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JOÃO VITOR PAULIN

**Structure-property correlations of synthetic eumelanin towards
bioelectronic applications**

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bioelectronic applications**

Tese apresentada como requisito à obtenção do título de Doutor à Universidade Estadual Paulista “Júlio de Mesquita Filho” – Programa de Pós-graduação em Ciência e Tecnologia de Materiais, área de concentração Ciência dos Materiais, sob a orientação do Prof. Dr. Carlos Frederico de Oliveira Graeff.

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE JOÃO VITOR PAULIN, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE MATERIAIS, DA FACULDADE DE CIÊNCIAS - CÂMPUS DE BAURU.

Aos 26 dias do mês de fevereiro do ano de 2021, às 14:30 horas, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de JOÃO VITOR PAULIN, intitulada **Structure-property correlations of synthetic eumelanin towards bioelectronic applications**. A Comissão Examinadora foi constituída pelos seguintes membros: Prof. Dr. CARLOS FREDERICO DE OLIVEIRA GRAEFF (Orientador(a) - Participação Virtual) do(a) Departamento de Física / Faculdade de Ciências - UNESP - Bauru, Profa. Dra. CLARA SANTATO (Participação Virtual) do(a) Génie physique / École Polytechnique de Montréal, Prof. Dr. CLAUDIO JOSÉ MAGON (Participação Virtual) do(a) Departamento de Física e Ciência Interdisciplinar / Instituto de Física de São Carlos / USP, Prof. Dr. NERI ALVES (Participação Virtual) do(a) Departamento de Física, Química e Biologia / UNESP/ Câmpus de Presidente Prudente, Prof. Dr. JOSE HUMBERTO DIAS DA SILVA (Participação Virtual) do(a) Departamento de Física / Faculdade de Ciências - UNESP/ Bauru. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: A PROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.



Prof. Dr. CARLOS FREDERICO DE OLIVEIRA GRAEFF

To my mom and grandparents, in particular,
Ana and Marcílio (*in memoriam*); they
are/he was probably more excited about this
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“It is not our part to master all the tides of the world, but to do what is in us for the succour of those years wherein we are set, uprooting the evil in the fields that we know, so that those who live after may have clean earth to till.”

— J.R.R. Tolkien; Lord of the Rings:
The Return of the King.

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ABSTRACT

The natural pigment known as melanin has become a very promising material for many technological applications, but especially in the emerging area of organic bioelectronics due to its charge transport properties and biocompatibility. Thus, this thesis aimed to shed light on the optoelectronic properties of a series of melanin derivatives and apply such materials in organic devices. With this in mind, synthetic melanin derivatives were investigated using structural, optical, magnetic resonance and electrical techniques to elucidate the basic behavior of the materials as well as their charge transport properties. These set of characterizations included depth-profile X-ray photoelectron spectroscopy, photoluminescence, UV-Vis-NIR and ellipsometry spectroscopy, electron paramagnetic resonance, DC conductivity and electrochemical impedance spectroscopy under hydration control and neutron scattering. It was possible to show that soluble melanin synthesized in water behaves similarly to standard (and insoluble) melanin with slight variations that could be related small changes in the oxidation state of the samples or to different film thickness. On the other hand, the sulfonated melanin behaves like the standard one, indicating that these materials can be considered part of the “melanin family” of compounds. However, there were at least two key differences; it appears to have a lower ionic conductivity, most likely due to sulfonated groups replacing available conductive protons. However, this is compensated by the fact that sulfonated melanins exhibit easier thin-film formation on different surfaces, making it more attractive for device applications. We further demonstrated that SMel (one of the sulfonated melanin derivatives) is sensitive to pH in a carbon/graphene screen-printed electrode, that is, a sensor. This device can be considered a proof of concept that sulfonated melanin can act as transduction material for bioelectronics applications.

Keywords: bioelectronic material, melanin, synthetic melanin, optoelectronics, charge transport, pH sensor.

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RESUMO

O pigmento natural conhecido como melanina tem se tornado um material bastante promissor para muitas aplicações tecnológicas, mas principalmente na área emergente da bioeletrônica orgânica devido as suas propriedades de transporte de cargas e biocompatibilidade. Desta forma, esta tese tem como objetivo estudar as propriedades optoeletrônicas de uma série de derivados de melanina e aplicar estes materiais em dispositivos orgânicos. Com isto em mente, derivados sintéticos de melanina foram investigados utilizando técnicas estruturais, ópticas, de ressonância magnética e elétricas para elucidar suas propriedades, em especial as de transporte de carga. Este conjunto de caracterizações inclui técnicas como a espectroscopia de fotoelétrons de raios-X com perfil de profundidade, UV-Vis-NIR, fotoluminescência e elipsometria, ressonância paramagnética eletrônica, condutividade elétrica DC e espectroscopia de impedância eletrônica com controle de hidratação e espalhamento de nêutrons. Mostramos que as melaninas solúveis sintetizadas em água se comportam de maneira similar a amostra padrão (e insolúvel) com pequenas variações que podem estar relacionadas a pequenas diferenças no estado de oxidação das amostras ou a diferentes espessuras dos filmes. Por outro lado, as melaninas sulfonadas se comportam como a amostra padrão, indicando que estes materiais podem ser considerados como parte de compostos da “família melanina”. Contudo, existem pelo menos duas importantes diferenças: ela aparenta ter uma menor condutividade iônica, provavelmente devido aos grupos sulfonados substituindo os prótons condutores disponíveis. Entretanto, isto é compensado pelo fato de que a melanina sulfonada apresenta maior facilidade de formação de filmes finos em diferentes superfícies o que a torna mais atrativa para aplicação em dispositivos. Demonstramos ainda que a SMel (um dos derivados de melanina sulfonados) é um material sensível ao pH em um eletrodo impresso de carbono/grafeno, ou seja, em um sensor. Este dispositivo pode ser considerado a prova de conceito de que as amostras sulfonadas podem atuar como um material para transdução para aplicações bioeletrônicas.

Palavra chaves: material bioeletrônico, melanina, melanina sintética, optoeletrônica, transporte de carga, sensor de pH.

