Arch Pharm Res Vol 24, No 2, 100-104, 2001

Electrochemical Behavior and Differential Pulse Polarographic Determination of Rifampicin in the Pharmaceutical Preparations

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(Received December 10, 2000)

Differential pulse polarographic(DPP) analytical procedure for the rifampicin antibiotic, which can be applied to monitor its synthetic process from the starting antibiotic of rifamycin B or rifamycin SV, has been developed based on the electrochemical reduction of an azomethine group. Rifampicin exhibited a cathodic peak due to the azomethine group in the side chain of 3-[(4-methyl-1-piperazinyl)imino]methyl moiety and another cathodic peak due to the carbonyl group in rifamycin SV by DPP. The experimental peak potential shift of an azomethine reduction was -73 mV/pH in the pH range between 3.0 and 7.5, agreeing with involvement of 4 e^- and 5 H^+ in its reduction. By the cyclic voltammetric(CV) studies, the azomethine and the carbonyl reductions in rifampicin were processed irreversibly on the mercury electrode. The plot of peak currents vs. concentrations of rifampicin ranging 1.0×10^{-7} M~1.0×10^{-5} M yielded a straight line with a correlation coefficient of 0.9996. The detection limit was 1.0×10^{-8} M with a modulation amplitude of 50 mV. DPP has been successfully applied for the determination of rifampicin in the pharmaceutical preparations.

Key words: Differential pulse polarography, Cyclic voltammetry, Rifampicin

INTRODUCTION

Rifampicin ($C_{43}H_{58}N_4O_{12}$: F.W. 823: 3-[(4-methyl-1-piperazinyl)imino]methyl rifamycin SV) is a semisynthetic antibiotic which is chemically derived from rifamycin SV(Korea Food and Drug Administration, 2000; British Pharmacopoeia, 2000; European Pharmacopoeia, 1997). Rifamycin SV is a substance obtained by chemical transformation of rifamycin B, which is produced during the growth of certain strains of *Streptomyces mediterranei* or a substance isolated directly from a culture medium of certain *S. mediterranei* mutants (EP, 1997). Rifamycin B and rifamycin SV which are the parent antibiotics for the synthesis of rifampicin as well as rifamycin S and rifamycin O which are the impurities of rifamycin SV (BP, 2000) differ in the chemical structure from rifampicin such that rifamycin antibiotics do not contain the side chain of 3-[(4-methyl-1-piperazinyl)imino]methyl group. Rifampicin has been officially determined by a microbial assay, the usual method for measuring the potency of the antibiotics and a spectrophotometric method based on its reddish-brown color measured at 475 nm. A high performance liquid chromatography with a UV detector (254 nm) has been recently adopted as the analytical method for rifampicin (KFDA, 2000; EP, 1997; BP, 2000; USP, 2000). Other spectrophotometric methods have been reported for the analysis of rifampicin by forming a chelate with cupric ion or forming the charge-transfer complexation with halogenated quinones (Galal *et al.*, 1992). The detection limit of this method was 10 μg/ml(=1.2×10^{-5} M) of the antibiotic, which was relatively high. Rifampicin has also been determined spectrophotometrically in the presence of its main degradation products by using Glenn's method or first derivative method (Walash *et al.*, 1993). High performance thin layer chromatography(HPTLC) has been employed for monitoring rifampicin and its degradation products, of which quantities were measured by densitometry using deuterium lamp (Jindal *et al.*, 1994). The detection limit for each component was ca. 10 ng per band with application of 20 μl volume. HPTLC was also used for simultaneous determination of rifampicin with other drugs for the medication of tuberculosis (Argekar *et al.*, 1996). Rifamycin SV which is the core structure of rifampicin and other rifamycin B, O, S have been studied by DC pola-

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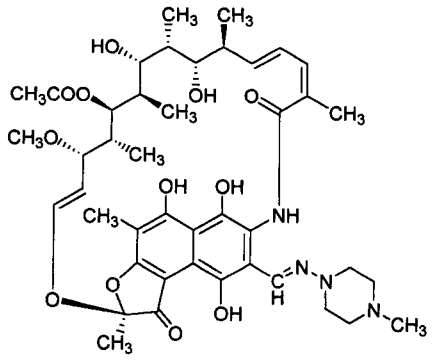

Fig. 1. The chemical structure of rifampicin

rography without information of their chemical structure (Gallo *et al.*, 1962). The electrochemical reductions of rifamycin antibiotics are thought to be due to carbonyl groups in the structure.

