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Short communication

A polyamidoamine dendrimer-streptavidin supramolecular architecture for biosensor development

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ABSTRACT

A novel polyamidoamine dendrimer-streptavidin supramolecular architecture suitable as a versatile platform for biosensor development is reported. The dendrimer was electrodeposited on a glassy carbon electrode via cyclic voltammetry. The dendrimer electrode was further modified with streptavidin by electrostatic attraction upon drop coating. The platform i.e. the dendrimer-streptavidin modified electrode was electrochemically interrogated in phosphate buffer, ferrocyanide and H_2O_2. The dendrimer-streptavidin platform was used in the preparation of a simple DNA biosensor as a proof of concept. The supramolecular architecture of dendrimer-streptavidin was stable, electroactive and thus lends itself as a versatile immobilisation layer for any biotinylated bioreceptors in biosensor development.

1. Introduction

Research on biosensors have been on the increase since the pioneering works of Clark and others [1,2]. To date, electrochemical biosensors (a subset of biosensors with electrochemical transducer) have been developed for various targets of biochemical, biological and environmental importance [3]. Despite the strides made in biosensor research, there are still challenges mainly around detection limit, selectivity, sensitivity, reproducibility and stability. To a large extent these challenges are related to the immobilisation step in biosensor design. Immobilisation optimisation encompasses the choice of an immobilisation layer/material and immobilisation chemistry. Since one of the main philosophy in biosensor design is biomimicry, the immobilisation process and the environment must therefore be as close to nature as possible. The materials of choice must be biocompatible and the chemistry of bonding the bioreceptor to the immobilisation layer must be 'gentle' enough to mimic the biological or natural environment of the bioreceptor. Furthermore, such linkage should not usurp the structural integrity of the bioreceptor so as to enhance the biorecognition event. The branch of chemistry or bonding principle that best mimics nature is supramolecular because it involves non-covalent bonds such as hydrogen bonding, and other van Der Waals forces. Supramolecular chemistry which has been defined as the chemistry of the molecule [4–6], is indeed the chemistry of nature. For example, the binding of DNA, the folding of protein etc. are supramolecular. Therefore, the quest and application of biocompatible materials and biomolecules with supramolecular features will open up more options for research in biosensor optimisation - a class of such materials are dendrimers.

Dendrimers [6] have been shown to be biocompatible as seen from their applications in sensors [7–9], drug and gene delivery [10]. They are also excellent candidates in supramolecular architectures [10,11], host-guest and click chemistry [12]. On the other hand, streptavidin has also been used as a biomaterial in biosensor design [13]. The application of streptavidin in biosensors is based on the high affinity between biotin and streptavidin. The biotin-streptavidin bond, which is supramolecular, is one of the strongest non-covalent biospecific interactions known. The affinity constant of biotin-streptavidin in solution has been reported to be as high as $Ka \approx 10^{13}\,M^{-1}$ with kinetic irreversibility [14]. In principle, as long as the bioreceptor can be biotinylated, a platform of streptavidin will be suitable for excellent immobilisation. The challenge however is the immobilisation of streptavidin onto the electrode or the modification of an electrode with streptavidin.

This paper reports a novel supramolecular approach to immobilising streptavidin on a polyamidoamine dendrimer (PAMAM) modified glassy carbon electrode. The preparation of a PAMAM modified carbon electrode by electrodeposition from an aqueous solution is also reported for the first time (to the best of our knowledge).

2. Experimental

Streptavidin, streptavidin-peroxidase, TE buffer and generation 3 (G3) poly(amidoamine) (PAMAM) dendrimer and all other chemicals

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were purchased from Sigma-Aldrich. A 5′ Biotin Probe 5′-GGTTGGTGTGGTTGG-3′ and its Complementary 5′-CCAACCACACCA ACC-3′ were obtained from Inqaba biotech (South Africa). All glassware was sterilised by autoclaving for 30 min. Electrochemical analysis was carried out at room temperature using Zahner Zenium electrochemical workstation running on Thales software (Zahner, Germany). A 3 mL electrochemical cell with a convectional three electrode setup was used with a bare or modified GCE as working electrode, a platinum wire as counter electrode and an Ag/AgCl (3 M KCl) as reference electrode.

