A Novel Electroanalytical Approach to the Measurement of B Vitamins in Food Supplements based on Screen-Printed Carbon Sensors

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Abstract

This paper describes the development of a novel electrochemical assay for the measurement of water-soluble vitamins in food and pharmaceutical products. The optimum conditions for the determination of vitamin B_1 (thiamine), B_2 (riboflavin) and B_6 (pyridoxine) in phosphate buffer were established using cyclic voltammetry in conjunction with screen printed carbon electrodes (SPCEs). The optimum current response for all three vitamins was achieved in 0.1 M phosphate buffer pH 11 using an initial potential of -1.0 V. Using square wave voltammetry, the linear ranges for thiamine, riboflavin, and pyridoxine were found to be: 15-110 µg/ml, 0.1-20 µg/ml, and 2-80 µg/ml respectively. The application of the method to a commercial food product yielded a recovery of 95.78 % for riboflavin, with a coefficient of variation (CV) was 3.38% (n=5). The method was also applied to a multi-vitamin supplement for the simultaneous determination of thiamine, riboflavin and pyridoxine. In both cases only simple dilution with buffer followed by centrifugation was required prior to analysis. The resulting square wave voltammetric signals were completely resolved with Ep values of -0.7 V, +0.2 V, and +0.6 V respectively. The recoveries determined for the vitamin B complex in a commercial supplement product were found to be 110 %, 114 %, and 112 % respectively (CV= 7.14%, 6.28 %. 5.66 % respectively, n=5).

Keywords: Cyclic, Square-wave, Voltammetry, Thiamine, Riboflavin, Pyridoxine

1. Introduction

Vitamins were first proposed by Funk in 1912 [1] when he demonstrated that a deficiency of 'some' substances resulted in disease, he termed these compounds vitamines; in more recent years the word vitamin has replaced the old terminology. Over a century later we understand a great deal more about the importance of these dietary components. They are grouped by their solubility and similarities in chemical structure; water soluble (vitamin B & C) and fat soluble (A, D, E and K). They all play important roles in the human body and the B-group vitamins have a diverse range of functions. Thiamine (vitamin B_1) is a co-enzyme precursor which indirectly contributes to the metabolism of carbohydrates, it has also been shown to play a role in neurological development and the immune system [2]. Riboflavin (vitamin B_2) has been linked to wide range of biological processes; some important processes include the oxidation of fatty acids and the transfer of electrons in the generation of ATP [3]. Pyridoxine (vitamin B_6) also has many proposed roles, one of which is the metabolism of amino acids as a component of a co-enzyme [4]. These important micronutrients are present in both processed and unprocessed foods; with some processed foods fortified to improve public health. Fortification has been in practice for over 90 years [5] and supplements are becoming more popular in an ever aging and increasingly health-conscious society. In 2006 a UK regulation on the addition of vitamins and minerals to food was published, this regulation became a law in 2007 therefore it is important that reliable analytical methods are established for the analysis of these compounds in commercial foods and pharmaceutical products.

One of the most commonly used methods for vitamin analysis in industry involves the use of high performance liquid chromatography [6]. However, this technique requires high operator skill, can be costly to operate on a routine basis and is time consuming. Simple, low cost, reliable methods for vitamin analysis are required in both the food and pharmaceutical industries and a promising approach is to employ disposable screen-printed carbon electrodes (SPCEs). These can be mass produced in a wide range of geometries at low cost since the working electrode material is carbon; consequently, they can be considered disposable. Most vitamins have been reported to possess electro-activity in media of a specific pH; these reports have involved various electrode materials such as diamond [7], glassy carbon (GCE) [8], and mercury [9]. Siddiqui and Pitre [10] used the latter material for the separate measurement of vitamins B1 B2 and B6, however the authors reported different experimental conditions for each vitamin therefore their simultaneous measurement was not achieved. A recent review of the electroanalysis of vitamins showed that no other publication has described the simultaneous measurement of the three vitamins of interest [11]. Only a few reports have appeared which describe the application of unmodified SPCEs to vitamin analysis, however, these devices have been shown to hold great promise for a wide range of chemical classes [12].

