

Novel biosensor fabrication methodology based on processable conducting polyaniline nanoparticles

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Abstract

This work investigates polyaniline (PANI) nanoparticles, (synthesised using dodecylbenzenesulphonic acid (DBSA) as a dopant), as a novel, highly processable, non-diffusional mediating species in an enzyme biosensing application. These nanoparticles are readily dispersed in aqueous media which helps overcome some of the processability issues traditionally associated with polyaniline. Modification of screen-printed electrodes was readily achieved with these aqueous nanoparticle dispersions, where the nanoparticles were simply cast by a drop-coating method onto the surface. After suitable pH adjustment, it was shown that horseradish peroxidase (HRP) enzyme could be added to the dispersion, and cast simultaneously with the conducting polyaniline. This effective fabrication method involves no electrochemical steps, and as such is easily amenable to mass production. The feasibility of casting enzyme with polyaniline nanoparticles is demonstrated in this short communication. More accurate deposition of protein-containing inks onto screen-printed carbon working electrodes could in the future transfer the drop-coating protocol from manual deposition to large-scale production by mechanical methods such as ink-jet printing.

Keywords: Nanoparticles; Polyaniline; Biosensor; Casting; Screen-printed electrodes

1. Introduction

Modification of electrodes with polyanilines and other conducting polymers is commonly carried out by means of electropolymerisation of the monomer aniline from aqueous media [1]. In many instances, modification of electrodes with polyaniline has been applied in biosensing applications [2–12], where polyaniline can act as an effective non-diffusional mediating species coupling electrons directly from the enzyme redox site to the electrode. This allows for very effective direct electrical

communication between the biomolecule and the electrode surface. This format has been described for many enzymes including glucose oxidase [6–8], horseradish peroxidase (HRP) [9], uricase [10], ascorbate oxidase [11] and sarcosine oxidase [12]. Polyaniline (PANI) exhibits good environmental stability, and its electrical properties can be modified by the oxidation state of the main chain and degree of protonation for different applications including the aforementioned biosensors, microporous electrically conducting materials, anti-corrosion protection of metals, chemical sensing, supporting material for catalysts, etc.

However, the poor processability of polyanilines has prevented their exploitation in commercial biosensor applications. Aniline is a carcinogenic monomer and

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must be distilled prior to use. It is insoluble in common solvents [13], seriously hindering its material processing. In addition, acidic conditions are required for the formation of the most highly conductive form of PANI, which does not lend itself to entrapment of pH-sensitive materials such as proteins. As such, proteins have to be subsequently deposited, adding complexity to the sensor fabrication. Processability issues, such as these need to be overcome for further successful exploitation of this electrically conducting polymer.

Much effort has been spent improving the processability of PANI. Dispersion of this polymer is one of the interesting ways to improve this. Problems with solubility can be overcome and its processability by conventional plastic processing methods improves. In addition, little or no aniline should be present in dispersions, thereby reducing its carcinogenic properties. These dispersions have been studied by many research groups [14–16]. Recently, Moulton et al. [17] used a micelle polymerisation method developed by Han et al. [18] to synthesise PANI nanoparticles, using dodecylbenzenesulphonic acid (DBSA), where DBSA plays the role of both dopant and surfactant in aqueous dispersions. These nanoparticles were characterised as spherical particles, 10 ± 2 nm in diameter with an electrical conductivity of 15 ± 3 S cm⁻¹ [17].

A recent paper has reported, the application of these nanoparticles in a biosensing application where the nanoparticles were electrodeposited on the surface of glassy carbon electrodes to form nanofilms [19]. HRP was subsequently electrostatically attached to these films. Although this served as an effective biosensor platform, the fabrication technique did not lend itself to mass production. In this short communication, a simpler method of depositing these nanoparticles is proposed, where the dispersions were cast onto disposable screen-printed electrodes by means of drop-coating. In addition, by casting these nanoparticles simultaneously with enzyme, a very quick, simple fabrication method for a biosensor was developed. This was shown to be an effective biosensor format. The signal-to-background (*S/B*) was comparable to the biosensor developed using electrodeposited nanoparticles [19] but has the added advantage of ease of fabrication; a method that would lend itself to techniques such as ink-jet printing [20,21] for mass production of biosensors.

