

Optimising amperometric pH sensing in blood samples: An iridium oxide electrode for blood pH sensing

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Abstract

Amperometric pH sensing in blood samples has been studied using iridium oxide electrodes. The iridium oxide electrodes are made by electrodeposition of iridium oxide onto iridium micro-disc electrode from alkaline solutions of iridium(III) oxide. The pH responses of the electrode are studied in aqueous solutions and authentic samples of sheep's blood employing both cyclic voltammetry and square wave voltammetry. Uncertainties of pH measurement in blood samples via cyclic voltammetry (± 0.07 pH units) were improved by a factor of two using square wave voltammetry (± 0.03 pH units). Limitations of amperometric pH sensing in blood samples are considered as caused by the uncertainty of the required reference measurements (via a conventional glass electrode) and also the use of matrix-free and low ionic strength buffers to calibrate a standard glass electrode for the measurement of blood pH.

1 Introduction

pH is the most frequently measured chemical parameter due to its importance in many areas including environmental monitoring, medical diagnosis, and quality control in food industry.¹⁻³ As recommended by IUPAC, pH is defined in terms of the single ion activity of the hydrogen ion in solution

$$\text{pH} = -\log a_{\text{H}^+} = -\log(m_{\text{H}^+}\gamma_{\text{H}^+}/m^\ominus)$$

in which a_{H^+} is the relative (molality basis) activity and γ_{H^+} is the activity coefficient of the hydrogen ion (H^+) at the molality m_{H^+} , and m^\ominus is the standard molality (1 mol kg^{-1}).⁴ It is noteworthy that the IUPAC-recommended definition of pH has been mentioned and argued as an ambiguous definition of the measured quantity. A detailed description of the historical development of pH and the complexity of pH measurement appears in a review by de Levie.⁵ The activity coefficient of individual ions cannot be defined uniquely or measured.⁶ From thermodynamic studies of electrolytic solutions one always obtains mean activity coefficients (γ_{\pm}) for combinations of ions. The validity of the experimentally derived values of the single ion activities is still under dispute. Significant work has focussed on the estimation of the single ion activity coefficients of H^+ and Cl^- from the mean activity of HCl , on the basis of the hydration model.^{7, 8} In 2011, Sakaida *et al.* proposed the experimental procedure to estimate the values of individual ion activity of H^+ and Cl^- to the extent of the accuracy of the liquid junction potential which is the use of ionic liquid salt bridge.⁹ The experimental values show the fairly good agreement with the recent theoretical predictions extending of Debye-Huckel theory proposed by Fraenkel.^{10, 11} Although considerable literature reported an experimental estimation and a theoretical prediction of single ion activities, IUPAC-recommendation of the single ion activity for the pH in aqueous solutions is based on the

Harned cell.⁴ The Harned cell (a hydrogen electrode and silver/silver-chloride electrode in a cell without a liquid junction) is a primary method of measurement in order to incorporate pH determinations into the SI system, based on a well-defined measurement equation in which all of the variables can be determined experimentally.¹² The activity coefficient is estimated by help of the Debye-Hückel theory. All measurements are restricted to samples with ionic strength $\leq 0.1 \text{ mol kg}^{-1}$.^{13, 14} At higher ionic strength ($I > 0.1 \text{ mol kg}^{-1}$), activity coefficient cannot be evaluated by the Debye-Hückel limiting law leading to an uncertain value of the activity of hydrogen ion. In terms of biological fluids it is noteworthy that the ionic strength of blood is normally about 0.15 M .^{15, 16} Consequently, pH measurements in blood samples become operationally defined. The measured values depend on the measurement procedure and have their own uncertainty. In terms of practical usage, the use of low ionic strength buffer to calibrate a glass electrode for high ionic strength solution pH measurements may lead to an erroneous reading from a pH meter and this value might not be a true representation of the measurand.

