

Electrochemical detection method

[0001] This invention relates to methodology and sensors for the electrochemical detection and quantification of disulphides. In a particular embodiment, the present invention relates to sensors and methodology for the electrochemical detection and quantification of disulphide components present in garlic (including particular forms of garlic (such as, for example, garlic purees, additives or extracts) and any garlic containing products).

BACKGROUND

[0002] Garlic is consumed in a variety of forms for its medicinal properties, and is also widely used world-wide as a cooking ingredient.¹⁻³ Data indicates that in 2007 at least 15.7 million tonnes of garlic was produced world-wide.² However, different batches of garlic vary significantly in the strength of flavour, therefore the 'garlic flavour' needs to be quantified before appropriate amounts of garlic can be added during industrial-scale food preparation. This fact, as well as variations caused by different cooking methods³ and the physiological and biochemical activity of the various components of garlic^{2, 3} has lead to interest in a variety of fields in the quantification of the characteristic flavour and fragrance molecules of garlic.

[0003] In the food industry garlic batches are quantified by organoleptic testing. One method is based upon the dilution of garlic in sour cream followed by taste testing; repeated testing can be used in order to monitor the stability of garlic during long term storage. The extraction, identification and quantification of the volatile flavour constituents of garlic has been described by a number of authors,² for example by solid-phase trapping solvent extraction,¹ headspace solid-phase microextraction,¹ steam distillation,¹ steam distillation/solvent extraction^{1, 3} and super-critical CO₂ extraction.⁴ It has been found that the majority of these volatile compounds are thiosulfinates, although the rich variety of compounds that contribute to the flavour and fragrance of garlic stem from the odourless precursor alliin. Upon rupture of the garlic cellular tissue alliin is converted enzymatically to allicin,¹ which makes up ca. 70 % of the thiosulfinates present in freshly chopped garlic (ca. 0.4 to 1.4 % w/w of the garlic itself).² However, allicin is also unstable and decomposes to form a variety of compounds; although the quantitative determination of allicin has been described,⁵ most studies determine diallyldisulfide to be the most overwhelmingly abundant extractant from processed, raw garlic,^{1, 3, 4} as well as garlic that has been cooked by a number of methods.^{3, 4}

[0004] A number of techniques have been described for the electrochemical quantification of disulfides.⁷ Disulfides such as cystine^{8, 9} and oxidised glutathione^{8, 10} have been quantified by their accumulation then stripping at hanging mercury drop electrodes. Pulsed electrochemical detection has also been used for the quantification of cystine and oxidised glutathione at gold electrodes.¹¹⁻¹⁴ However, such techniques utilise toxic or expensive metals, and are also likely to be susceptible to interference by the non-thiosulfinate constituents of garlic.

[0005] Accordingly, there is a need for improved methods and sensors for the detection and quantification of garlic strength in a sample.

10 [0006] More generally, there is also a need for improved methods and sensors for detecting disulphides. Examples of other disulphides that could usefully be detected in food products include cystine, lipoic acid, lenthionine (which is the active compound from shiitake mushrooms), and disulphide components present in onions.³⁰

15 BRIEF SUMMARY OF THE DISCLOSURE

[0007] The present invention provides a simple, effective and inexpensive means for the electrochemical detection and quantification of disulphides. In particular, the present invention provides a simple, effective and inexpensive means for the electrochemical detection and quantification of the disulphide components present in garlic and garlic-containing products, thereby providing a means for detecting presence and strength of garlic constituents of food and/or medicinal products.

[0008] The term garlic is used herein to refer to any form of garlic and includes garlic, garlic extracts, purees, additives and any other garlic-derived constituents or components of food products.

25 [0009] In one aspect the present invention provides a method of detecting and quantifying disulphides present in a sample, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution which comprises a mediator, and determining the electrochemical response of the working electrode to the sample.

30 [0010] The mediator may be any mediator compound giving rise to an enhanced electrochemical response by virtue of catalytic electron transfer with disulphides. In particular, the mediator is any compound capable of being converted into an oxidised form at the working electrode which then reacts with any disulphides present in the sample to regenerate the mediator (in a reduced/"non-oxidised" form). The regenerated mediator

can then be re-oxidised at the working electrode to give an enhanced electrochemical response when disulphides are present in the sample. The extent of the enhanced electrochemical response (current) can be correlated with the amount of the disulphide that is present.

5 [0011] In a particular embodiment of the invention, the mediator is selected from bromide or iodide.

[0012] The methodology of the present invention is based, at least in part, on the discovery that garlic (or more specifically, the disulphide components that are present in garlic) can be detected electrochemically when a mediator, such as bromide or iodide, is
10 present in the sample medium.

[0013] Thus, the present invention provides, in a particular aspect, a method of detecting the presence of garlic in a sample and quantifying its strength, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution comprising a mediator, such as bromide or iodide, and determining the
15 electrochemical response of the working electrode to the sample.

[0014] This, in an embodiment, the method of the present invention involves the oxidation of bromide (Br^-) or iodide (I^-) at the working electrode to generate bromine (Br_2) or iodine (I_2) respectively. The molecular bromine or iodine generated at the working electrode reacts with any disulphides present in the sample to regenerate bromide or
20 iodide ions respectively, which are then reoxidised at the working electrode. The regeneration of bromide or iodide as a consequence of the reaction between the disulphides and molecular bromine or iodine respectively, followed by the subsequent reoxidation of the bromide or iodide ions at the working electrode, generates a detectable electrochemical response that enables the detection and quantification of any disulphides
25 present in the sample. This process is similar to an EC' mechanism known in the art²⁰.