2. Materials and methods

2.1. Materials

Aniline was purchased from Aldrich (13,293-4), vacuum distilled and stored frozen under nitrogen. Dodecylbenzenesulphonic acid (DBSA) was purchased from Tokyo Kasei Kogyo Co., Ltd. (TCI). Horseradish per-

oxidase (HRP, 232-668-6) was purchased from Biozyme Laboratories (South Wales, UK). 30% (v/v) hydrogen peroxide solution was purchased from Merck. The silver/silver chloride (Ag/AgCl) reference electrode was purchased from Bioanalytical Systems Ltd. (Cheshire, UK). The platinum mesh auxiliary electrode (29,809-3) was purchased from Aldrich.

Unless otherwise stated, all electrochemical measurements were carried out in phosphate buffered saline (PBS), (0.1 mol dm⁻³ phosphate, 0.137 mol dm⁻³ NaCl and 2.7 mmol dm⁻³ KCl), pH 6.8.

2.2. Instrumentation

All electrochemical protocols were performed either on a BAS100/W electrochemical analyser with BAS100/W software, or a CH1000 potentiostat with CH1000 software, using either cyclic voltammetry or time-based amperometric modes. An Ag/AgCl reference electrode and a platinum mesh auxiliary electrode were used for bulk electrochemical experiments.

2.3. Screen-printed electrode fabrication

Screen-printed electrodes were fabricated in-house using a DEK 247 according to Grennan et al. [22]. Briefly, electrodes were screen-printed onto pre-shrunk PET substrate. Initially a layer of silver was deposited as the conducting path. A layer of Gwent carbon paste ink (C10903D14) was deposited as the working electrode. Finally, an insulation layer was deposited to eliminate cross-talk and to define the working electrode area (9 mm²).

2.4. Synthesis of nanoparticles

Polyaniline dodecylbenzenesulphonic acid nanoparticles (*nano*PANI/DBSA) were synthesised according to a previously published procedure [17]. A modified polymerisation procedure similar to Han et al. [18] was used. The oxidant-to-monomer ratio was increased to 1:1 in order to obtain PANI in the conducting emeraldine salt (ES) form. Polymerisation was carried out in a thermostated bath at 20 °C. Equimolar quantities (1.3 mol dm⁻³) of aniline and DBSA were added to 100 ml of water in a round-bottomed flask mixed under mechanical stirring for 1 h. 100 ml of 1.3 mol dm⁻³ ammonium persulphate (Aldrich) (equimolar with aniline and DBSA) was then added drop-wise to the milky white aniline/DBSA solution. The polymerisation was allowed to proceed for 2.5 h, at which time a dark green dispersion was formed.

Purification of the polymerised dispersion was achieved by dialysing against deionised water using a 12,000 Da molecular weight cut off dialysis membrane for 48 h. After dialysis, the dispersion was centrifuged

at 10,000 rpm for 10 min. The supernatant was retained for residual aniline analysis by HPLC. Approximately 100 ml of deionised water was added to the remaining solid. The water/solid mixture was shaken to redisperse the solid, followed by centrifugation at 10,000 rpm for 10 min and repeated four times to remove DBSA.

2.5. Electrode pre-treatment procedure

Screen-printed electrodes were placed in a solution of $0.2 \text{ mol dm}^{-3} \text{ H}_2\text{SO}_4$. A single voltammetric cycle was carried out between -1200 and $+1500 \text{ mV}$ at 100 mV s^{-1} vs. Ag/AgCl.

2.6. Preparation of nanoPANI/DBSA biosensors

NanoPANI/DBSA was dispersed in distilled water at the required concentration. pH adjustment was carried out at this point if necessary using conc. NaOH. HRP in PBS was then added at the required concentration to the dispersion, and the resulting nanoPANI/DBSA/HRP was drop-coated onto pre-treated screen-printed electrodes and allowed to dry overnight at 4°C over silica gel.