The purpose of the present study was to develop a novel voltammetric assay in conjunction with plain SPCEs (vs. Ag/AgCl) for the simultaneous measurement of vitamins thiamine (B_1), riboflavin (B_2), and pyridoxine (B_6) in a pharmaceutical product; we also wish to demonstrate the possibility of using a similar approach for measuring a vitamin in a food product, we selected vitamin B_2 for this purpose. We show that by judicious choice of the phosphate buffer solution, as well as the initial potential, all three vitamins can be measured in a single anodic scan, using square wave voltammetry, in only 8 seconds.

2. Experimental

2.1. Instrumental

All voltammetric measurements were carried out with a µAutolab III potentiostat interfaced to a PC for data acquisition via NOVA v1.10 (Metrohm, Netherlands). SPCEs were supplied by Gwent Electronic Materials Ltd (Pontypool, UK); the working electrode is fabricated using a carbon ink (C2030519P4) and the reference electrode is fabricated using a Ag/AgCl ink (C61003P7). All pH measurements were carried out with a Testo 205 (Testo Limited, Hampshire UK) pH meter.

2.2. Voltammetry

All voltammetric studies were carried out with a screen-printed strip, comprising the working and reference electrodes mentioned above, placed in a voltammetric cell containing a 10 ml aliquot of 0.1 M phosphate and 0.1 M sodium chloride (PBS). The possibility of using SPCEs more than once was investigated however there was a reduction in sensitivity on subsequent scans; consequently the sensors were disposed of after each analysis.

The initial cyclic voltammetric conditions used to study the effect of pH over the range 7-11 were as follows: (A) for thiamine initial potential 0.1V -1.0V; switching potential -1.0 V, final potential 0.1 V; (B) for riboflavin initial potential -1.1 V -0.0 V; switching potential -0.0 V, final potential -1.1V; (C) for pyridoxine initial potential 0.1 V - 1.2 V; switching potential 1.2 V, final potential 0.1 V. The scan rate chosen for all these studies was 100 mV/s. A further cyclic voltammetric study was performed with a phosphate buffer pH 11 using the following scan rates: 20, 50, 100, 150, and 200 mV/s. The data was used to determine the nature of the reactions occurring with our screen-printed carbon electrodes.

After deducing the voltammetric behaviour of each vitamin at the SPCEs (vs. Ag/AgCl) quantitative studies were performed using square wave voltammetry. Calibration studies were carried out at 250 mVs^{-1} with a step height of 0.005 V and an amplitude of 0.05 V scanning from an initial potential of -1.0 to a final potential of +1.0. The simultaneous analysis of all three vitamins in a pharmaceutical preparation was performed under the same conditions as those used in the calibration study. To do this a 10 ml extract of the sample containing the three vitamins was first subjected to square wave voltammetry using the conditions stated above with a screen printed strip; this was followed by the addition of standard solutions of the three vitamins after which the second square wave voltammogram was recorded. This process was continued with a further two additions of the individual vitamin solutions. Similarly the analysis of a Marmite® extract was performed at 250 mVs^{-1} with a step height of 0.005 V and an amplitude of 0.05 V; the initial potential was -1.0 and the final potential was 0.0. To do this a 10 ml extract of the sample containing riboflavin was first subjected to square wave voltammetry with the conditions stated above using a screen printed strip, this was followed by the addition of a riboflavin standard solution after which the second square wave voltammogram was recorded. This process was continued with a further two additions of the riboflavin standard solution.

2.3. Light Microscopy

Light microscopy was used to observe the SPCE topography. The SPCE surface was imaged with a Smartzoom 5 (Zeiss, Germany) light microscope at 70x and 300x magnification. The graphical abstract displays these surface images which demonstrate a homogenous carbon surface. The graphical abstract also shows the square wave voltammetric response observed for the standard addition study of a pharmaceutical preparation. The proposed electron-transfer mechanisms for vitamin B_1, B_2 and B_6 are also displayed in the graphical abstract; with a more detailed explanation of the mechanisms in section 3.1.