[0015] There are a number of advantages associated with this methodology. Firstly, the method does not require the use of metal electrodes, which can be prohibitively expensive. Furthermore, the methodology provides a specific means for detecting disulphides, (such as, for example, the disulphide components (e.g. diallyldisulphide)
30 present in garlic), and it is therefore less likely to be susceptible to interference from other non-disulphide components (such as, for example, the non-thiosulfinate constituents present in garlic). In addition, when bromide or iodide is used as the mediator, molecular bromine or iodine respectively is generated *in situ*, so there are no health and safety issues associated with the direct handling of molecular bromine or iodine.

[0016] It has previously been demonstrated that bromine can be electrochemically generated, reacted on column and the remaining bromine detected downstream, in order to indirectly quantify thiols (cysteine and reduced glutathione) and the dialkylmonosulfide methionine.¹⁵ Iodine was used in a similar manner to quantify cysteine and N-acetylcysteine in urine.¹⁶ Bromine has also been used as a mediator during electrosynthesis; cysteic acid was prepared by the reaction of electrogenerated bromine with cystine,¹⁷ while reaction with a range of substituted diaryldisulfides was reported to form sulfenium cations as intermediates.¹⁸ The reaction of molecular bromine with disulfides has also been used for the direct titration (as thus quantification) of solutions of dialkyldisulfides.¹⁹ However, the inventors are not aware of any published work relating to the amperometric quantification of disulfides *via* the electrogeneration of bromine.

[0017] In another aspect, the invention provides the use of a mediator as defined herein, such as bromide or iodide, for the electrochemical detection of garlic and/or disulphides.

[0018] In yet another aspect, the present invention provides an electrochemical sensor for detecting and quantifying disulphides in a sample, which comprises a working electrode, a counter electrode, a reference electrode and an electrolyte solution comprising a mediator, such as bromide or iodide.

[0019] During use of the sensor, the mediator is oxidised at the working electrode and then undergoes a reaction with any disulphide components present in the sample to regenerate mediator, and wherein the regenerated mediator is then reoxidised at the working electrode to generate a detectable redox couple.

[0020] In an embodiment, the mediator is bromide or iodide and, during use of the sensor, the bromide or iodide mediator is oxidised to bromine or iodine respectively at the working electrode and then undergoes a reaction with any disulphide components present in the sample to regenerate bromide or iodide ions respectively, and wherein the regenerated bromide or iodide ions are then reoxidised at the working electrode to generate a detectable redox couple.

[0021] In another aspect, the present invention provides a kit for the detection of garlic and/or disulphides in a sample, the kit comprising a working electrode, a counter electrode, a reference electrode and a bromide or iodide mediator.

[0022] The sensors and methodology of the present invention are useful for a number of potential applications, including (but not limited to): (i) detecting the presence of garlic in a sample; (ii) detecting the strength of garlic in a sample; (iii) detecting changes in the strength of garlic (e.g. during long term storage; batch to batch and seasonal variations). These particular uses form further aspects of the present invention.

[0023] The methodology and sensors of the present invention may also be used in other applications to detect the presence of disulphides. For example, the methodology of the present invention could also be used to detect and/or quantify the levels of cystine. Cystine is present in many food supplements and an excess of cystine can be dangerous for people suffering from cystinosis and/or cystinuria. The methodology could also be used to detect lipoic acid, lenthionine (which is the active compound from shiitake mushrooms), as well as disulphide components present in onions.³⁰ These particular uses also form further aspects of the present invention.

10 BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Particular embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 shows the cyclic voltammograms of a solution of 15 mM sodium bromide (NaBr) in 50:50 H₂O:acetonitrile (AcN) (0.1 M NaClO₄) at a surface printed carbon electrode (SPCE; diameter 4 mm), edge plane pyrolytic graphite (EPPG; 4 mm) and basal plane pyrolytic graphite (BPPG; 5 mm) electrodes. Recorded at 50 mV s⁻¹.

Figure 2 shows the cyclic voltammetry response for the oxidation of 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at SPCE with the standard addition of dipropyldisulfide (0, 0.33, 0.66, 1.00, 1.33, 1.66 and 1.99 mM). Recorded at 50 mV s⁻¹.

Figure 3 shows overlaid square wave scans for 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at a SPCE, displaying the forward current only (corresponding to the right-hand y-axis) and the resulting square wave voltammogram (forward current minus backwards current). Recorded at a frequency of 25 Hz, an amplitude of 0.9 mV and step potential of 1.95 mV.

Figure 4 shows:

(a) a square wave voltammogram of 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at SPCE, in the presence of PrSSPr (0, 0.33, 0.66, 1.00 and 1.33 mM). Scans smoothed and offset such that at E = 0.20 V, I = 0 A. The square wave voltammogram parameters are the same as in Figure 3.

(b) Plot of I_p vs. PrSSPr concentration for the peaks at ca. +0.95 V (○) and +1.25 V (■, gradient = 1.2 ± 0.2 μA mM⁻¹). Lines are linear best fits, error bars are assuming 10 % error.

