Iodine mediated electrochemical detection of thiols in plant extracts using platinum screen-printed electrodes

M.I.González-Sánchez[a], E. Valero[a]* and R.G.Compton[b]

[a]Department of Physical Chemistry, School of Industrial Engineering, University of Castilla-La Mancha, Campus Universitario s/n, 02071-Albacete, Spain.

[b] Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, United Kingdom.

Address correspondence to Edelmira Valero: Edelmira.valero@uclm.es.

ABSTRACT

The quantitative analysis of the ratio of reduced glutathione to total glutathione provides useful information for the evaluation of the oxidative stress levels in biological samples. We report an electro-analytical methodology for determining this ratio using cyclic voltammetry and platinum screen-printed electrodes. The method involves the use of the reaction of electro-generated iodine with biological thiols, using GSH as a model, which produces an increase in the current of the anodic peak from iodide oxidation, and allows its easy analytical quantification. Iodine reacts with thiols in its reduced state, but not with disulfides, making possible the discrimination of these two kinds of compounds in a mixture. For the total glutathione determination, sodium borohydride was added as a reducing agent. Calibration plots for the reduced and total glutathione were analyzed. It was demonstrated that this method is able to analyze these compounds in solutions spiked with reduced and oxidized glutathione. Finally, to test the effectiveness of the analytical method in real samples, plant extracts from *Pisum sativum* (pea) were analyzed by the developed methodology as a proof-of-concept and validated independently with a spectrophotometric method.

KEYWORDS

Thiol, disulfide, glutathione, iodine, screen-printed electrode, cyclic voltammetry.

1. INTRODUCTION

Thiols (-SH) are the main form of reduced sulphur in plants and can be found as protein thiols (thioredoxins) and low molecular weight thiols (cysteine, glutathione or phytochelatins) [1]. These compounds play important roles in the stress response of plants and their adaptation to the environment. Thiols can be oxidized to disulfides (-S-S-) and the oxidized/reduced couple ratio is regarded as an important biochemical marker for oxidative stress [2]. In particular, the tripeptide glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH), whose concentrations are reported to range up to millimolar in some tissues [3], and its corresponding oxidized form (GSSG), is the most abundant thiol/disulfide redox pair in plant cells. GSH is a critical factor in protecting organisms against toxicity and disease both in animals and plants [4]. In plants, this metabolite acts in the so-called ascorbate-glutathione cycle, helping to prevent damages caused by reactive oxygen species [5-7]. Under normal physiological conditions, the glutathione pool is mainly in its reduced form (GSH), while the rest is present as disulfide or thioether [1]. However the ratio GSSG/GSH is shifted towards GSSG upon stress, as a result of reactive oxygen species (ROS) scavenging pathways that can lead to programmed cell death [2]. Therefore, from a physiological point of view, the ratio of oxidized GSH with respect to total GSH (tGSH) is more relevant than the total concentration of GSH itself [8]. Monitoring these compounds is very useful in the study of plant metabolism, its growth and developmental processes, as well as its response to a variety of external stressors.

Nowadays there are different methods for the analysis of thiols such as chromatography [9,10] , colorimetry [11,12], fluorescence [13] or mass spectrometry [14]. However, these methods have some disadvantages such as high costs, long analysis times and often require laborious technical handling and chemical derivation [15]. The use of electroanalysis has its major advantages over the mentioned methods, being cheap, technically straightforward and having good sensitivity. Electrochemical techniques have been used as versatile tools to measure biologically relevant compounds and study biochemical pathways[16]. Significant effort has been placed upon finding electrochemical methods by which the glutathione content and/or thiol/disulfide ratio could be analytically determined [15]. Some of these methods use quinones or quinone analogs as electrochemical mediators [17-20] and have been applied in the measurement of thiols in commercial tissue culture media[21], plasma and saliva [22-24]. *Harfield et al* (2012) offers a critical review about the electrochemical determination of GSH for medical applications [15].

