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Polymer 46 (2005) 11505–11509

polymer

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Electrochemical synthesis of melanin free-standing films

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Received 12 April 2005; received in revised form 7 September 2005; accepted 1 October 2005

Abstract

Free-standing melanin films were successfully synthesised electrochemically from dopa. The optimum synthetic conditions such as pH, concentration and current were determined, and it was found that free-standing films could only be formed when ITO glass electrodes were used. The films were analysed by solid state NMR and XPS which showed the presence of indolic moieties characteristic of melanin-type macromolecules. The film showed higher conductivity than chemically synthesised melanin previously reported in literature and also exhibited photoconductivity.

Keywords: Melanin; Polyindolequinone; Electrochemical synthesis

1. Introduction

Melanin is a class of biofunctional macromolecule which is the predominant pigment in the surface structure of vertebrates [1,2]. It is most well known for its photoprotective role in humans, a property related to its strong absorbance in the UV and its antioxidant behaviour. There is also a suggestion that melanin may have some function in the nervous system due to its presence in the substantia nigra of the human brain [3–6].

Eumelanin and phaeomelanin are the two predominant forms in humans. Eumelanin is the more common black–brown pigment commonly found in the skin, and is formed naturally by the oxidation of tyrosine through the Raper–Mason scheme [1] (Fig. 1).

Synthetically, melanin is usually made by the autooxidation of dopa in alkaline aqueous solution. However, due to its insolubility in most common solvents, the resultant melanin powder cannot be easily processed with conventional polymer processing methods. As far as technological materials are concerned, melanin has excellent chemical and photostability, and being a natural material it represents the ultimate in potential biocompatibility. Despite this, melanin-based devices are not widely reported, and so the main applications studied concerning melanin has been their use as UV filters [7]. This is mainly due to the processing difficulty outlined above, and hence for melanin-based devices to be fabricated a better synthetic route is required. An alternative method for melanin synthesis that has been reported recently is electrochemical synthesis, which has been widely used in the fabrication of conducting polymer thin films.

The first published work on electrochemically synthesised melanin was in an abstract by Zielinski [8]. However, this was not followed by a full publication until Horak and Weeks [9] published a paper showing melanin synthesis electrochemically through the oxidation of 5,6-dihydroxyindole (DHI). Since then, other researchers have synthesised melanin thin films from dopa [10,11] or DHI [12–14]. However, these studies are concerned with melanin thin films as electrode coatings or modifiers, and none have so far synthesised melanin free-standing films, which is the next step towards the fabrication of melanin-based devices.

In this paper, we describe the fabrication of melanin free-standing films by means of electrochemical oxidation from basic aqueous solutions of dopa and the subsequent characterisation of these films.

2. Experimental

All chemicals were obtained from Sigma-Aldrich and used as received. Electrochemical synthesis was done using a Thandar TS3021S 30V/2A power supply connected to a Parameters 8040 multimeter in a two-electrode set-up, in which

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doi:10.1016/j.polymer.2005.10.068

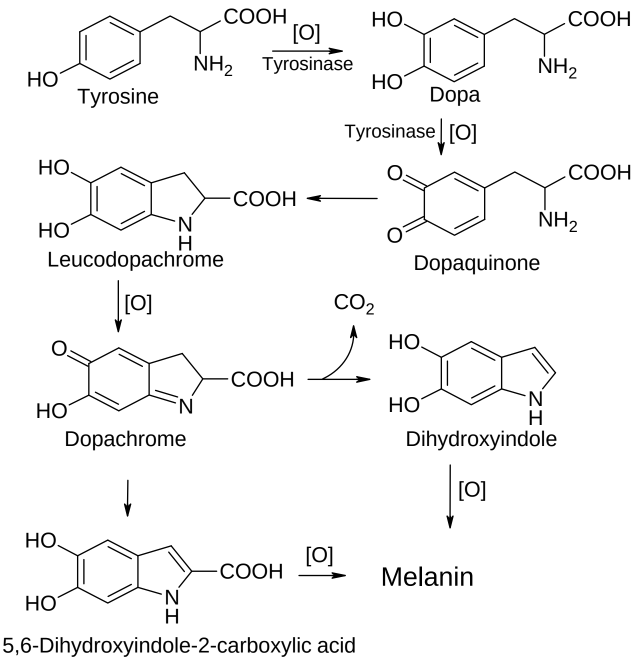

Fig. 1. The Raper–Mason scheme for melanin formation.

the potential was referenced against the counter electrode. The distance between the two electrodes was 3 cm.

