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**Amperometric micro pH measurements in oxygenated saliva**

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## Abstract

An amperometric micro pH sensor has been developed based on the chemical oxidation of carbon fibre surfaces (diameter of 9  $\mu\text{m}$  and length of *ca.* 1 mm) to enhance the population of surface quinone groups for the measurement of salivary pH. The pH analysis utilises the electrochemically reversible two-electron, two-proton behaviour of surface quinone groups on the micro-wire electrodes. A Nernstian response is observed across the pH range 2-8 which is the pH range of many biological fluids. We highlight the measurement of pH in small volumes of biological fluids without the need for oxygen removal and specifically the micro pH electrode is examined by measuring the pH of commercial synthetic saliva and authentic human saliva samples. The results correspond well with those obtained by using commercial glass pH electrodes on large volume samples.

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**1. Introduction**

Salivary pH measurement has gained attention over the past 10 years due to its possibility as a biomarker for diseases such as anxiety disorder, gingivitis, and tooth decay<sup>1-5</sup>. Among the biological fluids, saliva is one of the most preferable and practical specimens for health monitoring due to the possibility of non-invasive collection, especially in comparison to blood. Most research studies on salivary pH have focussed on oral diseases including dental caries and periodontal (gum) diseases<sup>5, 6</sup>. Salivary pH is normally maintained near neutrality (pH 6.7-7.3). When the pH of the saliva specifically on dental plaque or in dental cavity is below a critical value of 5.5, tooth enamel will demineralise resulting in dental decay. Therefore, pH measurement may provide a readily to identifying active or inactive caries<sup>7-9</sup>.

A great number of methods have been utilised for pH determination including voltammetry<sup>10-12</sup>, potentiometry<sup>13-15</sup>, and the use of fluorescent agents<sup>16, 17</sup>. The pH of bulk aqueous solutions are easily measured using traditional glass electrodes. However, measuring pH inside a small volume requires an appropriate micro-scale pH probe. In addition in the biological context, a number of disadvantages of the glass electrode have been recognised in terms of the “sodium error” and the “acid error”, the fragility of glass, the large probe size, and the lack of disposability<sup>18, 19</sup>. A normal bulb tip diameter of the commercial glass electrode is 10-12 mm which is not a practicable size for *intra-oral* use, while the smallest bulb tip that is manufactured is 1.3 mm<sup>20</sup>; however, this micro electrode has a high fragility and is non-disposable.

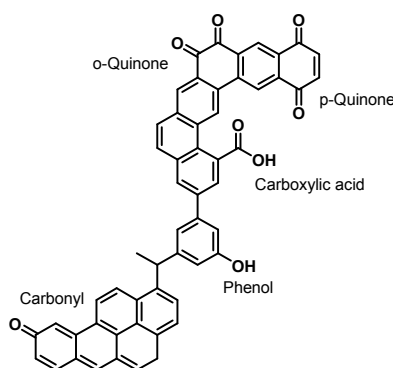
To address such limitations of glass electrodes, considerable interest has focused on the design and development of non-glass pH sensors. Examples include voltammetric electrochemical pH sensors utilising a pH sensitive layer of polymeric films<sup>13, 21</sup>, enzymes<sup>22</sup>, and organic redox species<sup>23</sup> added to the surface of electrodes. The approach of attaching

redox species often involves utilisation of the pH dependence of quinone/hydroquinone (Q/QH<sub>2</sub>) redox species<sup>23, 24</sup>. The Nernst equation can be used to quantify the voltammetric of these redox couples,

$$E_{mid} = E_{formal}^{\circ} - \frac{2.3RTm}{nF} pH$$

where m is number of protons and n is number of electrons involved in the redox process<sup>25</sup>.

Thus assuming a reversible redox process then there is a direct relationship between the peak potential and pH. In particular, for m and n = 2, slope of *ca.* 60 mV per pH unit is predicted at 25°C.



**Scheme 1** Representation of various functional groups present on graphitic carbon surfaces, adapted from Ref. 26.

In addition to pure graphitic structures, carbon surfaces contain of a great number of different surface functional groups including quinone moieties as shown in Scheme 1<sup>26, 27</sup>. In 2014, Min *et al.* reported using unmodified EPPG and glassy carbon electrodes for pH determination<sup>28, 29</sup>, where the pH sensitive nature of intrinsic surface quinone moieties was exploited. In the present paper, we extend the concept by enhancing the level of intrinsic quinone groups on a carbon microfibre (*ca.* 5-10  $\mu$ m) to develop a micro pH sensor for use with limited volumes of biological fluids or in vivo application, focussing specifically on measurements in saliva.

