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Electrochemical Tuning of Oxygen-containing Groups on Graphene Oxides: Towards Control of the Performance for the Analysis of Biomarkers

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Electrochemical Tuning of Oxygen-containing Groups on Graphene Oxides: Towards Control of the Performance for the Analysis of Biomarkers

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Graphene materials are very popular in the field of biosensing owing to their distinctive characteristics. However, oxygen-containing groups are known to exist intrinsically in graphene-related materials. These groups influence the electrochemical properties of graphene materials and therefore affect the sensing performance of graphene based electrodes when used to detect redox active biomarkers. A well-defined carbon/oxygen (C/O) ratio can be obtained upon applying different reduction potentials to graphene oxide (GO) films for a controlled removal of redox active oxygen functionalities. We show here that a precise control of the oxygen functionalities onto a graphene oxide films allow the tuning of the biosensing capabilities of the electrodes for the analysis of two significant biomarkers, uric acid and ascorbic acid, as well as two DNA bases, guanine and adenine. Both the catalytic properties and the sensitivity of the reduced GO films electrodes (ERGOs) are evaluated by measuring the oxidation potential and the peak current, respectively. We demonstrate that each biomarker requires different optimal conditions which can be easily matched by varying the electrochemical pre-treatment of the sensing GO film.

1. Introduction

Graphene and its related materials have been of great interest and significance in sensing and fuel cell applications in the recent years. Their unique properties such as high conductivity,[1] high mechanical strength[2] and fast heterogeneous electron transfer rates[3,4] have allowed them to be widely used in areas such as conductive composites[5] and electrochemical devices.[6,7]

The existence of oxygen functionalities in graphene can hinder the sensing abilities of graphene as they are usually present on the basal plane and at the edges of the graphene materials, which are the most sensitive parts of graphene materials for sensing. Following the removal of these oxygen functionalities, a partial restoration of the sp^2-carbon network of graphene is suggested to occur, allowing it to possess better electrical properties such as charge-carrier mobility which are very useful towards electronic applications.[8] Partial removal of oxygen-containing groups can be done via chemical, electrochemical or thermal reduction methods.[9-12] Chemical reduction is done with the aid of strong reducing agents such as sodium borohydride[13,14] and hydrazine;[15] electrochemical reduction can be performed by applying a negative set potential on the graphene oxide layers for a period of time, while thermal reduction subjects the graphene oxide layers to a high temperature treatment. Electrochemical reduction is popular due to its simplicity, to the fact that toxic chemical agents are not required,[16] and electrochemically reduced materials can be utilized for sensing as graphene nanoribbons and nanosheets[17] and energy storage.[18] More importantly, electrochemical reduction is able to control accurately the extent of oxidation/reduction as the number of electrons to be supplied to or taken from the system can be easily monitored.[19] It is noteworthy that different reduction potentials can eliminate different oxygen-containing groups, such as peroxyl,[20,21] aldehyde[22] and epoxyl,[23,24] which can be electrochemically reduced at –0.7 V, –1.0 V and –1.1 V, respectively (*vs.* Ag/AgCl). Therefore, the types of oxygen-containing groups removed, as well as the extent to which they are removed can

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be varied by applying different reduction potentials to the graphene oxides prior to the analyses of desired probes.

Previous research has shown how different reduction potentials influenced the oxidation of potassium ferro/ferricyanide, as substantiated by the varying carbon/oxygen (C/O) ratios of the electrochemically reduced graphene oxides (ERGOs) by tuning different extent of the reduction.[19] In this work, we wish to evaluate the biosensing capability of GO films for the electrochemical analysis of two significant biomarkers, uric acid and ascorbic acid, and two DNA bases, guanine and adenine in relation to the reduction pretreatment which precisely control the amount of surface oxygen functionalities. The reduction potentials applied ranged from –0.25 V to –1.50 V and parameters such as sensitivity (evaluated using peak current intensities) and catalytic property (evaluated by measuring the oxidation potential) of ERGOs were studied.

2. Experimental

2.1 Materials

Hydrochloric acid (37 %, w/w), fuming nitric acid (>90 %, w/w), potassium chlorate (98 %, w/w), sulphuric acid (95-98 %, w/w), potassium phosphate monobasic, sodium phosphate dibasic, potassium chloride, sodium chloride, uric acid, ascorbic acid, guanine, adenine were purchased from Sigma-Aldrich, Singapore. Screen-printed electrodes were purchased from CH Instruments. Milli-Q water with a resistivity of 18.2 MΩcm was used throughout the experiments.