The solution used was dl-dopa or l-dopa (Sigma-Aldrich) in 0.05 M borax (Sodium Tetraborate, Sigma-Aldrich) buffer (pH 9). The anode in the two-electrode set-up was ITO conducting glass (Pilkington, 15 Ω/cm^2) and the cathode used was a copper sheet. All polymerisation reactions were done under atmospheric conditions and the polymerisation solution was not degassed. The polymerisation was carried out at a current density of 0.5 mA/cm^2 for 6–10 days. The resultant film was washed by several immersions in deionised water, and then dried on a teflon sheet in a desiccator for a week. The air in the desiccator was kept humid for the first 3 days in order to slow down the drying process. The films were then transferred to a vacuum desiccator for further drying, and the final dried films were kept under argon in the dark.

SEM images were taken using a JEOL 35CF and JEOL 840A scanning electron microscope. The samples were sputter coated with gold before the images are taken.

Solid state NMR spectra were obtained using a MSL300 spectrometer. The parameters used were those outlined by Duff et al. [15] for the ^{13}C solid state NMR analysis of synthetic melanin. XPS analysis was performed on a Kratos Axis Ultra with a monochromated Al source.

Conductivity measurements were obtained using a four point probe connected to a PAR A270 Potentiostat/Galvanostat. Photocurrent measurements were performed in two-point contact mode using vacuum deposited platinum gold/-conducting epoxy contacts and a Keithley 2400 SMU. A 19 V bias voltage was applied for the photocurrent measurement and the sample was illuminated using an Oriel 100 W solar simulator. All measurements were performed under ambient conditions.

3. Results and discussion

Galvanostatic electropolymerisation of dl-dopa over a period of 6 days yielded thick, highly hydrated melanin films. The hydrated films were not uniform through their thickness, with the side attached to the electrode forming a film with reasonable mechanical stability while the side exposed to the electrolyte had the consistency of a thick paste. When dried, the melanin films reduced considerably in mass (the mass of dried film was about one-tenth the mass of the hydrated film) and became very brittle. To prevent cracking of the film hydrophobic surfaces were used when drying the film to prevent attachment of the film onto the drying surface which would result in cracking of the film due to mechanical stress.

Longer reaction times resulted in thicker films only up to 7 days at 0.5 mA/cm^2 since sufficiently thick films detached from the electrode surface due to their own weight and the electrode's vertical orientation. The melanin films, once dried, were very hydrophilic, but had poor solubility even in alkaline aqueous solution (pH ∼ 12), which is a commonly used solvent for melanin. This was expected since the polymer was formed (and hence insoluble) in pH ∼ 9 borax solution.

Galvanostatic polymerisation was preferred over potentiostatic due because as the film builds up on the electrode surface, the potential required to maintain the rate of reaction increases. Thus, galvanostatic method was deemed more efficient since it maintain the same rate of reaction throughout by maintaining a constant current density. As expected, the required potential to drive the galvanostatic polymerisation significantly increased as the film formed on the surface, presumably due to the less conductive nature of the polymer compared to the electrode material. As the polymerisation progressed, the potential required also reached a more stable value, presumably since the rate of film formation also slows down and a diffusion-limited state is reached.

The optimum current density which would provide maximum rate of dopa oxidation without initiating production of oxygen was found to be 0.5 mA/cm^2. This limit on the current density was due to the potential at which oxygen formation occurs. If a significant amount of oxygen was produced and hence bubbling occured on the anode, the newly formed polymer would be pushed away from the anode surface and no film formation would occur.

The polymerisation current density was also dependant on the type of anode used, with different anode materials having different oxygen overpotentials and hence different currents at which oxygen formation occurs. Doped indium–tin oxide conducting glass was the favoured anode substrate because it has a high oxygen overpotential which allows for higher polymerisation current.

