

**Electrochemical Detection and Quantification of Gingerol Species in Ginger
(*Zingiber officinale*) using Multiwalled Carbon Nanotube Modified
Electrodes**

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Abstract

We demonstrate the potential of electrochemical detection for the analysis of the 'strength' of ginger in ginger sample. This facile and fast detection method is aimed at the quality control in food industry. Specifically, we report adsorptive stripping voltammetry (AdsSV) as a technique for detection of gingerol compounds, the pungent components of ginger rhizome. Among the gingerols, 6-gingerol is the most abundant and is chosen as a model to characterise the behaviour of a wider range of related compounds. Multiwalled carbon nanotube modified basal plane pyrolytic graphite electrodes (MWCNT-BPPG electrode) are employed to enhance the sensitivity of the measurement. A linearity range from 1 μM to 50 μM with limit of detection of 0.21 μM and limit of quantification of 0.71 μM is obtained.

Further, the simple and rapid extraction procedure by simply vortexing the ginger sample with ethanol is developed for extraction of gingerol related species.

1. Introduction

Zingiber officinale (ginger) is widely used around the world as a spice. This culinary product contains several different pungent and active ingredients; predominantly these are gingerol, shogaol, zingerone, and paradol which have the chemical structures shown in Fig. 1.¹ These compounds all contain a 2-methoxyphenol group and differ only in the structure of their side chain. It is this aromatic group of species that have been reported to be the most pungent components in fresh ginger rhizome.² Of these 6-gingerol (compound A in Scheme 1) was identified as the most abundant (50-70% relative abundance with [8]- and [10]-gingerol).^{3, 4} Shogaol, a dehydrated form of gingerol mostly found in a dried or cooked ginger, is reportedly 'spicier' than gingerol (160,000 vs 80,000 Scoville Heat Units respectively).⁵ However, fresh ginger contains much more gingerol than the other pungent compounds⁶ and as such is used in this work as a model compound for the analysis of the 'strength' of ginger.

Beyond culinary uses gingerols have been shown to possess a variety of biological properties that can be beneficial for human health. They exhibit anti-inflammatory^{7, 8}, anti-platelet⁹, anti-tumor¹⁰, and high antioxidant properties.^{11, 12} In addition, these active compounds have the possibility for use in cardiovascular disease treatment¹³, and the prevention of nausea and vomiting during pregnancy and chemotherapy.¹⁴⁻¹⁶

The concentration of gingerol in ginger products has been shown to be related to the pungency level of ginger.⁶ The degree of pungency is an important determinant of the value of the ginger products. However, the concentration of gingerol is found to vary with growing

conditions, harvesting, processing of the ginger rhizome, and the extraction methodology.¹⁷ From the reported literature, the concentration of gingerol related species in a variety of ginger samples (fresh ginger, ginger dietary supplements, ginger powder, and ginger beverage) is normally found between the regions of *ca.* 0.20 – 15.92 mg g⁻¹.^{18,19}

Owing to their culinary and medicinal importance, a simple and accurate determination method of gingerol is desirable in the food industry. Hitherto, the major technique utilised for determine the concentration of gingerol is chromatography.¹⁹⁻²⁴ Gas chromatography (GC) has been used to analyse gingerol.^{22, 23} Nonetheless, GC showed several disadvantages for analysing gingerol for instance the temperatures in the GC column have been shown to result in significant conversion of gingerol to shogaol because of its thermal instability which limits the application of this method.²¹ High-performance liquid chromatography (HPLC) is by far the most popular technique to detect gingerol. However, the HPLC method is limited in terms of the high cost of the instrumentation and extensive sample preparation method rendering the method inapplicable for use by non-skilled personnel and also time consuming for frequent commercial use.

To address these problems of high cost instrumentation, complex sample preparation and to facilitate analysis by non-trained operators, we report a simple and sensitive electroanalytical detection of gingerol and related species at a multiwalled carbon nanotube modified basal-plane pyrolytic graphite electrode (MWCNT-BPPG electrode) using adsorptive stripping voltammetry (AdsSV). The adsorptive stripping voltammetry technique involves the accumulation of target compound(s) on the electrode surface. The electrochemical response obtained is highly dependent on the area of the electrode surface. Therefore, carbon nanotubes are chosen to modify the electrode due to their inherent high surface area (200 - 400 m² g⁻¹)²⁵ which significantly increases the AdsSV response. In

addition, we study real samples and develop a simple preparation method. Finally, we demonstrate the capability of the electrochemical detection to quantify gingerol related species in crushed ginger samples.

