*The A. C. Polarography of S-Benzoylthiamine O-Monophosphate**

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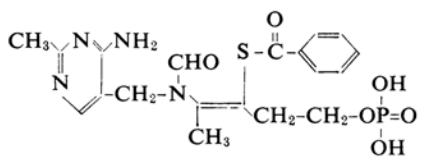
One of the new derivatives of thiamine, *S*-benzoylthiamine *O*-monophosphate (BTMP) (I), has been found to have such predominant pharmaceutical effects as the remarkable increase and prolongation of the thiamine blood level in administration. Many pharmaceutical, chemotherapic and physicochemical studies of on BTMP have, therefore, been reported.



(I)

Some of the polarographic studies of BTMP have been undertaken by the author[1] and by Tachi[2] in order to learn its physicochemical properties.

In a previous work[1] a conventional polarographic study of BTMP was reported, but no theoretical treatment of the d.c. reduction waves of BTMP could be made, because of their irreversibility and ill-defined forms.

Alternating current polarographic techniques were applied in order to make the nature of

* Polarographic Studies of Some Organosulfur Compounds. Part IV.

1) K. Okamoto, *Vitamine (Kyoto)*, **22**, 364 (1961).
2) I. Tachi, ibid., **21**, 423 (1960).

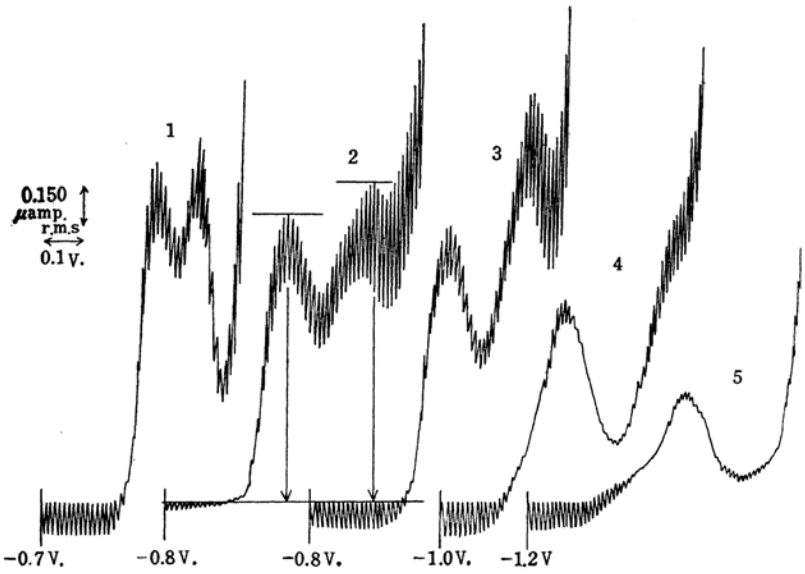


Fig. 1. Some of the a. c. polarograms of BTMP.
pH 1, 1.00 2, 3.00 3, 4.00 4, 7.00 5, 8.55
[BTMP] $=0.40\times10^{-3}$ mol./l., at 25°C, air free, capacitance $=0$ μF

the reduction waves of BTMP more definite. By the a. c. method, two very definite peak currents could be observed and it became possible to discuss the reversibility of its electrode reaction.

In this paper, the a. c. polarographic behavior of BTMP and some of the theoretical considerations of the reversibilty of its electrode reaction will be presented.

Experimental

Apparatus.—The polarograph used was Yanagimoto model PA-102 (d. c. and a. c.), a. c.; $\Delta E=$ 15 mV. r. m. s., 50 c. p. s., sinusoidal. The characteristics of the dropping mercury electrode were: $m=1.006$ mg. sec^{-1} (open circuit), $t=4.24$ sec. (open circuit), 4.03 sec. (at -1.164 V.) and 3.73 sec. (at -1.377 V.). The thermostat was Yanagimoto's water bath for polarography with an accuracy of ±0.5°C.

Reagents. — The BTMP used was of purified crystal, as in the previous work[1].

The *S*-*p*-nitrobenzoylthiamine *o*-monophosphate (NBTMP) (II) used was that synthesized in our laboratory by the same method as for BTMP; m. p. $=154\sim156$°C (decomp.).

Found: C, 41.73; H, 4.70; N, 12.89. Calcd. for $C_{19}H_{22}N_5O_8PS\cdot2H_2O$: C, 41.68; H, 4.79; N, 12.80%.

Results

The Effect of the pH Value on the Summit Potential, E_s, and the Peak Current, i_s.—In the a. c. polarographic technique, the use of comparatively concentrated buffer solutions is preferable in order to reduce the resistance of the electrolyte solution; therefore, 0.2 or 0.25 mol./l. buffer solutions were employed in the following experiments.