The PAMAM modified glassy carbon electrode (GCE) was fabricated by electrodeposition from a 10 mM G3 PAMAM dendrimer solution by cyclic voltammetry (15 scans) at a potential window of −200 mV to 1100 mV at 50 mV s^{-1} scan rate. This was followed by a gentle rinse in ultrapure water. The GCE/PAMAM electrode was further modified by dropping with 50 μL of 100 μg mL^{-1} streptavidin of pH 7.5. The electrodes (at each stage of the process) was electrochemically characterised in 10 mM phosphate buffer saline (containing 0.3 M KCl) of pH 7.5 and in 5 mM (1:1) $[Fe(CN)_6^{3-/4-}]$ redox probe prepared in 10 mM phosphate buffer saline (PBS) solution. Square wave voltammetric measurements were performed at a potential window of −100 mV to 900 mV with an amplitude of 25 mV and frequency of 15 Hz. All impedance measurements were performed with a 250 mV alternating current potential and a 10 mV voltage amplitude in a frequency range from 100 kHz to 100 mHz. The Randle's equivalent circuit was used to obtain the impedance fitting parameters.

For the immobilisation of probe DNA, the dendrimer-streptavidin electrode (GCE/PAMAM/Strept) was drop coated with 50 μL of 2 μM biotinylated probe DNA for 1 h at room temperature and subsequently rinsed gently with ultra-pure water and 10 mM PBS of pH 7.2 respectively to remove the unbound DNA probes. Thus, the biosensor (GCE/PAMAM/Strept/ssDNA) was fabricated and stored at 4 °C when not in use.

The biosensor was immersed into a hybridisation buffer containing different concentrations of the target complementary DNA (DNAc) ranging from 0.05 nM to 10 nM for 1 h at 38 °C. Subsequently the biosensor was washed gently with water and phosphate buffer solution respectively to remove the un-hybridised DNA and then characterised electrochemically.

3. Result and discussion

Generation 3 PAMAM was deposited onto a glassy carbon via electrooxidation of its peripheral primary amine (Supp Fig. 1) onto the carbon electrode via a C—N linkage [15] and labelled GCE/PAMAM. The portions highlighted by arrows in Fig. 1a shows the onset redox peaks suggesting the electroactive nature of PAMAM around 400 mV (vs Ag/AgCl) (see Fig. 1a inset) in phosphate buffer during electrodeposition. Similar results have been reported for the electrodeposition of poly(propylene imine) dendrimer with an electroactive response at around 200 mV (vs Ag/AgCl) [15,16]. The GCE/PAMAM (and other electrode modifications which will be discussed later) was characterised in

