Proton Conduction in Bio-macromolecules
The Search for the Dominant Charge Carrier within Melanin

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Declaration

This thesis is a study undertaken between February 2006 and October 2006 at the Department of Chemistry, in the School of Molecular and Microbial Sciences, in collaboration with the Department of Physics, in the School of Physical Sciences, both at the University of Queensland, Brisbane, Australia. The research was done under the supervision of Dr. Paul Meredith, A/Prof. Ian Gentle and Dr. Ben Powell.

Except where acknowledged in the customary manner, the material presented in this thesis is, to the best of my knowledge, original and has not been submitted in whole or part for a degree in any university, or any other publication.

A. Bernardus Mostert

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Acknowledgments

The work I present here is not solely the invention of my own mind nor did it come about purely due to my own abilities, for during the year I have had tremendous advice and encouragement. Therefore, I wish to take the opportunity presented in this acknowledgment section to thank the people who have helped and guided me throughout the year.

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Albertus Bernardus (Bernard) Mostert
“I hate melanin”

- A.B. Mostert, 11:35, 12/10/06
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Abbreviations and Symbols

Abbreviations

2D 2 Dimensional  
3D 3 Dimensional  
ATR-FTIR Attenuated Total Reflectance Fourier Transform Infrared  
BET Brunauer-Emmett-Teller theory of adsorption isotherms  
CPMP Condensed Phase Melanin Pellet  
DC Direct Current  
DHI 5,6-dihydroxyindole  
DHICA 5,6-dihydroxyindole-2-carboxylic acid  
dl-dopa dl-3,4-dihydroxy-phenylalanine  
ESR Electron Spin Resonance  
EPR Electron Paramagnetic Resonance  
FID Free Induction Decay  
ITO Indium Tin Oxide  
IV Current-Voltage  
MFT Mean Field Theory  
NMR Nuclear Magnetic Resonance  
PVC Poly Vinyl Chloride  
redox reduction-oxidation  
RH Relative Humidity  
SAXS Small angle X-ray scattering  
SMU Source Meter Unit  
std Standard deviation  
UV-Vis Ultraviolet - Visible Spectroscopy  
vdP van der Pauw

Symbols

$A_{\text{H}_2\text{O}}$ Area that one water molecule occupies  
$\chi^2$ Value associated with how well a linear regressed curve fits the data
Diameter of the pellet
DM The dry mass of CPMP in the thermodynamic limit
Δ Width of NMR peaks at half height (Chapter 5, Section 5.2)
Δ Denotes uncertainty within the variable that immediately follows this symbol
$\Delta E$ Enthalpy of adsorption, not uncertainty within energy
$E_A$ Average activation energy for amorphous semi-conduction
$e$ The fundamental charge, $1.602 \times 10^{-19}$ C
$e^-$ Electron
$\varepsilon_0$ The permittivity of free space, $8.854 \times 10^{-12}$ A$^2$ s$^4$ kg$^{-1}$ m$^{-3}$
$H^+$ Proton
$^1H$ NMR Proton NMR
$H_2O$ Water molecule
$HO - \phi - OH$ Fully reduce melanin indole unit
$\kappa$ Dielectric constant of environment around macromolecule
$\kappa_w$ Modified dielectric constant of macromolecule due to polarization
$k_B$ Boltzmann’s constant, $1.381 \times 10^{-23}$ J K$^{-1}$
$l$ Distance the contact span the circumference of a pellet
$M$ The mass of hydrated CPMP in the thermodynamic limit
$M_{H_2O}$ Molecular weight of water
$m$ Distance the contact intrudes toward the centre of the pellet
$\mu$ Critical exponent for percolated conductivity
$N_A$ Avogadro’s number
$n^\sigma$ Number of moles of adsorbate adsorbed unto adsorbent
$n^\sigma_g$ Specific monolayer coverage
$n^\sigma_m$ Number of moles required to give 1 monolayer coverage
$\nu$ Number of possible monolayers of adsorbate covering an adsorbent
$^{17}O$ Oxygen 17, isotope of oxygen
$O_2$ Oxygen
$O_2^*$ Super oxide anion
$OH$ Alcohol group
$O = \phi = O$ Fully oxydised melanin indole unit
$P$ Vapour pressure of water
$P_0$ Saturated vapour pressure of water
$p$ Percolation probability of a strand being connected
$p_c$ The percolation threshold
$R$ Cavity radius of the charge region (Chapter 1, Section 1.4.1)
$R$ Universal Gas Constant, $8.314$ J mol$^{-1}$K$^{-1}$ (Chapter 3, Section 3.2.3)
$R$ Resistance for a specific van der Paue configuration (Chapter 4, Section 4.2.1)
$R_H$ Average resistance for all van der Pauw horizontal configurations
\( R_V \) Average resistance for all van der Pauw vertical configurations
\( \sigma \) Conductivity
\( \sigma_0 \) Arbitrary conductivity constant, dependent on the material
\( SSA \) Specific Surface area of melanin
\( T \) Temperature in kelvin
\( T_2 \) Transverse relaxation, known also as spin-spin relaxation
\( T_2^* \) Effective transverse relaxation time
\( w \) Statistical weighting
\( wt(\%) \) Weight percent gained
\( wt(\%)_C \) The critical Weight percent gained
\( X \) Relative Water Pressure
\( X_C \) The critical relative pressure
\( \frac{X - X_C}{X_C} \) Reduced relative pressure
\( Z \) Boltzmann factor appearing in BET theory, \( \approx \exp \frac{\Delta E}{RT} \)
Abstract

Melanins are a class of functional bio-macromolecules ubiquitous throughout the biosphere. They possess a set of unique physico-chemical properties, including condensed phase electrical conduction. It has been suggested that they are bio-organic amorphous semiconductors, although recent results have called this paradigm into question. In particular, condensed phase melanin samples have been shown to exhibit a strong dependence of electrical conductivity on relative humidity, potentially associated with the dominant carrier being protonic rather than electronic at high humidity levels.

The motivation for understanding charge transport in melanin is twofold: Firstly, its an important bio-molecule, therefore characterizing the charge transport properties may lead to understanding of its function in vivo. The second reason for studying charge transport is that melanin has shown potential as a high-tech material. ‘Soft’ bio-organic materials are currently being looked at as an alternative to silicon based electronics since they are environmentally friendly and relatively cheap. Therefore, determining the nature of the water-melanin interactions and how they lead to different types of charge transport may open up melanin as a possible contender in this rapidly growing field.

To investigate these water dependent phenomena requires knowledge from both the fields of chemistry and physics. Chemistry explains how water molecules relate to bulk melanin and how possible proton charge carriers may emerge. From the field of physics one needs to have an understanding of charge transport. Using the resources and skills from both fields enables
a thorough investigation of the electrical properties of melanin.

In the following study this multi-disciplinary approach, with an emphasis on chemistry, is employed to probe melanin’s conductivity and the potential protonic charge carrier. The work presented here is part of an on-going effort to fully map the structure-property relationship of melanin. Specifically, this thesis focuses on experimental results documenting water-melanin behaviour. An adsorption isotherm for condensed phase melanin pellets is presented and indicates melanin’s strong affinity for water. This isotherm is a first for condensed phase melanin pellets. An electrical (DC) conductivity isotherm is also presented, which indicates that water has formed a percolated system within melanin. This result, together with observed Child’s law current-voltage dependence is consistent with protons as the dominant charge carrier at high humidity levels. Finally, a study of proton dynamics using solid state NMR is presented. This study is also the first of its kind for melanin, showing an increase in the mobility of protons as water content within melanin is increased.
CHAPTER 1

Introduction

Melanins are a class of functional bio-macromolecules found throughout nature(1) and possess an intriguing and unique set of physical and chemical properties(2). In humans, they serve as our primary photoprotectant and pigment, and it has been recently suggested that they may be useful as high tech functional materials(3).

Interest in the electrical properties of the melanins started with the discovery of switching behaviour in their resistive properties by McGinness et al.(4). They suggested that the melanins might be semiconductors, however, recent studies(5; 6) indicate this view may not be wholly accurate. The electrical behaviour of melanin is highly dependent on its water content. Moreover, the dominant charge carrier within melanin does not appear to be electronic but something else which has a positive charge. There is disagreement about what this positive charge carrier may be, but a recent proposal suggest that it may be protonic(6).

The purpose of this thesis is to investigate the nature of the positive charge carrier. Ultimately, because melanin’s electrical properties have been shown to be sensitive to water content, characterising the charge carrier becomes important if one wishes to build devices such as sensors based on melanin. Additionally, understanding the nature of the dominant charge carrier is also crucial if we are to gain a more complete understanding of the fundamental chemistry and physics of these systems.

Studying melanin’s electrical properties is notoriously difficult requiring a diverse range of
skills and techniques. Therefore a multi-disciplinary approach is needed which derives inspiration from both the fields of chemistry and physics. Using chemical knowledge, interactions between water can be melanin are considered, and a background in physics allows the study of charge carriers.

In this chapter a review of the literature concerning the charge carrier of melanin is presented, culminating in a hypothesis which suggests that melanin’s dominant charge carrier is dependent on its water content. However, to start, a basic introduction is given concerning the structure and chemistry of melanin.

1.1 The Chemistry of Melanin

In humans there are two main types of melanin, eumelanin that consists of brown-black pigments, and pheomelanin that is a yellow-red pigment(7; 8). Most experiments concerning the electrical properties of melanin have been carried out on synthetic eumelanin, therefore, following in the footsteps of previous researchers, this project will also use synthetic eumelanin. It should be noted that in this thesis, ‘melanin’ and ‘eumelanin’ are used interchangeably even though melanin covers a broad class of substances.

Eumelanin is derived from two chromophores, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA)(1; 3; 7) (Figure 1.1). These are formed from the oxidation of

![Figure 1.1: The monomer unit of eumelanin. The squiggly lines indicate attachment to other chromophores and possible proteins. The R group can be an H (for DHI) or COOH (for DHICA).](image)

Tyrosine via the Raper-Mason scheme (Figure 1.2) and go on to form melanin by a biosynthetic pathway known as melanogenesis(1; 7). The overall molecular structure of eumelanin
is considered to be a heterogeneous, chemically disordered substance derived from the indole units DHICA and DHI. However, how the indole units cross-link or aggregate is unknown and is the subject of intense research(2; 3; 7; 8; 9).

