*A. C. Polarography of Thiamine Hydrochloride in Weakly Acidic Buffer Solutions**

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A number of polarographic investigations on thiamine hydrochloride have been reported(2). Most of these studies have been carried out in highly acidic or basic solutions or in unbuffered solutions, because their D. C. reduction waves were not well-defined in weakly acidic or in neutral buffer solutions owing to the overlapping of hydrogen discharging currents or pre-sodium waves.

The stability of thiamine under such extreme conditions was not sufficient to carry out the polarographic measurements and in unbuffered solutions the real pH-values at the surface of D. M. E. could not be defined experimentally. Therefore, the investigations have to be done in buffered solutions in order to know the real polarographic reduction waves of thiamine in weakly acidic solutions.

When the A. C. polarographic method was applied, thiamine hydrochloride was found to show one or two well-defined peak currents in weakly acidic and in neutral buffer solutions. Especially the behavior of the first wave

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* Polarographic Studies of some Organo-sulfur Compounds, Part II(1).

1) Part I. K. Okamoto, This Bulletin, 34, 920 (1961).

2) M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy", Interscience Publishers, New York (1958), p. 382.

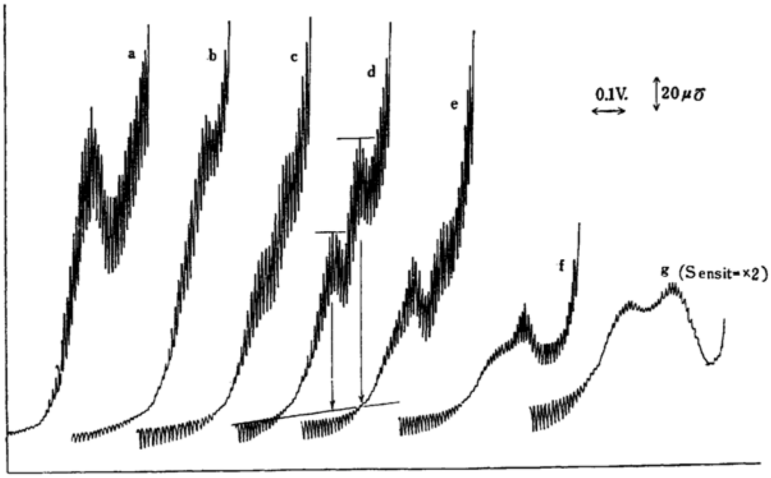

Fig. 1. Typical polarograms of B_1-HCl. a : pH 3.00, b : 4.75, c : 5.50, d : 6.00, e : 7.00, f : 7.90, g : 8.95. The applied voltage starts from −1.0 V. vs. S. C. E. $[B_1\text{-HCl}]=1\times10^{-3}$ mol./l. The solutions were air-free. Parallel capacitance is zero.

was distinctly observed in the solutions of pH 4.75∼7.0. These two waves were considered to be attributed to the reduction of two species of thiamine hydrochloride in a solution.

In this paper, these A. C. polarographic behaviors of thiamine hydrochloride will be reported.

Experiments and Results

The polarograph used was the same as that reported in the previous paper[1]. The characteristics of the dropping mercury electrode were: $m=0.917$ mg. sec^{-1}, $t=4.68$ sec. (open circuit), 3.92 sec. (at −1.30 V. vs. S. C. E.), 3.85 sec. (at −1.40 V.).

TABLE I. THE VARIATION OF THE SUMMIT POTENTIALS E_s AND THE PEAK CURRENTS i_s WITH pH
$[B_1\text{-HCl}]=1\times10^{-3}$ mol./l., 25°C

pH	Buffer	$-E_s$, V. vs. S.C.E.		i_s, μ℧	
		$-E_s^1$	$-E_s^2$	i_s^1	i_s^2
3.00	A	N.W.	1.273	N.W.	234.6
4.00	A	N.W.	1.275	N.W.	233.2
4.25	A	N.W.	1.299	N.W.	227.0
4.50	A	N.W.	1.316	N.W.	209.0
4.75	A	N.W.*	1.320	N.W.	199.2
5.00	A	1.275	1.335	ca. 120	185.6
5.25	A	1.290	1.357	114.9	178.8
5.50	A	1.300	1.370	111.3	165.0
5.75	A	1.307	1.390	102.8	157.7
6.00	B	1.310	1.392	94.0	147.0
7.00	B	1.351	1.445	85.4	82.0
7.90	B	1.320	1.399	45.0	61.9
8.95	C	1.317	1.453	28.0	29.3
10.00	D	N.W.	N.W.	N.W.	N.W.

