

Chemically Polymerized Polyaniline Films for the Mass-Production of Biosensor Devices

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Abstract

The widespread use of screen-printed electrodes in biosensor applications has meant that mass-production of disposable, inexpensive sensors has become feasible. However, the complexity of surface coatings that require difficult and time-consuming deposition procedures do not lend themselves to the same production conveniences. This article describes moves towards the development of an alternative to the electrochemical deposition of polyaniline on electrode surfaces for incorporation into an established sensor format. Chemical rather than electrochemical polymerization of the conducting polymer polyaniline enables a multitude of electrodes to be coated simultaneously, without the need for complex instrumentation. The aim of this work was to control the coating parameters such that a coating of polymer was deposited that displayed the optimum thickness and electrochemical properties of that previously optimized electrochemically. Deposition procedures were found to depend on the hydrophilicity of the underlying carbon paste electrode as well as on a variety of polymerization parameters.

Keywords: Polyaniline, Chemical polymerization, Mass-production, Screen-printed electrodes, Biosensor

Devoted to the Memory of Professor Robert (Bob) Osteryoung

1. Introduction

Conducting polymers such as polypyrrole, polythiophene and polyaniline (PANI) have merited applications in recent years in a great variety of fields such as anticorrosive coatings, energy storage systems, gas sensing, as well as electrochromic, electrochemiluminescent and electrocatalytic devices [1]. Over the last decade, these polymers have gained increasing applications in the development of biosensors. PANI has gained particular popularity in such applications, partly due to its favorable storage stability and ease of preparation [2]. PANI can fulfill several important roles in the biosensor including extension of the linear range of the sensor, as an immobilization platform for biocomponents and as the electron mediator. The latter role is possible due to the inherent electroactivity of PANI. Conducting polymer films have also been used to confer antifouling properties to the sensor, with the film serving to reject both electroactive interferents and other fouling species such as proteins [3].

Enzymes on their own are poor electrochemical communicators, due to the fact that a thick shell of protein insulates their redox centers. Conducting polymers are capable of penetrating this insulating shell and providing a means of direct electrical communication between the redox centre and the electrode surface [4]. They achieve this by delocalizing redox charges over a series of conducting polymer groups, thereby acting as self-contained electron transfer

mediators. Consequently, no additional diffusional mediators need be added to the sensing system in order for electron transfer to take place. This has advantages for the long-term stability of the sensor, since the confinement of the mediator to the sensor surface prevents it from leaching into solution, thus eliminating the requirement for a containment membrane. Such moves toward reagentless systems also facilitate the use of such sensors as stand-alone devices. Recent work by us has focused on the use of PANI for these purposes.

The use of the conducting polymer PANI also results in the enzyme becoming 'electrically-wired' to the electrode surface via the conducting polymer chains, which contain non-diffusionally mediated redox active species [5]. Since PANI films do not retain their conductive character in non-acidic media [6] (i.e. the neutral environments required for most proteins to function optimally), the electropolymerization process must be carried out in the presence of a dopant, in this case the poly(vinyl sulfonic acid) (PVS) ion. Its inclusion maintains electrical neutrality in the oxidized form of the polymer and also leads to increases in its structural stability and conductivity at a broader range of pH values [7].

PANI may be deposited onto electrode surfaces through chemical or electrochemical means. Acidic conditions are required in both cases in order to obtain the most highly conductive polymer films, since a low pH is necessary to solubilize the monomer [8]. With chemical oxidation, the

number of counter ions available is limited to those associated with the oxidant employed; the most common being FeCl_3 and $(\text{NH}_4)_2\text{S}_2\text{O}_8$. Electrochemical polymerization, through galvanostatic, potentiostatic or potentiodynamic means, offers the potential to incorporate a wider range of dopant ions, since the reaction is carried out in the presence of an appropriate electrolyte rather than a chemical oxidant. Electrochemical oxidation also gives better control over film properties, such as thickness and morphology. For these reasons, this has become the most common method of PANI film preparation for sensor applications and has been the method employed by our group to date in the development of a range of enzyme- and antibody-based biosensors [9–14]. Recent work by us has been on the optimization of the thickness of the PANI films deposited on electrode surfaces with respect to their influence on sensor performances [15]. It was found that sensors prepared from PANI/PVS grown potentiodynamically to a peak current of -2.8 mA for the first oxidation peak of polyaniline, representing the leucoemeraldine radical cation (LM^+), produced sensors with optimum performances in terms of catalytic, charging and signal to background (s/b) ratios and time taken to reach steady state [9].

Although electrochemical polymerization of aniline affords a means to deposit PANI layers of defined thickness onto surfaces, such as the screen-printed electrodes (SPEs) used in this work, the main drawback of this technique with the SPEs developed is that only a single electrode can be coated at any one time. In addition, this process can only be accomplished using electrochemical instrumentation. With increasing moves towards the development of disposable sensor devices, for example those based on screen-printed electrodes, the mass-production capabilities of all aspects of sensor construction are critical for cost-effectiveness. Electrochemical polymerization does not comply with these requirements of mass-production, especially of single-use sensor devices, with some authors being of the opinion that cyclic voltammetry is not a suitable deposition technique for industrial applications of PANI [16]. Consequently, a chemical method of polymerization was investigated in the present work, to assess its feasibility as a component of an established biosensor system. Such a method would enable a greater number of electrodes to be prepared in situ without the need for instrumental techniques.

