Arch Pharm Res Vol 27, No 1, 31-34, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Electrochemical Behavior and Square Wave Voltammetric Determination of Doxorubicin Hydrochloride

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(Received September 20, 2003)

The electrochemical behavior of doxorubicin hydrochloride was investigated by cyclic voltammetry (CV) and square wave voltammetry (SWV). From CV and SWV studies of doxorubicin hydrochloride in the acetate buffers of various pH values, it was found that protons were involved in the reduction of the antibiotic at the H^+/e^- ratio at one ($\Delta Ep/pH$ = -53 ~ -61 mV at 23°C), proposing the electrochemical reduction of the quinone moiety in its anthraquinone aglycone. Its electrochemical behavior was pseudo-reversible in the acetate buffer of pH 3.5 by exhibiting the well-defined single cathodic and anodic waves and the ratio of Ip^a/Ip^c at approximately one over the scan rates of 10~100 mV/s. Fast and sensitive SWV showing a single peak of doxorubicin has been applied for its quantitative analysis using an acetate buffer of pH 3.5. A linearity was obtained when the peak currents (Ip) were plotted against concentrations of doxorubicin in the range of 5.0×10^{-7} M~1.0×10^{-5} M with a detection limit of 1.0×10^{-7} M.

Key words: Doxorubicin hydrochloride, Cyclic voltammetry, Square wave voltammetry

INTRODUCTION

Doxorubicin is an anthracycline antibiotic isolated from *Streptomyces peucetius var. caesius* and has been clinically used in treatment of patients with leukaemias and tumors in the lung, or the breast. The anthracycline antibiotics are generally composed of the aminosugar linked to the anthraquinone aglycone as shown in the chemical structure of doxorubicin (Fig. 1). The sensitive and accurate techniques are required to control chemotherapy of doxorubicin, or other anticancer anthracyclines. For the assay of doxorubicin, the official methods (USP, 2000; EP, 1997) employ HPLC with UV detector set 254 nm. The Korean official assay methods for doxorubicin include the spectrophotometric method measured at 495 nm as well as a microbial assay using *Bacillus subtilis* ATCC 6633 (KFDA, 2000). Recently, the papers concerning the analysis of anthracycline contents in biological samples, or in pharmaceutical preparations have been reviewed (Zagotto *et al.*, 2001). Visible spectrophotometric methods (Sastry and Rao, 1996) and HPLC with fluorescence

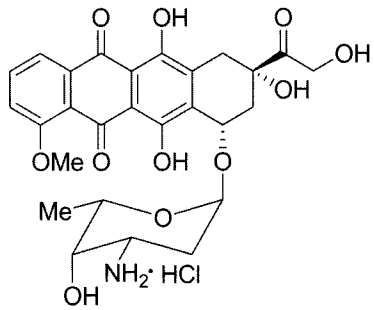

Fig. 1. The structure of doxorubicin hydrochloride

detector (Buehler *et al.*, 1999; Alvarez-Cedron *et al.*, 1999) have been employed for the determination of doxorubicin in pharmaceutical formulations, or in biological samples. Electrochemical detection combined with capillary zone electrophoresis (CE) (Hu. *et al.*, 2000), or with HPLC (Ricciarello *et al.*, 1998) has been developed for the determination of anthracyclines in biological fluids. The detecting system connected to CE was a carbon disk working electrode with an applied potential of 0.95 V vs. a Ag/AgCl (3 M KCl), which measured anodic currents due to the oxidation of two phenolic hydroxyls in the aglycone of daunorubicin (Hu *et al.*, 2000). Meanwhile, reductive detecting system measured at -0.30 V was preferred to oxidation in which the chromatographic profile suffered

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severe interference from substances that result from the biological matrix (Ricciarello *et al.*, 1998). Electrochemical assay often offers selectivity and sensitivity due to the selective detection of electroactive species among the complex samples. The chemical structure of doxorubicin contains a electrochemically a reducible quinone moiety in the aglycone which prompted us to study its electrochemical behavior by using mercury electrodes, followed by developing the fast and sensitive square wave voltammetric (SWV) procedure for the determination of doxorubicin hydrochloride in the present study.

MATERIALS AND METHODS

Instruments

Electrochemical measurements were made using a static mercury drop electrode (SMDE; PAR Model 303A) connected to a digital voltammetric analyzer (PAR Model 394). The reference electrode and the auxiliary electrode were a Ag/AgCl (sat. KCl) and Pt-wire, respectively.

Chemicals and solutions

Doxorubicin hydrochloride (97%) was kindly supplied by Dong A Pharmaceutical Company. All other chemicals were extra pure grade. The 0.10 M acetate buffer solutions were prepared by dissolving sodium acetate trihydrate (Shinyo Co.) in pure water and adjusting the pH value with glacial acetic acid (Aldrich Chem. Co.).