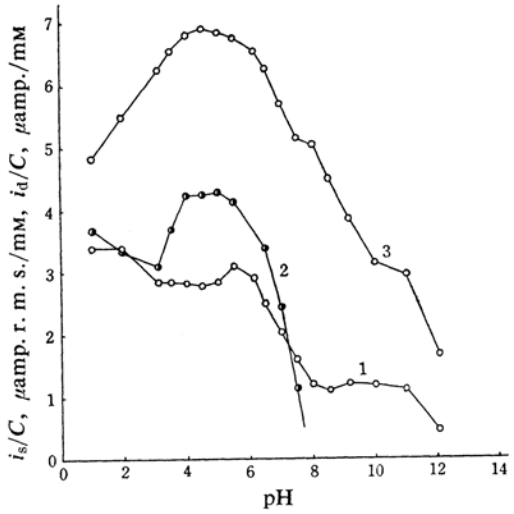


Fig. 2. The relations of pH to i_s^1, i_s^2 and i_d^1.
1, a. c. 1st wave 2, a. c. 2nd wave
3, d. c. 1st wave

No maximum suppressor was used, thus avoiding its unfavorable influences upon the electrode reaction.

All experiments, except for some special studies, were carried out at 25 ± 0.5°C, and air was removed by the bubbling of hydrogen.

All the values of potentials and of currents

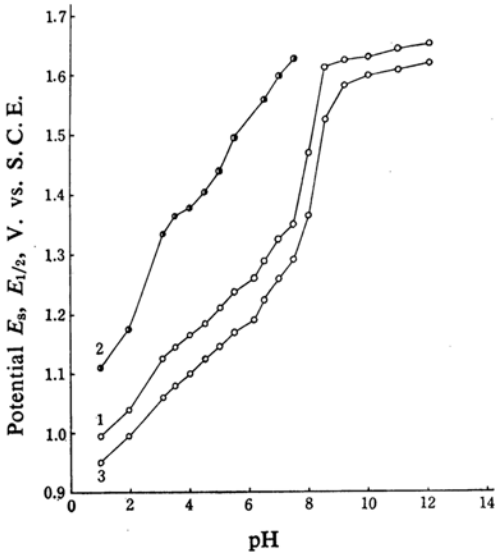


Fig. 3. The relations of pH to E_s^1, E_s^2 and $E_{1/2}^1$.
1: a. c. 1st wave 2: a. c. 2nd wave
3: d. c. 1st wave

TABLE I. THE VARIATION OF THE SUMMIT POTENTIALS E_s AND THE PEAK CURRENTS i_s WITH pH
[BTMP] $=0.40\times10^{-3}$ mol./l., 25°C

pH	Buffer†	i_s^1 μamp. r.m.s.	i_s^2	$-E_s^1$ V. vs. S. C E.	$-E_s^2$
1.00	A	1.36	1.47	0.994	1.110
1.95	A	1.37	1.34	1.040	1.176
3.10	B	1.13	1.23	1.125	1.335
3.50	B	1.15	1.48	1.143	1.364
4.00	B	1.13	1.70	1.164	1.377
4.50	B	1.11	1.70	1.186	1.404
5.00	B	1.14	1.72	1.212	1.441
5.50	B	1.25	ca. 1.65*	1.237	ca. 1.50*
6.15	C	1.17	NW**	1.262	NW
6.50	C	1.01	ca. 1.35*	1.288	ca. 1.56*
7.00	C	0.81	ca. 0.98*	1.325	ca. 1.60*
7.50	C	0.64	ca. 0.45*	1.350	ca. 1.63*
8.00	C	0.47	NW	1.468	NW
8.55	C	0.43	NW	1.615	NW
9.20	D	0.49	NW	1.627	NW
10.00	D	0.47	NW	1.632	NW
11.00	D	0.45	NW	1.644	NW
12.05	D	0.17	NW	1.653	NW

† Ionic strength was adjusted to 0.50 constant by the addition of KCl.
A, 0.2 M KCl+HCl
B, 0.25 M acetate buffer
C, 0.25 M phosphate buffer
D, 0.1 M borate buffer
* Shoulder
** No wave

are expressed in V. vs. S. C. E. and. μamp. r. m. s. respectively.

On account of the somewhat inferior reproducibility of a. c. polarograms, every measurement was repeated two or three times, and the values obtained were averaged.

Typical polarograms are shown in Fig. 1.

As it was necessary to compare the values obtained under the same conditions by the d. c. and a. c. methods, the d. c. polarograms were observed again.