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RESUMO EXPANDIDO

A revolução da eletrônica orgânica demonstrou que materiais orgânicos condutores têm características únicas e desejáveis (entre elas, transporte e incorporação de íons, alteração do comportamento eletrônico por caminhos sintéticos, flexibilidade e baixo custo) que possibilitam a criação de dispositivos para auxiliar no entendimento de fenômenos biológicos, assistência médica e na interface entre sistemas naturais e máquinas. Esta nova era ficou como *Bioeletrônica Orgânica*. Biosensores, *drug-delivery*, pele eletrônica, sistemas sensoriais artificiais, próteses de membros, exoesqueletos robóticos e interfaces neurais são áreas deste campo de pesquisa. Além disto, marcapassos e implantes cocleares são tecnologias amplamente já difundidas no mercado consumidor.

A maioria dessas aplicações combina dispositivos optoeletrônicos com o mundo biológico de forma que uma função fundamental da bioeletrônica orgânica é a transdução eficiente dos sinais iônicos dos meios biológicos com os sinais eletrônicos de um dispositivo tradicional e vice-versa. Contudo, tal tarefa não é fácil de ser obtida, uma vez que tanto íons quanto elétrons apresentam propriedades físico-químicas diferentes. Desta forma, utilizar materiais capazes de sustentar sinais eletrônicos e iônicos é de extrema importância. Um material promissor para esta aplicação e que será o foco deste trabalho é o pigmento natural melanina.

A melanina pode ser considerada o principal pigmento da biosfera e está amplamente difundida em humanos, animais, plantas e microrganismos. A melanina está presente no mecanismo de defesa de alguns cefalópodes e em nosso cotidiano, por exemplo, em manchas pretas em alimentos ou superfícies mofadas, em partes escuras de cascas de banana e no escurecimento de uma maçã. Também é responsável pela cor dos cabelos, olhos cinza-azul-verde-amarelo-avelã-marrom, complexações da pele humana e cérebro, apresentando diferentes funções biológicas como quelação de metais, propriedades antioxidantes e anti-inflamatórias e proteção contra radiação ultravioleta-visível.

O interesse tecnológico desta classe de pigmentos decorre de suas intrigantes propriedades físico-químicas como absorbância em toda a região UV-visível, forte relaxamento não radiativo de estados eletrônicos fotoexcitados, a presença de centros de radicais livres estáveis, biocompatibilidade, biodegradabilidade e condutividades variando entre 10^{-8} e

10^{-4} Scm^{-1} dependendo do seu estado de hidratação. Outra vantagem é possibilidade de produção de filmes com espessuras inferiores a 100 nm, característica atraente para dispositivos. Estas propriedades deram origem a uma série de potenciais aplicações tecnológicas como corantes, materiais fotoativos e fotoprotetores, transistores, sensores, dispositivos de armazenamento de energia, engenharia de tecidos e interfaces biomiméticas.

Melanina, contudo, foi considerada um material difícil de se trabalhar devido à dificuldade no processo de extração de suas fontes naturais sem a interferência de impurezas de proteínas e possíveis efeitos de degradação estrutural, mas principalmente sua elevada insolubilidade. Sua insolubilidade não só dificulta uma análise química mais completa a respeito de sua composição estrutural, como também dificulta o processamento de camadas homogêneas para aplicações em dispositivos.

Atualmente, diferentes alternativas foram utilizadas para solucionar este problema. A principal é a utilização de soluções alcalinas com amônia ou hidróxido de sódio. Este procedimento pode estar relacionado à dissolução de fragmentos da estrutura com grau limitado de polimerização ou mesmo aos efeitos da fissão oxidativa do anel indol. Outra abordagem é a modificação da estrutura química da melanina e/ou estado de agregação para aumentar sua solubilidade, porém mantendo as demais propriedades físico-químicas o mais inalterado possível.

No trabalho aqui desenvolvido, estas duas perspectivas foram exploradas do ponto de vista estrutural, óptico, de ressonância magnética e elétrico para elucidar suas propriedades, em especial as de transporte de carga. Além disto, também estudamos o uso de melanina em um sensor flexível e impresso de pH. Diferentes amostras foram analisadas: i) melaninas não-funcionalizadas (NF), padrão & insolúvel e derivados solúveis em água, e; ii) melaninas sulfonadas (S), solúveis em DMSO, DMF e NMP.

Devido a insolubilidade da melanina, espectroscopia de fotoelétrons de raios-X (XPS) é uma técnica amplamente utilizada para avaliar sua análise elemental. Contudo, XPS é uma técnica de superfície e sempre houve uma dúvida se o espectro obtido poderia ser uma representação do bulk do material, devido a possíveis contaminantes atmosféricos e oxidação parcial decorrente do contato com o ar. Desta forma, realizamos um estudo estrutural com a técnica de XPS em perfil de profundidade para examinar a composição química de pastilhas e filmes finos como um todo. Mostramos que XPS pode ser utilizado como uma caracterização básica da NF-Melanina e S-Melanina. Além disto, a superfície e o bulk são bastante compatíveis entre si, porém pequenas variações foram observadas e as relacionamos a impurezas adsorvidas, força de empacotamento π - π ou a química da estrutura do indol. Também, outros dois

importantes resultados foram: i) a adição de amônia para produção do final não altera de maneira significativa a estrutura básica da melanina, sendo observado principalmente um aumento da quantidade de nitrogênio, e; ii) os derivados sulfonados de uma razão de enxofre e nitrogênio de 0,5. Atribuímos esta razão a uma possível mistura de unidades funcionalizadas e não-funcionalizadas nas S-Melanina; isto é, o grupo sulfonado não está presente em todos os monômeros.

Outra caracterização básica da melanina refere-se as suas propriedades ópticas. Para isso, tais propriedades foram investigadas em solução e em filmes finos por UV-Vis-NIR, fotoluminescência e espectroscopia de elipsometria. Os resultados indicaram que ambos os grupos de amostras possuem um comportamento óptico similar. No entanto, uma diferença significativa é a S-Melanina apresentar uma eficiência do rendimento quântico de até duas ordens de magnitude maior em comparação com as não funcionalizadas. Propusemos que este comportamento seja decorrente das diferentes estruturas e um conjunto de mecanismos de *quenching* (empilhamento π - π , transferência de prótons, grupos carboxílicos e grau de conjugação da estrutura).