The use of carbon fibres for pH sensing has been reported by several research groups<sup>11, 30-32</sup>. In most cases, chemical compounds containing quinone moieties have been used to modify the carbon fibre surface<sup>31, 32</sup>. Others have reported the use of electrochemical activation of the carbon surface to increase the quinone population<sup>32</sup>. Chemical oxidation of carbon fibre surface is another interesting method for activation of surface functionalities especially quinone groups<sup>33</sup>, for example Mathur *et al.* reported the use of potassium permanganate solutions to modify carbon fibres to create quinone functionality<sup>34</sup>. Hitherto, some others oxidants which are oxidising acids, oxygen plasma, and hydrogen peroxide have been reported<sup>35, 36</sup>. However, none have used for measurement of pH in biological media such as saliva.

As aforementioned, the quinone/hydroquinone redox couple can be exploited for pH sensing. However, these species are known to be electrocatalytic towards oxygen reduction<sup>37-39</sup>. Owing to the presence of oxygen in general biological specimens, the use of quinone groups for pH sensing in biological samples contained oxygen could result in an error of measurement arising from mediated oxygen reduction. Consequently, the challenge for this work is to measure pH in oxygenated biological samples, especially for applications in the mouth where it is not possible to remove oxygen from the solutions.

In this paper, we investigate the use of carbon fibre electrodes for the measurement of salivary pH by highlighting the pH dependence of the reduction of quinone groups on a carbon fibre surface. In addition, we report a simple chemical modification of carbon fibre surface to enhance the population of quinone groups. The voltammetric measurement of different pH buffer solutions is studied using cyclic voltammetry and square wave voltammetry. The present paper also demonstrates pH measurement of non-degassed synthetic saliva solutions which we use as a model. The measurement of pH in oxygenated solution performs on the basis for applying to measure the pH in the mouth. Finally, we

evidence the capability of the carbon fibre micro-wire electrode to measure pH levels in oxygenated authentic human saliva.

## 2. Experimental

### 2.1 Reagents and solutions

All chemicals used in this work were of analytical grade and were used as received without further purification. Solutions were prepared using deionized water (Millipore) with a resistivity of 18.2 MΩ cm at 25 °C.

The buffer solution were prepared using citric acid/sodium citrate for the pH range 2.5-5.0 and monosodium phosphate/disodium phosphate for the pH range 5.0-9.0. All solutions contained 100mM KCl as supporting electrolyte. pH buffers were freshly made daily and measured using a Hannah pH213 pH meter with calibration using Duracal buffers (Hamilton) of pH 4.01 ± 0.01, 7.00 ± 0.01, and 10.01 ± 0.01. All pH buffers measurements were carried out in a degassed system where solutions were purged with pure N<sub>2</sub> gas (BOC, Guildford UK) prior to experiments for a minimum of 20 minutes.

Synthetic saliva (pH 7.5) was prepared with the composition listed in Table 1 (AFNOR standard: S90-701)<sup>40, 41</sup>. This prepared synthetic saliva solution was designated as *literature synthetic saliva*. 1 M HCl and 1 M NaOH were used for adjusting the pH of literature synthetic saliva to obtain the required pH. *Commercial synthetic saliva* was purchased from Synthetic Urine e.K. manufactured according to a currently standardised production process pursuant to DIN 53160-1<sup>42</sup>. For real saliva samples, informed consent was obtained from all the subjects in this research. Real saliva samples were collected no earlier than 30 minutes after a meal or drink using a salivette sampling device (Sarstedt, Germany). The small cotton swab from salivette was gently chewed for one minute. Recovery of saliva from the swab was carried out by centrifugation of the salivette for 15 min at 2400 rpm.

**Table 1** Composition of synthetic saliva (AFNOR standard S90-701)<sup>40, 41</sup>

Chemicals	Concentration	
Na <sub>2</sub> HPO <sub>4</sub>	0.260 g/L	1 mM
KH <sub>2</sub> PO <sub>4</sub>	0.200 g/L	1.5 mM
NaHCO <sub>3</sub>	1.500 g/L	18 mM
KSCN	0.330 g/L	3 mM
NaCl	6.700 g/L	115 mM
KCl	1.200 g/L	16 mM

**2.2 Apparatus**

Electrochemical experiments were performed using a three electrode system in a Faraday cage held at 25 °C using a commercial potentiostat, PGSTAT 101 (Metrohm-Autolab, Netherlands). 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 fabricated carbon fibre microwire electrodes were used as a working electrode (detailed in section 2.4 electrode preparation).

**2.3 Chemical modification of carbon fibre surface**

Carbon fibre used in this work was a pitch-based material (9 µm diameter, GoodFellow). The surface of the as-received fibres is coated with 1.0% epoxy in order to enhance their stiffness. The carbon fibre was boiled in acetone for 1 hour to remove the



coating and expose the carbon surface <sup>43</sup>. After boiling in acetone, the treated carbon fibre was immersed in room temperature acetone and left to dry.

Chemical modification of carbon fibre surface was carried out and optimised. The de-coated carbon fibre was oxidised with aqueous solution of 0.03 M potassium permanganate at 85°C for 5, 10, 20, and 30 minutes <sup>34</sup>. The samples were washed thoroughly with distilled water and then dried in an oven at 60°C for 2 hours.