Procedure

The 1×10^{-4} M doxorubicin solution was prepared by dissolving the accurate amount of the antibiotic weighed by the difference in the acetate buffer. The cyclic voltammetry (CV) and square wave voltammetry (SWV) was set up to use done using a hanging mercury drop electrode (HMDE). The electrode area was 0.0284 cm^2. The CV conditions were as follows: the initial potential of -0.1 V, the final potential of -1.0 V, and the varying scan rates of 10, 20, 40, 60, 80 and 100 mV/s. The SWV conditions were as follows: the initial potential of -0.1 V, a final potential of -1.0 V, frequency of 120 Hz, pulse height of 25 mV, and the scan increment of 2 mV.

RESULTS AND DISCUSSION

The CVs of doxorubicin in the acetate buffers of various pH values

As shown in Fig. 2, the CVs of doxorubicin in the acetate buffer of pH 3.5 showed the well defined cathodic and anodic waves due to the reduction of quinone moiety in its anthraquinone aglycone and the reoxidation of the hydroquinone moiety produced at the HMDE surface. Its

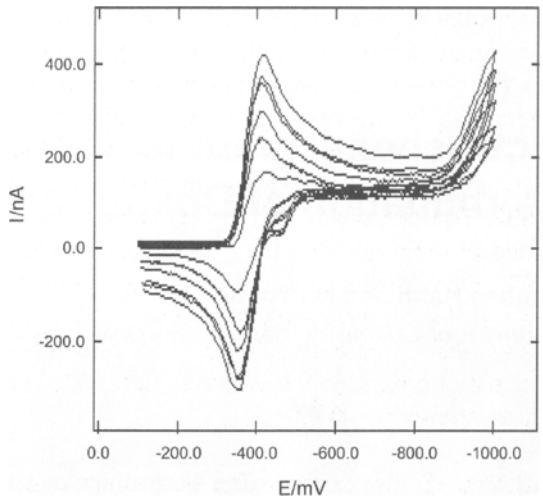

Fig. 2. The CVs of 1.0×10^{-4} M doxorubicin hydrochloride in the acetate buffer of pH 3.5. Working electrode: HMDE, Electrode area: 0.0284 cm^2, Equilibration: 20 sec, Initial potential: -0.1 V, Final potential: - 1.0 V, Temperature: 23°C. Scan rates: 10 mV/s, 20 mV/s, 40 mV/s, 60 mV/s, 80 mV/s, and 100 mV/s (As the scan rates were increased, Ip^c and Ip^a were increased).

electrochemical behavior was pseudo-reversible in the acetate buffer of pH 3.5. The CV data of doxorubicin in pH 3.5 and 4.5 media are listed in Table I and II. The reduced species of doxorubicin in the acetate buffer of pH 3.5 was stable by showing the ratio of Ip^a/Ip^c of approximately at one over the scan rates of 10~100 mV/s, while the ratio of

Table I. CV data of 1.0×10^{-4} M doxorubicin hydrochloride in a pH 3.50 acetate buffer with different scan rates

Scan rate (mV/s)	Cathodic		Anodic		ΔEp (mV)	Ip^a/Ip^c
	Ep^c(V)	Ip^c(μA)	Ep^a(V)	Ip^a(μA)		
10	-0.416	165.00	-0.349	215.00	67	1.30
20	-0.410	233.33	-0.357	286.67	53	1.23
40	-0.408	308.33	-0.351	341.67	57	1.11
60	-0.400	306.93	-0.349	396.04	51	1.29
80	-0.412	355.00	-0.357	400.00	55	1.13
100	-0.410	400.00	-0.349	433.33	61	1.08

Table II. CV data of 1.0×10^{-4} M doxorubicin hydrochloride in a pH 4.50 acetate buffer with different scan rates

Scan rate (mV/s)	Cathodic		Anodic		ΔEp (mV)	Ip^a/Ip^c
	Ep^c(V)	Ip^c(μA)	Ep^a(V)	Ip^a(μA)		
10	-0.484	203.64	-0.383	185.45	101	0.91
20	-0.468	280.70	-0.409	245.61	59	0.87
40	-0.468	350.00	-0.400	307.69	68	0.88
60	-0.472	394.74	-0.404	334.21	68	0.85
80	-0.476	463.16	-0.409	394.74	67	0.85
100	-0.470	480.45	-0.400	424.58	70	0.88

Ip^a/Ip^c was 0.9 in the supporting electrolytes of pH 4.5. As the pH values of supporting electrolytes were increased, the reduced species of doxorubicin became more unstable as indicated by the decreasing ratio of Ip^a/Ip^c shown in Fig. 3 and Table III. When the Ep^c was plotted vs. pH values, slope was -60.8 mV/pH with a correlation coefficient of -0.992. The plot of Ep^a against pH values yielded the the slope of -57.5 mV/pH with a correlation coefficient of -0.9997. From these data we confirmed protons were involved in the electrochemical reduction of doxorubicin and the ratio of H^+/e^- was one. The electroreduction mechanism of doxorubicin is proposed due to its quinone moiety as shown in Fig. 4.

