Exploring the electrical wiring of screen-printed configurations utilised in electroanalysis

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In this technical note the often overlooked issue of electrically connecting screen-printed electrode sensors is considered. The electrical connection of screen-printed electrodes to the potentiostat/electronics can be a main issue when a true electrochemical response is trying to be obtained and if one does not have a stable electrical connection, the results that are obtained may lack reproducibility and therefore maybe discredited as “another failed electrochemical probe/sensor”. This paper considers the case of electrically connecting screen-printed with crocodile clips and compares that to the use of edge-connectors demonstrating the precise connection of an electrode setup when utilising crocodile clips can result in extremely reproducible electrochemical outputs when applied correctly.

1. Introduction

The rise of screen-printed electrodes has become a major revolution in the world of electrochemical sensors. Such electrode systems allow laboratory developed experiments to be translated into the field allowing for reproducible and disposable on-site detection of analytes. In the pursuit of developing electrochemical sensing protocols that utilise screen-printed electrodes, some researchers have significant problems in achieving this, largely due to the poor choice of how to electrically wire/connect the electrode to the potentiostat. This is an often overlooked issue which can be the difference between gaining sufficient qualitative and quantitative electroanalytical signals. The involvement of electrochemistry on the front line of analytical detection will only advance with such comparisons of experimental setups shown in this technical note, which will allow for low costing, portable, quick, reliable and disposable sensors being used in everyday analytical scenarios; Fig. 1A shows a typical experimental set-up where a screen-printed electrode is connected via an edge connector to a potentiostat. Such an approach allows for excellent electrochemical signals rivalling that of the traditionally employed solid electrodes, such as glassy carbon, boron doped diamond and edge plane electrodes. These electrodes have the ability to be easily electrically wired to the potentiostat/controlling electronics via the use of crocodile clips, as shown in Fig. 1B. However it is well established that such solid electrodes cannot be easily translated to measurements outside of the laboratory due to the requirement of pre-treatment between measurements in the form of electrode polishing. A well-established approach is to utilise screen-printed electrodes such that for each measurement a new electrode/sensor can be utilised, giving rise to rapid and sensitive in-the-field measurements and avoids any problems from “memory effects” that might be encountered as is the case with solid electrodes and hence their requirement of electrode polishing/pre-treatment.

The utilisation of screen-printed electrodes is a highly pursued area within the field of electroanalysis. Many researchers have expressed the need for miniaturisation of electrochemical setups and for the continuation of electroanalytical studies. For example, Metters et al. have shown the constant diverse use of screen-printed sensors to many electrochemical targets, including chromium, hydrazine and atropine, to name a few. Other researchers such as Honeychurch et al. have focused upon analytes such as diazepam and nitrazepam. The ongoing pursuit within the electroanalytical field using screen-printed electrodes is extremely diverse with many research groups applying these in a range of analytical pursuits allowing “in-field” analysis where miniaturisation and portability can be realised. Not only do these screen-printed electrodes express a great portability aspect but demonstrate analytical robustness, for example, a report by Smith et al. has shown that electrochemical characterisation and detection of legal highs (synthetic cathinones) can compete with other favoured analytical methods, such as HPLC.

Researchers can clearly see the allure of utilising screen-printed electrodes and newcomers to the field often fail to obtain reproducible and useful electrochemical measurements which are typically blamed upon the screen-printed electrodes themselves. Others lay the problem with connection to the electrode being difficult, typically utilising crocodile clips and
thus obtain electrical noise in the electrochemical signals resulting in unreliable electroanalytical responses, in terms of the analytical sensitivity and limit of detection, towards the target analytical probe. Both such scenarios inevitably result in researchers abandoning the use of screen-printed electrodes.

Motivated by these problems, in this technical note the often overlooked factor of screen-printed electrode experimental setups is explored between the use of an edge connector and the crocodile clips both used in the electrical wiring of the SPEs to the potentiostat/electronics. This paper provides valuable advice of how to electrically screen-printed electrodes/sensors giving confidence to newcomers in the field.