As expected, film formation was best at high pH (∼9), which favours the formation of dopaquinone and suppresses reprotonation, providing a higher concentration of oxidisable species and therefore higher rate of polymerisation. If a very

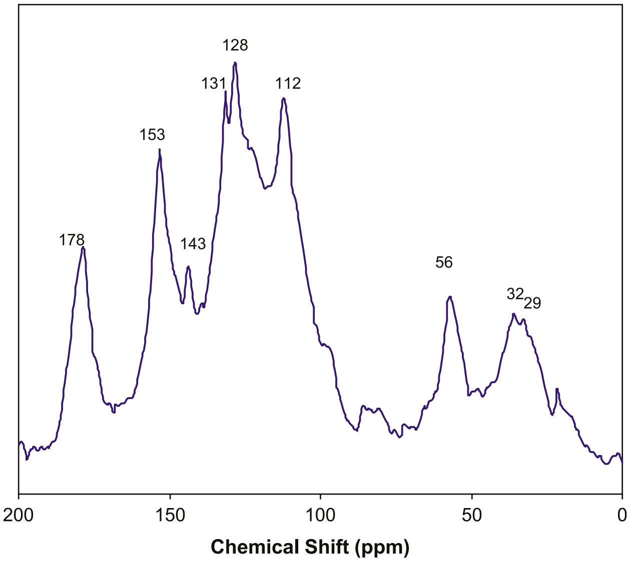

Fig. 2. ^{13}C Solid state NMR spectrum of the melanin sample. Melanin was prepared from an aqueous solution of 0.02 M dl-dopa in pH 9 borax buffer electropolymerised for 10 days at 0.5 mA/cm^2.

high pH (12–14) solution was used, the majority of the melanin formed remained soluble and hence only a very thin film was formed. Autooxidation would also proceed quite rapidly in the solution, therefore reducing the amount of dopa available for polymerisation on the anode, further hindering film formation. At neutral pH, filming was very slow and therefore only a very thin film formed as a coating on the anode for the equivalent polymerisation time. Theoretically, longer polymerisation times at neutral pH would result in a free standing film, however, we expect that most of the precursor would be consumed by autooxidation in solution before a free standing film would be formed, resulting in precipitation of melanin powder. At lower pH (1–3), polymerisation does not occur because the oxidation of dopa requires the deprotonation of the hydroxyl groups, and in acidic pH this is unfavourable.

Increasing the initial concentration of dopa in solution up to 50 mM helps facilitates film formation, however, solid state NMR results also showed that the melanin films made from solution of higher dopa concentration contains more unreacted dopa, which are believed to have merely been trapped within the polymer since vigorous washing of the film was not possible due to the lack of mechanical strength.

Film formation was favoured by hydrophilic anode surfaces, which is presumably due to the better attachment of the newly formed polymer to the anode surface. However, thick films were formed only when ITO conducting glass was used as the anode. When metallic anode such as stainless steel were used, thin films of melanin formed on the surface which did not appear to grow thicker even after being polymerised for 6 days. This is consistent with what Horak and Weeks [9] reported in that the melanin was formed as a semi-transparent bluish film that had good attachment to the electrode surface. This may be due to the melanin formed on the stainless steel anode creating a dense polymer layer, which prevents further diffusion of monomer onto the electrode surface, so the current was maintained only by diffusion of the smaller electrolyte ions and no further polymerisation occured on the electrode. The lone pair of electrons in the nitrogen or oxygen contained in the polymer would be attracted to the metal. The nitrogen or hydroxyl groups (and their corresponding quinone form) in the polymer would also chelate metal ions on the electrode surface and therefore bind the polymer to the metal.

Horak and Weeks [9] found that their films formed better when the potential was cycled from anodic to cathodic. They were, however, making thin films coating the electrode surface, and indeed when the potential was cycled the melanin seemed to form as a dense film which passivates the electrode.

It was also found that anodic pre-oxidation with mechanical stirring helped improve initial film formation. This is attributed to the increased concentration of dopachrome (as was observed with the increasingly deeper orange colour in the solution), which can be oxidised into DHI and further into melanin. It was also thought that the initial film, once formed, acts as a scaffold that helps prevent the intermediates from diffusing back into the solution, hence increasing the concentration of polymerisable species on the electrode surface. It was found that oxidation at 10–20 mA/cm^2 for 10–15 min with stirring before polymerisation was sufficient to increase the initial dopachrome concentration without producing melanin in solution. Shorter pre-oxidation time would produce less dopachrome in solution and hence slower initial film formation, while excess pre-oxidation results in the formation of low molecular weight species that are soluble in the pH 9 solution used. Excess melanin in solution seems to be detrimental to film formation, perhaps due to the fact that the larger oligomeric species would find it more difficult to diffuse through the film onto the electrode surface where oxidation occurs.

The choice of the cathode material does not affect polymerisation, and since the cathode is subject to reduction, leaching of metal ions into the solution would be negligible.

