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Short communication

Anodic adsorptive voltammetric determination of the vitamin B_1 (thiamine)

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1. Introduction

Thiamine is widely distributed in foods, the richest sources being whole grains, pulses, yeast, liver and milk. A deficiency of thiamine in the diet results in the disease known as Beri Beri [1]. Analytical methods for determination of the vitamin need to be both selective and sensitive owing to the presence of potential interferences and to the low concentrations present.

Several methods have been used for the determination of thiamine, e.g. liquid chromatography with electrochemical detection [2–5], coulometrics [6] and amperometrics [7], and other electrochemical methods have been carried out with dropping mercury electrode [8], a carbon past electrode [9], and a glassy carbon [10] electrode. The United States Pharmacopoeia describes a method for the determination of thiamine based on the spectrofluorimetric measurement of thiochrom; formed by oxidation of the vitamin with potassium hexacyanoferrate (III) [11]. The recommended procedure of the British Pharmacopoeia is based on the gravimetric precipitation of thiamine-silicotungstate [12].

There are also several methods for determining thiamine by polarography [13–15]. Differential pulse polarography was used to distinguish the reduction wave of thiamine [16], the peaks appearing after the reduction peak were mostly of a catalytic nature and adsorption type.

Many of these methods, however, suffer from lack of selectivity, are complicated and tedious procedures, and require the use of either expensive instrumentation or dangerous reagents.

The present paper describes an extremely sensitive stripping voltammetric procedure for trace measurements of thiamine, based on the coupling of adsorptive accumulation and catalytic effects. The dual (adsorptive/catalytic) amplification effects result in improved sensitivity of an adsorptive stripping procedure for thiamine. These improvements as well as detailed optimization and characterization are reported.

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2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade. Vitamin B_1 was obtained from Sigma (Poole, Dorset, UK) and used without further purification. Solutions of 0.1 mol dm^{-3} sodium nitrate, potassium chloride, sodium perchlorate, and a mixture of sodium monohydrogen phosphate and sodium dihydrogen phosphate as buffer [17], were used as supporting electrolytes. Sodium hydroxide solution was used to adjust the pH of the supporting electrolytes using a pH meter (Mv 87 digital pH-Messgerate), accurate to $t \pm 0.005$ U.

2.2. Apparatus

The instrumentation used was an EG&G Princeton Applied Research (PAR, Princeton, NJ, USA). Model 264A stripping analyzer, coupled with a PAR 303A mercury drop electrode. The polarographic cell was fitted with an Ag/AgCl-saturated KCl reference electrode and a platinum wire counter electrode. A PAR RE 0089 X–Y recorder was used for the collection of experimental data.

2.3. Procedure

Ten millilitres of 0.01 mol dm^{-3} supporting electrolyte were transferred into the cell and deaerated by passing nitrogen through for 10–16 min. An accumulation potential of -1.4 V and a scan rate of 100 mV s^{-1} were used. After the accumulation step and a further 15 s (equilibrium time), the voltammogram was recorded. The thiamine solution was introduced using an automatic pipetter (Volac 20–200 μl). The mixture was stirred while purging with nitrogen, and then the deposition and stripping step were followed as before.

All the results were obtained at room temperature (25 ± 1°C) with a nitrogen atmosphere maintained over the solution surface.

3. Results and discussion

Various supporting electrolytes such as, $NaNO_3$, $NaClO_4$, KCl, $Na_2HPO_4 + NaH_2PO_4$ mixture and Britton–Robinson buffer solution were tested to determine vitamin B_1 (thiamine) by linear sweep stripping voltammetry using a hanging mercury drop electrode. As can be seen from Fig. 1, in the presence of sodium perchlorate, a