2. Experimental

All chemicals were of analytical grade and were used as received without any further purification and were obtained from Sigma-Aldrich. All solutions were prepared with deionised water of resistivity not less than 18.2 MΩ cm. Voltammetric measurements were carried out using a Palmsens Emstat (Palmsens, Netherlands) potentiostat.

Experiments carried out throughout this study utilise Screen-Printed Electrodes (SPEs) (with the dimensions of 41 mm long × 7 mm wide) which comprise a three electrode configuration with a 3 mm graphite working electrode, a graphite counter and an Ag/AgCl reference electrode. These SPEs are fabricated in-house with appropriate stencil designs to achieve a 3 mm diameter working electrode respectively, using a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). Firstly, a carbon–graphite ink formulation (product code: C20000802P; Gwent Electronic Materials Ltd, UK) is printed onto a polyester (Autostat, 250 micron thickness) substrate. This layer is then cured in a fan oven at 60 degrees Celsius for 30 minutes. Next a silver/silver chloride reference electrode was included by screen printing Ag/AgCl paste (product code: C2040308P; Gwent Electronic Materials Ltd, UK) onto the plastic substrate. Finally, a dielectric paste (product code: D2070423D5; Gwent Electronic Materials Ltd, UK) was then printed onto the polyester substrate to cover the connections. After curing at 60 degrees Celsius for 30 minutes the screen-printed electrodes are ready to be used. The reproducibility of the batch of screen-printed sensors were found to correspond to less than 4% RSD towards the redox probe, [Ru(NH₃)₆]²⁺/³⁻/0.1 M KCl. Edge connectors can be obtained from any good electronic wholesalers and are mounted onto a circuit board such that the potentiostat connections can be easily applied at one end and the SPE at the other; see Fig. 1A.

3. Results and discussion

The differences between the edge connector and the crocodile setups have been reported to be a main issue of under-performing electrode systems, and in some instances resulted in researchers abandoning experiments; we explore the electrical connection of SPEs to the potentiostat/electronics using both edge connectors and crocodile clip connections towards a range of standard redox probes. Firstly, the electrochemical characterisation of the SPEs were carried out using the redox probe [Ru(NH₃)₆]²⁺/³⁻/0.1 M KCl. Fig. 2A and B depict typical cyclic voltammograms using both the edge connector and crocodile clip setups respectively. The analysis of this data is presented in Fig. 2C which shows the linear plots of peak current vs. square root of scan rate for both experimental setups (edge connector: \(I_p/\mu A = -1.28 \mu A/(V s^{-1})^{1/2} - 0.18 \mu A, R^2 = 0.99, N = 10\). Crocodile: \(I_p/\mu A = -1.27 \mu A/(V s^{-1})^{1/2} - 0.94 \mu A, R^2 = 0.99, N = 10\)) where it is apparent that there is no substantial difference between the two electrical connection set-ups in terms of peak currents, peak positions and quasi-reversibility. Secondly, attention was next turned to exploring the response of the redox probe [Fe(CN)₆]³⁻/⁴⁻/0.1 M KCl using the edge connector.
and crocodile clip connection, and as shown in Fig. 3 it is clear that the cyclic voltammograms are near identical with both experimental setups exhibiting similar electrochemical characteristics. Fig. 3C shows the analysis of the voltammetric signatures presented in Fig. 3A and B in the form of plots of peak current vs. square root of scan rate which are found to be linear (edge connector: \( I_p/\mu A = 1.68 \mu A/(V s^{-1})^{1/2} + 2.33 \mu A, R^2 = 0.99, N = 10 \). Crocodile: \( I_p/\mu A = -1.78 \mu A/(V s^{-1})^{1/2} + 2.33 \mu A, R^2 = 0.99, N = 10 \) in both cases. As observed above, (viz. Fig. 2C and 3C) there is no apparent deviation from using either an edge connector or the crocodile clip set-up.

