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Voltammetric Detection of Vitamin B1 (Thiamine) in Neutral Solution at a Glassy Carbon Electrode via *in situ* pH Modulation

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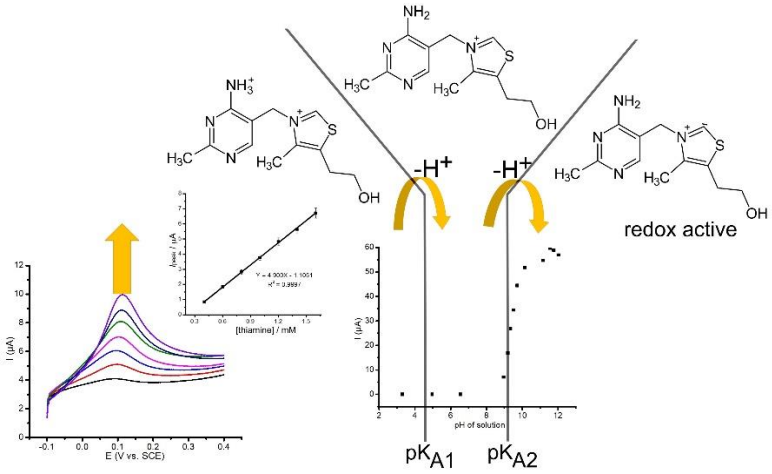
Abstract

Voltammetric analysis is often dependent on pH and on the addition of buffer reagents to optimise the analytical procedure. This approach is not always possible for *in situ* analytical measurements, for example when studying biological fluids or ingredients in food. Therefore, a method is proposed here employing the working electrode to do both, locally modulate the pH value as well as to measure the analytical response. As a model system, thiamine (vitamin B1) is detected in aqueous KCl with a pH modulation brought about with negative potentials applied to the working electrode. Interference from ascorbic acid and uric acid are considered. Exploratory data are presented and methods for improving the detection limit are suggested. Potential for applications in electroanalysis (and in a broader range of processes) are discussed and the detection of thiamine in rice is demonstrated.

Graphical Abstract



Keywords: diffusional gradients; transient species; food sensors; in field analysis; acid-base equilibria

Introduction

Controlling the solution pH is integral to many types of electrolytic processes, in particular for those involving organic or biological redox systems. However, when working with biological and analytical samples outside the laboratory, addition of reagents and buffers is not always convenient. As an alternative approach here *in situ* pH control is suggested for the detection of thiamine or vitamin B1.

Food safety is a very important aspect for dealing with threats to the human health and the well-being of the population.[1] One of the key challenges to both control food nutrients composition and detect chemical or biological contaminants in food is the availability of selective, sensitive, and reliable analytical methods.[2] Conventional methods for food safety detection, such as chromatography,[3] spectrometry,[4] and immunosorbent assays[5] for food analysis provides high reliability and very low detection limits but they are expensive, time-consuming, and require trained personnel and therefore they do not allow frequent monitoring of nutrients and contaminants of foods. There is also an increasing demand for robust, rapid, inexpensive alternative technology for monitoring in-situ and real-time analysis. In this context, the electrochemical methods offer attractive methods for the sensitive determination of

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analytes in terms of high sensitivity, selectivity, and long-term stability. The electrochemical methods also have advantages such as uncomplicated sample pretreatment and fast detection time also in some scenarios could be applied in developing countries and areas with limited equipped facilities and specialists.[6]

Vitamins are essential food nutrients for humans to maintain normal activity and play important roles in metabolism such as slowing the progression of diabetic nephropathy, preventing vascular events,[7] and reducing the concentration of homocysteine.[8] Vitamin B1 (thiamine) is a sulfur-containing compound, which participates in cellular processes as a coenzyme in the form of its pyrophosphate. The deficiency of the vitamin clinically leads to anorexia, neurological disorders followed ultimately by weakness, heart failure, and death.[9] Vitamin B1 can today be provided as a supplement in rice.