As is shown in Fig. 2, the effect of the pH value on i_s^1 was very different from that on i_d^1, but the effect on E_s^1 was not different from that on $E_{1/2}^1$, as is shown in Fig. 3.

Fortunately, the second wave, which had not been observed well by the d. c. method, could be obtained clearly by the a. c. method.

As is shown in Fig. 2-2, the behavior of the second wave in various buffer solutions is interesting.

All numerical values are shown in Table I.

The Effect of the Concentration on i_s^1 and i_s^2 and on E_s^1 and E_s^2.—For the theoretical considerations, it seemed to be rather simple to chose that condition in which an ionic form of BTMP in the solution had no charge, namely, the condition at the isoelectric point where the pH value=4.05[3]. For this reason, the following studies were carried out on a 0.25 mol./l. acetate buffer solution with a pH value of 4.0.

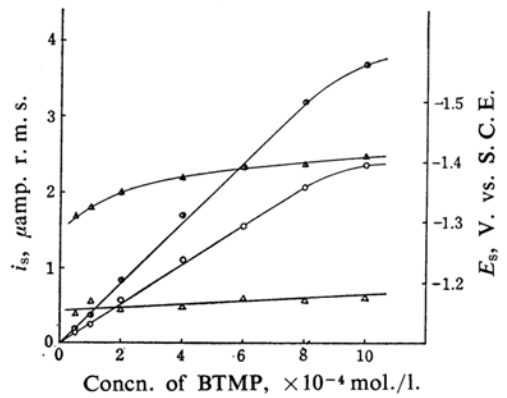


Fig. 4. The relations of the concentrations to i_s and E_s.
1, i_s^1 2, i_s^2 3, E_s^1 4, E_s^2
Base solution: pH 4.00 acetate buffer, 25°C

The relations between the concentrations of BTMP and i_s^1 and i_s^2 were both linear up to 0.8×10^{-3} mol./l. The saturating tendencies were observed over 0.8×10^{-3} mol./l., and they passed exactly through the zero point, as is shown in Fig. 4. Such relationships were

3) A. Ito, ibid, 22, 349 (1961).

considered to indicate that there was no remarkable adsorption or desorption phenomena at the interface of DME and the solution.

The effect of the concentration on E_s^1 and E_s^2 is shown in Fig. 4.

The Effect of the Mercury Reservoir Height on i_s^1 and i_s^2.—In a. c. polarography, the reaction current was not influenced by the height of the mercury reservoir, but it was proportional to the surface area of DME, so that i_s was usually independent of H.

A linear but slight change of i_s for BTMP was observed with these changes in H values:

$$i_s^1 = 0.007H + 0.687$$

$$i_s^2 = 0.004H + 1.404$$

on 0.25 mol./l. acetate buffer solutions of pH 4.0 containing 0.40×10^{-3} mol./l. of BTMP at 25±0.5°C.

The above relations coincide with the theoretical results in a. c. polarography.

Temperature Coefficients of i_s^1 and i_s^2.—In a. c. polarography, the temperature coefficient of i_s was usually smaller than of i_d, so that +1.0~1.3% at about 25°C were considered to be the ordinary values.

When some adsorption-desorption phenomena occur, a negative coefficient was obtained because of the decrease in the number of molecules adsorbed at DME with the rise in temperature. If the measurements of coefficients are carried out at various concentrations of depolarizer, a discontinuous change, such as from positive to negative values, was often observed according to the change in the adsorption state.

TABLE II. TEMPERATURE COEFFICIENTS OF i_s AT VARIOUS CONCENTRATIONS

Base solution: 0.25 M acetate buffer of pH=4.00

Concn. mol./l.	i_s, μamp. r. m. s.		Temp. coeff.
	10°C	25°C	%
8×10^{-5}	i_s^1=0.16	0.22	+1.97
	i_s^2=0.19	0.30	+2.37
4×10^{-4}	0.79	1.10	+1.85
	0.92	1.64	+2.91
8×10^{-4}	1.40	1.96	+1.89
	1.88	3.05	+2.55
2×10^{-3}	2.55	3.65	+2.01
	4.03	5.88	+2.10

The temperature coefficients of BTMP at various concentrations are summarized in Table II. In the range of concentrations of 8×10^{-5}~2×10^{-3} mol./l., the coefficients of both i_s^1 and and i_s^2 were positive, but they were larger than that of the theoretical one. No discontinuous change was obtained; the coefficient of the first wave was in 1.85~2.01%, and the second was in 2.10~2.91%. The i_s^1 and i_s^2 seemed not to be controlled by the adsorption process but by diffusion, and the values larger than the theoretical ones were attributed to the influence of the irreversibility of the electrode reaction.