Electrochemical techniques have been demonstrated to be useful tools for the determination of plant metabolites related on oxidative stress. For example, the electrochemical detection of extracellular hydrogen peroxide in *Arabidopsis thaliana* cell suspensions was successfully used for the evaluation of oxidative stress [25]. In addition, the first *in situ* detection of the hormone salicylate in *Ocimum basilicum* plant leaves via reverse iontophoresis in combination with cyclic voltammetry at disposable screen-printed electrodes has been recently published [26]. However little is found in the

literature about electrochemical quantification of thiols and disulfides in plant extracts. *Supalkova et al* (2007) [27] showed that the electrochemical techniques were useful in the analysis of plant thiols. These authors used maize extracts and studied the effect of cadmium on the concentration of GSH, GSSG and phytochelatins. Furthermore, *Ryant et al* (2008) [28] also performed the electrochemical determination of low molecular weight thiols content in potato extracts. However, in these papers, a mercury drop electrode was used as working electrode and a supporting electrolyte at high pH (> 9) was employed. Under a technical point of view, these are drawbacks due to the high toxicity of mercury and that, at this basic pH, the GSH spontaneous oxidation takes place [29]. Therefore the development of simple and less polluting electrochemical sensors, able to work at physiological pH values, is very important in the analysis of these compounds in plants and other biological samples.

The reactivity of halogens towards organosulfur compounds is well documented [30,31]. However, their potential electroanalytical utility as a method of detecting thiols in biological media has yet to be explored. Iodine under aqueous conditions is able to oxidize thiols to their corresponding disulfide [30]. The iodide/iodine pair redox, in contrast to the bromide/bromine pair redox that can react both with thiols and disulfides [32-34], is only sensitive to the presence of thiols in their reduced state [32]. Therefore, it is a very useful tool in the discrimination between the reduced and oxidized forms of GSH and other relevant biological thiols. Therefore, the aim of this paper was to use screen-printed electrodes for the electrochemical measurement of reduced and total GSH in plant extract samples using the iodide/iodine pair as a mediator. Screen printed electrodes offer a number of advantages versus conventional electrodes, for example, they are suitable for working with small volumes, which is highly needed in the analysis of biological samples. This technology has never been applied for thiol determination in plant extracts.

2. MATERIAL AND METHODS

2.1 Reagents

5-5'-dithiobis(2-nitro-benzoic acid) (DTNB), glutathione (GSH), glutathione reductase (GR), hydrochloric acid, oxidized glutathione (GSSG), potassium carbonate, sodium borohydride, sodium iodide, sodium perchlorate and 2-vinylpyridine (2VP) were purchased at their highest available purity from Sigma-Aldrich (Spain) and were used as received. All solutions were prepared with deionized water (resistivity ≥ 18.2 MΩ·cm at 25°C) (Millipore, Watford, UK). All solutions were freshly prepared every day prior to experiments.

2.2 Plant extracts

Plant extracts from *Pisum sativum* (pea) leaves were supplied by the Fruit Technology Group (http://www.cebas.csic.es/dep_spain/mejora/biotecnologia/biotec_lineas.html, CEBAS, CSIC, Murcia, Spain). Briefly, pea leaves (1g) were frozen in liquid nitrogen, then ground in a mortar and pestle in 3 mL of 1 M $HClO_4$ solution. Then, the homogenate was centrifuged at 12 000 rpm for 15 minutes at 4 ºC, and the supernatant removed and stored at -80ºC. The samples were neutralized with K_2CO_3 to a pH of 7 prior to the analytical measurements.

