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Direct oxidation of methionine at screen printed graphite macroelectrodes: Towards rapid sensing platforms

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ABSTRACT

The direct electrochemical oxidation of methionine has been achieved at bare carbon based electrodes and for the first time at screen printed graphite electrodes in aqueous solutions. Due to scales of economy and intended use as a potential point-of-care sensor, the quantification of methionine was explored at screen printed electrodes, allowing linear ranges over the range 0.05–5.0 mM with a detection limit of 95×10^{-6} mol L^{-1} possible in model solutions. Application of this sensor was used for the determination of methionine in a pharmaceutical product containing a complex mixture of vitamins, amino acids, chelated minerals and additional factors with the results agreeing with manufacturers' specification suggesting that this sensing platform holds promise as a rapid, sensitive and disposable sensor for methionine determination.

1. Introduction

Oxidative stress is a physiological condition produced by the imbalance of the concentration of reactive oxygen and nitrogen species (ROS and RNS) and antioxidant substances in the cells [1,2]. Many studies correlate oxidative stress with diseases such as cancer [3], diabetes, cardiovascular diseases, Alzheimer [4,5], neurodegenerative diseases [6] and even ageing [7]. Most of the reactive species, including radicals, react with biomolecules such as DNA, RNA, cholesterol, lipids, carbohydrates, proteins [8,9] and antioxidants such as vitamins or enzymes [10]. The extent of the oxidative damage depends on various factors: the concentration of the target molecule, the distance in between the localization of this target and the source of ROS/RNS, the formation of secondary oxidants, the intervention of trapping agents or the repair action of enzymes blocking the attack [11].

Proteins are the majority of the macromolecules of biological systems which are consequently the most favourable target macromolecules to be attacked by ROS/RNS [12,13]. The most easily oxidised amino acid residues are methionine (Met) [14], tryptophan (Trp), tyrosine (Tyr), cysteine (Cys), phenylalanine (Ala) and histidine (His). Among them Met, also known as 2-amino-4(methylthio)-butanoic acid, is the most facile to be oxidised for two reasons: the sulphur atom is very accessible and the methyl group can combine with active free radicals that converts methionine into the very best scavenger for ROS and RNS [15–19].

Methionine is a source of sulphur in the body as it is the precursor of other sulphur amino acids such as cysteine, taurine and glutathione and it is a methyl donor essential for the formation of DNA and RNA, helped by the action of cofactors such as vitamins B6, B12, choline, folic acid and magnesium. Methionine is important in the formation of blood proteins, globulins and albumins helping in the breakdown of lipids and acting as chelates for heavy metals assisting their removal from body. Methionine deficiencies have been attributed to toxaemia, muscle paralysis, hair loss, depression, schizophrenia, Parkinson's disease, liver deterioration and impaired growth [20]. Since methionine cannot be synthesized in the body it must be obtained from food supplies and pharmaceutical supplements are commercially available.

It has been put into evidence the role which ROS and RNS may play *in vivo* and therefore the determination of this species directly or indirectly has been widely studied. Methionine, in particular, but other amino acids as well can act as ROS/RNS scavengers such that the sensing of methionine is an indirect way of determining the oxidative stress *in vivo*. Traditional analytical techniques for amino acid measurement are complex laboratory procedures requiring a number of assay steps of derivatization via the reactions between the primary amino group and the o-phthalaldehyde in the presence of an alkyl thiol by pre- or post-column, additional chemicals and complex instrumentation [21,22]. Electrochemical techniques have