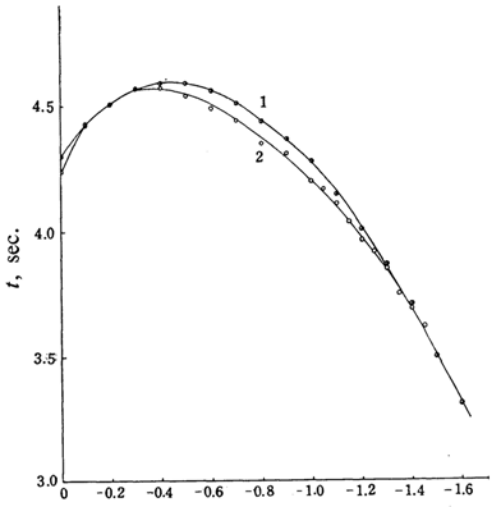


Fig. 5. Electrocapillary curve.
1 Base solution: acetate buffer solution of pH 4.00
2 0.40×10^{-3} mol./l. of BTMP in 1

Electrocapillary Curve.—The electrocapillary curve of 0.40×10^{-3} mol./l. BTMP in a 0.25 mol./l. acetate buffer solution with a pH value of 4.0 and of the base solution are shown in Fig. 5.

Upon the addition of BTMP, a slight decrease of t in the range of potential −0.3~−1.4 V. was observed, and there was no remarkable kink was at any poiential. The values of t were 0.05 sec. and 0.02 sec. smaller than that of the base solution at the potentials corresponding to the first and the second waves.

BTMP was considered to be weakly adsorbed at the positive side of the molecule at the surface of DME in the range of −0.3~−1.4 V., so that there were no predominant influences of adsorption phenomena on i_s^1 and i_s^2.

A Consideration of the Electrode Reaction.—As has been described in previous sections, two definite peak currents of BTMP were considered to be controlled by the electrode reaction, not by the adsorption.

The discussions, therefore, of the reversibility and the mechanism of the electrode reaction were thought to be possible.

The estimations of the diffusion coefficient, D, and the electron number, n, were carried out. Then the calculations of the transfer coefficient, α, and the rate constant, k_G, were also made.

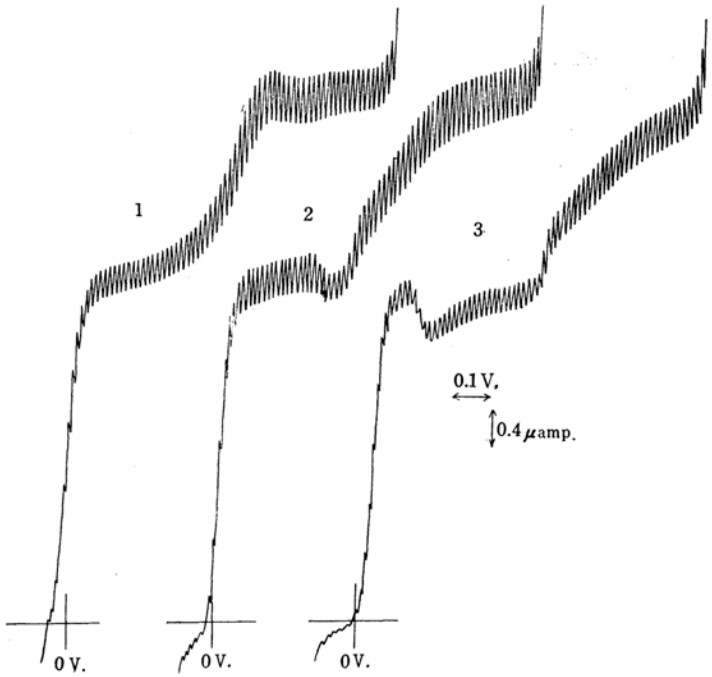
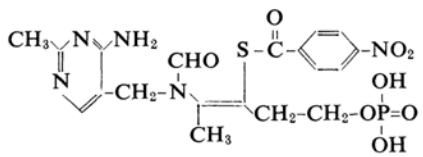


Fig. 6. Some of the d. c. polarograms of NBTMP.
pH 1, 0.81 2, 1.36 3, 1.73 [NBTMP] $=0.477\times10^{-3}$ mol./l., 25°C

The Estimation of the Diffusion Coefficient of BTMP.—The *n*-values and the diffusion coefficient of BTMP could not be obtained because of the irreversibility of the electrode reaction.