![Chemical Structures](image)

**Figure 1.2:** The Rapo-Mason scheme of melanin synthesis in vivo. The reaction involves the oxidation of tyrosine to melanin intermediates DHI and DHICA, which then polymerizes into melanin. Taken from Subianto et al.(10)

It has been observed that model systems for melanin are able to scavenge reactive oxygen species such as singlet oxygen(11) (1^2O_2), hydroxyl radicals(12) (•OH) and the superoxide anion(12) (O_2•). This has lead to the belief that melanin may act as a free radical scavenger and antioxidant(1) in vivo (See also a review by Hill(13)). This scavenging ability may be due to the numerous redox active moieties on the indole units assumed to be within melanin. These moieties may be oxidized as shown in Figure 1.3.

![Indole Structures](image)

**Figure 1.3:** The oxidation of indole hydroxy groups to ketones.

Melanin has a persistent ESR signal, which indicates the presence of free radicals centres,
which are exceptionally stable within the bio-macromolecule(14). The formation of these radicals in melanogenesis, and also synthetic methods, is accompanied by hydrogen peroxide production. Furthermore, the formation of melanin consumes O$_2$. From these observations it has been suggested that melanin may form according to the following reaction mechanism (See review by Blois(7))

\[
\text{HO} - \phi - \text{OH} + \text{O}_2 \rightarrow \text{HO} - \phi - \text{O}^\bullet + \text{O}_2^\bullet + \text{H}^+ \\
2\text{O}_2^\bullet + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]  

where \(\text{HO} - \phi - \text{OH}\) represents a fully reduced indole unit. The reduction of molecular oxygen (in its ground state) proceeds - because of spin restriction - in univalent free radical steps(15). These reactions form hydrogen peroxide, as required, and activates the indole units for the formation of melanin. Furthermore, the reaction showed in Equation 1.1 also demonstrates how the final redox active moieties within melanin may be oxidized as shown in Figure 1.3. It may also explain the chemical disorder of melanin since free radical mechanisms don’t impose a great deal of control during reactions(7). The presence of free radicals in the final product is most likely due to indole units that have been activated but are unable to react, which is very much like the presence of free radicals in a classical polymer.

The free radicals that are left behind within in the final melanin matrix are stabilized by:

\[
\text{HO} - \phi - \text{OH} + \text{O} = \phi = \text{O} \iff 2(\phi = \text{O})^\bullet + 2\text{H}^+
\]

which is known as the comproportionation equilibrium (See Review by Meredith & Sarna(16)). This equilibrium lies heavily towards the fully reduced/oxidized forms of the indoles, as was demonstrated by Froncisz et al.(17) who showed using EPR that there is 1 free radical in equilibrium with \(\approx 1000\) fully reduced and/or oxidized melanin indole units.

The above chemistry may also explain why melanin conducts electrons. Free radicals, because
they are unpaired electrons, will be easier to move using an external electrical field.

1.2 Evidence for Charge Transport

Many techniques can be used to demonstrate the sign of the dominant charge carrier of a material. However, to demonstrate that protons are the dominant charge carriers specifically is difficult (18; 19). The following critical phenomena are considered essential to indicate protons as the dominant charge carrier:

1. Decay in current over time under constant voltage (19). This is observed while conducting current-voltage (IV) measurements.

2. Ohmic to Child’s law dependence of the voltage on current, \( I \propto V^n \) where \( n > 1 \) (19). This should also be seen in IV measurements.

3. Transference number measurements (coulometric studies) can determine the percentage of the current passing through a sample that is protonic (19; 20). Gasses evolved in the reaction are compared to theoretical predictions as given by Faraday’s equivalence law and thus the ratio of the charge carriers can be determined.

4. The sign of the Hall voltage must be positive (19). This can be determined by Hall effect measurements.

5. The thermoelectric voltage should be positive (19). This can be determined from thermoelectric measurements (Seebeck effect).

6. Line narrowing in \(^1\)H NMR peaks with increased water content (20). The phenomena can be probed using solid state NMR.

7. The energy gain or loss in a material after inelastic neutron scattering. This provides information on the energy levels of the hydrogen bonded systems in the material (19; 20).
1.3 Melanin, the Conductor

McGinness et al. (4) found electrical resistance switching behaviour within melanin, which is similar to that of amorphous inorganic semiconductors, suggesting that melanin can be thought of as an amorphous organic semiconductor. They and Baraldi et al. (21) found conductivities of $10^{-5}$ S cm$^{-1}$ and $10^{-12}$ S cm$^{-1}$ respectively, which is a difference of seven orders of magnitude. Both studies found that loss of water dramatically changed the electrical properties of melanin. However, they did not report humidity levels. The different values for the conductivities obtained are most likely due to differing water content in their respective samples.

Baraldi et al. and Bridelli et al. (22) found evidence for two types of water in melanin: ‘structural’ water, probably within the structure of melanin; and ‘physical’ water, probably on the surface of melanin. Bridelli et al. also found that water is a major factor in the electrical properties of melanin.

Hall voltage measurements by Trukhan et al. (23) indicated that the charge carrier in melanin was positive. However, once again no hydration levels were recorded. It is most likely that the experiments were performed at ambient conditions; possibly indicating water’s role in the result. They suggested that the charge carriers were holes but conceded that protons were also a likely interpretation.

Coulometric studies by Powell et al. (24) suggested that synthetic melanin carried charges that were 65% protonic and 35% electronic over a hydration range of 12% to 35% weight gained in water.

Seebeck effect studies suggested a different dominant charge carrier at low hydration. Performed under vacuum by Osak et al. (25), thermoelectric tests gave negative thermoelectric voltages, suggesting a negative charge carrier. However, they found a Child’s law dependence
on the voltage at high voltages, and they also observed decay in current with applied voltage. Both these effects indicate ionic carriers. Due to the thermoelectric measurements, they tried to explain the conductivity of melanin in terms of conformational changes in melanin’s structure, which allows the electrons to hop more frequently. Water was never considered to play a role in the conductivity of melanin by Osak et al. Giacomantonio(6) explained the results by suggesting that electrons dominate at low water content. However, some water is still present indicating the presence of protons, but in a minor capacity.

Further studies by Strzelecka(26; 27) investigated the supposed semiconductor properties of both natural and synthetic eumelanin. In these studies, hydration levels of melanin were also not reported. It was implied that the dominant charge carrier was a positively charged hole(27). Child’s law dependence on the current(26) was also observed.

In 1995, Jastrzebska et al.(5) attempted to map out the DC conductivity of melanin systematically as a function of hydration. They assumed that the hydration of melanin was proportional to relative humidity (RH). For this reason, they used various salt solutions to control the RH over a range of values. Figure 1.4 shows the results obtained. They suggested

![Graph showing conductivity of melanin vs Relative Humidity](image)

**Figure 1.4:** The conductivity of melanin as a function of RH at 298K, by Jastrzebska et al.(5). Note that the y-axis is a log scale.

that at low hydration the main charge carriers were electrons, but at higher hydration levels
the carriers were positively charged polarons\footnote{Polarons are quasi-particles made up of electrons and crystal lattice distortions, which are induced by the electrical field of the electron. Positive polarons are related to holes.}.

Giacomantonio\cite{Giacomantonio} extended the work done by Jastrzebska \textit{et al.}, also employing salt solutions to control the RH around melanin. Her results can be seen in Figure 1.5a.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.5.png}
\caption{a) The conductivity of melanin as a function of RH at 24±2°C. b) The conductivity plotted as a function of wt(%). Note the fitted curves to the data, these are predicted by the mixed conduction hypothesis (Section 1.4). Note that the y-axis is a log scale. Taken from Giacomantonio\cite{Giacomantonio} and modified.}
\end{figure}

Overall the conductivities obtained by Giacomantonio were higher than those found by Jastrzebska \textit{et al.} This was most likely due to slightly differing experimental setups. Jastrzebska \textit{et al.} used a sandwich electrode setup and waited 1 hour for equilibrium. This did not leave a lot of surface area exposed to the environment and the one-hour wait did not necessarily allow the bulk of the samples to reach equilibrium with the RH. In contrast, Giacomantonio used the van der Pauw setup\cite{VanDerPauw}, which allowed more surface area for water adsorption. Further, more time for the samples to equilibrate with the surroundings was allowed.

An assumption made by Jastrzebska \textit{et al.} was that the amount of water contained in melanin was proportional to the RH in the surrounding air. This assumption is false, for there is not a linear relationship for the Brumauer, Emmett, Teller (BET) isotherm, the isotherm for RH
against water adsorbed by weight percent (wt(%)) of powdered melanin(6; 24). Therefore, Giacomantonio refitted her data so as to obtain a plot of wt(%) against conductivity (Figure 1.5b).

Giacomantonio postulated that her results and the literature indicated that melanin is a mixed conductor, instead of a semiconductor. At low water content electrons are the dominant charge carrier and at high water content protons would dominate. She suggested that the behaviour of the conductivity at low water content can be modelled by an Arrhenius type equation and at high water content that a percolation picture, using the Grotthuss mechanism can be employed. This hypothesis is discussed below.

1.4 The Mixed Conductor Hypothesis
1.4.1 Amorphous Semi-Conduction Theory

In an amorphous substance, electrons conduct via hopping (Figure 1.6) from one localized state to another(29; 30). These localized states can be individual atoms, molecules, or regions within a substance. The conductivity of amorphous semiconductors usually follows the Arrhenius equation

$$\sigma = \sigma_0 \exp \left( \frac{-E_A}{k_B T} \right)$$  \hspace{1cm} (1.3)

where \(\sigma\) is the conductivity, \(\sigma_0\) is a constant of the material, \(k_B\) is Boltzmann’s constant, \(T\) is temperature and \(E_A\) is the average activation energy needed to effect conductivity.

A theory developed by Rosenberg(24; 30; 31) accounted for the change in the conductivity of

![Figure 1.6: Two localised states with energy E separated by a potential barrier V. Electrons move from one state to the other (hopping) by thermal energy. This require high enough temperatures to move the electron over the barrier V(29; 30). Taken from Lewis(29) and modified.](image)
a bio-macromolecule due to its environment (e.g. water)

\[ \sigma = \sigma_0 \exp \left( -\frac{E_A - c}{k_B T} \right) \]  

(1.4)

where

\[ c = \frac{e^2}{4\pi\varepsilon_0 R} \left( \frac{1}{\kappa} - \frac{1}{\kappa_w} \right) \]

where \( e \) is the fundamental charge, \( \varepsilon_0 \) is the permittivity of free space, \( \kappa \) is the dielectric constant of the environment (e.g. water), \( \kappa_w \) is the modified dielectric constant of the bio-macromolecule due to polarization effects from the environment, and \( R \) is the cavity radius of the charged region.