A : 0.5 M acetate buffer
B : 0.5 M phosphate buffer
C : 0.1 M borate buffer+KH_2PO_4
D : 0.1 M borate buffer+NaOH
N. W. : No wave * Shoulder

The thiamine hydrochloride and other reagents used were of reagent grade.

Experiments were carried out 2 or 3 times on the same kind of sample at 25°C and the values obtained were averaged in general. All potentials and peak currents were expressed in V. vs. S. C. E. and μ℧, respectively.

The Influences of pH to i_s and E_s.—In the buffer solutions of pH 3.0∼10.0, containing 1×10^{-3} mol./l. thiamine hydrochloride (B_1-HCl), the variation of the wave forms with pH was observed, especially in the region of pH 4.00∼6.00.

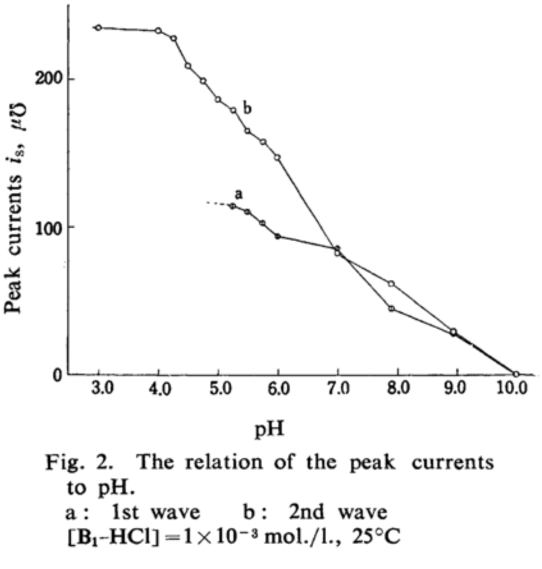

Fig. 2. The relation of the peak currents to pH.
a : 1st wave b : 2nd wave
$[B_1\text{-HCl}]=1\times10^{-3}$ mol./l., 25°C

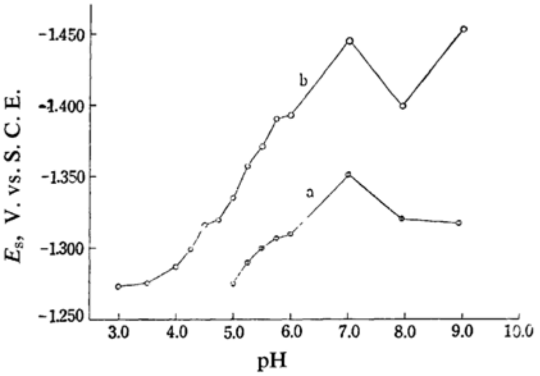

Fig. 3. The relation of E_s to pH.
a : first wave b : second wave
$[B_1\text{-HCl}]=1\times10^{-3}$ mol./l., 25°C

Some of the typical polarograms were shown in Fig. 1 and the relations of peak currents i_s or of summit potentials E_s to pH were summarized in Table I, Figs. 2 and 3.

Wave heights were measured following the method shown in Fig. 1.

Only one peak current was obtained in the region of pH 3.0∼4.75, two peak currents were observed in the region of pH 4.75∼9.0. They had the same wave heights at pH 7.0. In basic solutions of pH 7.0∼10.0, these two waves changed irregularly and completely disappeared at pH 10.0.

As shown in Figs. 1 and 2, the 1st wave began to be separated at pH about 4.75 and decreased gradually with pH-increase in the same manner as the second.

Gradual negative shifts of E_s of the first and the second waves with pH-increase were found, certain irregularities, howerver, being observed in basic solutions as shown in Fig. 3.

The Relations of the Concentrations of B_1-HCl to i_s and E_s.—In order to know whether these two peak currents were both due to the reduction of B_1-HCl itself, the relation between the concentrations and i_s was investigated in 0.5 M phosphate buffer of pH 6.00.

