26. **Tadashi Sasaki**: Polarographic Study of Nitrofuran Derivatives. I. Polarographic Behavior of Nitrofurfural Semicarbazone.*

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Since the electroreduction potential is believed to be a measure of electronegativity of a reducible radical, the measurement of a half-wave potential by means of polarography shows the extent to which a reducible radical could be reduced by electrolysis. The bactericidal action of nitrofuran derivatives is lost in the absence of the nitro radical[1]. This fact clearly shows that the nitro radical is a source of their bactericidal action in some way and that the knowledge on the behavior of the nitro radical could be useful means of expressing their bactericidal mechanism in microörganisms.

On the study of organic nitro compounds by means of polarography, many attempts have been made since nitrobenzene was reported by Shikata[2]. The application of polarography on furfural was carried out by Tachi[3], Korshunov[4], and later by Day[5]. On the nitrofuran derivatives, Cramer[6] reported on the behavior of Furacin against *Staph. aureus* by using this method and explained that its bactericidal action could be caused by its electrostatic character; on account of which the oxidation-reduction potential of the cultivation medium became unfavorable to the growth of microörganisms. Tachi[7] also reported on the polarography of Furacin and several simple nitrofuran derivatives in an aqueous solution.

In the present paper, the polarographic behavoir of Furacin in various solvents containing buffer solution is reported.

Experimental

The electrolytic reduction of Furacin at the dropping mercury cathode was carried out in water, in hydrated alcohol, and in dioxane containing various buffer solutions. As a buffer soultion, for pH 1, 0.1*N* HCl was used; for a range of pH 2.2～6.0, McIlvain's citric acid-NaH_2PO_4 solution; for pH 7.0～9.0, Kolthoff's borate-K_2HPO_4 solution; for pH 10～12, Sφrensen's NaOH-borate solution; for pH 13, 0.1*N* NaOH solution. In order to cancel the appearance of polarographic maximum, 1～2 drops of a 0.02% gelatine solution was added to the electrolyte solution. After the preparation of the solution, hydrogen gas was passed through this solution for more than 30 minutes and the solution was electrolyzed immediately at a room temperature of 20～25°. All the measurements were carried out by using the electrically operated photo-recording polarograph†, and the values of the half-wave potential vs. the saturated calomel electrode were measured from their polarograms.

Results

Analysis of Furacin Wave in an Aqueous Solution—To analyze the Furacin wave, experiments were carried out on furan (Table I), furfural (Table II), furfural semicarbazone (Table III), semicarbazide (Table IV), nitrofurfural (Table V), and nitrofurfural semicarbazone (Table VI). From

* Commercial "Furacin" from Eaton Laboratory, N.Y., U.S.A. Cf. N.N.R., 10, (1947). Presented partly before the Annual Meeting of the Pharm. Soc. of Japan in June, 1950.

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1) M. C. Dodd, W. B. Stillman: J. Pharmacol. Exptl. Therap., 82, 11(1944).
2) M. Shikata: J. Agr. Chem. Soc. Japan, 1, 38(1925).
3) I. Tachi: *Ibid.*, 14, 1317(1938).
4) I. A. Korshunov, S. A. Ermolaeva: C. A., 42, 41(1948).
5) R. A. Day Jr.: J. Am. Chem. Soc., 76, 280(1954).
6) D. L. Cramer: J. Bacteriol., 54, 119(1947).
7) I. Tachi: Japan J. Pharm. & Chem., 20, 38(1948).

† Polarograph, manufactured by the Yanagimoto Manufacturing Co., Kyoto.

these results it is clear that the first wave is due to the reduction of the nitro group and the second one due to that of the azomethine bond, -CH=N-, in the side chain.

Table I. Furan (1×10^{-4} mole)

There was no reduction wave in all of the pH ranges.

Table II. Furfural (2×10^{-4} mole)

pH	E_1*	i_{d1}**
2.2	−1.045	4.56
4.0	−1.136	4.56
7.0	−1.336	7.92
10.0	−1.472	7.92

Table III. Furfural semicarbazone; (9×10^{-5} mole)

pH	E_1	i_{d1}
2.2	−0.882	5.73
4.0	−0.996	8.20
7.0	−1.201	4.02
10.0	—	—
13.0	—	—

* E_i : Reduction potential vs. the standard calomel electrode in volt for i-th wave.
** i_{di} : Diffusion current in $\times10^{-7}$ ampere for i-th wave.