In a dry environment, \( \kappa_w = \kappa \), normal Arrhenius behaviour (Equation 1.3) is expected. However, when water is introduced, the \( c \) term becomes important and start to lower the activation energy needed to affect conductivity. As more water is introduced, the \( \frac{1}{\kappa_w} \) term becomes negligibly small, therefore the conductivity reaches a saturated value(24; 32; 30; 31). Essentially the presence of water increases the dielectric constant of melanin, making it easier for electrons to conduct. This theory was suggested to be consistent with the first part of Giacomantonio’s results (Figure 1.5b)(6).

The source of electrons for conduction in melanin is most likely from free radical electrons trapped within melamins’ structure (Section 1.1).

1.4.2 Percolation Theory and Proton Conduction

Consider an electrical network such as in Figure 1.7a. Each strand is either intact (conducting) or cut (insulating). The network has a voltage applied across it. It has been shown theoretically and experimentally that the conductivity of such a network, as a function of the probability \( p \) of a strand being connected, has the form of the graph in Figure 1.7b (33).

Percolation theory explains the relation between the conductivity and \( p \). Below some critical probability \( p_c \), known as the percolation threshold, there is no conduction as there is no con-
Figure 1.7: a) A random electrical network. b) The conductivity of the network as a function of probability $p$ of a strand being connected. Taken from Deutscher (33) and modified.

Continuous electrical path connecting the ends. At $p_c$, long-range conductivity suddenly occurs as a continuous path is found. Past $p_c$, more pathways are found and the conductivity increases rapidly. The percolated conductivity can be described, both in 2 and 3 dimensions by

$$\sigma = \sigma_0 (p - p_c)^\mu$$

(1.5)

where $\mu$ is a critical exponent and is equal to 1.3 in a 2D system and 2.0 in a 3D system (34). This model though does not explain the mechanism of conduction, only the relation between the conductive elements are considered. It is a macroscopic picture of matter, so one is at liberty to chose the microscopic mechanism as long as its consistent with the percolation model(6). It was assumed by Giacomantonio that the H$_2$O molecule is the conductive element, analogues to a strand in a network, and through adsorption onto melanin reside on an arbitrary site(6) within melanin with probability $p$. The charge carrier that would conduct is the proton since all the phenomena described in the literature are consistent with proton conduction and water is known not to conduct electrons but protons.

For protons to conduct through percolation would require the Grotthuss mechanism of proton conduction(9; 35; 36; 37). This mechanism requires a hydrogen bonded network in which H$^+$ are transfered from one H$_2$O molecule to another, followed by reorientation. This mechanism is for long range ordered networks of water, and should not be confused with centre of mass diffusion (vehicle mechanism(9; 36; 37)).
Protons for conduction purposes would be obtained by the dissociation of H$^+$ from the OH groups on the melanin indole units into the adsorbed water. Dissociation would be determined by the pKa of the OH moieties and the extent of conductivity determined by the concentration of dissociated H$^+$(6).

With this percolating model in mind Giacomantonio suggested that her results at high water content could be explained by percolation (Figure 1.5).

There are two problems with the theory put forward by Giacomantonio. The first is that upon fitting the percolation plot to her high hydration results, she determined a the critical exponent for Equation 1.5 to be 11±1. This value is much higher than the theoretical results of 1.3 and 2.0 in 2 and 3 dimensions respectively. Giacomantonio tried to explain the result by stating that percolation theory assumes that site elements don’t interact with one another, and since water interacts with itself strongly and with the melanin matrix, one may expect a larger exponent(6).

The second problem with Giacomantonio’s theory a decrease in her data near what appears to be the percolation threshold (Figure 1.5). It was suggested that this was due to a possible interaction between the competing electronic and percolation mechanisms proposed(6).

One further thing Giacomantonio noticed was that, upon performing Thermogravimetric analysis on hydrated melanin, the water content of melanin measured was at odds with the BET isotherm found by Powell et al(24). The reason for this discrepancy was most likely that the BET isotherm obtained by Powell et al. was for powdered melanin and Giacomantonio used pellet and film samples.

1.5 Aims of This Study

In the literature there is a lot of tantalizing evidence to suggest that melanin is a mixed conductor. There is no debate about electrons dominating at low water content, but there is
disagreement between authors on the nature of the charge carrier at high water content.

The first step to understanding melanin’s water dependent electrical behaviour requires knowledge of how much water content is within melanin’s structure. This can be determined with an adsorption isotherm. To date the only isotherm available is the one by Powell et al. (24) where they used powder melanin, which is a useless morphology for investigating the electrical properties of melanin. In this thesis a new adsorption isotherm for melanin as a function of water is shown, but where the melanin sample is a condensed phase pellet. The intricate experimental setup and the results obtained from it are shown in Chapter 3.

The next step in understanding the electrical properties of melanin is to map out the conductivity isotherm of melanin as a function of water content. Researches have now spent several decades trying to determine the charge carrier within melanin but only two groups, Jastrzebska et al. and Giacomantonio have mapped out the overall conductive behaviour. Both groups used salt solutions to control water content in the air. This is cumbersome method which does not eliminate the atmosphere, which includes the reactive chemical entity O2. Oxygen may have a significant impact on the conductivity of melanin due to its ability to produce free radicals (Section 1.1). In Chapter 4, a new and extensive methodology is demonstrated from which several conductivity isotherms were obtained. These results are rigorously analysed in terms of percolation theory, which no one has seriously attempted before.

There are many more experiments that can be conducted to investigate the charge transport properties of melanin (Section 1.2), but due to time constraints only one was attempted, which was determining the proton dynamics of water protons within melanin using NMR. This kind of study on the dynamics of the protons within melanin systems has not been done before. The results is shown in Chapter 5.
CHAPTER 2

Melanin Fabrication

As has been mentioned in Chapter 1, Section 1.1, in this project synthetic analogues to natural melanin were used for investigating the electrical behaviour of melanin. Two synthetic methods were used: the first is through electrochemical polymerization of 3,4-dihydroxyphenylalanine (dopa), which produces free standing films; the second method is through auto-oxidation of dopa.

The samples produced were then confirmed to be melanin using UV-Vis spectrometry and FTIR spectrometry. Melanin is insoluble in organic solvents, and only sparingly soluble in alkaline solutions(7), which makes characterization of the substance through the usual techniques impossible. Fortunately, melanin is known to have a broad band monotonic absorbance within the UV-Vis spectrum, which is certainly unique to an organic chromophore and is therefore a definite marker.

2.1 Methodology of Melanin Fabrication

2.1.1 Melanin Synthesis

Melanin was synthesized through electro-polymerization of dl-dopa according to the methodology as described by Subianto et al.(10). The dl-dopa was obtained from Sigma-Aldrich and used as received. Initially solutions of 50 – 80 mM of dl-dopa in sodium tetraborate buffer (0.1 M, pH 9) were prepared. Current was passed between two electrodes through the solution using a PHY-EWS-89 30V power supply. The cathode was a sheet of aluminium, and the
anode was conductive indium tin oxide (ITO) glass. Melanin formation was accelerated by stirring the solution at current densities of 10 – 15 mA cm$^{-2}$ for 10 – 15 min, after which stirring was stopped and the current density was dropped to 0.5 mA cm$^{-2}$. The suspension was left for a minimum of 7 days to polymerize. After 7 days a black solution as well as a film of black substance on the ITO glass was obtained. The film was scraped off back into the black solution using a scalpel blade. The solution was then brought to pH 2 using concentrated hydrochloric acid to precipitate completely the product. The solution was centrifuged at 3500 r/min for 10 min which produced a black precipitate. This precipitate was then washed twice with distilled water. Samples were dried in the oven at 80 °C, producing a powder that had the appearance of crushed obsidian.

Auto-oxidized melanin was produced according to the method described by Felix et al.(38). Instead of using 10 g of dl-dopa, 5 g of dl-dopa was dissolved in 2 L of deionized water. Ammonia solution was added to bring the solution to pH 8. Air was then bubbled through the dl-dopa solution for 3 days. During the 3 days, ammonia was periodically added to keep the solution at pH 8. The solution was then brought to pH 2 using 1 M hydrochloric acid which produced a black precipitate, which was then filtered and washed with deionized water. The filtrate was dried in an oven at 80 °C and the powder with the appearance of crushed obsidian was obtained.

2.1.2 Analysis - Confirmation of the Production of Melanin

The production of melanin by these standard methods was confirmed by UV-Vis and FTIR spectroscopy, ensuring that the characteristic markers of melanin were present.

UV-Vis spectrometry was done using a Varian built Cary 300 Bio spectrometer version 9 which had Scan Software version 3.00. Baseline corrections were obtained using a silica cell with a pathlength of 1 cm containing an ammonia solution (pH 8) and scanned over a range
of 220 – 800 nm with a scan rate of 600 nm min\(^{-1}\), with data intervals at 1 nm and with a beam source change over at 350 nm. The UV-Vis spectrometer’s SBW was 2 nm. A small amount of dried powder melanin samples were then ground and dissolved in pH 8 ammonium solution. The solution was then filtered to get rid of insoluble fractions. Afterwards, the filtered solution was diluted to a concentration such that the maximum absorbance was not more than 5, and placed within the same type of silica cell used for baseline corrections. The melanin solution was then scanned with the same parameters as for the baseline corrections. ATR-FTIR spectroscopy was conducted with a Perkin-Elmer built FTIR spectrometer, spectrum 2000 using Spectrum software version 2.00. The spectrometer had a DuraSampleIR II sample holder built by Smiths, which is used for ATR-FTIR spectrometry. The sample holder has a plunger to secure sample contact to a diamond window. To obtain a background spectrum, the diamond window was kept clear and the plunger brought down leaving just a small amount of space between it and the window. A range of 4000 to 550 cm\(^{-1}\) was then scanned 8 times at a resolution of 8 and data point intervals of 1 cm\(^{-1}\). Both J and B stops were fixed. To obtain the actual spectrum, melanin powder was ground into a fine powder. Enough powder to cover the diamond window was then placed on top of the window. The plunger then secured the sample to the window with a preset anvil pressure. A range of 4000 to 550 cm\(^{-1}\) was then scanned with same parameters as before.