2.3 Electrochemical measurements

Electrochemical measurements were carried out on three electrode platinum screen-printed electrodes (Dropsens, DRP-550), which consist of a platinum working electrode (diameter 4 mm), a platinum counter electrode and a quasi-silver reference electrode. Approximately 50 μL of the sample solution were deposited onto the chip covering the three electrodes prior to running the electrochemical experiment. The electrochemical experiments were performed using a computer-controlled potentiostat, AUTOLAB PGSTAT302N (Eco Chemie B.V., The Netherlands) in a Faraday cage. All solutions were previously incubated in a Thermomixer Comfort Eppendorf (Hamburg, Germany) at 25.0 °C before each measurement.

The method measures the total glutathione as GSH plus 2x GSSG. Firstly the GSH (reduced form) was measured by the electrochemically generated iodine at the platinum electrode surface. The total GSH was measured in the same way, but previously sodium borohydride to give a final concentration of 2% was added to the sample (solution containing GSSG or plant extract) to reduce GSSG to GSH. Then the solution was neutralized with hydrochloric acid 1M to pH 7 prior to the electrochemical analysis.

2.4 Spectrophotometric measurements

Spectrophotometric measurements were carried out using a UV/Vis Perkin-Elmer Lambda 35 (Perkin Elmer Instruments, Waltham, USA) spectrophotometer coupled to a PCB 150 Water Peltier System. Temperature was controlled at 25 °C.

GSH was measured by the recycling assay initially described by Tietze[35]. In it, GSH in its reduced state is oxidized by DTNB (producing GSSG and 2-nitro-5-thiobenzoate (TNB)) and subsequently reduced by glutathione reductase in the presence of NADPH. The appearance of TNB is monitored at 412 nm. The method measures the tGSH as GSH plus 2 x GSSG. Specific determination of GSSG was achieved by pretreatment of extract aliquots with 2VP [11]. For the total GSH measurements, triplicate aliquots of 25 μl of neutralized extract were added to a solution containing 0.5 mM NADPH and 0.6 mM DTNB. The reaction was started by the addition of GR (1 U/ml). The increase in absorbance at 412 nm was monitored for 3 min. GSSG was quantified by the same method after the incubation of 100 μl of the neutralized extract with 0.26M of2VP during 30 minutes at room temperature and subsequent centrifugation for 15 min (12 000 rpm). Calibration plots were prepared using GSH and GSSG standards at different concentrations. All solutions were prepared using 0.2 M sodium phosphate buffer pH 7.5 containing 10 mM EDTA.

2.5 Fitting the data

Data were fitted by using the SigmaPlot Scientific Graphing Software for Windows, version 13.0.

3. RESULTS AND DISCUSSION

3.1 Optimization of the analytical method

In this section, an optimized method for the measurement of reduced and total GSH is studied using commercially available platinum screen printed electrodes. This method is based on the oxidation of thiols by iodine electrochemically generated from an aqueous iodide solution. The thiol chosen was GSH, as the level of this compound

in plants is much greater than others such as cysteine [7]. The chemical structures of the reduced and oxidized GSH are shown in Fig 1. GSH, in its reduced form, is a tripeptide consisting of L-cysteine, L-glutamate and glycine. Two glutathione molecules can oxidize to form a disulfide (GSSG) via the cysteine thiol groups.

The oxidation of 1 mM iodide to iodine was recorded at a platinum screen printed electrode (Fig. 2, green line). A well-defined quasi-reversible diffusional redox wave occurred at approximately 0.7 V (*vs* Ag). This voltammetric peak corresponds to the one-electron oxidation of the iodide ion to iodine. Despite the fact that only a single process was observed in the voltammetry (Fig. 2, green line), the iodide oxidation process is complicated due to the triiodide ion formation [36]. This compound is very likely to be in the aqueous solution, although in a smaller concentration than iodine (approximately 5 times less) [32]. The standard potentials for the oxidation of iodide to iodine and iodide to triiodide only differ by less than a millivolt [37]. Therefore, it cannot be ruled out that the triiodide, although likely present in lower concentration, was the active oxidizing species [32].