Table IV. Semicarbazide·HCl (1×10^{-4} mole)

There was no reduction wave in all of the pH ranges.

Table V. Nitrofurfural (9×10^{-5} mole)

pH	E_1	i_{d1}	E_2	i_{d2}	E_3	i_{d3}	E_4	i_{d4}
2.2	−0.111	2.88	−0.391	2.55	−0.859	2.47	−1.207	3.63
4.0	−0.180	3.27	−0.467	3.00	−0.917	1.65	−1.310	3.96
7.0	−0.247	3.63	−0.428	3.09	−1.037	1.95	−1.528	2.10
10.0	−0.360	5.40	−0.580	0.75	−1.260	1.50	−1.643	1.59
13.0	—	—	—	—	—	—	—	—

Table VI. Nitrofurfural Semicarbazone (9×10^{-5} mole)

pH	E_1	i_{d1}	E_2	i_{d2}
1.0	?	?	−0.872	5.70
			−1.110	11.25
2.2	−0.140	8.07	−1.013	6.00
			−1.135	4.50
3.0	−0.150	8.73	−1.045	5.52
			−1.163	4.62
4.0	−0.205	8.13	−1.110	7.83
5.0	−0.214	10.08	−1.137	7.23
6.0	−0.365	7.50	−1.190	6.21
7.0	−0.277	9.54	−1.246	4.89
8.0	−0.348	9.57	−1.337	3.90
9.0	−0.400	9.87	−1.381	3.75
10.0	−0.461	9.33	−1.390	0.75
	−0.461†	9.30	—	—
11.0	−0.477	10.23	—	—
	−0.485†	9.18	—	—
12.0	−0.518	10.71	—	—
	−0.525†	9.75	—	—
13.0	−0.539	5.64	—	—
	−0.625	3.99	—	—
	−0.727	0.96	—	—
	−0.550	1.32	—	—

† Electrolysis was carried out one day after the preparation of the solution.

Polarography of Furacin in an Alcoholic Solution—Furacin was electrolyzed in 16.6% aqueous alcohol with 0.1*N* KCl added as an electrolyte. The result is given in Table VII.

Table VII. Nitrofurfural Semicarbazone
(8×10^{-4} mole in 16.6% alcoholic solution)

pH	E_1	i_{d1}	E_2	i_{d2}
2.3	−0.228	52.92	—	—
3.1	−0.391	48.60	—	—
4.0	−0.310	41.56	−1.155	50.04
4.9	−0.289	49.32	−1.332	37.98
6.1	−0.440	48.60	−1.351	33.12
7.1	−0.491	45.90	−1.385	26.10
8.0	−0.686	60.30	−1.520	26.55
9.0	−0.609	43.20	−1.417	15.30
10.0	−0.774	46.98	−1.619	18.45
11.0	−0.685	48.60	−1.650	9.00
12.0	−0.642	24.30	—	—
13.0	—	—	—	—

Table VIII. Nitrofurfural Semicarbazone
(saturated in 40% dioxane solution)

pH	E_1	i_{d1}	E_2	i_{d2}	E_3	i_{d3}
3.2	−0.365*	170.7	−1.038	66.3	−1.328	132.1
4.1	−0.436*	123.0	−0.956	38.4	−1.374	90.1
5.1	−0.508*	273.0	−0.928	57.0	−1.517	197.4
6.1	−0.579	79.8	−0.888	41.7	−1.486	81.0
7.0	−0.599	172.8	−0.880	222.0	−1.440	73.8
8.0	−0.618	82.5	−0.876	209.4	−1.411	84.6
9.0	−0.604	39.0	−0.849	117.0	−1.411	30.0
9.7	−0.604	21.6	−0.854	66.6	−1.259	10.6

*All these waves showed the maxmium phenomena; thus the data given here are approximate values.

Table IX. Furfural
(1×10^{-4} mole)

pH	E_1	i_{d1}
2.2	−1.068	3.66
4.0	−1.194	3.00
7.0	−1.389	3.09
	−1.539	2.25
10.0	−1.496	3.21
	−1.621	2.17

Table X. Furfural Semicarbazone
(saturated solution)

pH	E_1	i_{d1}	E_2	i_{d2}
2.2	−1.109	399.6	†	—
4.0	−1.162	120.0	−1.377	121.8
7.0	—	—	—	—
10.0	—	—	—	—

†Maximum appeared.