2.4. Reagents

All chemicals were obtained from Sigma Aldrich (Dorset, UK), unless otherwise stated. Deionised water was obtained from a Purite RO200 – Stillplus HP System (Oxon, UK). Stock solutions of disodium and trisodium were made at a concentration of 0.5 M by dissolving the appropriate mass in deionized water, these were then titrated to give the desired pH. Sodium chloride was prepared to a concentration of 1.0 M by dissolving the appropriate mass in deionised water; this was added to the working standard giving a final concentration of 0.1 mM sodium chloride. Primary stock solutions of thiamine hydrochloride and pyridoxine hydrochloride were prepared by dissolving the required mass in deionised water to give 0.02 M concentration solutions. Sodium hydroxide was prepared to a concentration of 0.1 M by dissolving the appropriate mass in deionised water; a primary stock solution for riboflavin was prepared to a concentration of 0.02 M by dissolving the appropriate mass in 0.1 M sodium hydroxide. Working standards for voltammetric studies were prepared by dilution of the primary stock solution with either phosphate buffer or water to give a final concentration of 0.1 M phosphate buffer.

2.3. Sample Preparation

The food product Marmite® was prepared by diluting a 2 gram quantity in 5 ml of 0.2 M trisodium phosphate buffer. This was prepared in a 15 ml centrifuge tube and gently warmed to 30°C for 10 minutes to allow the viscous sample to dissolve in the buffer. The sample was then vortexed and finally centrifuged in an MSE Centaur 2 (Fisons, UK) for 10 minutes at 2500 rpm. The final solution was prepared in a voltammetric cell with a 1.25 ml aliquot of the supernatant and 0.1 M phosphate buffer (pH 11) with 0.1 M sodium chloride taking the total volume to 10 ml.

The vitamin B tablet Ultra Vit B Complex™ by Vitabiotics© was prepared by crushing a total of 5 tablets with a pestle and mortar, 0.1 g of the powdered tablets was transferred to a centrifuge tube containing a 5 ml solution of 0.1 M (pH 11) phosphate buffer which was shaken,

vortexed, and finally centrifuged in an MSE Centaur 2 for 10 minutes at 2500 rpm. The final solution was prepared in a voltammetric cell with 0.25 ml of the supernatant and 0.1 M phosphate buffer (pH 11) with 0.1 M sodium chloride taking the total volume to 10 ml.

3. Results and Discussion

3.1. Cyclic Voltammetric Behaviour and Optimisation of Buffer Conditions

In order to determine the optimum conditions for the simultaneous electrochemical measurement of the water soluble vitamins thiamine, riboflavin, and pyridoxine at plain SPCEs, cyclic voltammetric studies were performed over a wide pH range at a fixed scan rate of 100 mV s^{-1}.

Fig. 1A clearly shows well defined cyclic voltammetric responses for thiamine in phosphate buffer (0.1 M) at pH 10 and pH 11; no significant oxidation peak was observed below pH 10 (Fig. 1D). Similar behaviour was observed by Hart and co-workers [13] using a GCE, who suggested that the electrochemically inactive thiamine is converted to an electro-oxidisable thiol derivative at pH values above 9. The proposed mechanism is shown in equation 1, and the proposed free-radical ($RS^{\cdot}$) production and subsequent dimerization are shown in equations 2 and 3. In order to deduce whether the mechanism obtained with a SPCE was the same as that obtained with the glassy carbon electrode mentioned above, we carried out wave analysis of the voltammogram in figure 1A. The αna value was calculated using the relationship: αna=0.048/Ep (V) - Ep_2 (V) [14]. The value obtained was 0.52 and as the value of α is usually close to 0.5 this implies that n is 1. Consequently this value is in agreement with that obtained using a GCE.

Riboflavin also exhibited well-defined cyclic voltammetric responses using the same buffers (Fig. 1B). The voltammetric oxidation and reduction peak currents were of similar magnitude over the whole pH range studied (Fig. 1D), suggesting that any of these buffers could be used for the analysis of this vitamin. It should be mentioned that riboflavin is initially in the oxidised form, therefore at the initial potential (-1.0 V) the vitamin is reduced prior to the scan and subsequently re-oxidised during the forward anodic scan. This behaviour is consistent with an electrochemically quasi-reversible redox couple and has also been reported to occur with other electrode materials and the proposed mechanism is shown in equation 4 [15]. The quasi-reversible nature of the redox reaction with a SPCE is evident from the separation of the Epa and Epc values; the expected value would be 59/n (mV) for a truly reversible electrode process [16].