The SWVs of doxorubicin in the acetate buffers of various pH values

The SWV of doxorubicin also showed the well-defined single peak which shifted towards negative potentials as the pH values of supporting electrolytes were increased as shown in Fig. 5. The plot of peak potentials (Ep) of doxorubicin vs. pH values of the supporting electrolytes resulted in linearity with a slope of -53.4 mV/pH (Fig. 6) confirming the proposed reduction mechanism. The peak

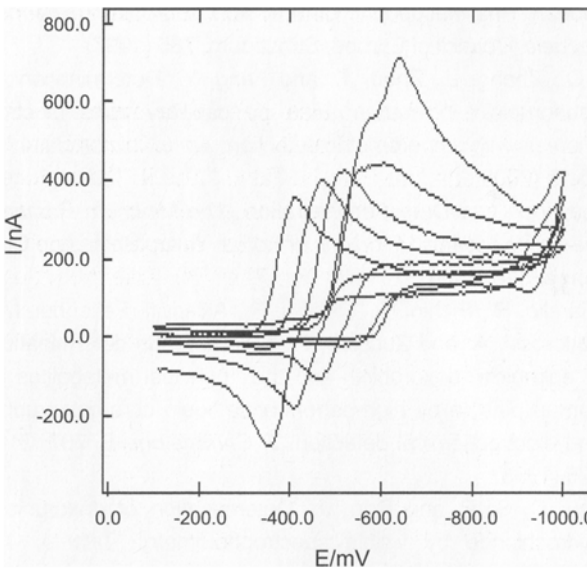

Fig. 3. The CVs of 1.0×10^{-4} M doxorubicin hydrochloride in the acetate buffers of different pH values. From left to right: pH 3.5, pH 4.5, pH 5.5, pH 6.5, pH 7.5. Working electrode: HMDE, Electrode area: 0.0284 cm^2, Equilibration: 20 sec, Initial potential: -0.1 V, Final potential: -1.0 V. Scan rate: 60 mV/s, Temp.: 23°C.

Table III. CV data of 1.0×10^{-4} M doxorubicin hydrochloride in the acetate buffers of different pH values. (scan rate = 60 mV/sec)

pH	Cathodic		Anodic		ΔEp (mV)	Ip^a/Ip^c
	Ep^c(V)	Ip^c(μA)	Ep^a(V)	Ip^a(μA)		
3.5	-0.400	306.93	-0.349	396.04	51	1.29
4.5	-0.472	394.74	-0.404	334.21	68	0.85
5.5	-0.514	424.00	-0.459	288.00	55	0.68
6.5	-0.604	428.57	-0.519	178.57	85	0.42
7.5	-0.638	670.39	-0.579	128.49	59	0.19

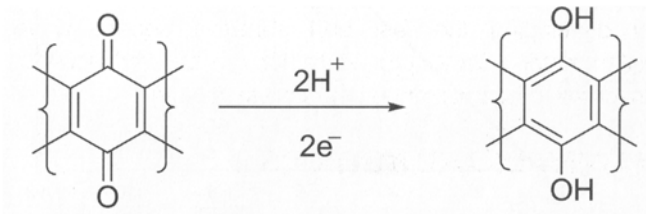

Fig. 4. Reduction mechanism of the quinone moiety in doxorubicin

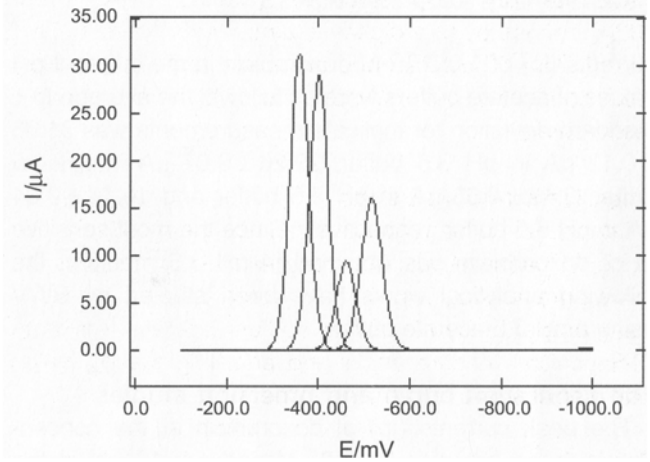

Fig. 5. The SWVs of 1.0×10^{-5} M doxorubicin hydrochloride in the acetate buffers of different pH values. From left to right: pH 3.5, pH 4.5, pH 5.5, pH 6.5. Working electrode: HMDE, Electrode area: 0.0284 cm^2, Equilibration: 20 sec, Initial potential: -0.1 V, Final potential: -1.0 V, Frequency: 120 Hz, Pulse height: 25 mV. Scan increment: 2 mV, Temp.: 23°C.