2.2 Apparatus

All cyclic voltammetry and differential pulse measurements were performed with a μAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a personal computer. The analyser is governed by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie). Cyclic voltammetry experiments were performed in an electrochemical cell using a three-electrode configuration, at room temperature. A platinum electrode served as an auxiliary electrode; an Ag/AgCl electrode was used as a reference electrode. Disposable screen-printed electrodes (SPE) were used as working electrodes for all measurements in this work. All electrochemical potentials in this report are stated *vs.* the Ag/AgCl reference electrode, and all measurements were carried out at a scan rate of 0.1 V/s.

2.3 Procedure

The graphene oxide used was prepared using the Staudenmaier method.[25] Sulfuric acid (95-98 %, 17.5 mL) and fuming nitric acid (9 mL) were added to a round-bottomed flask with a magnetic stir bar. The mixture was stirred and cooled in an ice bath for 15 min. Graphite (1 g) was then added to the mixture and a homogeneous suspension was obtained after vigorous stirring. Potassium chlorate (11 g) was then slowly added to the mixture to avoid formation of chlorine dioxide gas. Upon complete dissolution of potassium chlorate, the mixture was stirred at room temperature for 96 hours vigorously. The resultant mixture was poured into deionised water (1 L) and filtered. Graphite oxide was then re-dispersed and washed repeatedly in HCl (5 %) solutions to remove sulphate ions. After which, the mixture was washed with deionised water until a neutral pH was obtained for the filtrate. The graphite slurry was dried in a vacuum oven for 48 hours at 60 ºC before use.

A suspension of the graphene oxide material was prepared with a concentration of 1 mg mL^{-1} in water. After sonication of 2 hours, 8 μL of the required suspension was then deposited onto the SPE surface. Immobilisation of the graphene material onto the SPE surface is complete after evaporation of the solvent at room temperature. A randomly distributed film on the screen printed electrode surface is formed, before measurements were carried out. The various ERGOs were obtained by applying reduction potentials, between –0.25 V, and –1.50 V for 300 seconds to a graphene oxide-modified (GO-modified) screen printed electrode in a 50 mM phosphate buffer solution (pH 7.2). Three repeated experiments were performed each time, using 3 different screen printed electrode units to ensure the reproducibility of each measurement. All cyclic and differential pulse voltammetry measurements were performed in a 50 mM phosphate buffer solution with pH 7.2.

3. Results and Discussions

The application of different reduction potentials to graphene oxides modified electrodes between –0.25 V to –1.50 V can be used to form ERGOs bearing different amount of oxygen functionalities on the film surface. Quantification of the oxygen functionalities can be obtained by means of X-ray photoelectron spectroscopy (XPS) which allows the calculation of C/O ratios as a useful parameter to judge the extent of the oxygen-containing groups removal. Table 1 summarizes the calculated C/O ratios of the electrochemically reduced GO film electrodes in relation to the applied reduction potential.[19]

Table 1 C/O ratios calculated from XPS survey spectra corresponding to the reduction potential applied.[19]

Reduction Potential	C/O ratio
GO (untreated)	3.15 ± 0.31
–0.25 V	3.47 ± 0.35
–0.50 V	4.14 ± 0.39
–0.75 V	4.68 ± 0.37
–0.85 V	5.77 ± 0.65
–0.90 V	7.13 ± 0.60
–1.00 V	7.78 ± 0.73
–1.25 V	9.44 ± 0.35
–1.5 V	9.76 ± 0.35

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A higher C/O ratio indicates that a larger portion of oxygen-containing groups has been removed, expecting the graphene oxide to perform better for sensing applications. The C/O ratios displayed in Table 1 shows a steady increase from around 3 for the untreated GO film to approximately 10 when the reduction potential applied increased to –1.50 V.