Next, the effect of the electrical connection upon the standard heterogeneous rate constant, \( k^0 \), was next evaluated using the data obtained with the redox probes Ru(NH₃)²⁺/³⁺/₀.1 M KCl and [Fe(CN)₆]³⁻/²⁻/₀.1 M KCl. The Nicholson method is routinely used to estimate the observed, \( k^0 \), for quasi-reversible systems using the following equation:

\[
\varphi = k^0[\pi DnF/(RT)]^{-1/2}
\]

where \( \varphi \) is the kinetic parameter, \( D \) is the diffusion coefficient, \( n \) is the number of electrons involved in the process, \( F \) is the faraday constant, \( v \) the scan rate, \( R \) the gas constant, and \( T \) the temperature of the solution. The kinetic parameter, \( \varphi \) is tabulated as a function of peak-to-peak separation (\( \Delta E_p \)) at a set temperature (298 K) for a one-step, one electron process. The function of \( \varphi(\Delta E_p) \), which fits Nicholson’s data, for practical usage (rather than producing a working curve) is given by:

\[
\varphi = (-0.628 + 0.021X)/(1 - 0.017X)
\]

where \( X = \Delta E_p \) is used to determine \( \varphi \) as a function of \( \Delta E_p \) from the experimentally obtained voltammetry. From this, a plot of \( \varphi \) against \([\pi DnF/(RT)]^{-1/2}\) can be produced graphically allowing the standard heterogeneous rate transfer constant, \( k^0 \), to be readily determined, however \( \Delta E_p \) values that exceed 212 mV within the Nicholson table have to rely upon the following equation:

\[
k^0 = [2.18(DanFv/RT)^{0.5}] \exp\left[-((\alpha^2nF)/RT)\times\Delta E_p\right]
\]
where the constants are the same as described in eqn (1) however, \( a \) is assume to correspond to 0.5. The heterogeneous rate transfer constants were calculated for both redox probes using the above equations, with values found to correspond to \( 1.69 \times 10^{-3} \) and \( 1.93 \times 10^{-3} \) cm \( s^{-1} \) for the edge connector and crocodile clips respectively using the \([\text{Ru(NH}_3\text{)}_6]^{2+/3+}/0.1 \text{ M KCl}\) redox probe. The \( k^0 \) values were also calculated for the \([\text{Fe(CN)}_6]^{4-/3-}/0.1 \text{ M KCl}\) redox probe using both electrical setups, with values of \( 3.20 \times 10^{-4} \) and \( 3.60 \times 10^{-4} \) cm \( s^{-1} \) found. These results therefore reiterate that crocodile clips when used correctly, can produce useful electrochemical signals when utilising standard electrochemical redox probes and are comparable to using an edge connector such that there is no significant difference observed in the obtained voltammetric responses.