Chemical polymerization of aniline has the advantage that it may be carried out on a greater number of electrodes simultaneously, using basic laboratory materials. This advantage of mass-production is counterbalanced by the difficulties in controlling the thickness of deposited films, leading to poor reproducibilities and greater inter-batch and intra-batch variabilities. The most important factors influencing chemical polymerization of aniline are the concentration and oxidizing power of the oxidant used. Changes in these properties result in variations in the deposition rate and level of doping, provided that the concentration of monomer present in solution is sufficient to avoid diffusion-controlled deposition taking place [8]. If the concentration

of the monomer is too low, this can significantly affect the rate of polymerization, in that diffusion-controlled depositions dominate in these cases.

While the range of deposition conditions that can be varied is limited in comparison with electrochemical polymerization, aspects such as the deposition technique, volume and drying conditions could be varied in attempts to improve control over film thickness. Attempts to investigate production factors that might lead to greater control over the deposition process and hence produce reproducible sensors prepared from chemically polymerized PANI films are also described.

2. Experimental

2.1. Materials

Horseradish peroxidase (HRP) was 200 U/mg (Sigma P6782) and was from Sigma-Aldrich (Poole, Dorset, UK) as was poly(vinylsulfonic acid) sodium salt (27,842-4). Aniline was from Aldrich (13,293-4), vacuum distilled and stored frozen under nitrogen. 30% (v/v) hydrogen peroxide solution (108,597) was from Merck (Darmstadt, Germany).

Carbon paste (C10903D14) was from Gwent Electronic Materials Ltd. (Gwent, UK). Silver conductive ink (Electrodag PF-410) and dielectric polymer ink (Electrodag 452 SS BLUE) were from Acheson. Poly(ethylene) terephthalate (PET) substrates were Melinex (preshrunk) films from HiFi Industrial Film Ltd. (Dublin 6, Ireland). PVC insulating tape (494 360) was from Radionics (Dublin 12, Ireland). The silver/silver chloride reference electrode was from Bioanalytical Systems Ltd. (Cheshire, UK). The platinum mesh (29,809-3) was from Aldrich.

2.2. Buffers and Solutions

PBS is 0.1 M phosphate buffer, 0.137 M NaCl and 2.7 mM KCl, pH 6.8. This was prepared by mixing solutions containing 0.1 M Na_2HPO_4 , 0.137 M NaCl and 2.7 mM KCl and 0.1 M KH_2PO_4 , 0.137 M NaCl and 2.7 mM KCl to a pH of 6.8.

2.3. Instrumentation

Screen-printing was performed with a semi-automated DEK Albany 247 printing machine (Weymouth, UK). A nylon screen (Miller Group Ltd., Dublin 12, Ireland) with a mesh thickness of 77 T (filaments per cm on thick grade thread (35–40% open area) and mounted at 45° to the print stroke was employed. A single square blade rubber squeegee was employed. Carbon and silver layers were cured in a conventional laboratory oven. The ultraviolet (UV) lamp curing system was obtained from UV Process Supply, Inc. (Cortland, Chicago, IL, USA).

All voltammetric and amperometric electrochemical measurements were performed using a BAS100/W electrochemical analyzer with BAS100/W software, operating either cyclic voltammetry or time-based amperometric modes. An Ag/AgCl reference electrode and a platinum mesh auxiliary electrode were employed for bulk electrochemical experiments.

Scanning electron microscopy (SEM) was performed with a Hitachi S 3000N Scanning Electron Microscope. Images were obtained using the secondary electron (SE) detection mode. Acceleration voltages of 20 keV were employed. Resistance measurements were carried out using the two-probe technique and a HP 34401A Multimeter (Hewlett Packard, Leixlip, Co. Kildare, Ireland).

Water contact angle measurements on electrodes printed with no dielectric layer, were carried out by Annika Ainslie, Inverness Medical Ltd., Inverness, Scotland, UK. A 2 μ L droplet of distilled water was deposited on electrode surfaces and contact angle data was measured using 'Video Contact Angle Optima XE System' (Version 1.75) from AST Products Inc. (Billerica, MA, USA). Surface tension measurements were performed with the same system on the same droplets.

2.4. Production of Screen-Printed Electrodes

Screen-printed electrodes were designed and produced as described previously [12]. Electrodes were cut from the printed sheet leaving excess substrate on either side to a width of 14 mm. Electrodes were then pre-treated in 0.2 M H₂SO₄ solution as described previously [11].

2.5. Chemical Polymerization of Aniline on Electrode Surfaces

PVC insulating tape (140 μ m thickness) was employed to define the electrode area on a PET substrate base by masking. Circular holes of 3 mm diameter were punched in the tape, to define a sensing area of 7.07 mm² on the working electrode surface. The polymerization solution was prepared by mixing equal volumes of solutions containing 100 μ L aniline dissolved in 9.9 mL 1 M HCl (solution A) and 0.112 g ammonium persulfate dissolved in 10 mL 1 M HCl (solution B). Two deposition procedures were investigated as outlined below.

2.5.1. Drop Coating

One mL of solution A was mixed with 1 mL of solution B in a plastic vial. After swirling to mix, a small quantity (5–50 μ L), depending on the area of the electrode to be coated and the depth of the insulating layer, was immediately dropped onto the surface of the working electrode using a glass pipette. The solution was left to polymerize for 10 to 20 min, depending on the level of oxidant used. Electrodes were then rinsed in 0.1 M HCl and left to air-dry prior to use or further coatings.

