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Differential pulse polarographic study of thiamine (vitamin B_1) in neutral and acidic aqueous solutions

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Abstract. The differential pulse (dp) polarograms of thiamine in neutral aqueous solutions exhibited six peaks at low depolarizer concentration ($\lesssim 10^{-4}$ mol dm^{-3}) and only three peaks at concentrations $\geq 10^{-3}$ mol dm^{-3}. Only one of these was found to correspond to the diffusion-controlled reduction of this compound at the dme and this was shown to be an irreversible two-electron process. The kinetic parameters derived from the dp polarograms were found to be in good agreement with those calculated from classical polarograms and were: $E_{1/2} = -1{\cdot}261$ V *vs* SCE, $\alpha n_a = 0{\cdot}54$ and $D \sim 3{\cdot}5 \times 10^{-6}$ cm^2 sec^{-1} for 10^{-3} mol dm^{-3} thiamine in 0·1 mol dm^{-3} acetate buffer (pH 6·5). The reduction product has been identified as dihydrothiamine. The effect of pH on the dpp of thiamine was studied in the pH range 0–7. In the pH region 5·5 to 7·0 only one peak attributable to the B_1^+ form of thiamine is present. In the pH region 3·5–5·5 another dpp peak attributable to the protonated form (B_1H^{2+}) of thiamine was also observed. At pHs less than 3 only one peak was observed which could be attributed to the doubly protonated form ($B_1H_2^{3+}$) of thiamine. Surfactants like triton-X-100 and CTABr were found to inhibit the electroreduction of thiamine due to the strong adsorption of these compounds on the dme. Thiamine itself was found to have an inhibitory effect on its own electroreduction, although to a smaller extent.

Keywords. Thiamine ; vitamin B_1 ; differential pulse polarography ; electroreduction ; surfactant effect.

1. Introduction

Thiamine (vitamin B_1) which is present in neutral aqueous media as the monocation B_1^+ had hitherto been studied mainly by the classical polarographic technique. In neutral KCl medium three waves have been observed (Tikhomirova and Belenkaya 1962; Shkodin and Tikhomirova 1953) with $E_{1/2}$ at −1·2, −1·43 and −1·6 volts *vs* SCE of which only the first has been found to depend on the concentration of thiamine, and hence useful for its determination. In neutral and acidic media, the cathodic waves observed have been attributed to the reduction of B_1^+ and discharge of H^+ (Tachi and Koida 1951). In an a.c. polarographic study (Okomoto 1961) two peaks were observed in neutral media (e.g., at −1·351 and −1·445 V *vs* SCE at pH 7) which shifted anodically with decreasing pH until

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pH 5 below which only the more cathodic peak was observed. These peaks were assigned to B_1^+ and B_1^+ HCl (i.e., B_1H^{2+}). Oscillopolarographic square-wave polarographic study of thiamine has also been reported (Okomoto 1964) at different pHs. Here also the multiplicity of peaks confused the picture.

We have used the differential pulse polarographic technique to identify the electroreduction peak of thiamine in neutral aqueous solutions and estimate the kinetic parameters D, αn_a and $k_{f,h}$. The effect of surfactants and pH on the electroreduction of thiamine has also been studied.

2. Experimental

Thiamine chloride hydrochloride ($B_1^+ Cl^- HCl$) employed was from Sigma Chemicals 'A' grade and was used without further purification. The UV spectrum of its solution at pH 6·5 was identical to the one reported in the literature (Maier and Metzler 1957). The various surfactants, viz., Triton X-100 (Koch-Light scintillation grade), sodium lauryl sulphate (Fluka, Puriss), cetyl pyridinium chloride (E. Merck, G.R.) and cetyltrimethylammonium bromide (Fluka, Puriss) were used as such. All other chemicals employed were either BDH 'Analar' or S. Merck 'G.R.' grade. The compositions of buffers employed for neutral pH ($\sim$ 6·5) were as recommended by Brezina and Zuman (1958) except for acetate buffer. For reasons to be discussed in § 3 acetate buffer was used at this pH in spite of its poor buffer capacity at pH $\gtrsim$ 6·0.

In the study of pH effects, acetate buffer (Brezina and Zuman 1958) was employed for the pH region 4–6. Between 2·5–4, the pH was adjusted by addition of HCl to the acetate buffer of pH 4. Below 2·5 only HCl was used as the buffer and constant ionic strength was maintained by adding KCl.

Solutions were made in triply distilled water containing the appropriate buffer and were purged with oxygen-free argon gas prior to recording the polarograms on a Bruker Model E-310 modular polarograph, equipped with a mechanical drop time controller. Mercury was doubly distilled after washing with dilute HNO_3. The mass flow rate was $\sim 1 \cdot 6$ mg sec^{-1}. In the differential pulse polarographic experiments the drop time was 3 sec, the pulse being applied 55 m sec before the drop was dislodged. A natural drop time of $\sim$ 5 sec was used in the classical polarographic experiments. All polarograms have been recorded in the 3-electrode mode with SCE as the reference electrode. The uncompensated cell resistance was found to be less than 200 ohms. Controlled potential coulometry was carried out at a mercury pool electrode employing platinum foil as counter electrode and SCE as the reference electrode.

3. Results and discussion

The number of peaks, peak potentials (E_m), their widths, ($\triangle W_{1/2}$) and peak current (i_m) in the differential pulse (dp) polarograms of thiamine were found to be strongly dependent on its concentration and on the type and concentration of the buffer used. A few representative dp polarograms illustrating these dependences are shown in figures 1 and 2 and the variation of peak potentials of the different peaks