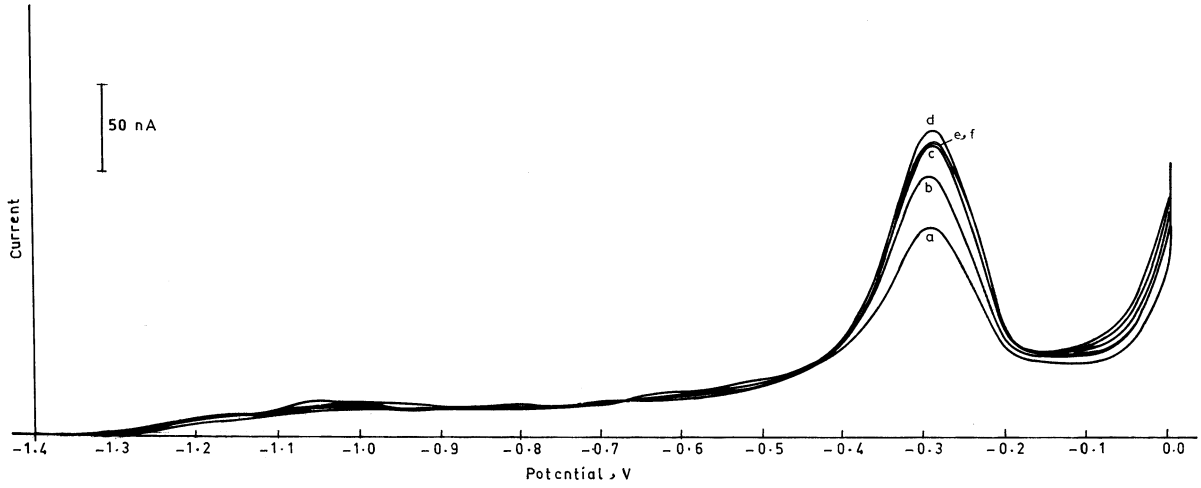
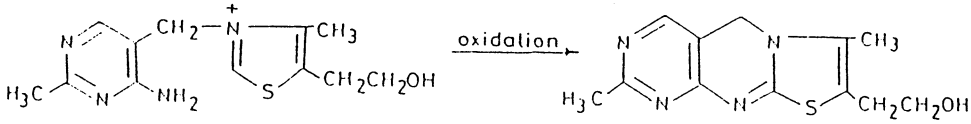


Fig. 1. Voltammograms for 1×10^{-5} mol dm^{-3} of thiamine (B_1) in the presence of 0.01 mol dm^{-3} sodium perchlorate (pH ~ 6.5), scan rate 100 mV s^{-1}, following the accumulation periods: curve a, 0 s; curve b, 5 s; curve c, 10 s; curve d, 30 s; curve e, 60 s; curve f, 90 s.

single anodic peak was observed, whereas in the other supporting electrolytes, two anodic peaks appeared: the first peak is a catalytic peak due to catalytic discharge of hydrogen ions ($E_p = -1.09$ V), and the second is due to oxidation of thiamine ($E_p = -0.31$ V), as shown in the following reaction:



thiamine thiochrome

From these studies, it is found that the highest signal was obtained in the presence of $NaHPO_4 + NaH_2PO_4$ mixture.

The effect of pH values from 6.0 to 10.0 on the peak height were tested in the mixture by the addition of carbonate-free sodium hydroxide. The optimum pH for studying the vitamin is about 6.2. Also, the effect of phosphate concentration at constant pH (~ 6.2) was examined at 0.01, 0.05 and 0.1 mol dm^{-3}. The phosphate concentration giving the highest signal is 0.01 mol dm^{-3}.

The influence of deposition potential on the peak height was examined using the linear sweep voltammetric technique, where the vitamin exhibits a strong adsorption at -1.4 V. The peak height decreases as the initial potential becomes more positive (-1.4 to 0 V). Therefore, a potential of -1.4 V was used as the accumulation potential for studying the adsorptive stripping voltammetry of thiamine in this work with 0.01 mol dm^{-3} phosphate solution (pH ~ 6.2) and an initial potential of -1.4 V.

