PROCESS FOR THE PRODUCTION OF THIN FILMS OF MELANIN AND MELANIN-LIKE MOLECULES BY ELECTROSYNTHESIS

1. DC Power Supply

2.

3. Cathode (Cu)

4.

5.

6.

(57) Abstract: The invention relates to a process for the production of thin films of melanin or melanin-like biomolecules, said process including the steps of: adding at least one melanin or melanin-like precursor to an electrolytic cell [1] containing a buffered electrolyte [2]; and polymerizing said at least one melanin or melanin-like precursor to form a thin film of melanin or melanin-like biomolecules [6] by passing a current of 0.1mA/cm² -1A/cm² through the electrolytic cell [1].
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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FIELD OF THE INVENTION

The invention relates to processes for the production of thin films of melanin and melanin-like biomolecules and applications therefor. In particular, although not exclusively, the invention relates to a method of forming thin films of melanin and melanin-like biomolecules by electropolymerisation.

BACKGROUND TO THE INVENTION

Melanin and melanin-like biomolecules have been found to have biocompatibility and exhibit good photoconductivity properties, lending them to use in photovoltaic, optoelectronic, semiconductor and other electronic applications. In addition, the materials are useful for contact lenses, prothesis and a range of other therapeutic applications.

Melanin and melanin-like biomolecules are typically synthesised by:

a) autiodxidation of precursors, such as dihydroxypyrenylalanine, dihydroxyindole, catechols, etc.;

b) controlled oxidation of protected precursors, such diacetoxyindole;

c) enzyme assisted oxidation of precursors, such as tyrosine; or

d) extraction and purification of melamins from native tissues.

M.D. Rubianes & G.A. Rivas, *Analytica Chimica Acta*, 440, pp99-108, (2001) and G.M. Robinson, E.I. Iwuoha & M.R. Smyth, *Electrochimica Acta*, 43(23), pp3489-3496, (1998) describe a number of studies which involve the formation of melanin or melanin-like composite films deposited on substrates and investigate the electrochemical properties of these films. In general these documents teach the polymerisation of melanin precursors and study of their electrochemical properties in alkaline conditions applying a range of potential differences to an electrode coated with a melanin polymer. These publications describe the formation of melanin or melanin-like films, which are not capable of being free-standing and/or continuous thin films, once removed from the electrode to which they are deposited.
The prior art methods of forming melanin and melanin-like biomolecules result in the formation of powders or discontinuous films, limiting the application that the melanin and melanin-like biomolecules can be used for, e.g. powders generally have lower conductivity and are difficult to utilise in electronic applications where thin continuous films are desirable.

**DISCLOSURE OF THE INVENTION**

In one form, although it need not be the only or indeed the broadest form, the invention resides in a process for the production of thin films of melanin or melanin-like biomolecules, said process including the steps of:

- adding at least one melanin or melanin-like precursor to an electrolytic cell containing a buffered electrolyte; and
- polymerizing said at least one melanin or melanin-like precursor to form a thin film of melanin or melanin-like biomolecules by passing a current of 0.1mA/cm\(^2\) to 1A/cm\(^2\) through the electrolytic cell.

The process of forming thin films of melanin or melanin-like biomolecules may further comprise the step of oxidizing the said at least one melanin or melanin-like precursor prior to or during the polymerization step.

The term melanin-like is used herein in relation to the invention to refer to melanin and to materials defined as oligomers or biopolymers derived from naturally occurring eumelanins such as sepia melanin, neuromelanin, or phaomelanin.

The melanin-like biomolecules may be natural or synthetic monomeric, oligomeric or polymeric analogues of eumelanins, sepia melanin, neuromelanin, or phaomelanin and may be formed from one or more of the following: an indolequinone, such as dihydroxyindole, dihydroxyindole carboxylic acid, dihydroxyacetoxindole, dimethoxyindole and their quinones, semiquinone and carboxylic acid analogs; dihydroxyphenylalanine (DOPA) or its quinone or quinones, tyrosine, a catechol, a catechol amine, cyteinylldopa, or derivatives thereof.

Preferably, the melanin-like biomolecules is a biopolymeric material, such as natural occurring eumelanins, such as sepia melanin, or neuromelanin, or synthetic derivatives, such as DOPA eumelanin or polyhydroxyindole.
The term melanin or melanin-like precursor is used herein in relation to the invention to refer to one or more monomers required to form the melanin or melanin-like biomolecules as described above. Preferably the melanin or melanin-like precursor are selected from dihydroxyindole (DHI), dihydroxyindole carboxylic acid (DHICA), dihydroxyphenylalanine (DOPA), dihydroxyacetoxindole (DAI), dimethoxyindole (DMI), methoxy-acetoxy indoles, methylated DHI, methylated DHICA, methylated DOPA, methylated DAI, methylated DMI or methylated methoxy acetoxy indoles.

