Pharmaceutical electrochemistry: the electrochemical oxidation of paracetamol and its voltammetric sensing in biological samples based on screen printed graphene electrodes.

FERNANDEZ, C., HEGER, Z., KIZEK, R., RAMAKRISHNAPPA, T., BORUN, A. and FAISAL, N.H.

2015

Int. J. Electrochem. Sci., 10 **(2015)** 7440 - 7452

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Pharmaceutical Electrochemistry: the Electrochemical Oxidation of Paracetamol and Its Voltammetric Sensing in Biological Samples Based on Screen Printed Graphene Electrodes

Carlos Fernandez[1,*] , *Zbynek Heger*[2,3], *Rene Kizek*[2,3], *Thippeswamy Ramakrishnappa*[4], *Agnieszka Boruń*[5] *and Nadimul H. Faisal*[6]

[1] School of Pharmacy and Life Sciences, Robert Gordon University, Garthdee Road, Aberdeen AB10 7GJ, United Kingdom.
[2] Department of Chemistry and Biochemistry, Laboratory of Metallomics and Nanotechnologies, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic
[3] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic.
[4] Center for Nano and Material Sciences, Global Campus, Jain University, Jakkasandra (P),Kanakapura (T), Bangalore -560001, Karnataka, India.
[5]University of Łódź, Department of Physical Chemistry of Solutions, 90-236 Łódź, Pomorska 163, Poland.
[6]School of Engineering, Robert Gordon University, Garthdee Road, Aberdeen AB10 7GJ, United Kingdom.
[*]E-mail: c.lledo-fernandez@rgu.ac.uk

Received: 5 June 2015 / *Accepted:* 11 July 2015 / *Published:* 28 July 2015

We present a sensitive, fast and unmodified sensor for the electrochemical detection of paracetamol. The electrochemical behaviours of paracetamol on screen printed graphene electrodes were investigated for the first time by cyclic voltammetry. The results showed that the screen printed graphene electrodes revealed exceptional electrocatalytic activity to paracetamol. The response showed by this sensor was enhanced when it was compared to the bare screen printed electrodes.
When Screen Printed Graphene Electrodes were compared to bare SPE, it was shown that the response with graphene was greater than without. This is due to its unique characteristics physical and chemical, π-π interactions and a strong adsorptive capability.
In this manuscript, the effect of supporting electrolyte, pH and scan rate were also investigated.
The oxidation peak current was linearly proportional to the concentration of paracetamol in the range from 0.1 to 50 μM with a limit of detection of 20nM based on (3-σ/slope), under the optimum conditions. The proposed method was successfully applied to paracetamol determination in in biological samples such as human oral fluid.

Keywords: Paracetamol, Electrochemical Sensor, Real Samples, Screen Printed Graphene Electrodes

1. INTRODUCTION

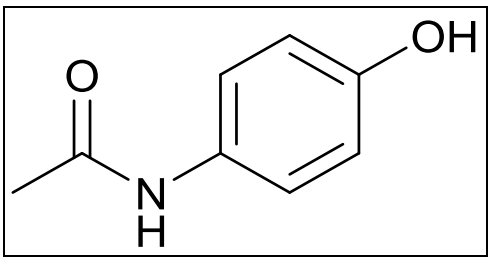


Figure 1. Chemical structure of paracetamol.

Paracetamol, also known as acetaminophenol, or APAP, chemically named N-acetyl-p-aminophenol which is depicted in Figure 1 is a commonly employed over-the-counter analgesic (pain reliever) and antipyretic (fever reducer) [1-5]. Paracetamol is classified as a mild analgesic [4-5]. It is generally utilised for the relief of headaches [4-6] and other minor aches and pains. Paracetamol is also the main ingredient in a great number of cold and flu remedies [2, 5] . When it is combined with opioid analgesics [5-6], paracetamol can also be employed to alleviate more severe pain such as post-surgical pain as well as offering palliative care in advanced cancer patients [5-6]. It is not usually categorized as a non-steroidal anti-inflammatory drug [5-6] **(**NSAID), although paracetamol is employed to treat inflammatory pain, as exhibits only weak anti-inflammatory activity. It is generally safe for use at recommended doses, however compared to other over-the-counter pain relievers, paracetamol is considerably more toxic. In overdose the accumulation may cause kidney and liver damage [5-6].

Therefore, a trustworthy method for the rapid and accurate detection of paracetamol it is crucial and beneficial to control the use and avoid the possible abuse of paracetamol. Most of the current analytical methods employed to analyse paracetamol are generally: high-performance liquid chromatography–mass spectrometry [7-11] and gas chromatography–mass spectrometry [12], ultra performance liquid chromatography-tandem mass spectrometry [13] and capillary electrophoresis [14] have also been reported for the determinations of paracetamol. However, the main drawbacks of these techniques are time-consuming manipulation steps, sophisticated instruments and special training.