Fig. 2 shows cyclic voltammograms for 1×10^{-5} mol dm^{-3} of thiamine in a medium containing sodium dihydrogen phosphate and sodium monohydrogen phosphate, total phosphate being 0.01 mol dm^{-3} (pH ~ 6.2), recorded at 0, 30 and 60 s accumulation periods at -1.4 V. Distinct catalytic and anodic peaks are observed at -1.09 and -0.31 V, respectively, during the positive-going scan; a smaller peak is observed upon scanning in the negative direction. This indicates that the oxidation process was irreversible and the reduction form of thiamine has lower adsorptive response. The catalytic response increases dramatically (Fig. 2) when a preconcentration period preceded the potential scan. The second peak response increases until a preconcentration period of 60 s. On further increase of preconcentration time, the peak current decreases. The fact that a well-defined cyclic voltammetric response is observed indicates the remarkable sensitivity associated with the coupling of the catalytic and adsorptive accumulation process. Full analytical exploitation of these enhancement effects is accomplished through the used of d.c. stripping operation, as illustrated later.

Cyclic voltammetry was also used to obtain αn_a values [7] (where α is the electron transfer coefficient and n_a is the number of electrons involved in the rate-determining step). The magnitudes of the αn_a values suggest that one electron is involved in the rate-determining step of the oxidation process.

Fig. 3 shows linear sweep stripping voltammograms for 0.17 μg/l (5×10^{-10} mol dm^{-3}) thiamine after different accumulation periods (0–300 s). Despite the extremely low (sub-p.p.b.) concentration and the short preconcentration times, well-defined catalytic peaks (-1.09 V) are observed, whereas the oxidizing peaks are not defined at lower concentrations of thiamine. The catalytic peak increases rapidly with increasing accumulation time, a 2-min accumulation period brought an approximately 18-fold enhancement of the peak (relative to that obtained without preconcentration, compare curves a and b in Fig. 3). From the presented results, it is found that the catalytic peak can be used for measuring ultra-trace amounts of thiamine, whereas the oxidizing peak cannot be used owing to the adsorption of the oxidizing form of thiamine on the electrode surface being low.