In this work, we focus on using an iridium oxide electrode for amperometric pH measurement of blood. This system is selected due to its long history of being developed and repeatedly used as potentiometric pH sensors in other applications.¹⁷⁻²⁰ In order to generate an iridium oxide layer on a conducting metal, iridium oxide can be deposited (a) thermally²¹⁻²³, (b) by sputtering^{24, 25}, (c) by sol-gel method²⁶, or (d) electrochemically²⁷⁻²⁹. The characteristics of iridium oxide are generally sensitive to its structure and composition, which depend on the fabrication method and conditions. Thermally and sputtering prepared iridium oxide give dry oxide films or anhydrous films that are less hydrous than electrochemically prepared iridium oxide deposited from an aqueous solution. Overviews of different preparation methods were provided by Huang *et al.*³⁰, Kakooei *et al.*³¹, and Yao *et al.*²¹ Consequently, it is important to investigate how accurate and precisely we can define pH

using this system. Note that the accuracy is the closeness between a measured value and true value of measurand, while precision is the closeness of agreement between independent measurements of a quantity under the same condition without reference to a theoretical or true value. In this work, the iridium oxide electrodes are made by electrodeposition of iridium oxide onto iridium micro-disc electrode and are tested in aqueous solutions of variable pH and in authentic samples of sheep's blood. Cyclic voltammetry and square wave voltammetry are employed to study the limitations of amperometric pH sensing of blood.

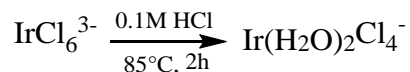
2 Experimental

2.1 Electrode preparations

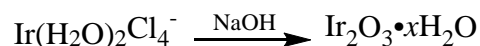
Paint deposition. An iridium wire (100 μm in diameter, GoodFellow, UK) was insulated using cathodic electrophoretic paint (Clearclad HSR, LSV Coating, UK). The paint coating procedure was adapted from Ellison *et al.*³² The wire was held vertically in a centre of a conducting metal cell with a diameter of 1 cm. A constant DC voltage of 30 V was applied for 2 min between the iridium wire and the metal cell. After deposition, the iridium wire was then heated at 120 $^{\circ}\text{C}$ for 30 minutes to cure the deposited insulating film. Repeated deposition and cure steps were performed 3 times to obtain insulated electrodes. The thickness of paint coating layers was $18.3 \pm 2.6 \mu\text{m}$ ($n=3$) as evaluated from microscope imaging (see Supporting Information section 1).

Iridium oxide deposition. The iridium containing deposition solution was prepared according to the method described by Baur *et al.*³³ and Salimi *et al.*³⁴ The preparation of this solution consists of two steps. The first step is the formation of the diaquatetrachloroiridate(III) ion ($\text{Ir}(\text{H}_2\text{O})_2\text{Cl}_4^-$) from K_3IrCl_6 . To prepare ca. mM solutions of

IrCl_6^{3-} , potassium hexachloroirridate(III) (K_3IrCl_6) (Aldrich) is dissolved in 0.1M HCl and the solution is heated at 80-85°C for 2 h:

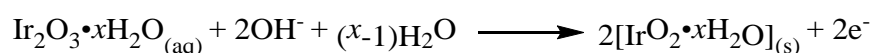


At this point, the prepared solution is reportedly stable and can be stored for several months³³. The second step is the formation of iridium(III) oxide from $\text{Ir}(\text{H}_2\text{O})_2\text{Cl}_4^-$ by making the solution of $\text{Ir}(\text{H}_2\text{O})_2\text{Cl}_4^-$ basic according to the following reaction:



Prior to addition of the base, oxygen must be removed from the acidic solution of $\text{Ir}(\text{H}_2\text{O})_2\text{Cl}_4^-$ due to the instability of iridium(III) oxide under oxygen. After a thorough degassing with nitrogen gas, the pH of solution is raised to 10.5-11.5 by adding 0.1M NaOH, and this solution is used for the deposition of iridium oxide onto the iridium wire micro-disc electrode.

An insulated iridium wire was cut at the tip to generate a micro-disc electrode. Then the clean electrode was immersed in the deposition solution containing 1mM iridium(III) complexes and the potential cycled from +0.2 to +1.2 V at scan rate of 50 mV s⁻¹ for 15 cycles (unless stated otherwise). Cyclic voltammograms of consecutive scans showed an increasing of the currents suggesting of the formation of an electroactive deposit on the electrode surface (section 2 of Supporting Information). The formation of iridium(IV) oxide layers is initiated electrochemically at the surface of iridium micro-disc electrode based on the following reaction which is modified from Baur and Spaine's work.³³



After deposition, the iridium micro-disc electrodes modified with iridium oxide films were cleaned with deionised water and dried with nitrogen gas.

