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TITLE: Electrochemical oxidation of acetyl salicylic acid and its voltammetric sensing in real samples at a sensitive edge plane pyrolytic graphite electrode modified with graphene.

YEAR: 2014

Publisher citation: KRUANETR, S., POLLARD, P., FERNANDEZ, C. and PRABHU, R. 2014. Electrochemical oxidation of acetyl salicylic acid and its voltammetric sensing in real samples at a sensitive edge plane pyrolytic graphite electrode modified with graphene. International journal of electrochemical science [online], 9(10), pages 5699-5711. Available from: http://www.electrochemsci.org/papers/vol9/91005699.pdf

OpenAIR citation: KRUANETR, S., POLLARD, P., FERNANDEZ, C. and PRABHU, R. 2014. Electrochemical oxidation of acetyl salicylic acid and its voltammetric sensing in real samples at a sensitive edge plane pyrolytic graphite electrode modified with graphene. International journal of electrochemical science, 9(10), pages 5699-5711. Held on OpenAIR [online]. Available from: https://openair.rgu.ac.uk

Int. J. Electrochem. Sci., 9 **(2014)** 5699 - 5711

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Oxidation of Acetyl Salicylic Acid and its voltammetric sensing in real samples at a sensitive edge plane Pyrolytic Graphite Electrode modified with Graphene

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Received: 5 May 2014 / *Accepted:* 17 June 2014 / *Published:* 16 July 2014

We present in this manuscript for the first time the electrochemical oxidation of acetyl salicylic acid and its voltammetric sensing in real samples at a sensitive edge plane pyrolitic graphite electrode (EPPGE) modified with graphene. The electrochemical response of the sensor was improved compared to edge plane pyrolytic graphite electrode and displayed an excellent analytical performance for the detection of acetyl salicylic acid .These characteristics were attributed to the high acetyl salicylic acid loading capacity on the electrode surface and the outstanding electric conductivity of graphene. A linear response was obtained over a range of acetyl salicylic acid concentrations from 10 nM to 100nM into a pH 4 buffer solution (N defined as the sample size N = 7) with a detection limit of 3 nM based on (3-σ/slope). The methodology is shown to be useful for quantifying low levels of acetyl salicylic acid in a buffer solution. The protocol is also shown to be applicable for the sensing of acetyl salicylic acid in human oral fluid samples. A linear response was obtained from 30nM to 150 nM into a human oral fluid solution (N = 7) with a detection limit of 17.3nM. Cyclic Voltammetry (CV) using EPPG modify with graphene has been employed in the proposed method for the determination of ASA in drug preparations and human oral fluid.

Keywords: Acetyl Salcylic Acid, Electrochemical, Sensor, Modified Edge Plane Pyrolytic Graphite Electrode and Graphene.

1. INTRODUCTION

Acetylsalicylic acid (ASA) which is depicted in Figure 1**,** is a salicylate drug, often used as an analgesic to relieve minor aches and pains [1-5], as an antipyretic to reduce fever [1, 6-8], and as an

anti-inflammatory medication [1, 9-10]. The active ingredient of ASA was first discovered from the bark of the willow tree in 1763 by Edward Stone [4]. (Figure 1)

Most of the current analytical methods employed to analyse ASA are generally: high-performance liquid chromatography–mass spectrometry [11-15] and gas chromatography–mass spectrometry [16], ultra performance liquid chromatograghy tandem mass spectrometry [17] and capillary electrophoresis [18] have also been reported for the determinations of ASA. However, many of these methods require several time-consuming manipulation steps, sophisticated instruments and special training.

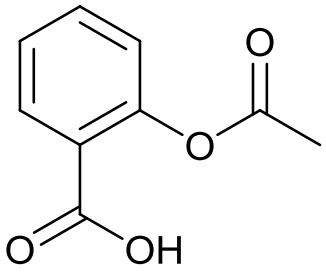


Figure 1. Chemical structure of Acetylsalicylic acid.