Electrochemical methods play an important role in health-related sensors.[10] Most work is performed on a single working electrode, but in some cases, dual-electrode methods have been developed[11] and exploited for pH-modulation.[12] Dual-disk electrode measurements have been performed for gold electrodes placed very close to each other. In this configuration, it was possible to employ one working electrode to generate pH "pulses" and simultaneously to maintain a constant oxidation potential on the second working electrode for the detection of glucose.[13] A related approach was proposed for quinols.[14] Compton and coworkers developed a generator-collector methodology based on a single electrode and rapid switching of the local pH at the electrode surface.[15] Electrochemical pH-modulation in microchannels was reported by Bohn and coworkers.[16] Modulation of the local pH at silica modified electrode surfaces was employed by Walcarius and coworkers for sensitive metal ion detection.[17]

Thiamine or vitamin B1 plays a crucial role in nature[18] and in food science. A substantial range of early studies addressed polarographic redox characteristics and in particular the reduction of thiamine.[19] The anodic behaviour of thiamine is more accessible with carbon electrodes but has been observed only in the presence of a base. The process has been attributed mainly to deprotonation, followed by ring-opening and S-based follow-up redox processes[20] (see Figure 1). The pK_A values for the thiamine hydrochloride have been reported as 4.8 and 9.3.[19] In highly dry organic solvent the deprotonation of the thiazolium ring can lead to persistent carbenes with high reactivity. However, the second proton removal occurs at the carbon to give a highly moisture-reactive intermediate.[21] Here, follow-up reactions lead to electron transfer and the analytical signal for thiamine detection.

Electroanalysis offers a powerful tool for the detection and quantification of redox active vitamins in biological samples.[22] The electroanalytical detection of vitamin B1 (thiamine) is often linked to food analysis and the simultaneous detection of multiple food ingredients. The detection is possible in alkaline aqueous media.[23] The mechanism for the electroanalytical detection was elucidated by Hart et al.[23] to occur via a thiol intermediate. A one-electron mechanism was observed with fast follow up a chemical reaction (dimerisation) to products.

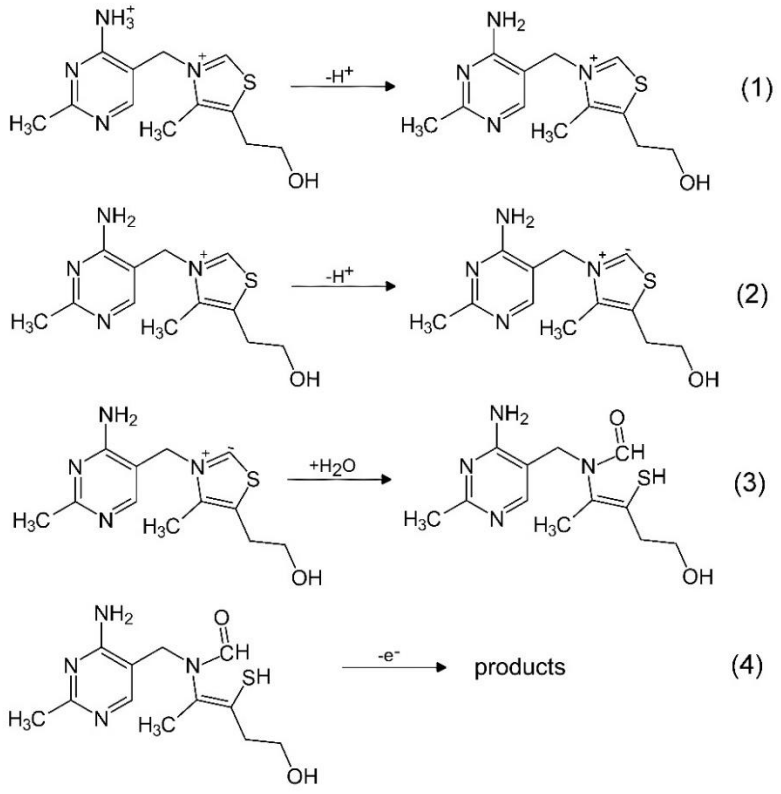

Figure 1. Reaction scheme summarising the two deprotonation steps for thiamine hydrochloride, which are followed by hydrolytic ring-opening and one-electron oxidation.

ARTICLE

In this report, the deprotonation of thiamine hydrochloride is studied with an electro-generated base directly at the working electrode surface. An *in situ* method is suggested to do both, generating the base (with negative applied potential) and detecting the analyte (with positive applied potential). Interference effects and real applications are considered.