The aqueous iodide oxidation at a platinum electrode has been previously studied by laser-activated voltammetry [38] and the following mechanism was proposed:

$$2I^-(aq) \leftrightharpoons I_2(aq) + 2e^- \quad \textbf{(1)}$$

$$I_2(aq) + I^-(aq) \leftrightharpoons I_3^-(aq) \quad \textbf{(2)}$$

$$I_2(aq) \leftrightharpoons I_2(s) \quad \textbf{(3)}$$

$$I^-(aq) + I_2(s) \leftrightharpoons I_3^-(aq) \quad \textbf{(4)}$$

Voltammograms of GSH and GSSG in the absence of the iodide show that the direct electrochemistry of GSH and GSSG is disfavoured (Fig. 2, black and red dashed lines, respectively). The addition of GSSG to the iodide solution had not effect on the voltammetric response (Fig. 2, red solid line). However when GSH was added to the solution containing iodide, the voltammogram showed an increase in the forward peak (Fig. 2, blue solid line) and a decrease in the back peak characteristic of a catalytic (EC') process. Therefore it is possible to discriminate between GSH and the disulfide GSSG in the presence of iodide. This analysis is possible because iodine (electrochemically produced) under aqueous conditions is able to oxidize thiols to their corresponding disulfide by means of the next reaction [39].

$$2RSH + I_2 \xrightarrow{I^-} RSSR + 2HI \quad \textbf{(5)}$$

Cyclic voltammograms were measured using different concentrations of GSH from 10 to 250 μM (Fig. 3). The inset of Fig. 3 shows a linear relationship between the GSH concentration and the increase in current, ($Ip-Ip_0$ (μA) = (0.033 ± 0.001) [GSH] (μM), n=3). The limit of detection was determined to be 14.06 μM using 3σ/S, where σ is the standard deviation and S is the sensitivity of the assay.

The determination of total GSH requires the reduction of the disulfide bond with a reducing agent. Sodium borohydride, a strong reductant, was selected to reduce the oxidized GSSG due to its lack of electrochemical activity and inability to foul the electrode surface [4,24]. To be sure that the $NaBH_4$ treatment has no interference in the iodide/iodine voltammogram, an experiment was carried out with and without the addition of $NaBH_4$ (Fig. 4). It should be taken into account that when the base $NaBH_4$ is used, an important increment in pH occurs, which was corrected using the appropriate quantity of aqueous HCl. Fig. 4 shows that there was no change nor in the forward and neither in the backward peak (black and red solid lines). In addition, the electrochemical activity of $NaBH_4$ itself was discarded (black dashed line), making ~~it~~ possible the use of this reducing agent for the analysis.

Solutions with different GSSG concentrations were treated with $NaBH_4$ to produce GSH (Fig. 5). The Figure shows the voltammetric behavior of the system iodide/iodine using different concentrations of GSSG. It is shown that with each increasing concentration, the forward peak increases with a linear relationship ($Ip-Ip_0$ (μA) = (0.066 ± 0.002)[GS-] (μM), n=3), being [GS-] the concentration of GSH generated from GSSG. The detection limit was 11.09 μM (3σ/S). In addition, the same experiment was performed using GSH (instead GSSG) in the presence of the reducing agent and a similar slope was obtained (data not shown). Therefore the effectiveness of $NaBH_4$ for the reduction of GSSG to GSH is demonstrated and therefore, the tGSH quantification is possible. In this case, the sensitivity of the GSH formed from GSSG (for tGSH calculation) is higher in comparison to the sensitivity of the direct detection of the reduced form of GSH. This is most likely due to the neutralization process using HCl since pH may slightly be different (the solution was a little more alkaline than the $NaClO_4$ solution in the absence of the reducing agent). Anyway this does not affect the GSH quantification because the determination of reduced GSH and total GSH were made separately using their respective calibration plots.