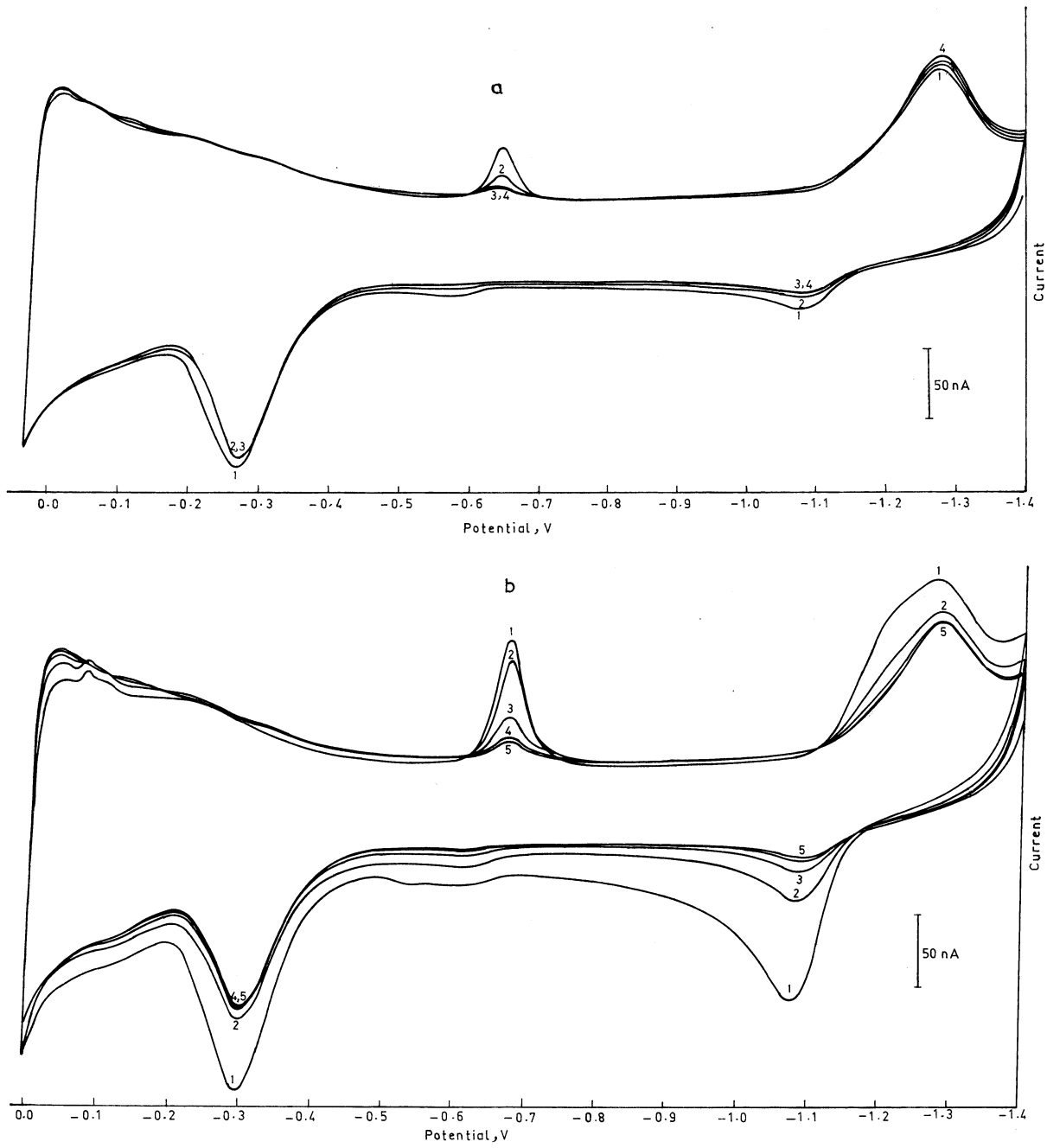


Fig. 2. Repetitive cyclic voltammograms in the presence of 1×10^{-5} mol dm^{-3} of thiamine (B_1) and 0.01 mol dm^{-3} sodium monohydrogen phosphate and sodium dihydrogen phosphate mixture (pH ~ 6.2), accumulation potential −1.4 V, scan rate 100 mV s^{-1}, and accumulation time (a) 0 s, (b) 30 s, and (c) 60 s.

The current versus accumulation time plot gives a straight line, indicating that the peak current increases linearly with the time (correlation coefficient, 0.994).

Fig. 4 shows linear sweep stripping voltammograms for 1×10^{-6} mol dm^{-3} thiamine for increasing periods of time; it is found that two well-defined peaks have appeared, the first is a catalytic peak ($E_p = -1.09$ V) and the second is an oxidizing peak at -0.31 V with a peak width at half-height of 100 mV. Hence, the number of electrons involved is one [18]. The current of this peak ($E_p = -0.31$ V) increases with time until 60 s, as shown in Fig. 5, whereas above this time, the current decreases. However, the first peak (catalytic peak) increases on increasing accumulation periods. From these results, trace levels of thiamine can be determined rapidly with a simple approach using the catalytic-adsorptive stripping peak of thiamine. Such coupling of interfacial and catalytic processes offers higher sensitivity and lower detection limit.

The effect of potential scan rate (v) on the peak current and peak potential was evaluated for the adsorbed thiamine. The log I_p versus log v plot is linear over the 10–200 mV s^{-1} range, with a slope 0.9 and 0.68 V for first and second peak, respectively, which is in agreement with the expected for irreversible reaction of surface species [18]. A 60 and 11 mV negative shift in the peak potential for first and second peak, respectively, was observed upon increasing the scan rate in the range given. The plot of E_p versus log v was also linear (correlation coefficient, 0.998).