2.5.2. Immersion

Electrodes were placed in a glass petri dish and immersed in a 10 mL solution of equal volumes of solutions A and B. Electrodes were rinsed and air-dried as per Section 2.5.1.

2.6. Electrochemical Polymerization of Aniline on Electrode Surfaces

A mixture of 7.8 mL 1 M HCl, 186 μ L aniline and 2 mL PVS, was prepared and degassed for 10 min. An argon or nitrogen atmosphere was maintained over the surface of the solution throughout. Aniline was polymerized on the surface of the working electrode using voltammetric cycles between –500 and 1100 mV (vs. Ag/AgCl) at 100 mV/s until the first oxidation peak, representing the leucoemeraldine radical cation, reached an anodic current value of –2.8 mA.

2.7. Immobilization of Horseradish Peroxidase

Following deposition of polyaniline/poly(vinylsulfonic acid) (PANI/PVS) onto the electrode surface, the electrode was transferred to a 2 mL batch cell as previously described [13]. The surface of the polymer was reduced in 2 mL of PBS (degassed for 10 min under nitrogen or argon) at –500 mV (vs. Ag/AgCl) using a sample interval of 500 ms over 1500 s.

HRP was prepared in PBS prior to use. Very quickly after reduction was complete, PBS was removed from the cell and quickly replaced with the protein solution, not under stirring or degassing. Again quickly, oxidation was performed at 700 mV (vs. Ag/AgCl) for 1500 s. During this oxidation, the protein became electrostatically attached to the polymer surface. After oxidation was complete, the protein solution was carefully recovered from the cell and restored for later use.

2.8. Cyclic Voltammetry

For cyclic voltammetric analysis, SPEs were cycled in 1 M HCl as supporting electrolyte, using a scan rate of 100 mV/s (vs. Ag/AgCl).

2.9. Amperometry

HRP at a concentration of 0.625 mg/mL was immobilized onto the working electrode, according to Section 2.7, which was then incorporated into a batch cell set-up. H₂O₂ at a concentration of 8 mM was added to the cell containing 2 mL PBS following the reaching of a steady state at –100 mV (vs. Ag/AgCl), with a sample interval of 500 ms.

Table 1. Influence of curing temperature and electrode pre-treatment on properties of electrode surfaces.

Curing temp. (°C)	Average contact angle [a] (Deg.)	Standard deviation (Deg.)	C. V. [b] (%)	Average surface tension [a] (dynes/cm)	Standard deviation (dynes/cm)	C. V. [b] (%)
24	84.33	6.54	7.75	33.67	4.37	12.98
40	82.74	9.63	11.64	35.17	5.01	14.24
80	78.83	6.76	8.58	37.33	4.01	10.74
120	75.67	10.83	14.31	39.33	6.53	16.59
150	75.34	10.54	13.99	39.50	6.54	16.55
Pretreated electrodes	67.11	5.28	7.86	44.33	3.25	7.34

[a] Average of 3 replicate measurements

[b] Coefficient of variation

2.10. Resistance Measurements

Measures of the base resistance of electrodes coated with polymer films were performed with a two-point probe and a digital voltmeter. Measurements were made from the edge of the silver conducting track to the centre-point of the sensing surface.

3. Results and Discussion

3.1. Chemical Polymerization of Aniline

Chemical polymerization was performed in order to produce polymer films of the same thickness optimized using electrochemically prepared PANI films [15]. In the present work, chemical polymerization of aniline was achieved through the deposition of a mixture of aniline and ammonium persulfate, each prepared in acidic media, on the surface of screen-printed electrodes. Two methods of deposition were investigated, in accordance with Section 2.5. The first involved immersion of electrodes in a petri dish containing equal volumes of solutions of aniline and ammonium persulfate oxidant, respectively. The second involved the deposition of a fixed volume of a mixture of the two solutions onto the working electrode surface.

In the case of the immersion procedure using SPEs, since the aim was to form a PANI coating on the carbon surface alone and not on the surrounding PET, insulating layer and conducting track surfaces, it was necessary to mask the remainder of the electrode with insulating tape. This deposition procedure produced polymer coatings of varying thicknesses, as demonstrated by background voltammograms in 1 M HCl (results not shown), with error values as high as 10% in some cases ($n = 5$). This could be attributed to the relative lack of control over the polymerization and the inability to confine the chemistry to the working electrode surface. Both the walls of the glass petri dishes and the remainder of the electrode coated in insulating layer also received coatings of polymer.

The drop coating deposition procedure offered a means to confine the polymerization event to a defined region of the electrode surface. However, certain electrodes proved difficult to coat using this procedure, since the mixture did

not adhere to the surfaces of the electrode. The insoluble polymer was instead suspended in the drop of polymerization solution. This effect was thought to have been caused by the hydrophobic nature of the carbon paste used to construct the screen-printed electrodes. Contact angle and surface tension measurements are important factors to be considered when the deposition of a chemical species onto the electrode surface is desired, as the bonding quality of the surface would determine the effectiveness of the surface coverage by solutions deposited on it.