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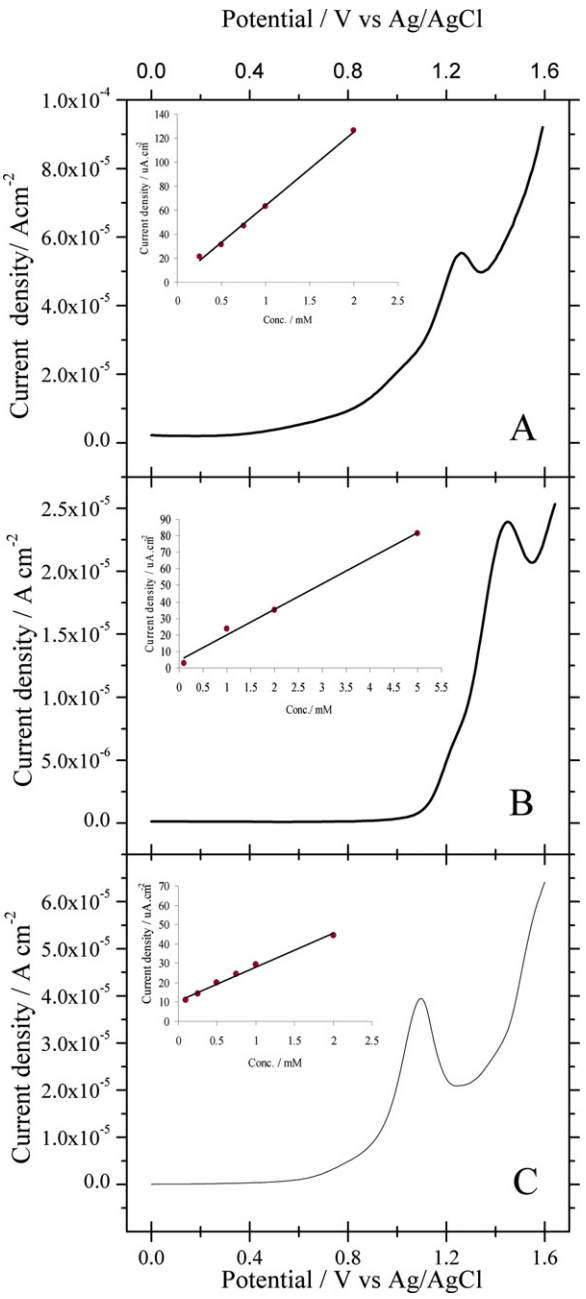

Fig. 1. Comparison of the DPV of different carbon based electrodes: GC (A), BDD (B) and SPGE (C) for the direct oxidation of 1 mM methionine in pH 7 0.1 M phosphate buffer solution. Inset figures, calibration plots for ranges between 0.25 and 2 mM for (A), 0.1 and 5 mM for (B), 0.1 and 2 mM for (C).

been recently added to the pool of techniques that have been used to detect ROS/RNS such as immunochemistry, colorimetry, fluorimetry, chemiluminescence, mass spectroscopy or Electro Spin Resonance (ESR) [23,24]. The detection of these species is often difficult due to the numerous cellular mechanism and pathways which they are involved in and their complicated nature and short lifetimes, although there are studies of different direct and indirect approaches for its detection [25–27]. ROS/RNS via direct or indirect sensing is a non-destructive approach allowing real time analysis which is selective and specific [28]. Studies with complex electrochemical techniques such Scanning ElectroChemical Microscope (SECM) have been performed for the intracellular detection of NO [29].

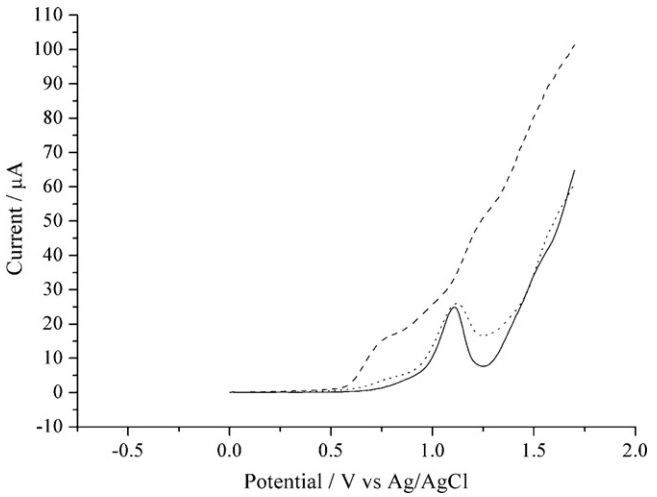

Fig. 2. Effect of the pH in the response for the direct oxidation of methionine 1 mM at phosphate buffer solution pH 2 (solid line), pH 7 (dotted line) and pH 12 (dashed line).

