

Decreasing the Polymerization Potential Improves the Selectivity of PPD Coated Disc Biosensors for Glutamate

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Decreasing the Polymerization Potential Improves the Selectivity of PPD Coated Disc Biosensors for Glutamate

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Abstract

Selectivity of glutamate microdisc biosensors coated with poly(*o*-phenylenediamine) (PPD) as the interference rejecting layer against ascorbic acid was observed to be very low. Enhancement in the selectivity was noticed when the electropolymerization potential for the polymerization of the *o*-phenylenediamine monomer was decreased from 0.65V to 0.40V vs. Ag/AgCl. The selectivity coefficient increased from $-34.93 \pm 3.75 \% (n = 5)$ to $53.05 \pm 4.33 \% (n = 3)$. Decreasing the polymerization potential decreases the rate of formation of the polymer and therefore improves the compactness of the polymer layer formed, thereby increasing the selectivity of the electrodes.

Introduction

Biosensors to monitor the levels of glutamate with applications towards food industry and health have been developed since the early 1990s. Biosensors utilize a biological element such as an enzyme or an antibody to detect electro-inactive or poorly electroactive substances (O'Neill and Lowry 2006; O'Neill, Lowry, and Mas 1998). Biosensors prepared from different electrode materials and enzymes have been used to detect a variety of analytes, with glucose getting considerable attention due to its importance in diabetes care. More recently, neurochemical monitoring has gained considerable attention and in particular, the excitatory amino acid neurotransmitter glutamate due to its abundant

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4 presence in the brain and its association to pathophysiological conditions such as
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6 neuronal synaptic plasticity, learning and memory, ischaemia, secondary brain injury,
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8 schizophrenia and epilepsy amongst others (Budd 1998; Danbolt 2001; Dingledine and
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10 McBain 1999; Lancelot and Beal 1998). *In vivo* monitoring of glutamate by
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12 electrochemical methods has been mentioned to be a difficult process due to the presence
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14 of several electro-active species in the brain extracellular fluid that could be oxidised at
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16 the electrodes, influencing the measured current (O'Neill and Lowry 2006); of particular
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18 importance is ascorbic acid which has been found to be present in approximately 50 times
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20 larger concentration than glutamate in the normal brain (O'Neill 1994). Therefore,
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22 particularly for *in vivo* applications, the selectivity of the biosensor is of utmost
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24 importance. First generation biosensor methods of H₂O₂ detection utilizing potentials of
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26 0.65V or 0.70V vs. Ag/AgCl reference electrode are more prone to interferences due to
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28 higher recording potentials. To improve the selectivity of the first generation glutamate
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30 biosensors, several techniques have been used such as polymer coatings on platinum,
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32 gold, palladium or glassified carbon electrodes (O'Neill et al. 2004) (*e.g.*, over oxidised
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34 polypyrrole (Hamdi et al. 2006), polyphenylenediamines (O'Neill, Lowry, and Ryan
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36 1997), polyurethanes (Yu, Moussy, and Moussy 2005), polynaphthalenes (Murphy 1998),
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38 Nafion (Gerhardt et al. 2002)).

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40 Electropolymerization of *o*-phenylenediamine (*o*-PD) at platinum electrodes have been
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42 shown to provide a high level of selectivity at platinum cylinders while maintaining a
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44 high sensitivity to the target analyte (O'Neill, Lowry, and Ryan 1997). Recently,
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46 McMahon and co-workers showed that platinum microdiscs showed increased oxygen
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48 tolerance when compared to platinum cylinders (McMahon et al. 2006). In addition, in
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50 our own study, it was found that the temporal stability of the glutamate response was
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52 higher for microdiscs than cylinders (results not shown). But the selectivity of poly(*o*-
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3 phenylenediamine) (PPD) covered microdiscs have been shown to be quite low when
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5 compared to 1mm cylinders and that the efficiency of the polymer formation decreases
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7 with decrease in the surface area of the electrode (McMahon et al. 2004). Therefore, in
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9 this study, we investigate the impact of decreasing the potential required for the
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11 electropolymerization of the monomer on the selectivity of the PPD coated disc
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13 electrodes.
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17 Teflon[®] coated platinum wire of 125 μ m diameter was used for the experiments.
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19 Microdiscs of 125 μ m diameter were obtained by cutting the insulated platinum wire
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21 transversely and leaving the Teflon[®] coating intact. The solutions for glutamate (Glut),
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23 ascorbic acid (AA) and H₂O₂ were prepared in distilled water, while the monomer
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25 solution (300mM *o*-PD + 0.5% (w/v) BSA) was prepared in PBS at pH 7.4.
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29 To determine the effects of polymerization potential on the selectivity of PPD covered
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31 disc electrodes, different polymerization potentials were examined; 0.40V, 0.45V, 0.50V
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33 and 0.65V vs. Ag/AgCl for 20 minutes. The polymerization was performed at bare
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35 platinum disc electrodes by applying a potential against Ag/AgCl electrode in a 3
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37 electrode cell with a stainless steel needle acting as the auxilliary electrode immersed in
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39 the monomer solution mentioned above. All calibrations were performed in a two
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41 electrode cell with the reference electrode acting as both reference and counter at 0.65V
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43 vs. Ag/AgCl in PBS at pH 7.4.
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50 51 **Results and Discussion**