Figure 5 shows a plot of I_p at ca. +1.15 V vs. concentration of diallyldisulfide (●, gradient = 224 ± 14 μA mM⁻¹, R² = 0.98) and dipropyldisulfide (■, gradient = 224 ± 10 μA mM⁻¹, R² =

0.99). Cyclic voltammograms (CVs) were recorded in 50:50 H₂O:AcN (0.1 M NaClO₄) containing 15 mM NaBr at SPCE and 50 mV s⁻¹.

Figure 6 shows:

(a) the cyclic voltammetry response of 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at an EPPG electrode, in the presence of PrSSPr (0, 0.10, 0.20 and 0.30 mM), recorded at 50 mV/s, and

(b) a plot of I_p in the presence of PrSSPr for 5 mM (■), 10 mM (◆) and 15 mM NaBr (▲).

Figure 7 shows the cyclic voltammetry response of 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at EPPG and 10 mV s⁻¹, in the presence of 0 mM PrSSPr (---), 0.15 mM (— · —) and 1.5 mM (— · · —). The inset displays the same data, with an additional larger peak for 15 mM PrSSPr (— · · —).

Figure 8 shows the cyclic voltammetry response for the oxidation of 15 mM NaBr in 16 mL of 50:50 H₂O:AcN (0.1 M NaClO₄) at SPCE, where the 8 mL of AcN was first shaken for 5 min with 0, 0.33, 0.67, 1.00 and 1.33 g of Spanish garlic puree. Recorded at 50 mV s⁻¹.

Figure 9 shows a plot of I_p at ca. +1.15 V vs. weight of garlic used in extraction step (recorded by CV using the parameters specified in Fig. 2), for Spanish garlic (■, gradient = $135 \pm 3 \mu\text{A g}^{-1}$, $R^2 = 0.999$) and Chinese garlic (◆, gradient = $59 \pm 5 \mu\text{A g}^{-1}$, $R^2 = 0.936$). Also shown are the values for 15 mM Br⁻ by itself (●). Error bars are based upon measurements in triplicate or more, and include both extraction error and SCPE batch-to-batch variation error.

DETAILED DESCRIPTION

[0025] As stated above, the present invention provides, in a particular aspect, a method of detecting and/or quantifying the strength garlic or garlic extracts present in a sample, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution comprising a mediator, such as bromide or iodide, and determining the electrochemical response of the working electrode to the sample.

[0026] In a particular embodiment the mediator is bromide or iodide.

[0027] The methodology relies on the reaction of molecular bromine or iodine (generated by the oxidation of bromide or iodide respectively at the working electrode) with disulphide components present in garlic. The primary flavour-component of raw garlic varies based upon the method used to extract and quantify it, although diallyldisulfide (4,5-dithia-1,7-

octadiene) consistently ranks as the predominate compound with its extracted abundance varying between 58 and 98 %, in addition to various other thiosulfinates and hydrocarbons.¹

5 **[0028]** Thus, in a particular embodiment, the present invention provides a method of detecting and/or quantifying disulphide components of garlic or garlic extracts present in sample.

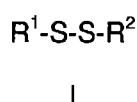
[0029] In a further embodiment, the present invention provides a method of detecting and/or quantifying diallyldisulphide (4,5-dithia-1,7-octadiene) in a sample.

10 **[0030]** Although the detection and quantification of the garlic content of a sample is one primary technical problem addressed by the present invention, a person skilled in the art will appreciate that the methodology described herein can also be used to detect the presence of other disulphides present in a samples.

15 **[0031]** Hence, in a further aspect, the present invention provides a method of detecting and quantifying disulphides present in a sample, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution which comprises a mediator, such as bromide or iodide, and determining the electrochemical response of the working electrode to the sample.

20 **[0032]** It shall be appreciated that any disulphide that can react with the oxidised mediator electrochemically generated at the working electrode can be detected by this methodology.

[0033] Disulphides will typically have the general formula I shown below:



wherein R¹ and R² are organic groups.

25 **[0034]** As stated above, one particular disulphide that is found as a major component of garlic is diallyldisulphide. Dimethyldisulfide, methylpropyldisulfide and methylallyldisulfide are typically minor components.

30 **[0035]** For electrochemical analysis, the sample will typically be in liquid form. In situations where the raw sample is a solid, it will be necessary to convert it into a suitable liquid form for analysis. This can be achieved by techniques well known in the art, such as, for example, homogenising the sample and/or carrying out a liquid phase extraction with a suitable solvent. It may be necessary, in certain circumstances, to supplement the sample solution with a suitable electrolyte.

[0036] The disulphide compounds may be detected using an electrochemical sensor containing a working electrode with which the sample is contacted. Typically, electrochemical sensors are based upon the standard configuration of an electrochemical cell, comprising a working electrode, a counter electrode and an electrolyte. The sensor
5 may further comprise a reference electrode. Suitable sensor designs are well known in the art.

[0037] The working electrode may be any suitable electrode known in the art, for example a metallic or carbon electrode.

[0038] Examples of metallic electrodes include gold, silver and platinum electrodes.
10 However, such electrodes tend to be expensive.