2.7. Real-time monitoring of protein interactions in a batch cell

NanoPANI/DBSA/HRP electrodes were placed in PBS buffer in a stirred batch cell. Amperometric experiments were performed at -100 mV vs. Ag/AgCl, with a sample interval of 100 ms and a sensitivity of $1 \times 10^{-4} \text{ A V}^{-1}$. H_2O_2 (8 mmol dm^{-3}) was added once the current had reached steady state.

3. Results and discussion

3.1. Drop-coating of nanoPANI/DBSA onto screen-printed electrodes

Drop-coating of the nanoPANI/DBSA dispersion onto electrodes was carried out as an alternative deposition method to the more commonly utilised electropolymerisation method [2–4,7–12]. Electropolymerisation is not a technique applicable to cost-effective mass production of biosensors. Drop-coating (or casting) is a simpler method of electrode modification that, combined with screen-printed electrodes, would be amenable to mass production. In addition, enzyme can be drop-coated either simultaneously or sequentially in order to develop a very simple method for fabrication of a biosensor.

In order to examine this approach, various compositions of nanoPANI/DBSA ($20 \mu\text{l}$) were first drop-coated onto screen-printed electrodes (SPEs) and allowed to dry overnight over silica gel. Fig. 1 shows the CVs in

HCl (1 mol dm^{-3}) of nanoPANI/DBSA compositions ranging from 1.39% w/v to 11.08% w/v. It can be seen that 1.39% w/v and 2.77% w/v compositions resulted in low currents. Increasing the concentration to 5.54% w/v resulted in much higher currents, where the electrochemistry of the PANI dominated. Increasing the concentration to 11.08% w/v resulted in only a negligible increase in current. Therefore, 5.54% w/v was chosen for all further work.

As can be seen from Fig. 1, high volumes of nanoPANI/DBSA ($20 \mu\text{l}$) resulted in slow electron transfer rates between the nanoparticles and the electrode surface, implied by the broadness of the voltammetric peaks. The volume of nanoPANI/DBSA used for the drop-coating was varied to see if this could be improved. Fig. 2 shows that smaller volumes of nanoPANI/DBSA (5.54% w/v) resulted in more defined electrochemistry resulting from higher electron transfer rates which was more optimal, despite losing magnitude in current. $5 \mu\text{l}$ showed the most well-defined peaks. This was attributed to the thin films that would result from using lower drop-coating volumes. However, even at these volumes, the electron transfer rates of these PANI films in HCl (1 mol dm^{-3}) was still not ideal. Drop-coating may not result in a continuous polymer chain as the nanoparticles may not aggregate on the electrode surface in an ordered fashion, as occurs for the electrodeposition method [19], which may reduce the quality of the charge propagation throughout the film and hence the electrochemistry. It was not possible to deposit films using lower volumes than $5 \mu\text{l}$, as these volumes did not cover the surface of the 3 mm diameter electrode adequately due to the low surface energy of the carbon paste electrode. However, thinner films could be physically created by

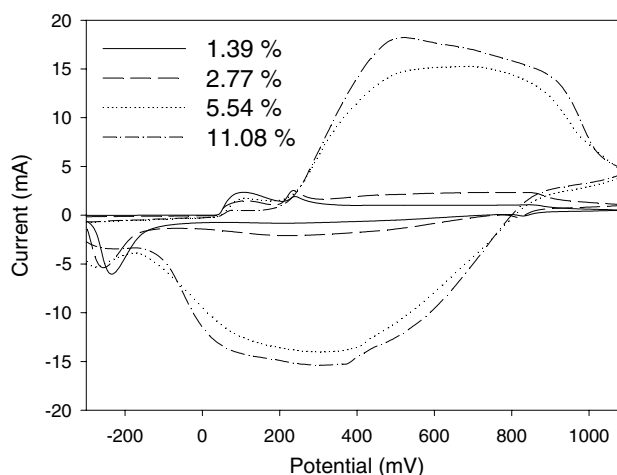


Fig. 1. Cyclic voltammograms in HCl (1 mol dm^{-3}) for various compositions (% w/v) of nanoPANI/DBSA drop-coated ($20 \mu\text{l}$) onto screen-printed electrodes (electrode area, 9 mm^2 ; scan rate, 100 mV s^{-1}).