2. Experimental

2.1 Chemicals and reagents

All chemicals were of analytical grade and were used as received without further purification. 6-gingerol was purchased from Carbosynth (98.9% purity, Berkshire, UK). The bamboo-like multiwalled carbon nanotubes, b-MWCNTs (30±10 nm diameter, 5-20 µm length, >95% purity) were purchased from NanoLab (Brighton, MA, USA). All solutions were prepared with ultrapure water at resistivity not less than 18.2 MΩ cm at 298K (Millipore, USA). TESCO Ingredients Crushed Ginger was purchased from Tesco (Oxford, UK). The Britton-Robinson buffer, 0.05 M at pH 1.8, was prepared using boric acid (99.5% purity, Aldrich), phosphoric acid (99.99% purity, Aldrich) and acetic acid (99.5% purity, BDH).

2.2 Apparatus

All electrochemical experiments were performed using a three-electrode system in a Faraday cage held at 25 °C with a potentiostat, PGSTAT 101 (Metrohm-Autolab, Netherlands). A basal-plane pyrolytic graphite (BPPG) electrode was produced from pieces of the highly ordered pyrolytic graphite (HOPG) (Le Carbone, Sussex, UK). The electrode was set in an insulating PTFE housing with an electrical connection made using a stainless steel core. The BPPG electrode is used as a substrate for the modified electrode discussed below. The electrochemical cell was completed using a saturated calomel electrode, SCE, as the

reference electrode (SCE +0.244 V vs SHE, BASi Inc., Japan) and a platinum mesh 99.99% (Goodfellow, UK) as the counter electrode.

The MWCNT modification of BPPG electrode was prepared using the drop casting method. The bamboo-like multiwalled carbon nanotubes were first dispersed in acetone (1 mg mL⁻¹). The solution was then sonicated in an ultrasonic bath for 10 minutes prior to drop casting. A 20 µL volume was drop-cast onto the BPPG electrode surface. This amount of MWCNT suspension yields the surface coverage which gives sufficiently enhanced electrochemical responses for detection of micromolar levels using AdsSV.²⁶ Evaporation of the acetone at room temperature then resulted in a nanotube modified electrode.^{26, 27} The MWCNT-BPPG electrode was gently rinsed with acetone after each voltammetric scan prior to repeated use of the same drop-cast electrode for further scans. Electrode refreshed in the manner could be used for 10-15 times before having to be replaced by a new drop cast. BPPG electrode surfaces were prepared by polishing the electrode surface on sand paper P2500 and P4000 grade respectively and then repeatedly pressing with cello tape (Henkel AG, Dusseldorf, Germany). The electrode was subsequently rinsed with acetone.²⁸

2.3 Sample preparation

To facilitate analysis of real samples, gingerol species were extracted from commercially available crushed ginger. 1.0 g of sample was weighed (unless otherwise stated) and placed into a 15 mL centrifuge tube. 5 mL of ethanol was added as a solvent and the sample was vigorously shaken by a vortex mixer for 1 minute. The tube was then sonicated in the range of 0 – 20 minutes by placed in an ultrasonic bath, and then the mixture was centrifuged at 4000 rpm for 10 minutes to remove solids. The supernatant was carefully collected.

2.4 Analytical procedure

A stock standard solution of 6-gingerol (2 mM) was prepared before diluting with a 0.05 M Britton-Robinson buffer at pH 1.8 to concentration of 1, 5, 10, 15, 20, 25, 30, and 50 μM as standard working solutions.

For voltammetric characterisation experiments using the MWCNT-BPPG electrode, the electrode was immersed in a solution containing the 50 μM 6-gingerol in a 0.05 M Britton-Robinson buffer at pH 1.8. In order to adsorb the 6-gingerol onto the electrode surface, the electrode was held at an open circuit condition for one minute in the well-stirred solution. The electrode was then gently rinsed with deionised water and transferred to the blank Britton-Robinson buffer solution at pH 1.8 for voltammetric analysis.

In order to investigate the influence of the scan rate on the voltammetric response of the gingerol, a 100 μM solution of 6-gingerol was used and analysed using the same procedure as discussed above. Note that, as will be seen later, for the voltammetric analysis two (or more) voltammetric cycles were performed as there is irreversible chemical change induced as a result of the fast scan, leading to reversible, and reproducible voltammetry on the second and subsequent scans.