Contact angle measurements were carried out on electrode surfaces in order to measure the degree of interaction between the electrode surface and a liquid droplet of distilled water deposited on the electrode. These measurements offered a means of determining the chemical bonding nature of the uppermost layers of the surface. This provided information on the wettability and adhesion of the electrode surface, as well the degree of surface hydrophobicity or hydrophilicity; properties which determine the coating properties of the electrode. Measurements of the interfacial tension between the surface and the liquid could be readily observed. Larger contact angles were indicative of surfaces with lower surface energies and the converse was true for lower contact angle values. The low surface energy of the dielectric insulating layer of the electrodes caused the 2 μ L distilled water droplet deposited on the electrode surface to “bead up” and form contact angles greater than 90°. This made it difficult to differentiate between electrodes with regard to accuracy. Consequently, contact angle measurements were performed on SPEs prepared without this layer.

All of the samples analyzed displayed high contact angles, indicating that the carbon surfaces of the SPEs displayed high hydrophobicity and poor wettability. However, this was reported to have been the case with other commercially available carbon based electrodes analyzed using the same system. The influence of the temperature of curing on such electrode surface behavior was established, with average contact angles observed to be dependent on the temperature of curing, as shown in Table 1. The average contact angle between the droplet of water and the electrode surface was found to decrease in a linear fashion with increasing curing temperature (equation of best fit trendline: $y = -0.0753x + 85.614$, $r^2 = 0.9585$). A value of 84.33° ($\pm 6.54^\circ$) was determined for electrodes cured at room

temperature and this decreased to $75.34^\circ (\pm 10.54^\circ)$ for electrodes cured at 150°C . Consequently electrodes cured at higher curing temperatures exhibited surfaces that were more hydrophilic in nature and hence these would be more accessible to deposition of chemically polymerized aniline solutions. This hydrophilicity can be associated with the breakdown of the polymeric binder component of the carbon paste ink, which may have added a degree of hydrophobicity to the electrodes. Previous work using SEM analysis has proven that the roughness of the electrode increased with increasing curing temperature, so this could also be a reason for the progressive decreases in contact angles [11].

Electrodes subjected to an electrochemical pretreatment procedure, namely performing a single cyclic voltammetric cycle on electrodes in $0.2\text{ M H}_2\text{SO}_4$ solution between -1200 and 1500 mV , displayed a lower average contact angle of $67.11^\circ (\pm 5.28^\circ, n=3)$. This decrease in contact angle after pretreatment illustrated that the interaction between the aqueous phase and the electrode surface had increased with the pretreatment procedure, as the electrode surface had become more hydrophilic in nature. Decreases in contact angle are known to be attributed to surface roughness [17] and previous work on SEM analysis of electrode surfaces such as these had already shown the physical profile of a pretreated electrode to be rougher than an un-treated one [11]. This roughness would make electrode surfaces more amenable to the deposition of chemically polymerized PANI solutions.

The linear relationship between contact angles and curing temperature was also complemented by measurements of the surface tensions of the water droplets on these electrodes. Larger values of surface tension are indicative of larger surfaces with more surface energy, such that water molecules tend to interact more strongly with these surfaces. Concurrently, lower values of surface tension indicate that the molecules did not interact as strongly and that these surfaces were more hydrophobic in nature. The relationship between surface tension and curing temperature, for electrodes cured at a range of temperatures is also illustrated in Table 1. Surface tensions were found to increase linearly with increasing curing temperature (equation of best fit trendline: $y = 0.0472x + 33.093, r^2 = 0.9487$). Electrodes cured at room temperature exhibited average surface tensions of $33.67 (\pm 4.37)$ dynes/cm, whereas electrodes cured at 150°C displayed average surface tensions of $39.5 (\pm 6.54)$ dynes/cm. As the temperature of curing was increased, the surface tension of the water droplets increased, indicating that the surface had become more hydrophilic in nature and the water molecules in the liquid were able to interact more strongly with the surface.

Electrochemically pretreated electrodes also exhibited decreased standard deviation and coefficient of variation (C. V.) values for contact angle measurements, which may be attributed to the particular test being very sensitive to any marks or scratches on the surface, or to the rougher nature of the surfaces. The average surface tension of the pre-treated SPE surface was $44.3 (\pm 3.25)$ dynes/cm ($n=3$), which was

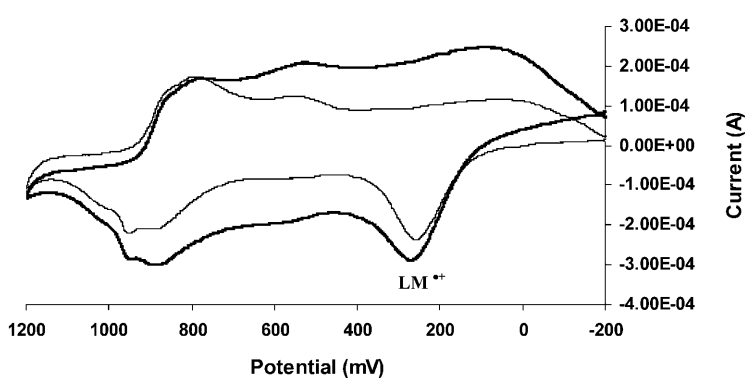


Fig. 1. Cyclic voltammograms of PANI, chemically deposited on SPEs using drop coating (thin line) and immersion (thick line) techniques. The oxidation peak of the $\text{LM}^{+\cdot}$ radical cation is shown. Voltammograms obtained in 1 M HCl (vs. Ag/AgCl , scan rate 100 mV/s).

greater than the $33.67 (\pm 4.37)$ dynes/cm ($n=3$) to $39.5 (\pm 6.54)$ dynes/cm ($n=3$) range exhibited by any of the untreated electrodes. Since contact angles were found to decrease and surface wettability was found to improve with the incorporation of a pre-treatment protocol into the electrode preparation procedure, this was performed on all electrodes prior to any subsequent deposition procedures.