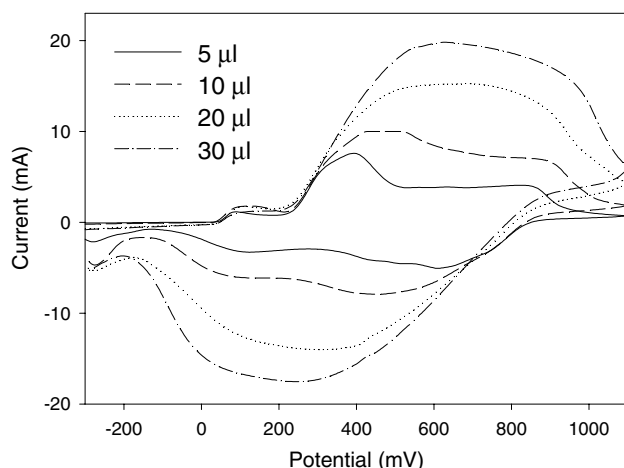


Fig. 2. Cyclic voltammograms in HCl (1 mol dm^{-3}) for various volumes of *nanoPANI/DBSA* (5.54% w/v) cast on screen-printed electrodes. All electrodes were dried overnight at 4°C over silica gel (electrode area, 9 mm^2 ; scan rate, 100 mV s^{-1}).

controlled deposition techniques such as ink-jet printing or spin-coating.

The drying conditions of the cast films needed to be investigated, as the films drop-coated using smaller volumes on screen-printed electrodes were more fragile, and formed cracks upon drying. Different drying conditions were investigated in order to try to strengthen the film. Drying under vacuum over silica gel at room temperature, and drying at 4°C , also over silica gel, were both explored. It was observed that the films dried at 4°C were most stable, i.e., exhibited minimal cracking, perhaps due to the slower drying process that would occur for these films compared to those dried under vacuum at room temperature.

3.2. Preparation of *nanoPANI/DBSA* biosensors

Incorporation of HRP into the films was carried out in order to construct a working biosensor that could respond to H_2O_2 substrate. Three HRP immobilisation methods were examined: (1) pre-mixing HRP with a dispersion of *nanoPANI/DBSA* for simultaneous casting, (2) pre-mixing HRP with a pH adjusted dispersion of *nanoPANI/DBSA* for simultaneous casting, and (3)

drop-coating of HRP (pH 6.8) onto a pre-cast dried *nanoPANI/DBSA* film. All of the electrodes were tested in a stirred batch format using amperometry where the potential was held at -100 mV vs. Ag/AgCl wire at pH 6.8. H_2O_2 (1 mmol dm^{-3}) was added once the current had reached steady state. (The potential of -100 mV was chosen as it is sufficiently negative to catalytically reduce H_2O_2 using PANI as the mediator [23,24], and sufficiently positive to eliminate any interferences that could arise in potential biological matrices). The immobilisation methods used, and the catalytic signals achieved upon addition of H_2O_2 are summarised in Table 1. Controls were carried out in each instance and no increase in current was observed.

Films of simultaneously cast *nanoPANI/DBSA* with HRP (no pH adjustment) gave no measurable catalytic signal in the presence of H_2O_2 . This was due to the acidic nature (pH < 3) of the nanoparticulate dispersion due to the presence of DBSA. Enzymatic activity of HRP decreases irreversibly at such low pH, so that it can no longer catalyse H_2O_2 efficiently. Films of simultaneously cast *nanoPANI/DBSA* and HRP (with pH adjustment using conc. NaOH, prior to addition of HRP) possessed the ability to catalytically reduce H_2O_2 , had high current responses and good signal-to-background ratios. Fig. 3 shows the effect of increasing the pH of the *nanoPANI/DBSA* casting dispersion (before addition of HRP) on the catalytic response of the cast films to H_2O_2 . Catalytic responses were low for films cast from dispersions where pH adjustments made were from pH 3 to 5. When the pH of the dispersion was increased to pH 6, the catalytic signal of the film to H_2O_2 experienced a dramatic increase, reflecting the fact that HRP remains active at this pH. Good catalytic responses were obtained for films cast from dispersions up to pH 8. No data was collected beyond pH 8. A pH adjustment of 7 was chosen for all further work. Passive adsorption of HRP (1 mg ml^{-1}) by casting on dried pre-cast films of *nanoPANI/DBSA* did not achieve such high catalytic currents (Table 1). The PANI-mediated mechanism of the catalytic reduction of H_2O_2 was much more pronounced when HRP and *nanoPANI/DBSA* were cast simultaneously. Hence, the simpler process of pre-mixing (with pH adjustment to 7.0) was used for all further