2.2 Chemicals and reagents

All chemicals and reagents used were of analytical grade. Solutions were prepared using deionised water (Millipore, resistivity not less than 18.2 M Ω cm at 25 °C). Buffer solutions 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 100 mM KCl as supporting electrolyte. The pH values of buffers were measured using a HACH LANGE Sension+ PH31 pH meter calibrated using standard buffers of pH 4.01 \pm 0.01, 7.00 \pm 0.01, and 10.01 \pm 0.01. Defibrinated sheep's blood was purchased from TCS Biosciences Ltd, UK and the pH adjusted using vapour withdrawal carbon dioxide gas (BOC, Guildford UK). Carbon dioxide gas was bubbling into sheep's blood samples for a few seconds to adjust the pH and the pH of the samples was recorded.

2.3 Apparatus

Electrochemical measurements were performed using a μ Autolab II potentiostat (Metrohm-Autolab BV, Utrecht, Netherlands). A standard three electrode set up was used, consisting of a saturated calomel reference electrode (SCE +0.244 V vs. SHE, BASi Inc., Japan) and a platinum wire counter electrode, and an iridium oxide micro-disc working electrode. The electrochemical set up was thermostated at a constant value of 25.0 \pm 0.2 °C.

3 Results and discussion

In the following sections, the electrodeposition of iridium oxide onto an iridium micro-disc electrode is demonstrated for use as a blood pH sensing probe. Defibrinated sheep's blood is used as a model for biological samples in general and blood samples in particular. Note that defibrinated sheep's blood is the blood without fibrin protein where fibrin is mechanically removed without the presence of anticoagulants or other additives. First, the pH response of the iridium oxide layers on iridium micro-disc electrodes is studied in buffer solutions. Second, the voltammetric responses of the developed probe in sheep's blood are investigated via both cyclic and square wave voltammetry. Last, the scope and limitations of amperometric pH sensing in real blood samples are critically considered.

3.1 pH response of the iridium oxide layers on iridium micro-disc electrodes in *buffer* solutions

The iridium oxide was investigated for voltammetric pH measurement. Cyclic voltammetry in a range of buffer solutions in the presence of 0.1M KCl supporting electrolyte was conducted using an iridium oxide micro-disc electrode. The voltammetric response was measured from 0.0 V to +0.8 V for a buffer solution of pH 2 at a scan rate of 100 mV s⁻¹. The potential window was adjusted as a function of pH accordingly to accommodate the shift in potential and to best observe the response of the oxidation and reduction peak. Note that a wider potential window consecutive scans from 0 V to +1.0 V is shown in Supporting Information section 10 where the onset of solvent breakdown is appeared. The CVs of iridium oxide supported on the iridium micro-disc electrode was recorded in buffer solutions of pH 2-8. Fig.1 shows the results over the pH range 2-8. The inset of Fig.1 depict plots of the oxidation, reduction and midpoint potentials *vs.* pH for iridium micro-disc electrode modified with iridium oxide films; the slope of the linear shown is about 62.7±1.2, 57.5±1.2, and 67.9±1.5 mV per pH for midpoint potential, oxidation/reduction peak potentials

respectively. The values obtained by extrapolating to pH 0, of midpoint potential, oxidation and reduction peak potentials are 596 ± 7 , 613 ± 7 , and 579 ± 9 mV respectively. Note that there has been some concerns that the presence of chloride ions might be caused instability of iridium oxide.³⁵ According to our experiments, all aqueous buffer solutions contained 100 mM KCl as a supporting electrolyte. There was no appreciable effect of chloride ion interference in any experiments.