Para análise de elipsometria, utilizamos um modelo que consiste em silício cristalino com uma camada de óxido como substrato e uma camada homogênea de melanina. Com este modelo, a melanina apresentou um índice de refração que aumenta conforme o comprimento de onda diminui, comportamento similar ao coeficiente de absorbância. O índice de refração foi então calculado utilizando a equação de Cauchy e obtemos um valor 1.54 ± 0.06 para NF-Melanina e 1.58 ± 0.04 para derivados sulfonados. Nós atribuímos este resultado para NF-melanina ao alto empacotamento de suas macromoléculas e no caso das S-melanina uma combinação da menor intensidade de empacotamento com átomos de enxofre dos grupos sulfonados. Cálculos teóricos da susceptibilidade óptica corroboram que unidades sulfonadas devem apresentar maior índice de refração, de forma que atribuímos a similaridade a mistura dos diferentes monômeros, conforme observado por XPS. Através dos espectros do índice de refração, também calculamos o número de Abbe que é associado ao grau de dispersão de uma amostra. As melaninas apresentam um valor equivalente a 17.86 ± 0.14 , sugerindo alto grau de dispersão e aberração cromática.

Considerando que os radicais livres estáveis apresentados na estrutura da melanina podem apresentar-se de forma proeminente como um portador de carga ou um marcador de spin para o transporte de carga, é altamente essencial compreender sua natureza. Atualmente, é aceito que a melanina possui dois tipos de radicais livres: radicais centrados em carbono (CCR) e radicais livres de semiquinonas (SFR). Embora se acredite que o segundo surja de estruturas de

semiquinona, a natureza do primeiro não é bem compreendida. Desta forma, utilizando simulações espectrais mostramos que realmente a melanina pode ser vista como um sistema com múltiplas espécies de radicais livres, porém mais importante mostramos que a origem do sinal CCR possa ser consequência de um defeito estrutural no nitrogênio com carga neutra (N_{def}^0). Além disso, comparando os espectros de ressonância paramagnética eletrônica (EPR) em estado sólido em banda-X ($\nu = 9,7$ GHz) da NF e S-melanina em condições secas ou em 80% de hidratação foi possível observar que o sinal de EPR dos dois grupos são similares, porém derivados sulfonados possuem uma menor sensibilidade de hidratação. As análises computacionais dos espectros da S-melanina sugerem a existência de pelo menos três espécies paramagnéticas que são compatíveis tanto com estruturas funcionalizadas quanto não funcionalizadas. Desta forma, atribuímos que a baixa variação com a umidade possa estar relacionada as propriedades hidrofóbicas dos grupos metílicos do grupo sulfonado e também ao fato de que as unidades sulfonadas possam prevenir as interações das unidades não funcionalizadas com o ambiente úmido. Baseado em cálculos de estrutura eletrônica, especulamos que o CCR da S-Melanina também pode ser atribuído à estrutura $S-N_{Def}^0$, enquanto que as SFR a estruturas aniônicas de quinona imina e indolquinona e também ao radical de semiquinona. Tais atribuições foram confirmadas com medidas de EPR em alto campo/alta frequência ($\nu = 263$ GHz). Não só isso, conseguimos mostrar que as espécies CCR e SFR devem apresentar simetria espectral rômica, porém baixa e alta anisotropia, respectivamente.

As propriedades eletrônicas dos derivados solúveis da melanina também foram estudadas. A forma de plote de Nyquist apresenta um semicírculo em alta frequências e uma linha quase linear em baixa frequência, geralmente atribuída a um elemento de Warburg. É interessante que para NF-melanina o plote de Nyquist é quase uma linha reta com um *slope* de aproximadamente 45° em quase toda a região de frequência, enquanto que para S-melanina existe uma separação clara entre o semicírculo e o Warburg. Utilizando o circuito de Randles como modelo mostramos que os dois grupos podem apresentar comportamentos de transporte de carga similares, porém é esperado uma diminuição da densidade de carga iônica para S-melanina, observado pela diferença da intensidade da componente de Warburg. Este comportamento também é observado pela menor histerese observado através das medidas de condutividade DC. Não só isso, as medidas de condutividade DC sugerem (de maneira similar as medidas de EPR) que S-melanina apresenta uma menor sensibilidade ao efeito de hidratação, o qual novamente a atribuímos a presença dos grupos funcionais. Por fim, através de uma análise mais detalhada do módulo e fase de cada uma das amostras, especulamos que a S-melanina deve apresentar um

aumento de processos eletrônicos mesmo em alto níveis de hidratação, em comparação com NF-melanina que é puramente iônica. Isto pode estar relacionado a substituição dos “prótons condutores” das hidroxilas fenólicas pelos grupos sulfonados ou a presença de impurezas de DMSO que impedem a solvatação dos hidrogênios disponíveis.

Espalhamento quase elástico de nêutrons (QENS) é uma ferramenta poderosa para estudar a dinâmica de átomos e íons, sendo particularmente interessante para o estudo das melaninas devido a importância dos fenômenos envolvendo movimentos de hidrogênio. A análise da componente elástica do espectro de QENS confirma uma proporção maior de hidrogênio móvel em amostras úmidas quando comparadas com as secas. Por outro lado, para temperaturas maiores que 270 K a componente inelástica mostra que NF-Melanina molhada apresenta uma maior quantidade de movimento de prótons do que a S-Melanina. Este resultado é compatível com a menor sensibilidade do transporte de carga observado com medidas de condutividade DC e a também a menor variação da quantidade de radicais livres da melanina observados com EPR.

O último assunto desta tese foi usar S-Melanina em um sensor de pH flexível e impresso. Sabe-se que a NF-Melanina padrão não adere bem a superfícies parcialmente hidrofóbicas e os filmes finos se dissolvem em condições alcalinas moderadas a altas, tornando-a inadequada para esta aplicação. Portanto, o objetivo principal foi utilizar um derivado sulfonado em um dispositivo para transdução de íon-elétron no qual a melanina padrão não pode ser usada. Foi demonstrado que o sensor de pH é capaz de funcionar em uma ampla faixa de pH, com desempenho excepcional em condições fisiológicas. Além disso, deve-se notar que a S-Melanina atuou como um elemento transdutor de íons para elétrons; com alta sensibilidade, ele pegou uma atividade iônica (pH) e a traduziu em um sinal eletrônico. Este estudo fornece a base para o desenvolvimento e otimização posteriores das propriedades sensíveis ao pH do eletrodo para aumentar ainda mais sua precisão e sensibilidade.