Experimental

Chemical Reagents

Thiamine hydrochloride ($C_{12}H_{17}ClN_4OS{\cdot}HCl$; molecular weight 337.27 g mol^{-1}; CAS: 67-03-8; purity 99%) were purchased from Himedia Ltd. Potassium chloride (KCl; molecular weight 74.55 g mol^{-1}; CAS: 7447-40-7) were obtained in analytical grade from Merck and used as received without purification. The solution was prepared under the ambient condition with ultrapure water with a resistivity of 18.2 MOhm cm (at 22 °C, from ELGA Purelab Classic System). Rice samples analyzed in this report were obtained from an Indonesian food market in Bogor.

Instrumentation

Voltammetric measurements were carried out using IVIUM Compactstat electrochemistry system. A single compartment cell with three-electrode configuration was used for electrochemical measurements. A saturated calomel electrode (SCE) and Pt wire electrode (from BASi Ltd, UK) were used as the reference and counter electrode, respectively. The working electrode was a 3.0 mm-diameter glassy carbon disk electrode obtained from IJ Cambria Scientific Ltd. All measurements were performed at a temperature of 20 ± 2 °C. All voltammetric data were processed using *OriginPro* 7.0 software.

Protocols

Oxidation of Thiamine in the Presence of Hydroxide. Thiamine hydrochloride was dissolved in 0.1 M KCl to obtain 5 mM of thiamine solution. For pH adjustment, various amounts of 1 M KOH were added into 25 mL of 5 mM thiamine solution prior to electrochemical measurement. The electrochemical measurements were carried out with pretreatment/conditioning of the working electrode at 0 V vs. SCE for 2 s. The pH of thiamine solutions after addition of KOH was measured using calibrated pH meter (Voltcraft pH-100ATC).

Oxidation of Thiamine with Localised Hydroxide Generation. Thiamine hydrochloride was dissoved in 0.1 M KCl to obtain 10 mM of thiamine solution. Negative polarization at various potentials vs. SCE was applied to the working electrode to provide localised hydroxide generation. This pretreatment was applied for 1 s (if not stated otherwise) prior to the electrochemical measurement of thiamine solution. Furthermore, thiamine solution in various concentrations was measured with localised hydroxide generation at selected potential and period of pretreatment.

Oxidation of Thiamine with Localised Hydroxide Generation: Interferences. Electrochemical measurements with 5 mM thiamine solution in the presence of 0.5 mM ascorbic acid were performed. The electrochemical measurement of 5 mM thiamine solution was also carried out with the presence of 0.25 mM of uric acid.

Oxidation of Thiamine with Localised Hydroxide Generation: Analytical Detection in Rice. Oxidation of thiamine with localised hydroxide generation was applied for the measurement of thiamine in brown rice. Brown rice was first milled in a blender (5 minutes) to give a fine brown powder. Next, the amount of 25 g of brown rice powder was soaked in 50 mL KCl 0.1 M and stirred for 30 minutes. Various amounts of thiamine hydrochloride solution (0.8 M) was added as standard into 20 mL sample prior to thiamine analysis with localised hydroxide generation.

Estimation of Errors. Reproducibility in voltammetric detection is sensitive to many parameters (including electrode area, concentration variations, interferences, and temperature). Here, temperature variation is estimated as 20 ± 2 °C to result in a major error component. We therefore estimate a realistic error based on temperature effects and based on the Arrhenius equation. Assuming a typical activation energy E_A for diffusion in aqueous media with 20 kJ mol^{-1},[24] and using R the gas constant, T the absolute temperature, ΔT the temperature error, the relative error in current is $E_A/RT \times \Delta T/T$ = +/- 6%.