Cyclic voltammograms of glassy carbon electrodes (GCEs) coated using the drop coating technique were then comparable to those prepared using the immersion technique, as shown in Figure 1 and these were characteristic of those obtained previously for electrochemical polymerization [9]. Slightly thicker films were formed in the case of the immersion procedure. However, films grown on SPE surfaces were several orders of magnitude thicker than those grown on GCE surfaces (results not shown).

3.2. Influence of Oxidant Concentration on Film Properties

Several parameters were investigated in order to optimize the reproducibility of the production of conductive films of defined thicknesses on electrode surfaces. Polymerization times were not found to affect the thickness or properties of the finished films, once the polymerization event had gone to completion. The concentration and age of oxidant, as well as the geometric proportions, numbers of coatings and volumes of the polymerization solutions employed were found to influence resulting film properties. The effects of varying the concentration of oxidant (in terms of the oxidant/monomer molar ratio) on the time taken for the completion of the polymerization process and on the resistance of the resulting films are shown in Table 2.

Increasing the oxidant/monomer molar ratio from 0.5 to 3 decreased the polymerization time (judged to be the time taken for the polymerization mixture to change color from clear to green or black) from 25 to 6.5 min. Additional increases in oxidant levels did not reduce polymerization

times further. Varying the oxidant concentration influenced the color of the films produced. Films prepared from solutions containing oxidant/monomer molar ratios of 3 and 4 no longer exhibited the characteristic green color of PANI. Instead a thin black layer of polymer was deposited on the sides of the tube in the case of the solution with the oxidant/monomer molar ratio of 3 and a lighter brown coating was deposited in the case of the solution with the oxidant/monomer molar ratio of 4. Since the rate of polymerization was highest for solutions containing increasing concentrations of oxidant, it appeared that complete polymerization could not be achieved in these cases, possibly due to over-oxidation of PANI.

The corresponding resistances of the PANI films grown on SPEs are also given in Table 2. These results illustrate that decreases in polymerization times due to increases in the oxidant levels, were at the expense of concomitant decreases in the conductivity of films. The resistance of sensors increased from 83 Ω to 268 Ω for films prepared using ratios of oxidant/monomer of 0.5 and 4, respectively. At oxidant/monomer ratios of 3 and over, the characteristic redox peaks associated with PANI were no longer clearly defined (Figure 2), which indicated that effective polymerization of a conductive polymer was not achieved in these cases. Decreases in conductivity of polyaniline-dodecylbenzene-sulfonic acid (DBSA) films have also been reported at oxidant/aniline-DBSA molar ratios of greater than 1.2 [18]. Similarly, for PANI films deposited on quartz crystal microbalances surfaces, oxidant/monomer molar ratios of greater than 1.13 resulted in degradation of the PANI films [19].

SEM analysis of PANI films prepared using increasing concentrations of oxidant revealed variations in surface topography are shown in Figure 3(A)–(D). The characteristic budding or cauliflower effect evident with electrochemically polymerized aniline, shown in Figure 3(E), was not observed for any surface. Rather, the polymer appeared to have formed an irregular sheet over the carbon paste particles of the electrode, resulting in smooth surfaces of continuous PANI films. In addition, the underlying mesh pattern of the screen-printed electrodes did not influence the morphology of deposited films, as was the case with electrochemically deposited films (results not shown).

As the oxidant/monomer ratio was increased, the level of cracking observed in the polymer films also increased. This could be attributed to increases in the rate of polymerization, which caused pressures on the polymer films, forcing them to crack during the polymerization process. Decreases in the conductivity of the polymer films outlined in Table 2 also manifested themselves during imaging through increased charging effects with increasing oxidant concentration. Increases in the level of cracking could also have led to interruptions in the conducting network, which could account for reduced current flows experienced in sensing applications. Film thickness differences could also have been a contributing factor.

Although the films exhibited a smoother appearance in comparison to electrochemically prepared PANI, within the

Table 2. Influence of oxidant/monomer ratio on rate of aniline polymerization and film resistance.

Oxidant/monomer molar ratio	Polymerization time (min)	Resistance (Ω)
0.5	25	83
1	18	112
2	8.5	165
3	6.5	177
4	6.5	268

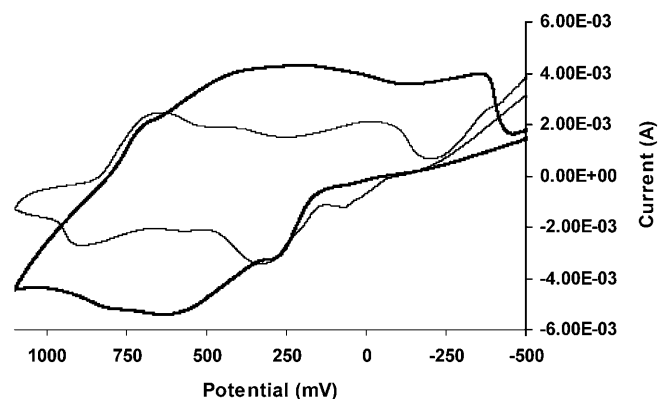


Fig. 2. Influence of oxidant concentration on redox peaks of PANI for 0.05 M (thin line) and 0.4 M (thick line) $(\text{NH}_4)_2\text{S}_2\text{O}_8$. Voltammograms obtained in 1 M HCl (vs. Ag/AgCl, scan rate 100 mV/s).

cracks in these films, the budding nature of the polymer could be seen, albeit at a much more microparticulate level, as shown in Figure 3(D). These results illustrate the importance of keeping the concentration of the monomer in excess when carrying out chemical polymerization procedures, since over-oxidation of films can have deleterious consequences for sensing purposes.