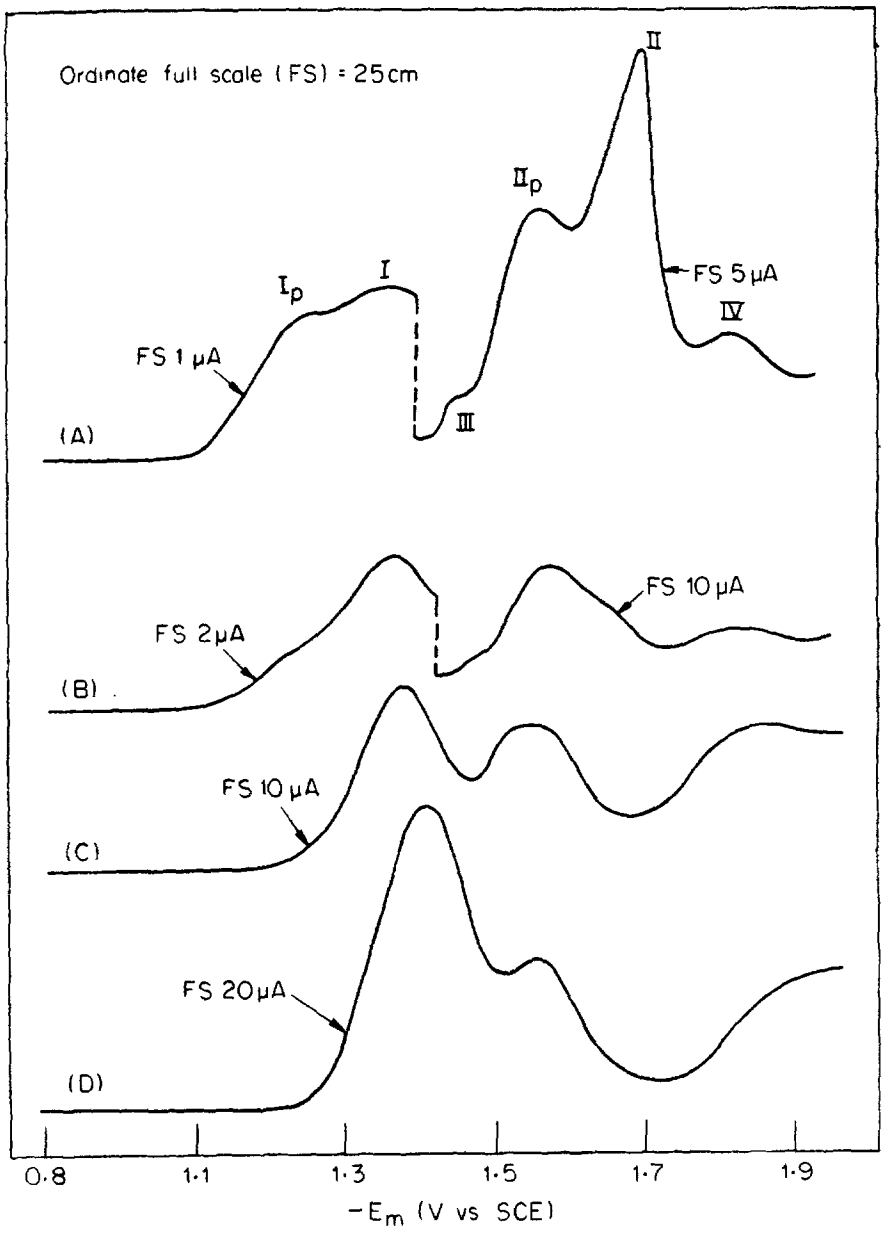


Figure 1. Differential pulse polarograms of thiamine at different concentrations in aqueous solution (pH 6·5), 0·1 mol dm^{-3} acetate buffer. Thiamine concentration mol $dm^{-3} \times 10^3$: 0·06 (A), 0·1 (B), 0·6 (C) and 2 (D).

with thiamine concentration are summarised in figure 3. Peaks are numbered in the order of increasing cathodic potentials corresponding to their peak positions. The subscript *p* is used to denote that the concerned peak is either an adsorption pre-peak or post-peak.

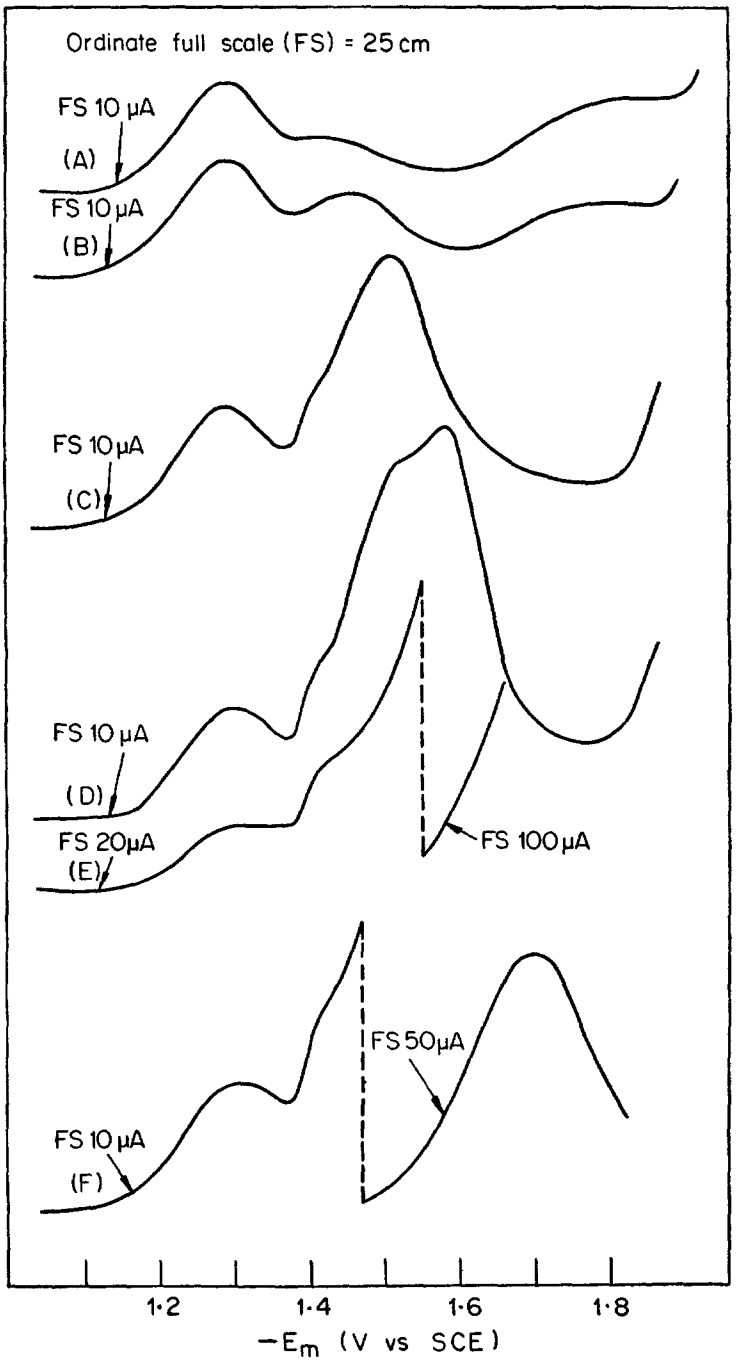


Figure 2. Influence of buffers and their concentrations on the differential pulse polarograms of 10^{-3} mol dm^{-3} thiamine in aqueous solutions (pH 6·5). Acetate—(A) 0·05, (B) 0·1, (C) 0·5, (D) 1·0, mol dm^{-3}; phosphate—0·1 mol dm^{-3} (E); phosphate 0·1 mol dm^{-3} + citrate 0·05 mol dm^{-3} (3 : 1 V/V) (F).

3.1. *Peaks I and I_p*

In the absence of surfactants, and in neutral medium (pH 6·5, 0·1 mol dm^{-3} acetate buffer) peak I was observed at thiamine concentration $\gtrsim 5 \times 10^{-5}$ mol dm^{-3} and exhibited a small negative shift with increasing concentration (figure 3). The peak current increased with concentration, the increase being not quite linear particularly at concentrations above $\sim 10^{-3}$ mol dm^{-3}. In this region $\Delta W_{1/2}$ also increased with concentration (table 1) and a plot of $i_m \times \Delta W_{1/2}/\Delta E$ *versus* concentration was found to be more closely linear.