Recent research has been performed to the determination of amino acids using bare screen printed electrodes [30], due to the numerous advantages of cost effective, mass produced, easy-to-use, micro-volumes of samples and its innovative customization. Alegret and co-workers demonstrated the direct oxidation of cysteine and tyrosine at screen printed electrochemical sensors based in a two-electrode strip configuration [31]. The introduction of metal nanoparticles [32–34] as well as carbon nanotubes [35] or metallodendrimers [36] has been used to decrease the overpotential of oxidation of amino acids [37,38]. Likewise, Ershad's group modified glassy carbon electrode with cobalt hydroxide nanoparticles for the oxidation of a range of amino acids in alkaline media [39]. Turner and co-workers demonstrated the development of biosensors for the measurement of L- and D-amino acids through the immobilization of enzymes on rhodinised carbon electrodes [40] and similarly, Hirata and co-workers developed a biosensor by attaching the enzyme to a polyion complex membrane onto glassy carbon [41]. Elegant work by Pingarrón and co-workers has reported gold nanoparticles cysteamine mediated carbon paste electrodes for the sensing of methionine [42]. Additionally, Tan et al. have reported the mediated oxidation of methionine by modifying a gold electrode with the fullerene-C_{60} [43].

In this paper, we report for the first time the direct oxidation of methionine at bare screen printed graphite electrodes (SPGEs). Note that no chemical or electrochemical pre-treatments are applied and the SPGEs are used 'as is'. The suitability of the use of screen printed electrodes for the measurement of the direct oxidation of methionine is demonstrated for the analysis of the content of this analyte in a pharmaceutical product.

2. Materials and methods

All chemicals used were of analytical grade and were used as received without any further purification. All solutions were prepared with deionised water of resistivity not less than 18.2 MΩ cm^{-1}. Methionine, L- (Acros Organics, 98%), sodium pyruvate (Fluka, >99%), ascorbic acid (vitamin C) (Fluka, >99%), cysteine (Lancaster Synthesis Ltd.). The support electrolyte solutions consisted of pH 2 and pH 7 potassium phosphate buffers.

The pharmaceutical product "Super Once a Day" (Quest Vitamins Ltd., Birmingham, United Kingdom), an orange suspension containing 60 mg of methionine per tablet (concentration as given on the label) in pH 2 phosphate buffer was filtered through