When some of the reducible radicals the electrode process of which had already been confirmed were substituted into a suitable position in BTMP, the polarographic diffusion coefficient of the substituted BTMP might be calculated from the reduction wave by the substituted radical.

The nitro radical, therefore, was substituted into the para-position of the phenyl group in BTMP to obtain *S*-*p*-nitrobenzoylthiamine *O*-monophosphate (NBTMP) (II).



(II)

Some of the d. c. polarographic waves of NBTMP are shown in Fig. 6. The first and the second waves were thought to be reduced to the reduction of the nitro group to hydroxylamine ($n=4$) and to amine ($n=2$) respectively. The third wave, which is not drawn in Fig. 6, was due to the reduction of the *S*-acyl bond, as was observed for BTMP.

A minimum at about −0.25 V. which did not disappear upon the addition of Triton-X-100 was thought to be caused by the strong adsorption of NBTMP to the DME surface.

The values obtained for the first wave of 0.239×10^{-3} mol./l. NBTMP in the buffer solution with a pH value of 1.36 were as follows: $i_d=1.76$ μamp., $n=4$, $C=0.239\times10^{-3}$ mol./l. and $m^{2/3}t^{1/6}=1.330$ mg$^{2/3}$ sec$^{-1/2}$.

The diffusion coefficient D could be obtained by Ilkovic' equation as $D=5.22\times10^{-6}$ cm^2 sec^{-1}.

The molar volumes of BTMP and of NBTMP were thought to be approximately equivalent, so that the D value could be applied to that of BTMP.

The number of electrons discharged for BTMP, therefore, at various pH-value conditions could be calculated using the above *D*-value. They are summarized in Table III.

The change in *n*-values with the change in pH values was found almost to coincide with the change in the wave height, with the largest value of 4 showing in the pH range of 4.5～5.5. These variations of *n*-values with pH-value change are thought to be due to the differences in the reduction process of the ionic species of BTMP in the solutions of various pH values.

Consideration of the Reversibility of the First Wave.—Theoretical equations for the reversible and irreversible a. c. polarograms have already been given by H. Matsuda[4] as follows:

4) H. Matsuda, *Z. Elektrochem.*, **62**, 977 (1958).

TABLE III. THE COMPARISON OF THE REVERSIBILITIES OF THE ELECTRODE REACTION OF BTMP AT VARIOUS pH VALUES

pH	$-E_{1/2}$	$-E_s$	$E_{1/2}-E_s$	$RT/\alpha nF$	i_d/C	i_s/C	i_s/i_d	n	αn	α	$k_G/D^{1/2}$	k_G
	V. vs. S. C. E.		mV.	mV.	μamp./mmol.	μamp.r.m.s./mmol.					$sec^{-1/2}$	$cm.sec^{-1}\times10^{-4}$
1.00	0.950	0.944	44	25	4.86	3.40	0.70	2.74	2.51	0.91_6	0.25_2	5.7_5
1.95	0.996	1.040	44	26	5.49	3.43	0.63	3.10	2.26	0.72_9	0.38_6	8.8_0
3.10	1.062	1.125	63	36	6.27	2.83	0.45	3.55	1.62	0.45_6	0.34_9	7.9_6
3.50	1.080	1.143	63	35	6.55	2.88	0.44	3.71	1.58	0.42_6	0.38_5	8.7_9
4.00	1.100	1.164	64	37	6.80	2.83	0.42	3.86	1.51	0.39_1	0.43_1	9.8_3
4.50	1.125	1.186	61	43	6.90	2.78	0.40	3.92	1.44	0.36_7	0.60_7	13.8_4
5.00	1.145	1.212	67	46	6.85	2.85	0.44	3.71	1.58	0.42_6	0.30_1	6.8_6
5.50	1.168	1.237	69	50	6.75	3.13	0.46	3.84	1.65	0.43_0	0.22_0	5.0_2
6.15	1.191	1.262	71	52	6.55	2.93	0.45	3.74	1.62	0.43_3	0.21_1	4.8_2
6.50	1.223	1.288	65	67	6.27	2.53	0.40	3.58	1.44	0.40_2	0.48_6	11.0_5
7.00	1.259	1.325	66	63	5.70	2.03	0.36	3.26	1.29	0.39_6	0.67_4	15.3_7
7.50	1.292	1.350	58	63	5.15	1.60	0.31	2.95	1.11	0.37_6	1.51_4	34.5_2
8.00	1.366	1.468	102	77	5.04	1.18	0.23	2.90	0.83	0.28_6	0.68_7	15.6_6
8.55	1.526	1.615	89	100	4.50	1.08	0.24	2.63	0.82	0.31_2	1.00_9	23.0_5
9.20	1.584	1.627	43	50	3.87	1.23	0.32	2.28	1.15	0.50_4	2.70_9	61.7_7
10.00	1.599	1.632	33	35	3.14	1.18	0.38	1.85	1.36	0.73_5	3.23_7	73.8_0
11.00	1.609	1.644	35	41	2.94	1.13	0.38	1.73	1.36	0.78_6	2.91_2	66.3_9
12.05	1.621	1.653	32	55	1.67	0.43	0.26	0.98	0.93	0.94_9	5.83_3	132.9_9