### 2.1.3 Fabricating Condensed Phase Melanin Pellets

From the synthesised melanin powders, approx. 200 mg of powder was placed within a custom made press head with a diameter 12.7 mm. This was placed within a hand press capable of pressing up to 10 t (approx. 770 MPa). Initially, enough pressure was applied so that the pressure meter just moved off the zero point. Vacuum pumping was then applied to get rid of any residual water and air. After 1 min the pressure was increased in increments of 1 t every
15 s for 1 min. The pressure was then increased to 10 t and left for 3 min. After the 3 min, the pressure was relaxed and the pellet taken out of the press. The condensed phase melanin pellets (CPMP) appeared smooth and black.

2.2 Spectroscopic Analysis Results

2.2.1 UV-Vis Spectra

A characteristic UV-Vis spectrum for the range 220 – 800 nm of the synthesised product (using both methods) is shown in Figure 2.1. The UV-Vis spectrum in Figure 2.1 has a broad-band monotonic absorbance, characteristic of melanin(39; 40). This type of absorbance is highly atypical of organic chromophores, which normally contain peaks corresponding to transitions between electronic states(39). The spectrum is so simple that an exponential fit to this particular figure gave a $\chi^2$ value of 0.9988 using $A = 13.333e^{-0.0051\lambda}$ where $A$ is the absorbance and $\lambda$ is the wavelength in nanometers.

The spectrum in Figure 2.1 is such a unique feature to melanin that it provides good evidence that melanin has been synthesised.
2.2.2 ATR-FTIR Spectra

A characteristic FTIR spectrum of synthesised melanin can be seen in Figure 2.2. The various broad features have been assigned and are in good agreement with various literature results cited in a review by Bilinska(41). The spectrum shows a hygroscopic aromatic substance containing carbonyl units, which is characteristic of melanin.

![Figure 2.2: The FTIR spectrum of melanin powder over a range of 4000 – 500 cm⁻¹. Peak assignment can be seen, but note, other potential OH peaks are washed out by the broad H₂O peak.](image)

2.2.3 Other Observations

According to Giacomantonio(6), the two synthetic methods used produce samples of different morphology. When subjected to high pressures in a press, pressure sintering occurs and the melanin samples start to flow and reform. The morphology one obtains for pellets therefore appear to be the same, whether or not the starting morphology was different(6).

Also, it was noticed using the auto-oxidized method produced far more melanin compared to the electro-polymerized method. Due to time constraints, most samples used in this project were obtained via the auto-oxidation method.

In conclusion, CPMP were fabricated from accepted synthetic techniques and shown to be melanin using spectroscopy.

20
CHAPTER 3

Adsorption Isotherm

The preferred morphology for investigating electrical behaviour of melanin is CPMP samples. As was mentioned in Chapter 1, Section 1.5, the isotherm obtained by Powell et al. is useless for CPMP samples, since it is applicable only to powders. In this chapter, a new adsorption isotherm, with an extensive experimental procedure, is shown for the relevant, pellet morphology. It should be noted that this is the first of its kind to be reported.

3.1 Methodology of Adsorption Experiment

3.1.1 Equipment

The determination of the adsorption behaviour of CPMP centred on the use of a vacuum microbalance. Figure 3.1 shows the entire apparatus that was used in the experiment. It consists of a CI, Electronics Ltd. vacuum microbalance with an MK 2 vacuum head, in which the CPMP were laid. The balance is connected to an external CI STABAL control unit where electronic signals were processed into a value for the mass and sent to a computer where the values are displayed. The microbalance unit is also connected by a vacuum line to a diffusion and rotary vacuum pump setup.

Melanin is extremely hygroscopic, which led to sudden large pressure drops in the system, resulting in pressure instability. In an attempt to keep the pressure relatively constant, the volume of the system was increased by attaching bulbs C and D (Figure 3.1). Water was introduced into the system from a water flask. The water was subjected to three
Figure 3.1: Schematic of the equipment used to determine the adsorption isotherm of melanin pellets. A) The balance arm holding the melanin sample, B) The reference balance arm, C) Empty chamber 1 (Approx. 1L), D) Empty chamber 2 (Approx. 4L), E) Cold trap 1, F) Cold trap 2, G) Baratron Gauge, H) Penning Gauge

freeze-thaw cycles that ensured that no dissolved gases were left in the water.

A MKS Baratron pressure transducer, which has a range of 0 – 1000 mbar, was employed to measure the water vapour pressure in the system. A Penning Gauge was used to monitor the pressure in the system during evacuation. This enabled checking for possible leaks.

Outside temperature was monitored by a thermometer that sat above the microbalance. The temperature was not controlled in the vacuum line, but because the equipment was located in an air-conditioned laboratory the outside temperature remained at 22 ± 2 °C.

Melanin powder was prepared from electro-polymerization of dl-dopa. The powder was then pressed at 770 MPa (Chapter 2) to form a CPMP. The pellet was then loaded into the microbalance.
3.1.2 Adsorption and Desorption Data Acquisition

Desorption data for the pellets were obtained by evacuating the system for at least 10 hours, while the change in mass was monitored.

To obtain the adsorption data, the system was isolated from the vacuum pumps immediately after the desorption procedure. Water vapour was then bled into the system to the desired pressure level. The system was then allowed to equilibrate for half an hour. If after 30 minutes, the pressure did drop in the system, more water vapour was let in up to the desired pressure level. The system was then allowed to equilibrate for the next 3.5 hours, while the change in mass was monitored with the microbalance. Afterwards, the melanin was dried by pumping for 1 hour and then wetted again with the same procedure, but at a different vapour pressure.

The whole procedure above was then repeated several times to obtain enough data to obtain a full adsorption isotherm.

3.2 Results for Adsorption Isotherm

3.2.1 Desorption Data

Some typical desorption data of the mass of melanin is shown in Figure 3.2. As can be seen the desorption data is a continuous curve to which it is possible to fit an equation. The equation is then evaluated in the thermodynamic limit \( (time \to \infty) \) since adsorption isotherms are only valid at thermodynamic equilibrium. This evaluated equation determined the dry mass of CPMP, \( DM \)\textsuperscript{1}.

Multiple desorption experiments were performed on a single pellet and the \( DM \) for each experiment determined. From these results a mean value for \( DM \) was obtained, with an associated error, which was taken as 2 times the standard deviation from the mean.

\textsuperscript{1}All masses were calculated in grams.
**Figure 3.2:** The typical desorption curve observed for CPMP pumped under vacuum. Time between each data point is 10 sec. The generic equation that fitted these graphs is displayed, where $y$ is the mass, $t$ is time and $a, b, c$ are positive constants and $d$ is a negative constant. The constants differed between data sets. The evaluation of the equation in the thermodynamic limit is also shown.

### 3.2.2 Adsorption Data

Typical adsorption data can be seen in Figure 3.3. The data also represent a continuous curve, to which an equation was also fitted. Again the equation was evaluated in the thermodynamic limit, allowing the determination of the equilibrated mass of wet melanin, $M$.

Values for $M$ could not be treated statistically since each new result was taken at a different water vapour pressure. Errors were assigned to the results by taking the difference between the mass at 4 hours and the mass in the thermodynamic limit $M$.

For a detailed analysis of the raw data and error analysis, see Appendix A.1.

### 3.2.3 BET Analysis

Adsorption data for two pellets were obtained, with the final isotherm data shown in Figure 3.4. The data were then analysed using the BET equation (42) to obtain surface area and
Figure 3.3: The typical adsorption behaviour observed for CPMP for any given water vapour pressure. Time between each data point is 10 sec. The equation displayed is the generic equation that fitted these graphs, where \( y \) is the mass, \( t \) is time and \( a, b, c, d \) are positive constants. The evaluation in the thermodynamic limit is also shown.

enthalpy of adsorption. The BET equation in linear form is given by

\[
\frac{P}{n^\sigma(P^0 - P)} = \frac{1}{Zn^\sigma_m} + \frac{Z - 1}{Zn^\sigma_m} \frac{P}{P^0}
\]  

(3.1)

where \( n^\sigma = \frac{M - DM}{M_{H_2O}} \) is the number of moles of adsorbate (i.e. water)\(^2\), \( n^\sigma_m \) is the number of moles of adsorbate required to give monolayer coverage, \( P \) is the vapour pressure of water, \( P^0 \) is the saturation vapour pressure of water and \( Z \approx \exp[(\Delta E)/RT] \). \( \Delta E \) is the enthalpy of adsorption. The BET equation is meant for an ideal system. Therefore, Barnes & Gentle(42) and Adamson(43) recommend that Equation 3.1 be used on data that ranges from 0.05 to 0.3 relative pressure, since data within this range for most systems behave in an ideal way. This recommendation was followed in a plot of \( \frac{P}{n^\sigma(P^0 - P)} \) vs \( \frac{P}{P_m} \) (Figure 3.5) from which the gradient and intercept were determined. These were then used to determine the unknown parameters \( Z \) and \( n^\sigma_m \). From \( Z \) and \( n^\sigma_m \) the values for \( \Delta E \), specific surface area and specific monolayer coverage of CPMP were calculated and can be seen in Table 3.1.

\(^2M_{H_2O} \) is the molar mass of water.
Figure 3.4: The adsorption isotherms for pellet 1 (blue circles) and pellet 2 (red dots), with their theoretical curves. The results for pellet 1 were obtained at 22.8 ± 0.6 °C with a saturation pressure of 26.4 ± 0.9 mbar. The results for pellet 2 were obtained at 22.0 ± 0.6 °C with a saturation pressure of 25.2 ± 0.9 mbar. Results were fitted to equation 3.4. The parameter \( \nu \) is indicated on the graph.