Solid state NMR analysis of the melanin free-standing film (Fig. 2) showed good agreement with the literature for melanin and a distinct difference with the literature value for the chemical shift of dopa [15] (Fig. 3). The peak at 110 ppm corresponds to a pyrrolic carbon, indicating that cyclisation of dopa has occurred. The peak at 178 ppm and the two peaks at 33 and 55 ppm indicates that some dopa is still present in the polymer, and is most likely trapped in the polymer and not chemically bound. This is evident when an increase in the

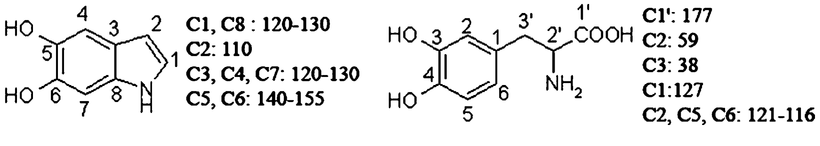

Fig. 3. Literature chemical shifts for dopa and dihydroxyindole [15].

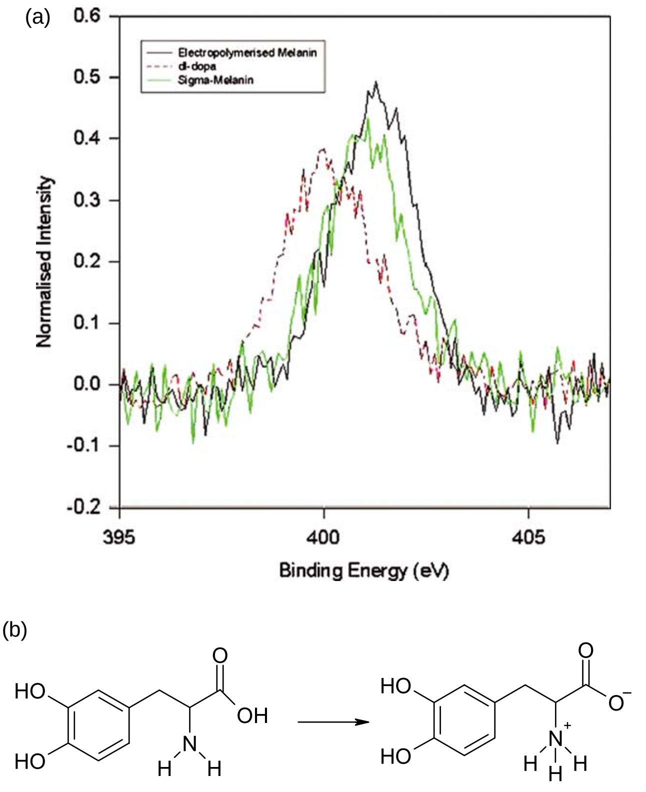

Fig. 4. (a) N 1s XPS spectra of the melanin samples. (b) Acid–base tautomerisation of dopa.

initial concentration of dopa in the solution results in an increase in the amount of dopa present in the polymer.

The peak at 178 ppm can also be due to the presence of 5,6-dihydroxyindole-2-carboxylic acid (DHICA), but peak fitting shows that the area under the carboxylic acid peak was roughly equal to the area under the aliphatic peaks of 33 and 55 ppm which is due to dopa alone, indicating that there was only a small amount of DHICA in the polymer.

XPS analysis of the melanin film revealed that the electrochemically synthesised melanin exhibits distinct differences to dopa and is similar to commercially obtained melanin powder. A 2 eV shift in the N 1s peak of dopa compared to the two melanin samples (Fig. 4) was observed for the carboxylic acid group in dopa adjacent to the amine, resulting in acid–base tautomerisation (Fig. 4). Further work is currently being done in this area using high-resolution XPS in order to determine the difference in oxidation states of the synthetic melanin. Elemental analysis using XPS revealed that the polymer also contains traces of sodium and boron due to the borax buffer used in the synthesis. The amount of sodium was generally stochiometrically higher than the amount of boron, which was thought to be due to excess dopa present.

The film was found to have a conductivity of 1.4×10^{-6} S/cm under ambient conditions, which is higher than the reported value in the literature of 6.4×10^{-8} S/cm [16] for melanin pellets at room temperature under ambient conditions. The increased conductivity is thought to be caused by increased ordering in the polymer grains. Indeed, SEM analysis of the cross section of the film showed ordered grain structure reminiscent of wood grains (Fig. 5) while previous literature studies of natural and chemically synthesised melanin showed a mainly spherical granular structure [17]. The grain structure is indicative of an underlying layered structure, however XRD analysis revealed that the melanin film was amorphous and therefore the layers are macroscopic features and not an indication of crystallinity.