[0039] In an embodiment of the invention, the electrode is a carbon electrode. Such electrodes include an edge plane pyrolytic graphite electrode, a basal plane pyrolytic graphite electrode, a glassy carbon electrode, a boron doped diamond electrode, a highly ordered pyrolytic graphite electrode, carbon powder and carbon nanotubes.

15 **[0040]** Suitably, the carbon electrode is a printed carbon electrode.

[0041] In a particular embodiment, the working electrode is a screen printed carbon electrode (SPCE). SPCEs are typically cheap, customisable and disposable.^{21, 22}

[0042] The working electrode may be a microelectrode or a macroelectrode.

[0043] The counter electrode may be any suitable counter electrode known in the art, for
20 example, a platinum or graphite electrode. In an embodiment, the counter electrode is a graphite electrode.

[0044] Suitably, the counter electrode is also a printed electrode, for example a screen printed carbon electrode.

[0045] The reference electrode may be any suitable reference electrode known in the art.
25 Examples of suitable reference electrodes include, by way of example, a saturated calomel electrode (SCE) or a silver electrode. In a particular embodiment, the reference electrode is a printed electrode. In a further embodiment, the reference electrode is an Ag/AgCl reference electrode.

[0046] In an embodiment, the sensor comprises three electrodes, a working electrode, a
30 counter electrode and a reference electrode.

[0047] In a further embodiment, the sensor comprises three printed electrodes (a working electrode, a counter electrode and a reference electrode) Suitably, the electrodes are printed onto a small strip which can be connected to the sensor apparatus. This makes

the sensor electrodes easy to handle and ideal for use in food quality control procedures.^{23, 24}

[0048] In a further embodiment, the sensor may comprise a working electrode and a joint reference/counter electrode.

- 5 **[0049]** Suitably, the concentration of bromide or iodide should be constant. In an embodiment, the bromide or iodide is present at concentration within the range of 3 mM to 15 mM.

[0050] In an embodiment, the mediator is a bromide mediator. Any suitable bromide mediator may be used in the methodology and sensors of the present invention.

- 10 Examples of suitable bromide mediators include bromide salts, such as potassium bromide or sodium bromide, which can be readily dissolved in the sample medium.

[0051] In a particular embodiment, the bromide mediator is sodium bromide.

[0052] In an alternative embodiment, the mediator is an iodide mediator. Any suitable iodide mediator may be used in the methodology and sensors of the present invention.

- 15 Examples of suitable iodide mediators include iodide salts, such as potassium iodide or sodium iodide, which can be dissolved in the sample medium.

- [0053]** The electrochemical response of the working electrode may be determined using any suitable technique known in the art. This typically involves applying a potential across the working and counter electrodes, and determining the response of the working
20 electrode to the sample. A potential may be applied across the electrodes using a potentiostat, and the response of the cell to the sample determined.

- [0054]** Various electrochemical techniques, for example voltammetry (e.g. cyclic voltammetry) and amperometry, are encompassed by the present invention. For determination of the voltammetric response, the applied potential is varied relative to a
25 reference electrode; in this way, a cyclic voltammogram may be obtained. Alternatively, the amperometric response of the cell can be determined by applying a fixed potential across the electrodes, optionally controlled relative to a reference electrode.

- [0055]** In one embodiment, the current is measured using linear sweep or cyclic voltammetry. In another embodiment, said current is measured using square wave
30 voltammetry. In an alternative embodiment, the current is measured using a pulsed voltammetry technique, e.g. differential pulse voltammetry.

[0056] The present invention also provides an electrochemical sensor for detecting and quantifying the strength of garlic and/or disulphides in a sample, which comprises a

working electrode, a counter electrode, a reference electrode and an electrolyte solution comprising a mediator, such as bromide or iodide, as defined herein before.

5 [0057] During use, the mediator is oxidised at the working electrode and then undergoes a reaction with any disulphides present in the sample to regenerate the mediator, and said regenerated mediator is then re-oxidised at the working electrode to generate a detectable redox couple.

10 [0058] In the case of bromide or iodide mediators, during use, the bromide or iodide mediator is oxidised to bromine at the working electrode and then undergoes a reaction with any disulphide components of garlic present in the sample to regenerate bromide or iodide ions, and said regenerated bromide or iodide ions are then re-oxidised at the working electrode to generate a detectable redox couple.

[0059] In another aspect, the present invention provides a kit for the detection of disulphides in a sample, the kit comprising a working electrode and a mediator, such as bromide or iodide, as hereinbefore defined.

15 [0060] In an embodiment, the kit further comprises a counter electrode, as hereinbefore defined.

[0061] In an embodiment, the kit further comprises a reference electrode, as hereinbefore defined.

[0062] In an embodiment, the kit further comprises an electrolyte solution.

20 [0063] In an embodiment, the kit further comprises means for preparing the sample for analysis. Such means may include, for example, solvents to dissolve a solid sample.

[0064] The following examples illustrate the invention.

EXPERIMENTAL

25 [0065] In order to illustrate the present invention, the inventors have investigated the quantification of two disulfides (diallyldisulfide and dipropyldisulfide) using the methodology of the present invention. Sodium bromide was used as a mediator. As discussed above, bromine is generated *in situ* at the working electrode, hence there are no health concerns arising as a consequence of the use and handling of molecular
30 bromine.

