Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPORTING INFORMATION

for

Design and development of an amperometric immunosensor based on screen-printed electrodes

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CONTENTS

Section 1 **Page No(s)**

Fabrication of SPEs

Section 2

Characterization of SPEs

Section 3

Section 4

Section 1

Fabrication of SPEs

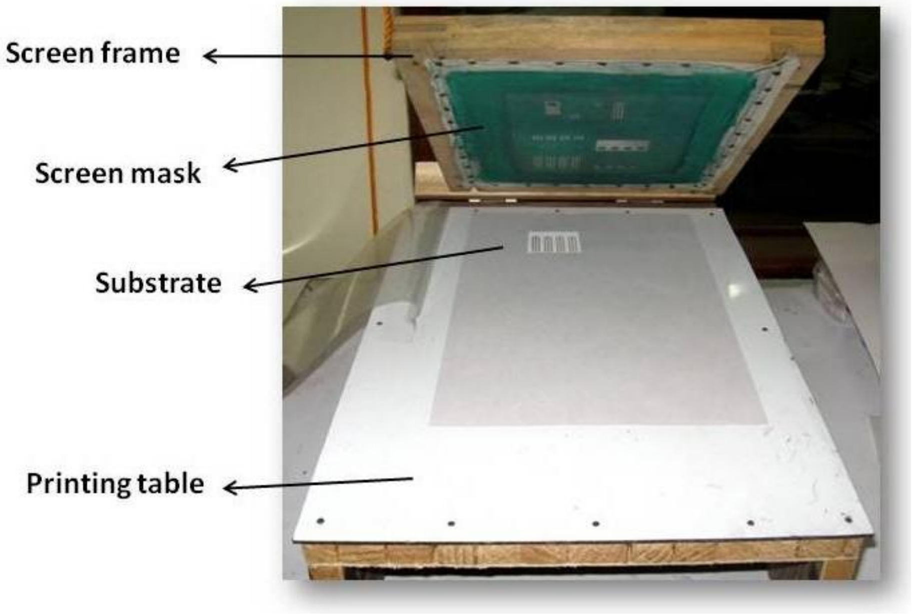


Fig. 1 Manual screen-printer as set up in laboratory

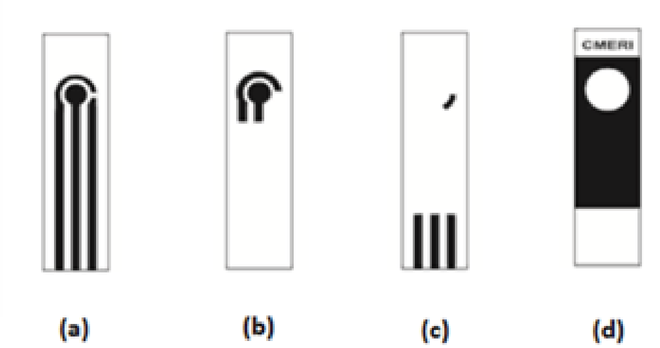


Fig. 2 Masks involved in the fabrication of SPEs (a) conducting tracking line (b) WE and AE (c) RE and (d) dielectric layer.

Fig. 3 Ready-to-use flexible SPEs.

Section 2

Electrochemical characterization of SPEs

a. Cyclic voltammetry (CV)