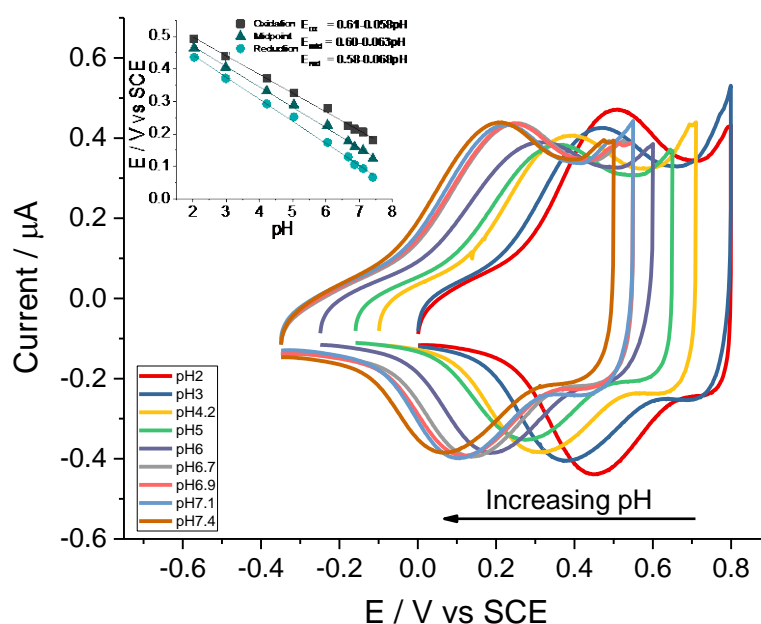
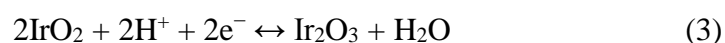
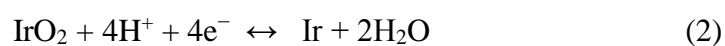
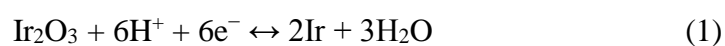


Fig. 1 Cyclic voltammetry responses of iridium oxide on iridium micro-disc electrode at different pH of *buffer* solutions; scan rate of 100 mV s^{-1} . Inset shows the variation of potential vs pH.

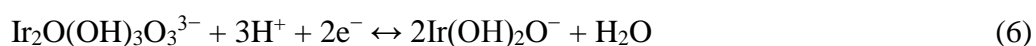
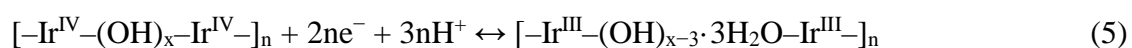
Three possible redox processes have been reported by Pourbaix^{36, 37} to be involved in the pH dependent redox response of an iridium oxide electrode as



and the Nernst equation is

$$E = E^0 - 2.303 \frac{mRT}{nF} pH \quad (4)$$

where m, n are number of protons and electrons involved in the redox process, respectively. The theoretical standard electrode potential (E^0) given by Pourbaix^{36, 37} for each equilibrium is identical with a value of 926 mV vs SHE or 681 mV vs SCE. From equation 1-3 Nernstian responses will be obtained with an expected slope of 59 mV/pH at 25°C. This type of response is reported for anhydrous iridium oxide film resulting from procedures involving heat-treatment or sputtering processes^{23, 38}. For the electrochemically grown iridium oxide, this process results in amorphous films with varying hydration and possibly the presence of a hydrated oxyhydroxide.³⁹ Many redox reactions are possible between the reduced and oxidised forms of oxide and oxyhydroxide where the exact stoichiometric composition of hydrous film is difficult to determine. The general equilibria investigated by Burke et al.⁴⁰ and Olthuis et al.³⁹ were



The equilibrium (6) indicates that the theoretical sensitivity may range from 59 mV/pH to *ca.* 90 mV/pH. Electrodes that exhibit super-Nernstian slopes are generally assumed to have a mixed iridium oxide composition and be operating under a correspondingly mixed reaction mechanism.^{41, 42}