Em conclusão, considerando as semelhanças entre a estrutura e as propriedades optoeletrônicas dos derivados sulfonados da melanina com a amostra padrão, é possível afirmar, sem dúvida, que tais amostras pertencem à vasta gama de compostos da “família melanina”. Os resultados apresentados nesta tese são um avanço no entendimento das propriedades básicas dos derivados solúveis da melanina e é uma base para o desenvolvimento de dispositivos bioeletrônicos à base de melanina solúvel, especialmente no que diz respeito às amostras sulfonadas.

LIST OF FIGURES

Figure 1. Organic bioelectronic scope. Biological moieties can be interfaced with organic electronic devices for electroceuticals purposes. Reprinted with permission from (INAL et al., 2018). Copyright© 2018, American Chemical Society.	31
Figure 2. Melanin's function and localization in vertebrates. Reprinted with permission from (DUBEY; ROULIN, 2014). Copyright© 2014, John Wiley & Sons A/S.....	34
Figure 3. Proposed monomeric unit of sulfonated melanin.....	42
Figure 4. Possible available positions for polymerization of melanin synthesized in DMSO from (a) traditional and (b) molecular oxygen pressure synthetic approaches.	43
Figure 5. Experimental setup of the steam delivery system.	49
Figure 6. Absorbance as a function of concentration for SMel in DMSO.	51
Figure 7. (a) Energy-level scheme of a simple $\frac{1}{2}$ spin system as a function of the magnetic field. (b) Microwave absorption of this system. (c) The derivative of the absorption spectrum (b), which corresponds to the typical EPR signal obtained experimentally.....	54
Figure 8. Enhanced spectral resolution by HFEPR of a two spins system with isotropic lineshapes and similar g-values. At ν_1/B_1 the two EPR lines are almost completely overlapping; as ν_i/B_i increases, the two lines gets more separated.	56
Figure 9. Enhanced orientational selectivity by HFEPR. Reprinted with permission from from (MÖBIUS; LUBITZ; SAVITSKY, 2013). Copyright© 2013 Elsevier B.V.....	57
Figure 10. A typical example of (a) Nyquist and (b) Bode plots. The equivalent circuit inserted in the Nyquist plot represents the model used to fit the data.....	58
Figure 11. Schematic of a scattering function $S(Q, \omega)$ as a function of energy exchanged $\hbar\omega$ with the representation of elastic, quasi-elastic and inelastic peaks. Inspired by (SAKAI, 2013).	61
Figure 12. Building blocks of melanin. R = H (for DHI-like species) or COOH (for DHICA-like species).	64
Figure 13. An example of the secondary structure following the heteropolymer model point of view. Inspired by (BLOIS, 1978; PROTA, 1992).....	66
Figure 14. Stacked oligomer model. Reprinted with permission from (CLANCY; SIMON, 2001). Copyright© 2001, American Chemical Society.	67
Figure 15. Main differences between the structure-property relationships of synthetic DHI-rich melanin and DHICA-rich Melanin. Reprinted with permission from (D'ISCHIA et al., 2015). Copyright© 2015, John Wiley & Sons A/S.	68
Figure 16. Atomic concentration quantification of carbon C1s in depth of (a) NFMel-I and (b) SMel pellets.	70
Figure 17. (a-c) High-resolution XPS depth profiling spectra of NFMel-I pellet. The legends indicate the time since start of depth profiling. 0 seconds is equivalent to a standard surface scan, whereas 600 s (after five etching procedure with 5 kV energy during 120 s each) is considered the pellet bulk. (d-f) High-resolution XPS spectra simulation for a surface pellet of NFMel-I. The same modeling was applied to the depth profile spectra. (g-i) Functional group distribution as a function of etching time normalized by the surface intensity.....	72
Figure 18. (a-c) C1s, O1s and N1s high-resolution XPS depth profiling for NFMel-I thin-film from standard water and ammonia procedure (H_2O+NH_3). Also indicated in the legends is the time since the start of depth profiling. 0 seconds, in this case, is equivalent to a standard surface scan. 600 s (equal to 5 different etching with 5 kV energy during 120 s each) represents the	

thin-film bulk. (d-f) High-resolution XPS spectra simulation of C1s, O1s and N1s regions for a surface thin-film of NFMel-I prepared using standard ammonia procedure ($\text{H}_2\text{O}+\text{NH}_3$). The same modeling was applied to the depth profile spectra. (g-i) Functional group distribution as a function of etching time normalized by the surface intensity.....	75
Figure 19. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P pellet (see legend). In the legends, 0 s corresponds to the equivalent of a standard surface scan and 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).	78
Figure 20. XPS functional groups distribution for NFMel-S and NFMel-6P pellets as a function of etching time. The surface intensity normalized the intensity of each group.....	79
Figure 21. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P thin-films (see legend) following the standard water and ammonia procedure. Time equal to 0 s corresponds to the equivalent of a standard surface scan, whereas 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).	80
Figure 22. XPS functional groups distribution for NFMel-S and NFMel-6P thin-film from water&ammonia procedure as a function of etching time. The surface intensity normalized the intensity of each group.	81
Figure 23. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P thin-films (see legend) using the water-only procedure. Time equal to 0 s corresponds to the equivalent of a standard surface scan, whereas 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).	82
Figure 24. XPS functional groups distribution for NFMel-S and NFMel-6P thin-film from water procedure as a function of etching time. The surface intensity normalized the intensity of each group.....	83
Figure 25. High-resolution (a) C1s, (b) O1s, (c) N1s, and (d) S2p XPS depth profiling of SMel pellet.	85
Figure 26. (a, b, c, g) C1s, O1s, N1s and S2p high-resolution XPS spectra simulation for the SMel pellet surface. The same modeling was applied to the depth profile spectra. For S2p high-resolution spectra, high intensity and low binding energy refer to S2p _{3/2} , whereas the opposite to S2p _{1/2} . (d, e, f, h) Functional group distribution as a function of etching time normalized by the surface intensity.	86
Figure 27. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel thin-film from DMSO (left column), DMF (middle column) and NMP (right column) solutions.....	90
Figure 28. XPS functional groups distribution for SMel thin-film from DMSO (left column), DMF (middle column), and NMP (right column) solutions as a function of etching time. The surface intensity normalized the intensity of each group.	91
Figure 29. Monoatomic profile of melanin absorption. The solid line represents the simulated absorbance spectrum, while dashed lines are the individual absorbances of several different chromophores. Reproduced from (MEREDITH et al., 2006) with permission from The Royal Society of Chemistry.	94
Figure 30. The photo-luminescence spectra for eumelanin plotted as a function of (a) wavelength or (b) energy of light for five excitation energy: from 360 nm (solid line) and in 5 nm increments to 380 nm (inner dashed line). Reprinted with permission from (MEREDITH; RIESZ, 2004). Copyright© 2004, John Wiley & Sons, Inc.	95
Figure 31. Molar absorption coefficient as a function of wavelength for SMel, SMel-4P e SMel-8P.....	97