The fabricated SPEs were characterized by CVs in both potassium ferricyanide and uric acid solutions respectively and their performances compared with commercially available Dropsens (C110) SPEs. The Dropsens electrodes are fabricated on ceramic substrate and the WE and AE are made of carbon whereas the RE is made of silver. The diameter of the WE is 4 mm and the surface is unmodified. [1] Four different concentrations of potassium ferricyanide solutions (0.25 mM, 0.5 mM, 0.75 mM and 1.0 mM) were freshly prepared in 0.5M KCl. A volume of 50 µl of each of these solutions was pipetted onto the WE area of four fresh fabricated SPEs respectively and an equal volume of these solutions were dispensed on four new Dropsens SPEs. In order to perform CV, a sweep potential from -0.2V to 0.5V with a scan rate of 50 mV/s was applied and the voltammogram recorded. In both cases the current intensity increased with the concentration of the analyte as expected (see Fig. 4 and Fig. 5). In case of our laboratory made SPEs, it was observed that the current response was about 2.5 times higher than that of commercial SPEs. The primary reason for this increase in sensitivity is attributed to the increase in surface roughness of the WE as evident from scanning electron microscopic images of the two SPEs and will be discussed later in details. A larger peak to peak separation (ΔE_P) was also obtained in case of our laboratory fabricated SPEs. This may be attributed to a presence of higher percentage of binder in the ink though the exact formulation of ink is unknown due to proprietary issues. [2] Carbon ink curing temperature has also been known to affect the electrochemical responses of SPEs. High curing temperatures lead to greater extent of electrochemical reversibility. This is because high temperatures lead to the removal of organic components and increase in carbon content which strongly correlates with high electron transfer rates. Also removal of surface contaminants and introduction of carbon oxygen functional groups are known to enhance electrochemical reversibility. [3] We tried to calculate the apparent heterogeneous electron transfer rate constant (k^o_{app}) of the two types of SPEs used in our study for potassium ferricyanide redox reaction based on Nicholson method. [4] Using ΔE_P values obtained from Fig. 4 and 5, k^o_{app} values obtained for fabricated SPEs and Dropsens SPEs were found to be 9.27×10^{-4} and 1.82×10^{-3} cm/s respectively (see Section 3). The faster kinetics at commercial SPEs may be facilitated by oxygen containing groups in ink which may interact electrostatically and/or chemically with the redox species. [5]

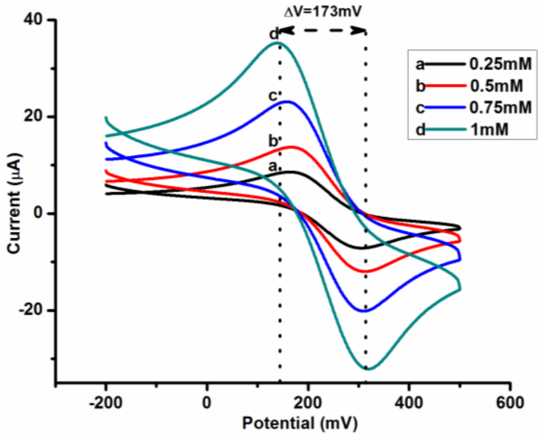


Fig. 4 CV response of in-house fabricated SPEs in a set of four different 0.25mM, 0.5mM, 0.76mM and 1mM potassium ferricyanide solution prepared in 0.5M KCl aqueous solution; applied potential scan rate is 50 mV/s.

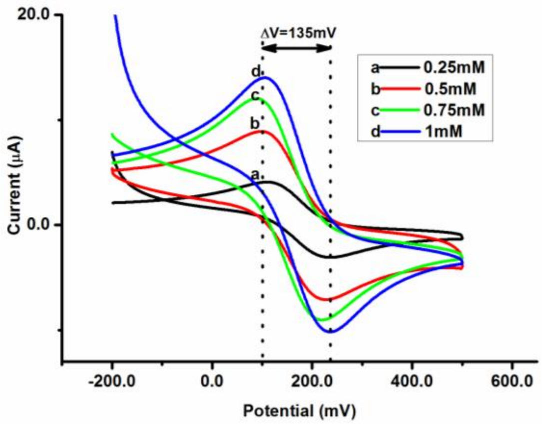


Fig. 5 CV response of Dropsens C110 SPEs in a set of four different 0.25mM, 0.5mM, 0.76mM and 1mM potassium ferricyanide solution prepared in 0.5M KCl aqueous solution; applied potential scan rate is 50 mV/s.

The electrochemical responses of both SPEs were further characterized in uric acid solutions (see Fig. 6 and Fig. 7). Similarly different concentrations of uric acid solutions were prepared in phosphate buffer solution (PBS) at pH 7 and were dispensed on the electrodes. The peak intensity was found to increase with the increase in uric acid concentration as expected. The current amplitude was found to be higher in case of our fabricated SPEs compared to the commercial ones reinforcing the fact that the effective surface area of the WE is higher in case of the former.