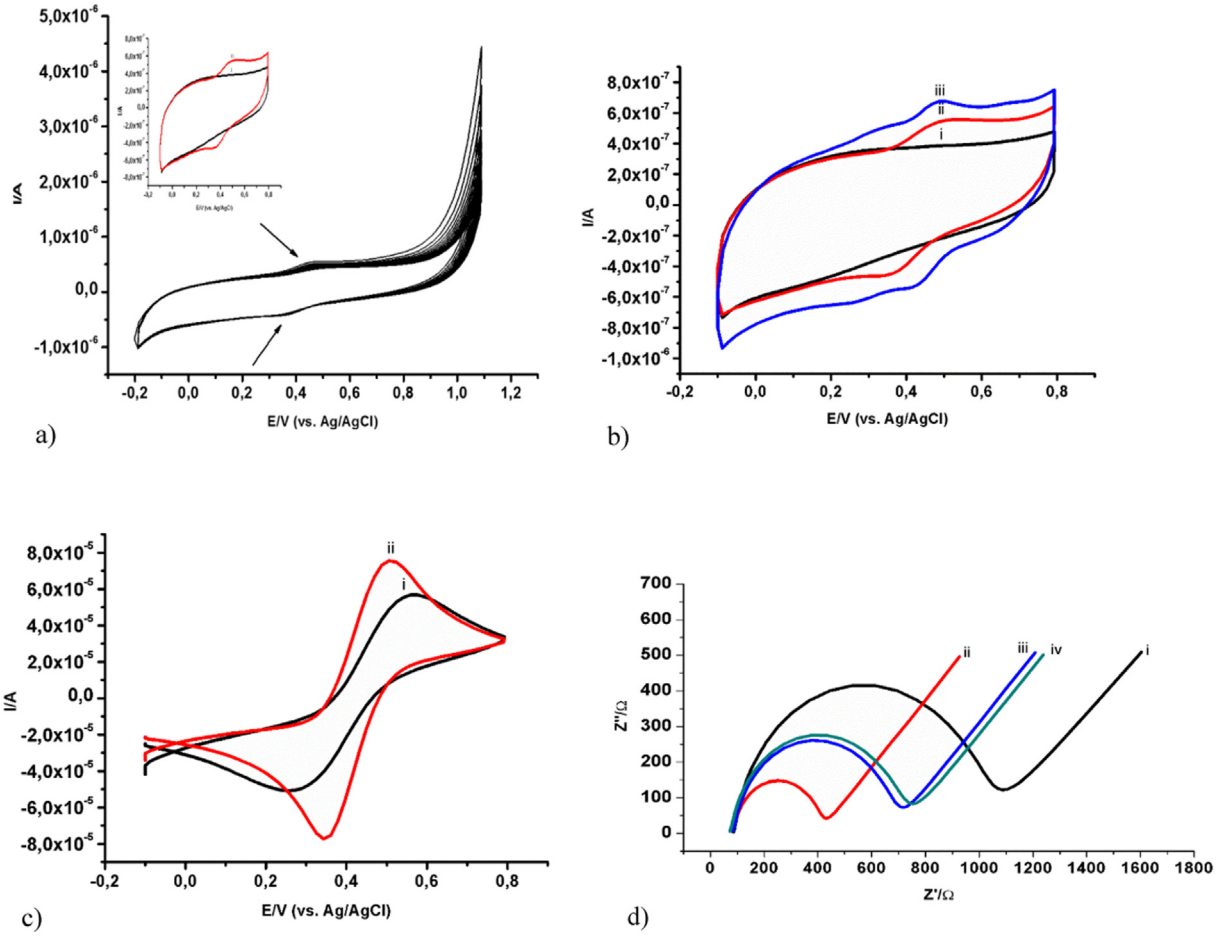

Fig. 1. (a) Cyclic voltammogram of the electrodeposition of 10 mM G3 PAMAM in 10 mM PBS. Inset is the overlay of bare GCE (i, black) and GCE/PAMAM (ii, red) in 10 mM PBS. (b) CV of the overlay of bare GCE (i, black), GCE/PAMAM (ii, red) and GCE/PAMAM/Strept (iii, blue) in 10 mM PBS. (c) Cyclic voltammograms of bare GCE (i, black) and GCE/PAMAM (ii, red) in the presence of 5 mM $[Fe(CN)_6^{3-/4-}]$. (d) Nyquist plot of bare GCE (i, black), GCE/PAMAM (ii, red), GCE/PAMAM/Strept (iii, blue) and GCE/PAMAM/Strept/ssDNA (iv, green) in the presence of 5 mM $[Fe(CN)_6^{3-/4-}]$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PBS by cyclic voltammetry (Fig. 1b) and square wave voltammetry (Supp Fig. 2a) where the redox peaks observed during the electrodeposition step can be seen clearly. This redox pair is not only a confirmation of the electrodeposition of PAMAM, but it also shows that PAMAM exhibits electroactive behaviour. The redox couple or faradaic current observed is as a result of the protonation and deprotonation of the amine functional groups within the dendrimer as influenced by the application of potential. PAMAM has primary, secondary and tertiary amine groups which can be protonated within its structure. The concept of dendrimer as polyelectrolyte has been studied [17–20] and we have reported the electroactive nature of poly(propylene imine) dendrimer which has similar amine groups to PAMAM [15]. The presence of amine on PAMAM is expected to produce a similar electrochemistry though the redox couple observed for PAMAM has a different formal potential in comparison to PPI owing to the differences in amine groups. Some organic polymers (such as polyaniline) with amine group susceptible to protonation and deprotonation, have also been found to be electroactive. The effect of PAMAM on the electron transfer kinetics of $[Fe(CN)_6^{3-/4-}]$ redox probe was investigated with cyclic voltammetry (Fig. 1c) and with square wave voltammetry (SWV) (Supp Fig. 2b) so as to further characterise the dendrimer modified electrode electrochemically. The presence of the dendrimer film on the GCE is confirmed by the following: 1) The increase in redox current after the deposition of the dendrimer. 2) The increase in the electron transfer rate at the GCE/PAMAM as denoted by the lower ΔE value for GCE/PAMAM. 3) The electroactive surface area of the electrode which increased from 0.1016 cm^2 for bare GCE to 0.1993 cm^2 for GCE/PAMAM as calculated from Randle's Sevcik equation $Ipa = (2.69 \times 10^5) n^{2/3} A_{ff} D^{1/2} v^{1/2} C_0$ [21].