For real sample analysis, extracts were diluted with 0.05 M Britton-Robinson buffer solution at pH 1.8 until the voltammetric response fell within the linear detection range (the dilution factors used were 25, 50, 100 and 200 for 0.25 g, 0.50 g, 0.75 and 1.0 g, and 2.0 g respectively). The detection of gingerol in the real sample was carried out by a standard addition method. The final standard solution concentrations of 6-gingerol in the diluted real sample solutions is 5, 10, 15, and 20 μM . The electrode was immersed in the solution and

allowed to accumulate under stirring for one minute at the open circuit potential. The voltammetric analysis of the real sample was then recorded at a scan rate of 100 mV s^{-1} .

3. Results and discussion

In the following, first, the voltammetric response of 6-gingerol is investigated and characterised in Section 3.1. As discussed previously 6-gingerol is the predominant aromatic compound present in ginger providing its pungency and is used here as a model compound to characterise the behaviour of the wider family of species present in real samples containing the 2-methoxyphenol moiety. Second, following the initial investigation of the electrochemical behaviour, in Section 3.2, the electrochemical redox response is evidenced to proceed via surface controlled processes. Third, in Section 3.3, the accumulation time is optimised and the adsorption of 6-gingerol on the electrode surface is investigated. Next, the analytical performance of the modified electrode towards 6-gingerol is studied (Section 3.4). Finally in Section 3.5, the extraction procedure of 6-gingerol from a real sample is optimised, followed by the use of the developed electrochemical detection to quantify the 6-gingerol and other structurally related 2-methoxyphenol content in a real sample.

3.1 Voltammetric characterisation

The cyclic voltammetric response and electroactivity of 6-gingerol was initially explored at a bare BPPG electrode. In order to characterise the voltammetric profiles, $50 \text{ }\mu\text{M}$ of 6-gingerol was adsorbed on the bare-BPPG electrode under open circuit conditions for one minute. Subsequently the oxidative voltammetric response of the electrode was recorded. The oxidative scan was started at 0.0 V and was initially scanned anodically to $+1.2 \text{ V}$ at which point the scan direction was subsequently reversed and the potential

returned to 0.0 V. After the first scan (dashed line) a second (solid line) voltammetric cycle was recorded, the results of which are shown in Fig. 2.

On scanning the first oxidative scan (dashed line) of the bare BPPG electrode a clearly defined peak is observed a peak at +0.68 V vs. SCE with peak current *ca.* 0.94 μ A labelled as peak I. In the absence of the adsorption of 6-gingerol no voltammetric peak was observed as shown in the inlay of Fig. 2(a). Upon reversing the scan direction from +1.2 V to 0.0 V, a reductive peak is observed at +0.37 V (peak II) with a peak current of *ca.* 0.44 μ A. On the second scan a new oxidative peak at +0.43 V is observed and labelled peak II' and a corresponding reductive peak recorded at +0.37 V with peak currents of 0.42 μ A and 0.44 μ A respectively. Notably on the second scan peak I is found to be absent. The voltammetric wave shapes associated with the redox processes indicates that the electroactive species and intermediates are surface bound; this will be further evidenced later in the paper.

In order to seek to enhance the sensitivity of the voltammetric response towards the presence of gingerol, a BPPG modified electrode with multiwalled carbon nanotubes (MWCNT-BPPG) was investigated. The modified electrode was immersed into a 50 μ M of 6-gingerol under open circuit conditions for one minute to allow the adsorption of 6-gingerol on the electrode surface. The voltammetric analysis was then recorded at a scan rate of 100 mV s⁻¹. The results shown in Fig. 2(b) illustrate qualitatively similar cyclic voltammograms, however the magnitude of the voltammetric responses is very significantly enhanced as compared to the bare electrode. As measured from the peak currents of the peaks I, II, and II', the voltammetric signal on the MWCNTs is approximately 200 times larger. This distinct enhancement of peak heights when using the MWCNT-BPPG electrode is ascribed to the larger surface area of the modified BPPG electrode with MWCNT. The surface area of the modified electrode which was *ca.* 20 cm² as estimated from the amount of MWCNTs

dropped on the BPPG electrode (see section S1, supplementary information) while the surface area of bare electrode was *ca.* 0.16 cm² which gives a ratio of surface areas of the same order of magnitude as the observed sensitivity difference of *ca.* 200. Consequently, all further experiments are reported on the MWCNT-BPPG electrode.