Table XI. Nitrofurfural
(1×10^{-4} mole)

pH	E_1	i_{d1}	E_2	i_{d2}	E_3	i_{d3}	E_4	i_{d4}
2.2	−0.225	10.8	−0.531	5.4	−0.014	5.2	−1.314	7.4
4.0	−0.712	7.6	−0.596	5.3	−1.035	3.5	−1.436	4.6
7.0	−0.433	7.2	−0.699	1.7	−0.931	4.4	−1.626	5.6
					−1.251	4.2		
10.0	−0.448	6.4	—	—	−1.438	3.2	−1.734	2.6

Table XII. Furacin
(30% alcoholic solution at pH 7)

Concentration of Furacin	Wave height of 1st wave	Wave height of 2nd wave
9.0×10^{-5} mole	53.2 mm. (8.0×10^{-7} amp.)	25.5 mm. (3.8×10^{-7} amp.)
7.5 ,,	41.5 (6.2 ,,)	19.2 (2.9 ,,)
6.0 ,,	32.6 (5.0 ,,)	16.5 (2.5 ,,)
4.5 ,,	24.9 (3.7 ,,)	12.4 (1.9 ,,)
3.0 ,,	16.5 (2.5 ,,)	10.0 (1.5 ,,)
1.5 ,,	8.3 (1.3 ,,)	5.4 (0.8 ,,)

Table XIII. Furacin
(40% dioxane solution at pH 6)

Concentration of Furacin	Diffusion-current of the 1st wave	Diffusion-current of the 2nd wave	Diffusion-current of the 3rd wave
1.6×10^{-4} mole	8.6×10^{-7} amp.	3.5×10^{-7} amp.	6.8×10^{-7} amp.
1.4 ,,	7.3 ,,	3.3 ,,	5.5 ,,
1.2 ,,	6.4 ,,	2.7 ,,	4.8 ,,
1.0 ,,	5.4 ,,	3.0 ,,	3.7 ,,
0.8 ,,	4.3 ,,	2.6 ,,	3.0 ,,
0.6 ,,	3.5 ,,	2.2 ,,	2.3 ,,
0.4 ,,	2.7 ,,	—	1.5 ,,
0.2 ,,	1.6 ,,	—	0.8 ,,

Polarography of Furacin in Dioxane—Furacin was electrolyzed in 40% dioxane solution; in this case the saturated solution was used because of Furacin not dissolving completely in this solution, and 0.1*N* $(CH_3)_4NBr$ was added to this solution as an electrolyte. This polarograph showed a very different wave from that of Furacin in water and in aq. alcohol as shown in Fig. 1. Therefore, furfural (Table IX), furfural semicarbazone (Table X), nitrofurfural (Table XI), and Furacin (Table VIII) were electrolyzed. The data are given in Tables VIII~XI.

Attempt on Quantitative Analysis—1.5 ~9×10^{-5} mole of Furacin was dissolved in 30% alcoholic buffer solution of pH 7 with 0.1*N* KCl as an electrolyte. The concentration-wave height curve was obtained as shown in Table XII and Fig. 2. The same procedure was applied to 1.6×10^{-4}~2×10^{-5} mole Furacin in dioxane, but in this case the concentration-wave height curve was not linear as shown in Table XIII.

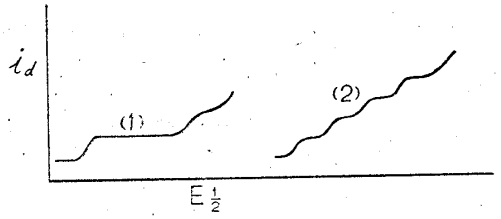

Fig. 1.
Polarogram of Furacin
(1) in water or alcoholic soln.
(2) in dioxane soln.