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53 Poly(*o*-phenylenediamine) electropolymerized at neutral pH has been shown to form a
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55 insulating, self-sealing polymer layer on Pt and other electrode materials (O'Neill et al.
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57 2004; O'Neill et al. 1994; O'Neill, Lowry, and Ryan 1997; McMahon et al. 2004). The
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59 selectivity of disc electrodes immobilized with glutamate oxidase (GluOx) and
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3 electropolymerized with PPD-BSA (Pt_D/GluOx/PPD-BSA) at 0.65V vs. Ag/AgCl
4 provided a mean selectivity of $-34.93 \pm 3.75 \%$ ($n = 5$) against ascorbic acid calculated as
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8 $S_{\text{Glut(AA)}} = (I_{\text{Glut}} - I_{\text{AA}}) * 100 / I_{\text{Glut}}$ (O'Neill, Lowry, and Ryan 1997). I_{Glut} and I_{AA}
9 represent the absolute steady-state currents obtained for $10 \mu\text{mol l}^{-1}$ glutamate and 500
10 $\mu\text{mol l}^{-1}$ AA respectively, as they correspond to the physiological levels of the substances
11 in the brain ECF. A negative selectivity value meant that the response for $500 \mu\text{mol l}^{-1}$
12 AA was greater than the response for $10 \mu\text{mol l}^{-1}$ Glut. A biosensor with a negative
13 selectivity value would be unusable and therefore further investigation was necessary in
14 order to attempt to enhance the selectivity of PPD coated discs towards glutamate.

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25 As it had been speculated by McMahan et al. that the decreased selectivity at disc
26 electrodes could be due to the highly efficient hemispherical transport of monomer
27 molecules resulting in a very rapid but non-compact polymer formation on the electrode
28 (McMahan et al. 2004), it was necessary to decrease the rate of polymer formation in
29 order to increase the selectivity. As it is well known that the rate of polymer formation is
30 affected by variations in monomer concentration, polymerization potential and the
31 polymerization time, amongst others, the effect of polymerization potential on the
32 selectivity of the PPD coated discs were considered.

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The effect of polymerization potential on the selectivity of the electrodes was studied by
performing electropolymerization at a series of different potentials on bare platinum
discs. The results showed a drastic improvement in the selectivity of the electrodes with
decreasing potential. Since the electrodes were devoid of the enzyme, H₂O₂ was used to
determine their selectivity. The selectivity of the PPD coated discs (Pt_D/PPD-BSA) were
calculated using the formula $S_{\text{AA(H}_2\text{O}_2)}} = I(\text{AA}) * 100 / I(\text{H}_2\text{O}_2)$, with $I(\text{AA})$ and $I(\text{H}_2\text{O}_2)$
representing the currents obtained at 1mM concentrations respectively (McMahan et al.
2004). As $I(\text{AA})$ should ideally be very small when compared to $I(\text{H}_2\text{O}_2)$, the electrode