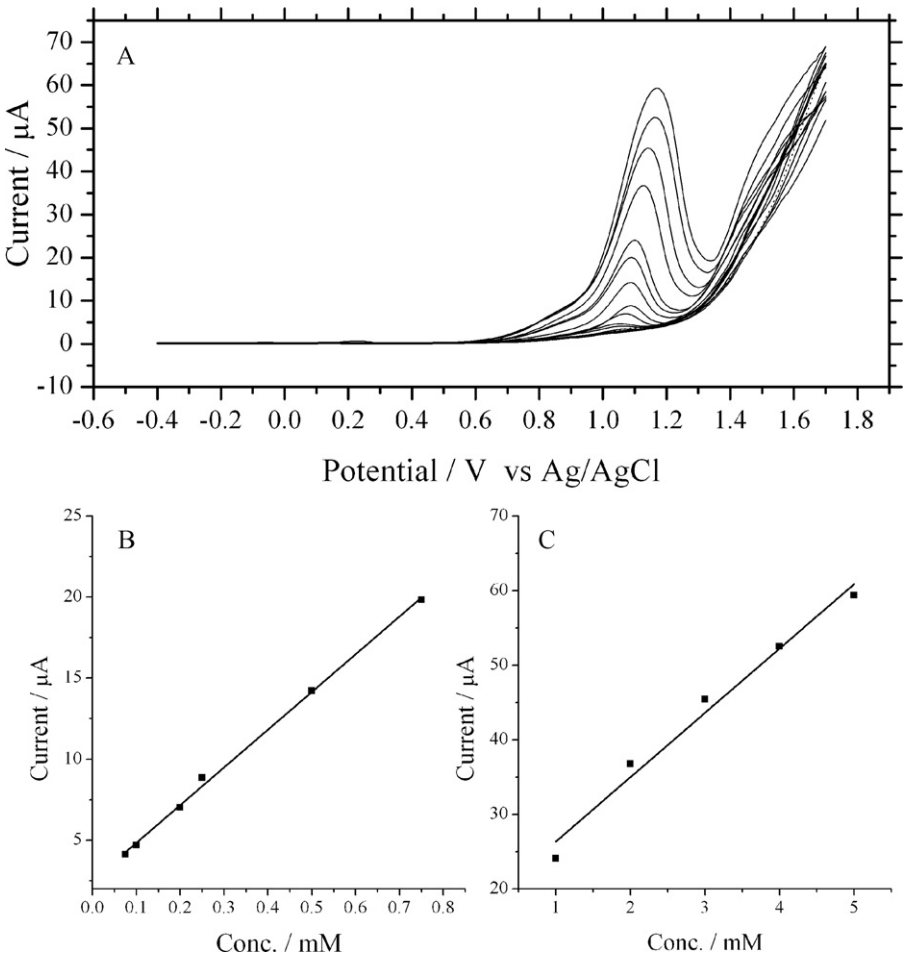

Fig. 3. Typical calibration plot for methionine (A) at a SPGE in pH 2 phosphate buffer solution with analysis of the peak current as a function of concentration shown in (B) and (C).

0.22 μm diameter PTFE filters. Additional extraction procedure was used by passing the sample through a LC-NH_2 solid phase extraction cartridge (Varian, Bond Elut). The tablet also contains vitamins: panthotenic acid (vitamin B5), biotin, folic acid, beta-carotene, alpha-carotene, cryptoxanthin, vitamin C, B6, B12, D, E, thiamine, zeaxanthin, lutein, riboflavin, niacin; other complements such as choline bitartrate, inositol, para-amino benzoic acid, bioflavonoids, lecithin, papain, rutin, betaine hydrochloride, hesperidin; other amino acids such as lysine hydrochloride and cysteine; and chelated minerals: Ca, P, Fe, Mn, Cu, Mg, Zn, I, Mo, Cr, Se and vanadium.

Voltammetric measurements were carried out using a μ-Autolab III (Eco Chemie, The Netherlands) potentiostat/galvanostat and controlled by Autolab GPES software version 4.9 for Windows XP. All measurements were conducted using a SPGE, which was fabricated as reported previously [44–46]. These electrodes consist of a graphite working electrode (3.1 mm diameter) with a carbon counter and silver/silver chloride pseudo-reference electrode on a flexible plastic base. The screen printed electrodes have been characterised electrochemically in a prior paper and have heterogeneous rate constants of 1.7×10^{-3} cm s^{-1} [47]. Connectors for the electrochemical connection of the screen printed electrodes were purchased from Kanichi research Services Ltd. (UK) [48]. Boron doped diamond mounted in PEEK (BDD, Windsor Scientific Ltd.) and glassy carbon electrode (GC) were also used with a 3-electrodes cell configuration with a platinum counter and a SCE as a reference electrode. Differential pulse voltammetry (DPV) measurements where the potential range was from −0.4 V to +1.7 V, the step potential was 0.01 V, the amplitude was 0.1 V and the scan rate 0.02 V s^{-1}. No preconditioning step was used.