Turning attention to the electrochemical oxidation/reduction mechanism of 6-gingerol, the first cycle shows oxidation peak I at +0.68 V. In light of the literature on the oxidation of 2-methoxyphenol moieties²⁹, this peak is ascribed as being due to the oxidation of the 2-methoxyphenol substructure in the 6-gingerol (compound A in Scheme 1). On the reverse scan of the first cycle, the reduction peak II was observed at +0.37 V which results from electron transfer to the product from the chemically irreversible hydrolysis of the intermediate cation (compound B in Scheme 1) which forms an o-benzoquinone moiety (compound C in Scheme 1).^{29, 30} After the first scan, peak I is no longer observable. Accordingly on the second scan, the reversible redox reaction (at +0.43 V and +0.37 V for oxidative and reductive peaks respectively) was exclusively seen and corresponds to the reversible redox process of o-benzoquinone and catechol (compounds C and D in Scheme 1) couple. This process is shown in the voltammograms as peaks II and II'.^{31, 32} The proposed mechanism is illustrated and summarised in Scheme 1. The above electrochemical results closely mirror those found for the electrooxidation of capsaicin. Capsaicin is closely structurally related to 6-gingerol and the electrochemical response has been previously reported by Kachoosangi *et al.*²⁶ However a notable contrast between the voltammetric responses of the two species arises upon consideration of the magnitudes of peak I and II as discussed below.

The charge transferred during the voltammetry of 6-gingerol was studied by integration of the peak areas of peaks I and II from the adsorptive stripping voltammogram

of 50 μM 6-gingerol measured after a 1 minute accumulation time. The charge transferred in peaks I and II was *ca.* 204 μC and 56 μC respectively. Hence, the ratio of peak I and II was found experimentally to be approximately 3:1. To enable direct comparison of these results with the electrochemical response of capsaicin, we conducted analogous experiments to those described above with capsaicin (data not shown). In contrast, the peak areas for peaks I and II from redox reaction of capsaicin were found to be close to unity *ca.* 116 μC for peak I and *ca.* 101 μC for peak II. These results indicate that in the case of adsorbed capsaicin the oxidative product of peak I is almost completely converted to the o-quinone species. Comparatively, from the result of 6-gingerol, we conclude that upon formation of the cation (compound B in Scheme 1) a second competing pathway exists in parallel to the irreversible hydrolysis reaction leading to the formation of the o-benzoquinone moiety. Moreover the products of this competing pathway are not electroactive in the electrochemical window being recorded since no other voltammetric peaks are observed experimentally. This second competing pathway included in the mechanism shown in Scheme 1.

3.2 Effect of voltage scan rate

The voltammetric method used in this work is adsorptive stripping voltammetry; this technique involves the accumulation of target analyte(s) on the electrode surface prior to conducting a voltammetric stripping analytical procedure. To confirm that the 6-gingerol is adsorbed on the MWCNT-BPPG electrode, the voltammetric response of the peaks II and II' were studied as a function of scan rate. The cyclic voltammograms were recorded in a 0.05 M Britton-Robinson buffer solution pH 1.8 at scan rates between 0.01 – 0.8 V s^{-1} . Note that before conducting this experiment the first scan was performed in each case in order to generate the redox couple prior to record the second voltammetric cycle. As shown in Fig. 3,

the wave shape of second scans is almost constant; however, at higher scan rates the peak-to-peak potential separation is larger. The magnitudes of the oxidative and reductive peak currents of 6-gingerol exhibit distinct deviations from linearity as plotted against the scan rate (Fig. 3 inlay). Linearity of a voltammetric peak current with scan rate is commonly used as diagnostic for the presence of a surface bound redox process. In the present case the deviation away from linearity likely predominantly reflects the non-idealities of the carbon nanotube modified electrode. In particular, the large capacitance of the substrate (arising from the large area) serves to distort the voltammetric response as partially reflected in the larger peak-to-peak separation observed at higher scan rates. It should be noted that integration of the redox peaks reveal the charge passed to be constant as a function of scan rate thus evidencing the surface bound nature of the redox couple.³³

3.3 Effect of accumulation time

Having studied the voltammetric response of the model compound 6-gingerol, here the accumulation time is optimised and the adsorption of 6-gingerol on the electrode surface is investigated. In order to optimise the accumulation time, we performed adsorptive stripping voltammetry of 6-gingerol in the range of 10 – 100 μM at various accumulation times. The physical reasoning for the use of these different concentrations will be discussed below. The electrode was immersed in the solutions of varying 6-gingerol concentrations with accumulation times ranging from 0 to 12 minutes at an open circuit potential in the well-stirred solution. After adsorption of the analyte onto the electrode surface, the electrode was rinsed with deionised water and transferred to an electrochemical cell containing 0.05 M Britton-Robinson buffer solution pH 1.8 for voltammetric interrogation.