Preferably the melanin or melanin-like precursor is DOPA. Suitably the DOPA is present within the buffered electrolyte at a concentration between 0.02-0.06 M.

The electrolytic cell comprises at least an anode and a cathode. The anode may be selected from fluorine-doped tin oxide (FTO), a metallic anode such as, stainless steel or platinum or indium tin oxide (ITO) conducting glass, or any other conductive or semi-conductive material which has a suitable work function for electropolymerisation of the melanin or melanin like precursors. The semi-conductive material may include titanium dioxide. The anode may also preferably have surface energy sufficient to allow the melanin thin film to be released upon drying. The cathode may be any metallic electrode, such as copper, stainless steel, and platinum. The polymerization step may take between several hours and several weeks. Preferably the polymerization step takes between 6 to 10 days.

The melanin or melanin-like precursor is preferably polymerized by passing a current of 0.2-0.5mA/cm² through the electrolytic cell.

The buffered electrolyte may be any buffer solution having a pH suitable to keep the melanin and melanin-like precursors in solution. Suitably the buffered electrolyte is an alkaline buffered electrolyte, which may be selected from borax, ammonia or phosphate buffers.

In another form, the invention resides in a thin film of melanin or melanin-like biocompounds when formed from the method above.

Further features of the present invention will become apparent from the following detailed description.
BRIEF DESCRIPTION OF THE DRAWINGS

To assist in understanding the invention and to enable a person skilled in the art to put the invention into practical effect preferred embodiments of the invention will be described by way of example only with reference to the accompanying drawings, wherein:

FIG 1 shows an apparatus for implementing the process of the present invention;

FIG 1A shows a flowchart of the process of the present invention;

FIG 2 shows the variation of potential applied to the electrolytic cell for the period of polymerisation to form a melanin-like biomolecule thin film;

FIG 3 shows the variation of potential applied to the electrolytic cell until current stabilisation is reached;

FIG 4 shows current-voltage characteristics of a free-standing electro-polymerised melanin film produced by the process of the present invention;

FIG 5 shows the photocurrent produced by the free-standing electro-polymerised melanin film produced by the process of the present invention;

FIG 6 shows a comparison of the XPS N1s spectra of the melanin film produced by the process of the present invention and that of dl-DOPA and commercially available powdered melanin;

FIG 7 shows a solid state $^{13}$C NMR analysis spectrum of the melanin film produced by the process of the present invention;

FIG 8A shows the structure of a theoretical monomer unit of the melanin produced by the process of the present invention;

FIG 8B shows literature values for $^{13}$C NMR analysis spectrum for the structure in FIG 8A;

FIG 9A shows the structure of the melanin precursor DOPA; and

FIG 9B shows literature values for $^{13}$C NMR analysis spectrum for the structure in FIG 9A.

DETAILED DESCRIPTION OF THE INVENTION

FIG 1 is a schematic representation of the electrolytic cell 1 used for electropolymerisation of melanin and melanin-like precursors to form thin films of melanin and melanin-like biomolecules of the invention. The electrolytic cell 1 contains a alkaline buffered electrolyte 2 containing a melanin and melanin-like
precursor. A copper cathode 3 and an FTO anode 4 are connected to a power supply 5. A current is passed through the electrolyte 2 initiating polymerization of the melanin and melanin-like precursors to form a melanin or melanin-like thin film 6 on the surface of the anode 4.

These steps are generally shown on the process flowchart of FIG 1A.

EXAMPLE 1

dl-DOPA is added at a concentration between 0.02-0.04 M to a borax buffer, as described in Robinson, G. M.; Iwuoha, E. I.; Smyth, M. R. *Electrochimica Acta* **1998**, *43*, 3489-3496., having a pH = 9. An electropolymerisation current density of 0.5 mA/cm² is applied to the electrolytic cell and the precursor is polymerized over 6-10 days.

In this example the potential difference on the anode oxidises the precursor dl-DOPA into the monomer DHI, which subsequently oxidise and polymerise to form a melanin film on the surface of the anode. The potential is varied through time to ensure that the current running through the system remains constant during the polymerization step. FIG 2 shows the variation of applied potential for the period of polymerization. FIG 3 shows the variation of applied potential for the initial polymerization period until current stabilization is obtained.