A typical differential pulse voltammetry experiment was employed to perform a standard addition of amino acids to the pharmaceutical product. This implied the addition of methionine in the range $(2–10) \times 10^{-5}$ mol L^{-1} from a standard 5.0×10^{-3} mol L^{-1} methionine in pH 2 0.1 M phosphate buffer solution.

3. Results and discussion

We first consider the direct oxidation of methionine at a range of carbon based electrode substrates. Fig. 1 depicts the voltammetric response of a GC (Fig. 1A), BDD (Fig. 1B) and SPGE (Fig. 1C) obtained in a solution of 1 mM methionine in pH 7 phosphate buffer. The DP voltammetric signatures of methionine at GC exhibit an oxidation peak at +1.26 V (vs. pseudo Ag/AgCl), compared to that obtained at BDD appearing at +1.44 V (vs. pseudo Ag/AgCl). In contrast, a bare screen printed electrode displays a well-defined peak at a slightly lower oxidation potential +1.1 V (vs. pseudo Ag/AgCl) and such a response is likely due to the higher global average of edge planes/like-sites/defects which constitute excellent conditions for this electrode as the basis of a methionine sensor. Insets depicted in Fig. 1 show the response of increasing additions of methionine into pH 7 phosphate buffer solution over the range 0.25–2 mM (Fig. 1A) for GC ($Ip/(\mu A\,cm^{-2}) = 61.264/mM + 2.5095$ ($\mu A\,cm^{-2}$); $R^2 = 0.9974$), over the range 0.1–5 mM (Fig. 1B)