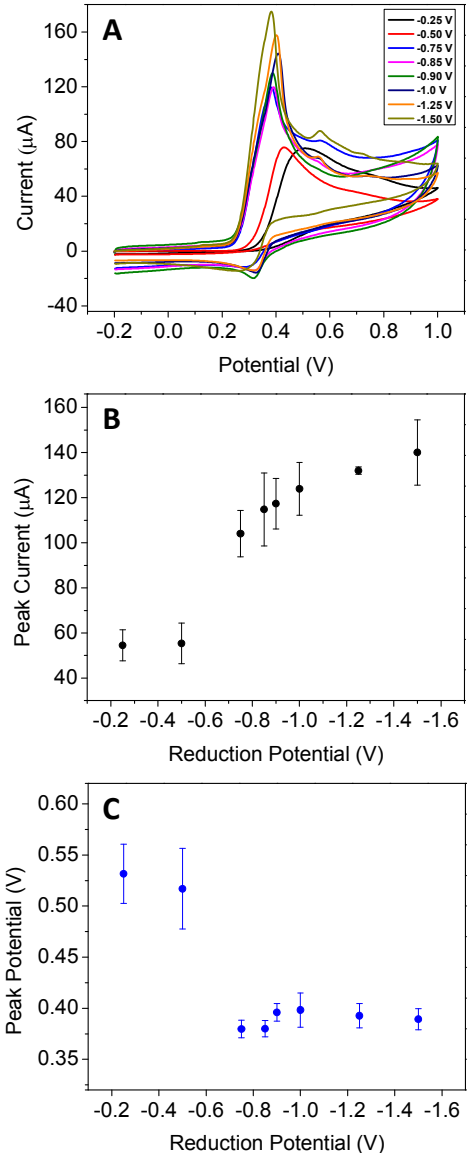

Fig. 1 (A) Cyclic voltamograms of 5 mM uric acid on graphene oxides reduced at different reduction potentials and comparison graphs of (B) peak height and (C) peak potential against reduction potential of graphene oxide. Conditions: 50 mM PBS background electrolyte, pH 7.2, scan rate 100 mV s^{-1}.

The effects of the amount of oxygen-containing groups on the sensitivity of the graphene oxides electrodes were firstly explored by carrying out cyclic voltammetry scans in the presence of ascorbic acid and uric acid and analysing the oxidation peak potentials as well as the peak current intensities. Figure 1A illustrates representative cyclic voltammograms of uric acid on graphene oxide electrodes previously treated at various reduction potentials. It is evident that varying the reduction potential applied to the film have a significant effect to the voltammetric signals with shifting of the oxidation peak as well as the peak intensity, owing to the amount of oxygen-containing groups present. The sensitivity of the graphene surfaces, as exemplified by the magnitude of the oxidation current is illustrated in Figure 1B. It can be seen that more intense peak currents were generated upon reducing the film

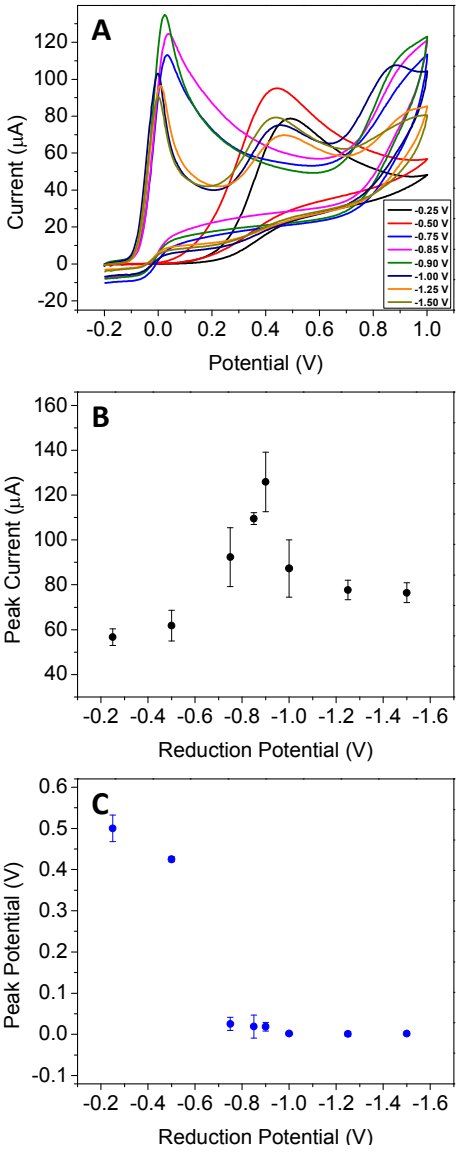

Fig. 2 (A) Cyclic voltamograms of 5 mM ascorbic acid on graphene oxides reduced at different reduction potentials and comparison graphs of (B) peak height and (C) peak potential against reduction potential of graphene oxide. Conditions: 50 mM PBS background electrolyte, pH 7.2, scan rate 100 mV s^{-1}.

with more negative potentials from –0.25 V to –1.50 V. It is hence evident that oxygen-containing groups on ERGOs strongly hindered the oxidation currents, and the absence of most oxygen functionalities resulted in larger oxidation currents and enhanced sensitivity. The improved performance of the electrode with increased C/O ratio is also manifested *via* the decrease of the oxidation potential of uric acid (Figure 1C).