Electrochemistry is an advantageous analytical tool, which is cost effective, portable and fast. It has been widely employed in biological matrixes [19-20], pharmaceutical [21] and some drugs containing tertiary amine functional group [19, 22-24] due to its continuance, sensitivity, reproducibility and selectivity towards many target analytes [19, 23, 25-26]. Electrochemical methods including voltammetric techniques have also been developed for the determination of ASA [27-30]. Srivastava et al. [27] used surfactant-modified multiwalled carbon nanotube paste electrode for the determination of ASA in pharmaceutical formulations, urine and blood samples by voltammetry. Tsai et al. [28] investigated the electrocatalytic oxidation of acetylsalicylic acid at multiwalled carbon nanotube-alumina-coated silica nanocomposite modified glassy carbon electrodes. Lu et al.[29] reported an electrochemical sensor based on PATP–AuNPs modified molecularly imprinted polymer film for the detection of ASA. Rynkowski et al. [30] reported Voltammetric studies of acetylsalicylic acid electro oxidation at platinum electrode. These reports showed good detection limits and sensitivity however need extra time consuming modification process that involves various steps in incorporation of the modifier to the substrate is the main drawback.

In the last ten years [31] graphene has attracted a great attention due to its large surface area, high thermal and electrical conductivities, impressive mechanical properties, and low cost [31]. Graphene has been greatly employed in many fields [32-36] due to the superior performances and its potential applications, including nanoelectronics, sensors, nanocomposites, catalysis, capacitors etc. Graphene sheets have extraordinary electronic transport properties and high electrocatalytic activities [37-39], and they have been investigated as electrode materials in optoelectronic devices [40], electrochemical super-capacitors [41], fabricated field-effect transistors [42], and constructed ultrasensitive chemical sensors [43], such as pH sensors [44], gas sensors [45], and biosensors [33].

The determination has been carried out at edge plane pyrolytic graphite sensor (EPPGs) owing to its wide potential window, strong adsorption tendency, low background current and easy

maintenance. Another reason for choosing EPPGs is that it exhibits a better response in comparison to other conventional electrodes like glassy carbon, indium tin oxide and gold electrodes [46-47].

This manuscript describes a novel electrochemical sensor that was fabricated with grapheme-modified EPPGE and the electrochemical properties of the sensor were investigated. To the best of our knowledge, there is no report based on using graphene edge plane pyrolytic graphite modified electrodes for the determination of ASA. It can be used for ultrasensitive determination of ASA in pharmaceutical products with Cyclic Voltammetry techniques. The results show that a grapheme modified electrode exhibits excellent performance for detecting ASA.

2. EXPERIMENTAL SECTIONS

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. Acetylsalicylic Acid containing tablets of different pharmaceutical companies such as Galpharm International Ltd. were purchased from the local market. Pristine Graphene Monolayer Flakes, Dispersion in ethanol was purchased from graphene Laboratories Inc. (Calverton, USA).

2.2 Apparatus

Voltammetric measurements were carried out using a PG 580 (Uniscan Instruments Ltd., UK) potentiostat/galvanostat and controlled by UiEchem software version with a conventional three-electrode system comprised of a platinum wire as auxiliary electrode, Ag/AgCl (saturated. KCl) as reference, and modified edge plane pyrolytic graphite electrode (EPPGE, diameter of 3 mm) with the graphene as working electrode

2.3 Preparation of Gr modified electrode

Before modification, the EPPGE was polished to a mirror finish using finer emery-paper and 0.5 μm alumina slurry followed by rinsing thoroughly with water. After successive sonication in 1:1 nitric acid, acetone, and doubly distilled water, the electrode was rinsed with doubly distilled water. The cleaned EPPGE was dried with nitrogen steam for the next modification. The Gr modified electrode was prepared by casting 5 μL of Gr solution (about 1.67 μg/mL) on the EPPG surface. After the solvent evaporated, the electrode surface was thoroughly rinsed with redistilled water and dried in the air. The obtained electrode was noted as Gr/EPPGE.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of Gr/EPPGE

The surface character of Gr/EPPGE was investigated 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.