The chemical structure of rifampicin is shown in Fig. 1. It contains a -C=N- functional group in the side chain of 3-[(4-methyl-1-piperazinyl)imino]methyl moiety. The azomethine(-C=N-) group is generally more easily reducible than the carbonyl group from which it is derived (Lund, 1991) and has been the basis of an electrochemical assay method for the class of 1,4-benzodiazepine compounds (Blaedel and Hahn, 1979). Differential pulse polarography has been applied to monitor a semisynthetic antibiotic of piperacillin sodium in the presence of penicillin G potassium (Hahn and Son, 2000). Since an electroanalytical method based on the azomethine reduction has not been reported for rifampicin antibiotic, its electrochemical behavior was investigated to develop a differential pulse polarographic analytical procedure, which can be applied to monitor its synthetic process from the starting antibiotic of rifamycin B or rifamycin SV. The suitability of this method was also evaluated on the pharmaceutical preparations.

MATERIALS AND METHODS

Instruments

Electrochemical measurements were made using a 303 Static Mercury Drop Electrode attached to a 174A Polarographic Analyzer (EG & G Prinston Applied Research Company) with a RE0074 Omnigraphic X-Y recorder[or a 394 Digital Voltammetric Analyzer (EG & G PARC) with a pentimum computer].

Chemicals and solutions

Rifampicin was obtained from Chong Kun Dang Pharmaceutical Company (purity: 99.76%). 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.). The other buffer solutions were made similarly as follows; phos-phate buffer solutions with sodium dihydrogenphosphate dihydrate (Kanto Co.) and sodium hydroxide (Shinyo Co); tris buffer solutions with tris·HCl (Aldrich Chem. Co.) and sodium hydroxide; tartarate buffer solutions with tartaric acid (Junsei Chem. Co.) and ammonia (Shinyo Co.). Pure water was prepared by passing doubly distilled water through a Milli-Q II deionizer (Millipore). The 1.0×10^{-3} M rifampicin stock solution was prepared by dissolving 0.0082 g of rifampicin in 0.5 ml methanol (Carlo Erba) completely and diluting up to 10 ml with buffer.

Procedure

Standard solutions of rifampicin were prepared by diluting the 1.0×10^{-3} M rifampicin stock solution with buffer. For differential pulse polarography (DPP) and cyclic voltammetry (CV), 10 ml of test solution was placed in a glass cell bottom and deaerated for 10 min using purified N_2 gas before the measurement. The DPP measurement conditions were usually as follows; a medium mercury drop size (area; 0.016 cm^2), a scan rate of -5 mV/s (or -6 mV/s) and a modulation amplitude of 25 mV (or 50 mV) were employed. The CV measurement was performed using a hanging mercury drop electrode (HMDE; area=0.016 cm^2).

RESULTS AND DISCUSSION

DPP behavior of rifampicin in different supporting electrolytes

DPP studies of rifampicin which was carried out in the acetate buffers (pH 3.0~7.5), phosphate buffers (pH 4.5 ~7.5), tartarate buffers (pH 3.0~7.5) and tris buffers (pH 5.5~7.5) resulted in two main reduction peaks which shifted toward the more negative potentials as the pH of the solution increased. Among the fifteen supporting electrolytes, the acetate buffer of pH 4.5 yielded the highest peak current for rifampicin, which was well defined as shown in Fig. 2. DP polarograms of rifampicin obtained in the other acetate buffers (pH range 3.0~7.5) showed similar wave patterns with two distinct peaks. Table I shows DPP data of two main peaks of rifampicin obtained in the acetate buffer system. As shown in Fig. 3, the peak potential shift ($\Delta Ep/\Delta pH$) was -72.5 mV for the first peak and -52.3 mV for the second peak at room temperature. Similar results were obtained with the phosphate buffer system; $\Delta Ep/\Delta pH$ of the first peak was -73.3 mV, while that of the second peak was -53.3 mV. The DC polarographic studies of rifamycin B have shown that the half wave potentials ($E_{1/2}$) of the principle waves shifted toward the negative direction as the pH values of 50% methanol-50% 1 M acetate buffer media in-