When reduction potentials were set between –0.25 V and –0.50 V, oxidation of uric acid occurred at around 500 mV. The peak potential then underwent a drop to around 370 to 390 mV when more negative potentials (–0.75 V to –1.50 V) were applied. A lower oxidation potential indicates improved electrocatalytic properties of the graphene film, whereby the rate and ease of electron transfer of uric acid increases with less oxygen containing groups present.

Cyclic voltammograms of oxidation of ascorbic acid on graphene oxides electrochemically reduced at different potentials are shown in Figure 2A. Compared to uric acid, the shape and peak position for the oxidation of ascorbic acid changed more dramatically following different reduction treatments. The oxygen groups present on the film surface remains to be a strong influence on the electrochemical oxidation of ascorbic acid. Considering the peak current intensities, as illustrated in Figure 2B, it is apparent that the optimal reduction potential which resulted in the most intense oxidation peak corresponds to –0.90 V. Contrary to uric acid which gave the most intense peak following a reduction treatment at the most negative value of –1.50 V, ascorbic acid seems to benefit from the presence of a certain amount of oxygen groups. Conductivity of the material could represent another important factor that affects the oxidation of ascorbic acid. As for the catalytic aspect, the peak position is however lowered with increasing reducing potential treatments. The oxidation potential of ascorbic acid, displayed in Figure 2C, experienced a significant decrease from around 425 mV to 35 mV when the reduction potential of graphene was increased from –0.50 V to –0.75 V. Thereafter, the oxidation potential remained between 0 to 40 mV. Hence, it is evident that in general, oxidation of ascorbic acid can occur at a much lower potential with higher reduction potential applied on the graphene oxide surfaces, due to the reduction of a large extent of oxygen functionalities initially present. This represents a piece of useful information as the change in oxidation potential of ascorbic acid is potentially significant for simultaneous detection of ascorbic acid with other redox active biomarkers such as dopamine and the uric acid with high resolution. Thus, altering the oxygen content of GO films electrodes electrochemically can allow a better separation of the oxidation signals of these three biomolecules.

Following the analyses of the effect of varied reduction potential of the graphene surfaces on the oxidation of the two biomarkers, oxidation of two DNA bases, adenine and guanine, were then carried out. Figure 3A illustrates the DPV measurements performed in the presence of adenine at different reduction potential treatment. While the oxidation peak potentials seem to remain almost unaffected by the electrochemical pre-treatment, the peak currents displayed substantial differences, as explained in Figure 3B. A gradual rise in the oxidation current of adenine was observed when the reduction potential was increased from –0.25 V to –1.0 V and this can be attributed to the weakening of interactions between adenine and the oxygen functionalities on the graphene surface which pose as a huge interference to the sensing capabilities of the graphene material. Beyond a reduction potential of –1.0 V, lower current intensities were recorded instead. Therefore, applying a reduction potential of –1.0 V allows the most sensitive detection of adenine. As observed from the Figures 3A and 3C, the oxygen functionalities have negligible influence on the oxidation potential of adenine. It remains, in fact, relatively constant at about 1.05 V following all the reduction treatments performed.