Figure 32. PL spectra at different excitation wavelengths at room temperature.	98
Figure 33. SMel, SMel-4P and SMel-8P PL spectra at $\lambda_{\text{ex}} = 425$ nm.	99
Figure 34. NH_4OH and CH_3COOH effect on the PL intensity at 487 nm with excitation at 425 nm of SMel.	100
Figure 35. NF and S-Melanin units considered in DFT calculations.	101
Figure 36. Transmittance (a, b) and reflectance (c, d) spectra of NF-Melanin (a, c) and S-Melanin (b, d). Similar features can be observed for other solvents.	104
Figure 37. Calculated absorbance spectra normalized by the film thickness of NF-Melanin and S-Melanin. Similar behavior was found for other samples (see Figure B3).	104
Figure 38. VASE spectra of (a) NFMel-I in $\text{H}_2\text{O}+\text{NH}_3$ and (b) SMel-T in DMSO.	106
Figure 39. Optical constants of all NF and S-Melanin derivatives for the different films (see legend). The solid line represents the refractive index and the dash line the extinction coefficient.	107
Figure 40. Refractive index values, following $n\lambda = n_{\text{Cauch}} + \xi_{\text{Cauch}} \lambda - 2$ with $\lambda \rightarrow \infty$, for NF- and S-Melanin. The left side legend indicates the refractive index scale; whereas the right side, the average value of the refractive index for NF-melanin (1.54 ± 0.06) and S-melanin (1.54 ± 0.04), represented by the pink- and violet-colored dashed lines.	108
Figure 41. Theoretical refractive indexes of melanin units. The dashed lines represent the average values: 1.73 and 1.93 for NF and S-units, respectively. 01 number refers to DHI units, whereas 02 to DHICA units (details in Figure 35).	108
Figure 42. Melanin X-band EPR spectrum simulation at pH (a) 1.6, (b) 6.9 and (c) 12.3. (d) Estimation of the spin densities of each component of the melanin EPR line for the different pH. The black line represents the experimental spectra while the red is the sim simulated one. Blue, pink and green lines and bars represent the three components used in the fit. Experimental data reprinted with permission from Chio <i>et al.</i> (CHIO; HYDE; SEALY, 1982) Copyright © 1982 Published by Elsevier Inc.	115
Figure 43. Energy levels around the frontier orbitals of melanin subunits. The color represents occupation: occupied orbitals (black), unoccupied orbitals (red), and semi-occupied orbitals (grey).	117
Figure 44. Melanin X-band EPR spectra simulation obtained from (a) acidic, (b) neutral and (c) alkaline solid-state samples in hydration-controlled conditions. (d) Spin densities estimation for each component of the different samples. Experimental data reprinted with permission from Mostert <i>et al.</i> (MOSTERT <i>et al.</i> , 2013). Copyright © 2013, American Chemical Society....	118
Figure 45. Melanin X-band EPR spectra simulation obtained from (a) DHI and (b) DHICA solid-state samples. (c) Estimation of the spin densities of each component of the EPR line for the different samples. Experimental data reprinted with permission from (PANZELLA <i>et al.</i> , 2013) Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.	120
Figure 46. CW X-band EPR spectra normalized to the positive peak for the four S-melanin under (a) dry (0% RH) or (b) wet (80% RH) hydration conditions, at low and high microwave power (see legend).	124
Figure 47. SMel-T CW X-band EPR spectra simulations at a microwave power of 0.03 mW under dry condition (0% RH). Black and red lines represent the experimental and simulated spectra, while blue, pink and green the three components used in the fitting. SMel-T simulations for wet spectra are shown in Figure C7, whereas for the other samples, please see Figures C8-C10, in Appendix C.2. The key differences in the parameters are shown in Figure 48.	126