The adsorption of the vitamin without stirring was investigated by measuring the peak current versus preconcentration time for 1×10^{-5} mol dm^{-3} of thiamine in quiescent solution. A linear dependence of the peak current on the square root of the preconcentration time, which can be repre-

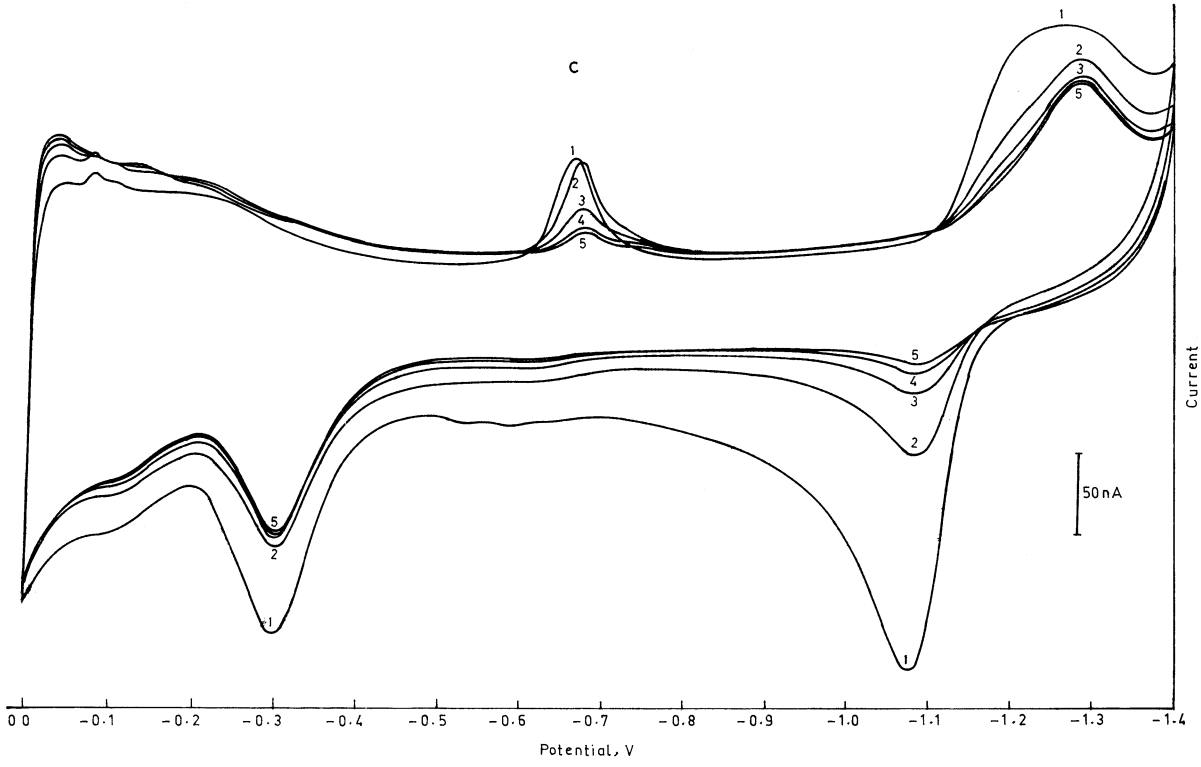


Fig. 2. (*Continued*)