Different solutions spiked with GSH and increasing concentrations of GSSG were prepared to check if the system is able to analyze the GSH and tGSH separately. Firstly the reduced GSH content was determined using the calibration plot obtained for GSH without $NaBH_4$ treatment. Subsequently, the mixed solution sample was treated with $NaBH_4$ and neutralized with HCl (pH 7) to determine the total GSH content using its respective calibration plot at pH 7. Figure 6 shows the comparison between the expected and measured values for reduced and total GSH in the different mixtures. The proposed method acceptably matches to the expected values with the average relative standard errors of 1.9 and 3.9 % for reduced and total GSH, respectively. Therefore the quantification and discrimination of these compounds can be performed using this analytical method.

3.2 Application of the method in the measurement of reduced and total GSH in plant extracts (from pea leaves).

One of the most important interests of electrochemical methods is the obtainment of micro-molar limits of detection in complex physiological and biological

matrices [15]. For that reason, the aim of this work was to apply the method previously optimized in a real sample. Plant extracts obtained from pea leaves were analyzed as a proof-of-concept by the developed methodology and validated with a traditional spectrophotometric method.

Fig. 7 shows the voltammograms of a plant extract (40% in $NaClO_4$) in the absence and presence of 1 mM NaI. The sample revealed some electroactive species, possibly ascorbate and/or other antioxidant compounds, but the voltammetric signal of NaI was considered enough to be used in GSH quantification by the standard additions method.

To check the applicability of the sensor in plant real samples, the proposed method was applied to the determination of GSH and tGSH in two plant extracts from pea leaves. Fig. 8 shows an example in which the addition of increasing GSH concentrations leads to an increment in the anodic peak current, making possible the analysis. Standard additions of GSH were carried out without (Fig. 8A) and with (Fig. 8B) previous $NaBH_4$ treatment to determine the GSH and tGSH respectively.

The concentrations calculated by the standard additions method for the two plant extracts were compared to those obtained by the classic spectrophotometric method with DTNB and GR (reference method) and the results are shown in Table 1. The ratio GSSG/tGSH was calculated by the two methods and a relative standard deviation (RSD) < 7.5 % was obtained, revealing a good precision of the electrochemical method.

Table 1. Reduced and total GSH in pea leaves extracts by the electrochemical and spectrophotometric (reference) method.

	Electrochemical method			**Spectrophotometric method**		
Sample	**Reduced GSH (nmoles/gFW)**	**Total GSH (nmoles/gFW)**	**GSSG/total GSH**	**Reduced GSH (nmoles/gFW)**	**Total GSH (nmoles/gFW)**	**GSSG/total GSH**
1	114.6±13.8	139.5 ±9.3	0.09	95.7 ± 17.4	119.4±18.6	0.10
2	71.1±4.5	113.7±22.5	0.19	63.0± 5.1	107.7±11.4	0.20

The determination of thiols concentration in plant extracts, particularly GSH, can be used to quantify the plant stress response to environmental factors. From data shown in Table 1 we can conclude that the sample 1 was affected by a lower oxidative stress level than sample 2 since its GSSG/total GSH ratio was lower. On the other hand, sample 1 revealed a higher content of both reduced and total GSH that probably could be related with a better protection of the plant against damaging factors.

4. Conclusions

Herein a simple, fast and easy method for determining reduced and total GSH is described. The method exploits the redox reaction between the electrochemically generated iodine with thiols in their reduced state. The fact that iodine is not able to react with disulfides allows the discrimination between the reduced GSH and the oxidized species GSSG. For total GSH, sodium borohydride was used as a reducing agent. Cyclic voltammetry and commercially available screen-printed electrodes (using Pt as a working electrode) were used for these studies. The sensitivity of the sensor was (0.033±0.001) μA/μM for reduced GSH and (0.066± 0.001) μA/μM for total GSH, with a limit detection of 14.06 μM and 11.09 μM respectively. The applicability of the methodology here proposed was tested with real plant extracts samples as a proof-of-concept and validated with a spectrophotometric method, obtaining similar results in the oxidized GSSG/tGSH ratio. Since the analysis of thiols is a potential marker for the evaluation of oxidative stress in plants, this approach could contribute to the monitoring of plant metabolism under normal and oxidative stress conditions.