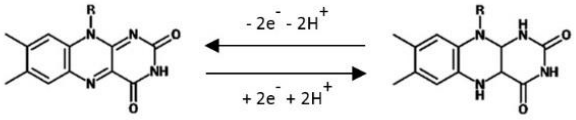


Fig. 1C shows the cyclic voltammetric behaviour of pyridoxine using the same two buffers mentioned above; clearly there is an influence of pH on the anodic response of the vitamin. As shown in Fig. 1D a break point occurs in the plot of peak current vs. pH at a value of pH 9.0. This is consistent with the occurrence of a pKa value at the pyridine moiety of the vitamin [17]. From the data shown in Fig. 1D we deduced that a pH of 11 was optimum for the voltammetric measurement of all three vitamins.

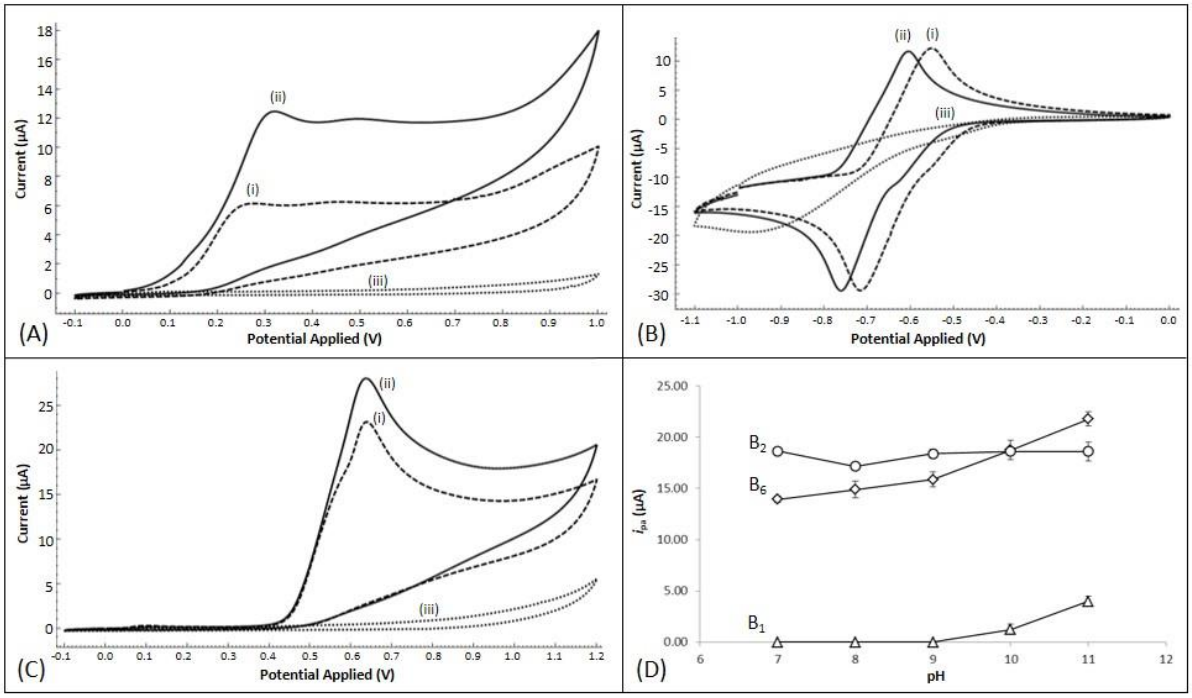
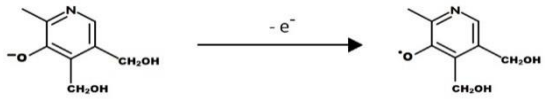


Fig. 1. Cyclic voltammograms were obtaining using a SPCE (vs. Ag/AgCl) with a solution containing 0.1 M sodium chloride with 0.1 M phosphate buffer at pH 10 (i) and pH 11 (ii): (A) 1 mM thiamine (B) 1 mM riboflavin (C) 1 mM pyridoxine hydrochloride. Blank voltammetric scans (iii) were obtained with the pH 11 buffered solution. (D) Shows a summary of the anodic peak currents (i_{pa}) obtained over the range pH 7 – 11. Conditions: (A) Initial/final potential 0.0 V, switching potential +1.0 V & -0.1 V; (B) Initial/final potential -1.0 V, switching potential 0.0 V & -1.1 V; (C) Initial/final potential 0.0 V, switching potential +1.2 V & -0.1 V; Scan rate: 100 mV s^{-1}.