Figure 48. Spin concentration for each component of S-melanin at a microwave power of 0.03 mW. The uncertainty bars (propagated from the difference between modelled and experimental data) are smaller than the data point resolution.	126
Figure 49. Distribution of <i>g</i> -values obtained for charged (anion and cation) and radical units of S-melanin with unpaired spins (i.e., EPR active species). The left side legend indicates the <i>g</i> -values scale, whereas, on the right side, the <i>g</i> -values estimated via spectral simulation (Table 13).	129
Figure 50. Frontier energy levels of neutral S- and NF-melanin building blocks.	130
Figure 51. DAM of S and NF-melanin monomers.	131
Figure 52. Normalized 263 GHz EPR absorption spectra at 293 K and different microwave power for (a) NFMel-S, (b) NFMel-6P and (c) SMel.	134
Figure 53. Experimental HFEPR spectra (black line) and their simulation (red line) for (a) NFMel-S, (b) NFMel-6P and (c) SMel at 0.1 mW microwave power. Simulation parameters are displayed in Table 14.	135
Figure 54. Different <i>g</i> -values obtained for anionic, cationic and radical NF-melanin units. 01 and 02 refer to NF-DHI and NF-DHICA species, respectively.	137
Figure 55. The plot of the rhombicity as a function of <i>g</i> -value anisotropy for the different (a) NF-melanin and (b) S-melanin radical species. The theoretical radicals are represented by the square, circle and triangle symbols; the crosses are each of the experimental radicals. A purely isotropic signal would have $\Delta g = 0$ and a completely axial symmetry signal would be obtained with rhombicity equal to 1.0 or 0.0.	138
Figure 56. Anisotropic <i>g</i> -values of anionic, cationic and radical S-melanin monomers. 01 refers to S-DHI and 02 to S-DHICA species.	139
Figure 57. Standard melanin conductivity, in pellet, as a function of relative humidity. Reprinted with permission from (JASTRZEBSKA et al., 1995). Copyright© 1996, Taylor & Francis.	143
Figure 58. Anionic charge hopping through a hydrogen-bonded network following the Grötthuss mechanism. Dashed grey arrow indicates the electron pathway, whereas the solid-colored arrow the hydrogen pathway.	145
Figure 59. Current as a function of voltage for (a) NFMel-I in H ₂ O-NH ₃ and (b) SMel-T in DMSO at different hydration levels. Each IV curve is an average of at least six different pairs of gold interdigitated electrodes. Similar behavior was found for other samples.	147
Figure 60. (a, b) NF-Melanin and (c, d) S-Melanin conductivity as a function of hydration. The conductivity was calculated through the inverse of the sample resistivity normalized by the electrode geometry.	148
Figure 61. Nyquist plots from EIS measurements on (a) NFMel-I and (b) SMel-T at 100% hydration level. Scatter colored plots represent experimental data, while continuous grey lines the fit using the Randles circuit as a model. Scatter colored plots represent experimental data, while straight gray lines the fit using the Randles circuit (insert in part a) as a model. The dashed green line is set at 45°.	150
Figure 62. (a, b) Modulus and (c, d) phase angle as a function of frequency for NF-Melanin films prepared using (a, c) water&ammonia and (b, d) water solution. Scatter colored plots represent experimental data, while continuous light grey lines the theoretical fit using a Randles circuit as a model.	151
Figure 63. (a, b) Modulus and (c, d) phase angle as a function of frequency for S-Melanin films prepared using DMSO solution. Scatter colored plots represent experimental data, while continuous light grey lines the theoretical fit using a Randles circuit as a model.	153

Figure 64. (a, b) NF-melanin and (c, d) S-melanin frequency dependency AC conductivity at 100% hydration level.....	154
Figure 65. Equilibrium adsorption isotherm for water adsorption of NFMel-I and SMel-T powder. The dashed green line indicates the humidity level of 75%.	156
Figure 66. QENS spectra for (a) dry NFMel-I, (b) wet NFMel-I and (c) wet SMel-T at different temperatures.....	158
Figure 67. (a, b) Elastic and (c, d) inelastic window scans (Q-summed) for NF and S-melanin derivatives.....	159
Figure 68. Steps of the healing process on skin wound: (a) inflammation, (b) new tissue formation, and (c) remodeling. Alteration of pH as a function of time in (d) acute and (e) chronic wounds. (a-c) Reprinted with permission from (GURTNER et al., 2008). Copyright© 2008, Springer Nature. (d, e) Reprinted with permission from (SCHNEIDER et al., 2007). Copyright© 2007, Springer Nature.	163
Figure 69. Screen-printed electrodes from (a) top and (b) cross-section point of view. (c) Photograph of the flexible sensor. Reprinted with permission from (TEHRANI et al., 2020). Copyright© 2020, IOP Publishing.	165
Figure 70. (a) High-resolution N 1s XPS spectra for SMel coated electrode (Black), carbon/graphene ink on a bare electrode (Blue) and SMel (Red). The nitrogen peaks around 401 on the coated electrode indicate that melanin is present at the surface of the sensor working electrode.....	166
Figure 71. (a) pH sensing performed of the pH sensor in a wide range of pH, from 2 to 12. (b) The ability of the SMel coated electrode to recover following exposure to high pH solution.	167
Figure 72. The sensitivity of (a) bare and (b) SMel coated electrodes under physiological conditions.	168

LIST OF SCHEMES

Scheme 1. Eumelanogenesis pathway. Adapted with permission from (BRONZE-UHLE et al., 2016). Copyright© 2016, Society of Chemical Industry.....	35
Scheme 2. The comproportionation equilibrium reaction. Quinone and hydroquinone moieties interact together to obtain an intermediate redox state like the negatively charged semiquinones and protons.	111
Scheme 3. Redox transformations of hydroquinones blocks within melanin in solid-state (pathway I and II) and aqueous environment (pathway III, IV and V). Reprinted with permission from (MOTOVILOV et al., 2019). Copyright© 2019, Royal Society of Chemistry.....	169

LISTA OF TABLES

Table 1. Examples of different technological applications with melanin and melanin-like materials.	39
Table 2. Information regarding the melanin derivatives.	47
Table 3. Neutron scattering cross-section of elements present in NF-Melanin and S-Melanin.	62
Table 4. The atomic composition (%) and atomic ratios determined from pressed powder pellets of standard synthetic melanin samples.	71
Table 5. The atomic composition (%) and atomic ratios determined from the spin-coated thin-films of standard synthetic melanin samples.	74
Table 6. The atomic composition (%) and atomic ratios determined from pressed powder pellets of soluble non-functionalized melanin samples.	77
Table 7. The atomic composition (%) and atomic ratios determined from spin-coated thin-films of soluble non-functionalized melanin samples from H ₂ O+NH ₃ solutions.	79
Table 8. The atomic composition (%) and atomic ratios determined from spin-coated thin-films of soluble non-functionalized melanin samples from H ₂ O solutions.	81
Table 9. The atomic composition (%) and atomic ratios determined from pressed powder pellets of sulfonated melanin samples.	84
Table 10. The atomic composition (%) and atomic ratios determined from the surface of spin-coated thin films of sulfonated melanin samples.	88
Table 11. The atomic composition (%) and atomic ratios determined from the bulk of spin-coated thin films of sulfonated melanin samples.	89
Table 12. Spectral parameters of the simulated signals of NF-Melanin.	116
Table 13. The spectral parameters of the simulated signals for all four S-melanin samples under both dry and wet conditions.	127
Table 14. Experimentally Determined g-Values for Melanin Derivatives.	136