On the other hand electrochemistry offers a number of very attractive advantages such as low cost, easy to manipulate, portable and fast. It has been widely employed in biological matrixes [15], pharmaceutical [16-17] and some drugs containing tertiary amine functional group [15, 18-20] due to its continuance, sensitivity, reproducibility and selectivity towards many target analytes [15, 19, 21-22]. Electrochemical methods including voltammetric techniques have also been developed for the determination of paracetamol. Some of the examples reported in literature are: the use of glassy carbon electrodes (GCEs) modified with carbon-coated nickel magnetic nanoparticles [23], GCEs modified with graphene–chitosan composite [24], C60-modified GCE [25], GCEs modified with Nafion/TiO_2–graphene [26], SPE modified with CNT [27] and pencil graphite electrode [28]. These reports showed good detection limits and sensitivity however the main drawback is the need of extra time through the consuming modification process which usually involves several steps to incorporate the modifier to the substrate and also the costs. Our work is based on disposable and cost effective

screen printed graphene electrodes without any prior modification which exhibit an accurate and effective response in comparison to other electrodes like indium tin oxide and gold electrodes [4, 29]. The enhancement of the response exhibited is due to its wide potential window, strong adsorption tendency, low background current and easy maintenance [30].

Over the last two decades, [31] a great attention has been focused on graphene due to its large surface area, high thermal and electrical conductivities, impressive mechanical properties, and low cost [31]. Due to graphene excellent properties, it has been greatly utilized in many areas [32-36] such as nanoelectronics, sensors, nanocomposites, catalysis, capacitors etc. This manuscript describes the electrochemical oxidation of paracetamol in aqueous solution (buffers) and its voltammetric sensing in real samples based on novel screen printed graphene electrodes. Screen printed electrodes exhibits a great number of advantages when compared to normal electrodes such as low cost, single-shot disposable as well as easy to manipulate, reliable and reproducible [30].

The electrochemical properties of the sensor were also investigated in this manuscript. To the best of our knowledge, there is no report based on using novel screen printed graphene electrodes for the determination of paracetamol. The unique benefits of this approach are that no-sample pre-treatment or surface modifications are required and that is fast, reliable and cost effective.

2. EXPERIMENTAL

2.1. Reagents

All chemical reagents used to prepare solutions were purchased in their purest commercially available forms from Aldrich. All aqueous solutions were made up with water (of resistivity of not less than 18 MΩ cm) taken from an Elgastat filter system (Vivendi, Bucks., UK). All experiments were undertaken at 23±2 ºC. Paracetamol tablets of different pharmaceutical companies such as Galpharm International Ltd. were purchased from the local market. A stock solution of paracetamol was prepared by dissolving the required mass in buffer to give a concentration of 1 mM. Working standards, for initial voltammetric studies, were prepared by dilution of this solution with phosphate buffer to give a final concentration of 0.1μM. In the same manner a stock solution of paracetamol was prepared by dissolving the required mass in human oral fluid to give a concentration of 1 mM.

2.2. Electrodes

Screen Printed Graphene Electrodes (SPGrE) and Screen Printed Graphite Electrodes (SPGE) which both have a 3 mm diameter for the working electrode were commercially available from Gwent Electronic Materials Ltd. (Pontypool, Cardiff, UK). Two sets of SPE were employed to obtain the results. One SPE system was composed of three electrodes with graphite as a working and counter electrode and silver/silver chloride for the reference electrode. The other system was composed of three electrodes with graphene as a working electrode and graphite as a counter electrode and silver/silver chloride for the reference electrode. The electrodes have been characterised electrochemically and have found to exhibit a heterogeneous electron transfer rate constants of ~1.7 ×

10^{-3} cm·s^{-1} using the ferrocyanide redox couple in 1 M KCl which are in agreement with our previous work [17].

2.3. Electrochemical measurements

Voltammetric measurements were carried out using a μ-AutolabIII (Eco Chemie, Amsterdam, The Netherlands) potentiostat/galvanostat and controlled by Autolab GPES software version 4.9 for Windows XP as previously used in similar works [17]. The cyclic voltammetric parameters were as follows: initial potential of 0 V, vertex potential 1.5 V, end potential 0 V, step potential 5 mV as previously used in similar works [17].

2.4. Sample preparation and measurement procedures

Under optimal conditions, a series of carbonate buffer (CBS) and phosphate buffer solutions (PBS) (0.1M) containing different concentrations of paracetamol were analyzed using screen printed graphite and screen printed graphene electrodes. One tablet of paracetamol (equivalent to 500 mg) pharmaceutical formulation were accurately weighed and finely powdered in a mortar. An adequate amount of the powders was weighed and transferred into a 100 mL volumetric flask and dissolved with the appropriate buffer and pH.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of SPGrEs vs SPEs

We first investigate the surface character of SPGrE, SPE, GCE/Gr and GCE in the electrochemical probe 1.0×10^{-3} mol L^{-1} [$K_3Fe(CN)_6$] containing 0.1 mol L^{-1} KCl, using cyclic voltammetry (CV) technique at a scan rate of 100mV/s which is depicted in Figure 2.