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3 with a $S_{AA(H_2O_2)}$ value closer to zero would a lower relative permeability to AA compared
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6 to H_2O_2 and hence have a higher selectivity towards H_2O_2 and glutamate. By performing
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8 cyclic voltammetric experiments it was determined that a polymerization potential lower
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10 than 0.4V (vs. Ag/AgCl) would result in a non-compact polymer layer as the threshold
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12 potential was identified to be close to 0.35V. Performing H_2O_2 and AA calibrations at
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14 PPD coated electrodes electropolymerized at 0.40V, 0.45V, 0.50V and 0.65V vs.
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16 Ag/AgCl, selectivity values of 0.16%, 0.18%, 0.37% and 0.72% were obtained
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18 respectively; a 400% increase in selectivity could be seen when the electrode was
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20 polymerized at 0.40V (vs. Ag/AgCl) when compared to 0.65V (vs. Ag/AgCl) which was
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22 common until now. A graphical representation of the AA calibration at electrodes
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24 polymerized at different potentials along with the respective selectivity coefficients have
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26 been shown in Figure 1.
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32 By closer observation of the currents obtained during polymerization at different
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34 potentials reveals the nature of polymer of formed. From Figure 2, the magnitude of the
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36 initial spike currents obtained immediately following the start of the polymerization
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38 process can be seen to decrease with decrease in the polymerization potential. This results
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40 in a slower self-sealing polymer formation leading to a more compact polymer layer,
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42 thereby increasing the electrode selectivity.
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46 Following the observation of improvement in selectivity with decrease in polymerization
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48 potential, enzyme incorporated disc electrodes were prepared with the polymerization
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50 performed at 0.45V (vs. Ag/AgCl). The selectivity $S_{Glut(AA)}$ could be seen to have
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52 improved to $53.05 \pm 4.33 \%$ ($n = 3$) from $-34.93 \pm 3.75 \%$ ($n = 5$) obtained for electrodes
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54 polymerized at 0.65V (vs. Ag/AgCl). Figure 3 shows the selectivities obtained for
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56 glutamate against ascorbic acid with the electropolymerization potential maintained at
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58 0.65V and 0.45V (vs. Ag/AgCl) respectively. Since all the other measurement parameters
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3 were similar during the experiments, the improvement in selectivity for glutamate can be
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5 directly attributed to the decreased electropolymerization potential.
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10 **Conclusion**

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12 In this study we observed that decreasing the polymerization potential for the formation
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14 of PPD at platinum microdiscs, increases the compactness of the polymer structure due to
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16 slower diffusion of the monomer towards the electrode, thereby increasing the selectivity
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18 of the disc electrodes to glutamate and H₂O₂. Additional lipid coatings incorporated into
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20 the biosensor matrix enhanced the selectivity of glutamate microdiscs above 85% (results
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22 not shown).
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30 **Acknowledgement**

31
32 We thank Prof. R.D. O'Neill (Department of Chemistry, University College Dublin,
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34 Ireland) for his guidance and support.
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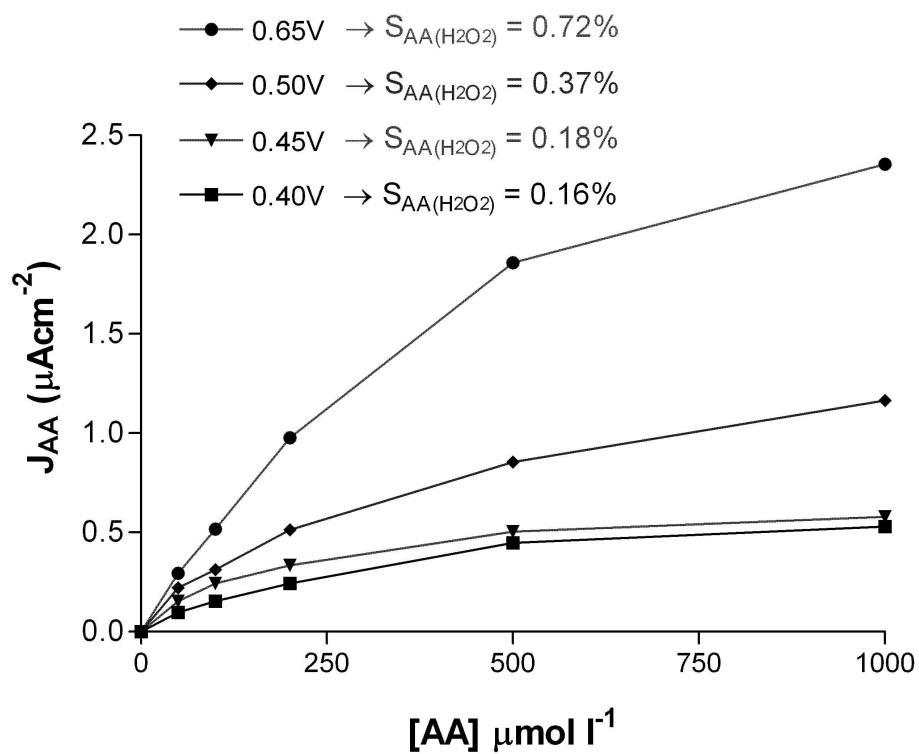


Figure 1: Ascorbic acid calibrations obtained at Pt_D/PPD-BSA electrodes polymerized at different potentials. The values are represented as current densities. The selectivity values for AA obtained against H_2O_2 at the different polymerization potentials are shown in the legend.