[0066] Furthermore, investigation of the mechanism has demonstrated that following electron transfer to form bromine, the disulfides react with several equivalents of bromine

to regenerate bromide, which is then reoxidised at the electrode (similar to an EC' mechanism²⁰).

[0067] The applicability of the methodology of the present invention for detecting and quantifying the strength of garlic in a sample has also been demonstrated. Samples of raw garlic puree were shaken in acetonitrile for 5 min followed by voltammetric investigation using cheap, disposable screen-printed carbon electrodes. The correlation between the electrochemical response detected and the quantity of garlic present was linear, and is predicted to relate to the strength of the garlic.

10 Materials and general methodology

[0068] All chemicals were purchased at the highest grade available and used directly without any further purification. Dipropyldisulfide (98 %), diallyldisulfide (tech., 80 %) and NaClO₄ (ACS Reagent, ≥98 %) were sourced from Sigma Aldrich, UK; NaBr was sourced from May and Baker Ltd, UK. All solutions were prepared with acetonitrile (HPLC Gradient grade, Fisher Scientific) and deionized water of resistivity not less than 18.2 MΩ·cm⁻¹ at 298 K (Millipore UHQ, Vivendi, UK).

[0069] Garlic samples were kindly donated by Beacon Foods Ltd (Brecon Powys, UK), and consisted of two batches of garlic sourced from China and Spain. The garlic cloves were peeled and chopped in Beacon Foods Ltd kitchens then posted under refrigerated conditions. The garlic was stored at 4 °C, and all experiments performed within five days of receipt. Extraction was performed as described in the Results & Discussion section.

[0070] All voltammetric measurements were recorded using a μ-Autolab III computer-controlled potentiostat (EcoChemie, Utrecht, The Netherlands). The DRP-110 Screen-Printed Carbon Electrodes (SPCE) were manufactured by Dropsens Ltd (Spain) and obtained through their UK distributor (IJ Cambria Scientific Ltd, UK). The edge plane pyrolytic graphite (EPPG, Le Carbone Ltd., Sussex, UK) and basal plane pyrolytic graphite (BPPG, Le Carbone Ltd., Sussex, UK) electrodes were prepared by securing the material inside an insulating PTFE surround to leave an exposed circular geometric surface with a diameter of 4 mm (EPPG) or 5 mm (BPPG). The EPPG electrode surface was renewed by polishing with alumina slurries (1.0–0.3 μm, Buehler Ltd., USA). The BPPG electrode surface was renewed by firmly pressing the surface on cellotape then pulling off to remove the surface layers, which was repeated five times before thoroughly rinsing the surface with acetone and then dionised water.

[0071] Experiments were performed using a three-electrode set-up, with a graphite rod and leak-free Ag/AgCl (Warner Instruments, USA) as counter and reference, respectively.

A beaker containing 16 mL or 25 mL of electrolyte was employed at ambient temperature; at no stage was degassing found to be necessary.

[0072] Further specific experimental details are provided in the brief description of the figures set out above.

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Example 1 - Comparison of the oxidation of bromide at edge plane pyrolytic graphite (EPPG), basal plane pyrolytic graphite (BPPG) and DropSens screen printed carbon electrodes (SPCE)

[0073] As stated above, Figure 1 shows the cyclic voltammetric response of a solution of 15 mM sodium bromide (NaBr) in 50:50 H₂O:acetonitrile (AcN) (0.1 M NaClO₄) at a surface printed carbon electrode (SPCE; diameter 4 mm), edge plane pyrolytic graphite (EPPG; 4 mm) and basal plane pyrolytic graphite (BPPG; 5 mm) electrodes. The cyclic voltammograms were recorded at 50 mV s⁻¹.

[0074] Oxidation of Br⁻ at BPPG occurred as a well-defined peak at ca. +1.33 V, while oxidation at the EPPG and SPCE both gave rise to two overlapping features, at ca. +0.95 and ca. +1.15 V. The first oxidative feature at the EPPG and SPCE is attributed to the oxidation of Br⁻ to Br₂, followed by rapid reaction with Br⁻ to form [Br₃]⁻, while the second feature corresponds to the oxidation of [Br₃]⁻ to yield Br₂. Such behaviour is well established in acetonitrile solutions.^{25, 26} The similarity of the EPPG and SPCE responses also agrees with the previous findings by Fanjul-Bolado *et al.*²⁷ and Kadara *et al.*,²⁸ namely that DropSens SPCE have a large area of edge planes exposed to solution due to the high density and likely random orientation of the graphite platelets of which the electrode consists.

Example 2 - Mediated oxidation of dipropyldisulfide (PrSSPr) with bromine at SPCE: Concentration, square wave voltammetric analysis

[0075] Figure 2 shows the cyclic voltammetry response for the oxidation of 15 mM NaBr in 50:50 H₂O:AcN (0.1 M NaClO₄) at SPCE with the standard addition of dipropyldisulfide (0, 0.33, 0.66, 1.00, 1.33, 1.66 and 1.99 mM). The CVs were recorded at 50 mV s⁻¹.