In terms of accuracy of pH measurement in buffer solutions using iridium oxide modified iridium micro-disc electrode, to evaluate the measurement uncertainty, a linear regression was applied. The linear plot of midpoint potentials of iridium oxide tested in aqueous buffer

solutions against pH (inset of Fig.1) was used to calculate uncertainties of pH measurement in buffer. Standard deviation of potential values deviated from the linear plot were calculated and converted to a pH unit using the calibrated sensitivity of the linear plot (section 3 in Supporting Information). According to the estimation, uncertainties of pH measurement in buffer using iridium oxide electrode were found to be approximately ± 0.1 pH units. This value is consistent with the literature where previous work has reported an accuracy of developed iridium oxide electrodes tested in aqueous solutions to be in the range of 0.02 – 0.2 pH units obtained from potentiometric measurement.^{18, 43, 44} Note that in these literature reports the reading from the glass electrode against which solutions are themselves calibrated was assumed to be absolute. However, as with any analytical technique the use of a glass electrode for the measurement of pH also has its own uncertainty. Significant work has been focussed on the assessment of uncertainty of pH measurements using glass electrode.⁴⁵⁻⁴⁸ The main errors that limit the accuracy of pH measurement are errors arising from the standard buffers and from junction potentials which limit the accuracy with the glass electrode to ca. ± 0.02 pH units.^{49, 50} Apart from the limitations from the uncertainty of pH measurement of the glass electrode in buffer solutions, quantitative interpretation of measured pH values is limited to dilute aqueous solutions of simple solutes. This requirement results in limitations of the glass electrode for use in non-aqueous media, suspensions, colloids, and aqueous solutions of ionic strength greater than 0.1 mol/kg. Note that the uncertainty of pH measurement of the glass electrode used in this study was found to be ca. ± 0.01 pH units for aqueous buffer solutions and ca. ± 0.02 pH units for blood samples. Briefly the uncertainty of pH the measurement using a glass electrode is determined by measuring pH of solutions either buffer or sheep's blood 10 times and the standard deviation of the measured values is calculated (section 4 of Supporting Information).

3.2 Investigating the measurement of pH in *sheep's blood* samples

3.2.1 Cyclic voltammetric measurement of pH in sheep's blood

Having demonstrated the pH dependence of iridium oxide film voltammetric response from measurements in buffer solutions, the capabilities of the iridium oxide electrode for blood pH sensing were investigated by studying the electrode in sheep's blood of different pH values. First the cyclic voltammetric response of iridium oxide at iridium micro-disc electrode in sheep's blood is considered. A sample of defibrinated sheep's blood was used as received without dilution. Carbon dioxide gas was employed to control and alter the pH of the sample. Iridium oxide electrode was set up in a cell together with a glass electrode to perform real time pH monitoring. The electrode was cleaned by DI water and dried between tests. The CV response was first measured from -0.5 V to +0.5 V for a sheep's blood solution of pH 7.4-7.5 at a scan rate of 100 mV s^{-1} , then the potential window was adjusted as a function of the pH. According to the first test in sheep's blood, the CV measured in sheep's blood is more distorted compared to the CV tested in aqueous buffer solution (see Figure SI7). Therefore, the thickness of iridium oxide film was varied by changing the number of deposition cycles in order to improve the peak to peak separation (section 5 in Supporting Information). Note that the number of deposition cycles may change the iridium oxide composition and consequently affect the y-intercept at pH=0. After the optimisation of iridium oxide thickness, the cyclic voltammetric response for iridium oxide on the iridium micro-disc electrode was recorded in sheep's blood solutions of pH 6.5-7.5. Fig. 2(A) shows the overlaid representative CVs of iridium oxide supported on the iridium micro-disc electrode in sheep's blood solutions of different pH at a scan rate of 100 mV s^{-1} . It can be observed that by increasing the pH, peak potentials shift in the cathodic direction towards more negative values. The oxidation, reduction, and midpoint potentials were recorded (n=22). The plots of

peak potentials against pH monitored by the pH meter are shown in Fig. 2(B). The gradients of the slopes were 90.4 ± 4.6 , 81.2 ± 7.7 , and 99.5 ± 8.6 mV per pH unit for midpoint potential, oxidation/reduction peak potentials respectively. The uncertainty of the pH measurement from the midpoint potential is ± 0.07 pH unit calculated using the same method as mentioned in section 3.1 (see section 3 in Supporting Information). Considering voltammetric responses obtained in sheep's blood, the peaks were not as well defined as and are more distorted compared to the CVs of buffer solutions. This leads to difficulty on determining the peak position which may cause uncertainty of peak measurement. In terms of peak to peak separation as mentioned earlier the obtained CV tested in sheep's blood became less "reversible" with a peak to peak separation of ca.200 mV compared to CV of electrode tested in buffer solutions where peak to peak separation is 50 mV (section 5 in Supporting Information). The peak to peak separation is larger in sheep's blood partly because the presence of complex matrix in blood may have an effect on the electron transfer process of Ir/IrOx electrode.