Acknowledgements

This work was funded by the Spanish Ministry of Economy and Competitiveness (MINECO, http://www.mineco.gob.es/portal/site/mineco/idi), Project No. BFU2013-44095-P (cofunded with FEDER funds, EU). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Figure captions

Figure 1. Chemical structures of reduced and oxidized glutathione.

Figure 2. Voltammetric response of 0.2 M $NaClO_4$ (black solid line), 100 μM GSH (red dashed line), 100 μM GSSG (black dashed line), 1 mM NaI (green solid line), 1 mM NaI in the presence of 100 μM GSH (blue solid line) and 1 mM NaI in the presence of 100 μM GSSG (red solid line). All solutions were prepared in 0.2 M $NaClO_4$. The scan rate was 100 mVs^{-1}.

Figure 3. Voltammetry (scan rate=100 mVs^{-1}) of 1 mM NaI in the absence (red line) and presence of increasing concentrations of GSH (10, 20, 30, 40, 50, 100, 150, 200 and 250 μM, black lines). Inset: Peak current increment *vs* GSH concentration, where Ip_0 is the oxidative peak height of the iodide in the absence of GSH.

Figure 4. Cyclic voltammetry (scan rate=100 mVs^{-1}) at Pt screen printed electrodes: 2% $NaBH_4$ (black dashed line), 1 mM NaI without the $NaBH_4$ treatment (red solid line) and 1 mM NaI after the $NaBH_4$ treatment (black solid line). All solutions containing $NaBH_4$ were neutralized with appropriate volumes of HCl 1M.

Figure 5. Cyclic voltammetry (scan rate=100 mVs^{-1}) at Pt electrode of 1 mM NaI with different concentrations of GSH from GSSG after the $NaBH_4$ treatment. Inset: Peak current increment *vs* total GS- concentration where Ip_0 is the oxidative peak height of the iodide in the absence of GSSG.

Figure 6. Expected and experimental values obtained of reduced and total GSH at different prepared solutions (n=3).

Figure 7. Cyclic voltammetry (scan rate=100 mVs^{-1}) of a plant extract sample solution (40% in $NaClO_4$, red line) and the same sample solution containing 1 mM NaI (black line).

Figure 8. Cyclic voltammograms (scan rate=100 mVs^{-1}) of 40 % plant extract (in $NaClO_4$) with 1 mM NaI after the addition of 0, 10, 20, 30, 40, 50 and 75 μM of GSH: A) without $NaBH_4$ treatment to measure only the reduced GSH and B) with $NaBH_4$ treatment to measure total GSH.

References

1. Tausz M, Gullner G, Komives T, Grill D. The role of thiols in plant adaptation to environmental stress. In: Abrol YP, Ahmad A, eds. *Shulfur in plants*: Springer; 2003:221-244.

2. Zagorchev L, Seal CE, Kranner I, Odjakova M. A Central Role for Thiols in Plant Tolerance to Abiotic Stress. *International Journal of Molecular Sciences.* Apr 2013;14(4):7405-7432.

3. Kruusma J, Benham AM, Williams JAG, Kataky R. An introduction to thiol redox proteins in the endoplasmic reticulum and a review of current electrochemical methods of detection of thiols. *Analyst.* 2006 2006;131(4):459-473.

4. Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clinica Chimica Acta.* Jul 2003;333(1):19-39.

5. Valero E, Gonzalez-Sanchez MI, Macia H, Garcia-Carmona F. Computer Simulation of the Dynamic Behavior of the Glutathione-Ascorbate Redox Cycle in Chloroplasts. *Plant Physiology.* APR 2009 2009;149(4):1958-1969.