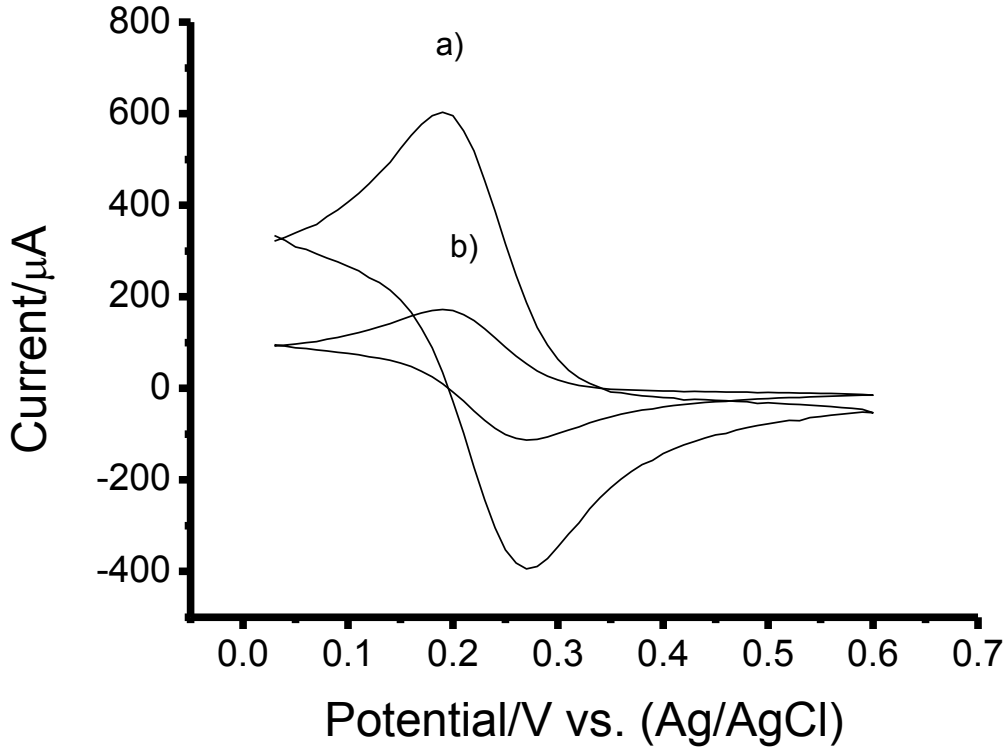


Figure 2. Cyclic voltammograms response detected for (a) EPPGE modified with Gr in 1.0 10^{-3} mol L^{-1} [$K_3Fe(CN)_6$] containing 0.1 mol L^{-1} KCl and b) bare EPPGE. Scan rate: 100 mV/s.

Figure 2 shows the voltammograms obtained at a EPPGE modified with Gr (curve a), and a bare EPPGE (curve b) respectively. A well shaped CV which is depicted in Figure 2, voltammogram b) with a peak-to-peak separation of approximately 70 mV was observed at the bare EPPGE. This shows a reversible redox one electron transfer voltammogram of [$K_3Fe(CN)_6$] with peak currents at Epa (anodic peak potential) = 0.198V and Epc (cathodic peak potential) = 0.273 V. When the EPPGE is modified with Gr (Figure 2a), the current increases from approximately 180 to 600μA. This increase in the peak current is owed to the excellent electric conduction of graphene compared to graphite. There is also a shifting in the peak potential, with peak currents at Epa (anodic peak potential) = 0.213V and Epc (cathodic peak potential) = 0.288 V compared to bare EPPGE.

3.2 Electrochemical behaviors of Acetyl Salicylic Acid on Gr/EPPG

A CV was used to investigate the electrochemical behavior of 10 μM acetyl salicylic acid on a graphene/EPPG and a bare EPPG in phosphate buffer pH4 at a scan rate of 100mVs^{-1}which were recorded and shown in Figure 3.