## 2.4 Electrode preparation

The oxidised carbon fibres were attached to a conducting wire (0.2 mm diameter) of *ca.* 6 cm in length, using electrically conducting silver loaded epoxy adhesive (RS Components Ltd.) to allow the microfiber working electrode to be connected for electrochemical measurements. The adhesive was set by heat treatment in the oven at *ca.* 80 °C for 20 minutes. The microfiber was then threaded through a plastic pipette tip leaving only the carbon fibre protruded out. The plastic tip was sealed with cyanoacrylate adhesive in order to prevent electrical leakage and the carbon fibre was cut to a length of *ca.* 1 mm <sup>44</sup>.

## 2.5 Electrochemical measurements

A range of pH 2-8 buffer solutions was prepared and their pH measured using a commercial glass electrode prior to experiments. “*Literature synthetic saliva*” was made up and its pH adjusted to obtain a desired pH using 1.0 M HCl or 1.0 M NaOH in the range from 3 to 8.5. The pH range investigated was chosen on the basis that most biofluids will fall within pH 3 to pH 9 <sup>45, 46</sup>. A small volume (*ca.* 5 mL) of each solution was transferred to an electrochemical cell. For buffer solutions, the bubbling of nitrogen gas was used to remove dissolved oxygen. Note that for synthetic saliva and real saliva sample, the electrochemical measurements were carried out without degassing the solutions with nitrogen in order to demonstrate the possibility of application to pH

measurement in an oral cavity or in vivo. All the electrochemical measurements were performed in a Faraday cage which was thermostatted to maintain temperature of  $25 \pm 2$  °C. The platinum mesh (counter electrode) was flamed before the experiment to ensure a clean set up. At least 3 experimental scans were carried out for each experiment. For the measurement of cyclic voltammetry, current integration CV was used in this work. Note that in current integration CV the total current is sampled during the whole potential step, while in contrast the current from a normal staircase CV is measured at a specific moment during the step. In systems involving time dependent process and surface species, there is a difference between the voltammetric responses for a reversible redox process from staircase CV and current integration CV<sup>47</sup>. Specifically the voltammetric response of surface bound species may be completely unobservable via staircase CV if the reaction takes place at the very beginning of a step or at different point that the current is sampled. In this work, surface quinone redox reaction is the process of interest. Therefore, current integration CV was used in all cyclic voltammetry experiments to avoid any problems from missing or reducing current measurements from a surface bound reaction.

3. Results and discussions

First, chemical modification of the carbon fibre is optimised to obtain a procedure which maximises the surface coverage of quinone functionality. Second, the voltammetric measurement of buffer solutions is studied in a degassed system using both cyclic voltammetry and square wave voltammetry. Lastly, the carbon fibre micro-wire electrode is carried out to measure the pH of synthetic saliva and then applied to human saliva samples.

3.1 Chemical modification of carbon fibre surface

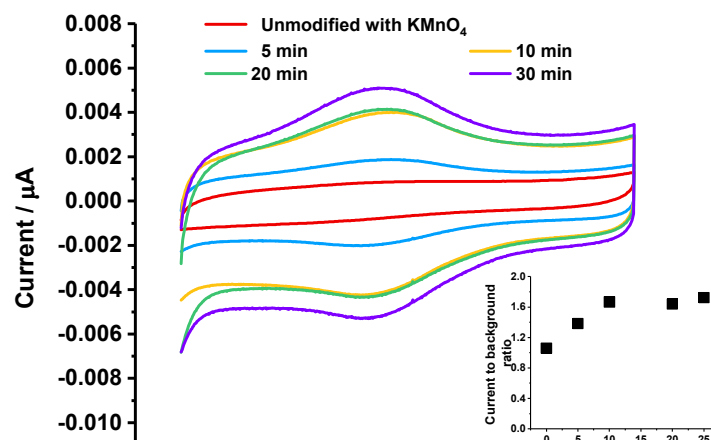
As discussed above, carbon substrates intrinsically exhibit quinone moieties on their surfaces. Cyclic voltammograms of unmodified carbon fibres were recorded in 0.01 M HNO<sub>3</sub> (pH 2.2) with 100 mM KCl as a supporting electrolyte at 100 mV s<sup>-1</sup> (Supporting

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information, SI1). The voltammetric response shows oxidation/reduction peaks for the quinone/hydroquinone (Q/QH<sub>2</sub>) redox couple. Specifically an oxidation peak potential occurs at 0.196 V and a reduction peak potential is seen at *ca.* 0.127 V vs. SCE. However, the magnitude of the peak from an *unmodified* carbon fibre is very small (0.46 nA and 0.07 nA for the oxidation and reduction peaks respectively). This suggests that the population of quinone groups on the unmodified carbon fibre is low. Therefore, our aim has been to increase the surface coverage of quinone moieties through the chemical oxidation of the carbon fibre surface. Several oxidants, including oxygen plasma, oxidising acids (HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and H<sub>3</sub>PO<sub>4</sub>), potassium permanganate (KMnO<sub>4</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), have been reported previously<sup>34-36</sup>.