For reversible reaction:

$$[(k_G \cdot f/D^{1/2})/\sqrt{\omega} > 40\alpha^{\alpha}\beta^{\beta}]$$

$$\bar{i}_s = 0.183\bar{i}_d(\tau\omega)^{1/2}(nF\Delta E/2RT) \quad (3)$$

$$E_s = E_{1/2} \quad (4)$$

For irreversible reaction:

$$(k_G \cdot f/D^{1/2})\sqrt{\tau} < 10^{-(1+2\alpha)}$$

$$\bar{i}_s = (2/\pi)\bar{i}_d(\alpha nF/RT)\Delta E[1-1.24(\tau\omega)^{-0.223}] \quad (5)$$

$$E_s = E_{1/2} + (RT/\alpha nF)\,[\ln k_G f/D^{1/2} - (1/2)\ln\omega - \ln\{1+1.67(\tau\omega)^{-0.490}\}] \quad (6)$$

where: $\bar{i}_d$ = diffusion current (d. c.).

$\bar{i}_s$ = peak current (a. c.).

$\omega = \pi/\theta$, angular frequency of superimposed alternating current

τ = drop time

ΔE = amplitude of superimposed a. c.

α = transfere coefficient ($\alpha+\beta=1$)

k_G = rate constant

D = diffusion coefficient of depolarizer

f = activity coefficient of depolarizer

$E_{1/2}$ = half-wave potential (d. c.)

E_s = summit potential (a. c.)

When the electrode raction is reversible, the half-wave potential $E_{1/2}$ and the summit potential E_s should coincide perfectly, according to Eq. 4, and the rate of $\bar{i}_s/\bar{i}_d$ is 0.183 $(\tau\omega)^{1/2}$ $(nF\Delta E/2RT)$ according to Eq. 3.

The slope of the d. c. wave ($=RT/\alpha nF$) indicates the reversibility of the d. c. electrode reaction. A comparison of the values of $RT/\alpha nF$, $E_{1/2}-E_s$, i_s/i_d, etc. of the first wave at various pH values is shown in Table III and in Fig. 7.

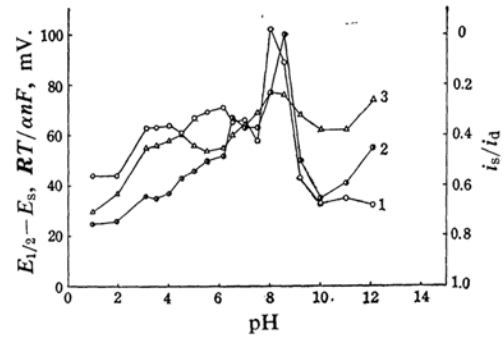


Fig. 7. The relations of pH to $E_{1/2}-E_s$, $RT/\alpha nF$ and i_s/i_d.
1, $E_{1/2}-E_s$ 2, $RT/\alpha nF$ 3, i_s/i_d

As is shown in Fig. 7, the reversibility of the electrode reaction seemed to change in rather a complicated way with the change in the pH-value. The smallest values for $E_{1/2}-E_s$, i_d/i_s and $RT/\alpha nF$ were each obtained at about pH 2~0 and 10 while remarkably large values were obtained at pH 8.0~8.5.

If these three values are taken as indicating the apparent reversibility of the electrode reaction, it is found that the smaller the pH value, the more reversible the electrode reaction in the acidic pH value region.

The stability of the *S*-acyl bond in BTMP was not sufficient in the basic medium, for it therein decomposed to give thiamine *O*-monophosphate, thiamine and other unknown decomposition products. The discussion, therefore, of the electrode reaction of BTMP in the basic medium was thought to be rather impossible. The discontinuous change on the curve in Fig.

7 at pH values 8.0～8.5 seemed to be caused by the decomposition process of BTMP.

In order to calculate the reaction rate constant, Eqs. 5 and 6 were thought to be useful, but they were defined under the condition that the superimposed a. c. amplitude, ΔE, was smaller than 5 mV. r. m. s. In this experiment $\Delta E=15$ mV. r. m. s.; therefore, the accurate value of k_G could not be obtained. However, it seemed possible to estimate k_G and α approximately by the use of the above equations.