Results and Discussion

Oxidation of Thiamine in the Presence of Hydroxide

The electrochemical oxidation of thiamine hydrochloride has previously been reported by Hart and coworkers and is shown in Figure 2 for cyclic voltammetry experiments in aqueous 0.1 M KCl. No discernable voltammetric feature is observed in neutral solution, but when adding KOH gradually (by addition of 1 M KOH into 25 mL of 5 mM thiamine hydrochloride), a peak for the oxidation of thiamine hydrochloride appears (see Figure 2A peak 1 at 0.26 V vs. SCE). Upon more careful inspection, more than one equivalent of KOH is consumed for the removal of the first proton from the hydrochloride (see Figure 2B) before the voltammetric response emerges. When continuing the addition of KOH the voltammetric peak response continues to increase up to a total of 4 equivalents of KOH to give the maximum peak current. Therefore, the oxidation of thiamine hydrochloride does seem linked to (i) the removal of two protons for oxidation to occur and (ii) the formation of up to two additional protons during the process to reach full oxidation. As this process occurs locally at the electrode surface and the diffusion of protons is fast, the reaction scheme in Figure 1 is consistent with this observation.

The behaviour during oxidation is confirmed when plotting the voltammetric peak current versus pH (measured, see Figure 2C). The removal of the first proton from the hydrochloride (with pK_{A1} 4.8)

remains invisible. However, the removal of the second proton (at pK_{A2} 9.3) clearly causes the voltammetric oxidation peak to emerge.

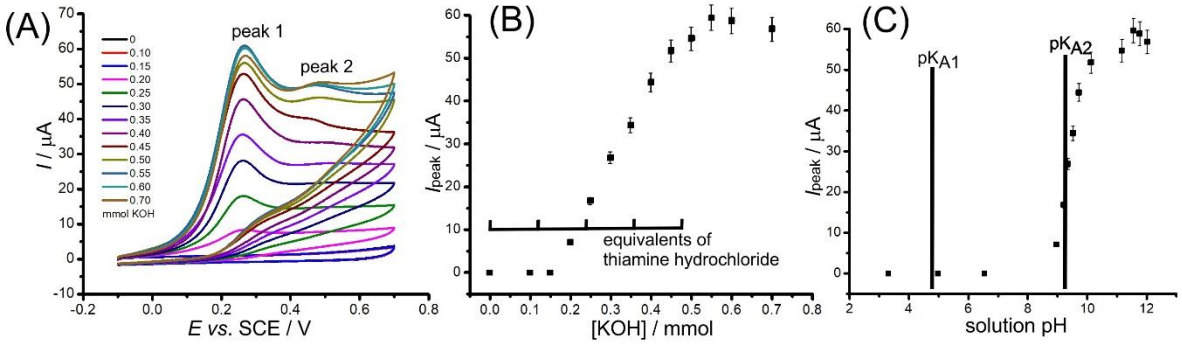

Figure 2. (A) Cyclic voltammogram (scan rate 100 mV s^{-1}) for 5 mM thiamine hydrochloride in 0.1 M KCl at a 3 mm diameter glassy carbon disk electrode with gradual additions of 1 M KOH (preequilibration of the working electrode at -0.1 V for 2 s). (B) Correlation between mmol KOH added to 5 mM thiamine hydrochloride in 0.1 M KCl and oxidation peak 1 current. (C) Correlation between pH of the solution and the oxidation peak 1 current for thiamine hydrochloride. Error bars estimated based on standard deviation ± 6%.

Note that the pH scale in Figure 2C reflects the pH in bulk solution and not the pH during oxidation at the electrode surface, which varies due to localised proton generation in essentially unbuffered conditions.

The oxidation of the thiamine does result in two distinct peaks denoted peak 1 and peak 2 (see Figure 2A at 0.26 V vs. SCE and at 0.48 V vs. SCE). Consistent with literature reports, the first peak (limiting at 61 µA) is assumed here to be associated with a one-electron transformation of the ring-opened thiamine. The Randles-Sevcik equation for the peak current I_p for a fully chemically irreversible one-electron process can be written as a function of the electrode area A, the bulk concentration c, the Faraday constant F = 96487, the scan rate v, the diffusion coefficient D, the gas constant R = 8.31 JK^{-1}, and the absolute temperature T = 293 K (see equation 5).[25]