In this work, KMnO<sub>4</sub> was chosen as oxidising agent to modify the carbon fibre surface due to the simple chemical procedure involved compared to other strong oxidising acids. The modification of carbon fibre surface was accomplished by simply oxidising its surface in aqueous 0.03 M KMnO<sub>4</sub> solution. Note that this concentration of KMnO<sub>4</sub> was reported by Mathur *et al.* for oxidation of polyacrylonitrile-based carbon fibre<sup>34</sup>. Oxidation of the carbon fibre generates structural defects which contain oxygen functional groups<sup>34, 35</sup>.

In order to obtain the best conditions for the modification of carbon fibre surface, an optimisation of the time of oxidation was made. Briefly, carbon fibres were boiled in aqueous 0.03 M KMnO<sub>4</sub> solution at 85 °C for 5, 10, 20, and 30 minutes. After the modification process, the micro-wire electrodes were constructed as described in section 2.4.



**Fig. 1** Optimisation of the chemical modification time in aqueous solution of 0.03 M  $\text{KMnO}_4$ . CVs at unmodified and modified carbon fibre electrodes in 0.01 M  $\text{HNO}_3$  in 100 mM KCl supporting electrolyte (pH 2.2) at a scan rate of  $0.1 \text{ V s}^{-1}$ .

In the first step, the voltammetric response of carbon fibre micro-wire electrode without treatment in  $\text{KMnO}_4$  was evaluated in 0.01 M  $\text{HNO}_3$  (pH 2.2) with 100 mM KCl as supporting electrolyte at  $0.1 \text{ V s}^{-1}$ . A typical CV signal can be seen as a red line in Fig. 1, with an oxidation peak potential of *ca.*0.211 V and a reduction peak potential of *ca.*0.136 V vs. SCE. Further experiments with the modified carbon fibres were performed using the same procedure as above in 0.01 M  $\text{HNO}_3$  with 100 mM KCl. Fig. 1 shows the overlaid CVs of the unmodified carbon fibre compared with the  $\text{KMnO}_4$  modified carbon fibres. Well-defined peaks were obtained in all cases. The magnitude of the peak current was observed to increase as a function of the oxidation time. However, not only does the peak current increase, so also does the background capacitive response. In order to select the optimal oxidation time, the current to background ratio was considered. As shown in an inset of Fig. 1, on increasing the oxidising time from 0 to 10 minutes, the current to background ratio enhanced significantly. For longer oxidation times the current to background ratio slightly increased. Specifically, the current to background ratio reaches a near plateau at *ca.* 10 minutes. Therefore, 10 minutes oxidation time was selected as the preferred chemical modification for the carbon fibre surface. Note that, it is known that  $\text{KMnO}_4$  prepared in acid (such as  $\text{H}_2\text{SO}_4$ ) is a stronger oxidising agent, and so we tried to modify the carbon surface using  $\text{KMnO}_4$  prepared in different concentrations of  $\text{H}_2\text{SO}_4$ . Accordingly the modification of the carbon fibre surface

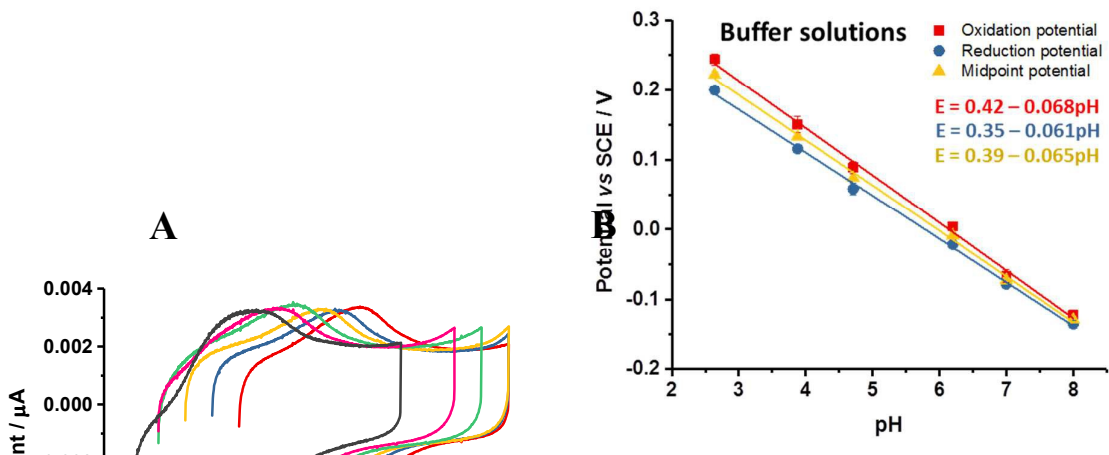
was also carried out in 0.03 M  $\text{KMnO}_4$  solution prepared in low concentrations (range of 5-20 mM  $\text{H}_2\text{SO}_4$ ) or concentrated  $\text{H}_2\text{SO}_4$  (18 M) and cyclic voltammograms recorded in 0.01 M  $\text{HNO}_3$  with 100 mM KCl as a supporting electrolyte at  $100 \text{ mV s}^{-1}$  (Supporting information, SI2). At low concentrations of  $\text{H}_2\text{SO}_4$  (5-20 mM), the results show that the current to background ratio did not greatly improve. For a modification of carbon fibre in  $\text{KMnO}_4$  prepared in concentrated  $\text{H}_2\text{SO}_4$  (18 M), the carbon fibre surface was destroyed. Consequently, the mild conditions of aqueous  $\text{KMnO}_4$  solution was utilised as the chemical oxidising agent. Reproducibility and stability of micro-wire electrodes were also investigated. A reproducibility of responses between three different constructed micro-wire electrodes shows a small standard deviation ( $\pm 0.006 \text{ V}$ ), indicating a good reproducibility of the electrodes. In terms of stability of electrodes, we performed the CV measurements of the same micro-wire electrode over multiple scans. The oxidation potential, reduction potential, and midpoint potential remained at same position of each potential, showing the excellent stability of micro-wire electrode; see Supporting Information (SI3) for the raw data.