Table 1

Summary of the conditions used for the biosensor fabrication methods with their respective catalytic signals and signal-to-background (*S/B*) ratios for catalytic reduction of H_2O_2 accordingly

<i>nanoPANI/DBSA</i> drop-coating conditions	Immobilisation method of HRP	Catalytic signal (μA)	<i>S/B</i>	<i>n</i>
5.54% w/v, 20 μl	Pre-mixed with nanoparticles (1 mg ml^{-1})	–	<i>S/B</i> < 1	3
5.54% w/v, 20 μl , pH adjusted to 7.0 using conc. NaOH	Pre-mixed with nanoparticles (1 mg ml^{-1})	32.02 ± 7.16	8.81 ± 4.13	3
5.54% w/v, 20 μl	Drop-coated onto pre-cast nanoparticle films (pH 6.8, 1 mg ml^{-1} , 10 μl)	6.46 ± 0.94	3.22 ± 1.06	3

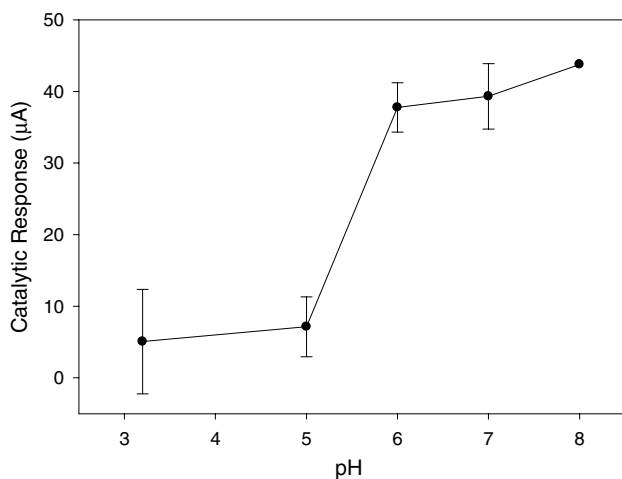


Fig. 3. Dependence of catalytic response of drop-coated *nanoPANI*/DBSA/HRP biosensor to H_2O_2 (1 mM) on the pH of the *nanoPANI*/DBSA/HRP casting solution. (pH adjustment carried out before the addition of HRP). Catalytic response measured by subtraction of background signal from total amperometric signal. (Potential held at -100 mV.)

investigations. This method, as well as yielding the highest catalytic signals, also has the advantage of being the simplest method for incorporating protein, and could be developed further with techniques such as ink-jet printing [20,21].

Using the simultaneous casting method (with pH adjustment) for biosensor fabrication, the concentration of HRP was varied over the range 0 – 50 mg ml^{-1} in order to determine the optimum working concentration. A plot of catalytic response from H_2O_2 (8 mmol dm^{-3}) against HRP concentration in the cast films (mg ml^{-1}) is shown in Fig. 4. Catalytic responses increased as the concentration was varied from 0 to 20 mg ml^{-1} . Above HRP concentrations of 20 mg ml^{-1} , the catalytic response was seen to decrease due to excess HRP. At these high concentrations, diminished amperometric responses may be due to inhibited diffusion of hydrogen peroxide to enzyme which is in electronic communication with the electrode surface, or impeded electron transfer. This demonstrated that the HRP was responsible for the catalytic reduction of H_2O_2 and that when immobilised within the *nanoPANI*/DBSA film could yield measurable signals. Although 20 mg ml^{-1} was seen to be the optimum concentration, this level is too high to work with in terms of enzyme consumption. Therefore 5 mg ml^{-1} was used for all further work. The catalytic signal at this level of HRP was 34.23 ± 10.03 μA , with a corresponding S/B ratio of 24.94 ± 3.4 . Recently, our group used this methodology to characterise a similar biosensor platform where a more complex, electrodeposition technique was used for the immobilisation of *nanoPANI*/DBSA films on carbon electrodes [19]. The average catalytic signal from the cast films was comparable to these electrodeposited *nanoPANI*/DBSA on