Considering the apparent standard electrode potential of the redox reaction ($E^{0'}$),^{51, 52} the y-intercepts at pH = 0 of linear plot of midpoint potential vs pH indicated that $E^{0'}$ was 596 ± 7 mV vs SCE for electrodes studied in buffer solutions (Inset Figure 1). However, the intercepts from plots of midpoint potentials against pH from CVs tested in sheep's blood were 765 ± 32 mV (Figure 2B). Possibilities of the differences of $E^{0'}$ may cause by irreproducibility of the electrochemical measurements or a matrix effect of the blood samples. To study the reproducibility of the electrochemical measurements, iridium oxide electrodes were tested in buffer and sheep's blood again to study the reproducibility of measurements in different samples. Considering $E^{0'}$ for electrodes studied in buffer solutions, values were slightly shifted between experiments to experiments in the range of 586 ± 4 mV -

596±7 mV (data shown in Supporting Information section 6). However, the y-intercept from the sheep's blood samples experiments shifted in the range of 752±39 – 817±47 mV. This result shows that the differences of E^0 value is not caused by the irreproducibility of the electrodes, but by a matrix effect of blood samples, probably specific chemical or medium effects as reflected in the proton activity coefficient. Electrodes in this work were stored in ambient air and can be used within a period of month. Note that the magnitude of the peaks decreases with storage time, but the peak positions do not change. To examine possible fouling effects on the electrodes, the electrodes were repeatedly tested in blood samples. On completion of the blood sample testing cyclic voltammograms were re-measured in aqueous buffer solution (pH=2). They were essentially identical to those obtained before the use of electrode in blood pH sensing experiments (data not shown) suggesting the reusability and robustness of the electrode.

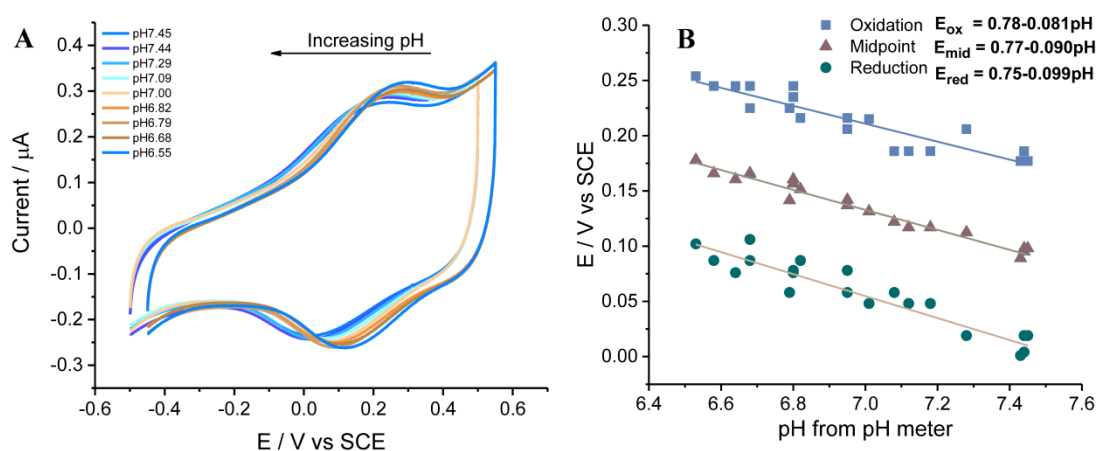


Fig. 2 (A) Cyclic voltammetry responses of iridium oxide on iridium micro-disc electrode with varying pH of *sheep's blood* samples ranging from 6.5 to 7.5. (B) Plots of potentials against pH value reading from pH meter.