Fig. 4(a) shows the plot of the responses of oxidative peak I as a function of time of adsorption 6-gingerol on the electrode surface. Considering the result from 10 μ M 6-gingerol, on increasing the accumulation time from 0 to 5 minutes, the signal enhanced significantly. At longer adsorption times the peak area increases more slowly and reaches a plateau at *ca.* 8 minutes. The magnitude of this plateau region is sensitive to the concentration of gingerol in solution. Accordingly we conclude that at long adsorption times (>8 minutes) the extent of adsorption of gingerol on electrode surface is thermodynamically and not kinetically limited. As can be seen from the result, an accumulation time for 8 minutes would yield the highest sensitivity towards the presence of gingerol. However, in terms of facilitating the analytical procedure, a 1 minute accumulation time was selected for the further experiments as a compromise between a shorter analysis time and the sensitivity of the procedure.

Next the adsorption of 6-gingerol is analysed in terms of the Langmuir isotherm. The equilibrated surface coverage of the 6-gingerol is measured after 8 minutes accumulation. Using the Langmuir adsorption isotherm, the fractional occupancy of the adsorption sites can be expressed as

$$\theta = \frac{B[C]}{1+B[C]} \quad (1)$$

where B is the equilibrium constant which is $\frac{k_{ads}}{k_{des}}$ where k_{ads} and k_{des} are rate constant of adsorption and desorption respectively, and [C] is the concentration of 6-gingerol.³⁴ Derivation of the equation (1) gives rise to the following equation which maybe expressed as (see S2, supplementary information)

$$\frac{1}{Q} = \left(\frac{1}{BQ_{max}} \right) \left(\frac{1}{[C]} \right) + \frac{1}{Q_{max}} \quad (2)$$

where Q is the voltammetrically measured charge transferred during oxidation of the species at the concentration studied and Q_{\max} is maximum charge transfer at saturation of the surface with gingerol.

The Langmuir isotherm predicts that a plot of $1/Q$ vs. $1/[C]$ should be linear. From this double reciprocal plot, the equilibrium constant and maximum charge transfer can be obtained from the slope and intercept respectively. As shown in Fig 4(b), the plot is linear ($R^2 = 0.990$) and the equilibrium constant and maximum charge transfer can be calculated *ca.* $0.023 \mu\text{M}^{-1}$ and $1035 \mu\text{C}$ respectively. Hence we conclude that the adsorption of gingerol is well-described by the Langmuir isotherm. The value of surface coverage (Γ) of the gingerol can be calculated using Faraday 1st law $\Gamma = Q/nFA$, where Q is the maximum charge transfer, n is the number of electrons ($n=2$), F is Faraday constant (96485 C mol^{-1}), and A is the surface area of the modified BPPG electrode (*ca.* 20 cm^2 , refer to section S1 supplementary information). The surface coverage was calculated to be *ca.* $2.7 \times 10^{-10} \text{ mol cm}^{-2}$ (or $1.6 \times 10^{14} \text{ molecules cm}^{-2}$) which is fully consistent with monolayer formation of 6-gingerol on the MWCNT-BPPG electrode surface.³⁵

In the study of accumulation time, we used the different concentrations of 6-gingerol. From the experimental Langmuir plot, the charge transfer corresponding maximum surface coverage is calculated to be *ca.* $1000 \mu\text{C}$. From the charge transfer obtained from $100 \mu\text{M}$ 6-gingerol, the value of *ca.* $700 \mu\text{C}$ suggests that we achieve almost the maximum coverage which expected to make the surface of electrode become saturated at the higher concentration. Therefore, the analytical range studied needs to be below $100 \mu\text{M}$.