A scan rate study was performed next in order to further deduce the nature of the electrode reaction for each of the water soluble vitamins at the optimum pH. The cyclic voltammetric behaviour for thiamine (Fig. 2A), riboflavin (Fig. 2B), and pyridoxine (Fig. 2C) were studied over the scan rate range 20-250 mV s^{-1}. The current function was plotted against the square root of the scan rate ($V^{½}$) for each vitamin (Fig. 2D). The linear profiles observed in Fig. 2D indicate the anodic current for both thiamine and riboflavin is controlled by diffusion. However a slight negative slope is observed for pyridoxine which could be a result of adsorption phenomena of the oxidation product at the electrode surface. At the pH used for the analytical determinations (pH 11) the anionic species would be expected to undergo oxidation of the dissociated phenolic group as shown in equation 5. In a similar manner to that described above for thiamine, we calculated the αna value to be 0.52, which implies that the oxidation step involves the transfer of one electron from the anion.



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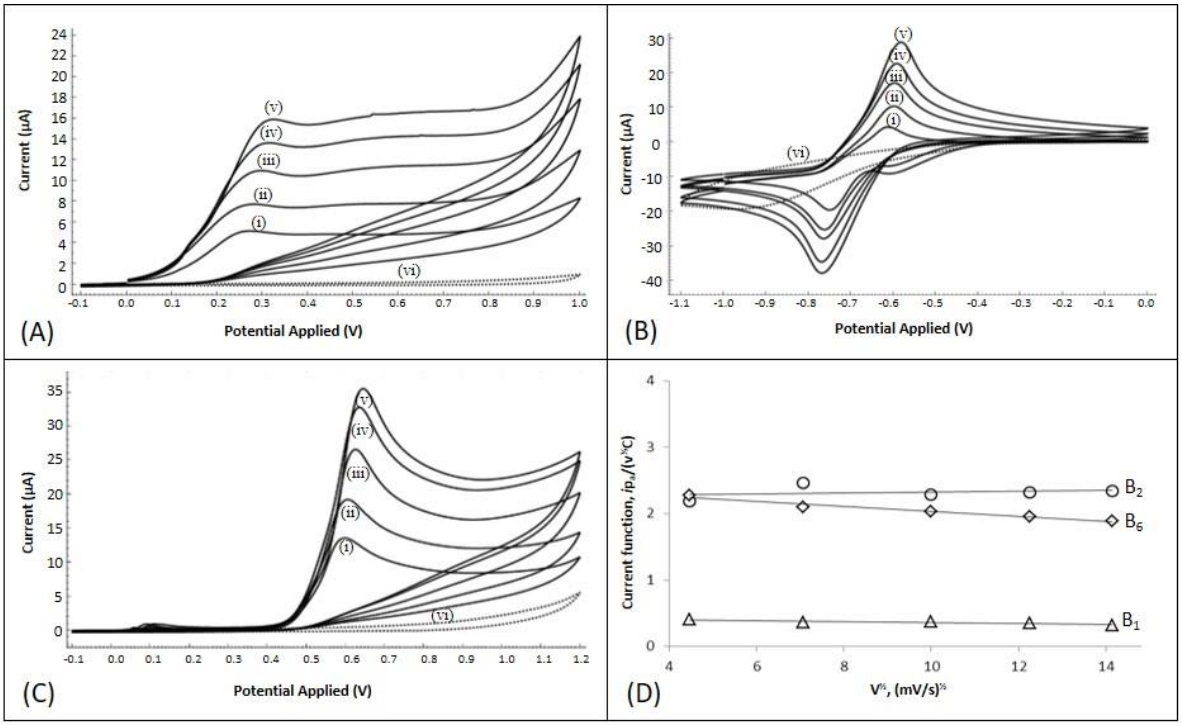