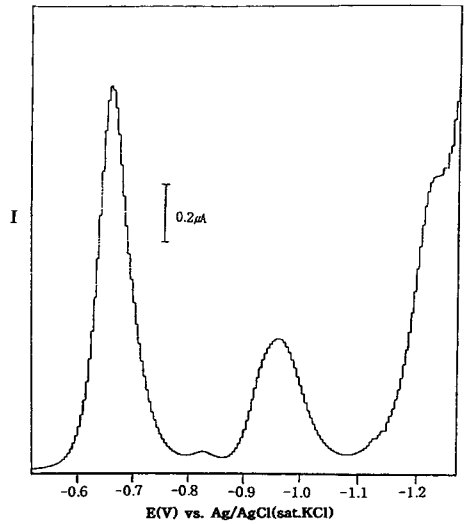

Fig. 2. DP polarogram of 1.0×10^{-5} M rifampicin in the acetate buffer of pH 4.5. drop size: medium (A=0.016 cm^2), drop time: 1 sec, scan rate: -5 mV/sec, modulation amplitude: 25 mV

Table I. DPP data of 1.0×10^{-5}M rifampicin in the acetate buffer

pH	Ep(V)	Ip ± SD(μA)(RSD)	$W_{1/2}$(mV)
3.0	Ep_1: -0.64	Ip_1: 1.46 ± 0.04(2.74%)	p_1: 81
	Ep_2: -0.93	Ip_2: 1.01 ± 0.01(0.99%)	p_2: 73
4.5	Ep_1: -0.70	Ip_1: 2.90 ± 0.00(0.00%)	p_1: 56
	Ep_2: -1.01	Ip_2: 0.78 ± 0.01(1.28%)	p_2: 92
5.5	Ep_1: -0.79	Ip_1: 1.97 ± 0.03(1.52%)	p_1: 70
	Ep_2: -1.08	Ip_2: 0.49 ± 0.01(2.04%)	p_2: 77
6.0	Ep_1: -0.83	Ip_1: 1.57 ± 0.04(2.55%)	p_1: 75
	Ep_2: -1.10	Ip_2: 0.37 ± 0.01(2.70%)	p_2: 75
7.5	Ep_1: -0.96	Ip_1: 1.46 ± 0.03(2.06%)	p_1: 102
	Ep_2: -1.16	Ip_2: 0.32 ± 0.00(0.00%)	p_2: 65

Ep: peak potential, Ip: peak current, SD: standard deviation, $W_{1/2}$: peak half width, Modulation amplitude: 25 mV, Drop time: 1s, Scan rate: -5 mV/s

creased (Gallo *et al.*, 1962). When the $E_{1/2}$ values of rifamycin B are converted to the Ep values, they agree with the Ep values of the second rifampicin peaks of our results within 10 mV in the pH range between 4 and 6. Thus, the second main peak of rifampicin is considered as the reduction peak of a carbonyl group which is contained in the structures of both rifampicin and rifamycin B. According to Lund's review (Lund, 1991), the reduction of azomethine compounds (RR'C=N-Y) is processed as follows, if Y is a heteroatom like 4-methypiperazine group in rifampicin.

$$RR'C{=}N\text{-}Y + H^+ \Leftrightarrow (RR'C{=}N\text{-}Y)H^+$$
$$(RR'C{=}N\text{-}Y)H^+ + 2e^- + 2H^+ \rightarrow RR'C{=}N^+H_2 + HY$$
$$RR'C{=}N^+H_2 + 2e^- + 2H^+ \rightarrow RR'CH\text{-}N^+H_3$$

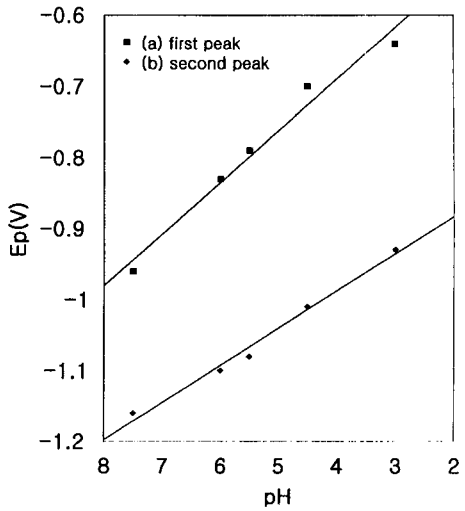

Fig. 3. The plots of peak potential vs. pH of the rifampicin solution. (a) the first peak, (b) the second peak

The ratio of H^+/e^- is 1.25 in the above mechanism and so a peak potential shift of -74 mV/pH is expected at 25°C. The experimental ΔEp/ΔpH of the first rifampicin peak agrees well with the theoretical ΔEp/ΔpH for the reduction of -C=N- in a 3-[(4-methyl-1-piperazinyl) imino]methyl group. If a carbonyl group is reduced, the ratio of H^+/e^- is one and the theoretical ΔEp/ΔpH is -59 mV at 25°C. The experimental ΔEp/ΔpH of the rifampicin second main peak confirms that the second main peak is due to the reduction of the -C=O group. In addition to the results, an azomethine group is generally more easily reducible than the carbonyl group (Lund, 1991), assigning the first peak as the azomethine reduction and the second peak as the carbonyl reduction is thought to be reasonable. The peak currents (Ip) listed in Table I are the average of three measurements within a day. The relative standard deviation of Ip are ranged between 0.00%~2.74%. The first peak was more sensitive than the second peak in every pH of buffer. And a pH 4.5 acetate buffer yielded the highest peak current with high precision which could be utilized for the quantitative analysis of rifampicin.