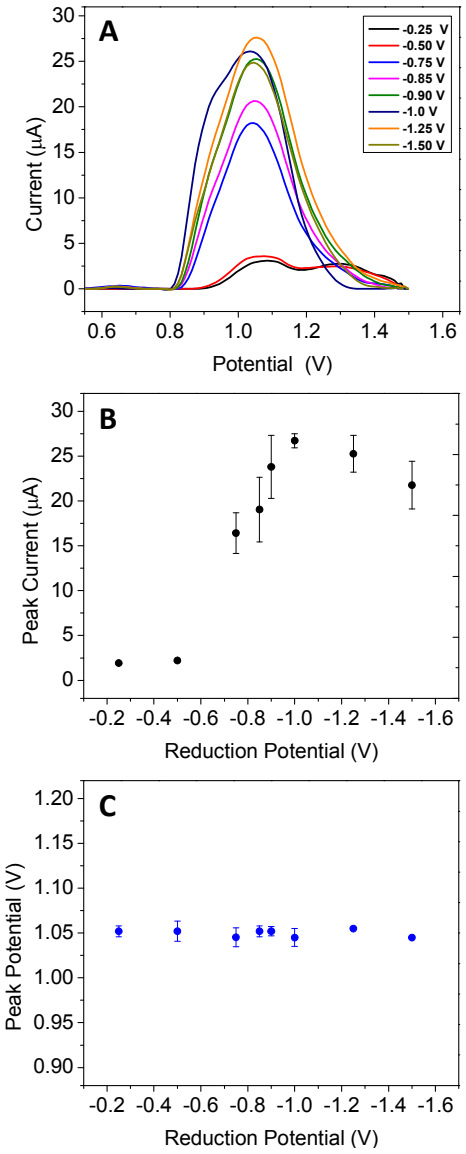

Fig. 3 (A) Differential pulse voltamograms of 5 mM adenine on graphene oxides reduced at different reduction potentials and comparison graphs of (B) peak height and (C) peak potential against reduction potential of graphene oxide. Conditions: 50 mM PBS background electrolyte, pH 7.2.

Oxidation of guanine showed similar results as adenine, as exhibited in Figure 4. Varying the reduction potential applied has resulted in significant differences in the peak intensities and some observable shifts in the oxidation potentials of guanine, as displayed in Figure 4A. From the comparison of peak intensities in Figure 4B, it is evident that the oxidation current

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of guanine displayed increments when the reduction potential applied to the graphene surfaces was increased gradually from –0.25 V to –1.0 V. A slight decrease was then recorded for reduction potential of –1.25 V and –1.50 V. Similar to the oxidation of adenine, applying a reduction potential of –1.00 V generated the optimal surface conditions for the most sensitive detection of guanine. For the oxidation potential trend displayed in Figure 4C, a gradual decrease in the oxidation potential from about 0.78 V to 0.66 V was firstly observed

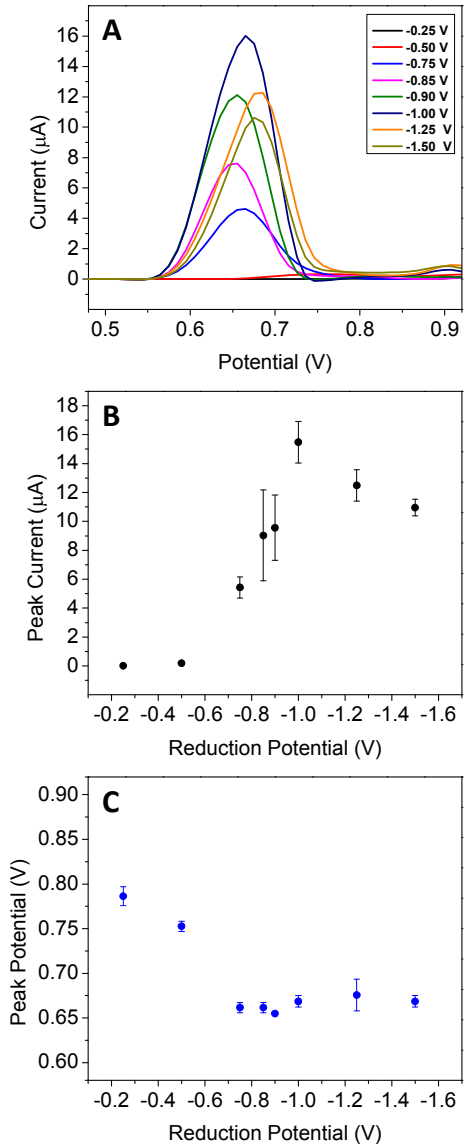

Fig. 4 (A) Differential pulse voltamograms of 5 mM guanine on graphene oxides reduced at different reduction potentials and comparison graphs of (B) peak height and (C) peak potential against reduction potential of graphene oxide. Conditions: 50 mM PBS background electrolyte, pH 7.2.

when reduction potentials of between –0.25 and –0.75 V were applied. However, this trend did not continue as no further decrease in oxidation potentials was recorded when more negative reduction potentials were applied. From the analyses of the DNA bases, it seems evident that the oxygen functionalities have little catalytic effect on the oxidation of both guanine and adenine since marginal potential shift was recorded upon altering the C/O ratio. Nonetheless. major effects were experienced in terms of sensitivity, with considerable changes in peak intensities observed when the amount of oxygen groups was varied.

From the analyses of the four biomarkers as aforementioned, the extent of electrochemical pre-treatment, governed by the reduction potential, enables the analytical performance of the GO electrodes to be tuned. The optimal reduction pre-treatment conditions also varied for each biomarker.