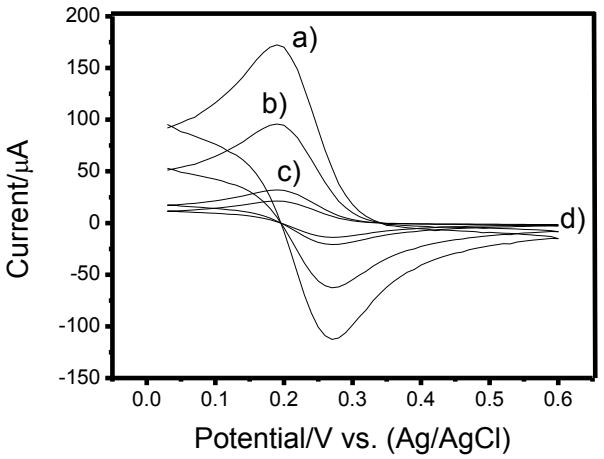
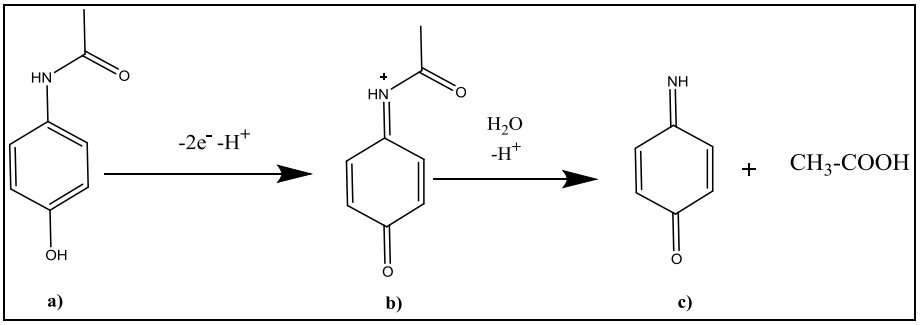


Figure 2. Cyclic voltammograms responses observed for (a) SPGrE, b) GCE/Gr c) SPE and d) GCE in 0.1 M phosphate buffer pH 7 in 10^{-3} mol L^{-1} [$K_3Fe(CN)_6$] containing 0.1 mol L^{-1} KCl, Scan rate: 100 mVs^{-1}

Figure 2 illustrates the voltammograms (CVs) obtained at SPGrE (curve a), GCE/Gr (curve b), SPE (curve c), and GCE (curve d) respectively. At the bare GCE Figure 2d shows a quasi-irreversible behaviour with relatively weak redox current peaks at Epa (anodic peak potential) = 0.1999 V and Epc (cathodic peak potential) = 0.285 V. At the bare SPE Figure 2c the voltammogram shows a greater response compared to GCE. The effect caused by the graphene is shown in Figure 2b with GCE/Gr and in Figure 2a with SPGrE, the peak current increases dramatically compared with that observed at bare GCE (Figure 2d) and bare SPE (Figure 2c). The enhancement in the peak current is also observed when SPGrE is compared to GCE/Gr due to the strong adsorption tendency and low background current of the SPGrE.

It can be seen from Figure 2c and 2d that there is a large background current at the Screen Printed Graphene Electrodes and GCE/Gr, which is caused by a larger surface area of the graphene [31, 37].

3.2. Mechanism of the Electrochemical Oxidation of paracetamol at SPGrE



Scheme 1. Mechanism of the Electrochemical Oxidation of paracetamol on the SPGrE.

Next, a suggested mechanism for paracetamol oxidation which is shown in Scheme 1 was investigated. Paracetamol in aqueous media oxidizes to *N*-acetyl-*p*-quinoneimine and acetic acid as depicted in Scheme 1.

Scheme 1 part b shows a two electron exchange process which is the first step of paracetamol electro-oxidation mechanism. The second step in the mechanism illustrated in Scheme 1c includes an exchange of one proton. In Figure 3, the voltammograms response only shows the involvement of one electron process, as the second electron is employed in the acetic acid formation.

At basic pH's the electro-oxidation of paracetamol is more favourable as indicated in Scheme 1a). This is in agreement with the pka value for paracetamol reported in literature to be 9.5 [2] which will be explained in detail in Section 3.3.2.

3.3. Electrochemical Behaviour of Paracetamol on SPGrE

3.3.1. The effect of the scan rate

In order to investigate the reaction kinetics, the effect of scan rate on the redox of paracetamol at the SPGrE was investigated by cyclic voltammetry (Figure 3).

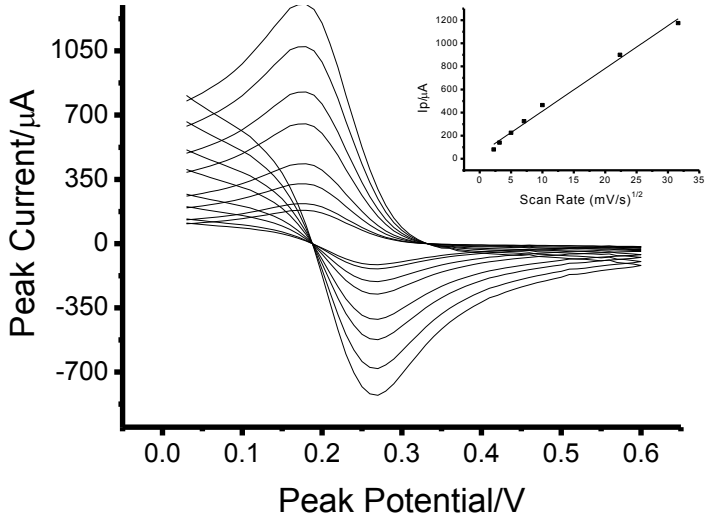


Figure 3. Cyclic voltammograms response observed for SPGrE in 1.0 10^{-3} mol L^{-1} of paracetamol in 0.1 M Phosphate buffer pH 7 at different scan rates (from inner to outer): 5, 10, 25, 50, 75, 100, 500 and 1000 mVs^{-1}. Inset the plot of peak current vs scan rate.