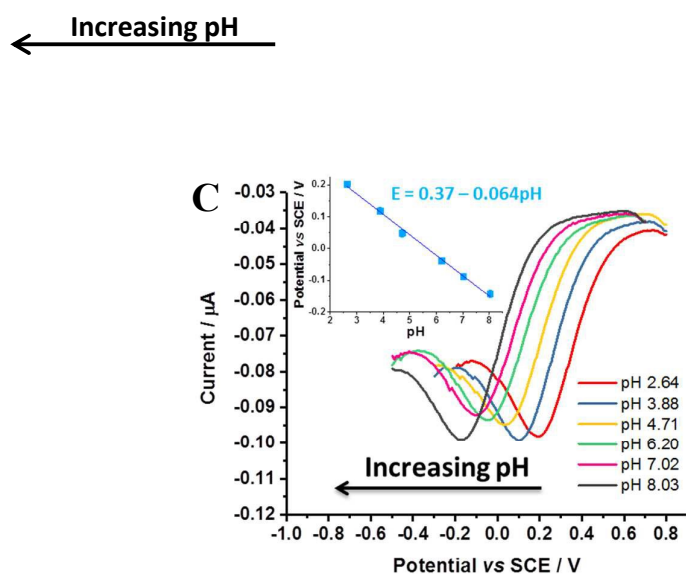
### 3.2 Voltammetric measurement of the pH of buffer solutions

The surface quinone/hydroquinone redox couple was investigated for voltammetric pH measurement. Cyclic voltammetry in a range of buffer solutions in the presence of 100 mM KCl supporting electrolyte was performed using carbon fibre micro-wire electrodes. The CV response was first measured from -0.2 V to +0.7 V for a buffer solution of pH 2 at a scan rate of  $0.1 \text{ V s}^{-1}$ , and then the potential window was adjusted as a function of the pH. The cyclic voltammetric response for quinone groups on the carbon fibre surface was recorded in solutions of pH 2 – 8. All measurements in this section were performed in a degassed system by bubbling nitrogen gas to remove dissolved oxygen in solutions; measurements without degassing are discussed later in the paper. Fig. 2(A) shows the overlaid cyclic

voltammograms of quinone groups on carbon fibre surface in different pH buffer solutions at scan rate of  $0.1 \text{ V s}^{-1}$ . It can be observed that by increasing pH, peak potentials shift in the cathodic direction towards more negative values. For analysis of the data, the oxidation, reduction, and midpoint potentials were all noted. The corresponding plots against pH are shown in Fig. 2(B). The gradient of the slopes were 68 mV, 61 mV, and 65 mV per pH reflecting an almost Nernstian response corresponding to a two-electron, two-proton electrochemical process.

After performing cyclic voltammetry and observing a linear response between the peak potential and pH, further studies using square wave voltammetry (SWV) were carried out using optimised SWV parameters: frequency 75 Hz, step potential 10 mV, and amplitude 100 mV, over the entire pH range studied. As can be seen in Fig. 2(C), a single SWV peak was observed by scanning from positive to negative potential. It can be observed that the peak potentials shift in the cathodic direction towards more negative potentials on increasing the pH. By measuring the reduction peak potentials and plotting against pH, a linear response was obtained in the pH 2-8 range, as shown in the inset of Fig. 2(C). The gradient of the slope was 64 mV per pH, consistent with the Nernst equation for a temperature of  $25^\circ\text{C}$  for a two-proton, two-electron process. From this, we can see that both CV and SWV can be exploited for measurement of pH in buffer solutions.