$$I_p = 0.4956\, A\, c\, (F^3\, v\, D\, R^{-1}\, T^{-1})^{0.5} \qquad (5)$$

With this information, the experimental peak current, and assuming one-electron oxidation, the diffusion coefficient for thiamine is evaluated as $D = 0.33 \times 10^{-9}$ m^2 s^{-1}. This can be compared to the estimated D value from the Wilke-Chang expression, $D = 0.45\times 10^{-9}$ m^2 s^{-1}.[26] The agreement is good (considering the neglected effect of the charge) and confirms the one-electron nature of the process. The presence of a second electron transfer for each molecule diffusing to the electrode surface can be inferred from the presence of the second oxidation peak (see Figure 2A, peak 2) and from the observation that in total two protons are released during the oxidation (see Figure 2B). The nature of the product will depend on (i) the applied potential, (ii) the presence of a base in the bulk solution, (iii) the depletion of a base (hydroxide) within the diffusion layer of the electrode as well as time and follow-up chemistry. Products have not been isolated for this study.

Oxidation of Thiamine with Localised Hydroxide Generation

Although no discernible voltammetric response is observed for the oxidation of 10 mM thiamine hydrochloride in aqueous 0.1 M KCl, an oxidation peak does emerge with a short cathodic pretreatment at negative applied potentials. Figure 3A shows the presence of an oxidation peak at 0.11 V vs. SCE which is shifted when compared to the oxidation peak detected with the addition of hydroxide (compared Figure 2A). Also, the shape of the new peak feature is more sharp and symmetric, when compared to the oxidation peak in the presence of hydroxide (Figure 2). A key difference in these experiments is the presence of an electro-generated base (hydroxide) diffusing away into the bulk. Protons generated during the oxidation therefore consume base (simultaneously to diffusional losses of base at the electrode surface). Therefore, the voltammetric peak appearance is shifted to more negative potentials and more symmetric (without the typically diffusional tail seen in conventional electrode reactions).

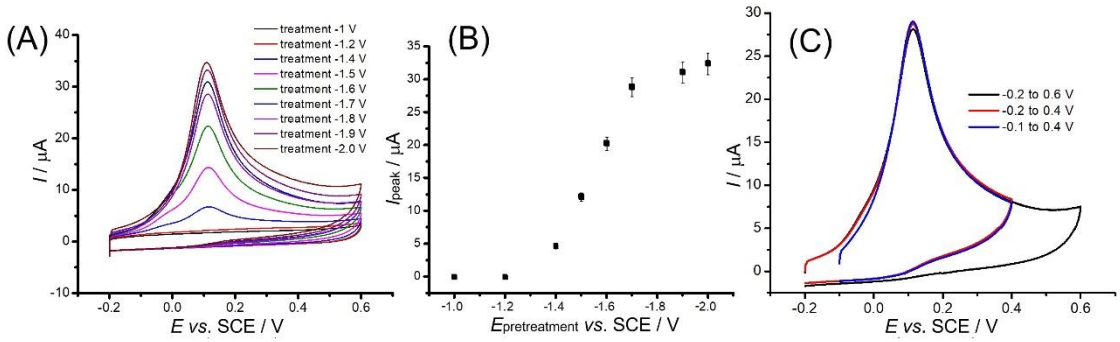

Figure 3. (A) Cyclic voltammograms (scan rate 100 mV s^{-1}) for 10 mM thiamine hydrochloride in 0.1 M KCl at a 3 mm diameter glassy carbon disk electrode with pretreatment for 1 s at various negative potentials. (B) A plot of the pretreatment potential versus thiamine oxidation

peak current. Error bars estimated based on standard deviation ± 6%. (C) Cyclic voltammograms over different potential ranges (scan rate 100 mV s^{-1}) for 10 mM thiamine hydrochloride in 0.1 M KCl with 1 s pretreatment of the working electrode at -1.7 V vs. SCE.

When generating base for one second and gradually moving the pre-electrolysis potential more negative, the peak for thiamine oxidation increases (Figure 3A). A limit is reached at -1.7 V vs. SCE producing an oxidation peak with I_p = 35 µA (Figure 3B). Data in Figure 3C demonstrate that the observed peak is reproducible and relatively insensitive to the potential window selection.

When changing the time of pre-electrolysis (see Figure 4), a further enhancement in the peak current can be achieved. Figure 4A shows data for thiamine oxidation after pre-electrolysis at -1.7 V vs. SCE as a function of time. For 4 s to 5 s pre-electrolysis, the peak current reaches 55 µA (Figure 4B).