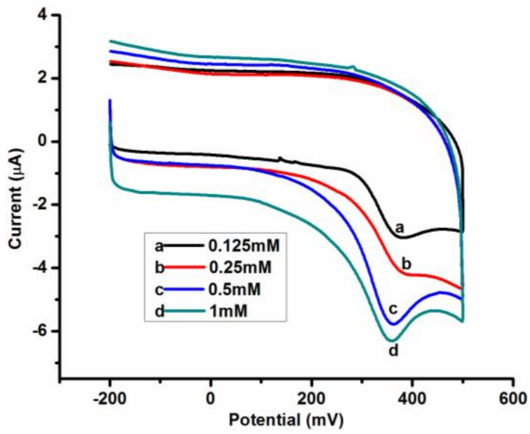


Fig. 6 CV response of in-house fabricated SPEs in a set of four different 0.125mM, 0.25mM, 0.5mM and 1mM uric acid (UA) solution, prepared in PBS at pH=7; applied potential scan rate is 50 mv/s.

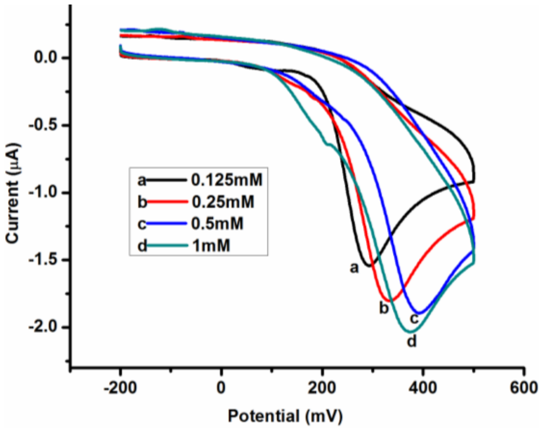


Fig. 7 CV response of commercial SPE ,Dropsens C110 in a set of four different 0.125mM, 0.25mM, 0.5mM and 1mM uric acid (UA) solution, prepared in PBS at pH=7; applied potential scan rate is 50 mv/s.

b. Field emission scanning electron microscopy-energy dispersive X-ray spectroscopy (FESEM-EDS)

The morphology of the electrodes was studied using field emission scanning electron microscopy (FESEM). Fig. 8a and 8b shows the FESEM images of the substrate of fabricated SPEs and Dropsens C110 SPEs respectively. The surface of the former shows nano sized particles interspaced with some larger sized particles. Though the substrate of fabricated SPEs is essentially polyester, it is assumed from (EDS) analysis (see Fig. 9a) that a coating comprised of composite nano oxides of aluminium, silicon and titanium cover the surface. This coating compensates for the low adhesiveness of the pristine polyester film towards inks as well as renders the surface non-tearable and mechanically strong. The surface of Dropsens C110 SPEs reveals pebble sized features which are in fact alumina particles as evident from EDS and XRD analysis (see Fig. 9b and Fig. 13). Comparing the two FESEM images we can conclusively infer that the effective surface area of fabricated SPEs is definitely higher than that of the commercial one though the geometrical areas are equal. This means that the former substrate provides a larger effective surface area for the same geometric area for the fabrication of WE which in turn increases the current response.