The peak position, the width and current of peak I were virtually unaffected by the concentration of the acetate buffer at the same pH (6·5). The same was true for other buffers such as phosphate and phosphate-citrate, but the resolution of peak I from its next more negative neighbour was the best in acetate buffer. It is for this reason that acetate buffer has been employed in the rest of the present study although it is not the most optimum for this pH.

Peak I_p positively shifted from −1·23 V at 3×10^{-6} mol dm^{-3} thiamine to −1·12 V at 10^{-4} mol dm^{-3}; its height was a maximum at 6×10^{-5} mol dm^{-3} and it disappeared beyond $\sim 10^{-4}$ mol dm^{-3}. This and another peak II_p are interpreted as adsorption pre-peaks corresponding to the normal peaks I and II. As evidence of this it was observed that the heights of these adsorption peaks relative to the corresponding normal peaks were considerably enhanced at lower drop times.

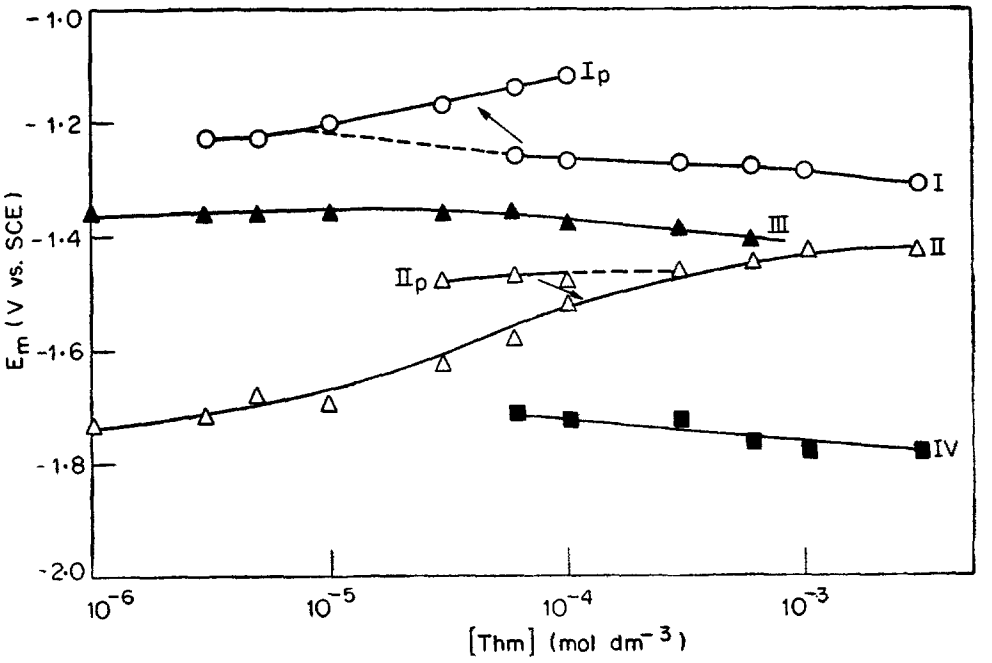


Figure 3. Dependence of the peak potentials on thiamine concentration in the differential pulse polarograms of thiamine in aqueous solution (pH 6·5, 0·1 mol dm^{-3} acetate buffer).

Table 1. Effect of thiamine concentration on its differential pulse polarographic parameters in 0·1 mol dm^{-3} acetate buffer, pH 6·5. $t_d = 3$ sec, $\Delta E = 50$ mV. Data refer to peak I (see text).

[Thiamine] (mmol dm^{-3})	$-E_m$ (V *vs* SCE)	$\Delta W_{1/2}$ (mV)	αn_a	i_m (μA)	$i_m \times \Delta W_{1/2}/C\,\Delta E$ (μA dm^3 m $mole^{-1}$)
0·06	1·26	*	..	0·04	..
0·1	1·27	*	..	0·22	..
0·3	1·27	144	0·58	1·32	12·7
0·6	1·28	144	0·58	2·40	11·5
1·0	1·29	152	0·55	3·88	11·8
3·0	1·31	160	0·505	8·96	9·56

* Not measurable as the peak is not well-defined.

3.2. *Other peaks*

Peak II was found to shift anodically with increasing thiamine concentration (figure 1) from $-1{\cdot}7$ V at 10^{-6} mol dm^{-3} to $-1{\cdot}43$ V at 3×10^{-3} mol dm^{-3}, a pre-peak II_p was first seen at $\sim 3 \times 10^{-5}$ mol dm^{-3} but it merged with II beyond $\sim 10^{-4}$ mol dm^{-3}. Peak III showed a rather small negative shift with increasing concentration and eventually merged with peak II at $\sim 10^{-3}$ mol dm^{-3} and beyond. Peak IV was first observed at 6×10^{-5} mol dm^{-3} and showed a small negative shift with thiamine concentration. In the region 6×10^{-5}–10^{-4} mol dm^{-3} all the peaks (I, I_p, II, II_p, III and IV) were observable whereas at 10^{-3} mol dm^{-3} and above only peaks I, II and IV were observed.

The height of peak II (or combined heights where it was merged with II_p and/or III) reached a limiting value beyond $\sim 5 \times 10^{-4}$ mol dm^{-3} thiamine). Because of merging of peaks no definite conclusion could be made regarding the variation of $\Delta W_{1/2}$. The height of peak IV also appeared to reach a limiting value beyond 3×10^{-4} mol dm^{-3} thiamine, but this inference as well as any conclusion regarding its width are not unambiguous because of its progressive negative shift and merger with the hydrogen current at still higher concentrations.

With increasing concentration of acetate buffer (pH 6·5) peak IV progressively shifted cathodically and eventually merged with the hydrogen current whereas peaks II, II_p and III got resolved from each other. The combined height of the latter three increased markedly with the buffer concentration. In phosphate-citrate buffer peaks II, II_p and III were better resolved at low buffer concentration (0·02 mol dm^{-3} disodium phosphate and 0·01 mol dm^{-3} citric acid), but at a higher buffer concentration (0·1 mol dm^{-3} phosphate, 0·05 mol dm^{-3} citric acid) their mutual resolution was poorer and peak I was also distorted considerably. The same was also true of phosphate alone as the buffer.

Among the various peaks only I, II and IV were observed at all concentrations, and only I was found to exhibit systematic increase of i_m with increase in thiamine concentration. The attainment of a limiting height in the case of peaks II–IV and the strong dependence of their peak positions and currents on buffer

concentration would suggest them to be of catalytic origin. They were not studied further.