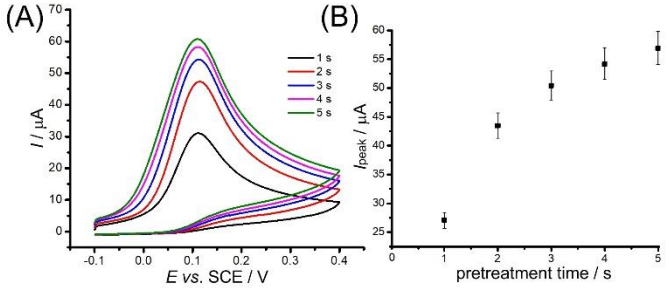

Figure 4. (A) Cyclic voltammogram (scan rate 100 mV s^{-1}) of 10 mM thiamine hydrochloride in 0.1 M KCl at a 3 mm diameter glassy carbon electrode with pretreatment at -1.7 V vs. SCE for different pretreatment periods. (B) A plot of the oxidation peak current *versus* pretreatment period at -1.7 V vs. SCE (currents baseline-corrected). Error bars estimated based on standard deviation ± 6%.

Perhaps interestingly, even under these conditions a full scale anticipated peak (I_p = 120 µA expected for 10 mM thiamine hydrochloride) is not observed and also the second oxidation process (peak 2) is not observed. The process is likely to be limited to a one-electron oxidation with the release of protons during oxidation further limiting (halving) the maximum peak current. Effectively, only half of the redox active species undergoes a one-electron conversion.

Based on these results, it was decided to perform analytical measurements with a protocol based on a 2 s pre-electrolysis at -1.7 V vs. SCE followed by a cyclic voltammetry sweep with a scan rate of 100 mVs^{-1} (see Figure 5A). The plot of the peak current for this voltammetric signal is plotted *versus* thiamine concentration in Figure 5B. A linear relationship of peak current to thiamine hydrochloride concentration confirms the analytical potential for this procedure.

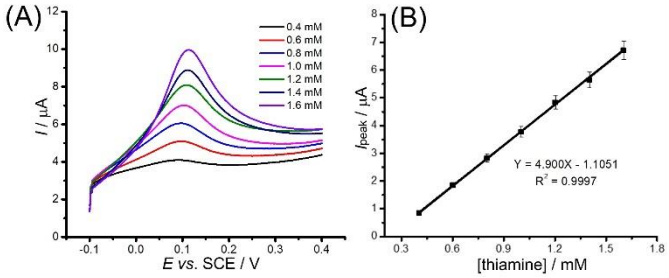

Figure 5. (A) Anodic voltammetry scan (scan rate 100 mV s^{-1}) for thiamine hydrochloride in 0.1 M KCl at a 3 mm diameter glassy carbon electrode (pretreatment at -1.7 V vs SCE for 2 s). (B) A plot of oxidation peak current *versus* concentration of thiamine (currents baseline-corrected). Error bars estimated based on standard deviation ± 6%.

Oxidation of Thiamine with Localised Hydroxide Generation: Interferences

Interferences in analytical measurements suppress or obscure the desired peak signals and in the case of the thiamine detection possible interference effects are expected due to (i) oxidation signals for other species in the same potential range (e.g. ascorbate), (ii) buffer systems and base consuming reagents that compete with the electrolytic hydroxide formation, (iii) other hydroxide consuming reactions that occur at a more negative working electrode potential, or (iv) the reduction of the target analyte under conditions of hydroxide generation at the working electrode. Here, the effects of ascorbate and uric acid are investigated with thiamine in the same solution.