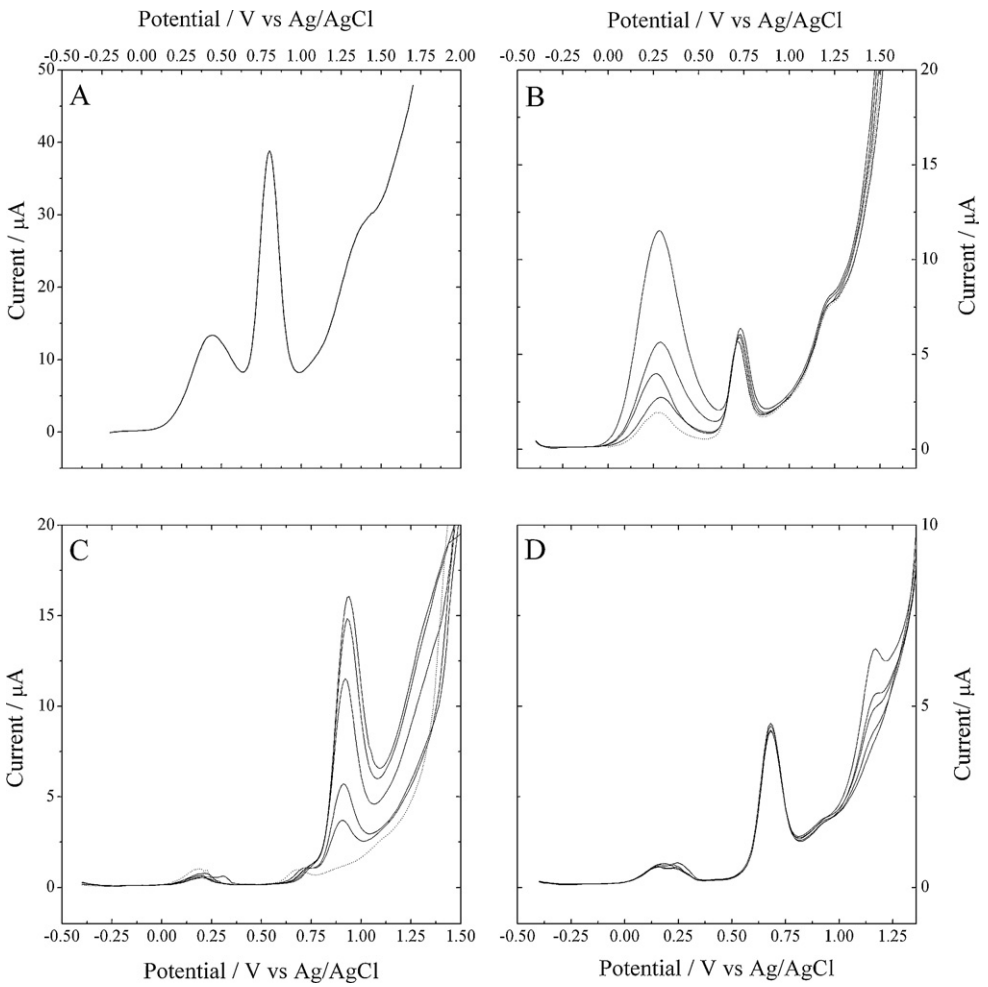

Fig. 4. (A) Pharmaceutical product dilution 1:10 in pH 2 phosphate buffer solution. (B) Standard additions of ascorbic acid (vitamin C). (C) Standard additions of pyridoxine hydrochloride (vitamin B6). (D) Standard additions of methionine using an extraction procedure.

for BDD ($Ip/(\mu A\,cm^{-2}) = 15.501/mM + 4.4298$ ($\mu A\,cm^{-2}$); $R^2 = 0.9923$) and over the range 0.1–2 mM (Fig. 1C) for SPGE ($Ip/(\mu A\,cm^{-2}) = 17.533/mM + 10.396$; $R^2 = 0.9917$).

The effect of scan rate was explored over the range 4–100 mV s^{-1} using a fixed concentration where the peak potential was observed to gently shift with increasing scan rates. A plot of peak height against scan rate reveals a linear response ($Ip/A = 1.97 \times 10^{-4}\,A/(V\,s^{-1}) - 2.27 \times 10^{-5}$ A; $R^2 = 0.9703$). Additionally the peak current was found to increase with increasing scan rates with a plot of $Ip/\upsilon^{1/2}$ vs. log υ clearly indicated the electrochemical process is adsorption controlled [49]. Experimental adsorption of the methionine on the SPGEs was electrochemically performed by measuring with DPV of 1 mM methionine in pH 7 phosphate buffer solution. After adsorption of methionine, the SPGE was gently rinsed with Millipore water and dried, after this the electrode was cycled with pH 7 phosphate buffer solution and a decrease of ~69% of the current peak height was obtained.

Next, attention was turned to exploring the effect of solution pH upon 1 mM methionine. Fig. 2 depicts the absence of a pH dependence response for measurements carried out, as can be observed from the voltammetric response obtained in pH 2 (solid line) and pH 7 (dotted line) phosphate buffer solutions. Experiments for methionine oxidation at pH 12 (dashed line) did not show a well-defined oxidation peak so consequently no more trials at intermediate pHs were performed. No reduction waves are observed in the accessible potential window consistent with the methionine likely undergoing a chemically irreversible reaction. Previous research from Pingarron's group have reported that at gold modified carbon paste electrodes pre-adsorption on the gold surface occurs followed by the oxide-catalyzed oxidation to the sulfone occurs where the mechanism has a pH dependant response involving 4 electrons and 4 protons [42]. In our work we observe a pH non-dependence for the oxidation of methionine at the SPGE and adsorption is noted and we surmise that the electrochemical mechanism might be as described as:

$$CH_3SR'COOH + H_2O \rightarrow CH_3SR'COO^- + H_3O^+ \quad (1)$$

$$2CH_3SR'COO^- \rightarrow CH_3SR' + CO_2 + 2e^- \quad (2)$$

which is similar to that suggested by Tan and Goh [43] at C_{60}-fullerene modified gold electrodes. Reproducibility of the response of methionine in SPGEs was checked by performing repetitive measurements of 1 mM methionine at phosphate buffer, yielding a % relative standard deviation of 2.04% ($n = 5$).