<table>
<thead>
<tr>
<th></th>
<th>Pellet 1</th>
<th>Pellet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( SSA (m^2 g^{-1}) )</td>
<td>540 ± 160</td>
<td>560 ± 200</td>
</tr>
<tr>
<td>( n_g^\sigma (\text{mol} g^{-1}) )</td>
<td>((4.7 \pm 1.4) \times 10^{-3})</td>
<td>((4.9 \pm 1.7) \times 10^{-3})</td>
</tr>
<tr>
<td>( \Delta E (\text{J mol}^{-1}) )</td>
<td>7760 ± 530</td>
<td>8470 ± 640</td>
</tr>
</tbody>
</table>

Table 3.1: The Specific surface area, \( SSA \), specific monolayer coverage, \( n_g^\sigma \), and energy of adsorption, \( \Delta E \), for CPMP. Results were obtained from the gradients and intercepts of the plots in Figure 3.5 using Equation 3.1.

The error in the relative pressure was found using

\[
\frac{\Delta X}{X} = \sqrt{\left(\frac{\Delta P}{P}\right)^2 + \left(\frac{\Delta P^0}{P^0}\right)^2} \tag{3.2}
\]

where \( X = \frac{P}{P^0} \) is the relative pressure. The error in \( \frac{P}{n_g^\sigma(P^0-P)} \) was found using

\[
\Delta \left( \frac{P}{n_g^\sigma(P^0-P)} \right) = \sqrt{\left(\frac{\Delta P}{P}\right)^2 + \left(\frac{\Delta n_g^\sigma}{n_g^\sigma}\right)^2 + \left(\frac{\Delta P^0 + \Delta P}{P^0 - P}\right)^2}. \tag{3.3}
\]

The errors in the gradient and the intercept were found using a multiple linear regression program.
**Figure 3.5:** Plot of the adsorption data using Equation 3.1. Values for the gradient and intercepts are shown. Data points were taken from a range of 0.05 to 0.3 relative pressure as recommended by Barnes & Gentle(42) and Adamson(43).

One of the assumptions in the BET equation is that an infinite number of monolayers is possible for adsorption. This assumption is not necessarily accurate for complex solids. To take into account a finite number of layers, the BET equation can be modified to

$$\frac{n^\sigma}{n^\sigma_{m}} = \frac{ZX}{1 - \frac{1}{X}} 1 + (Z - 1)X - ZX^\nu + \nu X^{\nu+1}$$

(3.4)

where $\nu$ is the number of monolayers possible(42). Equation 3.4 was used as the theoretical fit to the final adsorption data in Figure 3.4. From the fitted curves it was determined that for the data of pellet 1, $\nu = 4$ and for the data of pellet 2, $\nu = 3$. This indicates that there is only limited space for water to adsorb onto CPMP.

Errors for $wt(\%) = \frac{(M - DM) \times 100}{DM}$, for each data point were assigned using

$$\frac{\Delta wt(\%)}{wt(\%)} = \sqrt{\left(\frac{\Delta M + \Delta DM}{M - DM}\right)^2 + \left(\frac{\Delta DM}{DM}\right)^2}.$$  

(3.5)
3.3 Discussion of Adsorption Isotherm

3.3.1 Adsorption and Desorption

It is clear from the adsorption data that after 4 hours a reasonable amount of time has passed in order to analysis the data\(^3\), because at 4 hours the data is already deep into its asymptotic region.

The desorption data was not as clear since it appears that it may still continue to decrease significantly. But by performing multiple experiments, it was noticed that \(DM\) changed very little. This justifies the data analysis and the amount of time allowed to equilibrate.

From the desorption data it can be seen that drying melanin is difficult and also that water has a very high affinity for melanin.

3.3.2 BET Analysis

As can be seen from Figure 3.4 the water initially adsorbs strongly onto melanin to form the first monolayer, indicated by the ‘knee’\(^{42; 43}\) in the graph. Then a flattening of the curve occurs, which can be modeled by Equation 3.4. The experiment was not taken beyond 0.85 relative pressure since at these high pressures the pellets start to deform, and would likely go on to collapse at higher pressures.

It was found that the BET equation did not fit the data in Figure 3.4, therefore necessitating the use of the modified BET equation, Equation 3.4 as the theoretical fit to the data. Furthermore Equation 3.4 is a more realistic picture of what is happening molecularly, since it implies that one can’t adsorb an infinite number of monolayers within a sample.

To get a feel for \(\Delta E\) (Table 3.1), their values are compared to a result obtained for nitrogen adsorption by Crippa et al.\(^{44}\). Their work on synthetic melanin powders, found a value for

\(^3\)The adsorption profile seen Figure 3.3 shows that the major part of the adsorption occurred in the first 1.5 hours followed by a flattening of the curve. This behaviour was seen for all the different water vapour pressures employed, even for the high vapour pressures.
Z around 301. This leads to a $\Delta E$ of approx. 3500 J mol$^{-1}$. Even though powdered melanin is not CPMP, one would expect powders to adsorb more readily. But the very different values for $\Delta E$ indicate that water has a very strong affinity for melanin, even if the melanin is a CPMP. But the calculated values for $\Delta E$ in Table 3.1 are only indicative, as the calculations were based on water being a non-interacting adsorbent, which is inaccurate. Overall one can say that the numbers indicate that water has a high affinity for melanin as compared to nitrogen. This is most likely due to polar interactions between the water molecules and the polar moieties in melanin, and is exactly as one would expect.

The specific surface area values in Table 3.1 were calculated by assuming a surface area of 19 Å$^2$(45). This means a radius of 2.46 Å if one assumes a perfect circle. However, in liquid water, if one assumes the molecules to be perfect spheres, one obtains a radius of 1.93 Å. This indicates that water is capable of very efficient packing, which the literature result seems to downplay. The value of $\approx$ 500 m$^2$ g thus represents an upper bound on the surface area of melanin.

### 3.3.3 Comparison with Literature

Comparing the current isotherms with those of Powell et al. in Figure 3.6, it can be seen that Powell’s data show greater absolute adsorbance. This is easily explained since the surface area of powdered melanin is greater than that for the CPMP. This greater surface area allows more space for water adsorption, resulting in a greater increase in wt(%). The greater surface area also explains the delayed ’knee’(42; 43) in the literature curve. This ’knee’ occurs around 0.3 relative pressure and indicates the formation of the first monolayer4. In contrast the results for the pellet sample suggest a knee occurring around 0.1, indicating a smaller total surface area.

---

4One has to keep in mind that these observations are all qualitative observations and not quantative.
Figure 3.6: Comparing the results obtained from experiment to those obtained by Powell et al. (24). The black line is the literature work and the blue and red lines are the results from pellets 1 and 2. Figure taken from (24) and Figure 3.4 and modified.

Another observation is that Powell et al.’s isotherm is a classic type 2 isotherm, whereas the isotherms for CPMP is not. Again it is morphology that makes the difference. For powdered melanin there is more space for water to form a possible ‘infinite’ number of monolayers. In contrast the condensed phase melanin pellet has far less space and thus only up to 4 monolayers may be possible. However, quantitative comparisons are not possible because Powell et al. did not included their own analysis of the data. There is no fit to any theory, rather it seems that a line of best fit was hand drawn. Moreover, no error analysis is apparent, making it even harder to compare results. At best the data produced by Powell et al. should be used for qualitative comparisons.
3.3.4 Other Observations

It has been demonstrated that there are two types of water in melanin(6; 21; 22), water bound intrinsically with melanin (structural water), and water adsorbed unto the surface through physical interactions (physical water). Removal of the structural water requires the melanin to be heated to 100°C and beyond(6). At these high temperatures melanin starts to degrade, therefore it is impractical to fully dry melanin. From these considerations the experiment described above and results calculated largely concerns physical water.

3.4 Conclusions for Adsorption Isotherm

A first complete and detailed adsorption isotherm of CPMP is presented, which can be fitted to a modified BET equation. Quantities calculated from the BET analysis are only indicators since they neglect the interactive nature of water, but they do indicate that water has a strong affinity for melanin. Also it was found that the isotherm was different to the published powder isotherm because of different morphologies.

This adsorption isotherm is critical for full analysis of the conductivity data presented in the next chapter.
CHAPTER 4

Conductivity Measurements

As stated in Chapter 1, Section 1.5, there are no reports of the full mapping of the water dependent conductive behaviour of melanin, from which the surrounding atmosphere has been eliminated. Therefore, in this chapter a new experimental setup is introduced to fully map out the conductivity isotherm for CPMP as a function of water adsorbed.

4.1 Experimental for Conductivity Isotherm

4.1.1 Pellet Sample Preparation for Conductivity

CPMP samples were prepared from auto-oxidised dl-dopa (Chapter 2) and pressed at 770 MPa and 400 MPa. Pellets were contacted by evaporating gold, using a thermal deposition unit (Dynavac, High vacuum system), through a custom made shadow mask onto the pellets. The evaporative conditions were: 20 V, 18 A current through a tungsten basket containing the gold. Evaporations from the basket to the samples were done at a distance of approx. 10 cm for 3 min. The contact pattern geometry was selected according to a van der Pauw\(^1\)(vdP) configuration (Figure 4.1), leaving 2 mm of bare melanin between two adjacent gold contacts as the ‘active’ area.

Copper wires were then glued on top of the gold contacts with the use of conductive epoxy glue. Care was taken to ensure that the entire area covered by the gold was covered by conductive

\(^1\)This configuration allows one to expose a larger surface area for water adsorption as compared to the sandwich setup employed by Jastrzebska et al.
epoxy since the gold contacts by themselves did not yield discernible conductivities. This is most probably due to the gold film being too thin to be ‘percolating’ in the traditional sense since it was deposited on a rough surface.

The contacted pellets were then glued down using Arildite epoxy on top of a square plate of PVC, which had a hole in the center of it. Furthermore, the copper wires were also glued down unto the PVC plate. The Araldite was used to ensure that the contacts on the pellets didn’t come loose during the experiment. PVC was used to ensure a minimum of degassing would occur under the applied vacuum. A schematic of the final prepared pellet can be seen in Figure 4.1. It should be noted that one of the advantages of using the vdP configuration is

![Diagram](image)

**Figure 4.1:** Schematic of melanin pellets prepared for conductivity experiments. Melanin is contacted according to the vdP configuration and then mounted for stability. The hole in the PVC increases the surface area of melanin for water adsorption.

that it eliminates contact resistance from IV data one collects. Still, the individual contacts had a resistance of \( \approx 0.03 \, \Omega \), which is negligible compared to melanin’s resistance of approx. Mega Ohms. This combined with the vdP setup allows us to ignore any effects on the results due to contact resistance.
4.1.2 Equipment

The equipment used for controlling water content in the environment was the same as used for the adsorption isotherm (Figure 3.1), except the microbalance section was adapted for IV experiments. One of the microbalance glass bulbs had tungsten rods inserted in order to create a conducting pathway from the inside of the vacuum line to the outside. On the inside of the adapted bulb the prepared pellet was connected to the tungsten rods\(^2\), and on the outside a source meter unit (SMU) was connected to the tungsten\(^3\).