[0076] The largest oxidative peak (corresponding to Br₂ generation) increased in a linear manner with the amount of PrSSPr added, while the reverse peak (reduction of Br₂) decreased. The oxidation peak grew and shifted more positive with each addition of PrSSPr, with the oxidation peak eventually becoming indistinguishable from the solvent oxidation at ca. 5 mM PrSSPr (only shown up to 2 mM PrSSPr in Fig. 2). The PrSSPr

displayed no voltammetric features of its own on the SPCE in the investigated potential range. Such change in the behaviour of Br^- is qualitatively indicative of an electron transfer to form Br_2 , followed by chemical reaction to catalytically regenerate the initial species (either Br^- or $[\text{Br}_3]^-$), abbreviated as an EC' reaction²⁰ and discussed in more detail later.

- 5 [0077] Square wave voltammetric (SWV) analysis of the system was also carried out. First the parameters were optimised in order to give the largest current response and most clearly resolved peaks. During this process it was observed that the oxidation processes were relatively irreversible, such that the forwards and backwards currents were similar and the resulting SWV had a significantly smaller current than the forward current alone.
- 10 [0078] Figure 3 shows an overlay of the forward current with the resulting SWV for 15 mM NaBr in 50:50 $\text{H}_2\text{O}:\text{AcN}$ (0.1 M NaClO_4) at a SPCE, using the optimised SWV parameters. A frequency of 25 Hz, an amplitude of 0.9 mV and a step potential of 1.95 mV was utilised. The oxidation of Br^- to $[\text{Br}_3]^-$ (ca. +0.95 V) and Br_2 (ca. +1.25 V) are clearly resolved in the SWV. An additional oxidative feature is also observed at ca. +0.75 V. This
- 15 process cannot be observed in the forward current alone as it is masked by the double layer charging, and likely relates to the adsorption of Br^- at the rough carbon surface.

- [0079] SWV were recorded for SPCE in solutions containing NaBr (15 mM) and PrSSPr (0 to 1.33 mM). It was observed that subtle changes in the SPCE (such as different double layer capacitance), which did not obviously affect the CVs, had significantly more influence
- 20 on the response recorded using SWV due to the much smaller currents. Most notably the absolute current values were shifted by a different value for each SPCE. Fig. 4(a) displays SWV of Br^- in the presence of varying amounts of PrSSPr that have been smoothed and then 'referenced', by making a consistent minima in the SWV at +0.20 V equal to $I = 0$. Fig. 4(b) displays a plot of I_p vs. PrSSPr concentration for the peak at ca. +0.95 V ($\circ$) and
- 25 the peak at ca. +1.25 V ($\blacksquare$). The results clearly demonstrate that with the addition of PrSSPr the peak corresponding to the formation of $[\text{Br}_3]^-$ remains unchanged while the peak corresponding to Br_2 increases in a linear manner, demonstrating that Br_2 is the only species that reacts with the PrSSPr. It also highlights that in this case SWV offers no significant advantage over CV in terms of data collection, the increased resolution
- 30 frequently associated with SWV being counteracted somewhat by batch-to-batch variation in the SPCE.

Example 3 - Comparison of diallyldisulfide with dipropyldisulfide

- [0080] The primary flavour-component of raw garlic varies based upon the method used
- 35 to extract and quantify it, although diallyldisulfide (4,5-dithia-1,7-octadiene) consistently

ranks as the predominate compound with its extracted abundance between 58 and 98 %, in addition to various other thiosulfates and hydrocarbons.¹ Since diallyldisulfide could not be sourced in high purity the structurally similar dipropyldisulfide (98 % purity), which is essentially a saturated analogue of diallyldisulfide, was employed for the precise work described throughout the paper. However, examination of the two species was required in order to compare their relative responses, and a technical grade sample of diallyldisulfide with quoted purity of 80 % was investigated (primary contaminants anticipated to be the tri- and polysulfide derivatives²).

[0081] Experiments similar to those described in Fig. 2 were repeated, whereby CVs were recorded for the oxidation of 15 mM NaBr in 50:50 H₂O:AcN at SPCE, in the presence of 0 to 2 mM of either dipropyldisulfide or diallyldisulfide, with the diallyldisulfide being treated as 80 % diallyldisulfide v/v. Fig. 5(a) displays a plot of I_p for the peak at ca. +1.15 V vs. the concentration of the relevant disulfide species. It can be observed that both species gave closely similar linear responses with respect to concentration of the disulfide compound; both gave identical gradients of 224 $\mu\text{A mM}^{-1}$, although visually the data points for diallyldisulfide are also consistently lower than those representing equivalent concentrations of dipropyldisulfide. Therefore the two compounds are assumed to have an identical response, with the slight difference in the absolute current-concentration ratio being attributed to the actual diallyldisulfide concentration in the supplied sample being slightly lower than 80 % v/v. Different diffusion coefficient values could also account for the variation, although the diffusion coefficients of diallyldisulfide and dipropyldisulfide are not expected to differ significantly.

[0082] Voltammetry in the presence of 0.005 to 2 mM PrSSPr was investigated at SPCE, and a LOD value (based upon 3σ) of 0.067 mM was determined.

Example 4 - Confirmation of the EC' mechanism by scan rate study and simulation at EPPG

[0083] In order to investigate the electrochemical process further, the voltammetry of Br⁻ and PrSSPr was investigated further. An EPPG electrode was used in order to ensure that a well-defined, reproducible electrode surface was used for each scan.