3.3. Influence of Other Deposition Parameters on Film Properties

Through the use of increasing numbers of layers of insulating tape to define the electrode surface areas, the depth of the deposition well could be increased accordingly. Through the deposition of the polymerization solution described in Section 2.5, the effect the deposition well thickness had on the resulting peak currents for the LM^{+} oxidation peak and consequently the thickness of the PANI films is illustrated in Figure 4(A). Since the electrochemical system employed for these determinations could not measure anodic current levels below -10 mA, peak currents lower than this were approximated as -10 mA. As is evident from these results, the thickness of a PANI film coated using a single layer of insulating tape was greater than that obtained electrochemically using 10 voltammetric cycles. This thickness increased with increasing depth of the deposition well. C. V. values were below 6% for all electrodes ($n = 3$). For further

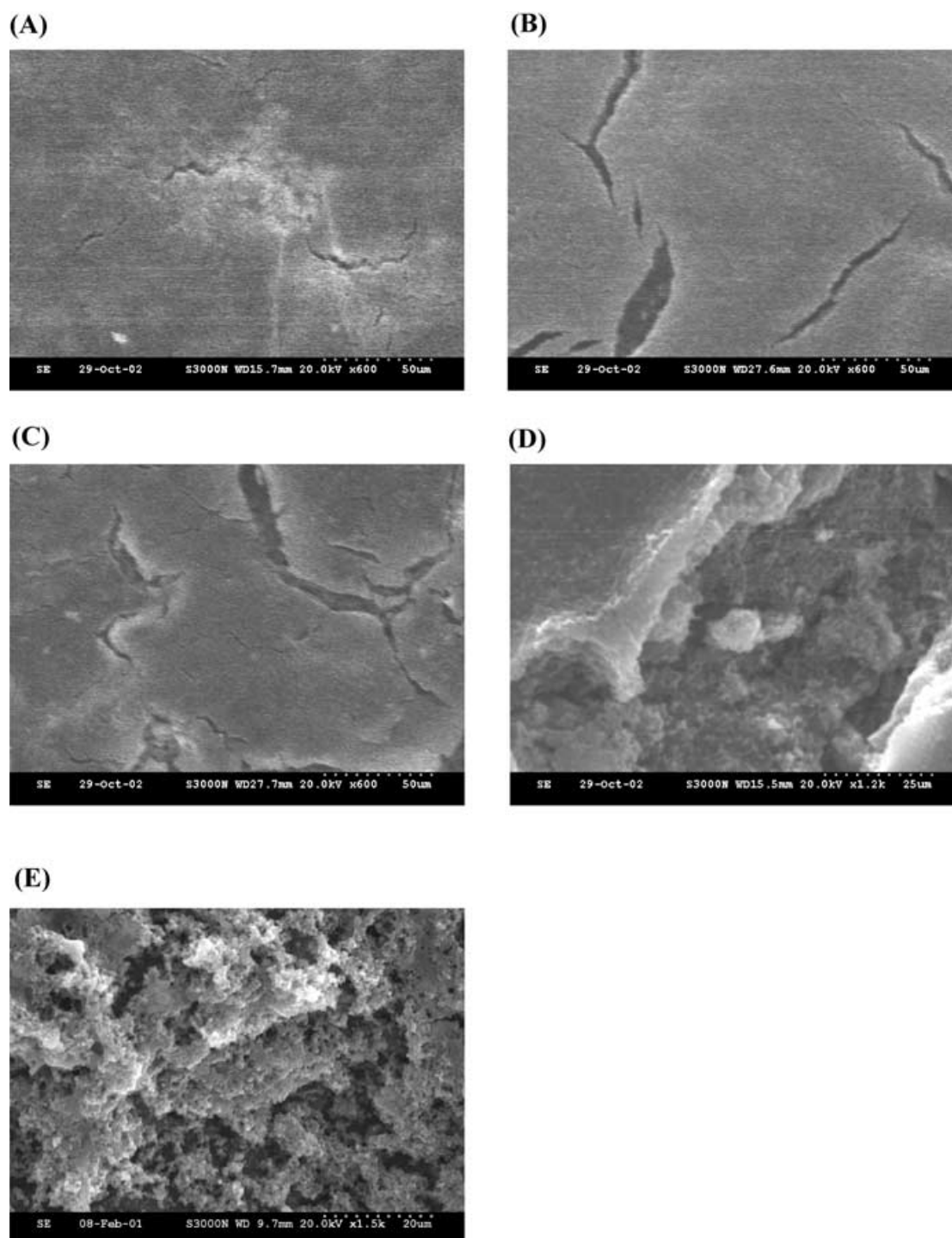


Fig. 3. SEM images of SPEs coated with chemically polymerized aniline using A) 0.05 M, B) 0.2 M and C) 0.4 M $(\text{NH}_4)_2\text{S}_2\text{O}_8$. Higher magnification images D) of the sample imaged in C), revealed nodes of PANI that lay within the grooves of the smooth polymer film surface. An SEM image of an SPE coated with electrochemically polymerized aniline (E) revealed buds or nuclei of polymer (600 and 1200 \times magnifications, 20 keV, SE detection mode).

studies, a single layer of tape was employed for the deposition well and other methods to reduce the thickness of the polymer film were sought.