Figure 3 shows a reversible one electron transfer mechanism for paracetamol with an oxidation peak potential value at 0.19 V and a reduction peak potential value at 0.26 V. The peak to peak separation was found to be approximately around 70 mV. The values of the scan rate (range from 5 to 1000 $mV \cdot s^{-1}$) increases linearly with the redox peak currents at the SPGrE in the paracetamol solution. Figure 3 inset graph illustrates the linear relationship between the peak current and the scan rate. The linear regression equation was given by: Ipa = 21.234 + 0.5134*v*, R = 0.995. This shows that the SPGrE reaction with paracetamol is a surface confined process.

3.3.2. The effect of pH

Next the effect of pH in the reaction of paracetamol and SPGrE was investigated. We note that the pKa of paracetamol is reported to be 9.5 within the literature [27] therefore paracetamol was prepared in a carbonate buffer solution at pH 9. Figure 4 illustrates the behaviour of 10 μM paracetamol using a) SPGrE compared to b) SPE at pH 9 at a sweep rate of 100 $mV \cdot s^{-1}$.

The voltammogram in Figure 4 a) compared to 4b) shows a well-defined oxidation peak value at 0.1 V which can be assigned to the oxidation of paracetamol to *N*-acetyl-*p*-quinoneimine, as reported in Literature [26-27] on SPGrE.

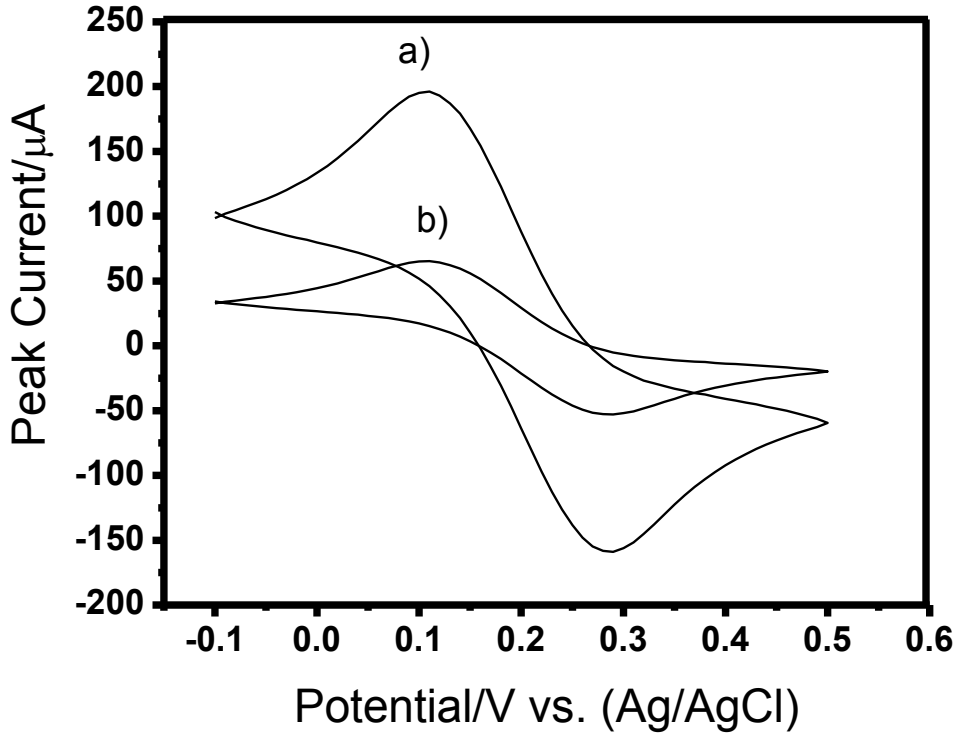
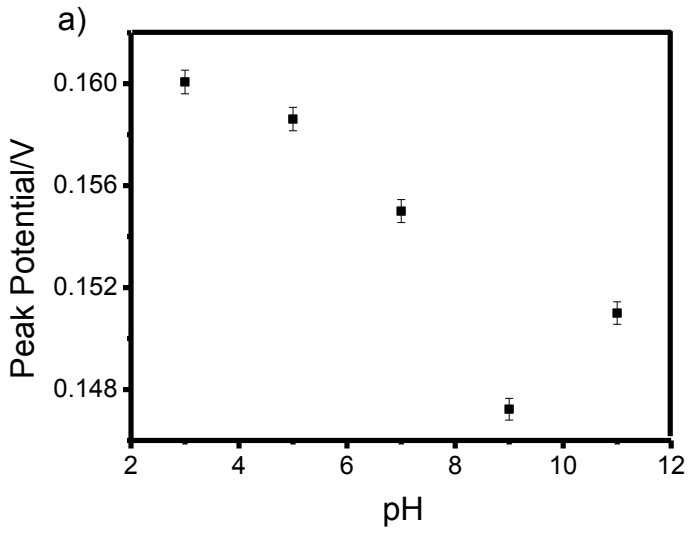


Figure 4. Cyclic voltammetric response recorded at a) SPGrE b) SPE in 10 μM paracetamol in pH 9 carbonate buffer solution (0.1 M). Scan rate: 100 100 $mVs^{-1.}s$

The reactivity of the SPGrE illustrated in Figure 4a) compared with a bare SPE 4b) can be greatly improved due to the electrochemical oxidation process of paracetamol, in the presence of graphene. The SPGrE response was higher compared to a bare SPE, this higher response can be associated to the stability of the product. This is due to the easily formed π–π interaction between the graphene sheets of sp^2-bonded carbon atoms with strengthening adsorption and the product molecules, which is in agreement with literature [37-38].