The procedure of the calculation may be described in the case of solution with a pH value of 4.00 as follows:

[BTMP] $=1.000\times10^{-3}$ mol./l., $T=25°C$, $\Delta E=$ 15 mV. r. m. s., $i_d=6.80\mu$amp., $i_s=2.83\ \mu$amp. r. m. s., $f=1$, $\omega=100\pi$ (rad. sec^{-1}), $\tau=4.03$ sec. (at the summit potential of the first wave), $E_{1/2}=-1.100$ V. vs. S. C. E., $E_s=-1.164$ V. vs. S. C. E., $n=4$.

Equation 6 was rewritten into Eq. 6-1 using the above values:

$$-1.083=1/\alpha n(\log k_G/D^{1/2}-1.2697) \qquad (6\text{-}1)$$

On the other hand, αn was obtained from Eq. 5.

$$\alpha n=1.51\ ;\quad \text{therefore,}\quad \alpha=1.51/4=0.38$$

When αn was introduced into Eq. 6-1, Eq. 6-2 was obtained:

$$k_G/D^{1/2}=4.31_1\times10^{-1} \qquad (6\text{-}2)$$

The $D^{1/2}$ value was obtained, as in the previous section for NBTMP, as 2.284×10^{-3} cm. sec$^{-1/2}$, so that

$$k_G=0.43_1\times2.284\times10^{-3}=9.83\times10^{-4}\ \text{cm. sec}^{-1}$$

The k_G and α-values of the a. c. first wave of BTMP at various pH values calculated by the above procedure are both shown in Table III and Fig. 8.

In the acidic pH value region, the variation of α and k_G with the pH value in Fig. 8 indicates that the more acidic the medium, the larger the transfer coefficient α, but the k_G-values did not change remarkably.

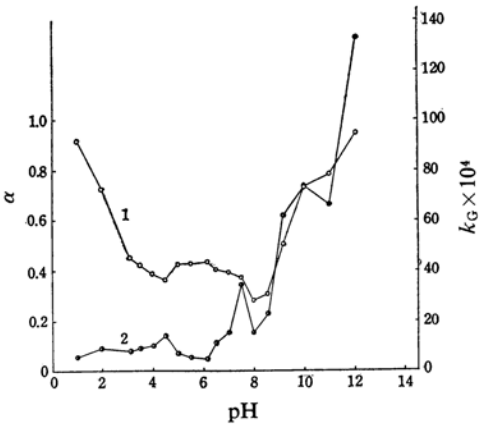


Fig. 8. The relations of pH to α and k_G.
1, α 2, k_G

The Estimation of the Electrode Reaction of BTMP.—The outline of the electrode reaction of BTMP was described in the previous paper[1].

In this paper, the approximation of the *D*-values of BTMP and the *n*-values of the electrode reaction at various pH values was carried out, it therefore being thought possible to estimate some of the processes of the electrode reduction.

If the reduction in the acidic solutions take place only at the acyl bond in BTMP and if the reduction at the such other reducible parts of the molecule as the pyrimidine ring is disregarded, the next reactions for the first wave (Fig. 9) may probably be estimated on the basis of the number of electrons, is shown in Table III.

The number of electrons is controlled by the ways in which III split; the alcohol type V is, therefore, produced by a 4-electron discharge, and the pinacol type VI, by a 3-electron discharge.

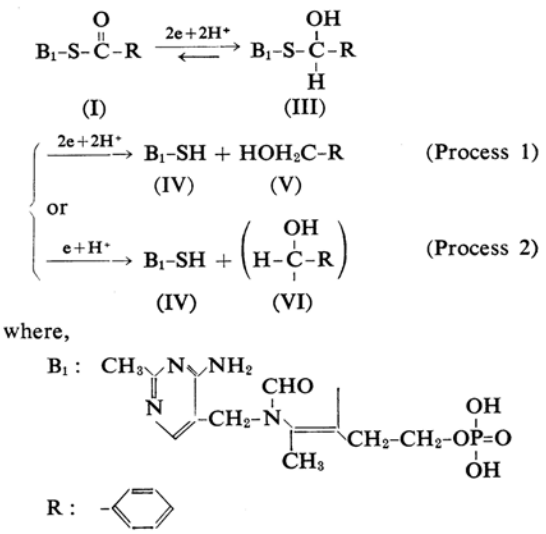


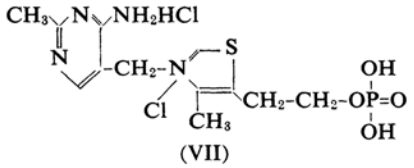
Fig. 9. The reduction processes estimated on BTMP.