The electrodeposition of PAMAM and the electrode build up was also characterised with electrochemical impedance spectroscopy (EIS) as depicted in Fig. 1d. A faster interfacial electron transfer of $[Fe(CN)_6^{3-/4-}]$ occurred at the GCE/PAMAM interface owing to the decrease in charge transfer resistance (R_{ct}) from 1050 Ω (bare GCE) to 332 Ω (GCE/PAMAM). The reduction in the R_{ct} value suggests the enhancement of charge flow (better conductivity) at the electrode interface.

The GCE/PAMAM was further modified with streptavidin in the bid to create a robust platform suitable for the immobilisation of a myriad of biotinylated bioreceptors. The immobilisation strategy is based on the exploitation of the isoelectric points of molecules. The isoelectric point of streptavidin is ca 5 and thus negative in the presence of PBS of pH 7.5. At this pH, the charge on the GCE/PAMAM is positive and thus electrostatic attraction between the PAMAM and the streptavidin ensued. This electrode modification step is supramolecular and should thus maintain the structural integrity of streptavidin suitable for its natural binding to biotin. It is interesting to note that the reversible redox peaks due to the PAMAM were still present after the immobilisation of streptavidin with $E^{\circ\prime} = 450$ mV (vs Ag/AgCl) and $I_{pa}/I_{pc} \approx 1$ (Fig. 1b). The square wave voltammogram also shows a similar trend (Supp Fig. 2a). The authors presume that the PAMAM and the streptavidin alignment did not hinder the flow of charge at the electrode interface owing to the supramolecular chemistry of immobilisation. The electrode building steps were also interrogated in the presence of $[Fe(CN)_6^{3-/4-}]$ redox probe with EIS (Fig. 1d) and SWV (Supp Fig. 2b). After the immobilisation of streptavidin the charge transfer increased (Table 1) as a resulted of the charge repulsion between streptavidin and the redox probe. In addition, the protein streptavidin is not as conducting as the PAMAM and thus hampered the flow of electron at the electrode interface. A similar trend can be observed from voltammetry in $[Fe(CN)_6^{3-/4-}]$ (Supp Fig. 2b) where the anodic peak current decreased after the supramolecular immobilisation of the streptavidin on PAMAM.