6. Valero E, Macia H, De la Fuente IM, Hernandez J-A, Gonzalez-Sanchez M-I, Garcia-Carmona F. Modeling the ascorbate-glutathione cycle in chloroplasts under light/dark conditions. *Bmc Systems Biology.* Jan 22 2016;10.

7. Noctor G, Mhamdi A, Chaouch S, et al. Glutathione in plants: an integrated overview. *Plant Cell and Environment.* Feb 2012;35(2):454-484.

8. Rellan-Alvarez R, Hernandez LE, Abadia J, Alvarez-Fernandez A. Direct and simultaneous determination of reduced and oxidized glutathione and homoglutathione by liquid chromatographyelectrospray/mass spectrometry in plant tissue extracts. *Analytical Biochemistry.* Sep 15 2006;356(2):254-264.

9. Diopan V, Shestivska V, Zitka O, et al. Determination of Plant Thiols by Liquid Chromatography Coupled with Coulometric and Amperometric Detection in Lettuce Treated by Lead(II) Ions. *Electroanalysis.* Jun 2010;22(11):1248-1259.

10. Chwatko G, Kuzniak E, Kubalczyk P, Borowczyk K, Wyszczelska-Rokiel M, Glowacki R. Determination of cysteine and glutathione in cucumber leaves by HPLC with UV detection. *Analytical Methods.* 2014 2014;6(19):8039-8044.

11. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem.* Jul 15 1980;106(1):207-212.

12. Cappiello M, Peroni E, Lepore A, et al. Rapid colorimetric determination of reduced and oxidized glutathione using an end point coupled enzymatic assay. *Analytical and Bioanalytical Chemistry.* Feb 2013;405(5):1779-1785.

13. Chen CY, Liu W, Xu C, Liu WS. A colorimetric and fluorescent probe for detecting intracellular GSH. *Biosensors & Bioelectronics.* Sep 2015;71:68-74.

14. MacCoss MJ, Fukagawa NK, Matthews DE. Measurement of homocysteine concentrations and stable isotope tracer enrichments in human plasma. *Analytical Chemistry.* Oct 1999;71(20):4527-4533.

15. Harfield JC, Batchelor-McAuley C, Compton RG. Electrochemical determination of glutathione: a review. *Analyst.* 2012;137(10):2285-2296.

16. Lawrence NS, Beckett EL, Davis J, Compton RG. Advances in the voltammetric analysis of small biologically relevant compounds. *Analytical Biochemistry.* Apr 2002;303(1):1-16.

17. Lowinsohn D, Lee PT, Compton RG. Towards Detection of Total Antioxidant Concentrations of Glutathione, Cysteine, Homocysteine and Ascorbic Acid Using a Nanocarbon Paste Electrode. *International Journal of Electrochemical Science.* Jul 2014;9(7):3458-3472.