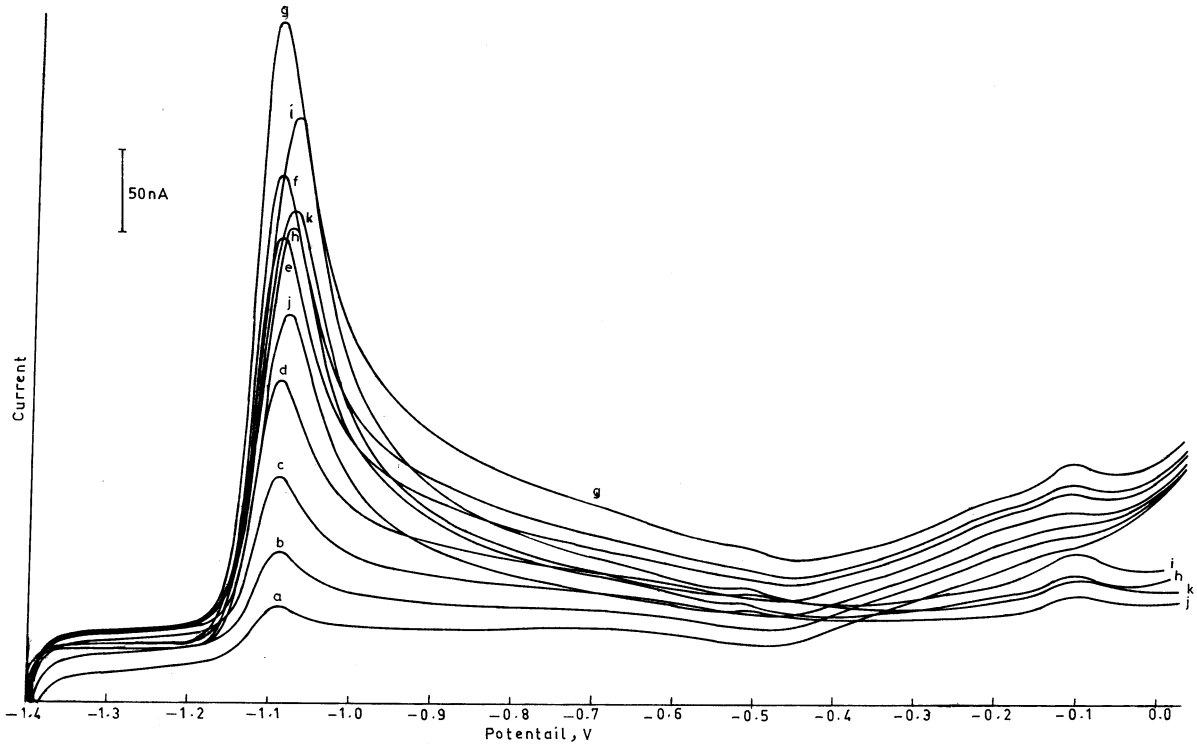


Fig. 3. Voltammograms for 5×10^{-10} mol dm^{-3} of thiamine (B_1) in the presence of 0.01 mol dm^{-3} sodium monohydrogen phosphate and sodium dihydrogen phosphate mixture (pH ~ 6.2): curve a, 0 s; curve b, 5 s; curve c, 15 s; curve d, 30 s; curve e, 50 s; curve f, 60 s (500 nA); curve g, 90 s; curve h, 120 s; curve i, 160 s (1 μA); curve j, 220 s; curve k, 300 s (2 μA).

sented by $I_p = F\sqrt{t}$, was obtained with a correlation coefficient of 0.985 and a slope of 0.96. This behaviour is good agreement with the prediction of Delahay and Fike [19] for the case of semi-infinite linear diffusion.

As low a concentration as 1×10^{-9} mol dm^{-3} of thiamine with relative standard deviation 1.6% ($n = 5$; accumulation time, 90 s) was determined.

The stability of thiamine during the analysis was tested by repeating the same experiment (1×10^{-6} mol dm^{-3} of the drug) after 2 h at a preconcentration time of 30 s. It was found that no change on the peak height was observed.

Table 1 summarizes the characteristics of calibration plots. The extension of the limit of linearity, even up to 1×10^{-7} mol dm^{-3} thiamine, indicates strong adsorption behaviour of the compound. This fact could also be shown by the peak heights obtained for various preconcentration times.

The linear sweep anodic stripping voltammetry is the best method for such determination of thiamine at lower concentration, where values as low as 1×10^{-7}mol dm^{-3} (33.7 μg/l) was estimated with 30 s pre-concentration time using standard additions (relative standard deviation, 2.3%).

The reproducibility of the adsorption process was tested by repeating 10 experiments on 1×10^{-6} mol dm^{-3} thiamine with 90 s preconcentration time. The relative standard deviation was calculated to be 1.2%.