CV behavior of rifampicin

The CVs of 1.0×10^{-4} M rifampicin in the acetate buffer of pH 3.0 were run in the potential range between -0.40 and -1.3 V. Although cathodic peaks were observed, no anodic peak was observed at any scan rate ranging 20 and 500 mV/s, which indicated the reduced forms of rifampicin were electrochemically irreversible. The first and the second cathodic peaks which appeared at the scan rates ranging 20~200 mV/s were combined to one peak at the scan rate of 500 mV/s (Fig. 4a). When three consecutive CVs were run from -0.40 to -0.95 V at the

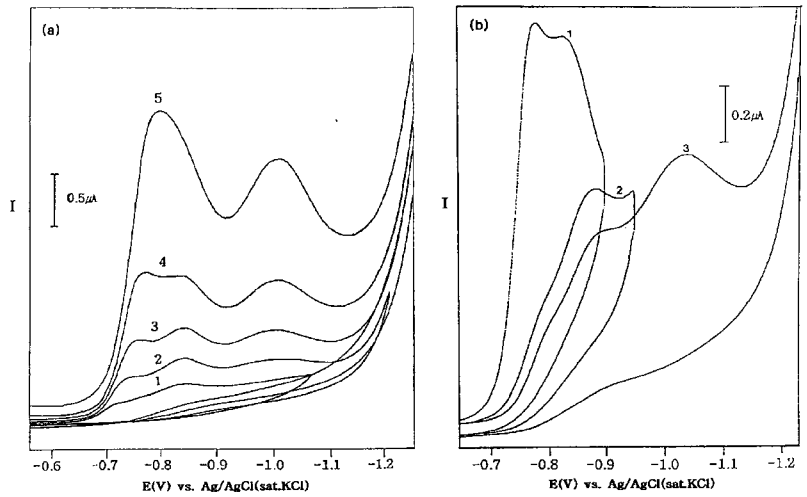

Fig. 4. Cyclic voltammograms of 1.0×10^{-4} M rifampicin in the acetate buffer of pH 3.0. a: at different scan rates of 1: 20 mV/sec, 2: 50 mV/sec, 3: 100 mV/sec, 4: 200 mV/sec, 5: 500 mV/sec b: at different cycles of 1: 1st cycle, 2: 2nd cycle, 3: 3rd cycle, scan rate: -200 mV/sec

scan rate of 100 mV/s, the first and the second cathodic peaks disappeared from the second cycle as well as no anodic wave was observed at any cycle, concluding an irreversible electrode process of the RR'CH-N^+H_3 form in rifampicin. Fig. 4b shows three consecutive CV cycles which were scanned at 200 mV/s and were reversed at the different potentials. When the first CV was reversed at -0.83 V, the anodic wave was not shown. In the second cycle ranged -0.4~-0.95 V, the first cathodic peak disappeared but the second cathodic peak appeared assuming due to reduction of the RR'C=N^+H_2 form in a rifampicin molecule. No anodic wave was shown in the second cycle, either. In the third cycle ranged -0.4~-1.3 V, the intact third cathodic peak and the less profound second cathodic peak, but no anodic wave appeared. Thus the reduction of carbonyl group in rifampicin is also considered as an irreversible electrode process. When three consecutive CVs were run in the potential range between -0.4 and -1.3 V at the scan rate of 500 mV/s, the combined first and second peak as well as the third peak decreased rapidly as the cycles increased. From the above CV results of rifampicin, it may be explained that the first and the second cathodic peaks are originated from the two steps of 2 e^- reduction of an azomethine group and the third peak results from the electrochemical reduction of carbonyl group.

