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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) of 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).

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

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[9]. Siddiqui and Pitre (2001) [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 s.

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 0.1 V −1.0 V; 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 −1.1 V; (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^{-1}. 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^{-1}. 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 mV s^{-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 70× and 300× 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.

2.5. Sample preparation

The food product Marmite® was prepared by diluting a 2 g 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 min 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 min 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 min 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.