Fig. 2. Cyclic voltammograms were obtained using SPCEs (vs. Ag/AgCl) with a solution containing 0.1 M sodium chloride with 0.1 M phosphate buffer (pH 11) for: (A) 50 mM thiamine; (B) 20 mM riboflavin (C) 20 mM pyridoxine. Scan rates (i) 20 (ii) 50 (iii) 100 (iv) 150 (v) 200 mV s^{-1}. Blank voltammetric scans (vi) were performed at 20 mV s^{-1} with the buffered solution. (D) Current function versus $v^{½}$ for the anodic peak of thiamine, riboflavin and pyridoxine under the above conditions. Conditions: (A) Initial/final potential 0.0 V, switching potential +1.0 V & -0.1 V; (B) Initial/final potential -1.0 V, switching potential 0.0 V & -1.1 V; (C) Initial/final potential 0.0 V, switching potential +1.2 V & -0.1 V.

3.2. Calibration Studies

The cyclic voltammetric studies in section 3.1. led to the optimisation of the electrolyte solution for the analysis of thiamine, riboflavin, and pyridoxine. Quantitative studies performed to determine the linear working range of the SPCE's (vs. Ag/AgCl) in the presence of the three analytes were performed in the optimised pH 11 0.1 M phosphate buffered solution with 0.1 M sodium chloride. These studies used the more sensitive voltammetric technique square wave voltammetry, where the anodic peak potentials (Ep_a) were found to be -0.7 V, +0.2 V, +0.6 V for riboflavin, thiamine, and pyridoxine respectively. The linear ranges for these vitamins were as follows: thiamine 15-110 µg/ml (R_2 = 0.987); riboflavin 0.1-20.0 µg/ml (R_2 = 0.999); and pyridoxine 2-80 µg/ml (R_2 = 0.999). The sensitivities were thiamine 0.0298 µA µg/ml, riboflavin 4.105 µA µg/ml, and pyridoxine 0.375 µA µg/ml. The precision of the method was calculated as the coefficient of variation (n=4), the calculated values were: thiamine 6.05 % (50 µg/ml); riboflavin 6.26 % (7.5 µg/ml); pyridoxine 5.42 % (32 µg/ml). The limit of detection was calculated from the sensitivity in conjunction with three times the baseline noise for a blank solution at the peak potential of the individual vitamins. The detection limits were: 0.1 µg/ml (riboflavin); 3.5 µg/ml (thiamine); 0.4 µg/ml (pyridoxine).

3.4. Analytical Applications

3.4.1. Food Analysis

The successful measurement of riboflavin in the food spread Marmite® was achieved using the standard addition method. The square wave voltammograms obtained for a typical standard addition study are shown in Fig. 3A. The riboflavin content for each sample was calculated using the method of standard addition [14]; a typical plot is shown in Fig. 3B. The average recovery for riboflavin was calculated to be 95.8 % with the precision (coefficient of variation) calculated to be 3.38 % (Table 1) n=5. The recoveries agree well with the declared content provided in the manufacturer's specification and the method could have application for quality control analysis of food products.