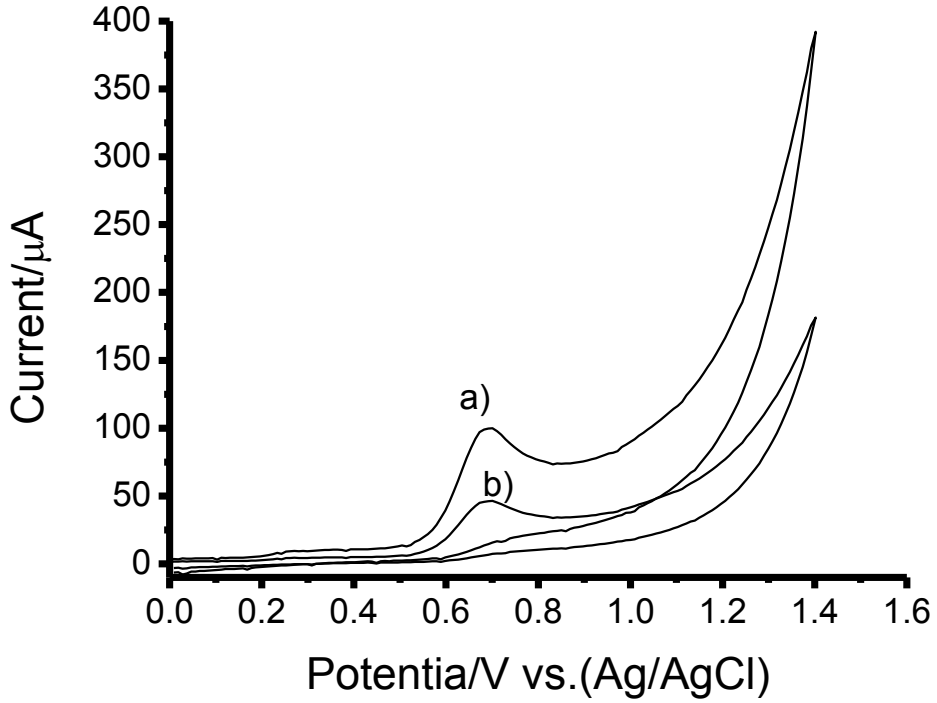


Figure 3. Cyclic voltammetric response recorded at EPPGE a) with Gr and b) without Gr in 10 μM ASA in pH 4 phosphate buffer solution. Scan rate:100 mV/ s.

At the bare EPPGE (curve b), ASA shows an irreversible behavior with relatively weak redox current peak approximately Ip=50μA and an anodic peak potential of Epa = 700mV. However, as can be seen from curve a), ASA exhibits on the graphene-modified EPPG a well-defined oxidation peak at Epa = 720 mV which shifted approximately 20mV compared to curve b) which can be assigned to the oxidation of ASA to Salycilic Acid, as reported in literature[30]. The redox peak currents for Gr/EPPG are higher than that at the bare EPPG.

3.3 Optimization of the experimental conditions

3.3.1 The effect of the scan rates

The effect of the scan rates is studied by using Cyclic Voltammetry for a EPPGE modified with Gr in 1.0 10^{-3} mol L^{-1} [$K_3Fe(CN)_6$] containing 0.1 mol L^{-1} KCl at different scan rates (from inner to outer): 5, 10, 25, 50, 75, 100, 500 and 1000 mV/s which is recorded and shown in Figure 4.

According to the Randles–Sevcik equation $Ip = 2.69\times10^5\ n^{3/2}AD_0\ ½\ C_0\nu^{1/2}$, the scan rates can be obtained from a plot of the voltammetric peak current (Ip) vs. the square root of scan rate ($\nu^{1/2}$).