The results obtained were shown in Table II and Fig. 4. Good linearities were found for two waves and the saturation tendencies occurred usually in cases where adsorption or desorption waves were not recognized at all.

TABLE II. THE VARIATIONS OF i_s AND E_s WITH THE CONCENTRATIONS OF B_1-HCl
Supporting electrolyte; 0.5 M phosphate buffer (pH 6.00), 25°C

Concn. (×10⁻³ mol./l.)	i_s, μ℧		$-E_s$, V. vs. S.C.E.	
	i_s^1	i_s^2	$-E_s^1$	$-E_s^2$
0.20	16.2	33.6	1.295	1.361
0.40	41.5	74.7	1.300	1.369
0.60	68.2	105.2	1.307	1.386
0.80	93.2	137.0	1.307	1.384
1.00	119.0	174.5	1.309	1.390

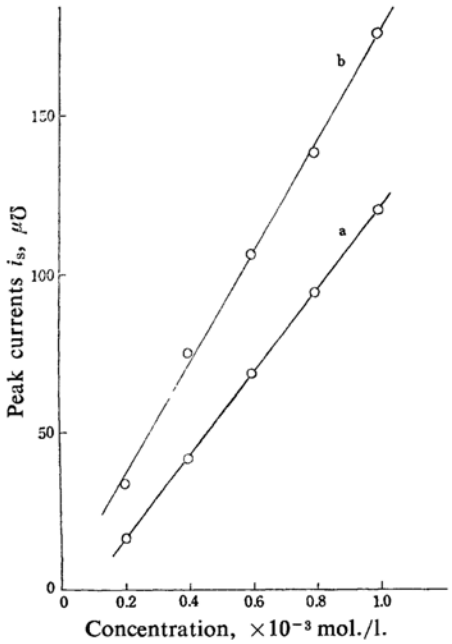

Fig. 4. The relation between the concentrations and the peak currents in 0.5 M-phosphate buffer of pH 6.00.
a : first wave b : second wave

Therefore, both of the waves seemed to be the real reduction waves of B_1-HCl itself.

The summit potential E_s fairly shifted to negative potentials with the increase of the concentrations of B_1-HCl.

Temperature Coefficients of i_s.—Temperature coefficients of the first and the second wave heights were obtained as 1.64 and 1.56% respectively on 0.5 M phosphate buffer solutions of pH 6.0 containing 1×10^{-3} mol./l. of B_1-HCl.

These coefficients coincided with the theoretical value of 1.6% of the diffusion currents.

The Influence of Mercury Heights H to i_s.—The relationship of H to i_s was studied using the method of the plotting $\log H$ (corrected with the reverse pressure of mercury height) against $\log i_s$. The sample offered was 1×10^{-3} mol./l. of B_1-HCl dissolved in 0.5 M phosphate buffer of pH 6.00.

The following equations were obtained:

first wave; $i_s{}^1=H^{0.34}+K_1$

second wave; $i_s{}^2=H^{0.29}+K_2$

where K_1 and K_2 are constants.

The peak currents were not proportional to the square root of H.

Electrocapillary Curve.—When strong adsorption-desorption phenomena occurred at the

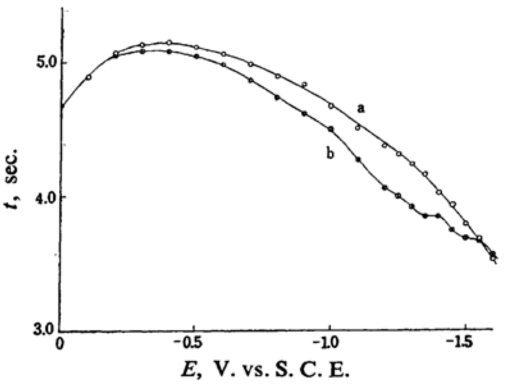

Fig. 5. Electrocapillary curves.
a: Reference; 0.5 M-phosphate buffer pH 6.00
b: 1 mmol B_1-HCl in reference soln. a.

surface of D. M. E., the electrocapillary curve was known to show certain irregularities.

The electrocapillary curve of B_1-HCl in pH 6.00 phosphate buffer solution was shown in Fig. 5.