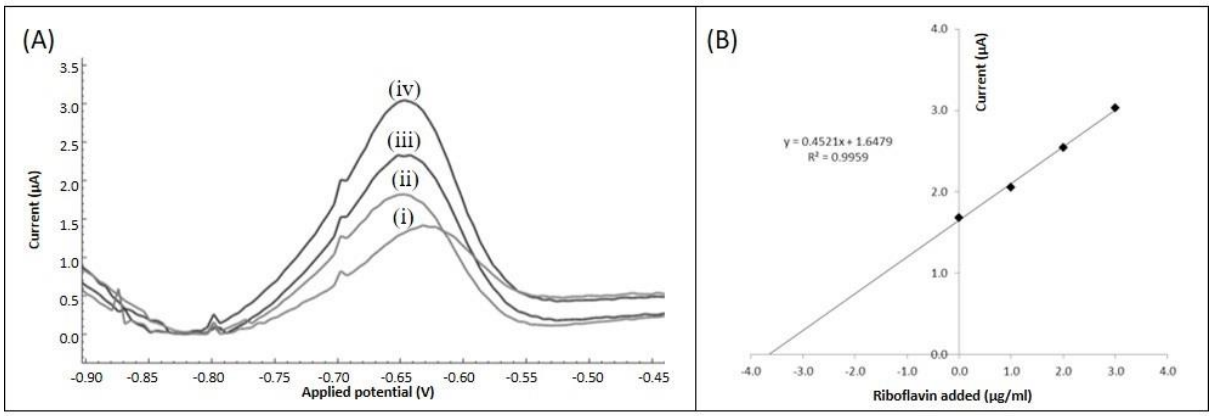

Fig. 3. (A) Square wave voltammograms were obtained using SPCEs (vs. Ag/AgCl) with a solution containing Marmite® extract in a pH 11 0.1 M phosphate buffer with 0.1 M sodium chloride with the following standard additions of riboflavin (i) 0.0, (ii) 1.0, (iii) 2.0, (iv) 3.0 µg/ml. Initial potential -1.0 V, final potential 0.0 V. Scan rate 250 mV s^{-1}. (B) Typical standard addition plot for riboflavin additions in a Marmite® sample using the conditions described in part A (n=5).

Table 1

Recovery data for vitamin B_2 in Marmite

Vitamin B_2 Sample	Declared (µg/g)	Measured (µg/g)	Recovered (%)
1	70.0	69.3	99.0
2	70.0	69.3	95.9
3	70.0	64.7	92.5
4	70.0	66.3	94.7
5	70.0	64.6	92.3
		Average recovery (%)	95.8
		Standard deviation	3.24
		Coefficient of variation (%)	3.38

3.4.2 Pharmaceutical Analysis

The simultaneous measurement of the three water soluble vitamins has been successfully achieved for a commercially available pharmaceutical preparation, (Ultra Vit B Complex™ by Vitabiotics©) using square wave voltammetry in conjunction with unmodified SPCEs. Fig. 4A shows well defined peaks for all three vitamins in the sample extract with no interference from the other components of the sample matrix. The standard addition plots for thiamine, riboflavin, and pyridoxine are shown in Fig. 4B, 4C, and 4D respectively; the recoveries were calculated to be 110% thiamine; 114% riboflavin; 112 % (pyridoxine) and precision data (coefficient of variation) for the same three vitamins were 7.14 % (thiamine), 6.28 % (riboflavin), 5.66 % (pyridoxine) (Table 2). The data shows that the method hold promise for the analysis of pharmaceutical products.