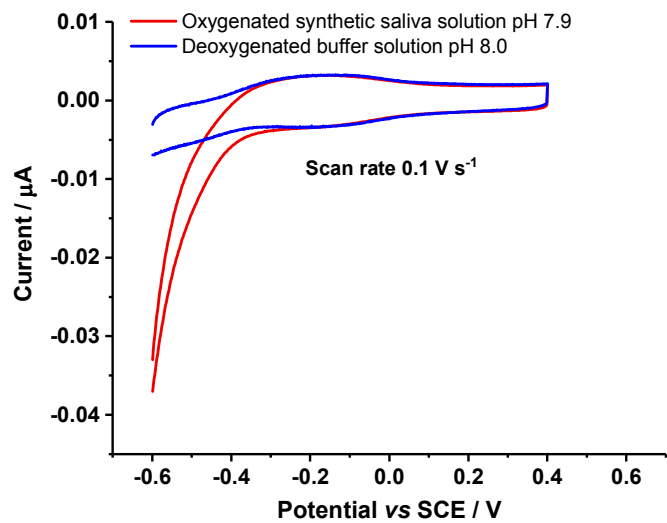


**Fig. 2** Quinone peaks in cyclic voltammetry at a scan rate of  $0.1 \text{ V s}^{-1}$  (A) and quinone reduction peaks in square wave voltammetry (C) on chemical modified pitch-based carbon fibre obtained in buffer solutions, ranging in pH from 2 to 8. Calibration plots of peak potential against pH obtained from CV (B) and SWV (inset of Fig 2C). All of the buffer solutions were degassed before the performed electrochemical measurement.

### 3.3 Investigating the measurement of pH in saliva

### 3.3.1 pH measurement of synthetic saliva

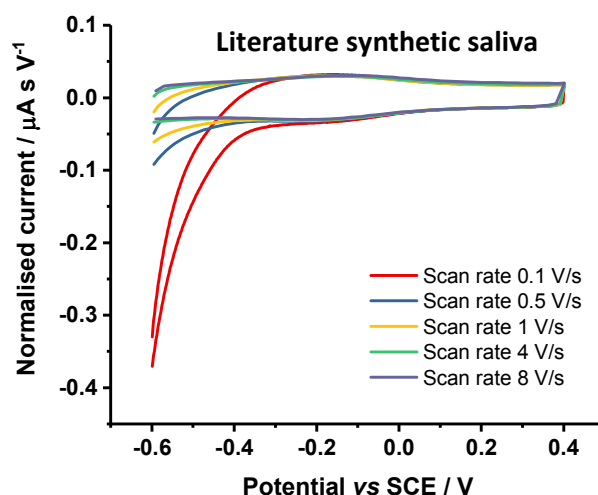
Synthetic saliva was prepared according to the composition and concentrations specified in literature<sup>40, 41</sup> (*‘literature synthetic saliva’*). To partially mimic the use of the micro-wire electrode in situations such as in the mouth or in vivo, we performed CV in solutions without removing dissolved oxygen. We first carried out CV in *literature synthetic saliva* of pH 7.9 at scan rate of 0.1 V s<sup>-1</sup> to observe the surface quinone behaviour in solutions without any degassing. An oxygen reduction peak was observed while the surface quinone oxidation/reduction peaks also appeared as shown in Fig.3 (red line). The slight overlaying of the two peaks can be observed as revealed by comparison with deoxygenated buffer (blue line). Quinone groups present on the surface of carbon electrode have been known under certain conditions to catalyse oxygen reduction<sup>37-39</sup>. Consequently, this together with the peak due to direct reduction of the oxygen at the carbon surfaces overlapping with the quinone signal may result in the error of peak potential measurement if CV at 0.1 V s<sup>-1</sup> is used for the pH measurement.



**Fig. 3** CV of chemical modified carbon fibre in literature synthetic saliva pH 7.9 without degassing compares (red line) with CV of chemical modified carbon fibre in buffer solution with degassing (blue line) at scan rate of 0.1 V s<sup>-1</sup>, showing a presence of oxygen reduction peak in oxygenated solution (red line).