3.3. *Classical polarography*

In the classical polarograms of thiamine in acetate buffer waves corresponding to all of the above peaks were not observed, but only those corresponding to peaks I and II (or IV) were observed at all concentrations. Waves corresponding to other peaks were not observed because of poorer resolution of this technique. The limiting current plateau of wave I was sufficiently well-defined only at high thiamine concentrations ($\sim 10^{-3}$ mol dm^{-3} and above). Under this condition the wave I limiting current varied linearly with $h^{1/2}$ suggesting it to be diffusion-controlled. Controlled potential electrolysis on the plateau of this wave gave an n value close to 2. The electrolysed solution exhibited an anodic wave with $E_{1/2} = -0{\cdot}4$ V. The electrolysis product, on extraction with $CHCl_3$ and dissolution in water, gave a UV spectrum with λ_{max} at 237 nm and 280 nm (OD ratio = 1·27). The above anodic $E_{1/2}$ and the UV spectral features were in agreement with those for dihydrothiamine (B_1H) prepared by chemical reduction of thiamine according to the procedure of Karrer and Krishna (1950). The mass spectra of both the electrolytically and chemically prepared products revealed a parent ion peak at $m/e = 266$ as expected for this compound. Therefore thiamine wave I (and hence also peak I in dpp) can be inferred to be due to an overall two-electron reduction of thiamine, the reduction occurring on the thiazole moiety.

The plot of wave I limiting current *versus* thiamine concentration was linear as expected of diffusion current, but did not pass through the origin. This is because of the increasing relative contribution from the foot of the succeeding catalytic wave with decreasing concentration of the depolarizer. The slope of the above linear plot, in conjunction with $n = 2$ gave a value of 4×10^{-6} cm^2 sec^{-1} for the diffusion coefficient of thiamine in 0·1 mol dm^{-3} acetate buffer. As will be seen later, this value was in agreement with the one derived from the differential pulse polarograms.

A log plot analysis of thiamine wave I in pH 6·5 acetate buffer gave $E_{1/2} = -1{\cdot}261$ V (*vs* SCE) at $t_d = 5{\cdot}1$ sec, and an αn_a value of 0·54 which, as will be seen later, was also in agreement with the value inferred from dpp measurements. From these and the above value of the diffusion coefficient the rate constant for electron transfer from the dme to thiamine (at the above $E_{1/2}$) is calculated to be $7{\cdot}15 \times 10^{-4}$ cm sec^{-1}. The reduction of thiamine at the dme in the above medium is therefore inferred to be irreversible.

3.4. *Effect of pH*

The dp polarograms of 10^{-3} mol dm^{-3} thiamine at different pH are shown in figure 4. Among the peaks I, II and IV observed at this concentration the latter two were poorly resolved below pH 6 and eventually merged with the hydrogen current, whereas E_m for the first was independent of pH in the region 5-6·5 and was not observable below pH 4·5. At pH $\leq$ 5·5 a new peak I_a appeared which shifted anodically with decreasing pH, the shift being $\sim$ 40 mV per pH unit in the region 3·5-5·5 and 122 mV per pH below pH 3 (figure 5). Because of the progressive cathodic shift of I_a with increasing pH and eventual

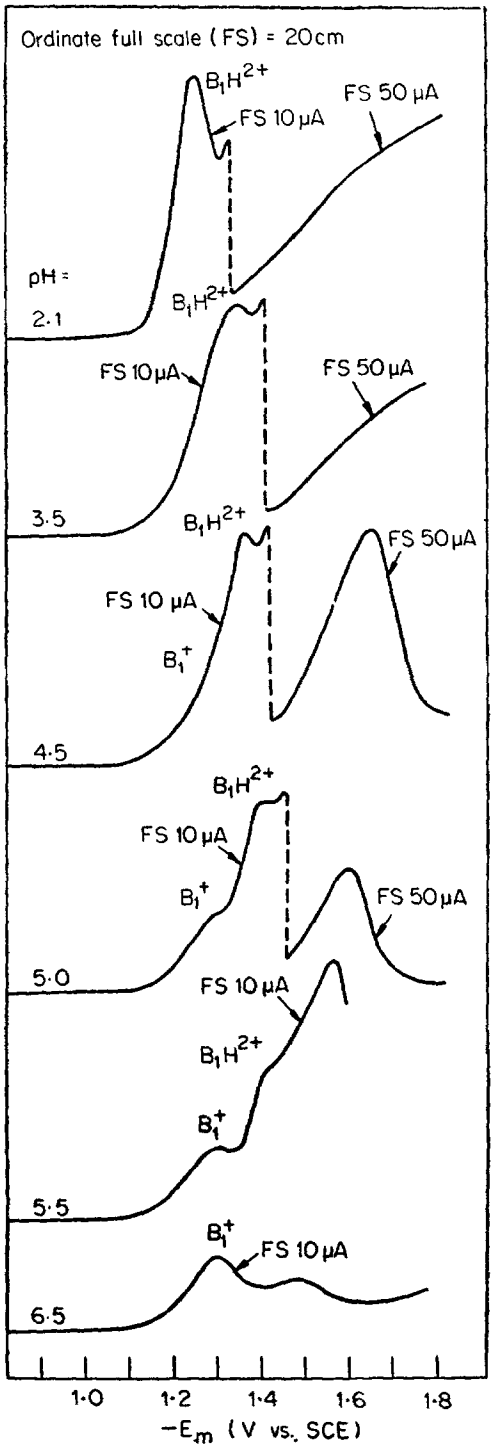


Figure 4. Effect of pH on the differential pulse polarograms of thiamine in aqueous solutions (10^{-3} mol dm^{-3} thiamine).

merger with peaks II-IV, the average slope of 40 mV per pH unit observed for the region 3·5-5·5 may be somewhat erroneous. However, if we consider the E_m values for peak I_a only at pH 3·5 and 4 where it is well resolved from the more cathodic peaks, the shift is 66 mV per pH unit (see table 2) close to what is expected for a reduction process involving one proton. The anodic shift of 122 mV per unit decrease of pH in the region of pH < 3 is indicative of the involvement of two protons in the electroreduction process.

Thiamine has a $pK_a = 4·8$ for the equilibrium (1)

$$B_1^+ + H^+ \rightleftharpoons B_1H^{2+} \qquad (1)$$

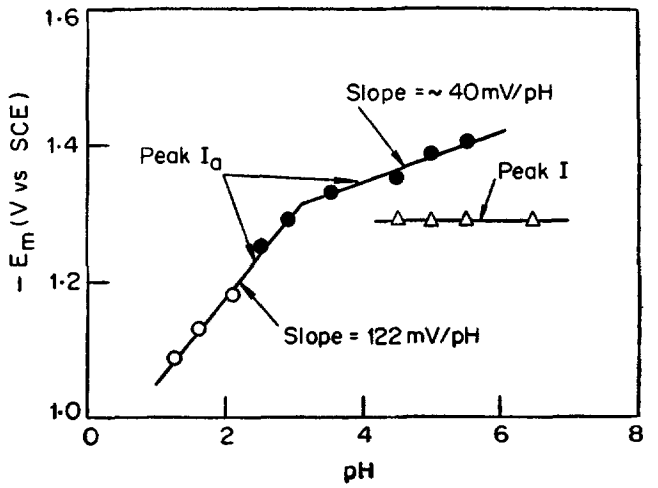


Figure 5. Effect of pH on the E_m of peak I_a in the differential pulse polarograms of 10^{-3} mol dm^{-3} thiamine. (● 0·1 mol dm^{-3} acetate pH adjusted with HCl ; O HCl + KCl, total electrolyte ~ 1F).