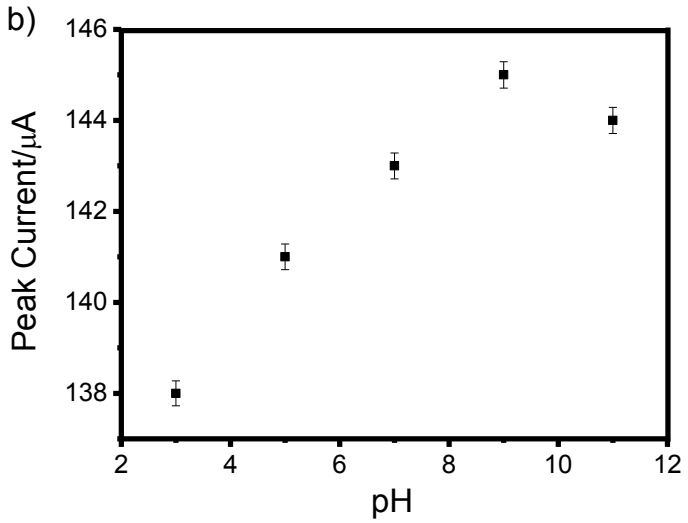


Figure 5. (a) A plot of peak potential as a function of pH from 3 to 11 for the electrochemical oxidation of 10 μM paracetamol in 0.1M CBS using SPGrE. Scan rate: 100 100 mVs^{-1}. (b) A plot of peak current (Ip) as a function of pH for the electrochemical oxidation of 10 μM paracetamol using SPGrE. Scan Rate: 100 mVs^{-1}.

The effects of the CBS solution pH in the range from 3.0 to 11.0 on the oxidation response of 10 μM paracetamol were investigated on 0.1 M CBS by CV and the results were exhibited in Figure 5. The relationships of the oxidation peak current/potential with the solution pH are depicted in Figure 5 a and b respectively.

Interestingly, the anodic peak potentials shift to more negative values with increasing the pH values as shown in Figure 5a. This observation can be explained by the proton staking part in the electrochemical reactions [39-40]. The peak potential (Ep) vs. pH plot which is depicted in Figure 5a is linear and dependence of anodic peak potential of analyte on the pH of supporting electrolyte and can be presented by the relation: E_p/V (pH 3-11) = −0.015 V/pH + 0.798 versus Ag/AgCl for paracetamol with correlation coefficients of 0.99. Note that the value obtained for pH 9 was not included in the linear equation. The electro-oxidation of paracetamol is more favourable at basic pHs in particular 9 compared to more acidic pHs as indicated in Figure 5a).

The peak height current values (Ip) versus a range of pHs from 3.0 to 11.0 is plotted and depicted in Figure 5b. The linear response over the range of pHs from 3.0 to 9.0 can be presented by the following equation: Ip/μA=-11.5x+196 798 versus Ag/AgCl for paracetamol. At pH 9 the peak height reaches its highest value and decreases gradually until pH 4 value. Those values well agree with the pKa of the paracetamol which is 9.5 where the electro-oxidation of paracetamol is more favourable. Considering the determination sensitivity, the pH value of 9 in the CBS solution was chosen in the following investigation as optimal.

3.3.3. Effect of concentration

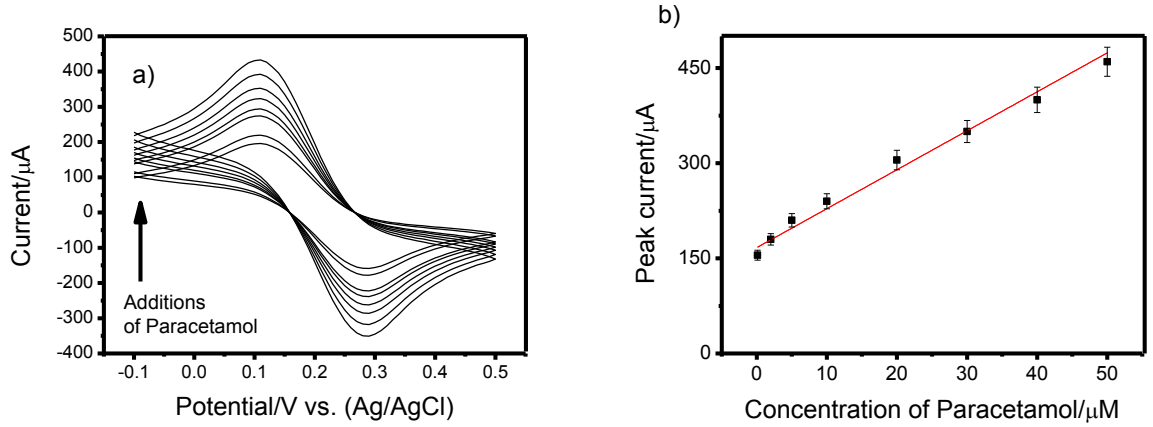


Figure 6. A) Cyclic voltammogram response observed for 0.1 M carbonate buffer solution pH 9 at SPGrE over a range of paracetamol concentrations from 0.1 μM to 50 μM Scan Rate: 100 mVs^{-1}. B) A plot of peak height (Ip), as a function of paracetamol concentration using SPGrE. Scan Rate: 100 mVs^{-1}.