The conductivity was also highly dependant on humidity, with an increase of conductivity over several orders of magnitude observed when water was present in the atmosphere. This observation is consistent with the literature [18] and further investigation into the mechanism for these electrical properties is ongoing.

Interestingly, the film also exhibited photoconductivity (Fig. 6). This is currently under further investigation, however, we believe that this behaviour is either partly due to the semiconducting nature of the material, or a change in conductivity due to photo-oxidation of the material.

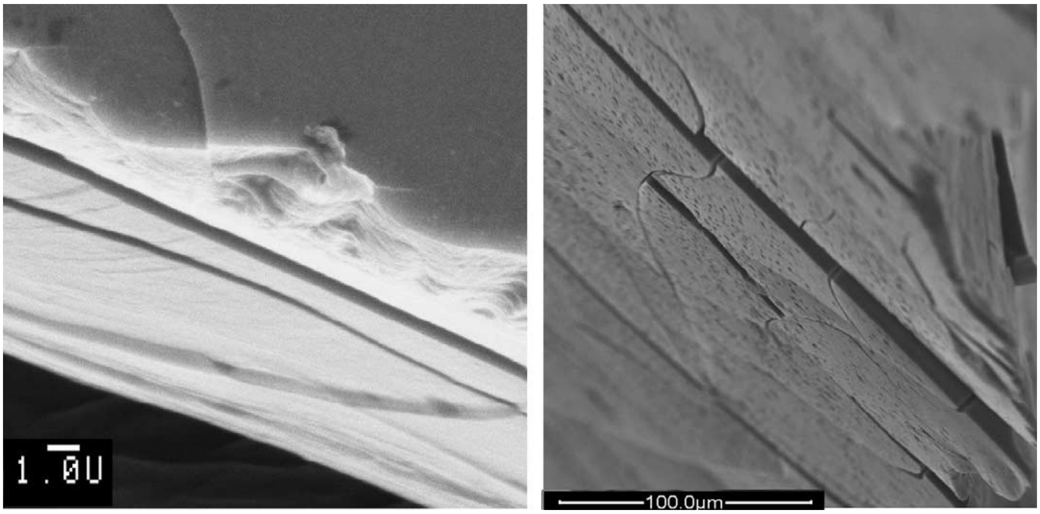

Fig. 5. SEM images of the cross section of melanin free-standing films. Both films were electropolymerised from 0.02 M l-dopa solution in borax buffer for 8 days at 0.5 mA/cm^2.

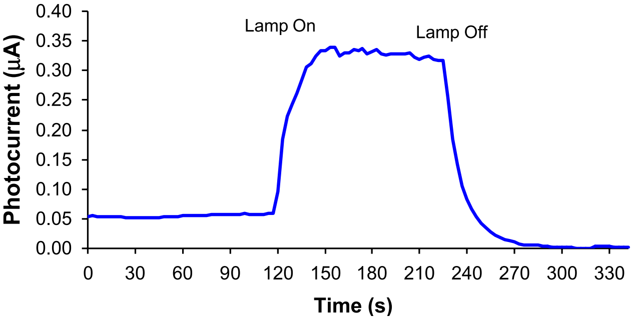

Fig. 6. Photoconductivity of the free-standing melanin film.

4. Conclusion

We have demonstrated the electrochemical synthesis of a free-standing melanin film. The optimum synthetic conditions were determined, and it was also found that films thick enough to be free standing can only form when ITO glass electrodes were used, with metallic electrodes resulting in a thin, dense film which does not grow thicker upon further oxidation. The solid state NMR and XPS analysis of the film showed distinct difference to its precursor dopa and confirmed the presence of the indolic units formed from dopa oxidation. The film showed higher conductivity than chemically synthesised melanin previously reported in literature. The film also exhibited photoconductivity which is a subject of further investigation.

Acknowledgements

The author would like to thank the group in the Inorganic Materials Research Program (Queensland University of Technology), Mr Loc Duong (analytical electron Microscopy Facility, Queensland University of Technology), Eric Tavenner (Soft Solid State Physics, University of Queensland), Dr Barry Wood (Surface Analysis Facility, University of Queensland), and Dr Andrew Whittaker (Centre for Magnetic Resonance, University of Queensland) and the ARC for funding.

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