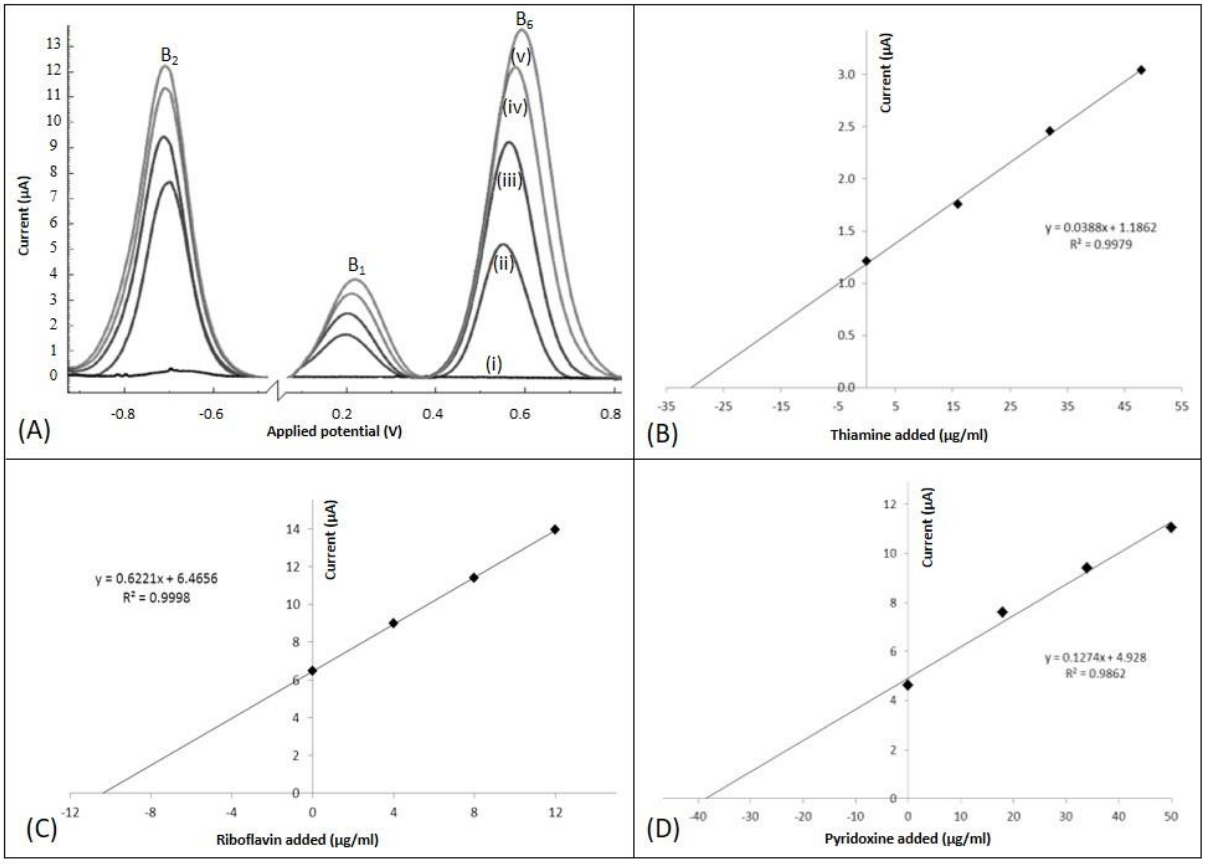

Fig. 4. (A) Square wave voltammograms were obtained using SPCEs (vs. Ag/AgCl) with a solution containing an extract from a vitamin B tablet (Ultra Vit B Complex™ by Vitabiotics©) in a pH 11 0.1 M phosphate buffer with 0.1 M sodium chloride (i) with the following standard additions of thiamine (ii) 0, (iii) 16, (iv) 32, (v) 48 µg/ml, riboflavin (ii) 0, (iii) 4, (iv) 8, (v) 12 µg/ml, & pyridoxine (ii) 0, (iii) 18, (iv) 34, (v) 50 µg/ml. Initial potential -1.0 V, final potential +1.0 V, scan rate 250 mV s^{-1}. Typical standard addition plots in a Vitabiotics© Ultra Vit B Complex™ sample for (B) thiamine (C) riboflavin (D) pyridoxine.

4. Conclusions

In this paper we have described the development of a novel voltammetric assay for the simultaneous measurement of thiamine, riboflavin and pyridoxine using SWV in conjunction with unmodified SPCEs. It was possible to perform the analysis in a single voltammetric scan, in only 8s, owing to the judicious choice of the optimum phosphate buffer to allow conversion of thiamine to its electroactive thiol form; in addition, the initial negative potential allowed the conversion of the oxidised form of riboflavin to its reduced form which is re-oxidised in the anodic scan. We have also shown that a simple sample pre-treatment step could be used prior to the quantification of these vitamins in both a food and pharmaceutical product. It should be readily feasible to develop similar methods based on this approach for other important vitamins in a variety of food and pharmaceutical matrices

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Table 2

Recovery data for vitamin B_1, B_2, & B_6 in supplement tablets

Vitamin B_1 Sample	Declared (mg/tablet)	Measured (mg/tablet)	Recovered (%)
1	5.00	5.78	118
2	5.00	5.55	109
3	5.00	5.94	102
4	5.00	5.19	110
5	5.00	6.12	114
		Average recovery (%)	110
		Standard deviation	7.88
		Coefficient of variation (%)	7.14
Vitamin B_2 Sample	Declared (mg/tablet)	Measured (mg/tablet)	% Recovered
1	1.40	1.65	116
2	1.40	1.52	111
3	1.40	1.43	119
4	1.40	1.53	104
5	1.40	1.59	122
		Average recovery (%)	114
		Standard deviation	7.18
		Coefficient of variation (%)	6.28
Vitamin B_6 Sample	Declared (mg/tablet)	Measured (mg/tablet)	% Recovered
1	5.00	5.31	106
2	5.00	5.76	115
3	5.00	6.05	121
4	5.00	5.40	108
5	5.00	5.37	107
		Average recovery (%)	112
		Standard deviation	6.32
		Coefficient of variation (%)	5.66

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