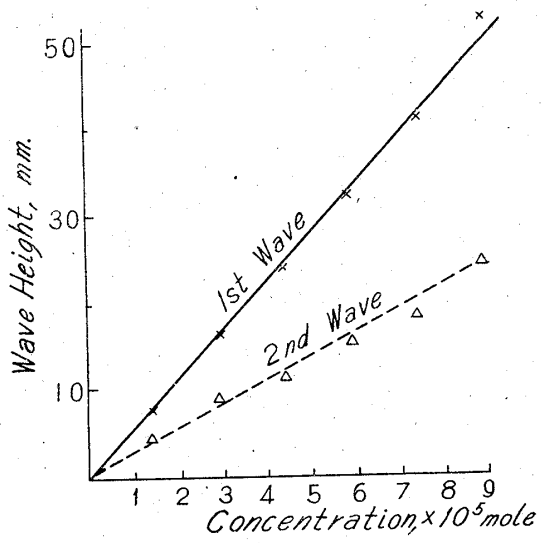

Fig. 2.
Concentration-Wave Height Curve of Furacin
(from Table II)

Discussion

1) Calculation of Electrons Transferred in the Reduction of Furacin in an Aqueous Solution.

The theoretical equation, derived by Ilkovic, for the diffusion current at the dropping mercury electrode is

$$i_d = 605 \cdot n \cdot D^{1/2} \cdot C \cdot m^{2/3} \cdot t^{1/6} = K \cdot C$$

where i_d is the average current in microamperes, n is the Faraday's electron number transferred per mole, D is the diffusion coefficient in cm^2/sec., C is the concentration of the reduced ion in millimole/L., m is the rate flow of mercury from the dropping mercury electrode in mg./sec., t is the drop time in second, and K is the proportionality

* $D=3.32\times10^{-5}/V^{1/3}$; V is the molecular volume. Cf. Kolthoff, Lingane: "Polarography," International Pub., N.Y., 48(1952). V for Furacin is approximately calculated by using the formula V=molecular weight/density =184/1.5, where, the density of Furacin is assumed to be 1.5.

constant. Although the diffusion coefficient D of Furacin is not known, it was computed by means of the Stock-Einstein diffusion equation*, to be about 8.36×10^{-6} cm²/sec. Substitution of the experimental values to the equation yielded a value of n of approximately 4; e.g., using the mercury electrode of characteristics of m=1.085 mg./sec. and t=2.60 sec., 0.9 millimole of Furacin (Table XII) showed the diffusion current of 8.0 microamperes. Then,

$$n=i_d/605\cdot D^{1/2}\cdot C\cdot m^{2/3}\cdot t^{1/6}=8.0/605\cdot(8\times10^{-3})^{1/2}\cdot0.9\ (1.085)^{2/3}.\ (2.6)^{1/6}$$
$$=4.44$$

A composite plot of $\log i/(i_d-i)$ vs. E for the first wave of Furacin in an aqueous solution at pH 7 (Table VI) gave a straight line with a value dE/d $\log i/(i_d-i)$ being 39 millivolt involving 1.5 electrons. This is not in accordance with the above calculted number 4 and it clearly shows that the reduction of the nitro group of Furacin is irreversible to hydroxylamine**.

2) **pH-$E_{1/2}$ Curve**—A pH-$E_{1/2}$ curve of Furacin was obtained in an aqueous solution on the basis of the data in Table VI, which showed a sigmoid having an inflexion point in a pH range of 5~8 as shown in Fig. 3 and which demonstrated that Furacin could be dissociated in this pH range.***

The author is much indebted to Mr. T. Kushita, who provided most of the data and who carried out the experiments for his graduation thesis under direction of Professor Tachi, Agricultural Department of Kyoto University. At the same time he wishes to thank Professors I. Tachi and H. Saikachi for their advices and criticisms.

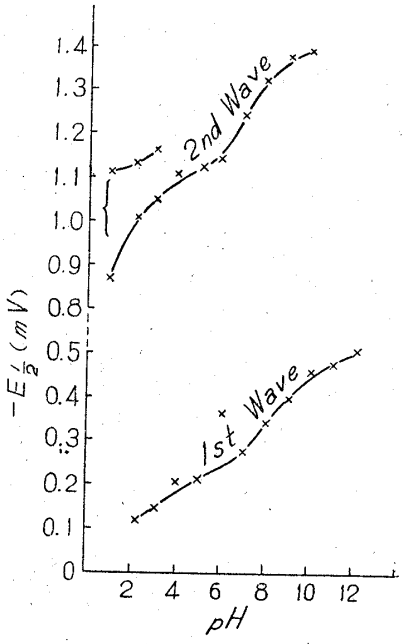

Fig. 3.
pH—$E_{1/2}$ Curve of Furacin
(from Table VI)

Summary

1) The polarogram of Furacin in an aqueous or alcohol solution shows two waves (Fig. 1). The first wave is due to the reduction of the nitro radical and the second one is due to that of the azomethine –CH=N– bond in another side chain of Furacin.