[0084] With the addition of small quantities of PrSSPr to solutions of Br⁻ at EPPG a small prepeak was formed. Fig. 6(a) displays CVs of 15 mM NaBr in the presence of 0 (---), 0.1, 0.2 and 0.3 mM PrSSPr, in 50:50 H₂O:AcN at 50 mV s⁻¹. Fig. 6(b) displays plots of I_p vs. [PrSSPr] at three different NaBr concentrations, demonstrating that I_p was linear with [PrSSPr] except when [PrSSPr] = 0.

[0085] Fig. 7 displays scans recorded for 15 mM Br⁻ at 10 mV s⁻¹, in the presence of 0 (---), 0.15 (—) and 1.5 mM (····) of PrSSPr, where the presence of the small prepeak is also clearly observed. This behaviour is consistent with a chemical reaction subsequent to the electrogeneration of Br₂. The removal of electrogenerated Br₂ by chemical reaction makes the initial electron transfer more thermodynamically favourable, and Br₂ is produced at lower potentials until the PrSSPr in the vicinity of the electrode is depleted.^{20, 29} As the concentration increased, or if the scan rate was increased above 50 mV s⁻¹ then the two distinct processes merged to form a single observable peak.

[0086] The inset in Fig. 7 displays the same data overlaid with a scan of 15 mM Br⁻ in the presence of 15 mM PrSSPr. The oxidative current increases significantly, while the reverse peak corresponding to reduction of Br₂ is absent. This significant change in oxidative current is consistent with the chemical reaction of Br₂ ultimately regenerating Br⁻ in the vicinity of the electrode surface where it is reoxidised again on the time scale of the scan (a so-called catalytic or EC' mechanism²⁰). The reaction is relatively rapid, as demonstrated by the complete absence of Br₂ on the reverse scan.

[0087] The presence of the prepeak on EPPG, and its absence on the SPCE is attributed to the relatively more well-defined, planar geometry of the EPPG facilitating the observation of such relatively small features. In the case of the SPCE the two distinct processes (EC' and E) merge over the whole range of investigated concentrations, with a resulting simplification of the voltammetry.

[0088] In order to confirm the EC' mechanism, the CV of 15 mM Br⁻ in the presence and absence of 15 mM PrSSPr at 200 mV s⁻¹ was simulated. A Randle-Sevcik plot derived from a scan rate study gave $D_{Br_2} = 1.06 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, a Tafel plot indicated $\alpha \approx 0.56$, while simulation indicated the Br⁻/Br₂ couple possessed $E^0 \approx +0.95 \text{ V vs. Ag/AgCl}$ and $k^0 \approx 0.0005$. The reaction of dialkyldisulfides have been reported to go to the alkanesulfonyl bromides,¹⁹ which presumably hydrolyse to form the alkanesulfonic acids,¹⁷ ultimately consuming 5 eq. of Br₂ per dialkyldisulfide.^{17, 19} The voltammetry in the presence of 15 mM PrSSPr could be simulated using each PrSSPr consuming between 1 to 8 eq. Br₂ simply by varying k_f ; however, for 1 to 4 equivalents of Br₂ the peak was relatively sharp due to rapid depletion of the PrSSPr. Satisfactory fits of I_p and the current at $E = +1.50 \text{ V}$ (the +ve vertex potential) could only be obtained for reactions consuming 5 to 8 eq. Br₂, with a value of $k_f \approx 375 \text{ L mol}^{-1} \text{ s}^{-1}$ required for best fit when 5 eq. Br₂ were consumed.

Example 5 - Quantification of the strength of real samples of garlic using SPCE

[0089] Two real samples of raw garlic puree were investigated in order to demonstrate proof-of-concept, namely that the quantity of garlic present could be electrochemically quantified using the electrochemistry of NaBr as a probe, and therefore the 'strength of garlic' could be inferred. Such techniques have direct implication for the monitoring of garlic during long term storage, quantifying the ingredients of medicinal garlic supplements, as well as a range of other quality control applications. The garlic samples were sourced from Spain and China; personal communication from the supplier indicated that the Spanish garlic could be classed as 'strong' and the Chinese garlic as 'less strong', but could not be quantified further.

[0090] The extraction process used to investigate the garlic was as follows; first a certain amount of garlic was weighed into a 15 mL centrifuge tube, 10 mL of acetonitrile was added, and the tube manually shaken by hand for exactly 5 min. During this process the puree was observed to aggregate to form a slightly desiccated-looking ball, from which the acetonitrile could easily be decanted. For extractions employing large quantities of garlic (> 2g) the neck of the tube was loosely packed with glass wool, the tube inverted and the acetonitrile drained out. This extraction process proceeded smoothly, although presumably other forms of mild agitation, as well as other non-aqueous solvents (such as ethanol) would also be effective.

[0091] After extraction 8 mL of the acetonitrile was combined with 8 mL of aqueous solution to form a solution containing 15 mM NaBr and 0.1 M NaClO₄. The resulting solutions were investigated voltammetrically, and qualitatively no differences were observed between the garlic-extract samples and those previously investigated containing dipropyldisulfide or diallyldisulfide. Fig. 8 displays an overlay of CVs after extracting various quantities of Spanish garlic. Fig. 9 displays a plot of I_p for the oxidative peak at ca. +1.15 V vs. the quantity of garlic used during the extraction. It can clearly be observed that the stronger Spanish garlic generated a larger signal with increasing weight, corresponding to more dialkyldisulfides present, and clearly demonstrates the potential of this electrochemical technique to rank the garlic samples in order of their strength.