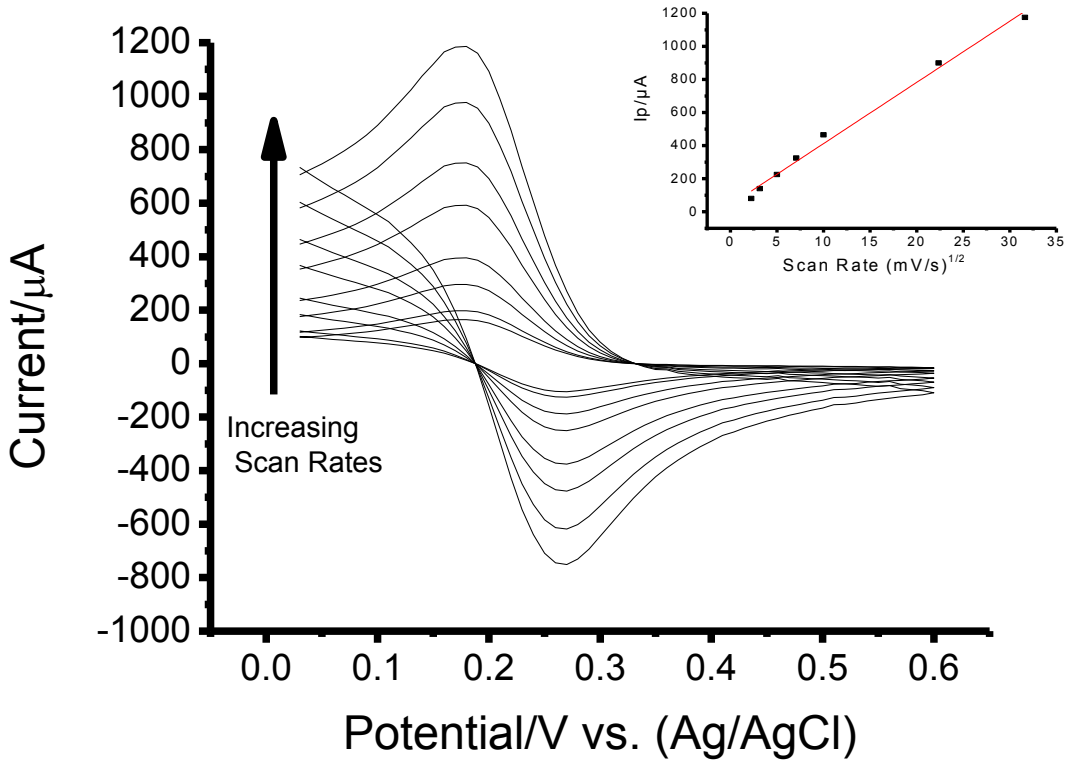


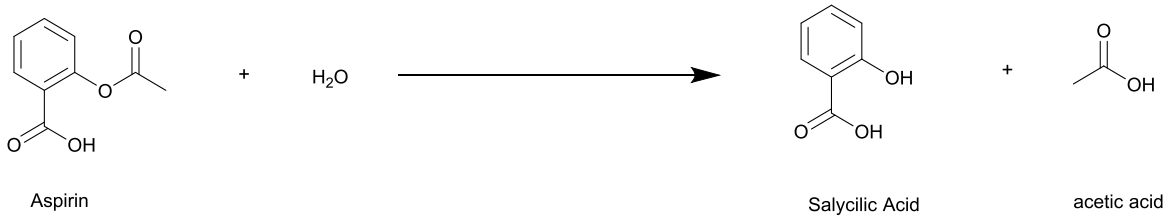
Figure 4. Cyclic voltammograms response observed for EPPGE modify with Gr in 1.0 10^{-3} mol L^{-1} $[K_3Fe(CN)_6]$ containing 0.1 mol L^{-1} KCl at different scan rates (from inner to outer): 5, 10, 25, 50, 75, 100, 500 and 1000 mV/s.

The redox peak currents at the graphene-modified EPPGE increased linearly with the scan rate in the range from 5 to 1000mV/s (inset, Figure 4; linear regression equation: Ip = 43.786 + 47.654*v*, R = 0.993). This indicates that the modified-electrode reaction is a surface confined process.

3.3.2 The effect of Ph

ASA in aqueous media oxidizes Salicylic Acid and Acetic Acid as depicted in Scheme 1.

We note that the pKa of ASA is reported to be 3.49 within the literature[48],therefore, ASA was prepared in a phosphate buffer solution at pH 4 as depicted in Figure 4. At pH 4, ASA is easily oxidised compared to more basic pHs as is demonstrated in Figure 5 a) and b).



Scheme 1. Mechanism of the Oxidation of Aspirin.

The effect of Peak Current vs. pH and Peak Potential vs. pH was investigated. The experiment results showed that electrochemical responses of ASA are highly dependent on the pH over a solution range of pH 4 to pH 10 as illustrated in Figure 5 a) and b).

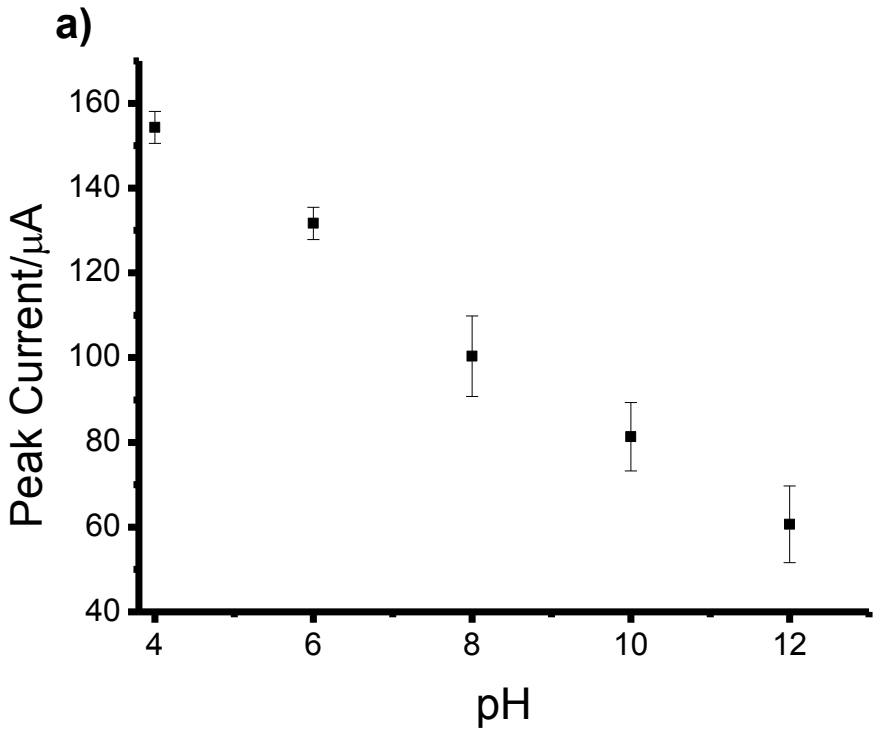
The pH of the supporting electrolyte is an important factor that affects redox behaviour of biomolecules and drugs. The influence of buffer pH on the electrochemical response of ASA was recorded and the data were shown in Figures 5a and 5b respectively (in pH range from 4 to 10). The results indicated that the peak current (Ipa) and peak potential (Epa) were affected by the pH of the solution. As it can be seen the maximum current response of ASA was obtained at pH 4.0. The oxidation peak potential of ASA increases linearly (from pH 4 to 10) with increase in pH which indicates that the electro-oxidation process becomes easier at lower pHs as shown in Figure 5b. At pH 4 there is a drop of the potential which indicates that pH 4 is the optimum pH for the ASA oxidation. Therefore, pH 4.0 was chosen for the subsequent analytical experiments.