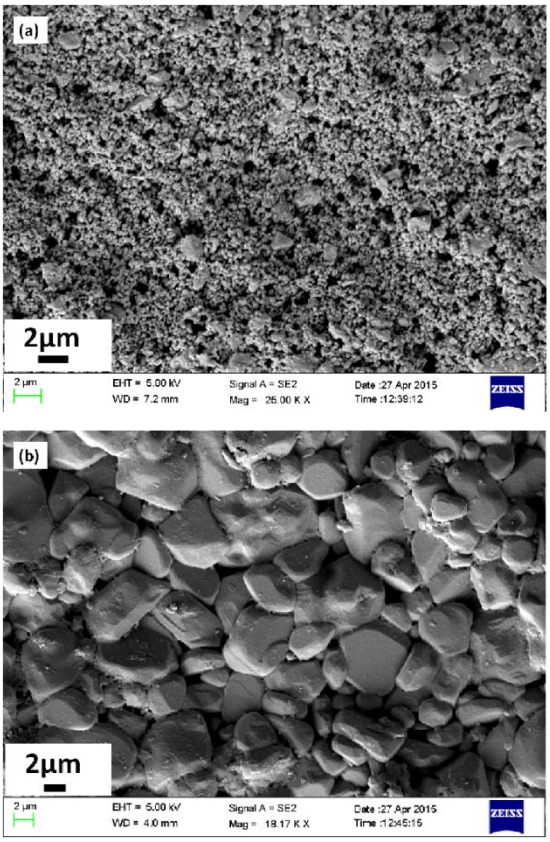
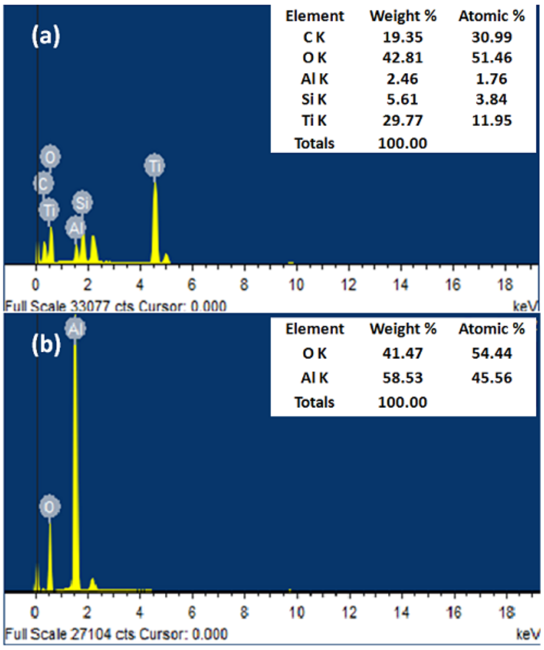


Fig 8 FESEM of the substrate of (a) laboratory made SPEs and (b) Dropsens C110 SPEs.



Element	Weight %	Atomic %
C K	19.35	30.99
O K	42.81	51.46
Al K	2.46	1.76
Si K	5.61	3.84
Ti K	29.77	11.95
Totals	100.00	

Element	Weight %	Atomic %
O K	41.47	54.44
Al K	58.53	45.56
Totals	100.00	

Fig. 9 EDS of the substrate of (a) laboratory made SPE and (b)Dropsens C110 SPE.

For a better understanding, we have also recorded the FESEM image of the bare WE of the laboratory made SPEs (see Fig. 10). The WE revealed the presence of nanosized features and hence a rough surface area. This surface roughness helps in strong immobilization of the analyte and therefore do not require any further surface modifications.

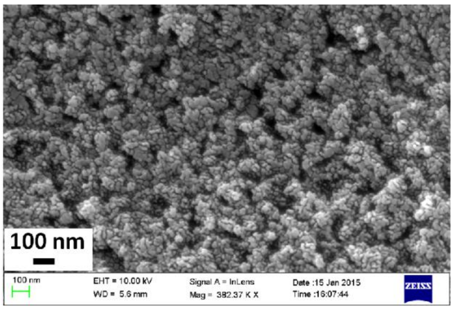


Fig 10 FESEM of the printed carbon WE of laboratory made SPEs.

c. X-ray diffraction (XRD)

The XRD planes of the substrate used for printing SPEs in laboratory predominantly reveal a semi crystalline polyethylene terephthalate (PET) material with a single high intensity peak centred around 26° and a minute broad peak around 45-50° (Fig. 11a) [6] Some low intensity peaks were also visible and on higher magnification revealed the XRD planes of rutile phase of titania corresponding to JCPDS file no. 88-1175. (Fig. 11b). [7] The presence of titania was also evident from the EDS analysis of the substrate as discussed earlier. XRD peaks corresponding to compounds containing aluminium and silicon were not visible which might be due to their very low weight percent in the substrate.