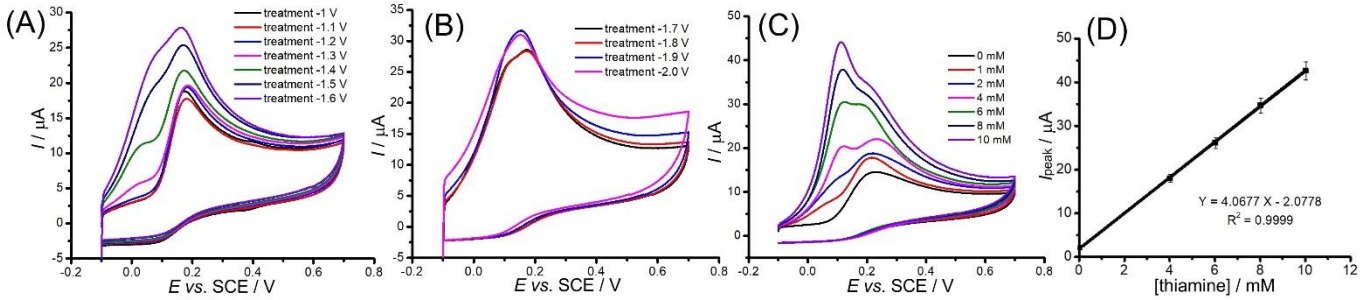

Figure 6. (A) Cyclic voltammogram (scan rate 100 mV s^{-1}) for 5 mM thiamine hydrochloride and 0.5 mM ascorbic acid in 0.1 M KCl at a 3 mm diameter glassy carbon electrode with pretreatment at various negative potentials for 2 s. (B) As in A but with pretreatment more negative. (C) Cyclic voltammograms (scan rate 100 mV s^{-1}) for various concentrations of thiamine hydrochloride and 0.5 mM ascorbic acid in 0.1 M KCl with pretreatment at -1.7 V for 2 s. (D) Correlation between oxidation peak current and thiamine hydrochloride concentration (currents baseline-corrected). Error bars estimated based on standard deviation ± 6%.

ARTICLE

Interference from Ascorbic Acid. Ascorbic acid is an anti-oxidant and widely present in food items. The oxidation of ascorbic acid is known to interfere with that of thiamine.[27] Figure 6A shows cyclic voltammetry data for the oxidation of thiamine hydrochloride in 0.1 M KCl in the presence of 0.5 mM ascorbic acid. Without negative potential pretreatment, no signal for thiamine is detected. When applying the negative potential pretreatment a new oxidation peak emerges at 0.03 V vs. SCE approximately 0.1 V before the peak for ascorbic acid oxidation. An optimum peak is observed with -1.7 V vs. SCE applied for 2 s before cyclic voltammetry. The resolution of the thiamin peak is clearly difficult as both peaks overlap (Figure 6C). A plot in Figure 6D suggests that thiamine concentration information can be obtained.

Interference from Uric Acid. Another important interfering redox species is uric acid. Figure 7A shows cyclic voltammetry data for the oxidation of 5 mM thiamine hydrochloride in the presence of 0.25 mM uric acid. A well separated oxidation peak responses are observed. By selecting the pretreatment potential more negative a well-defined oxidation peak for thiamine emerges without too strongly affecting the uric acid oxidation peak (a slight shift to more negative potential is observed). Cyclic voltammetry data for different thiamine hydrochloride concentrations are shown in Figure 7B. The plot of oxidation peak current *versus* thiamine hydrochloride concentration (Figure 7C) confirms a linear concentration-dependent response.