Ip vs. concentrations of rifampicin

Because the acetate buffer of pH 4.5 yielded the most sensitive current for rifampicin as shown in Table I, it was chosen as the supporting electrolyte. The plot of Ip vs. concentrations of rifampicin ranging 1.0×10^{-7} M 1.0×10^{-5} M yielded a straight line with a correlation coefficient of 0.9996 as shown in Fig. 5. The calibration curve was plotted with the first peaks of DPP obtained with a modulation amplitude(MA) of 25 mV. Fig. 6 shows the DP polarograms of rifampicin at the lower concentrations (1.0×10^{-8} M~6.0×10^{-7} M) in the acetate buffer of pH 4.5 based on the first peak. However, it was difficult to detect the antibiotic at the concentrations < 1.0×10^{-8} M, even with a MA of 50 mV. The lower detection limit than that of piperacillin sodium (Hahn and Son, 2000) was expected because 4 e^- was involved in the first DPP peak of rifampicin which was attributed to the electrochemical reduction of an azomethine group.

DPP determination of rifampicin in the pharmaceutical preparations

The contents of rifampicin capsules manufactured by

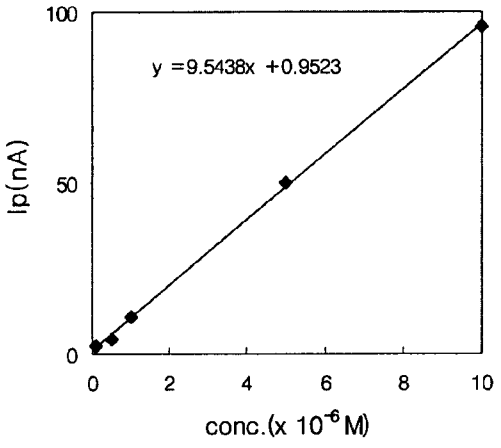

Fig. 5. The plot of Ip vs. concentrations of rifampicin. drop size: medium (A=0.016 cm^2), drop time: 1 sec, scan rate: -6 mV/sec, modulation amplitude: 25 mV

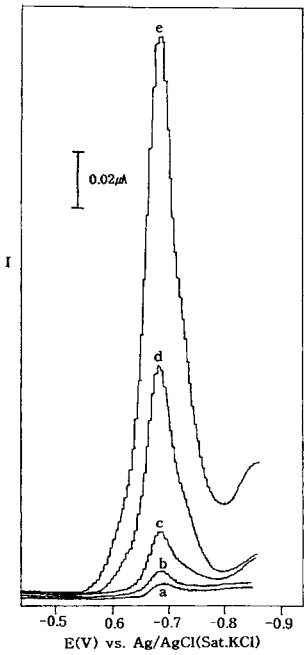

Fig. 6. DP polarograms of rifampicin at the lower concentrations. a: 0.01 μM, b: 0.02 μM, c: 0.05 μM, d: 0.30 μM, e: 0.60 μM. drop size: medium (A=0.016 cm^2), drop time: 1 sec, scan rate: -5 mV/sec, modulation amplitude: 50 mV

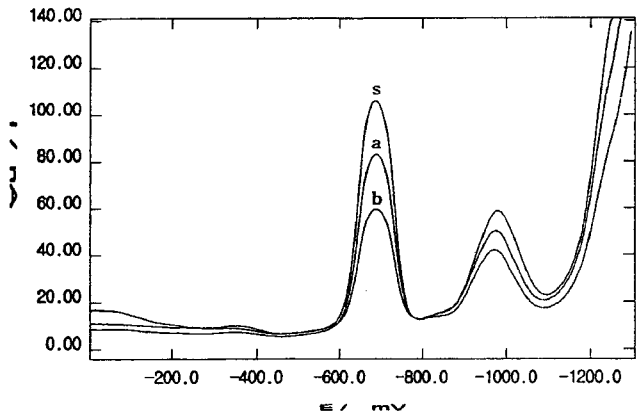

Fig. 7. DP polarograms of rifampicin in the pharmaceutical preparations. s: standard rifampicin, a: b: rifampicin capsules manufactured by two pharmaceutical companies, drop size: medium (A=0.016 cm^2), drop time: 1 sec, scan rate: -6 mV/sec, modulation amplitude: 25 mV

two pharmaceutical companies were compared with standard rifampicin (purity: 99.76%) obtained from Chong Kun Dang Pharmaceutical Company. The amount of 8.2 mg of each rifampicin powder was dissolved in 0.5 ml methanol, which was then diluted to 10 ml with the acetate buffer of pH 4.5 in order to make the sample solution for DPP analysis. Fig. 7 shows DP polarograms of rifampicin sample solutions. All of them exhibited two peaks; the first peak at -0.684 V and the second peak at -0.978 V (or -0.972V). Based on the first peak of standard rifampicin, purities of rifampicin capsules were calculated as 78% (a) and 56% (b), respectively.

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