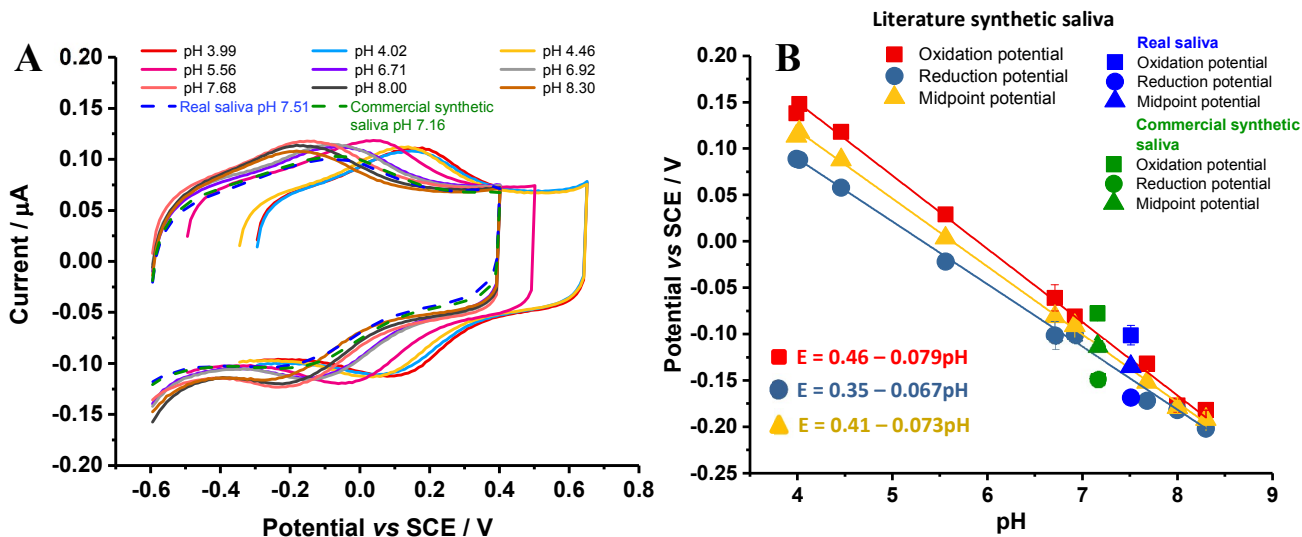


To address this problem, pH measurement in 'literature synthetic saliva' without degassing using a micro-wire electrode was next investigated through a variable scan rate study. Different scan rates ( $0.1 - 8 \text{ V s}^{-1}$ ) were applied to 'literature synthetic saliva' at pH 7.9 without degassing. The aim was to remove the influence of the oxygen reduction peak. Fig. 4 shows overlaid CVs of micro-wire electrode in literature synthetic saliva at different scan rates. The figure shows no oxygen reduction peak at the high scan rate in the potential range presented ( $0.5 - 8 \text{ V s}^{-1}$ ). This indicates that interference from oxygen reduction on micro-wire electrode can be effectively avoided by using high voltammetric scan rates. This reflects both the irreversibility of the  $\text{O}_2/\text{H}_2\text{O}_2$  couple and the likely "outrunning" of any catalytic reduction of oxygen mediated via the quinone. Note that the oxygen reduction process is electrochemically irreversible and hence shifts to more negative potentials with increasing scan rates in contrast to the electrochemically reversible quinone reduction. Therefore, interference from the oxygen reduction can be removed by using high scan rates without disturbing quinone signals. We also note that such a procedure is unlikely to be successful when applied to electrode of macroscopic proportions. For further studies, measurements of pH in saliva were all carried out using scan rate of  $4 \text{ V s}^{-1}$ .



**Fig. 4** CVs of chemical modified carbon fibre in prepared synthetic saliva of pH 7.93 without degassing at different scan rates, showing a removal of oxygen reduction peak at high scan rates.

Using the higher scan rate for pH measurement in 'literature synthetic saliva' without degassing, varying pHs of this synthetic saliva were tested at the micro-wire electrode (Fig. 5(A) solid line). Well-defined cyclic voltammograms were obtained from the whole range of pH from 3.99 to 8.30. The oxidation, reduction, and midpoint potential were measured and plotted against pH. The linear response from the plot of peak potential against pH was observed from all three lines as shown in Fig. 5(B). The gradients obtained from oxidation, reduction, and midpoint potential were 79 mV, 67 mV, and 73 mV per pH unit respectively. These shifts in the voltammetric peak position are greater than that predicted by the Nernst equation. However, this most likely arises due to the quinone voltammetric waveshape as a function of pH, leading to a greater apparent shift in the peak position at the higher scan rates.



**Fig.5** (A) CVs of chemical modified pitch-based carbon fibre in prepared synthetic saliva pH ranging from 3.99 to 8.30 (solid line), with real saliva and commercial synthetic saliva (dash line) at a scan rate of  $4 \text{ V s}^{-1}$ . (B) Calibration plots of peak potential against pH in prepared synthetic saliva without degassing, including results of real saliva and commercial synthetic saliva.