On completion of the polymerization the melanin film coated anode is dipped into water for 5-10 minutes to remove any excess electrolyte. The melanin film may be removed from the anode by peeling the melanin film from the anode with the assistance of a scalpel or the like. If a thick film is required the film may be folded to achieve the desired thickness.

The film may be transferred onto a hydrophobic surface, such as a Teflon or glass modified acrylease slide and placed in a humidified desiccator for several days. The film may subsequently be placed in a dry desiccator for 7-10 days to finish drying.

It will be appreciated that if the melanin or melanin-like biomolecule thin film is to be applied to a device, if the device is conductive the film may be polymerized directly onto the device to be coated.
The melanin film produced by the process of Example 1 is tested for conductivity and chemical characterisation. FIG 4 graphs the current-voltage profile for the melanin film of Example 1 under ambient conditions in a two (2) point contact mode using vacuum deposited platinum gold / conducting epoxy contacts and a Keithley 2400 SMU. The film appears ohmic, and has a resistance of 36MΩ, which corresponds to an electrical conductivity of $1.3 \times 10^{-6}$ S cm$^{-1}$. These conductivity results are two (2) orders of magnitude higher than those reported in the literature for amorphous powders measured under similar conditions, see for example, Jastrzebska et.al., J. Biomater. Sci. Polymer Edn., 7(7), pp577-586, (1995).

FIG 5 is a graphical representation of the photocurrent produced by the melanin film under ambient conditions (room temperature and relative humidity) in a two (2) point contact mode using vacuum deposited platinum gold / conducting epoxy contacts and a Keithley 2400 SMU. A bias voltage of +19V was applied across the film, and it was illuminated with white light from a mercury (Hg) -vapour arc lamp (300mW/cm$^2$). The electopolymerised thin films of melanin formed by Example 1 display pronounced photoconductivity, as shown in FIG 5. In the example given, a saturation current of 0.3μA was obtained with an electric field of +19V and an illumination power density of 300mW/cm$^2$. Although it is not possible to make a direct qualitative comparison between this photoconductive performance and those reported in the literature for pressed amorphous melanin powders, due to different field strengths, testing conditions and illumination powers, it is clear that the thin films of melanin formed by Example 1 show equivalent, if not enhanced, photoconductivity.

The thin films of melanin formed by Example 1 are chemically and physically characterised using XPS and $^{13}$C NMR analysis. The results are summarised in FIGs 6 and 7, respectively.

FIG 6 is the XPS Analysis comparing the melanin film of Example 1, dl-DOPA and commercially available powdered melanin obtained from Sigma Aldrich. The XPS analysis shows that the binding energy of the 1s electron of the nitrogen in the melanin film of Example 1 differs quite significantly from that of dl-DOPA, and is similar to the commercially available melanin.
FIG 7 is a solid state $^{13}$C NMR analysis of the melanin film of Example 1. The spectra shows a peak at 110 ppm which was not present in the literature value for the solid state NMR of dl-DOPA. There were also some aliphatic and carboxylic peaks present, but it is currently not known whether this was due to the polymer or DOPA trapped in the polymer matrix.

FIGs 8A and 8B show the chemical structure and theoretical $^{13}$C NMR values for the monomer DHI. FIGs 9A and 9B show the chemical structure and $^{13}$C NMR values for the monomer DOPA.

The XPS and $^{13}$C NMR analysis, with comparison to the theoretical values for $^{13}$C NMR shown in FIGs 8A to 9B shows that the melanin films of Example 1 are poly-DHI melanin.

The films of Example 1 composed of polyindolequinone melanins may also be described as biopolymers, and therefore possess the key advantages of biocompatibility and low biotoxicity.