4.1.3 Experimental Procedure for Conductivity Isotherm Data

After a pellet was connected, the vacuum line was evacuated for 1 hour using a rotary pump. The line was then isolated from the pump and water vapour bled in up to a pressure of 7.5 mbar \(^4\). The pellet was then left for 4 hours to equilibrate, at the end of which IV measurements\(^5\) were taken using the SMU (Section 4.1.4).

Following the IV measurements the water pressure in the line was increased by 1.5 mbar. Another set of IV measurements were taken once the sample had been allowed to equilibrate for 2 hours. The procedure was then repeated until the measurement for 22.5 mbar was completed.

4.1.4 Conductivity Measurements

The vdP technique requires that 8 different configurations (Figure 4.2) be used for obtaining IV data. The IV data are obtained using a Keithly 2400 SMU which drove the voltage from 0 to 5 V in a single sweep, taking 51 data points. The vdP procedure, as laid out by NIST, requires the current to be driven and the voltage to be measured. However, driving the voltage

\(^2\)Tungsten rods expand at the same rate as glass, this ensures a vacuum tight seal.

\(^3\)The SMU was used to drive voltage through the melanin pellet.

\(^4\)Pressures below 7.5 mbar were found to give no discernable conductivity.

\(^5\)The bulb holding the melanin samples was wrapped in a thick black cloth to minimize photoconductivity.
does not effect the results and since the SMU made it easier, the voltage was driven instead.

4.2 Results for Conductivity Measurements

4.2.1 Determining Conductivity

The vdP technique was designed to be employed on any sample with an arbitrary configuration\cite{46}, but if one has a highly symmetrical sample, like the circular pellets used in the above experiment then one obtains

\[
\exp(-\pi R_H d\sigma) + \exp(-\pi R_V d\sigma) = 1
\]  

(4.1)

where \( R_H \) is the average resistance for all horizontal configurations, \( R_V \) is the average resistance for all vertical configurations (Figure 4.2), \( d \) is the thickness of the pellet and \( \sigma \) is the conductivity. The values for \( R_H \) and \( R_V \) were found by obtaining three IV curves for each vdP configuration. These curves were averaged and a graph of voltage against current was then plotted, from which a resistance could be found from the gradient. A straight line was fitted to the initial linear region of the data, an example of which can be seen in Figure 4.3. This was done since any blocking behaviour (Child’s Law behaviour) is expected for high voltages. Using the above procedure a resistance of each of the 8 configurations was obtained.
Figure 4.3: Representative voltage against current data. The straight line is fitted to the first available straight part of the curve, and the gradient determined to yield a value for the resistance. Note also the behaviour of the data: the straight line clearly shows the non-linear, Child’s law dependence at higher voltages.

For each of the curves, a $\chi^2$ value was obtained and used to determine a weighting, $w$, for each curve through

$$w = 1/ \left( \left( 1 - \chi^2 \right) R \right)^2$$  \hspace{1cm} (4.2)

where $R$ is the resistance obtained for a specific configuration. To obtain the average resistance for all the horizontal configurations, $R_H$, a weighted average was taken of all the resistances of all the horizontal configurations

$$R_H = \frac{\sum_i w_i R_i}{\sum_i w_i}$$  \hspace{1cm} (4.3)

where $i$ specifies a horizontal configuration. The same method was used to determine $R_V$.

The determined values for $R_H$ and $R_V$ were then used in Equation 4.1. It should be noted that Equation 4.1 is impossible to solve analytically. Therefore, the conductivity was found numerically by plotting values for Equation 4.1 against a range of values for the conductivity, from which the corresponding value for the conductivity was obtained for where Equation 4.1 has a value of 1.
The relative error associated with the vdp setup is
\[
\frac{\Delta \sigma}{\sigma} = \sqrt{4 \times \left( \frac{l^2}{16D^2 \ln 2} \right)^2 + \left( \frac{m^2}{4D^2 \ln 2} \right)^2}
\]  
(4.4)

where \( l \) is the distance the contacts span the circumference of the pellet, \( m \) is the distance the contact intrudes toward the centre of the sample and \( D \) is the diameter of the pellet. The error was found to be 15 \%\(^6\).

The final conductivity isotherms as a function of relative pressure, \( X \), can be seen in Figure 4.4a. The errors in the relative pressure, were calculated the same way as for the adsorption isotherm results (Chapter 3, Section 3.2.3).

![Figure 4.4: Conductivity Isotherms of CPMP. Figure a) are the isotherms as a function of relative pressure of water. Figure b) are the isotherms as a function of wt(\%). The blue data was obtained at a temperature of 23.5 ± 0.1 °C, black data at a temp. of 22.9 ± 0.1 °C and red data at a temp. of 22.5 ± 0.1 °C.](image)

The conductivity isotherm as a function of water adsorbed by weight percent (wt(%)) can be seen in Figure 4.4b. The values of wt(\%) were determined using the modified BET equation (Equation 3.4, Section 3.2.3), where it was assumed that the number of layers \( \nu \) is 3. The error in wt(\%) was determined by
\[
\Delta \text{wt}\% = \sqrt{\left( \frac{\partial \text{wt}\%}{\partial Z} \Delta Z \right)^2 + \left( \frac{\partial \text{wt}\%}{\partial X} \Delta X \right)^2 + \left( \frac{\partial \text{wt}\%}{\partial n_\gamma} \Delta n_\gamma \right)^2}.
\]  
(4.5)

\(^6\)For the complete error analysis and the derivation of Equation 4.4, see Appendix A.2.1
4.2.2 Child’s Law Dependence

During the experiment a great deal of IV data was obtained, consequently allowing a detailed search for Child’s law dependence, \( I \propto V^n \), on the voltage (Section 1.2).

Approximately half of all the IV curves obtained were linear, indicating Ohm’s law dependence, but the other half had non-linear dependence. The curve in Figure 4.3 is a representative figure of Child’s law dependence in the data acquired. The most probable reason for only half of the curves being non-linear is the order in which the IV data for each vdP configuration was obtained. When one applies a potential field, charges aggregate around the electrodes and thus it exhibits non-linear behaviour. This aggregation can be effectively eliminated by applying the potential field in the opposite direction. However, aggregation would not occur for the second run since not enough time is allowed for the charges to move to the other electrode, thus one will only see ohmic behaviour.

4.3 Discussion of Conductivity Isotherms

4.3.1 Conductivity Isotherm, General Form & Percolation

Looking at the conductivity isotherms in Figures 4.4a and b it can be seen that initially the conductivity doesn’t change, but then a critical amount of water is adsorbed and the conductivity increases suddenly. The shape of the curves is highly reminiscent of percolated conductivity (Figure 1.7). Furthermore the behaviour around the critical area for the different sets of data is very similar. This suggests that the behaviour of the conductivity may be a continuous phase transition\(^7\), with a critical point at 0.41 relative pressure or 12.5 % weight percent gained(Figure 4.5). These observations suggest a percolating conductivity, but to determine whether the data is consistent with percolation theory an approximate critical

\(^7\)The discussion on phase transitions and critical exponents relies heavily on knowledge taken from the text Chaiken & Lubesky(47)
Figure 4.5: Determining the critical relative pressure in the conductivity. A straight line is drawn through the first few data points using the blue data from Figure 4.4a. Another curve is then fitted to the rest of the data. Where the two curves diverge is the critical relative pressure, $X_C$, and is $0.41 \pm 0.01$.

exponent was determined. According to percolation theory the conductivity should be dependent on water in melanin, i.e. $\sigma \propto (wt(\%) - wt(\%)_C)^\mu$ where $wt(\%)_C$ is the critical water adsorbed by weight percent. But, it can be seen the errors in $wt(\%)$ in Figure 4.4b are large. Instead, the data from Figure 4.4a was used. This is justified since the values for the relative pressures around 0.41 is essentially linear in the adsorption isotherm (Figure 3.4). Thus $X \propto wt\%$, and therefore $\sigma \propto (X - X_C)^\mu$ where $X_C$ is the critical relative pressure. Taking the log of both sides we obtain

$$\ln \sigma \propto \mu \ln(X - X_C).$$

(4.6)

Plotting the values for Equation 4.6 near the critical relative pressure (Figure 4.6) gives a critical exponent $\mu$ of $2.3 \pm 0.2$. The data is consistent with 3-dimensional percolation for which we expect $\mu = 2.0$ but to be sure one would require more data points which are much closer to $X_C$. The points chosen in Figure 4.6 ranges from 0.4 to 0.5 relative pressure, which is equivalent to a range of reduced relative pressure $\frac{X - X_C}{X_C}$ of 0.22. For comparison, a typical experiment for determining critical exponents have reduced value ranges of $10^{-2}(48)$. It
Figure 4.6: Determining critical exponent of the conductivity for hydrated melanin. The red line is the line of best fit, from which the gradient has the value of the exponent. The gradient has a value of $2.3 \pm 0.2$.

is clear from this comparison that the results presented in this thesis are not close enough to the critical point to get an accurate value for the critical exponent. However, a model experiment(49) that was done on percolated conductivity used a reduced range of 0.22 as well, and it was found that the samples used had a critical exponent of 3, consistent with mean field theory (MFT). Our result, which has a value of $2.3 \pm 0.2$, indicates that we may have passed beyond the MFT region and may actually be within the relevant region for obtaining critical exponents for 3D percolation.

Another consideration for the critical exponent obtained above is that the critical exponent for 3-dimensional percolation assumes that the conductive elements, i.e. water, are non-interacting. This is an inaccurate assumption since water forms strong hydrogen bonding networks, therefore this interaction may give rise to a higher critical exponent than predicted by percolation theory(6).

Comparing the result for the critical exponent to $11 \pm 1$ obtained by Giacomantonio, there is certainly a large difference. This difference is most probably due to the fact that Giacomantonio calculated the exponent using a reduced relative pressure range of 0.95, and as been mentioned above, one needs to be very close to the critical point to estimate a valid exponent.
This shows that the results obtained in our experiment probes the critical region with more accuracy.