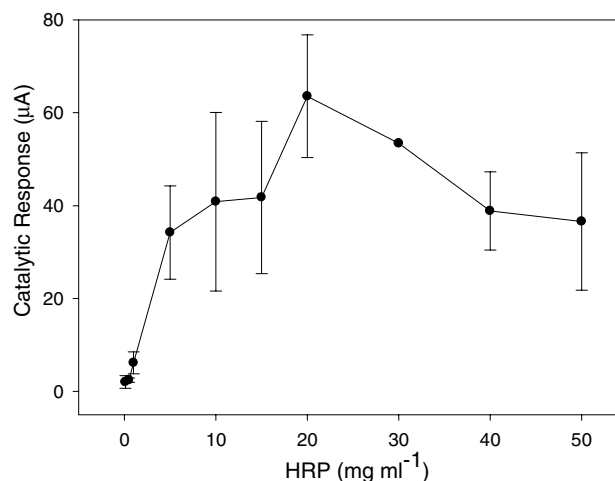


Fig. 4. Dependence of catalytic response of drop-coated *nanoPANI*/DBSA/HRP biosensor to H_2O_2 (1 mM) on HRP concentration. The nanoparticle dispersion (5.54% w/v) was adjusted to pH 7.0 to which different concentrations of HRP (0 – 50 mg ml^{-1}) were added. These were drop-coated onto SPEs (5 μl), and allowed to dry overnight at 4 $^\circ\text{C}$ over silica gel ($n = 3$). Catalytic response measured by subtraction of background signal from total amperometric signal. (Potential held at -100 mV.)

glassy carbon, where the HRP was electrostatically immobilised (42 ± 11 μA) [19]. However, the S/B of the cast film was only about half that of the electrosynthesised biosensor (61 ± 3 μA). This considerable decrease in S/B was attributed to the thickness of the cast films compared with the electrodeposited films. However, the cast films have the advantage of being much simpler and cheaper to fabricate. The electrodeposited films were found by profilometry to be approximately 300 nm thick [19]. No profilometry studies were carried out on the cast films, but could be estimated to be approximately 1000 times thicker, from the voltammetric peak heights in HCl (1 mol dm^{-3}). By decreasing the thickness of the films, by using alternative casting techniques such as ink-jet printing, would serve to increase the S/B , and hence the overall sensitivity of the biosensor.

4. Conclusion

Casting of conducting polyaniline nanoparticles and enzyme simultaneously by drop-coating onto disposable screen-printed electrodes served as an effective, facile method for biosensor fabrication. This biosensor was easy to prepare compared to the electrosynthesised biosensor, also using *nanoPANI*/DBSA as a non-diffusional mediator [19]. Incorporation of HRP was carried out prior to casting. The pH of the aqueous *nanoPANI*/DBSA dispersion was adjusted to pH 7 and HRP, at the required concentration, was added at this point. The *nanoPANI*/DBSA/HRP dispersion was then cast

onto electrodes. In terms of analytical parameters, the cast biosensor possessed reasonable analytical characteristics. However, the inherent thickness of the polymer film due to this casting method inhibited its potential. A more sophisticated method is required to deposit thinner, more homogeneous films. Ink-jet printing is an established technique that allows fluid to be deposited with low volume, great accuracy, and at high speed. Although this short communication reports drop-coating as a preliminary deposition method, the authors are currently working on ink-jet printing as an alternative, more powerful casting method. Successful manipulation of ink-jet printing for this system would bring this sensor to a more sophisticated fabrication level, lending itself to single-step mass production of enzyme biosensors.

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