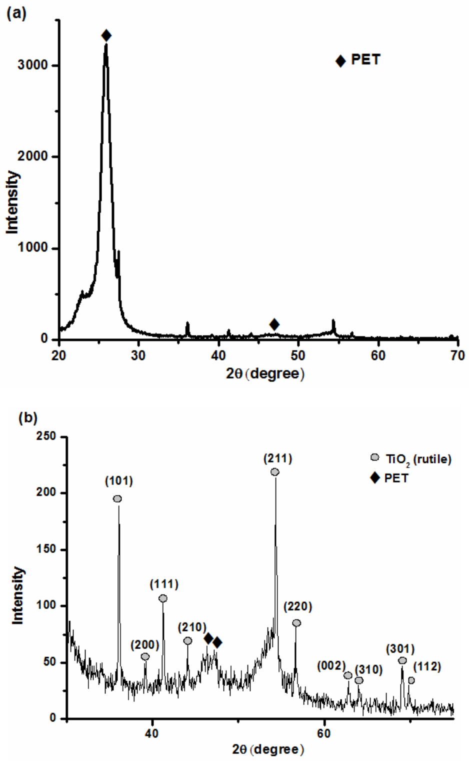


Fig. 11 XRD pattern of (a) substrate used for fabricating SPE and (b) magnified portion of the same from 30°. (the diamonds represent the phases of PET and the spheres indicate the XRD planes of rutile phase of titania.)

Fig. 12 shows the XRD planes of the carbon ink used for fabricating the WE and CE of our laboratory made SPEs. The sharp intense peak at 27.4° is characteristic of 002 plane of graphitic particles corresponding to JCPDS file no. 41-1487. [8] Fig. 13 shows the XRD pattern of Dropsens C110 SPE substrate. The peaks clearly indicate the presence of alumina (JCPDS file no. 46-1212) in the substrate. [9] This finding is also complimented by EDS analysis which reveal the presence of aluminium and oxygen solely in the substrate.

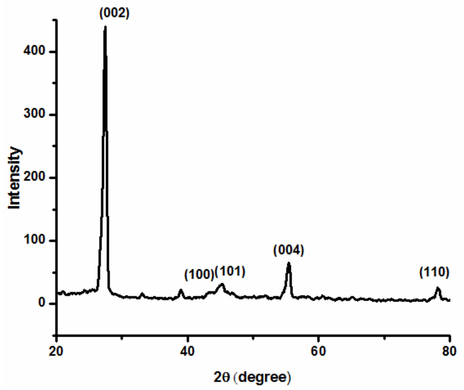


Fig. 12 XRD pattern of the carbon ink used for fabricating WE and CE of SPE.

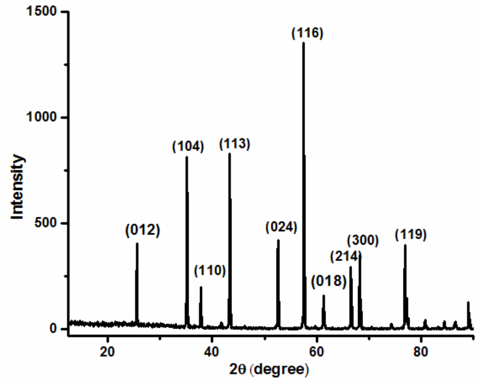


Fig. 13 XRD pattern of the substrate of Dropsens C110 SPE.

Section 3

Evaluation of apparent heterogeneous electron transfer rate constant (k^{o}_{app}) for SPEs in potassium ferricyanide solution based on Nicholson method

The oxidation of potassium ferrocyanide to potassium ferricyanide in aqueous solution involves the transfer of a single electron and exhibits close to ideal quasi-reversible outer sphere kinetic behaviour, especially at carbon electrodes where there is negligible interaction between the electrode material and reactant. [10] The apparent heterogeneous rate constant for electron transfer (k^{0}_{app}) is related to the peak separation ΔE_P in cyclic voltammetry and can be evaluated by Nicholson method [4] using the equation:

$$\Psi = \frac{\left(\frac{D_O}{D_R}\right)^{\frac{\alpha}{2}}}{\left(\frac{\pi D_O nFv}{RT}\right)^{1/2}} k^0{}_{app} \qquad \text{Eq. (1)}$$

where,

Ψ= dimensionless kinetic parameter which influences the shape of voltammetric curve

D_O and D_R are the diffusion coefficients of the oxidised and reduced species respectively in cm^2/s; for simplicity both are considered equal in case of potassium ferricyanide.