In conclusion, the above observations strongly indicate that a percolated system is present. This in turn would be consistent with proton conduction which employs the Grotthuss mechanism (Section 1.4.2, Chapter 1).

### 4.3.2 Conductivity Isotherms, Other Consideration

The overall change in the conductivity is approximately one order of magnitude whereas the results obtained by Giacomantonio (6) and Jastrzebska et al. (5) changed several orders of magnitude. Upon consideration, our results may be different to Giacomantonio and Jastrzebska et al. because of significant differences in experimental setup in the three cases. These differences are:

1. Sample geometry. The thinner samples in our experiment had the highest conductivity. Considering that the pellets by Giacomantonio and Jastrzebska et al. were even thinner still, suggests that future samples should be made thinner.

2. Sample difference. The samples used by Giacomantonio were from electro-polymerization which is suspected to have a higher ratio of DHICA to DHI. This would allow more protons for solvation and hence there would be more charge carriers for conduction.

3. Sample preparation. Another potential reason may be the physical preparation of the pellet samples. Samples pressed at higher pressures may create smaller pores, thus water entering the melanin matrix may be hindered and therefore lower the conductivity.

4. Experimental setup. The experimental setup used in Section 4.1 is different to Giacomantonio and Jastrzebska et al.. Their results were obtained using salt solutions to control the water content at atmospheric conditions, whereas our experiment subjected...
melanin to vacuum, and kept the samples at essentially vacuous conditions throughout the experiment. It may be that the vacuum changes the morphology of melanin to the extend that the positive charge carrier is retarded.

5. Presence of O$_2$ in experimental setup. As discussed in the introduction, Chapter 1 Section 1.1, O$_2$ is most important in the chemistry of melanin. Oxygen helps with the formation of free radicals, which may be used in conduction. Considering that Giacomantonio and Jastrzebska et al. performed their experiments in atmosphere in the presence O$_2$, as opposed to our experiment which had no O$_2$ present, it is proposed that O$_2$ may also be crucial in producing additional charge carriers for conduction. By isolating samples from the atmosphere, our samples could not be oxidized by O$_2$ and thus the observed conductivity was lower in magnitude. It is suggested that O$_2$ may oxidise melanin and thus shift the comproportionation equilibrium (Chapter 1, Equation 1.2) towards more radicals, and as it turns out, may also increase the number of available protons for conduction. Unfortunately, no work has been done on the possible link between O$_2$ and conductivity in melanin within the literature. This link should certainly be investigated.

This current work has established that synthesis, morphology, experimental conditions and possible reactions play important roles in determining the conductivity of melanin. Further, detailed controlled studies will be required to understand the full effects of these different factors. However, it is important to note that the form of the conductivity isotherm is very similar to Giacomantonio, but it is significantly more accurate and has enabled the calculation of a more realistic critical exponent.
4.3.3 Child’s Law Dependence

In Figure 4.3 there is clear evidence for Child’s law dependence in the IV data. Usually this dependence is due to ions in a sample, which are unable to transfer successfully into the oppositely charged electrode(19). These results confirm data obtained by Giacomantonio and Osak et al.(25). Since there are no other ions in melanin able to conduct except H⁺, the data is highly suggestive of proton conduction within melanin.

4.4 Conclusions for Conductivity Data

Using a new experimental setup, conductivity isotherms for melanin pellets as a function of water were obtained and compared to the literature. The data indicates that a percolated system may be present and alongside Child’s law dependence suggests that protons is the dominant charge carrier at high hydration levels. We have also established that synthesis, morphology and experimental conditions have significant effects on melanin conductivity. This is the most complete mapping of the DC conductivity of CPMP ever obtained, and furthermore we have normalized the data to the appropriate adsorption isotherm. It must be stressed that, although the absolute conductivities we measured are lower than previously reported, the form of the dependence upon water content is very similar to that reported by Giacomantonio.
CHAPTER 5
Proton Dynamics

For proton conductivity to be a reality, protons must be free to move within a material. One of the best, non-intrusive ways to study proton dynamics is through NMR. The study of relaxation times of proton spin can be used to understand proton motion and behaviour. No previous study on proton dynamics within melanin has been done before, therefore, this chapter describes the first experimental forays using solid state $^1$H NMR experiments to understand proton behaviour within melanin.

### 5.1 Methodology for NMR Spectroscopy

CPMP were fabricated from auto-oxidized melanin as described earlier. These were prepared by applying a pressure of 400 MPa. The samples were then dried in an oven at 80°C for 3 hours. Immediately after they were dried, they were placed in an adapted petri dish setup, in which the humidity was controlled with salt solutions (Figure 5.1). Different salts were used to obtain different humidities. The salts used and their corresponding humidities can be seen in Table 5.1.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Desiccator Pellets</th>
<th>MgCl$_2$</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>33%</td>
<td>75%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.1:** The RH each salt produces in surrounding atmosphere. The values for the chloride salts were taken from ASTM E 104-02(50).

The petri dishes were wrapped in parafilm to ensure minimum of exposure of the inside environment to the outside atmosphere. The samples were kept in the dishes for 3 days
**Figure 5.1:** Salt solution setup for hydrating melanin. The melanin sample is placed within a cap. This cap is then floated on top of a saturated salt solution, which controls the water content in the surrounding atmosphere.

To ensure that the samples did not gain or lose significant amounts of water while NMR data was being collected, the melanin samples were taken out of their petri dishes and wrapped in Teflon tape. Immediately afterwards the samples were placed within the NMR spectrometer and data collected. Wide-line $^1$H NMR experiments were conducted on a Bruker DRX-300 spectrometer operating at a proton frequency of 300.13 MHz. A simple pulse and collect pulse sequence was used to acquire the data. The 90° pulse time was 5 s, and pulse repetition time was 3 s. The spectrum width was 100 kHz. A receiver dead time of 5 s was used. Each free induction decay (FID) was accumulated for 64 scans and consisted of 1k complex data points. The FID was then zero filled to a total of 4k points to prior to Fourier transformation. No Spin-Echo NMR results were obtained since initial experiments could not find signals from which values for $T_2$, the transverse spin relaxation time could be determined.

### 5.2 Results of NMR Studies

The NMR spectra obtained can be seen in Figure 5.2. As water content in melanin increases,
the intensity of the peaks increase indicating that more water is present. Also, the width of the peaks decrease as water increases. Finally, it can be observed that a change in the chemical shift occurs as water content increases.

The widths of the peaks at half height were found, since this characterises the effective transverse relaxation of protons within the system according to $\Delta = \frac{1}{\pi T_2^*}$ where $T_2^*$ is the effective transverse relaxation and $\Delta$ is the width of the peaks at half height(51). The values for $T_2^*$ obtained are plotted in Figure 5.3a against water content within the melanin. Also, in Figure 5.3b the chemical shift is plotted against water content.

5.3 Discussion of NMR Results

As can be seen in Figure 5.3a the $T_2^*$ values are of order $\approx 10^{-4}$ s. Also, the values of $T_2^*$ increase as water content in melanin increases. This clearly indicates that the protons are moving faster as more water is added since an increase in the transverse relaxation time shows increase molecular movements(51). However, these results are most likely indicating that water molecules are moving faster via rotational and translational movements, and not
protons moving via a Grotthuss like mechanism. If we assume that the values for $T^*_2$ are not much smaller than for $T^*_2$, then the results are similar to water diffusion results in hydrated polymers (See review by McBrierty(52)). Here we assume that hydrated polymers are a reasonable approximation to hydrated melanin macromolecules.

Upon further literature investigation, it was found that the timescales involved for proton movement in liquid water due to the Grotthuss mechanism are of order $\approx 100$ ps(53; 54), which is much faster than the time involved in diffusion of water molecules. This would imply a much larger value for $T^*_2$ than is observed. Furthermore, this pico second timescale was observed by determining coupling between $^{17}$O and $^1$H, and not proton-proton coupling. This method would indicate whether protons have moved away from the oxygen centre as is assumed by the Grotthuss mechanism. In contrast proton-proton coupling as observed in our experiments would include this Grotthuss mechanism as well as the more dominant rotational and translational diffusion of water. Thus proton-proton coupling is an insensitive tool for

---

1. This assumption is correct in as far as that $T^*_2 > T^*_2$. The more fundamental assumption that is made is that the magnetic field inhomogeneity is small, since $\frac{1}{T_2} = \frac{1}{T_2} + \frac{1}{T_{inhom}}$, where $T_{inhom}$ is the value added to the transverse relaxation due to field inhomogeneity.
determining Grotthuss like movements since any such movement would be washed out by more dominant effects such as diffusion and rotational motions.

The change in the chemical shift with water content in Figure 5.3b was unexpected. However, upon consideration it can be explained: as water is added to melanin, the effective dielectric constant of melanin changes, which would mean that the magnetic susceptibility of the substance is changing(55). Therefore, the effective magnetic field within the substance will also change, giving rise to the change in chemical shift. It should be noted that it looks like the chemical shift is flattening out as more water is added, which implies that the dielectric value of the system is reaching a maximum limit. This is expected since it supports Rosenberg’s theory of electron conductivity within melanin (Chapter 1, Section 1.4.1). However, three data point are not enough to determine a trend, and thus more data needs to be obtained.

5.4 Conclusion of NMR Results
The very first study of proton dynamics within hydrated melanin using NMR was performed. It showed that as water is added to melanin the motion of the protons increase. Furthermore, it was found that proton coupling is not the way to study Grotthuss like movement of protons since such effects would be washed out by more dominant effects such as rotational and translational movements of water. A better experiment to do would be to obtain H₂O enriched with ¹⁷O, hydrate melanin with it and search for coupling between protons and ¹⁷O.

Also, it was found that the dielectric constant of melanin changes due to water in the environment, thus causing a chemical shift within the spectra. This supports the theory proposed by Rosenberg.

However, it is also clear that more data will have to be obtained since three data points are not enough to make reasonable quantitative assessments.
CHAPTER 6

Conclusion

In this thesis a detailed study of melanin’s electrical properties as a function of water content is presented. This includes what we believe to be the first, comprehensive water adsorption isotherm for CPMP which can be modeled according to a modified BET model, which suggests that melanin has a high affinity for water and that there is only limited space for water adsorption.