Solutions of aniline and oxidant could be stored for limited periods of time at 4 °C and reused for polymerization purposes. Solutions were stored in a sealed container in the

dark, in an attempt to minimize the light-induced formation of aniline oligomers. It was noted that the longer these solutions were stored, the quicker the polymerization procedure was initiated on mixing the two solutions. For example, freshly prepared solutions required ca. 20 min for the reaction to complete, whereas solutions stored for 3 days

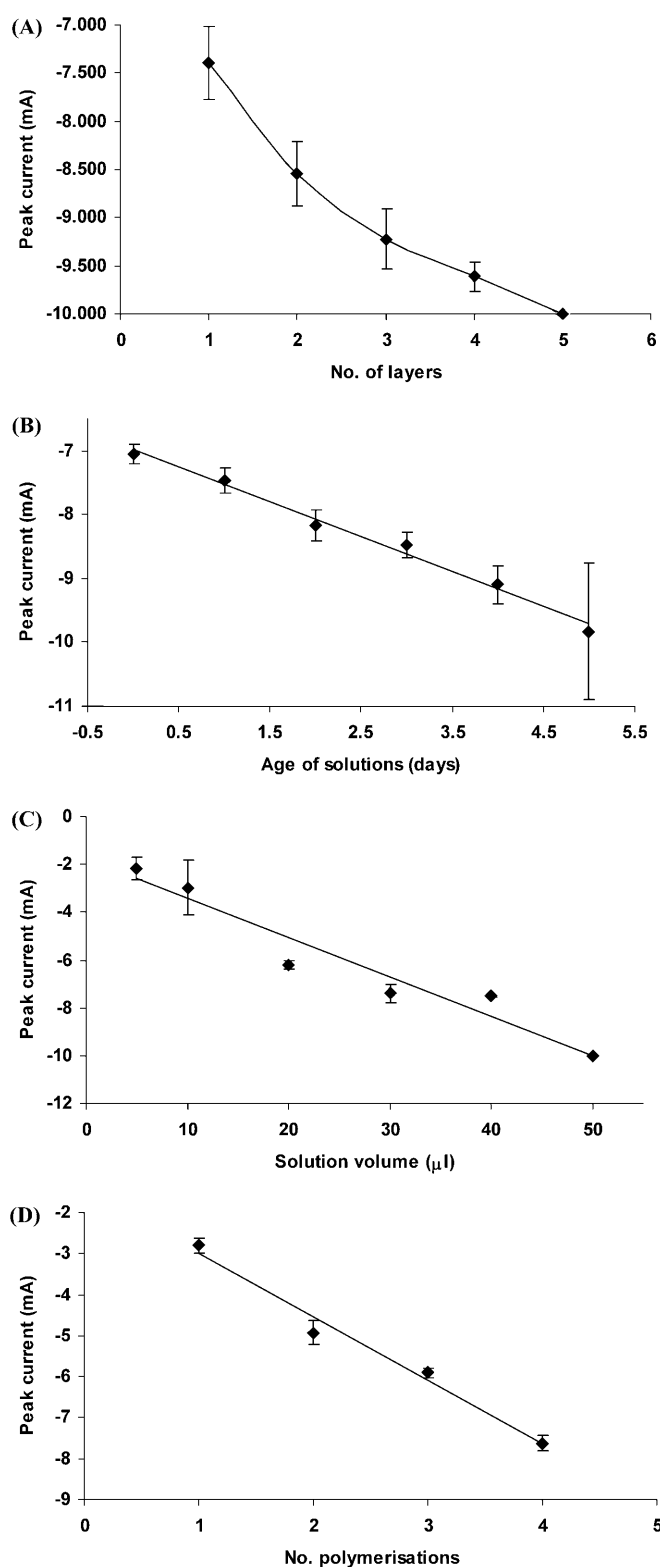


Fig. 4. Influence of A) depth of deposition well (number of insulation layers), B) the age of aniline and oxidant solutions, C) the volume of polymerization solution, and D) the number of polymerizations performed, on the peak current of the LM⁺ oxidation peak ($n=3$). Voltammograms obtained in 1 M HCl (vs. Ag/AgCl, scan rate 100 mV/s).

polymerized in less than 10 min. A linear relationship between the age of these solutions on the thickness of the films produced was also found and is outlined in Figure 4(B) (equation of best fit trendline: $y = -0.5455x - 6.9856$, $r^2 = 0.9891$). Save for a single datum, C. V. values below 3% were reported for solutions stored up to and including 4 days after preparation. Solutions stored for any longer resulted in films with great variations in thickness and so the maximum lifetime of the polymerization solutions was set at 4 days.

The volume of solution deposited into a deposition well formed by a single layer of insulating tape had a pronounced influence on the anodic peak currents and consequently on the thickness of the deposited PANI films, as shown in Figure 4(C). A pseudo-linear relationship between volume and film thickness was present (equation of best fit trendline: $y = -0.165x - 1.7771$, $r^2 = 0.936$). By increasing the volume of solution deposited from 5 to 50 μL, the anodic current of the LM⁺ peak increased from of the deposited film increased from -2.16 mA to -10 mA. The use of smaller solution volumes also produced films with thicknesses approaching those optimized for electrochemically polymerized films.