Table 2. pH effect on the differential pulse polarographic parameters of thiamine (10^{-3} mol dm^{-3}) in acidic solutions. Total $[Cl^-] = 1$ mol dm^{-3}. $t_d = 3$ sec. $\Delta E =$ 20 mV.

[HCl]	pH	$-E_m$ (V *vs* SCE)	$\Delta W_{1/2}$ (mV)	an_a
(*a*)	4·0	1·364	206	0·41
(*a*)	3·5	1·331	200	0·42
0·01	2·1	1·180	76	1·1
0·032	1·6	1·128	92	0·91
0·1	1·25	1·086	96	0·87

(*a*) 0·1 mol dm^{-3} acetate, pH adjusted with HCl, peak I_a.

As there are no dissociable protons in the B_1^+ form the pH independence of the E_m of peak I in the region 5·5-7 (figure 5) where it is present almost exclusively in this form is understandable. In this pH region, the value of αn_a in the absence of surfactants varied between 0·5 and 0·6. As under this condition α is expected to be close to 0·5, it may be inferred that n_a the number of electrons involved in the rate-detrrmining step is unity. From the controlled potential coulometric experiment the number of electrons involved in the overall reduction was found to be 2. We may therefore conclude that the addition of the second electron and a proton to give the product dihydrothiamine are fast :

$$B_1^+ + e^-(Hg) \rightarrow B_1 \tag{2}$$

$$B_1 + H^+ + e^-(Hg) \rightarrow B_1H \tag{3}$$

The radical species formed in the first reduction step has been produced in the past (Moorthy and Hayon 1977) by one-electron reduction of thiamine with hydrated electrons and other strongly reducing free radicals such as CO_2^- and found to undergo a second order dismutation reaction:

$$2B_1 + H^+ \rightarrow B_1^+ + B_1H \tag{4}$$

This might as well be an alternate pathway by which the one-electron reduction product formed in the electroreduction (reaction 2) can give rise to dihydrothiamine and account for a value of $n = 2$ for the overall reduction.

At lower pHs in the region 4-5 where both B_1H^{2+} and B_1^+ are present in equilibrium, the dp polarograms reveal two peaks corresponding to the electroreduction of these two conjugate forms. The pH dependent peak I_a can be assigned to the process:

$$B_1^+ + H^+ \rightleftharpoons B_1H^{2+} \xrightarrow{e^-(Hg)} B_1H^+ \tag{5}$$

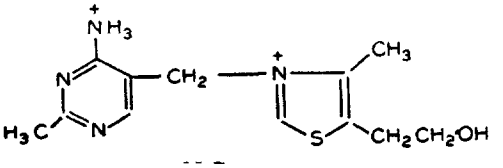
in agreement with the observed shift of E_m. From the $\triangle W_{1/2}$ of this peak at pH 3·5 and 4 a value of $\sim 0{\cdot}41$ is obtained for αn_a (table 2) indicating the process to be irreversible.

The single peak I_a observed at pH < 3 was much narrower than the ones observed at the higher pHs, and the value of αn_a calculated from the $\triangle W_{1/2}$ was close to unity (table 2). The log plot analysis of classical polarograms also gave a value of αn_a close to unity. From the anodic shift of the peak potential with decreasing pH (122 mV/pH unit) the reduction process is inferred to be:

$$B_1^+ + 2H^+ \rightleftharpoons B_1H_2^{3+} \xrightarrow{e^-(Hg)} B_1H_2^{2+} \tag{6}$$

From the intersection of the two linear plots of E_m *versus* pH for peak I_a we can infer a pK_a of ~ 3 for the equilibrium involved in process (6), although there is no report of this in the literature. However, the thiamine molecule has three sites for protonation, viz., the $-NH_2$ group and the two pyrimidine ring

nitrogen atoms. As the amino nitrogen is more basic than the ring nitrogens, we may, in agreement with Okomoto (1961), assign the B_1H^{2+} species to:



and hence the $B_1H_2^{3+}$ species to one with the pyrimidine N also protonated.

The similarity between the results of the present study and those reported by Okomoto (1961) is worth noting. In the pH region 5-6 two diffusion-controlled reduction peaks are observed, the more cathodic of which is attributed to the protonated form (B_1H^{2+}). The anodic shift of this with decreasing pH is close to that expected for a one H^+ one e^- process, and this is more clearly evident in Okomoto's data. However, the pH dependence of the electroreduction peak of the unprotonated form B_1^+ by this author is surprising, whereas we find it to be independent of pH as expected. The non-observance of this peak below pH 4 is in agreement in the two studies. As Okomoto's study does not cover the pH region below 3, our observation of a single peak due to reduction of a highly protonated form $B_1H_2^{3+}$ in which the $-NH_2$ group as well as both the pyrimidine nitrogens are protonated is a new result. The controlled potential coulometry at a Hg pool gave an n value of 2 for the electroreduction of both species B_1H^{2+} (pH 3·5, −1·3 V) and $B_1H_2^{3+}$ (pH 2, −1·2 V).

3.5. *Evaluation of parameters from differential pulse polarograms*

Differential pulse polarograms have the appearance of the first derivatives of classical polarograms. In their earlier paper, Parry and Osteryoung (1965) in fact derived expressions for the peak potential, half width and peak current of the differential pulse polarogram of a reversible wave based on the approximation.

$$\Delta i \simeq \Delta E\, di/dE \tag{7}$$

where ΔE is the pulse amplitude (which is kept constant throughout the scan) and Δi the sampled current at any point on the polarogram. di/dE is the first derivative of the classical polarographic equation:

$$i = i_d/[1 + \exp\{nF(E - E_{1/2})/RT\}]. \tag{8}$$

This approximation was considered valid under the condition that $\Delta E \ll \Delta W_{1/2}$ and led to:

$$E_m = E_{1/2}, \tag{9}$$

$$i_m(\mu A) = 7{\cdot}92\, n^2\, m^{2/3}\, t_d^{2/3}\, C\,(D/\pi\delta)^{1/2}\, \Delta E, \tag{10}$$

and
$$\Delta W_{1/2}(mV) = 91{\cdot}12/n \text{ at } 300\,\text{K}, \tag{11}$$

where n is the total number of electrons involved in the electroreduction, t_d the drop time (sec), m the mass flow rate of mercury (mg sec^{-1}), C the bulk con-

centration of the reducible species (mmol dm^{-3}), D its diffusion coefficient (cm^2 sec^{-1}), δ the pulse duration (sec) and $\triangle E$ is in mV. A more exact treatment (Keller and Osteryoung 1971) led to:

$$E_m = E_{1/2} - \triangle E/2, \tag{12}$$

$$i_m = 815{\cdot}19\, n\, m^{2/3}\, t_d^{2/3}\, C(D/\pi\delta)^{1/2}\, (\sigma - 1)(\sigma + 1), \tag{13}$$

where $$\sigma = \exp\ (nF\triangle E/2RT). \tag{14}$$

This is applicable even when $\triangle E > 2RT/nF$ and reduces to (10) when $\triangle E \ll 2RT/nF$.