Furthermore, a full and extensive conductivity isotherm has been presented which is qualitatively consistent with those reported by Giacomantonio and Jastrzebska et al. However, the data obtained in our study was obtained under far more controlled conditions than either Giacomantonio and Jastrzebska et al. achieved in their experiments. We believe, the conductivity isotherm data in Chapter 4 maps out more accurately the water dependent electrical behavior of melanin than anything previously presented.

This work was motivated by observations in the literature of a positive charge carrier, which is thought to be protonic. To this end, several experiments were performed to probe for protons, such as an in depth IV mapping which has shown blocking phenomena leading to Child’s law behaviour, which is consistent with protonic charge carriers.

Also, preliminary NMR studies are strongly indicative that we have mobile protons within hydrated melanin, with mobility increasing with increased water content.

Furthermore, the conductivity isotherms were analysed in terms of percolation theory, which
is postulated to be consistent with protonic conductivity. The data is complete enough to extract a critical exponent which has moved beyond the MFT region to where it is highly suggestive of 3D percolation.

The work presented may be comprehensive, but it has raised many more questions concerning the electrical behaviour of melanin. These questions need to be answered if we wish to fully understand the structure-property relationship within these systems. Therefore the following experiments are suggested to further our knowledge of melanin:

1. Obtaining a desorption isotherm for CPMP may give an indication of the nature of the pore structure within CPMP(42). To accomplish this an adsorption isotherm up to 0.8 relative pressure should be obtained, but without any drying in-between, and then a desorption procedure should follow which mirrors the adsorption procedure.

2. SAXS can also help determine the nature of the pore structure within melanin(56).

3. Difference in pellets geometry and preparation should be investigated. It is proposed that conductivity data for different thickness samples, differently pressed samples and differently synthesised samples should be investigated since the evidence in this thesis suggest that all these factors are important.

4. The NMR data suggested moving protons. However, the study of $^{17}$O - $^1$H coupling in NMR should be investigated. This may help determine whether anything like the Grotthuss mechanism within hydrated melanin is occurring.

5. Finally, but probably the most important consideration for conductivity of melanin, the link between O$_2$ in the atmosphere and increased conductivity. Conductivity isotherms should be performed within the presence of O$_2$ in the environment.
A.1 Adsorption Data

A.1.1 Experimental Errors in Adsorption Isotherm

Temperature was monitored during the experiment. The final value for temperature was taken at 4 hours. The error in the temperature was taken as two times the standard deviation (2std) of all the data taken during the 4 hours.

Pressure was monitored continuously during the experiment. The final value for the pressure was taken at 4 hours and the error in the pressure was taken as 2std.

The saturation pressure of water was determined by taking the final temperature, and reading the appropriate value from a saturation pressure curve found in (57). The error in the saturation pressure was determined from the error in the temperature. The upper and lower bounds for the temperature error was taken and the corresponding saturation pressures determined. The differences between these pressures and the actual saturation pressure was determined and the result that gave the largest difference was taken as the error.

A.1.2 Determining Weight Percent Gained in Adsorption Isotherm

The adsorption of water into melanin was expressed in weight percent gained. This was calculated using \( wt(\%) = \frac{(M - DM)}{DM} \times 100 \), where \( wt(\%) \) is the weight percent gained, \( M \) is the mass of hydrated melanin and \( DM \) is the dry mass of melanin.
The error in the weight percent gained is given by 
\[
\frac{\Delta\text{wt}(\%)}{\text{wt}(\%)} = \sqrt{\left(\frac{\Delta M + \Delta DM}{M - DM}\right)^2 + \left(\frac{\Delta DM}{DM}\right)^2},
\]
where \(\Delta\) denotes the error in the given quantity. This quadrature form was used since the results were obtained independently of one another and are therefore uncorrelated.

The number of moles of water, \(n^w\), can be related to \(\text{wt}(\%)\) by 
\[
n^w = \frac{DM \times \text{wt}(\%)}{M_{\text{H}_2\text{O}} \times 100}
\]
where \(M_{\text{H}_2\text{O}}\) is the molecular weight of water. The associated error is 
\[
\frac{\Delta n^w}{n^w} = \frac{1}{100} \sqrt{\left(\frac{\Delta DM}{DM}\right)^2 + \left(\frac{\Delta \text{wt}(\%)}{\text{wt}(\%)}\right)^2}.
\]

### A.1.3 BET Analysis

The BET equation is an equation based on an ideal system. Therefore, this equation is only valid for certain areas of an adsorption isotherm, usually for relative pressures ranging from 0.05 to 0.3 (42; 43). Taking into account this limitation, equation 3.1 was applied using a weighted linear regression program in Microsoft Excel to determine the values and errors for the intercept \(c\), and gradient \(m\). Errors in the parameters \(Z\) and \(n^w_m\) were found using
\[
\frac{\Delta Z}{Z} = \sqrt{\left(\frac{\Delta m}{m}\right)^2 + \left(\frac{\Delta c}{c}\right)^2} \quad \text{and} \quad \frac{\Delta n^w_m}{n^w_m} = \sqrt{\left(\frac{\Delta c}{c}\right)^2 + \left(\frac{\Delta Z}{Z}\right)^2}
\]
respectively.

The specific surface area for each sample was then calculated by
\[
SSA = \frac{n^w_m N_A A_{\text{H}_2\text{O}}}{DM},
\]
where \(SSA\) is the specific surface area, \(N_A\) is Avogadro’s number, \(A_{\text{H}_2\text{O}}\) is the area one water molecule occupies and \(DM\) is the dry mass of the sample in grams. \(A_{\text{H}_2\text{O}}\) was taken as \(19\AA^2\) (45). The error in the specific surface area was calculated using 
\[
\frac{\Delta SSA}{SSA} = \sqrt{\left(\frac{\Delta n^w_m}{n^w_m}\right)^2 + \left(\frac{\Delta DM}{DM}\right)^2},
\]
where the error in \(N_A\) is assumed to be negligible.

The error was calculated by 
\[
\frac{\Delta(\Delta E)}{(\Delta E)} = \sqrt{\left(\frac{\Delta Z}{Z}\right)^2 + \left(\frac{\Delta T}{T}\right)^2}.
\]

### A.2 Conductivity Data

#### A.2.1 Error Analysis for Conductivity

The final relative error for the conductivity from Equation 4.4 was obtained by considering the individual errors for the sizes of the contacts on the sample. According to van der Pauw (46),
the errors are due to contacts being non-ideal. The first error is

\[
\frac{\Delta \sigma}{\sigma} = \frac{l^2}{16D^2 \ln 2}
\]  

(A.1)

where \( l \) is the distance the contact spans around the circumference of the sample and \( D \) is the diameter of the sample. Keeping in mind that the thickness between two contacts is 2 mm, and the diameter of the sample is 12.7 mm, the value for the error for each contact from equation A.1 is 4%.

The second error is due to the size of the contact on top of the sample and is given by

\[
\frac{\Delta \sigma}{\sigma} = \frac{m^2}{4D^2 \ln 2}
\]  

(A.2)

where \( m \) is the distance the contact intrude from the edge of the sample to the centre. For each contact this error turns out to be 7%.

Assuming these two errors are introduced independently the error per contact becomes

\[
\frac{\Delta \sigma}{\sigma} = \sqrt{\left(\frac{l^2}{16D^2 \ln 2}\right)^2 + \left(\frac{m^2}{4D^2 \ln 2}\right)^2}
\]  

(A.3)

by again employing quadrature. The total error is the quadrature sum of all four contacts, and since these contacts are all the same, the final equation for the error is

\[
\frac{\Delta \sigma}{\sigma} = \sqrt{4 \times \left(\frac{l^2}{16D^2 \ln 2}\right)^2 + \left(\frac{m^2}{4D^2 \ln 2}\right)^2}
\]  

(A.4)

which is equation 4.4 and has the value of 15%.

**A.2.2 Error Analysis for Water Adsorbed by Weight Percent**

To calculate the error in the water adsorbed by weight percent, \( wt(\%) \), the following relation, the chain rule, for the uncertainty in an arbitrary function was used(58)

\[
\Delta P = \left[ \left( \frac{\partial P}{\partial x_1} \Delta x_1 \right)^2 + \ldots + \left( \frac{\partial P}{\partial x_n} \Delta x_n \right)^2 \right]^{1/2}
\]  

(A.5)
where \( p \) is your arbitrary function, the \( x \)'s are the variables of \( p \) and \( n \) is the number of variables. Equation A.5 led to equation 4.5, and the partial derivatives in equation 4.5 equate to

\[
\frac{\partial \text{wt}\%}{\partial Z} = - \frac{100 \times n_\sigma^g \times X (X - X^4) (1 - 4X^3 + 3X^4) Z}{(1 - X) (1 + X (Z - 1) - ZX^4)^2} \\
+ \frac{100 \times n_\sigma^g \times X (1 - 4X^3 + 3X^4)}{(1 - X) (1 + X (Z - 1) - ZX^4)}
\]

\[
\frac{\partial \text{wt}\%}{\partial X} = - \frac{100 \times n_\sigma^g \times ZX (1 - 4X^3 + 3X^4) (Z - 1 - 4ZX^3)}{(1 - X) (1 + X (Z - 1) - ZX^4)^2} \\
+ \frac{100 \times n_\sigma^g \times ZX (-12X^2 + 12X^3)}{(1 - X) (1 + X (Z - 1) - ZX^4)} \\
+ \frac{100 \times n_\sigma^g \times Z (1 - 4X^3 + 3X^4)}{(1 - X) (1 + X (Z - 1) - ZX^4)} \\
+ \frac{100 \times n_\sigma^g \times ZX (1 - 4X^3 + 3X^4)}{(1 - X)^2 (1 + X (Z - 1) - ZX^4)} \\
(\text{A.6})
\]

\[
\frac{\partial \text{wt}\%}{\partial n_\sigma^g} = \frac{ZX (1 - 4X^3 + 3X^4)}{1 - X (1 + X (Z - 1) - ZX^4)} \times 100.
\]

\[
\text{(A.7)}
\]

It should be noted that the variable \( \nu \) in the modified BET equation (Chapter 3, Equation 3.4) was assumed to be perfectly known and was taken as equal to a value of 3.


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