3.2.2 Square wave voltammetric measurement of pH in sheep's blood

Having investigated the pH measurement of sheep's blood using cyclic voltammetry, square wave voltammetry was next utilised to determine whether this technique can be employed to improve the measurement uncertainty. Owing to the ability of SWV to improve signal-to-noise ratios relative to those of in particular, cyclic voltammetry, one may expect an improvement of defined peaks facilitating peak position measurements.⁵³ The optimisation of SWV in sheep's blood sample including frequency, step potential, and amplitude was performed to obtain best quality square wave voltammetry for sheep's blood pH measurements (section 7 in Supporting Information). The optimised SWV parameters (frequency 15 Hz, amplitude 10 mV, and step potential 1 mV) were used to measure the pH of sheep's blood. SWV was conducted in variable pH of sheep's blood adjusted pH by bubbling carbon dioxide gas into blood samples (as above). The glass electrode and the iridium oxide probe were immersed in blood samples to monitor the pH of the solution simultaneously. Therefore, electrochemical measurements data (peak potential) obtained are reported relative to the pH monitored by the pH meter. Square wave voltammetry scans were initially to increasingly positive potentials to obtain an oxidative peak and then scans were reversed in direction. Representative square wave voltammograms of iridium oxides in variable sheep's blood pH solutions are shown in Figs. 3A and 3B. A single SWV peak was seen in both scan directions. It can be observed that the peak potentials shift in a cathodic direction towards more negative potentials on increasing the pH both for oxidative and reductive scans. For analysis of the data, the oxidation/reduction peak potentials and midpoint potential of iridium oxide supported on iridium micro-disc electrode were all recorded (n=19). The plots of potentials against pH reading from pH meter are shown in Fig. 3C. The gradients of the slopes were 101.3 ± 3.2 , 82.0 ± 4.2 , and 120.8 ± 3.0 mV per pH unit for for midpoint potential, oxidation/reduction peak potentials respectively. In terms of an

uncertainty of pH measurement in sheep's blood using iridium oxide electrode, the same method as mentioned in section 3.1 and SI section 3 was applied. The uncertainty from midpoint potential was calculated to be ± 0.03 pH unit which is an improvement of a factor of two compared to results from cyclic voltammetry. Moreover, the uncertainty from the glass pH meter itself is ± 0.02 pH unit. The improvement of the pH measurement by using SWV is evidenced in terms of sensitivity and uncertainty of pH measurement.