In order to confirm the attachment of streptavidin onto the surface of the dendrimer platform, a horseradish peroxidase enzyme tagged streptavidin (Strept-HRP) was used to modify the GCE/PAMAM electrode in the same manner as pristine streptavidin. An electrocatalytic reduction of H_2O_2 at this electrode will be used to confirm the presence of streptavidin on the GCE/PAMAM/Strept electrode in the PBS

Table 1
Fitting parameters obtained from the Randle's equivalent fitting of Fig. 1d.

Circuit element	R_s (Ω)	R_{ct} (Ω)	CPE (nF)	W_s (DW)
GCE	88	959	879	576
GCE/PAMAM	82	332	444	562
GCE/PAMAM/Strept	84	601	582	572
GCE/PAMAM/Strept/ssDNA	73	649	679	569

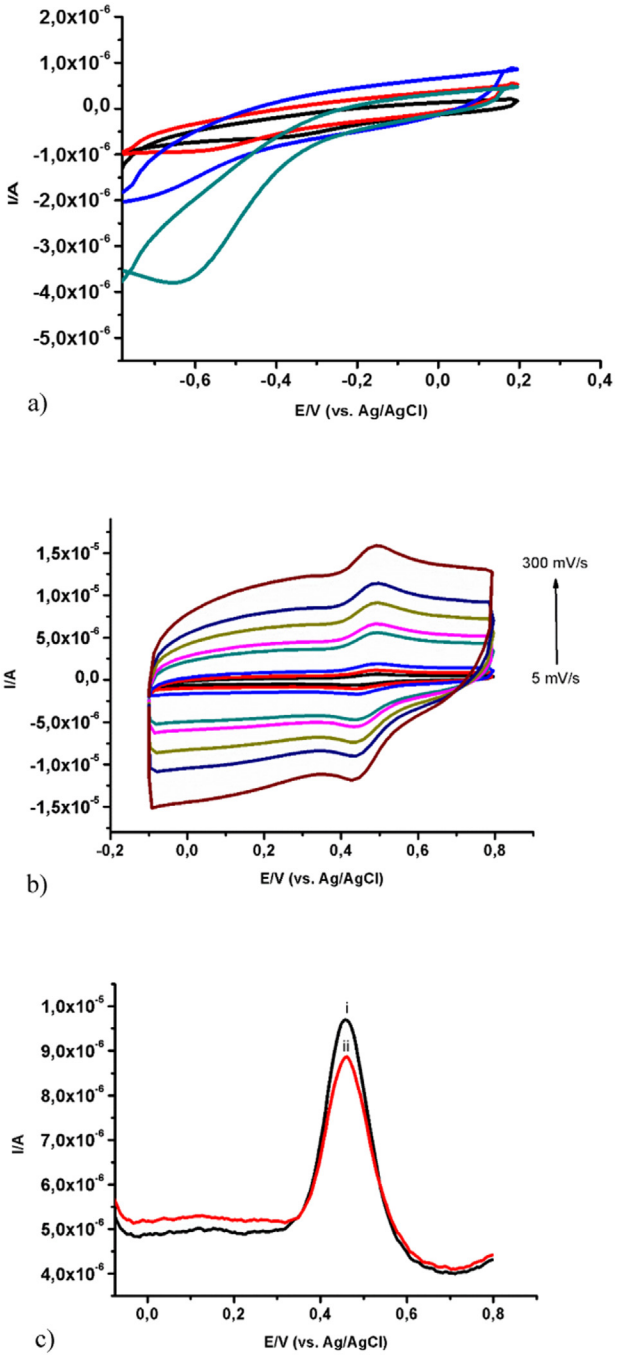

Fig. 2. (a) Bare GCE (i, black), GCE/PAMAM (ii, red), GCE/PAMAM/Strept (iii, blue) and GCE/PAMAM/Strept-HRP (iv, green) responses towards 1.0 μM H_2O_2 using CV (b) CV of GCE/PAMAM/Strept at different scan rates (mV s^{-1}) in 10 mM PBS (c) SWV of GCE/PAMAM/Strept in 10 mM PBS before (i, black) and after 30 days (ii, red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electrolyte [22]. When a 1.0 μM solution of H_2O_2 was added to the PBS solution (Fig. 2a), the GCE/PAMAM/Strept-HRP electrode showed a remarkable increase in reduction peak current as a result of the electrocatalysis between HRP and its H_2O_2 substrate. This indicates that streptavidin was indeed immobilised and that the immobilised peroxidase tagged streptavidin retained its bioelectrocatalytic activity. In the absence of HRP (Fig. 2a), the reduction peak was barely observed. This observation also suggests that the GCE/PAMAM/Strept-HRP electrode can be applied as a peroxide biosensor. The importance of streptavidin on the supramolecular platform is shown in the control experiment where HRP was immobilised on the PAMAM electrode directly. This method did not yield a similar magnitude of catalytic current in comparison to the streptavidin supramolecular platform (Supp 3). In essence, the presence of streptavidin aids in the immobilisation of HRP on to the electrode. It is also believed that the streptavidin anchor will reduce leaching of biotinylated bioreceptors from the biosensor surface.

The stability of streptavidin on the GCE/PAMAM (i.e. the GCE/PAMAM/Strept) was investigated in two ways. Firstly, the modified electrode was cycled at different scan rates in phosphate buffer (Fig. 2b). The fact that the anodic and cathodic peak potentials were not shifting with scan rate increase indicates that the streptavidin platform is stable and electroactive. Secondly, the GCE/PAMAM/Strept current responses in PBS were monitored at various times over 30 days. The electrode retained its electroactivity with only a current loss of 8.2% on day 30 (Fig. 2d). This observation further supports the stability of the GCE/PAMAM/Strept and its potential as a suitable platform in biosensor design.