Further refinements of the theory of differential pulse polarography have been made by Birke (1978). With this treatment an analytical solution of the equations was possible only in the case of reversible processes and the expression so obtained for E_m is the same as (12) and i_m as given by (13) was modified by a correction term. In the absence of analytical solutions to the differential pulse polarographic current-potential relations of quasi-reversible and irreversible systems based on rigorous theory, we follow here, as a rough approximation, the original procedure of Parry and Osteryoung (1965) making use of (15) for the current in an irreversible process (Meites 1965)

$$i = i_d/[1 + \exp\{\alpha n_a F(E - E_{1/2})/0{\cdot}916\, RT\}]. \tag{15}$$

This leads to : $E_p = E_{1/2} - \triangle E/2$ as in the reversible case and,

$$i_m(\mu A) = 8{\cdot}64\, nm^{2/3}\, t_d^{2/3}\, C(D/\pi\delta)^{1/2}\, \triangle E\, \alpha n_a, \tag{16}$$

and $$\triangle W_{1/2}(mV) = 83{\cdot}49/\alpha n_a \text{ at } 300\text{ K}. \tag{17}$$

From (10) and (16) which are applicable for $\triangle E \ll 2RT/\alpha n_a F$, it can be seen that i_m should vary linearly with $\triangle E$. As $\alpha n_a \simeq 0{\cdot}5$ for thiamine we can expect this to hold for $\triangle E < 103$ mV, and this was found to be the case for the I peak in the differential pulse polarograms of this compound in acetate buffer at pH 6·5.

The values of αn_a calculated from (17) in the case of dp polarograms and from the slopes of plots of log $(i_d - i)/i$ *versus* potential in the case of classical polarograms, as also the E_m and $E_{1/2}$ values are compared with each other in table 3. It is seen that E_m is very nearly equal to $E_{1/2} - \triangle E/2$ and the αn_a values by the two methods agree with each other at different thiamine concentrations. In fact at low concentrations of thiamine the diffusion current plateau in the classical polarograms was too poorly defined to allow an accurate log plot analysis whereas at thiamine concentrations $> 2 \times 10^{-3}$ mol dm^{-3} and in the absence of surfactants the classical polarograms exhibited maxima, again affecting the accuracy of log plots, and hence the E_m and αn_a values obtained from dp polarograms may be considered more reliable.

Although (16) suggests that for a given pulse amplitude i_m varies linearly with concentration, this is true only if αn_a does not vary. In the case of thiamine these were dependent on concentration (table 1). The variation was somewhat erratic at concentrations $\lesssim 5 \times 10^{-4}$ mol dm^{-3} because the peak was not fully developed at these low concentrations in the absence of surface active agents. At higher concentrations αn_a decreased with increasing concentration of thiamine.

Table 3. Comparison of $E_{1/2}$ and an_a values for thiamine in 0·1 mol dm^{-3} acetate buffer (pH 6·5) obtained by classical and differential pulse polarographic techniques.

[Thiamine] (m mol dm^{-3})	Classical polarographic values*		Differential pulse polarographic values**	
	$-E_{1/2}$ (V *vs* SCE)	an_a	$-E_{1/2}$ (V *vs* SCE)	an_a
0·60	1·263	0·55	1·255	0·58
0·84	1·261	0·54	1·263	0·58
1·20	1·263	0·54	1·263	0·57
2·0	1·267	0·53	1·279	0·52
3·2	1·265	0·51	1·291	0·49

* From log plot ; ** $E_{1/2} = E_m + \Delta E/2$; $an_a = 83{\cdot}49/\Delta W_{1/2}$.

In such cases where an_a is dependent on concentration the parameter $i_m \times \Delta W_{1/2}/\Delta E$ was found to exhibit a better linear dependence on concentration than was the case with i_m. This is evident from (16) by substituting for an_a in terms of $\Delta W_{1/2}$ to give:

$$i_m \Delta W_{1/2}/\Delta E = 721{\cdot}7\, nm^{2/3} t_d^{2/3} (D/\pi\delta)^{1/2} C. \qquad (18)$$

A plot of $i_m \times \Delta W_{1/2}/\Delta E$ *versus* C was found to be linear as expected from (18). From the slope of this plot we could calculate D knowing m, t_d and δ. These were respectively 1·2 mg sec^{-1}, 2 sec and 55 m sec in our experiments, and the slope was 11·6 μA (m mol)$^{-1}$ dm^3. These gave a value of $3{\cdot}6 \times 10^{-6}$ cm^2 sec^{-1} for D which is in agreement with the value derived from the slope of the i_d *versus* concentration plot of classical polarograms. The D values similarly evaluated (from dp polarograms) for the B_1H^{2+} (pH 3·5) and $B_1H_2^{3+}$ (pH 2·0) forms are $2{\cdot}4 \times 10^{-5}$ and $3{\cdot} \times 10^{-5}$ cm^2 sec^{-1} respectively. It would appear that protonation markedly enhances the diffusion of the species in the solution.

Although the dp polarogram of thiamine in aqueous solutions exhibited a number of peaks, only one of these could be attributed to a two-electron reduction of the compound. The parameters for this electroreduction, viz., an_a, $E_{1/2}$ and D derived from the dp polarograms and classical polarograms agreed well with each other. This agreement is quite good considering the fact that the electroreduction of thiamine at the dme is irreversible and there are no rigorous theoretical equations available to evaluate these parameters from dp polarograms in the case of irreversible processes. Procedurally it is much simpler to derive these parameters from dp polarograms as the peak positions, currents and widths are very easily measurable from such polarograms and no recourse to the tedious log plot analysis is necessary as in the case of classical polarograms. It may be noted that Aoki and Osteryoung (1980) have obtained analytical solutions to the differential pulse polarographic currents at expanding plane electrode, on the basis of which they have suggested a new procedure for obtaining kinetic parameters from experimental data. This, however, requires measurements at different drop times and

pulse times. As variable pulse time is not a feature in our instrument, as in fact is the case in all commercial instruments, we have not been able to perform such measurements and compare the values of the kinetic parameters with the ones derived from the simpler and approximate formalism adopted here.