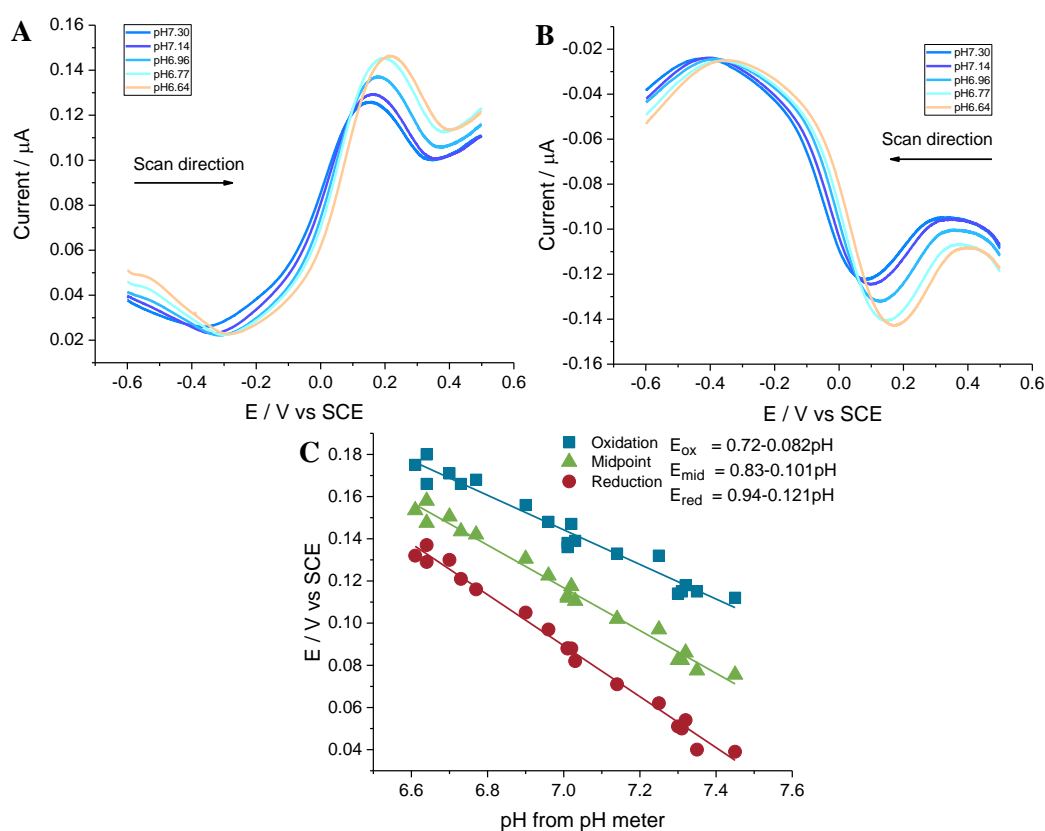


Fig. 3 Square wave voltammetry (frequency 15 Hz, step potential 1 mV, and amplitude 10 mV) response of iridium oxide on iridium micro-disc electrode with varying pH of *sheep's blood* samples ranging from 6.5 to 7.5(A) oxidation and (B) reduction. (C) Plots of potentials against pH value reading from pH meter.

3.3 Limitations of amperometric pH sensing in sheep's blood

Having investigated the measurement of pH in sheep's blood samples employing both CV and SWV, limitations of amperometric pH sensing in blood are next considered. First, precision of pH measurement of sheep's blood using a standard glass electrode is considered because of its use as a reference pH value for our developed method. Fundamentally, the absolute value of pH is not defined under these conditions. Due to the definition of pH, the activity coefficients of individual ions cannot be defined uniquely or measured. Consequently, one cannot accurately know the pH value. Because, the accuracy is the closeness between a measured value and true value of measurand, therefore to assess the accuracy of measurement the true value is required. However, according to the way of measurement we can tell how precisely we can define the pH of sheep's blood from measurement. Note that as mentioned in section 3.1 an uncertainty of pH measurement of sheep's blood using a glass electrode in this study is about ± 0.02 pH units. In consequence, precision of our developed method for pH measurement is considered next. The present findings show that an uncertainty of pH measurement in sheep's blood using CV is ± 0.07 pH units and can be reduced to ± 0.03 pH units using SWV. However, the apparent uncertainty of our measurements should also consider the uncertainty of reference measurement (glass electrode) and the uncertainty from our method. Because both methods have their own uncertainty, therefore, the apparent uncertainty is necessarily more than what we obtain from any new method itself. Uncertainties reported in this work assumed that pH reading from the pH meter was absolute. Moreover, another important point to consider is the effect of the matrix in different samples both in term of buffers versus blood and also blood sample to blood sample. There are the practical difficulties in using matrix-free buffers of low ionic strength to calibrate a glass electrode for blood pH measurement. Consequently, pH readings when measuring the pH of blood might not be an exact value of blood pH. To address the

problem of using matrix-free buffers to calibrate a standard glass electrode, the standard reference buffer solution of synthetic biological fluids or blood sample in particular may need to be developed to reduce an erroneous of the measured values. However, development and testing of a suitable model for the activity coefficients of acid-base components in such a blood medium need to be considered.

4 Conclusions

In this work, an iridium oxide electrode supported on an iridium micro-disc electrode is used as a pH probe for studying amperometric blood pH measurements. We have evidenced the possibility of iridium oxide electrode as a blood pH sensing. The scope and limitations of amperometric pH sensing in real blood samples resulting in an uncertainty of pH measurement are considered as follows: First, to estimate an uncertainty of our proposed method, the uncertainty of reference measurements which is a conventional glass electrode need to be taken into account because both methods have their own uncertainty. This may lead to a higher of the apparent uncertainty than the uncertainty obtained from any new method itself. Second, the effect of the matrix in samples can cause an uncertain measurement resulting from the use of matrix-free and low ionic strength buffers to calibrate a standard glass electrode for measurement of high matrix blood pH.

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