LISTA OF ABBREVIATION AND SYMBOLS

(D)L-DOPA	3,4-dihydroxy-phenyl-(D)L-alanine
DHI	5,6-dihydroxy-indole
DHICA	5,6-dihydroxy-indole-2-carboxylic acid
ε and k	Absorption coefficient or extinction coefficient
CH ₃ COOH	Acetic acid
NH ₃	Ammonia
NH ₄ OH	Ammonium hydroxide
CCR	Carbon-centered radicals
DFT	Density functional theory
g	Dimensionless constant called g-factor/g-value
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EIS	Electrochemical impedance spectroscopy
EPR	Electronic paramagnetic resonance
Z''	Imaginary component of impedance
Z	Impedance
S-Melanin	Melanin synthesized in DMSO
Functionalized melanin	Melanin synthesized in DMSO
Sulfonated melanin	Melanin synthesized in DMSO
NF-Melanin	Melanin synthesized in water
Non-functionalized melanin	Melanin synthesized in water
Ndef ⁺	Nitrogen-protonated of indole structure
NMP	N-methyl-2-pyrrolidone
IQ	<i>Ortho</i> -(indole)quinone
QH ₂	<i>Ortho</i> -hydroquinone
QI	<i>Ortho</i> -quinone(imine)
SQ*	<i>Ortho</i> -semiquinone
O ₂	Oxygen gas
ΔH_{pp}	Peak-to-peak linewidth
ΔY_{pp}	Peak-to-peak signal amplitude
PL	Photoluminescence
PET	Polyethylene terephthalate
QENS	Quasi-elastic neutron catering

Z'	Real component of impedance
n	Refractive index
RL	Resonant line
SFR	Semiquinone-free radical
SMel	Soluble melanin synthesized in DMSO following a standard procedure
SMel-T	Soluble melanin synthesized in DMSO heated at 100 °C
SMel-4P	Soluble melanin synthesized in DMSO using 4 atm of O ₂ pressure.
SMel-8P	Soluble melanin synthesized in DMSO using 8 atm of O ₂ pressure.
NFMel-S	Soluble melanin synthesized in water following a standard procedure
NFMel-6P	Soluble melanin synthesized in water using 6 atm of O ₂ pressure.
NFMel-I	Standard and insoluble melanin synthesized in water
UV-Vis-NIR	Ultra-violet to visible and near infra-red spectral regions
VASE	Variable angle spectroscopic ellipsometry
XPS	X-ray photoelectron spectroscopy

SCIENTIFIC CONTRIBUTIONS

Peer-reviewed papers related to this thesis:

- I. Structural and optical properties of soluble melanin analogues with enhanced photoluminescence quantum efficiency. **J. V. Paulin**, A. G. Veiga, Y Garcia-Basabe, M. L. M. Rocco, C. F. O. Graeff. *Polymer International*. v. 67, p. 550-556, 2018.
- II. Identification of common resonant lines in the EPR spectra of melanins. **J. V. Paulin**, A. Batagin-Neto, C. F. O. Graeff. *The Journal of Physical Chemistry B*. v. 123, p. 1248-1255, 2019.
- III. Printable and Flexible Graphene pH sensors utilising Thin Film Melanin for Physiological Applications. Z. Tehrani, S. Whelan, A. B. Mostert, **J. V. Paulin**, M. Ali, E. Daghighahmadi, C. F. O. Graeff, O. J. Guy and D. T. Gethin. *2D Materials*. v. 7, 024008, 2020.
- IV. Shedding light on the free radical nature of sulfonated melanins. **J.V. Paulin**, A. Batagin-Neto, P. Meredith, C.F.O. Graeff, A.B. Mostert. *The Journal of Physical Chemistry B*. v. 124, p. 10365–10373, 2020.
- V. Melanin system composition analyzed by XPS depth profiling. **J.V. Paulin**, J.D. Mcgettrick, C.F.O. Graeff, A.B. Mostert. *Surfaces and Interfaces*. v.24, p.101053, 2021.
- VI. Melanin thin-films: a perspective on optical and electrical properties. **J.V. Paulin**, A. P. Coleone, A. Batagin-Neto, P. Meredith, C.F.O. Graeff, A.B. Mostert. In preparation.
- VII. High-Field/High-Frequency EPR spectroscopy on synthetic melanin. **J.V. Paulin**, C.J. Magon, B. Naydenov, A. Batagin-Neto, K. Lips, C.F.O. Graeff. In preparation.
- VIII. Quasi-elastic neutron scattering for probing proton diffusion of melanin. I. P. Silverwood, **J.V. Paulin**, H. Cavaye, K. A. Motovilovd, C.F.O. Graeff, P. Meredith, A.B. Mostert. In preparation.

Peer-reviewed papers not related to this thesis:

- IX. Melanin synthesis under oxygen pressure. E. S. Bronze-Uhle, **J. V. Paulin**, M. Piacenti-Silva, C. Battocchio, M. L. M. Rocco, C. F. O. Graeff. *Polymer International*. v. 65, p. 1339-1346, 2016.