The melanin and melanin-like biomolecules formed by the process of the invention are mechanically stable, robust, thin, flexible films. In particular the films produced by the process are free standing, molecularly continuous thin films of melanin or melanin-like biocompounds. The films may have a thickness in the range of a single molecular layer to approximately 1mm. The thickness and composition of the films can be varied to provide the properties required in their final application. Throughout the specification the aim has been to describe the invention without limiting the invention to any one embodiment or specific collection of features. Persons skilled in the relevant art may realize variations from the specific embodiments that will nonetheless fall within the scope of the invention.
CLAIMS
1. A process for the production of thin films of melanin or melanin-like biomolecules, said process including the steps of:
   adding at least one melanin or melanin-like precursor to an electrolytic cell containing a buffered electrolyte; and
   polymerizing said at least one melanin or melanin-like precursor to form a thin film of melanin or melanin-like biomolecules by passing a current of 0.1mA/cm² - 1A/cm² through the electrolytic cell.
2. The process of claim 1, wherein the method further comprises the step of oxidizing said at least one melanin or melanin-like precursor prior to or during the polymerization step.
3. The process of claim 1, wherein the melanin-like biomolecules are selected from natural or synthetic monomeric, oligomeric or polymeric analogues of eumelansins, sepia melanin, neuromelanin, or phaomelanin.
4. The method of claim 3, wherein the melanin and melanin-like biomolecules are selected from one or more of the following: an indolequinone, such as dihydroxyindole and its quinone; semiquinone and carboxylic acid analogs; dihydroxyphenylalanine (DOPA) or its quinone or quinoles; tyrosine; a catechol; a catechol amine; cyteinyldopa, or derivatives thereof.
5. The method of claim 1 wherein the melanin and melanin-like biomolecules are a biopolymeric material.
6. The method of claim 5, wherein the melanin and melanin-like biomolecules are selected from natural occurring eumelansins such as sepia melanin, or neuromelanin, or synthetic derivatives thereof, such as DOPA eumelanin or polyhydroxyindole.
7. The method of claim 1, wherein melanin or melanin-like precursors are selected from one or more monomers required to form melanin or melanin-like biomolecules from natural or synthetic monomeric, oligomeric or polymeric analogues of eumelansins, sepia melanin, neuromelanin, or phaomelanin.
8. The method of claim 7, wherein the melanin or melanin-like precursors are selected from dihydroxyindole (DHI), dihydroxyindole carboxylic acid
(DHICA), or dihydroxyphenylalanine (DOPA), dihydroxyacetoxyindole (DAI), dimethoxy indole (DMI), methoxy-acetoxy indoles, methylated DHI, methylated DHICA, methylated DOPA, methylated DAI or methylated DMI.

9. The method of claim 1, wherein the melanin or melanin-like precursor is DOPA.

10. The method of claim 9, wherein the DOPA is present at a concentration between 0.02-0.06 M.

11. The method of claim 1, wherein the electrolytic cell comprises;

   at least an anode, selected from fluorine-doped tin oxide (FTO); a metallic anode; or any other conductive or semiconductive material which has a suitable work function for electropolymerisation of the melanin or melanin like precursors; and

   a metallic cathode.

12. The method of claim 11, wherein the anode also has surface energy sufficient to allow the melanin thin film to be released upon drying.

13. The method of claim 11, wherein the anode is a metallic anode selected from stainless steel or platinum or iridium titanium oxide (ITO) conducting glass.

14. The method of claim 1, wherein the polymerization step is carried out over several hours to several weeks.

15. The method of claim 13, wherein the polymerization step is carried out over 6 to 10 days.

16. The method of claim 1, wherein the buffered electrolyte is an alkaline buffered electrolyte selected from borax, ammonia or phosphate buffer solutions.

17. A thin film of melanin or melanin-like biocompounds when formed from the method of claim 1.
Adding at least one melanin or melanin-like precursor to an electrolytic cell [1] containing a buffered electrolyte [2]

Polymerizing - passing a current of 0.1mA/cm² -1A/cm² through the electrolytic cell [1].

Form a thin film of melanin or melanin-like biomolecules [6]
FIG 3

FIG 4
FIG 5
XPS N1s Spectra

Binding Energy (eV)

Normalized Intensity

Electropolymerised Melanin
di-dopa
Sigma-Melanin

FIG 6
FIG 8A

FIG 8B

FIG 9A

FIG 9B
**INTERNATIONAL SEARCH REPORT**

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<td>X</td>
<td>MARX, A. K et al &quot;Comparative study of electropolymerisation versus adsorption of tyrosine and the decyl ester of tyrosine on platinum electrodes&quot;; Journal of Electroanalytical Chemistry (2002), 521 (1-2), p53-60</td>
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* Further documents are listed in the continuation of Box C  
X See patent family annex

| **Date of the actual completion of the international search** | 28 October 2004 |
| **Date of mailing of the international search report** | 4 NOV 2004 |
| **Name and mailing address of the ISA/AU** | AUSTRALIAN PATENT OFFICE  
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<td>A</td>
<td>US 4835076 A (HEINZE et al.) 30 May 1989 whole document</td>
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<td>A</td>
<td>US 5578188 A (MERTENS et al.) 26 November 1996 whole document</td>
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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