3.4 Analytical response of MWCNT-BPPG electrode

308 Having investigated the adsorption of the model compound 6-gingerol we turn next
309 to investigation the analytical response and analytical performance of the modified
310 electrode towards 6-gingerol. With regard to study the analytical performance of the
311 MWCNT-BPPG electrode towards 6-gingerol, we further performed the adsorptive stripping
312 voltammetry of 6-gingerol of various concentrations. The experiment was conducted by first
313 immersing the MWCNT-BPPG electrode in standard solutions of 6-gingerol with
314 concentrations in the range 1 – 50 μM using the optimised 1 minute accumulation time at
315 an open circuit potential with stirring. After adsorption of the analyte, the same procedure
316 as above was used before commencing the voltammetric scanning step. The cyclic
317 voltammetric response as a function of the concentration of the 6-gingerol species is
318 depicted in Fig. 5. Notably, the small peak II' present on the overlaid voltammograms
319 appears due to the repeated use of the electrode without renewal of the drop-cast carbon
320 nanotubes. This repeated use of the electrode leads to the presence of the adsorbed o-
321 benzoquinone and catechol couple from the previous experiment. In order to investigate
322 the effect of repeatedly using the same MWCNT-BPPG electrode, the adsorptive stripping
323 voltammetry of μM 6-gingerol obtained using a fresh modified electrode was compared to
324 that obtained using the electrode which has been used for three voltammetric scans. The
325 peak areas of the oxidative peak I were investigated and compared (data not shown). A *ca.*
326 5% difference in the charge transferred was observed. Therefore, the result indicated that
327 there is no significant effect on the charge transferred of peak I that we use to quantify
328 gingerol and related species. The inset depicts the calibration plot which is using the
329 response of oxidative peak I of the first scan. The MWCNT-BPPG electrode reveals a linearity
330 range of 1 - 50 μM ($Q/\mu\text{C} = 2.97 \pm 0.07[6\text{-gingerol}] (\mu\text{M}) - 1.05 \pm 0.67 (\mu\text{C})$, $N=8$, $R^2 = 0.995$).
331 Using this procedure the limit of detection and limit of quantification were 0.21 and 0.71

μM respectively. The limit of detection was calculated based on $3\sigma/s$ and the limit of quantification was calculated based on $10\sigma/s$ where σ is the standard deviation and s is the sensitivity of the calibration curve.³⁶

3.5 Analysis of a real sample on MWCNT-BPPG electrode

In order to assess the applicability of the method for the determination and quantification of the presence of the gingerol related species in a real sample, we first investigated the method for extraction of the analytes from a sample of ginger purchased from a local supermarket. In recent years, there have been various reports on extraction methods to obtain gingerol from ginger such as reflux³⁷, high-pressure Soxhlet extraction³⁸,³⁹ and ultrasonic extraction.³⁹⁻⁴¹ Among these mentioned methods, ultrasonic extraction is suggested to be the most rapid and simple technique in comparison with the other mentioned techniques. Moreover, it has been reported that gingerol species was converted to shogaol which is also pungent compound in ginger at 80°C.⁴² For reflux and high-pressure Soxhlet extraction, the process heats the solvent to boiling temperature which is equal to or higher than 80°C.⁴³ On the other hand, the heat caused by sonication is far less than that temperature (*ca.* 30°C measured from the experiment). Therefore, we selected ultrasonication to extract the real sample in our work. The extraction procedure as fully described in experimental section 2.3 was used. The extraction used the crushed ginger as purchased without any further processing prior to addition of the ethanol followed by mixing the sample and solvent using a vortex mixer. We studied the extracted yield at various sonication times which were 0, 5, 10, and 20 minutes. Note that 0 minute means that the sample was mixed with ethanol using a vortex mixer without sonication. The result showed that the concentration of gingerol related species from each sonication time was

found in the range of 483 - 587 μM in the extracted solvent which was in the same range even with 0 minute sonication time, as shown in Fig. 6(a). Although the result showed a slight decrease in the concentration of gingerol related species with time, the value still lie in the same range of the detection. From this result, we inferred that we can simplify the extraction method from using the sonication assisted extraction by simply shaking the sample with selected organic solvent, ethanol. The simplified extraction procedure we obtained consists of two steps which are mixing the samples with ethanol on a vortex mixer for 1 minute and centrifuge to remove solids. In the following experiment the simplified extraction method was used for the further extractions of a real sample.