3.6. *Effect of surfactants*

The dp pulse polarograms of 6×10^{-5} mol dm^{-3} thiamine at pH 6·5 (0·1 mol dm^{-3} acetate buffer) in the presence of varying concentrations of triton X-100 are shown in figure 6. Qualitatively similar results were also obtained with cetyltrimethylammonium bromide as the surfactant. In the case of cetylpyridinium chloride and sodium lauryl sulphate, however, the tensammetric peaks of these compounds interfered with the thiamine peaks. With increasing triton concentration peak I shifted cathodically, and the $\triangle W_{1/2}$ decreased, the former effect being more marked at lower thiamine concentrations (table 4). Whereas this peak was poorly developed in the absence of triton at a thiamine concentration of 6×10^{-5} mol dm^{-3}, it was well defined in the presence of the surfactant even at a thiamine concentration as low as 10^{-6} mol dm^{-3}. The peak current increased with triton concentration but $i_m \times \triangle W_{1/2}/\triangle E$ was almost constant. Unlike the normal peak I, the prepeak I_p showed an anodic shift in the presence of the surfactant and was found to disappear even at as low as 0·002% triton. Also peak III merged with II_p at ~ 0·001% triton, and the latter peak merged with peak II at ~ 0·002% triton. In the region of high triton concentration, up to ~ 0·04% only peaks I, II and IV were observed, and these were well defined. At still higher triton concentrations (up to ~ 0·2%) although peak I was unaffected, peaks II and IV were severely distorted. This distorting effect of triton was not discernible at high concentration of thiamine (say 10^{-3} mol dm^{-3}); here, in fact peak IV was better defined at ~ 0·2% triton than at say 0·02%.

It may be noted here that in classical polarography (Meites 1965) it is often the practice to add a very small amount of surfactants (0·001% or less) as maximum suppressors. On the contrary, in our present study of thiamine by the differential pulse polarographic technique it was found advantageous to add a relatively large amount of the surfactant. The reason for this as will be seen later is that thiamine itself is adsorbed at the electrode and a high concentration of a strong surfactant such as triton X-100 is necessary to displace all the adsorbed thiamine from the dme surface. Thus at 0·04% triton well defined dp polarograms of thiamine, consisting of only peaks I, II and IV are observed over more than three decades of thiamine concentration from 10^{-6} mol dm^{-3}. The $\triangle W_{1/2}$ (and hence αn_a) of peak I was found to vary systematically with thiamine concentration (table 5) and the plot of $i_m \times \triangle W_{1/2}/\triangle E$ *versus* concentration was linear over several decades of thiamine concentration. The analytical utility of this finding has been reported earlier (Kamal Kishore *et al* 1979). The slope of this plot was the same as in the absence of triton. This, together with the observation that for a given concentration of thiamine, $i_m \times \triangle W_{1/2}/\triangle E$ is independent of triton concentration would suggest that the diffusion coefficient of thiamine is not affected by triton (within $\simeq$ 10%).

The neutral surfactants like triton have been observed in the past to inhibit the electroreduction of metal ions such as Cd^{2+} at the dme due to adsorption

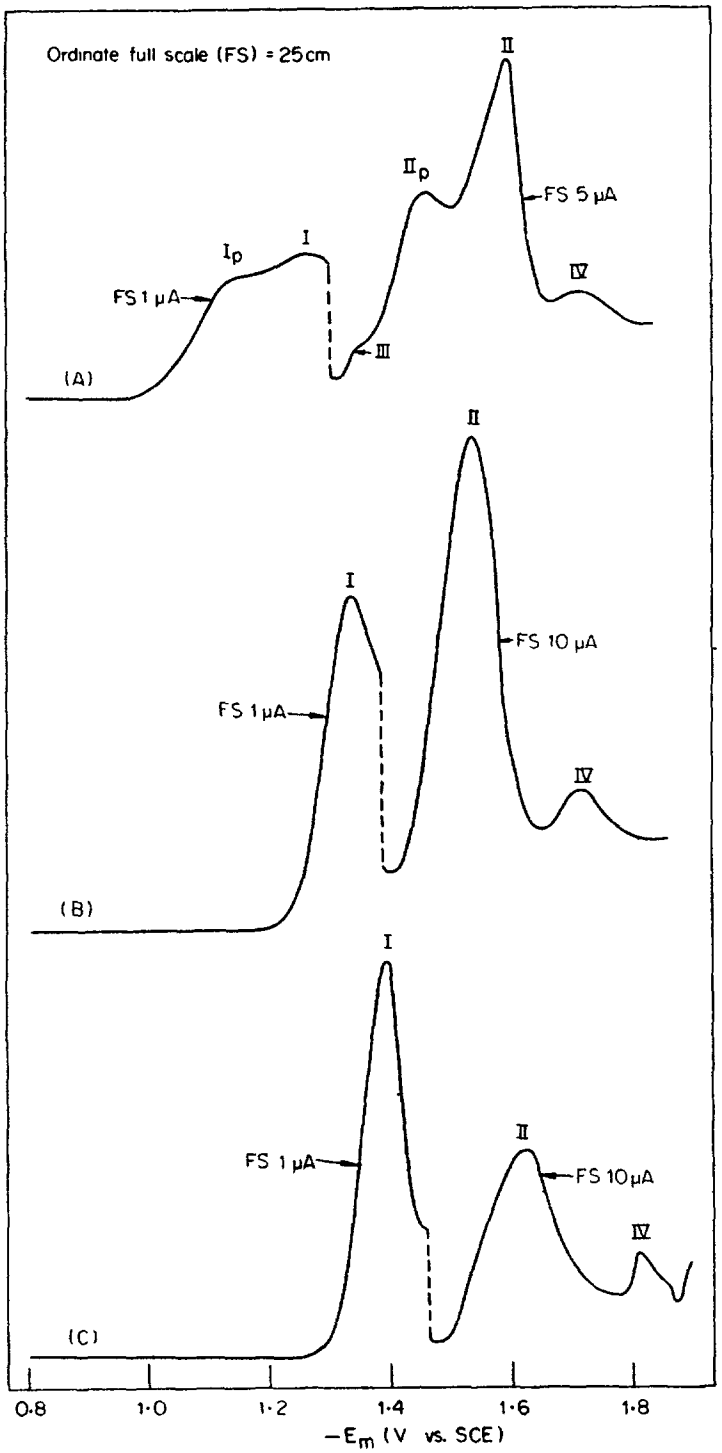


Figure 6. Effect of triton X-100 on the differential pulse polarograms of 6×10^{-5} mol dm^{-3} thiamine in aqueous solutions (pH 6·5, 0·1 mol dm^{-3} acetate), Percent triton X-100 : 0 (A), 0·01 (B), 0·15 (C).

Table 4. Effect of triton X-100 on the differential pulse polarographic parameters of peak I of thiamine in 0·1 mol dm^{-3} acetate buffer (pH 6·5). $t_d = 3$ sec $\Delta E = 50$ mV.