The analytical compatibility of both electrical setups were next analysed using the electrochemical oxidation of NADH (dihydronicotinamide adenine dinucleotide reduced form) to the corresponding oxidized form \([\text{NAD}^+\])}. This electrochemical probe receives considerable attention owing to its very important role as a cofactor in many naturally occurring enzymatic reactions, and mainly because of the potential application in over 300 NAD+/NADH\(^+\) dependent dehydrogenase-based biosensors\(^{23-27}\) and consequently its electrochemical characteristics are hugely important.\(^{28}\) Fig. 4A shows the successful electrochemical oxidation of 100 \( \mu \text{M NADH} \) within pH 7.4 PBS using both electrical setups, with both expressing similar oxidation peaks at \( \sim 0.40 \text{ V} \). The electroanalytical detection of NADH was also explored, as shown within Fig. 4B, over the concentration range of 10 \( \mu \text{M} \) to 100 \( \mu \text{M} \), with exceptional linearity in both cases (edge connector: \( I_p/\mu \text{A} = 0.02 \mu \text{A} \mu \text{M}^{-1} + 0.03 \mu \text{M}, R^2 = 0.99, N = 10 \). Crocodile: \( I_p/\mu \text{A} = 0.02 \mu \text{A} \mu \text{M}^{-1} + 6.80 \times 10^{-3} \mu \text{M}, R^2 = 0.98, N = 10 \). The limit of detection (LOD) \((3\sigma)\) for both electrical setups were found to correspond to 1.7 \( \mu \text{M} \) and 2.3 \( \mu \text{M} \) for the edge connector and crocodile clips respectively, which are comparable to previous carbon screen-printed electrode systems\(^6\) and demonstrates no concernable dependence of the electroanalytical performance characteristics upon the choice of electrical connection of the SPEs.
Attention was next directed to exploring the electrochemical detection of L-ascorbic acid (AA) which has been successfully reported previously at carbon based SPEs. AA is a naturally occurring molecule which plays a vital part within mammalian metabolism as an antioxidant. The detection of AA is vital for medical diagnosis of scurvy, therefore the electrochemical oxidation of AA was used as a model electroanalytical analyte.

Fig. 5A depicts cyclic voltammograms of the electrochemical oxidation of AA, it is apparent that there is a clear oxidation peak when utilising both electrical setups ($+0.30$ V). Note that there is a slight deviation in the peak potential that might be due to the resistivity of the crocodile clips. However such deviation does not affect the overall detection of AA, which are possible over the micromolar range as shown in Fig. 5B. The linearity of both calibration plots are excellent, with both setups exhibiting similar characteristics (edge connector: $I_p/\mu A = 2.57 \times 10^{-3} \mu A \mu M^{-1} + 0.39 \mu M, R^2 = 0.99, N = 10$. Crocodile: $I_p/\mu A = 2.45 \times 10^{-3} \mu A \mu M^{-1} + 0.36 \mu M, R^2 = 0.99, N = 10$). The LOD for both setups was found to correspond to 15.7 $\mu M$ and 15.5 $\mu M$ for the edge connector and crocodile clips respectively, which is in agreement with previous LODs studied utilising similar carbon electrodes and demonstrating no difference in the electroanalytical performance of the SPEs through choice of electrical connection.

Throughout many studies using SPEs, the downfall of most electrodes configurations are due to the poor electrical connections when one utilises crocodile clips. Fig. 6A shows the setup used throughout (obtaining near identical results to that of edge
connector) with Fig. 6B showing an inappropriate way to connect the electrode using crocodile clips, resulting in the noisy and unreliable cyclic voltammogram shown in Fig. 6D, unlike the typical and expected voltammetric signature which is shown in Fig. 6C. We systematically explored the distance between connecting crocodile clips and found that the only way to obtain the voltammetric response in Fig. 6D is to have the connections effectively touching which results in a short circuit and no meaningful voltammetric/analytical data to be obtained. It was also found that when utilising the crocodile clips, light damage of the carbon tracks can occur and can be observed visually but was found to not affect the overall cyclic voltammograms presented towards the target analyte. This usually happens when the crocodile clips are not securely clamped and the weight of the wires pull and drag the crocodile clips across the carbon surface resulting in light, but not detrimental damage.

**4. Conclusions**

The experimental setup for the utilisation of screen-printed sensors has been explored, with the main focus upon the electrochemical comparison between the two setups of the traditional approach of crocodile clip connection with that of the edge connector. It is important to note that throughout this study, the employment of crocodile clips for electrochemical analysis within a laboratory environment can produce near identical responses with that of an edge connector. When one diligently connects the crocodile clips, as shown in Fig. 6A and not like that in Fig. 6B, the latter gives rise to useless voltammetric signatures while the former gives near perfect and expected voltammetric responses; some researchers obtain such results as presented in Fig. 6B and unfortunately blame the electrode itself without further explanation.

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**References**