Having simplified the extraction procedure we next turn to quantify the concentration of gingerol and related species in a real ginger sample. In order to determine the amount of analyte in the real sample, the mass of crushed ginger used during the extraction process was varied from 0.25 g to 2.0 g. For each sample amount, three separate extractions were carried out and three repeat voltammograms were performed. The results showed that the amount of gingerol and related species extracted (μM) into the 5 mL ethanol increased proportionally to the amount of sample, as can be seen in Fig. 6(b). We converted the concentration in extracted solvent to a concentration per a gram of sample. The concentration of gingerol related species extracted from the samples in the unit of μmol per gram of sample is illustrated in the inset of Fig. 6(b). However, some small variation is observed between the different extractions as shown by the error bar in Fig. 6(b). The likely cause of this variability occurs is variation in the extraction efficiency. Therefore, we further studied the extraction efficiency by multiple re-extractions from the extracted sample and calculated the extraction efficiency of the first extraction. The results showed that the extraction method gave *ca.* 80-85% extraction efficiency (data shown in section S3,

supplementary information). From the result, we suggest that the variation of the result caused by the variability of different extraction efficiency. From the quantification result, we reported that the amount of gingerol related species found in the crushed ginger sample based on the first extraction was $2.64 \pm 0.23 \mu\text{mol g}^{-1}$ or $0.78 \pm 0.07 \text{ mg g}^{-1}$ (n=3).

4. Conclusions

A MWCNT-BPPG electrode has been successfully utilised for the voltammetric determination of gingerol species in standard and real ginger samples. A good linearity range of 1 – 50 μM was obtained with a detection limit of 0.21 μM and a limit of quantification of 0.71 μM . The extraction of gingerol related species from real sample ginger can be achieved via a simple methodology using the ethanol and a vortex mixer, leading to a time saving sample preparation. To the best of our knowledge, this proposed method is the first reported electrochemical method using for the detection of gingerol species. This fast and facile method of detection will be useful towards application in quality control of ginger products in the food industry.

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Figure captions

Fig. 1 Chemical structure of pungent and active compounds in ginger.

Fig. 2 The overlaid first (dashed line) and second (solid line) cyclic voltammograms for the adsorptive stripping voltammetry of 50 μM 6-gingerol on (a) a bare-BPPG electrode and (b) a MWCNT-BPPG electrode recorded in a 0.05 M Britton-Robinson buffer solution pH 1.8, after open-circuit accumulation for 1 minute; scan rate 100 mV s^{-1} . Inset (a): cyclic voltammograms for no adsorption of 6-gingerol on a bare-BPPG electrode.

Scheme 1 The structure of 6-gingerol and the proposed mechanism for the electrochemical reaction of 6-gingerol.

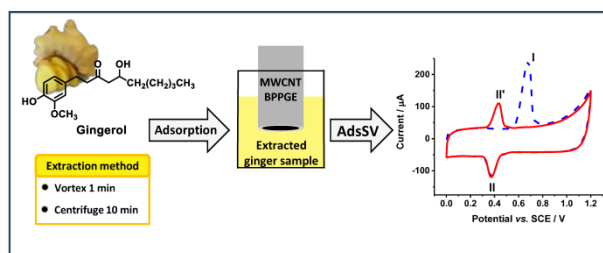
Fig. 3 The second scans of cyclic voltammetry of 100 μM 6-gingerol on a MWCNT-BPPG electrode at varying scan rates in a 0.05 M Britton-Robinson buffer solution pH 1.8. Inset: plot of peak current versus scan rate.

Fig. 4 (a) The responses of oxidative peak I as a function of accumulation time in 10 μM (---■---), 25 μM (---●---), 50 μM (---◆---), and 100 μM (---▲---) of 6-gingerol. (b) The data plotted according to the linearised Langmuir isotherm.

Fig. 5 Adsorptive stripping cyclic voltammetric responses (first scans) of a MWCNT-BPPG electrode to increasing 6-gingerol concentrations from 1 to 50 μM in a 0.05 M Britton-Robinson buffer solution pH 1.8, after open-circuit accumulation for 1 minute; scan rate 100 mV s^{-1} . *Inset*: the corresponding calibration curve plot using the oxidation peak (I).

Fig. 6 (a) Effective concentration of gingerol related species (μM) in the extracted solvent at different ultrasonic extractions time from 0 to 20 minutes. (b) Concentration of gingerol related species in the extracted solvent at different sample amounts. *Inset*: the plot of gingerol related species concentration after conversion to the concentration per gram of the sample and the amount of sample.

Graphical abstract



Electrochemical determination and quantification of gingerol species by adsorptive stripping voltammetry.