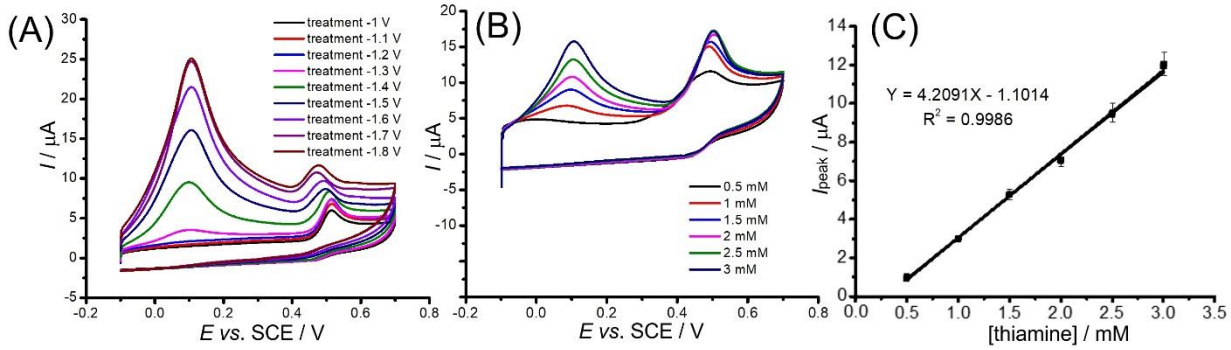

Figure 7. (A) Cyclic voltammogram (scan rate 100 mV s^{-1}) for 5 mM thiamine hydrochloride and uric acid in 0.1 M KCl at a 3 mm diameter glassy carbon electrode with pretreatment at the various negative potential for 2 s. (B) Cyclic voltammogram (scan rate 100 mV s^{-1}) for different concentrations of thiamine hydrochloride and 0.25 mM uric acid in 0.1 M KCl with pretreatment at -1.7 V vs. SCE for 2 s. (C) Correlation between oxidation peak current and thiamine concentration (currents baseline-corrected). Error bars estimated based on standard deviation ± 6%.

Oxidation of Thiamine with Localised Hydroxide Generation: Analytical Detection in Brown Rice Samples

The measurement of thiamine in a natural food sample was carried out using brown rice (from an agricultural market in Bogor, Indonesia) as an example of a protocol with a natural sample. After milling (in a blender) the rice powder sample was stirred for 30 minutes in 0.1 M KCl. The filtered solution was employed in electroanalytical detection experiments. The natural content of thiamine in this rice was just below the current detection levels. Figure 8A shows a very small peak at 0.05 V vs. SCE possibly associated with ca. 100 µM or less thiamine. The concentration of thiamine in rice milk was reported as 11.95 mg/L or equal to 35 µM in agreement with this estimate.[28] The sample was then spiked with a known amount of thiamine and the oxidation peak measured. The plot in Figure 8B shows the increase in the signal with concentration. For a quantitative determination of thiamine in rice the measurement needs to be improved into a lower range of detection, although the signal for thiamine in the natural rice sample is visible even with the methodology reported here.

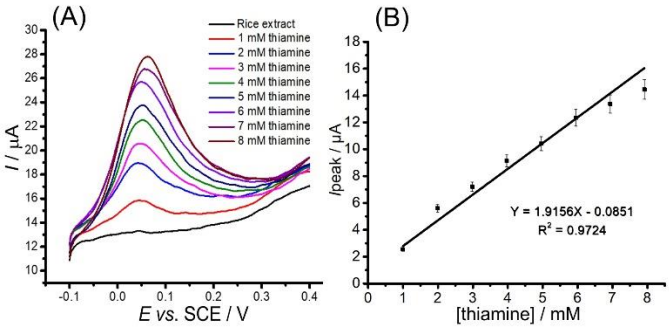

Figure 8. (A) Anodic voltammetry scan (scan rate 100 mV s^{-1}) for thiamine hydrochloride in rice extract in 0.1 M KCl at a 3 mm diameter glassy carbon electrode (pretreatment at -1.7 V vs. SCE for 2 s). (B) A plot of oxidation peak current *versus* concentration of thiamine (currents baseline-corrected). Error bars estimated based on standard deviation ± 6%.

Conclusions

A new methodology has been developed for the detection of redox active analytes that require the presence of base. Thiamine hydrochloride has been selected as a model system and shown to require two deprotonation steps before the electrochemical signal can be observed. Next, the in situ generation of base was developed to allow detection without adding base into the sample. Hydroxide is generated only locally at the electrode surface. The oxidation of thiamine is observed and the changes in peak shape and appearance have been explained. Interferences have been investigated.

Detection in a brown rice sample has been attempted. Ultimately, the methodology works and it needs to be improved to access lower detection limits. The improvements is methodology could be based, for example on the way the voltage is applied. New pulse sequences could be developed to better link the hydroxide production with the thiamine oxidation. The type of electrode may also have an important role and therefore in future other types of electrodes (e.g. screen printed electrodes) should be tested to further improve the analytical performance. A further benefit in the *in situ* base generation could be in the ability of this method to discriminate different chemical species and interferences. Sinusoidal modulation of the base electro-generation could be coupled to lock-in detection of the analyte to give further sensitivity.

Conflicts of interest

There are no conflicts of interest to declare.

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