The most linearly consistent relationship between any of the polymerization properties investigated and the thickness of the PANI film produced, was found to be the number of subsequent polymerizations that were performed on the same electrode. This relationship is illustrated in Figure 4(D), which shows that with increasing numbers of polymerization steps, the thickness of the deposited film increased accordingly (equation of best fit trendline: $y = -1.5475x + 1.4418$, $r^2 = 0.9819$). Since the goal was to produce a film with a thickness approximating that optimized for electrochemical polymerization experiments, a single polymerization step using 10 μL volumes of solution was employed. These were deposited on electrodes coated with 1 layer of insulating tape forming the deposition well, which produced a peak current of approximately -2.66 mA for the LM⁺ peak, was used for further studies.

3.4. Influence of PVS on Polymerization

Once the aniline had been successfully polymerized chemically onto electrode surfaces, PVS was incorporated into the mixture in order to exploit its advantages for biosensing outlined previously. Incorporating the equivalent level of PVS as employed for electrochemical polymerizations did not impede the chemical polymerization process or the binding of PANI to the electrode surface. A drawback of this method of polymerization in contrast to the electrochemical equivalent was the time taken for the polymerization reaction to complete and also the time for the solution to dry after the reaction. The polymerization time increased from 20 min to ca. 30 min with the inclusion of the dopant and drying times of several hours at room temperature were required, presumably due to the less volatile nature of the mixture deposited.

In an attempt to accelerate the drying process while still maintaining optimal adhesion of PANI/PVS films to electrode substrates, a variety of drying conditions of varying humidity and temperature were investigated; 4 °C, 24 °C, 24 °C in a desiccator and 50–75 °C in a laboratory oven. As explained previously, films left to air dry at room temperature required a drying time of several hours before they could be employed. Films left at 4 °C did not dry, even after 4 days storage. Drying at 50 °C produced brittle films that were not suitable for further solution-phase usage. The optimum drying conditions that produced stable films were at a temperature of 24 °C in a desiccator. Such films were dry and ready for use after 20 min drying.

3.5. Biosensors Developed from Chemically Polymerized Polyaniline Films

Electrodes coated with chemically polymerized PANI films and doped with PVS were coated with HRP and subjected to the electroanalytical procedures outlined in Section 2.9. A typical amperometric output obtained is given in Figure 5, which includes a background amperogram of a PANI/PVS film prepared with no HRP deposited. The catalytic currents of ca. 109 μA were greater than those normally attained using the electrochemically-polymerized system on carbon-based electrodes. *S/b* ratios of 12 were typically obtained for these sensors. In comparison to sensors prepared using electrochemically polymerized aniline, where *s/b* ratios of 24 were obtained (results not shown), optimization work still remains to be carried out on these systems if chemically polymerized aniline films are to replace electrochemically prepared films in sensor production. Electrodes could also be prepared from PANI/PVS alone on a variety of substrates, without the need for underlying tracks. However, the present electrical contact method limited the choice of substrates to those with suitable adhesive properties towards the polymer films; indium tin oxide (ITO), fluorine tin oxide (FTO) and frosted glass. Such substrates were not ideal for the current sensing instrumentation or production techniques and so chemically prepared PANI/PVS sensors based on polymeric PET substrates necessitated the use of the underlying silver conducting track.

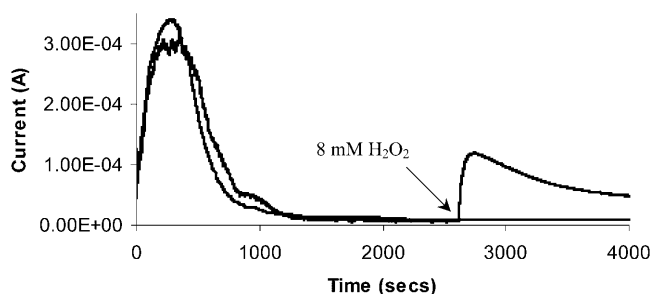


Fig. 5. Steady state amperometric responses of an unmodified chemically prepared PANI/PVS electrode (thin line) and one coated with 0.625 mg/mL HRP (thick line) (-100 mV vs. Ag/AgCl).

4. Conclusions

Chemically prepared PANI films have shown promise for biosensing purposes. In comparison to electrochemically polymerized films, chemically polymerized films displayed much flatter morphologies, with levels of cracking increasing with increasing ammonium persulfate oxidant concentration. Increased concentrations of oxidant were found to decrease polymerization times, but this over-oxidation produced polymer films of reduced conductivity. Deposition of polymerization solutions by drop coating was found to result in films of a more reproducible thickness, than those prepared using immersion techniques. Parameters such as the depth of the deposition well, the age and volume of the polymerization solutions and the number of coatings were found to influence film thicknesses. One layer of insulating tape and a single deposition of 5 μL of freshly prepared aniline and oxidant solutions produced films approaching the optimal thickness determined electrochemically.

With adequate automation coupled to individual electrode calibration, control over inter-electrode variabilities and film thickness could be achieved, thereby making chemically polymerized PANI films a viable prospect. Such electrodes could be prepared in bulk in a single step, allowing the mass-production of sensors based on PANI to be realized in short time periods.

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