[Triton] (Vol. %)	$-E_m$ (V *vs* SCE)	$\Delta W_{1/2}$ (mV)	an_a	i_m (μA)	$k_{f,h}$ (10^{-4} cm sec^{-1})
(*a*) [Thiamine] $= 6 \times 10^{-5}$ mol dm^{-3} :					
0	1·26	*	..	*	..
0·001	1·268	*	..	*	..
0·002	1·290	128	0·65	0·34	4·37
0·004	1·332	100	0·83	0·56	0·92
0·01	1·352	92	0·91	0·71	0·36
0·02	1·360	88	0·95	0·68	0·23
0·04	1·380	84	0·99	0·68	0·09
0·07	1·384	82	1·02	0·68	0·07
0·15	1·388	80	1·04	0·66	0·05
(*b*) [Thiamine] $= 10^{-3}$ mol dm^{-3} :					
0	1·284	148	0·56	5·2	5·54
0·001	1·292	136	0·61	5·3	4·37
0·004	1·316	128	0·65	5·7	2·27
0·008	1·320	120	0·70	5·6	1·83
0·02	1·332	112	0·75	5·9	1·14
0·04	1·352	112	0·75	6·8	0·64
0·08	1·376	112	0·75	7·0	0·32
0·12	1·376	112	0·75	7·5	0·32
0·20	1·376	112	0·75	7·0	0·32

* Peak not well developed.

Table 5. Dependence of the differential pulse polarographic parameters of peak I of thiamine on its concentration in 0·1 mol dm^{-3} acetate buffer (pH 6·5) containing 0·04% triton X-100. $t_d = 2$ sec. $\Delta E = 50$ mV.

[Thiamine] (mmol dm^{-3})	$-E_m$ (V *vs* SCE)	$\Delta W_{1/2}$ (mV)	an_a	$k_{f,h}$ (10^{-4} cm sec^{-1})
0·01	1·384	80	1·04	0·08
0·10	1·376	92	0·91	0·19
0·30	1·364	100	0·83	0·26
1·0	1·358	116	0·72	0·74
1·86	1·364	132	0·63	0·90
2·68	1·358	144	0·58	1·26

of the surfactant and the electron transfer rate constant decreased by several orders of magnitude at high concentration of the surfactant (Guidelli and Foresti 1977).

From the experimentally measured values of E_m and αn_a for peak I in the differential pulse polarograms of thiamine the $k_{f,h}$ values were calculated employing equations:

$$E_{1/2} = E_m + \triangle E/2 = 0{\cdot}2412 + (RT/\alpha n_a F)\ln\,[1{\cdot}349\;\; k^\circ_{f,h}\;\; t_d^{1/2}/D^{1/2}], \tag{19}$$

and $$k_{f,h} = k^\circ_{f,h}\exp\{-\alpha n_a F(E' + 0{\cdot}2412)/RT\}, \tag{20}$$

(*cf* equations 4.56 and 4·60 in Meites 1965). Following Guidelli and Foresti (1977) the reference potential E' is taken to be the $E_{1/2}$ ($= E_m + \triangle E/2$) of peak I at an uncovered Hg surface. The E_m at the uncovered surface was inferred to be 1·267 V *versus* SCE by extrapolating the plot of E_m *versus* thiamine concentration. The results are given in tables 4 to 6. It may be noted that $k_{f,h}$ calculated as above is very insensitive to variations in αn_a and is only marginally affected by D. The latter has been inferred to be unaffected by the surfactant, but even if it shows a small variation this would not greatly affect the calculated $k_{f,h}$ values. It is on $E_{1/2}$ that $k_{f,h}$ is markedly dependent and this is evaluated accurately from the peaks of differential pulse polarograms. It is seen from the data given in table 4 that $k_{f,h}$ progressively decreases with increasing concentration of triton, the inhibiting effect being more marked at the lower depolarizer concentration. The other surface active agent tried, viz., CTABr also had a similar inhibiting effect. The cathodic shift of $E_{1/2}$ and hence the inhibitory effect was not observed once the Hg drop surface was fully covered. This was the case at surfactant concentrations beyond $\sim 0{\cdot}5$ mmol dm^{-3} ($\sim 0{\cdot}03\%$ triton) and agrees with the findings of Guidelli and Foresti (1977). It is also seen that with increasing concentration of triton the value of the heterogeneous rate constant markedly decreased making the electroreduction of thiamine more irreversible than in the absence of the surface active agent. It may be inferred that although the activation controlled charge transfer is poten-

Table 6. Dependence of the differential pulse polarographic parameters of peak I of thiamine on its concentration in 0·10 mol dm^{-3} acetate buffer (pH 6·5) t_d = 3 sec, $\triangle E$ = 50 mV.

[Thiamine] (mmol dm^{-3})	$-E_m$ (V *vs* SCE)	$\triangle W_{1/2}$ (mV)	αn_a	$k_{f,h}$ (10^{-4} cm sec^{-1})
0·2	1·264	144	0·58	8·51
0·4	1·267	140	0·60	7·91
0·7	1·276	144	0·58	6·50
1·0	1·281	148	0·56	5·91
1·5	1·287	152	0·55	5·24
2·0	1·295	160	0·52	4·61
2·8	1·30	166	0·505	4·27

tially more facile (an_a approaching unity) the observed rate decreases due to a blocking effect of the adsorbed surfactant on the electrode surface.

In the absence of a surface active agent such as triton a negative shift of the E_{p} of peak I was observed with increasing thiamine concentration and decreased an_a (as inferred from the $\triangle W_{1/2}$ value). The $k_{f,h}$ values also showed a decrease with increasing tniamine concentration (table 6). It would appear that thiamine inhibits its own electroreduction by adsorption on the electrode surface. As this molecule has a $-CH_2OH$ group like the alcohols which are known to inhibit charge transfer reactions at the dme due to adsorption on the mercury surface (Guidelli and Foresti 1977) the inhibitory effect of thiamine can be understood. Further, it must be inferred that the molecule is oriented at the electrode surface in such a way that the reducible site, viz., the thiazole moiety is away from the surface so that the rest of the adsorbed molecule exerts a blocking effect for electron transfer from the surface. It is because of the adsorption of thiamine that the inhibiting effect of a second surface active agent such as triton X-100 is less marked at higher thiamine concentrations. Conversely in solutions containing a high concentration of triton an acceleration of the charge transfer rate was observed with increasing thiamine concentration (table 5). This can be inferred as due to the replacement of the adsorbed surfactant by thiamine which is less blocking than triton. At low thiamine concentrations ($< 10^{-4}$ mol dm^{-3}) its adsorption on the electrode surface is rather small and its reduction to the more strongly adsorbed product dihydrothiamine gives rise to the prewave (see e.g., Flanagan *et al* 1977). Surface tension measurements by the drop-time method revealed that at comparable concentrations the order of increasing adsorption at the dme is thiamine < dihydrothiamine < triton X-100. With increasing concentration when thiamine itself gets appreciably adsorbed, the electrode reaction is the reduction of adsorbed reactant to give adsorbed product giving rise to the normal wave. This exhibits the inhibition discussed above due to adsorption of thiamine which behaves just like any other surface active agent such as triton.

In the past it has been observed (Jacobsen and Lindseth 1976) that surfactants with the same charge as the depolarizer suppress the dp peak currents whereas oppositely charged surfactants enhance the peak currents. The results of the present study are contrary to this, as we have found that both a neutral surfactant such as triton X-100 and the cationic surfactant CTABr enhance the peak current in the case of thiamine as the depolarizer. It would therefore appear that no generalizations can be made regarding the effect of surfactants on the peak currents of depolarizers in differential pulse polarography.

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