4. Conclusion

We investigated the influence of oxygen functionalities on the sensing performance of GO-modified electrodes for the electroanalysis of four biomolecules, uric acid, ascorbic acid, adenine and guanine. The amount of oxygen functionalities was precisely tuned by applying a controlled reduction potential for a fixed period of time. By doing so, we were able to control the sensing capability of the electrode accurately, not only in terms of peak intensity, but also by altering the catalytic properties of the carbon surface which results in oxidation peak shifting. The alteration of the catalytic properties of the GO film was significant particularly for the case of ascorbic acid which gave oxidation potentials which were strongly affected by the amount of oxygen functionalities. Precise control of the oxygen groups on the surface of GO-modified electrodes is thus promising in biosensing applications with the ability to determine more accurate conditions that are optimal for specific analyte targets.

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Notes and references

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1 Du, X.; Skachko, I.; Barker, A.; Andrei, E. Y. *Nat. Nanotechnol.* 2008, **3**, 491.
2 Bunch, J. S.; van der Zande, A. M.; Verbridge, S. S.; Frank, I. W.; Tanenbaum, D. M.; Parpia, J.M.; Craighead, H. G.; McEuen, P. L. *Science.* 2007, **315**, 490.
3 Pumera, M. *Chem. Rec*. 2012, **12**, 201.
4 Kampouris, D. K.; Banks, C. E. *Chem. Commun*. 2010, **46**, 8986.
5 Hirata, M.; Gotou, T.; Ohba, M. *Carbon*. 2005, **43**, 503.
6 Pumera, A.; Ambrosi, A.; Bonanni, A.; Chng, E. L. K.; Poh, H. L. *Trends Anal. Chem*. 2010, **29**, 954.
7 Pumera, M. *Electrochem. Commun*. 2013, **36**, 14.
8 Chen, H.; Műller, M. B.; Gilmore, K. J.; Wallace, G, G.; Li, D. *Adv. Mater*. 2008, **20**, 3557.
9 Ambrosi, A.; Bonanni, A.; Sofer, Z.; Cross, J. S.; Pumera, M. *Chem. Eur. J*. 2011, **17**, 10763.
10 Allen, M. J.; Tung, V. C. Kaner, R. B. *Chem. Rev*. 2010, **110**, 132.

11 Chua, C. K.; Pumera, M. *Chem. Soc. Rev.* 2014, 4**3**, 291.
12 Mathkar, A.; Tozier, D.; Cox, P.; Ong, P.; Galande, C.; Balakrishnan, K.; Reddy, A. L. M.; Ajayan, P. M. *J. Phys. Chem. Lett.* 2012, **3**, 986.
13 Gao, W.; Alemany, L. B.; Ci, L.; Ajayan, P. M. *Nat. Chem.* 2009, **1**, 403.
14 Chua, C. K.; Pumera, M. *J. Mater. Chem. A*. 2013, **1**, 1892.
15 Hofmann, U.; König, E. *Z. Anorg. Allg. Chem.* 1937, **234**, 311.
16 Zhou, M.; Wang, Y.; Zhai, Y.; Zhai, J.; Ren, W.; Wang, F.; Dong, S. *Chem. Eur. J.* 2009, **15**, 6116.
17 C. S. Lim, C. K. Chua, M. Pumera, *Analyst*, 2014, **139**, 1072.
18 L. Buglione, A. Bonanni, A. Amboris, M. Pumera, *ChemPlusChem* 2012, **77**, 71.
19 Ambrosi, A. Pumera, M. *Chem. Eur. J.* 2013, **19**, 4748.
20 Stuart, E. J. E.; Pumera, M. *J. Phys. Chem. C.* 2011, **115**, 5530.
21 Ferdousi, B. N.; Islam, M. M.; Okajima, T.; Ohsaka, T. *Electrochim. Acta.* 2007, **53**, 968.
22 Baymak, M. S.; Bover, W. J.; Celik, H.; Zuman, P. *Electrochim. Acta.* 2005, **50**, 1347.
23 Xiao, L.; Wildgoose, G. G.; Crossley, A. Compton. R. G. *Sens. Actuators B.* 2009, **138**, 397.
24 Lee, L.; Villalba, M. M.; Smith, R. B.; Davis, J. *Electrochem. Commun.* 2009, **11**, 1555.
25 Staudenmaier, L. *Ber. Dtsch. Chem. Ges.* 1898, **31**, 1481.

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