116x93mm (600 x 600 DPI)

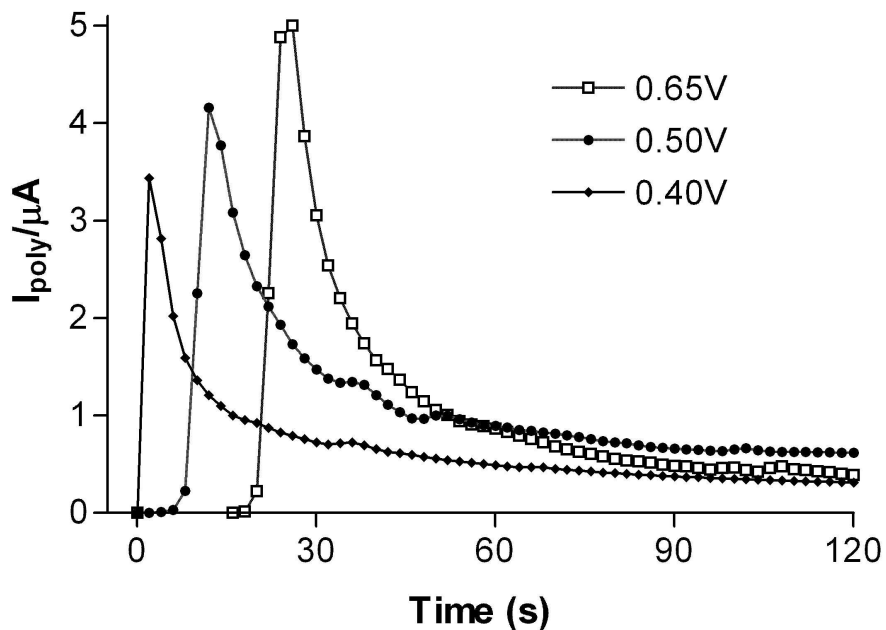
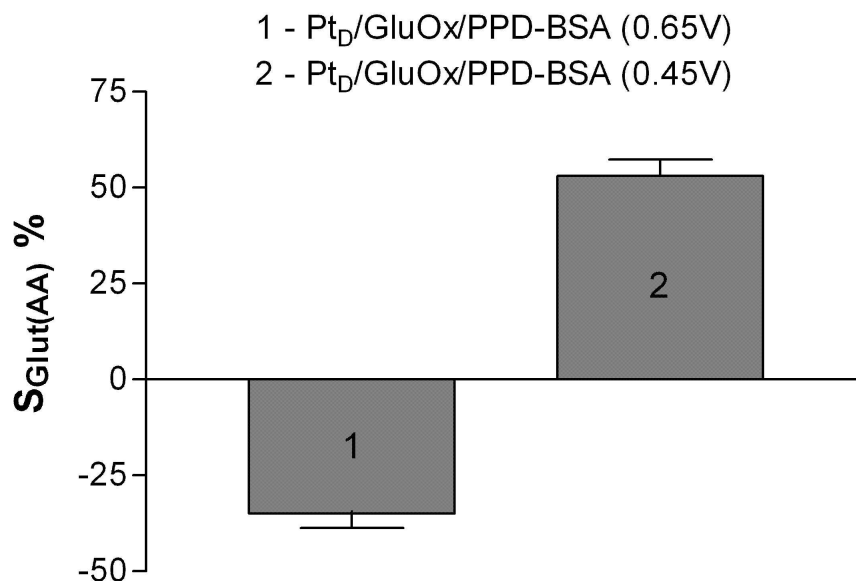


Figure 2: Currents obtained during the first 2 minutes after the initiation of electropolymerization of bare platinum discs at 0.40V, 0.50V and 0.65V (vs. Ag/AgCl). The currents have been shown with a 5 second time shift for better readability. The lower the polymerization potential, the lower the initial spike, leading to slower and more compact polymer deposition.
102x73mm (600 x 600 DPI)



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Figure 3: Selectivity values obtained for Pt_D/GluOx/PPD-BSA biosensor with the PPD-BSA polymerization performed at 0.65V and 0.45V vs. Ag/AgCl respectively have been shown. 1 - Pt_D/GluOx/PPD-BSA (0.65V) with a S_{Glut(AA)} value of -34.93 ± 3.75 % (n = 5) and 2 - Pt_D/GluOx/PPD-BSA (0.45V) with a S_{Glut(AA)} value of 53.05 ± 4.33 % (n = 3). The bars indicate Mean ± SEM values.
105x65mm (600 x 600 DPI)