[0092] One extracted sample was investigated using three SPCE, and the resulting variation in I_p was found to have an error of 1.7 %. The extraction process was also repeated separately three times and each solution investigated with a new SPCE, with the combined extraction/SPCE error found to be 3.1 %. The error bars in Fig. 9 come from a number of experiments in which the extractions were repeated in triplicate.

[0093] By comparing the gradient obtained for diallyldisulfide in Fig. 5 ($224 \mu\text{A mM}^{-1}$) with those obtained in Fig. 8 for Spanish ($135 \mu\text{A g}^{-1}$) and Chinese ($59 \mu\text{A g}^{-1}$) garlic, it can be determined that the extracted disulfide species corresponded to roughly 0.14 and 0.06 w/w %, respectively. These are reasonable values, with the total weight of the precursor
5 alliin being found between 0.5 to 1.4 % w/w in fresh garlic.²

Summary

[0094] The electrochemistry of bromide at edge plane pyrolytic graphite (EPPG) and screen printed carbon electrodes (SPCE) in 50:50 water:acetonitrile has been shown to be
10 a highly sensitive probe for dialkyldisulfide concentration. Dipropyldisulfide was detected with an LOD value of 0.18 mM at SPCE. The response of diallyldisulfide was shown to be identical to that of dipropyldisulfide.

[0095] Garlic puree was shaken in acetonitrile for 5 min, then the resulting voltammetry of 15 mM bromide recorded using cheap, disposable SPCE. The voltammetry was able to
15 quantify the dialkyldisulfides extracted into the acetonitrile, providing proof-of-principle that the strength of the garlic purees can be voltammetrically determined.

[0096] Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not
20 intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0097] Features, integers, characteristics, compounds, chemical moieties or groups
25 described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the
30 steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings),
35 or to any novel one, or any novel combination, of the steps of any method or process so

disclosed.

[0098] The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

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CLAIMS

1. A method of detecting and quantifying disulphides present in a sample, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution which comprises a mediator, and
5 determining the electrochemical response of the working electrode to the sample, wherein said mediator is capable of being converted into an oxidised form at the working electrode which then reacts with any disulphides present in the sample to regenerate the mediator; wherein the mediator is a bromide or iodide mediator.
- 10 2. A method according to claim 1, wherein said sample is a sample comprising garlic and said method provides a means for detecting and quantifying disulphide components present in said garlic.
3. A method according to claim 2, wherein said disulphide components are selected
15 from one or more of the group consisting of diallyldisulphide, dimethyldisulfide, methylpropyldisulfide and methylallyldisulfide.
4. A method according to any preceding claim, wherein the mediator is bromide.
- 20 5. A method according to any preceding claim, wherein the mediator is iodide.
6. A method according to any one of the preceding claims, wherein the working electrode is a carbon electrode.
- 25 7. A method according to claim 6, wherein the carbon electrode is a printed carbon electrode.
8. A method according to any one of the preceding claims, wherein the counter electrode is a printed carbon electrode.

9. A method according to any one of the preceding claims, wherein a reference electrode is also provided.
- 5 10. A method according to claim 9, wherein the reference electrode is a printed silver electrode.
11. A method according to any one of the preceding claims, wherein electrochemical response is determined using voltammetry.
- 10 12. A method according to any one of claims 1 to 10, wherein the electrochemical response is determined by amperometry.
13. An electrochemical sensor for detecting and quantifying the presence of
15 disulphides in a sample comprising a working electrode, a counter electrode, a reference electrode and an electrolyte solution comprising a mediator, wherein said mediator is capable of being converted into an oxidised form at the working electrode which then reacts with any disulphides present in the sample to regenerate the mediator; wherein the electrolyte comprises a bromide or iodide mediator.
- 20 14. A sensor according to claim 14 or claim 15, wherein the working electrode, counter electrode and reference electrodes are all printed electrodes.
15. A sensor according to claim 14, wherein the working electrode and counter
25 electrodes are printed carbon electrodes and the reference electrode is a printed silver electrode.
16. A kit for the detection and quantification of disulphides in a sample, the kit
30 comprising a working electrode, a counter electrode and a mediator which is capable of being converted into an oxidised form at the working electrode during use and then reacts

with any disulphides present in the sample to regenerate the mediator; wherein the mediator is a bromide or iodide mediator.

5 17. A kit according to claim 16, wherein the kit further comprises a reference electrode.

18. A method of detecting and quantifying disulphides present in a sample, said method comprising the steps of contacting the sample with working and counter electrodes in the presence of an electrolyte solution which comprises a mediator, and
10 determining a detectable change in the sample,
wherein said mediator is converted into an oxidised form at the working electrode which then reacts with any disulphides present in the sample to regenerate the mediator,
and wherein said detectable change is a means of monitoring the reaction between the mediator and the disulphide

15 wherein the electrolyte comprises a bromide or iodide mediator.

19. A method according to claim 18, wherein said detectable response is selected from a detectable colorimetric change or a detectable spectroscopic change in the sample.