The peak height (Ip) vs. pH plot which is depicted in Figure 5a is linear (from pH 4 to pH 10) and can be presented by the following equation:

Ip/μA (pH 4-10)=-8.36x+172.16 versus Ag/AgCl for ASA with correlation coefficients of 0.99.

The peak potential (Ep) vs. pH plot which is shown in Figure 5b is linear (from pH 4 to pH 10) and dependent on the anodic peak potential of the analyte on the pH and can be presented by the relation:

E_p/V (pH 4-10) = 0.0063 V/pH + 0.7321 versus Ag/AgCl for ASA with correlation coefficients of 0.99.



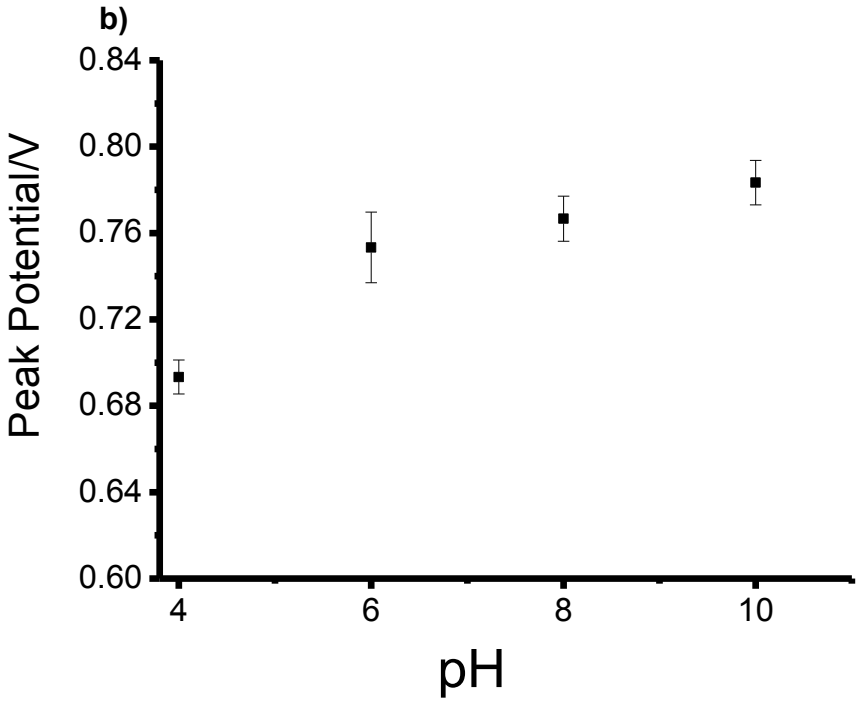


Figure 5. (a) A plot of peak current as a function of pH for the electrochemical oxidation of 10μM ASA using EPPGE modified with Gr. Scan rate: 100mV/s. (b) A plot of peak potential (Ep) as a function of pH for the electrochemical oxidation of 10μM ASA using EPPGE modified with Gr at Scan Rate: 100mV/s.