Next, after determining that the optimum experimental pH for the electro-oxidation of paracetamol using SPGrE was that of pH 9, we explored a range of concentrations of paracetamol. The dependence of the oxidation peak current of paracetamol and its concentration was investigated in CBS (0.1 M) buffer solution at pH 9 by CV.

The electro-oxidation of paracetamol occurs at 0.1 V potential as is depicted in Figure 6a. The oxidation peak current values increase in magnitude upon the addition of paracetamol concentrations over the range from 0.1 to 50 μM.

The oxidation peak current values versus concentration of paracetamol plots showed a good linearity for paracetamol in the concentration range of 0.1–50 μM as depicted in Figure 6b. The linear response observed is given by the equation: Ip (μA) = 5.8345x+172.93 (μM) with the correlation coefficient 0.9903 based on N =8 (being N the number of concentrations investigated over the analytical range studied 0.1 μM to 50 μM into a pH 9 CBS buffer solution). The detection limit was found to be 20 nM based on (3-σ/slope).

3.4. Electroanalytical Applications of the proposed method to detect Paracetamol in Human Oral Fluid

Following confirmation that successful determination of paracetamol was possible in ideal conditions utilising a standard 0.1M pH 9 carbonate buffer solutions, the feasibility of the analytical protocol was verified towards detection of paracetamol within relevant media. Paracetamol is clinically employed for analgesic and antipyretic effects [2] and it is taken via oral therefore, it is considered worthwhile to determine the concentration of paracetamol in human oral fluid. The attention was turned to investigate the analytical sensing of paracetamol in human oral fluid. Additions of

paracetamol were made into human oral fluid (collected from healthy individuals) solution over the concentration range from 10 to 100 μM.

As is shown in Figure 7 the calibration plot resulting from the addition of paracetamol is linear over the concentration range 10 to 100μM. *Ip/μA = 0.7092x+103.92*, R^2 = 0.9879 and *N* = 10) with a detection limit of 8.7μM (based on 3-sigma) studied. The main advantage of this electrochemical sensor is the lack of sample pre-treatment as well as the lack of modification of the human oral fluid solution employed. Next, we show a summary and a comparison of our method depicted in Table 1 of current analytical approaches for the sensing of paracetamol.