Fig. 3A depicts the linear calibration graph of methionine at pH 2 phosphate buffer solution obtained by DPV in the $(0.05–5.0) \times 10^{-3}$ mol L^{-1} concentration range, showing two different linear ranges, $(0.05–0.75) \times 10^{-3}$ mol L^{-1} with a slope of 24.11 ± 1.13/mM ($R^2 = 0.9982$) (95% probability) Fig. 3B and $(1.0–5.0) \times 10^{-3}$ mol L^{-1} with a slope of 5.375 ± 0.114 ($R^2 = 0.925$) Fig. 3C. A limit of detection was found, based on 3-sigma, to corre-

spond to 95 μM ($n = 4$). This is in comparable with literature reports, for example with that obtained by Pingarrón and co-workers who reported a limit of detection of ∼59 μM using gold nanoparticles modified electrodes.

An interference study for analytes that might likely appear was executed. DP voltammograms of the possible interferents were explored. These were: citrate, lactate, glucose, pyruvate; amino acids: glycine, alanine, glutamate, aspartate, asparagine, cysteine; vitamins: vitamin B6, vitamin C, vitamin B5. When applying the same conditions as above for the electrochemical oxidation of methionine it was found that out of these possible interferents, only cysteine at ∼+1.34 V and pyruvate at ∼+0.96 V were found to exhibit electrochemical responses close to that of the direct oxidation of methionine in pH 7 phosphate buffer solution while in pH 2 phosphate buffer solution there is no response of pyruvate and cysteine shown a non-defined oxidation peak around +1.40 V; consequently pH 2 was chosen as the optimum solution pH.

We now turn to exploring towards the analytical utility of the SPGEs in the determination of methionine in real samples as the electrode was found to be the most suitable in terms of not only sensitivity, repeatability, reproducibility but also due to its disposable and easy-to-use nature for the direct oxidation of methionine. Fig. 4A depicts typical DPV of the pharmaceutical product diluted to a factor of 10 in pH 2 phosphate buffer without any extraction procedures. It was found that three constituents of the tablet exhibited voltammetric responses which were ascorbic acid (vitamin C) at ∼+0.3 V, pyridoxine hydrochloride (vitamin B6) at ∼+0.9 V and methionine and at ∼+1.1 V; clearly there is sufficient resolution between the peaks to allow quantitative measurements to be performed. Fig. 4B shows DP voltammetric profiles resulting from the additions of ascorbic acid and Fig. 4C from the additions of pyridoxine hydrochloride, vitamin B6, (both at a dilution factor of 1000 in phosphate buffer solution pH 2) and Fig. 4D from the additions of methionine after the extraction procedure (see Section 2 for details; dilution factor of 50 using phosphate buffer solution pH 2). The matrix effect of the tablet was demonstrated by comparing the slopes from the result of standard additions for the case of just pH 2 buffer solution exhibiting a response of 29.8 (±0.8) A/mM while for the case of the tablet solution standard (no extraction) additions of methionine revealed a value of 28.5 (±0.3) A/mM, while in the case where extraction is performed a value of 22.4 (±2.8) A/mM is observed. Clearly, the matrix effect of the tablet results in a loss of sensitivity and hence a higher than expected value to be derived and hence to obtain meaningful results an extraction procedure needs to be implemented. As shown in Fig. 4D, the concentration of the tablet was determined by the addition standard method for 3 replicates yielding a methionine concentration of 6.3 ± 0.7 mM which is in good agreement with the manufacturers value (8 mM, 60 mg per tablet). The R.S.D. obtained for this determination was 8%.

4. Conclusions

We have explored the electrochemical behaviour of a range of carbon based electrode substrates for the direct oxidation of methionine and the use of bare SPGEs is reported for the first time. We have demonstrated that screen printed graphite electrodes is a suitable tool for the development of a potentially commercial sensor in terms of economy and analytical characteristics. The use of this SPGE is demonstrated to be possible for the determination of methionine in real samples such as pharmaceutical products and culture media. Further applications would be performed for the sensing of methionine in real biological media and peptides as an indirect sensing for the oxidative stress grade.

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Biographies

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