3.3.3. Effect of concentration

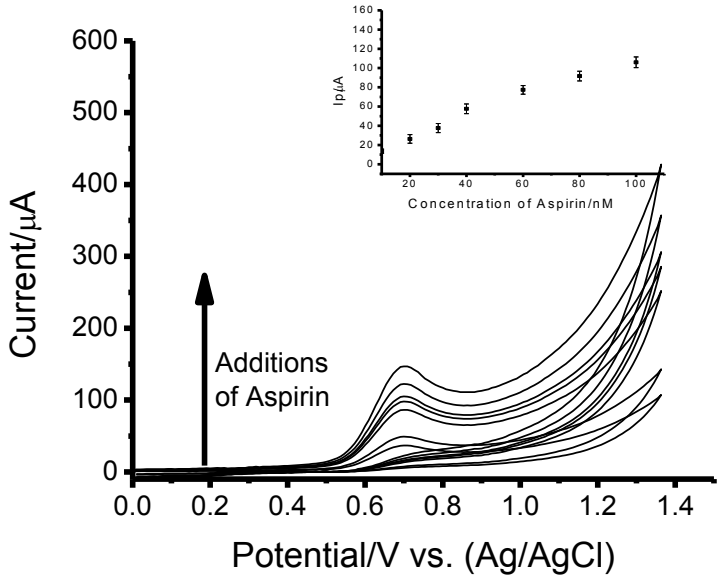


Figure 6. A) Cyclic voltammogram response observed for phosphate buffer solution pH 4 at EPPGE modified with Gr over a range of ASA concentrations from 10nM to 100nM. Scan Rate: 100mv/s. B) A plot of peak height (ip), a function of ASA concentration using EPPG modified with Gr at a scan rate: 100mV/s.

After determining that the optimum experimental pH for the oxidation of ASA using the EPPGE modified with Gr was that of pH 4, a range of concentrations of ASA was explored. The oxidation of ASA occurs at high positive potential as is illustrated in Figure 6a where the oxidation peak observed depicted in Figure 6b.

The Ipa was linearly related to the ASA concentration in the range of 10–100nM as depicted in Figure 6b. The linear regression equation was (Ip/μA= 1.7610x+18.901 with N=7, R^2 = 0.9914) over the analytical range studied 10 nM to 100nM into a pH 4 buffer solution (*N* = 7) with a detection limit of 3 nM (based on 3-σ/slope).

3.4 Analytical Applications

3.4.1 Pharmaceutical Analysis

The method that was developed was used to determine ASA (0.075g/tablet) commercial tablets (UK, Galpharm International Ltd.). The tablets were ground to powder and dissolved in buffer solution. Using the proposed method, the concentration of ASA of the pharmaceutical preparation was detected (0.069 g/tablet), which is in good agreement with the content of ASA provided by the manufacturer. The recoveries of the tests were in the range from 95.2% to 101.6% .The sensor was also validated with ASA - spiked human oral fluid samples, and the recovery of the spiked sample was 102.9%. These results indicate that the sensor developed in this work has high sensitivity and selectivity for detecting ASA in commercial tablets and human oral fluid samples

3.4 Electroanalytical Applications of the proposed method to detect Aspirin in human oral fluid

Following confirmation that successful determination of ASA was possible in ideal conditions utilising a standard pH 4 phosphate buffer, the viability of the analytical protocol was tested in relation to detection within analytically relevant media.

Aspirin is clinically employed for analgesic and antipyretic effects and it is taken orally, therefore, it is considered worthwhile to determine the concentration of ASA in human oral fluid. First, attention was turned to exploring the analytical sensing of ASA in human oral fluid. Additions of ASA were made into human oral fluid solution over the concentration range of 30 to 150nM which is depicted in Figure 7.

As is shown in Figure 7, the calibration plot resulting from the addition of ASA is linear over the concentration range 30 to 150nM. *(*Ip/μA = 0.8132x+120.132, R^2 = 0.9838 and N = 7) with a detection limit of 17.3 nM (based on 3-sigma) was studied. Returning to the observed analytical response, critically the human oral fluid solution employed was not modified in any way prior to use. The lack of sample pretreatment highlights the truly useful nature of the analytical protocol when utilised for real-world applications