Possible interference from co-existing trace metals (Pb(II), Cd(II), Zn(II) and Cu(II)) was investigated. No effect on the peak height was observed when Pb(II), Cd(II), Cu(II) and Zn(II)

ions was added to thiamine (2×10^{-6} mol dm^{-3}) until 1×10^{-8} mol dm^{-3} concentration of these ions using 30 s preconcentration time; above this concentration (1×10^{-8} mol dm^{-3}), the peak height was affected with increasing the concentration of the ions.

3.1. Application of the DCSV method for assay and a pharmaceutical formulation

3.1.1. In urine

The measurement of thiamine in urine sample was demonstrated as follows. The sample was diluted (1: 100) with supporting electrolyte (0.01 mol dm^{-3} phosphate buffer, pH ~ 6.2) and increasing thiamine concentration. At 1×10^{-7} mol dm^{-3}, a well-defined peak was observed at − 0.31 V (second peak), corresponding to oxidation of thiamine. The peak current increased with increasing thiamine concentration from 1×10^{-7} up to 1×10^{-6} mol dm^{-3} ($r = 0.9965$).

The reproducibility of the results was tested and the relative standard deviation was found to be 3.2% ($n = 6$).

The influence of ascorbic acid and some amino acids such as L-valine, L-serine and β-alanine, which are potent interfering compounds present in biological samples, were also investigated. It was found that an equimolar concentration of each of them has no effect on the peak response of thiamine. It was also found that Zn(II), Pb(II) and Cu(II) had no effect on the peak height of thiamine at lower concentrations of these ions.

3.1.2. In ampoules

The content of the ampoule (1 ml l^{-1}) 200 mg B_1, 50 mg B_6 and 1000 mg B_{12} (Neurorubine, mepha) can be determined using the method described. No pretreatment for the sample was done except for diluted with bidistilled water.

The DCS voltammogram was recorded after preconcentration time for 30 s. The content of the