In a very wide potential range $-0.20\sim-1.55$ V., the drop time t was smaller in a solution of B_1-HCl than in a solution without it, and two small minima were observed at potentials -1.35 and -1.50 V. corresponding to the slightly negative potentials of each two peak current.

Weak adsorption seemed to take place at the surface of D. M. E., but it could have no remarkable effects upon A. C. polarographic waves; therefore, two A. C. waves were both considered to be not entirely so-called tensammetric waves.

Discussion

As described in the above section, comparatively-well defined two A. C. polarographic waves of B_1-HCl observed in weakly acidic buffer solutions, were not the simple tensammetric, but the reduction waves with a few tensammetric properties.

When the aqueous solution of B_1-HCl was titrated with sodium hydroxide solution, two equivalent points were observed[3], the first point corresponding to 1 mol. equivalent of sodium hydroxide and to the process I→II (Fig. 6), another to 3 mol. equivalent of sodium hydroxide and to the process V→VI, while no point corresponding to 2 mol. of sodium hydroxide was detected. The values of pK_1 and pK_2 were $4.70\sim4.80$ and $9.30\sim9.37$, respectively at 25°C[2].

On the other hand, a new polarographic wave began to be observed at about pH 4.75; therefore, this (first wave) was considered to correspond to a reduction of species II that would increase from pH about 3.5 up to neutral range, then the second wave was to correspond to a reduction of species I that gradually decreased with pH-increase.

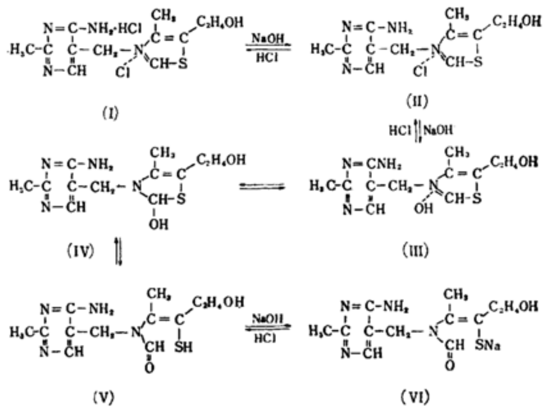

Fig. 6. The variation of B_1-HCl when titrated with HCl or NaOH solutions[3].

The first wave seemed to overlap with the second in the region of pH smaller than 4.75, but as the negative shift of the second was larger than of the first, it came to appear at pH 4.75.

At pH $4.70\sim4.80$ the ratio of the polarographic wave heights of the first and the second should be 1:2 and at pH 7.0 should be 1:1 theoretically. Similar results were obtained experimentally as shown in Fig. 2.

The gradual decreasing of both wave heights with pH-increasing was explained as follows by the above dissociation processes (Fig. 6). If the polarographic reduction of thiamine took place at the double bond $\geqslant$N=C- in thiazole ring, IV, V and VI could not be reduced at D. M. E. Although these formes were not detected in the titration curve, they were considered to exist together in the solutions of pH about $5\sim9$, so that the gradual decrease of wave heights were mainly due to this gradual increase of non-reductive IV or V.

The difference between the summit potentials of the first and second waves was up to about 100 mV., the influence of hydrochloric acid combining to pyrimidine ring or in other words to whole molecule of B_1, might produce this difference.

Summary

Although in ordinary D. C. polarography no well-defined polarographic reduction waves of thiamine in weakly acidic or in neutral buffer solutions have been reported, it was found that A. C. polarographic methods would be suitable for these studies.

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[3] A. Watanabe and Y. Asahi, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), 75, 1046 (1955).

A. C. polarographic waves of thiamine appeared at $-1.27\sim-1.35$ V. in buffer solutions of pH 3.0~4.75, and in the region of pH 4.75~7.0 a new wave began to be observed at more positive potentials than $-1.27\sim1.35$ V., while another wave was observed at $-1.32\sim-1.44$ V. in the same pH region. The heights of two waves decreased with the increase of pH.

In basic buffer solutions the wave forms varied irregularly and the waves disappeared completely at pH 10.0.

According to the relation of i_s to temperature, mercury heights and concentrations, these two waves were both recognized as diffusion currents with a few tensammetric properties.

The nature of the first and second waves were attributed to the reductions of thiamine with Cl^-, and with hydrochloric acid and Cl^- to each dihydrothiamine, respectively.

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