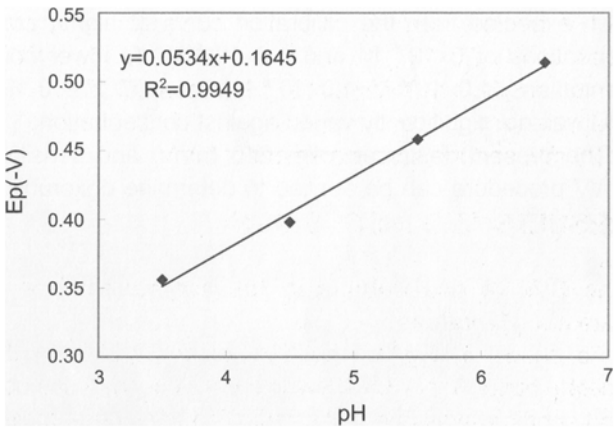

Fig. 6. The plot of peak potentials (Ep) of doxorubicin vs. pH values of the supporting electrolytes.

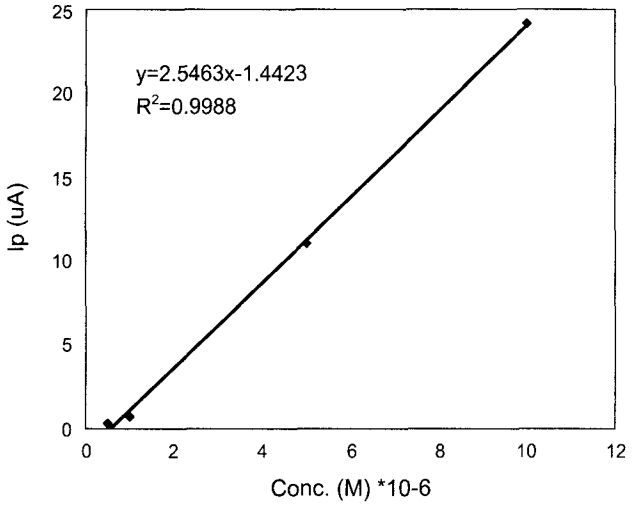

Fig. 7. The plot of peak currents vs. concentrations of doxorubicin hydrochloride in the acetate buffer of pH 3.5.

currents (Ip) of 1.0×10^{-5} M doxorubicin in the different pH values of acetate buffers were as follows: the average Ip ± standard deviation for triplicate measurements was 31.36 ± 0.12 μA in pH 3.5 buffer, 29.26 ± 0.07 μA in pH 4.5 buffer, 9.45 ± 0.03 μA in pH 5.5 buffer and 16.18 ± 0.04 μA in pH 6.5 buffer, respectively. Since the most sensitive Ip of doxorubicin was obtained in pH 3.5 medium, the following analytical works have been studied by SWV using a pH 3.5 acetate buffer.

The calibration curve and precision studies

The peak currents (Ip) of doxorubicin at the concentration range between 5.0×10^{-7} M and 1.0×10^{-5} M in the acetate buffer of pH 3.5 were measured by SWV and the calibration curve is shown in Fig. 7. For triplicate measurements at each concentration, the relative standard deviation ranged from 0.2% to 4%. The peak potentials (Ep) at these concentrations appeared at -0.354 ± 0.007 V. However, the Ep moved to more negative potentials of -0.385 V and -0.399 V, respectively and the Ip was less than expected from the calibration curve at higher concentrations of 5×10^{-5} M and 1.0×10^{-4} M. At lower concentrations (1.0×10^{-7} M~1.0×10^{-8} M), the Ip (0.218~0.190 μA) was not significantly varied against concentration.

The present, fast(scan rate; 240 mV/s) and sensitive SWV procedure can be applied to determine doxorubicin in the pharmaceutical preparations by simply dissolving the antibiotic in the acetate buffer of pH 3.5.

ACKNOWLEDGMENT

This work was supported by the academic research fund of The Institute of Natural Sciences of Sangmyung University in 2002.

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