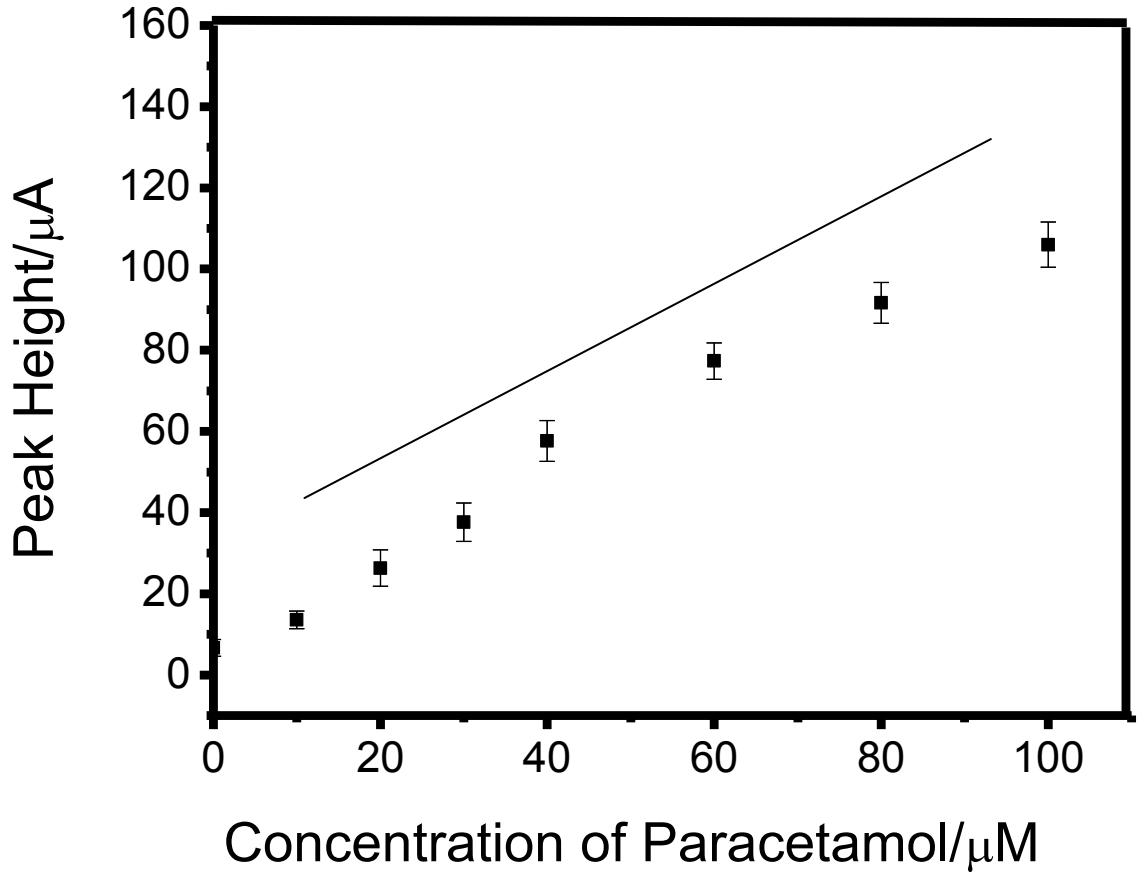


Figure 7. A calibration plot of peak height values (Ip), as a function of paracetamol concentration corresponding to the addition of paracetamol into human oral fluid sample solution over the concentration range 10-100 μM using SPGrE. Scan Rate: 100 $mV \cdot s^{-1}$.

Table 1. Comparison of our proposed method with other modified electrodes reported in the determination of paracetamol.

Electrochemical sensors	Technique	Sensitivity ($\mu A\ \mu M^{-1}$)	Linear range (μM)	Detection Limit (nM)	References

CNTs[a] /MPs[b]/SPE[c]	CV	0.15–0.30	0.5–5 and 5–100	200	[27]
R-GC-ABE[d]	NPV[e]	2.75μA/ mg L^{-1}	0–50 mg L^{-1}	4 μg L^{-1}	[40]
MWCNT/graphene/GCE	DPV[f]	0.0908	0.80–110.0	90	[39]
Graphene/GCE	SWV	4.055	0.1–20	32	[1]
Boron-doped diamond electrode	DPV	0.6	0.5–83	490	[38]
MWCNT/PPGE[e]	ASV[p]	no	0.1–25	45	[3]
pyrolytic carbon electrode	DPV	0.33	15–225	1400	[41]
SPGrE	CV		0.1–50 and 10–100	20	This work

[a] CNTs: carbon nanotubes; [b] MPs: magnetic microparticles; [c] SPE: screen-printed electrodes;
[d] R-GC-ABE: renewable glassy carbon annular band electrode
[e]PPGE: plane pyrolytic graphite electrode; [p] ASV: adsorptive stripping voltammetry.

When our proposed method is compared with other electroanalytical protocols reported for the sensing of paracetamol the analytical response exhibited is competitive.

The other important advantage of this method which is worthwhile mentioning is the lack of electrode surface modification as graphene was printed in-situ in the SPE so on-site determination and rapid testing times are possible.

This sensor replicates the real-world conditions encountered in nature so it could be directly applied to detect paracetamol.

4. CONCLUSIONS

The proposed method demonstrates for the first time the successful application of SPGrE for the determination of paracetamol in pharmaceuticals products as well as human oral fluid samples with excellent sensitivity and selectivity. A linear response is observed for a buffered solution given by the equation: Ip/μA= 5.8345x+172.93 (μM), R^2 = 0.9903 and N = 8 over the range from 0.1 μM to 50 μM onto a pH 9 buffer solution with a detection limit of 20 nM (based on 3-sigma/slope). In human oral fluid paracetamol also exhibits a linear behaviour over the concentration range 10 to 100 μM. The linear equation is given by: $Ip/\mu A = 0.7092x+103.92$, R^2 = 0.9879 and N = 10 with a detection limit of 8.7 μM (based on 3-sigma). This sensor exhibits important characteristics such as low cost, flexibility and reliability. In addition, we have replicated the real-world conditions encountered in nature so it

could be directly employed to detect paracetamol in routine analysis. Furthermore, the niche of this electro-analytical method is the lack of any sample pre-treatment and electrode surface modification as well as time effective.

ACKNOWLEDGEMENTS
C. L.-F. expresses gratitude to Robert Gordon University (School of Pharmacy and Life Sciences) and IDEAS research institute for financial support via a start-up grant. Further, support from SIX CZ.1.05/2.1.00/03.0072 is gratefully acknowledged.