### 3.3.2 pH measurements in authentic human saliva

Having investigated the voltammogram response of the quinone for pH measurement of 'literature synthetic saliva' at different pH values, here application of the micro-wire electrode to measure pH was next applied to 'commercial synthetic saliva' and 'real saliva samples'. The pH of these saliva solutions were measured using a commercial glass electrode before voltammetric measurements. The CVs of the micro-wire electrode were recorded in commercial synthetic saliva (pH 7.16) and real saliva samples (pH 7.51) at scan rate of  $4 \text{ V s}^{-1}$  without degassing. The dashed lines in Fig. 5(A) show well-defined CVs from both types of saliva solution. Again, the oxidation, reduction, and midpoint potentials were measured. The obtained potential values were plotted against pH and were shown in the same calibration plots as the 'literature synthetic saliva' (Fig. 5(B)). Good agreement is seen especially for the midpoint potential.

In Table 2 the results of pH measurements on commercial synthetic saliva and real saliva are given. The determinations were performed both with a micro-wire electrode and a glass electrode and the pH values of the saliva sample using a micro-wire were calculated from the midpoint potential and pH. The agreement between the results obtained with the two electrodes is good.

**Table 2** Determinations on saliva samples with a glass electrode and a micro-wire electrode

Saliva	Glass electrode	Micro-wire electrode
<i>Commercial synthetic saliva</i>	$7.16 \pm 0.04$	$7.19 \pm 0.08$
<i>Real saliva</i>	$7.51 \pm 0.05$	$7.48 \pm 0.07$

4. Conclusions

In summary, by chemical modification of carbon fibre surface, a micro pH electrode was obtained (diameter of 9  $\mu\text{m}$  and length of 1 mm). A Nernstian response observed across a pH range 2-8 which is the pH range of many biological fluids. The presence of surface quinone groups on the micro-wire electrode, which shows electrochemically reversible behaviour, facilitates a simple voltammetric pH sensor. The pH measurement in oxygenated biological samples, synthetic saliva and real saliva in this case, can be performed using high scan rate CV to remove the influence of any oxygen reduction reaction catalysed by surface quinone or the overlapping direct oxygen reduction. In particular, the micro-wire is validated for pH measurements in real saliva. In this work we illustrate a proof of concept of using surface quinones on micro-wire electrode for pH sensing in real saliva. Note that to realise a fully ‘micro device’, an electrochemical system without an external reference but with an internal reference (such as a ferrocene modified carbon fibre surface) should be further investigated<sup>23</sup>.

Acknowledgement

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## Figure captions

**Scheme 1** Representation of various functional groups present on graphitic carbon surfaces, adapted from Ref. 26.

**Fig. 1** Optimisation of the chemical modification time in aqueous solution of 0.03 M  $\text{KMnO}_4$ . CVs at unmodified and modified carbon fibre electrodes in 0.01 M  $\text{HNO}_3$  in 100 mM KCl supporting electrolyte (pH 2.2) at a scan rate of  $0.1 \text{ V s}^{-1}$ .

**Fig. 2** Quinone peaks in cyclic voltammetry at a scan rate of  $0.1 \text{ V s}^{-1}$  (A) and quinone reduction peaks in square wave voltammetry (C) on chemical modified pitch-based carbon fibre obtained in buffer solutions, ranging in pH from 2 to 8. Calibration plots of peak potential against pH obtained from CV (B) and SWV (inset of Fig 2C). All of the buffer solutions were degassed before the performed electrochemical measurement.

**Fig. 3** CV of chemical modified carbon fibre in literature synthetic saliva pH 7.9 without degassing compares (red line) with CV of chemical modified carbon fibre in buffer solution with degassing (blue line) at scan rate of  $0.1 \text{ V s}^{-1}$ , showing a presence of oxygen reduction peak in oxygenated solution (red line).

**Fig. 4** CVs of chemical modified carbon fibre in prepared synthetic saliva of pH 7.93 without degassing at different scan rates, showing a removal of oxygen reduction peak at high scan rates.

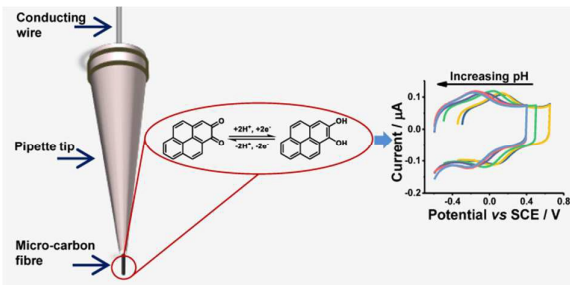
**Fig.5** (A) CVs of chemical modified pitch-based carbon fibre in prepared synthetic saliva pH ranging from 3.99 to 8.30 (solid line), with real saliva and commercial synthetic saliva (dash line) at a scan rate of 4 V s<sup>-1</sup>. (B) Calibration plots of peak potential against pH in prepared synthetic saliva without degassing, including results of real saliva and commercial synthetic saliva.

Table captions

**Table 1** Composition of synthetic saliva (AFNOR standard S90-701)<sup>40, 41</sup>

**Table 2** Determinations on saliva samples with a glass electrode and a micro-wire electrode

Graphical abstract



Amperometric micro pH measurements exploiting quinone groups on carbon fibre surfaces



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