18. Lowinsohn D, Lee PT, Compton RG. Electrocatalytic Detection of Glutathione - the Search for New Mediators. *Journal of the Brazilian Chemical Society.* Sep 2014;25(9):1614-1620.
19. Lee PT, Lowinsohn D, Compton RG. The Use of Screen-Printed Electrodes in a Proof of Concept Electrochemical Estimation of Homocysteine and Glutathione in the Presence of Cysteine Using Catechol. *Sensors.* Jun 2014;14(6):10395-10411.
20. Lee PT, Lowinsohn D, Compton RG. Simultaneous Detection of Homocysteine and Cysteine in the Presence of Ascorbic Acid and Glutathione Using a Nanocarbon Modified Electrode. *Electroanalysis.* Jul 2014;26(7):1488-1496.
21. Lawrence NS, Davis J, Compton RG. Electrochemical detection of thiols in biological media. *Talanta.* Jan 2001;53(5):1089-1094.
22. Lee PT, Compton RG. Selective Thiol Detection in Authentic Biological Samples with the Use of Screen-printed Electrodes. *Analytical Sciences.* Jul 2015;31(7):685-691.
23. Lee PT, Compton RG. Selective electrochemical detection of thiol biomarkers in saliva using multiwalled carbon nanotube screen-printed electrodes. *Sensors and Actuators B-Chemical.* Mar 31 2015;209:983-988.
24. Lee PT, Goncalves LM, Compton RG. Electrochemical determination of free and total glutathione in human saliva samples. *Sensors and Actuators B-Chemical.* Dec 2015;221:962-968.
25. Gonzalez-Sanchez MI, Gonzalez-Macia L, Perez-Prior MT, Valero E, Hancock J, Killard AJ. Electrochemical detection of extracellular hydrogen peroxide in Arabidopsis thaliana: a real-time marker of oxidative stress. *Plant Cell and Environment.* APR 2013 2013;36(4):869-878.
26. Gonzalez-Sanchez MI, Lee PT, Guy RH, Compton RG. In situ detection of salicylate in Ocimum basilicum plant leaves via reverse iontophoresis. *Chemical communications (Cambridge, England).* 2015-Nov-5 2015;51(92):16534-16536.
27. Supalkova V, Huska D, Diopan V, et al. Electroanalysis of plant thiols. *Sensors.* Jun 2007;7(6):932-959.
28. Ryant P, Dolezelova E, Fabrik I, et al. Electrochemical determination of low molecular mass thiols content in potatoes (Solanum tuberosum) cultivated in the presence of various sulphur forms and infected by late blight (Phytophora infestans). *Sensors.* May 2008;8(5):3165-3182.
29. Tai CY, Ho CT. Influence of glutathione oxidation and pH on thermal formation of Maillard-type volatile compounds. *Journal of Agricultural and Food Chemistry.* Jun 1998;46(6):2260-2265.
30. Danehy JP, Doherty BT, Egan CP. The oxidation of organic divalent sulfur by iodine. The equilibrating thiol-iodine-disulfide-hydrogen iodide system in acetic acid and evidence for sulfenyl iodide intermediates. *The Journal of Organic Chemistry.* 1971;36:2525-&.
31. S. O, J. D. Organic Sulfur Chemistry: Structure and Mechanism: Taylor & Francis 1991.
32. Valero-Ruiz E, Gonzalez-Sanchez MI, Batchelor-McAuley C, Compton RG. Halogen mediated voltammetric oxidation of biological thiols and disulfides. *Analyst.* 2016 2016;141(1):144-149.
33. Hall EM, Tschulik K, Batchelor-McAuley C, Compton RG. Electrochemical bromination of organosulfur containing species for the determination of the strength of garlic (A. sativum). *Food chemistry.* 2016-May-15 2016;199:817-821.
34. Martindale BCM, Aldous L, Rees NV, Compton RG. Towards the electrochemical quantification of the strength of garlic. *Analyst.* 2011 2011;136(1):128-133.
35. Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Applications to mammalian blood and other tissues. *Analytical Biochemistry.* 1969;27(3):502-522.
36. Bentley CL, Bond AM, Hollenkamp AF, Mahon PJ, Zhang J. Voltammetric Determination of the Iodide/Iodine Formal Potential and Triiodide Stability Constant in Conventional

and Ionic Liquid Media. *Journal of Physical Chemistry C.* Oct 2015;119(39):22392-22403.

37. Bard AJ, Parsons R, Jordan J. *Standard potentials in aqueous solution*: CRC Press; 1985.

38. Akkermans RP, Qiu FL, Roberts SL, Suarez MF, Compton RG. Laser-activated voltammetry. Mechanism of aqueous iodide oxidation at platinum electrodes: Theory and experiment. *Journal of Physical Chemistry B.* Sep 30 1999;103(39):8319-8327.

39. Danehy JP, Doherty BT, Egan CP. The oxidation of organic divalent sulfur by iodine. The equilibrating thiol-iodine-disulfide-hydrogen iodide system in acetic acid and evidence for sulfenyl iodide intermediates.1971, The Journal of Organic Chemistry.