References

1. X. Kang, J. Wang, H. Wu, J. Liu, I.A. Aksay, Y. Lin, *Talanta*, 81 (2010) 754.
2. R.M. de Carvalho, R.S. Freire, S. Rath, L.T. Kubota, *J. of Pharm. Biomed. Anal.*, 34 (2004) 871.
3. R.T. Kachoosangi, G.G. Wildgoose, R.G. Compton, *Anal. Chimica Acta*, 618 (2008) 54.
4. R.N. Goyal, V.K. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commun.*, 7 (2005) 803.
5. G. Graham, M. Davies, R. Day, A. Mohamudally, K. Scott, *Inflammopharm.*, 21 (2013) 201.
6. M. Li, L. Jing, *Electrochim. Acta*, 52 (2007) 3250.
7. D.D. Shinde, K.-B. Kim, K.-S. Oh, N. Abdalla, K.-H. Liu, S.K. Bae, J.-H. Shon, H.-S. Kim, D.-H. Kim, J.G. Shin, *J. Chrom. B*, 911 (2012) 113.
8. S.R. Polagani, N.R. Pilli, V. Gandu, *J. Pharm. Analysis*, 2 (2012) 206.
9. E. Yamamoto, S. Takakuwa, T. Kato, N. Asakawa, *J. Chrom. B*, 846 (2007) 132.
10. M.S. Elmasry, I.S. Blagbrough, M.G. Rowan, H.M. Saleh, A.A. Kheir, P.J. Rogers, *J. Pharm.Biomed. Anal.*, 54 (2011) 646.
11. J.T. Franeta, D. Agbaba, S. Eric, S. Pavkov, M. Aleksic, S. Vladimirov, *Il Farmaco*, 57 (2002) 709.
12. D. Tsikas, K.S. Tewes, F.-M. Gutzki, E. Schwedhelm, J. Greipel, J.C. Frölich, *J. Chrom. B: Biomed. Sci. Applic*, 709 (1998) 79.
13. S.M. Wabaidur, Z.A. Alothman, M.R. Khan, *Spectrochim. Acta Part A: Molec. Biomolec. Spectr.*, 108 (2013) 20.
14. M.C. Marra, R.R. Cunha, D.T.R. Vidal, R.A.A. Munoz, C.L.d. Lago, E.M. Richter, *J. Chrom. A*, 1327 (2014) 149.
15. J.L. Adcock, C.J. Barrow, N.W. Barnett, X.A. Conlan, C.F. Hogan, P.S. Francis, *Drug Test Analysis*, 3 (2011) 145.
16. D. Yuan, S. Chen, R. Yuan, J. Zhang, W. Zhang, *Analyst*, 138 (2013) 6001.
17. S. Kruanetr, P. Pollard, C. Fernandez, R. Prabhu, *Int. J. Electrochem. Sci.*, 9 (2014) 5699
18. C. Qihong, C. Lifen, L. Fang, Q. Bin, L. Zhenyu, C. Guonan, *Anal Bioanal Chem.*, 400 (2011) 289.
19. Z. Cai, Z. Lin, X. Chen, T. Jia, P. Yu, X. Chena, *Luminesc.*, 24 (2010) 367.
20. H. Dai, Y. Wang, X. Wu, L. Zhang, G. Chen, *Biosens. Bioelectr.*, 24 (2009) 1230.
21. C.S. Haslag, M.M. Richter, *J. Luminesc.*, 132 (2012) 636.
22. H.-J. Li, S. Han, L.-Z. Hu, G.-B. Xu, *Chin. J. Anal. Chem.*, 37 (2009) 1557.
23. S.-F. Wang, F. Xie, R.-F. Hu, *Sens. Actuators B: Chem.l*, 123 (2007) 495.
24. H. Yin, Q. Ma, Y. Zhou, S. Ai, L. Zhu, *Electrochim. Acta*, 55 (2010) 7102.
25. R.N. Goyal, S.P. Singh, *Electrochim. Acta*, 51 (2006) 3008.
26. Y. Fan, J.-H. Liu, H.-T. Lu, Q. Zhang, *Coll. Surfaces B: Biointerfac.*, 85 (2011) 289.
27. R. Olivé-Monllau, F.X. Muñoz-Pascual, E. Baldrich, *Sensors and Actuators B: Chemical*, 185 (2013) 685.
28. A. Özcan, Y. Şahin, *Anal. Chim. Acta*, 685 (2011) 9.
29. K. Kerman, D. Ozkan, P. Kara, B. Meric, J.J. Gooding, M. Ozsoz, *Anal. Chim. Acta*, 462 (2002) 39.

30. J.P. Smith, J.P. Metters, D.K. Kampouris, C. Lledo-Fernandez, O.B. Sutcliffe, C.E. Banks, *Analyst*, 138 (2013) 6185.
31. M. Pumera, A. Ambrosi, A. Bonanni, E.L.K. Chng, H.L. Poh, *TrAC Trends in Anal. Chem.*, 29 (2010) 954.
32. Y. Sui, J. Appenzeller, *Nano Lett.*, 9 (2009) 2973.
33. C. Shan, H. Yang, J. Song, D. Han, A. Ivaska, L. Niu, *Anal. Chem.*, 81 (2009) 2378.
34. S. Guo, S. Dong, E. Wang, *ACS Nano*, 4 (2009) 547.
35. B. Seger, P.V. Kamat, *J. Physic. Chem. C*, 113 (2009) 7990.
36. Y. Wang, Z. Shi, Y. Huang, Y. Ma, C. Wang, M. Chen, Y. Chen, *J. Physic. Chem. C*, 113 (2009) 13103.
37. K.S. Novoselov, A.K. Geim, S.V. Morozov, D. Jiang, Y. Zhang, S.V. Dubonos, I.V. Grigorieva, A.A. Firsov, *Science*, 306 (2004) 666.
38. B.C. Lourenção, R.A. Medeiros, R.C. Rocha-Filho, L.H. Mazo, O. Fatibello-Filho, *Talanta*, 78 (2009) 748.
39. M. Arvand, T.M. Gholizadeh, *Coll. Surfac. B: Biointerfac.*, 103 (2013) 84.
40. B. Baś, A. Bugajna, M. Jakubowska, W. Reczyński, A. Smalec, *Electrochim. Acta*, 99 (2013) 190.
41. G.P. Keeley, N. McEvoy, H. Nolan, S. Kumar, E. Rezvani, M. Holzinger, S. Cosnier, G.S. Duesberg, *Anal. Meth.*, 4 (2012) 2048.