2) Half-wave potential of a nitro radical in Furacin shifts to more negative values at generally regular intervals with increasing pH values and is in a range of an aromatic nitro radical. The wave height, the diffusion current, is not influenced so much by pH values and is constant at pH 4~12 values. These show that the nitro radical of Furacin has an aromatic character.****

3) Polarograms of Furacin in an aqueous and alcoholic solutions are similar although differing from that in dioxane solution. This shows that the reduction in dioxane proceeds seemingly by a different mechanism from that in aqueous or alcoholic

** This result is in accordance with the experiment carried out by Paul, *et al.* by using xanthine oxidase *in vitro*. Cf. J. Biol. Chem., 191, 223 (1951).

*** The antibacterial action of Furacin is known to be stable in a pH range of 5~7 and to fall in more acidic or alkaline ranges. Cf. Shibata: Japan J. Pharm. & Chem., 20, 32 (1948).

**** As generally believed, the reduction potential of an aromatic nitro radical is more positive than that of an aliphatic nitro radical and the reduction-wave height of the former compound is not influenced so much by the variation of pH as the latter which decreases with increasing pH values.

solution.*

4) Polarography can be applied to the quantitative analysis of Furacin in aqueous or hydrated alcoholic solution but not in dioxane soultion.

5) The nitro radical of Furacin is reduced to hydroxylamine by polarography and this reduction is an irreversible change.

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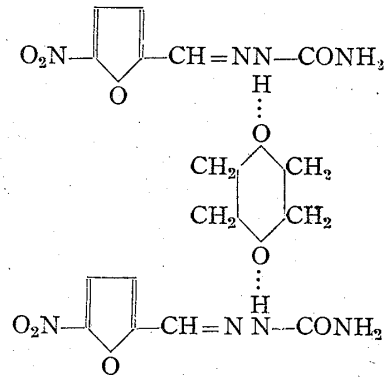
* This reduction machanism may be caused by the dissociation of Furacin in dioxane where Furacin acts as a proton donor and dioxane acts as a proton acceptor as follows:



27. **Tadashi Sasaki**: Polarographic Study of Nitrofuran Derivatives. II[1]. Reduction Potential of Nirofuran Derivatives and Nitrobenzene Analogs.

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In Part I of this study[1], the reduction curve of Furacin was analyzed completely and the first wave was explained as being due to the reduction of the nitro radical. In the present series, experiments were enlarged to include several other nitrofuan derivatives and nitrobenzene analogs having a similar structure as Furacin in order to discover relationship, if any, between the reduction·potential of the nitro group in nitrofuran derivatives and their bactericidal action.

Experimental

Experiments were carried out essentially in a similar way as in Part I of this study[1]. The preparation of the sample to be electrolyzed was as follows: After dissolving 10^{-4} mole of the sample in 15 cc. of alcohol**, 1.5 cc. of this solution was mixed with a buffer solution to make a total volume of 10 cc. As a buffer solution McIlvain's citric acid-Na_2HPO_4 mixture[2], pH ranging from 4.0 to 8.0 was used. Electrolysis by using the dropping mercury cathode was carried out at a room temperature on nitrofurfural semicarbazone (Table I), nitrofuran (Table II), *p*-nitrobenzal semicarbazone (Table III), nitrofurfural aminoguanidine hydrochloride (Table IV), nitrofurfural phenylsemicarbazone (Table V), dinitrofuran (Table VI), *p*-nitrobenzal aminoguanidine hydrochloride (Table

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1) Part I: This Bulletin, 2, 99(1954). Part of the paper read before the Annual Meeting of the Pharmaceutical Society of Japan, June, 1950.

2) McIlvain: J. Biol. Chem., 49, 183(1921).

** When a sample did not dissolve completely in alcohol, a saturated soultion was used. Cf. References to Tables V, XXIII, XXIV, XXV, XXVII, and XXVIII.