Process 1 seemed to take place in the pH range of about 4.5～5.5, and process 2, in the range of pH<2. These two processes were thought to occur at a pH value of about 3.

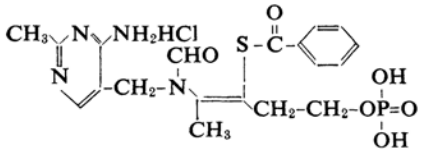
The decrease of *n* in pH values higher than 5.5 could not be explained by the chemical splitting of the *S*-acyl bond.

The second wave of BTMP seemed to be due to the reduction of the thiamine *O*-monophosphate (TMP) (IV) that was produced by the reduction of BTMP to give the first wave.

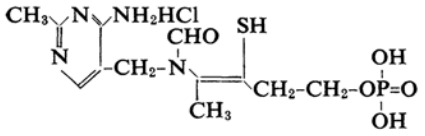
The discontinuous decrease in the wave height was observed for the second wave at pH<4, as is shown in Fig. 2-2, while the first wave of TMP did not show such a decrease[5]. This difference could be explained by a consideration of their species under the same pH value conditions.



(VII)



(VIII)



(IX)

At a pH value of about 3, TMP almost existed as VII, and BTMP as VIII. When VIII was reduced at DME, thiol type TMP (IX) was initially produced. Then IX gradually changed to thiazol type VII.

The second reduction process, therefore, was mainly the reduction of the thiol type IX, because the rate of the thiazol-ring formation seemed to be not so sufficiently rapid as to be comparable to or predominant over the potential sweeping rate.

As has been described in the previous paper[5], the a.c. wave heights of TMP without chlorine at the thiazol ring was about 74% that with chlorine.

The approximate ratio of the wave heights of BTMP and TMP[5] at the pH value of 3.0 was $i_s(\text{BTMP})/i_s(\text{TMP})=60\%$, which was thought not to be different from the result obtained for TMP. The second wave of BTMP, therefore, was considered to be due to the reduction of the thiol type TMP, while the first wave of TMP was that of the thiazol type TMP.

Summary

The polarographic behavior of BTMP was studied using the a.c. polarographic method.

The two peak currents of BTMP in a wide pH value range could be observed definitely. Especially, the observation on the second wave, which was not clearly observed by the d.c. method, could be made.

The variations of the two peak current with the change in pH value were not similar to that of the diffusion currents in that the d.c. first wave height gave the largest value at about the pH value of 5, while the a.c. first wave height was largest at the pH value of 2 and then decreased with the increase in pH value.

The second a.c. wave height showed a maximum value at the pH values between 4 and 5, a minimum at a pH value of about 3, and increased at pH values <3.

The two a.c. waves were recognized to be of the diffusion current, not adsorption or kinetic waves.

The approximation of the diffusion coefficient of BTMP was carried out using the d.c. reduction wave of *S*-*p*-nitrobenzoylthiamine *O*-monophosphate (NBTMP) to give $D=5.22\times10^{-6}\ \text{cm}^2\ \text{sec}^{-1}$.

The estimation of the electron number, n, at various pH values on the d.c. first wave of BTMP was made using the value of D, so that it was found that 3 or 4 electrons were discharged in the reduction of BTMP in the acidic solutions.

The calculation of the transfer coefficient, α, and the rate constant, k_G, for the first a.c. wave was undertaken by Matsuda's method, giving, $\alpha=0.91_6$, $k_G=5.7_5\times10^{-4}\ \text{cm.sec}^{-1}$ at a pH value of 1.00, and $\alpha=0.43_0$, $k_G=5.0_2\times10^{-4}\ \text{cm.sec}^{-1}$ at a pH value of 5.5, etc.

The reaction processes of BTMP were estimated again according to the results obtained in this work. For the first wave two splitting mechanisms of the *S*-acyl bond which produced benzyl alcohol and benzpinacol consuming 4 and 3 electrons respectively, were considered. For the second wave, as no definite discussion could be undertaken on account of the ill-defined form of the d.c. wave, it can only be suggested that it is the reduction of the reduction products, TMP. Some of the differences between the second wave of BTMP and the first wave of TMP observed in their pH value $-i_s$ curves can be explained by the difference in the wave heights of the thiazol type and of the thiol type of TMP and also by the rather slow rate of the thiazol-ring formation of the latter.

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5) K. Okamoto, This Bulletin, 36, 366 (1963).