $I_{pc}$  vs.  $v^{1/2}$  indicates the prevailing diffusion-controlled process in the electrochemical reduction of piperacillin sodium at



**Fig. 4.** CV of  $0.88 \times 10^{-4}$  M piperacillin sodium in a pH 3.4 phosphate buffer. scan rate: (a) -5 (b) -10 (c) -20 (d) -50 (e) -100 (f) -200 mV/sec, drop size: medium ( $A=0.015$  cm<sup>2</sup>)

**Table II.** CV of  $0.88 \times 10^{-4}$  M piperacillin sodium

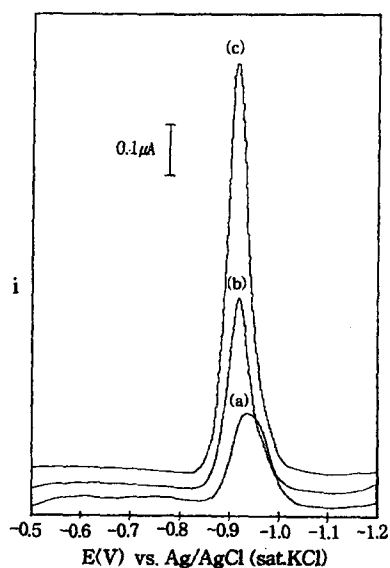
$v$ (mV/sec)	$E_{pc}$ (V)	$I_{pc}$ ( $\mu$ A)
5	-0.860	0.23
10	-0.865	0.33
20	-0.875	0.50
50	-0.890	0.82
100	-0.905	1.16
200	-0.920	1.44

supporting electrolyte: pH 3.4 phosphate buffer, drop size: medium ( $0.015$  cm<sup>2</sup>)

the HMDE. No anodic wave was observed at the experimental scan rates. In conjunction with the DPP studies of piperacillin sodium at different pH media, it may be proposed that one of the adjacent carbonyl groups in the side chain is reduced to a hydroxyl group via a  $2e^-/2H^+$  step. This is subsequently converted to the electro-inactive species by a chemical process.

#### Cablibration and precision studies

Using a phosphate buffer solution (pH 4.3), DP polarograms of piperacillin sodium at the concentration range between  $1.0 \times 10^{-7}$  M and  $1.0 \times 10^{-5}$  M were obtained yielding the results shown in Fig. 5 and Table III. As can be seen by the figure, the  $E_p$  of the  $1.0 \times 10^{-7}$  M piperacillin sodium solution was shifted to a negative potential by 20 mV. A concern was that piperacillin at higher concentrations might partially adsorb on the mercury electrode. However, the correlation between the  $I_p$  and the concentration was excellent ( $r=0.999$ ), which allows quantification of the antibiotic from the calibration curve. At a piperacillin



**Fig. 5.** DP polarograms of piperacillin sodium at the lower concentrations. (a)  $1.0 \times 10^{-7}$  M (b)  $5.0 \times 10^{-7}$  M (c)  $1.0 \times 10^{-6}$  M. drop size: medium ( $A=0.015 \text{ cm}^2$ ), drop time: 1 sec, scan rate:  $-2 \text{ mV/sec}$ , modulation amplitude:  $50 \text{ mV}$

**Table III.** Calibration and precision studies of piperacillin sodium

concentration(M)	$E_p(\text{V}, n=3)$	$I_p(\mu\text{A}, n=3)$
$1.0 \times 10^{-7}$	$-0.944 \pm 0.004$	$0.183 \pm 0.003$
$5.0 \times 10^{-7}$	$-0.923 \pm 0.003$	$0.382 \pm 0.003$
$1.0 \times 10^{-6}$	$-0.925 \pm 0.000$	$0.820 \pm 0.000$
$5.0 \times 10^{-6}$	$-0.918 \pm 0.003$	$3.150 \pm 0.000$
$1.0 \times 10^{-5}$	$-0.922 \pm 0.003$	$5.642 \pm 0.014$

$E_p$  and  $I_p$  values are ave.  $\pm$  S.D. for 3 measurements. supporting electrolyte: pH 4.3 phosphate buffer, drop size: medium ( $A=0.015 \text{ cm}^2$ ), drop time: 1sec, scan rate:  $-2 \text{ mV/sec}$ , modulation amplitude:  $50 \text{ mV}$ .

concentration of  $1.0 \times 10^{-7}$  M, the relative standard deviation (RSD) of the  $I_p$  was found to be 1.6%, but it was difficult to detect the antibiotic at concentrations  $\leq 5.0 \times 10^{-8}$  M. At higher concentrations the RSDs were  $<0.8\%$ , indicating excellent precision. When compared with the detection limit of  $0.01 \text{ mg/ml}$  ( $=1.85 \times 10^{-5}$  M) by the UV

**Table IV.** DPP data of the antibiotic mixture

volume ratio(ml)		experimental $I_p(\mu\text{A})$ of the antibiotic mixture	calculated $I_p(\mu\text{A})$ as piperacillin sodium
$0.88 \times 10^{-5}$ M piperacillin sodium	$0.99 \times 10^{-5}$ M penicillin G potassium		
10	0	1.35	1.35
8	2	1.09	1.08
5	5	0.70	0.68
3	7	0.41	0.41
0	10	0.02	0.00

drop size: medium ( $A=0.015 \text{ cm}^2$ ), drop time: 1 sec, scan rate:  $-5 \text{ mV/sec}$ , modulation amplitude:  $25 \text{ mV}$

method (Pajchel and Tyski, 1999), the present method is 185 times more sensitive.

### No Interference from penicillin G potassium

Piperacillin sodium is chemically synthesized from penicillin G potassium, the starting antibiotic. In order to monitor the synthetic process, the concentration of piperacillin sodium should be monitored without interference from penicillin G potassium over a short time period. As shown in Table IV, piperacillin sodium and penicillin G potassium at concentrations of  $0.88 \times 10^{-5}$  M and  $0.99 \times 10^{-5}$  M, respectively, were mixed with different volume ratios. The DPP of the antibiotic mixture yielded peak currents corresponding to piperacillin sodium only. According to *Standards and assay methods of antibiotics* (Ministry of Health and Welfare, Korea, 1986), the concentration of ampicillin in piperacillin sodium should be  $<0.5\%$ . Since a hydrogen of the phenyl acetamido moiety in penicillin G is replaced by an amino group in the chemical structure of ampicillin, DPP will thus ignore the presence of ampicillin. Therefore, DPP can be applied as a fast and simple technique for determining the concentration as well as monitoring the synthetic process of piperacillin sodium without a long incubation time and pretreatment procedure.

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