As a proof of concept, the applicability of the PAMAM-Strept as a supramolecular platform in biosensors was investigated by developing a simple biosensor based on hybridisation of a single stranded DNA probe with its complementary strand. A biotinylated probe ssDNA was immobilised on the GCE/PAMAM/Strept platform via the well-known streptavidin-biotin interaction to form the biosensor labelled GCE/PAMAM/Strept/ssDNA. In the presence of $[Fe(CN)_6^{3-/4-}]$ probe, the immobilisation of the DNA probe was confirmed by the slight decrease in the peak current (Supp Fig. 2b) and increase in charge transfer resistance (Fig. 1c). However, in PBS, the electrode showed electroactivity without any significant loss in current even after the immobilisation of the ssDNA probe (Supp Fig. 2a). Thus, biosensors prepared using this platform can be interrogated by both voltammetry and electrochemical impedance spectroscopy.

Hybridisation with the complementary target (DNAc) of 15 bases was performed by immersing the biosensor into the target solution and incubating for 1 h at 38 °C. A wide range of DNAc concentrations (ranging from 0.05 to 10 nM) was tested. The electrochemical detection of DNAc was directly monitored in PBS using SWV (Fig. 3a). The SWV plot shows that the current peak increases with increase in DNAc concentrations. This increase in current and electroactive behaviour in buffer facilitated by the dendrimer-streptavidin supramolecular architecture, are important because the biosensor can be used for "signal on" sensing where the current is directly proportional to the amount of the target. The biosensor in buffer solution also present the advantage of sensing in a more biocompatible PBS medium rather than the $[Fe(CN)_6^{3-/4-}]$ medium where sometimes the anionic charge repulsion between the negatively charged $[Fe(CN)_6^{3-/4-}]$ and DNA (or other negatively charged bioreceptors) can limit interfacial electron transfer during voltammetric measurements. The increase in current can be explained by the more conducting nature of double stranded DNA (formed during hybridisation) over single stranded DNA [15,23,24]. More so, the absence of a significant shift in the anodic peak potential of the square wave voltammogram can be an indication of the stability of supramolecular immobilisation layer.