α = transfer coefficient = 0.5

F = Faraday constant= 96485.33 coulombs/mole

R = Universal gas constant= 8.314 Joules/mole/°K

n = no. of electrons transferred =1

v = potential scan rate in V/s

T= Temperature in °K

So, Eq. (1) takes the form:

$$\Psi = \frac{k^0{}_{app}}{\left(\frac{\pi D_O Fv}{RT}\right)^{1/2}} \qquad \text{Eq. (2)}$$

The exact variation of peak potentials with Ψ was shown by a graph in Nicholson's classical work. [11] The experimental $\Delta E_P \times n$ values can be obtained from cyclic voltammetry and the corresponding value of Ψ is obtained from the graph. For a known scan rate, k^0_{app} can then be obtained using Eq.(2). However because of the lack of data in the working range, in a much later work, Lavagnini *et al.* [12] formulated an empirical equation (Eq.3) which enabled the evaluation of Ψ for $\Delta E_P \times n$ values upto 200 mV and which can practically replace the Nicholson's working curve.

$$\Psi = (-0.6288 + 0.0021X)/(1-0.017X) \qquad \text{Eq. (3)}$$

where, $X = \Delta E_P \times n$ in mV

For cyclic voltammetry of potassium ferricyanide in water, n=1, D_O is taken to be 7.6×10^{-6} cm^2/s [13], $v = 50\ \mathrm{mV/s} = 0.05\ \mathrm{V/s}$ and temperature is fixed at 298°K.

For our laboratory made SPEs, $\Delta E_P \times n$ value obtained was 173 mV from the cyclic voltammetry of potassium ferricyanide in water.

Using Eq.(3), the value of Ψ comes out to be 0.136.

Now, putting the value of Ψ in Eq. (2) we get

$k^o_{app} = 9.27 \times 10^{-4}$ cm/s

Similarly, for Dropsens C110 SPEs, $\Delta E_P \times n$ value obtained was 135 mV from the cyclic voltammetry of potassium ferricyanide in water.

The value of Ψ obtained was 0.267 which yielded k^o_{app} of 1.82×10^{-3} cm/s.

Section 4

References

1 Please visit http://www.dropsens.com/en/screen_printed_electrodes_pag.html#unmodified_spes for more details.
2 E. P. Randviir, D. A. C. Brownson, J. P. Metters, R. O. Kadara and C. E. Banks, *Phys.Chem.Chem.Phys.*, 2014, **16**, 4598-4611.
3 J. Wang, M. Pedrero, H. Sakslund, O. Hammerich and J. Pingarron, *Analyst,* 1996, **I21**, 345-350.
4 R. S. Nicholson, *Anal. Chem.*, 1965, **37**, 1351-1355.
5 S. Ko, T. Tatsuma, A. Sakoda, Y. Sakai and K. Komori, *Phys.Chem.Chem.Phys.*, 2014, **16**, 12209-12213.
6 B. Demirel, A. Yaraş and H. Elçiçek, *BAÜ Fen Bil. Enst. Dergisi Cilt*, 2011, **13**, 26-35.
7 K. Thamaphat, P. Limsuwan and B. Ngotawornchai, *Kasetsart J. (Nat. Sci.)*, 2008, **42**, 357-361.
8 G. Sun, X. Li, Y. Qu, X. Wang, H. Yan and Y. Zhang, *Mater. Lett.*, 2008, **62**, 703-706.
9 A. Bourbia, S. Boulkhessaim, H. Bedboudi and M. Draissia, *Phys. Scr.*, 2012, **85**, 055601.
10 Q. Zhu, R. Yuan, Y. Chai, J. Han, Y. Li and N. Liao, *Analyst*, 2013, **138**, 620-626.
11 1. M. E. G. Lyons 1 and G. P. Keeley, *Sensors*, 2006, **6**, 1791.
12 I. Lavagnini, R. Antiochia and F. Magno, *Electroanalyis*, **16**, 505.
13 W. J. Blaedel and R. C. Engstrom, *Anal. Chem.*, 1978, **50**, 476.