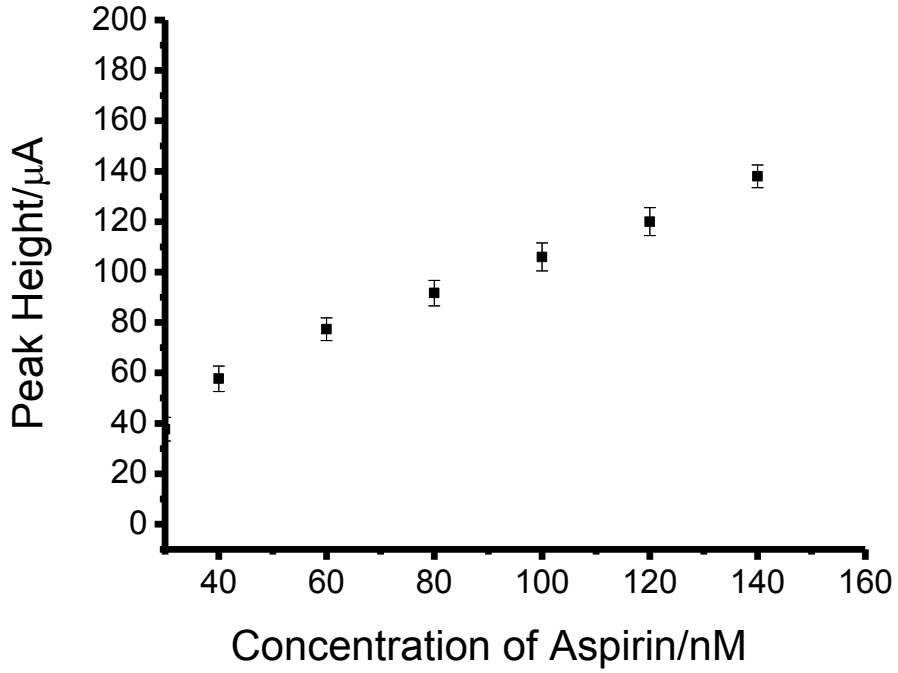


Figure 7. A calibration plot of peak height (Ip), as a function of ASA concentration corresponding to the addition of ASA into human oral fluid sample solution over the concentration range 30–150nM using EPPGE modified with Gr. Scan Rate: 100mV/s.

3.5 Summary and comparison of current analytical approaches for the sensing of acetyl salicylic acid

A summary and a comparison of our method depicted in Table 1 of current analytical approaches for the sensing of acetyl salicylic acid are shown. (Table 1)

It is important to pay special attention to the work reported by Tsai et al[28] who investigated the electrocatalytic oxidation of acetylsalicylic acid at multiwalled carbon nanotube-alumina-coated silica nanocomposite modified glassy carbon electrodes This report exhibits good detection limits and sensitivity however the drawbacks are the need of extra time consuming modification process that involves various steps in incorporation of the modifier to the substrate.

Table 1. Comparison of the proposed sensor with the other techniques earlier reported for the determination of acetyl salicylic acid in real samples and buffer.

Analytical Method	Analytical Linear Range	Limit of Detection	Matrix	Reference
This method	30-150nM	17.3nM	Human Oral Fluid	
This method	10-100 nM	3 nM	Buffer	
MWCNT–ACS/GCE	15-65μM	3.77 μM	Buffer	[28]
UPLC-MS	0.1-1μg/g	Not report	Buffer	[17]

Bare EPPGE	0.02-100μM	0.01 μM	Buffer	[49]
UPLC	32-98μg/mL	Not report	Buffer	[50]
GC-MS	0–1 μg/mL plasma.	Not report	Human Plasma	[16]
GCE/Gr		20.2nM	Buffert	[51]

4. CONCLUSIONS

The proposed protocol demonstrates for the first time the successful application of EPPGE modified with Gr for the determination of ASA in pharmaceuticals products as well as human oral fluid samples with excellent sensitivity and selectivity. Long term stability and excellent reproducibility of the proposed sensor with essentially no pretreatment or maintenance offers a good possibility for extending the method for routine analysis of ASA. A linear response is observed for a buffered solution (Ip/μA= 1.7610x+18.901, R^2 = 0.9914 and N = 7) over the range 10 nM to 100nM into a pH 4 buffer solution with a detection limit of 3 nM (based on 3-sigma). ASA is also linear over the concentration range 30 to 150nM. (Ip/μA = 0.8132+120.132, R^2 = 0.9838 and N = 7) with a detection limit of 17.3nM (based on 3-sigma) studied for a human oral fluid. The additional advantage of the approach is that no sample pretreatment is required and rapid testing times with on-site determination are possible.

ACKNOWLEDGEMENTS
C. F. expresses gratitude to Robert Gordon University and S_3C (Scottish Sensor Systems Centre) for financial support via a start-up grant and S. Beddoes for her advice and comments. CF,P.P and R.K would like to thank the support of the IDEAS Research Institute, RGU. R.P. would also like to thank the support of the IDEAS Research Institute, RGU. S. K. would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC) for financial support and Mahasarakham University for facilities.

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