The bio-recognition process was also monitored by EIS. Fig. 3(b) show the changes at the impedance of the biosensor associated with increase target DNA concentration. The Randle equivalent circuit (Fig. 3b inset) consisting of electrolyte resistance between working and reference electrodes (R_s); Warburg impedance (Z_w), resulting from the diffusion of ions to the interface from the bulk of the electrolyte; charge transfer resistance (R_{ct}); and electrode/electrolyte interface capacitance(C) was used to fit the impedance parameters. As shown in Fig. 3b the values of R_{ct} increase in direct proportionality to the concentration of the target DNA. This approach is commonly used in impedimetric biosensors [15]. The fitting results were plotted (Fig. 3b inset) and a detection limit of 5 picomolar was calculated.

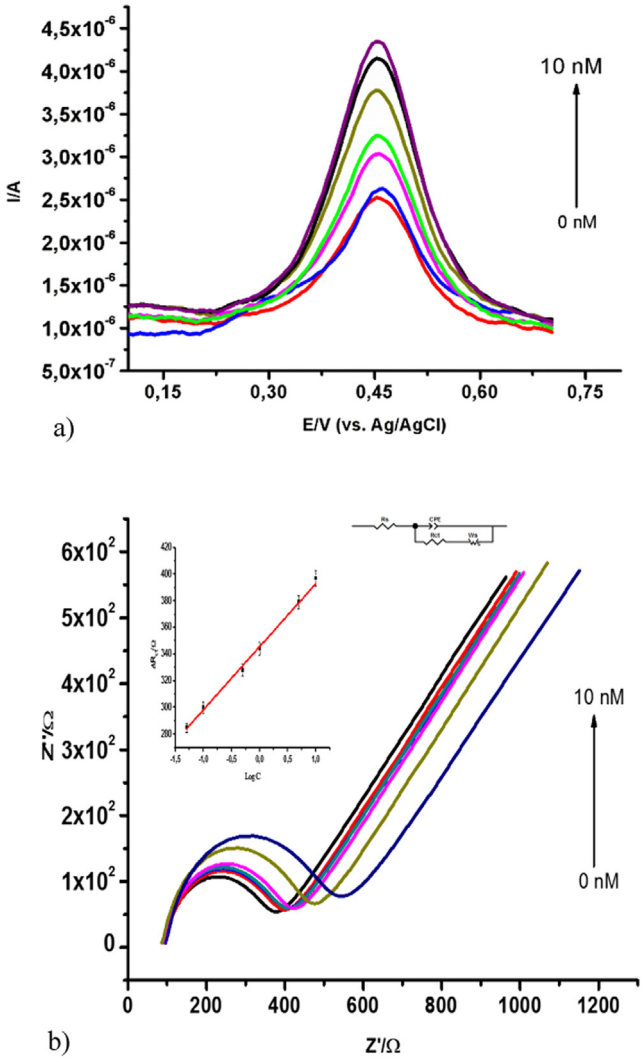

Fig. 3. (a) SWV of hybridisation of the biosensor with different concentration of target DNA (0.05 nM to 10 nM) in 10 mM PBS. (b) Nyquist plots of the biosensor responses to different concentration of target DNA in the presence of 5 mM $Fe(CN)_6^{3-/4-}$ redox probe.

4. Conclusion

In conclusion, we have reported a novel approach of functionalising a carbon electrode with PAMAM dendrimer to yield an electroactive modified electrode. This platform lends itself to further supramolecular modification with streptavidin and biotinylated DNA probe. The streptavidin platform, which was prepared by supramolecular immobilisation, can be used in principle as a versatile platform for the immobilisation of a myriad of biotinylated bioreceptors such DNA, aptamers, enzyme and so on. The supramolecular architecture reported can be used to monitor bio-recognition processes in buffer solution – an approach that may improve detection limit. Owing to the results obtained in this approach, further investigation into the development of a target specific biosensor is under study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2017.06.012.

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