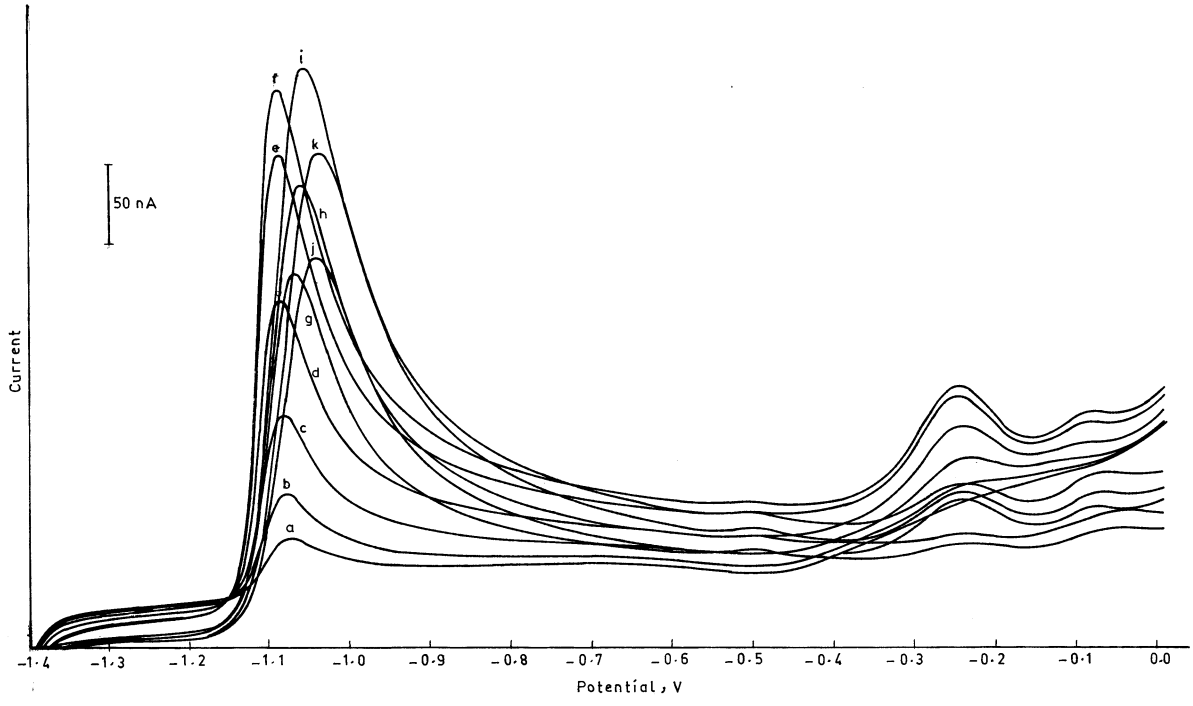


Fig. 4. Voltammograms for 1×10^{-6} mol dm^{-3} of thiamine (B_1) in the presence of 0.01 mol dm^{-3} sodium monohydrogen phosphate and sodium dihydrogen phosphate mixture (pH ~ 6.2): curve a, 0 s; curve b, 5 s; curve c, 15 s; curve d, 30 s; curve e, 50 s; curve f, 60 s; curve g, 90 s (500 nA); curve h, 120 s; curve i, 160 s (1 μA); curve j, 220 s; curve k, 300 s (2 μA).

Table 1
Characteristics of thiamine calibration plots (0.01 M phosphate buffer, pH ~ 6.2)[a]

Range of concentration	Equation	Correlation coefficient	Relative standard deviation (%) ($n = 5$)
First peak			
1×10^{-9} to 1×10^{-8} M	$Y = 2.26X + 252$	0.9998	1.6
1×10^{-8} to 1×10^{-7} M	$Y = 1.95X + 220$	0.9996	1.5
1×10^{-7} to 1×10^{-6} M	$Y = \text{constant}X + 300$	0.9997	1.8
Second peak			
1×10^{-7} to 1×10^{-6} M	$Y = 1.98X + 0$	0.9996	1.8
1×10^{-6} to 1×10^{-5} M	$Y = 2.58X + 47$	0.9995	1.4

[a] Peak height (Y) in nA, concentration X in mol dm^{-3}.

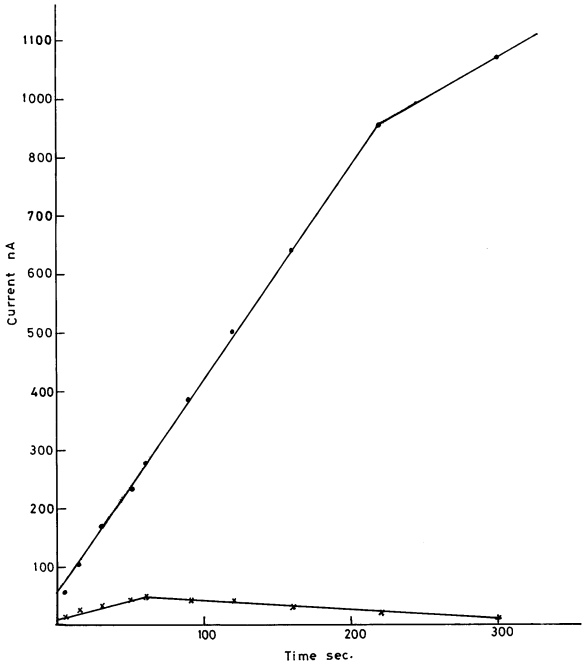


Fig. 5. Peak current versus accumulation time in the presence of 1×10^{-6} mol dm^{-3} of thiamine (B_1) and 0.01 mol dm^{-3} sodium monohydrogen phosphate and sodium dihydrogen phosphate mixture (pH ~ 6.2), scan rate 100 mV s^{-1}: curve a, first peak; curve b, second peak.

ampoule in the cell was determined by the standard addition method. The results obtained found that the analysis of ampoule claimed as 200 mg/ml gave a result of 219 mg/ml with an error of 9.5%. This means that the other vitamins (B_6 and B_{12}) do not interfere in the determination of thiamine in ampoule.

The reproducibility of the results was tested and the relative standard deviation was found to be 2.4% ($n = 5$).

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