- X. Biocompatibility investigations of synthetic melanin and melanin analogue for application in bioelectronics. M. Piacenti-Silva, A. A. Matos, **J. V. Paulin**, R. A. S. Alavarce, C. F. O. Graeff. *Polymer International*. v. 65, p. 1347-1354, 2016.
- XI. UV-protective thin film based on PVA-melanin/rod-coating silver nanowires and its application as a transparent capacitor. L. G. S. Albano, **J. V. Paulin**, L. D. Trino, S. L. Fernandes, C. F. O. Graeff. *Journal of Applied Polymer Science*. v. 136, p. 47805, 2019
- XII. Biosensor EGFET of fungal β -1,3-glucanase obtained from vinasse for application in the diagnosis of fungal infections. G. M. de Queiroz-Fernandes, **J. V. Paulin**, B. L. Martins, M. R. Batistuti, L. H. S. Nunes, H. J. N. P. D. Mello, M. Mulato, C. F. O. Graeff. In preparation.
- XIII. Microbiological culturing applications for a flexible and printable pH sensor based on graphene-melanin. S. P. Whelan, Z. Terhani, **J. V. Paulin**, O. Guy, D. Gethin. In preparation.
- XIV. Surface functionalization of 4-6 nm carbon nanodiamonds with conjugated ligands. B. A. Bregadiolli, **J. V. Paulin**, C. F. O. Graeff, L. C. Silva-Filho, M. A. Zaghet, A. Sellinger. In preparation.
- XV. Structural, optical and electrical properties of melanin-polyaniline nanocomposites. M.B.S.M. dos Reis, B.A. Bregadiolli, **J.V. Paulin**, L.M. Martins, C.F.O. Graeff. In preparation.
- XVI. Integration of sulfonated melanin and rGO towards the engineering of its physicalchemical properties. B. A. Bregadiolli, **J. V. Paulin**, L. G. S. Albano, L. C. Silva-Filho, C. C. B. Bufon, C. F. O. Graeff. In preparation.

Other contributions direct (or indirect) related to this thesis

- How a pigment might hold the key to cybernetic implants in humans. Available at <<https://stfc.ukri.org/news-events-and-publications/features/how-a-pigment-might-hold-the-key-to-cybernetic-implants-in-humans/>>.
- Bioeletrônicos podem substituir medicamentos em tratamentos de doenças. Available at <<https://www.youtube.com/watch?v=L-YD13H4pw8&t=235s>>.

TABLE OF CONTENTS

CHAPTER I.....	29
1.1 INTRODUCTION TO ORGANIC BIOELECTRONICS.....	30
1.2 INTRODUCTION TO MELANIN	33
1.2.1 Physical-chemical properties and biological functions.....	36
1.2.2 Technology issues and synthetic derivatives	38
1.3 MOTIVATION AND GENERAL CONSIDERATIONS	44
CHAPTER II	45
2.1 METHODOLOGY	46
2.1.1 Nomenclature and general information of the synthetic melanin derivatives.....	46
2.1.2 Chemical synthesis.....	46
2.1.3 Setup for hydration control	49
2.2 CHARACTERIZATION TECHNIQUES	50
2.2.1 X-ray photoelectron spectroscopy.....	50
2.2.2 UV-Vis-NIR spectroscopy	50
2.2.3 Ellipsometry	51
2.2.4 Photoluminescence.....	52
2.2.5 Electron paramagnetic resonance.....	53
2.2.6 DC conductivity measurements	57
2.2.7 Electrochemical impedance spectroscopy.....	57
2.2.8 Adsorption isotherm.....	59
2.2.9 Quasi-elastic neutron scattering	60
CHAPTER III.....	63
3.1 INTRODUCTION	64
3.2 EXPERIMENTAL PROCEDURE	68
3.2.1 Sample preparation.....	68
3.2.2 XPS Measurements	69
3.3 RESULTS AND DISCUSSION	70
3.4 CONCLUSION.....	92
CHAPTER IV	93
4.1 INTRODUCTION	94
4.2 OPTICAL PROPERTIES OF S-MELANIN IN SOLUTION.....	96
4.2.1 Methodology	96
4.2.2 Results and Discussion.....	97
4.3 OPTICAL PROPERTIES OF MELANIN DERIVATIVES IN THIN-FILMS	100
4.3.1 Methodology	100
4.3.2 Results and Discussion.....	103
4.4 CONCLUSION.....	109
CHAPTER V	110
5.1 INTRODUCTION	111
5.2 THE NATURE OF INTRINSIC AND EXTRINSIC FREE-RADICAL OF NF-MELANIN	113

5.2.1	Methodology	113
5.2.2	Results and Discussion.....	114
5.3	X-BAND CW-EPR OF S-MELANIN UNDER HYDRATION CONTROL	121
5.3.1	Methodology	121
5.3.2	Results and Discussion.....	124
5.4	HIGH-FIELD/HIGH-FREQUENCY EPR SPECTROSCOPY FOR NF AND S-MELANIN	133
5.4.1	Methodology	133
5.4.2	Results and Discussion.....	133
5.5	CONCLUSION.....	139
CHAPTER VI.....		141
6.1	INTRODUCTION	142
6.2	COMPARISON OF NF AND S-MELANIN CHARGE TRANSPORT.....	146
6.2.1	Methodology	146
6.2.2	Results and discussion.....	147
6.3	QUASI-ELASTIC NEUTRON SCATTERING FOR PROBING PROTON DIFFUSION.....	155
6.3.1	Methodology	155
6.3.2	Results and Discussion.....	157
6.4	CONCLUSION.....	160
CHAPTER VII		162
7.1	INTRODUCTION	163
7.2	EXPERIMENTAL PROCEDURE	164
7.2.1	Device assembly.....	164
7.2.2	Characterization Techniques	165
7.3	RESULTS AND DISCUSSION	166
7.3.1	Characterization of melanin-coated pH sensor	166
7.3.2	Mechanism of action	168
7.4	CONCLUSION.....	170
CHAPTER VIII		171
8.1	CONCLUSIONS	172
8.2	POSSIBLE FUTURE WORKS	173
REFERENCES		176
APPENDIX A		198
APPENDIX B.....		210
APPENDIX C.....		214
APPENDIX D		241

CHAPTER I

BACKGROUND

1.1 INTRODUCTION TO ORGANIC BIOELECTRONICS

Bioelectronic dates back to the 18th century at the University of Bologna with the now-famous experiment of Luigi Galvani and the frog leg. In this experiment, by applying a small voltage to a detached leg muscle of a frog Galvani induced muscular activity. Such leg movement revolutionized the understanding of muscular and nerve function and the use of the biological electrical current to study living organisms' tissues (PICCOLINO, 1997; ADEE, 2019).

Nowadays, with the solid-state organic electronics revolution that has demonstrated that conducting organic materials have unique and desirable features including transportation and incorporation of ions, high tunability of electronic behavior via synthetic paths, flexibility (in comparison to silicon) and cost-effective manufacturing; many research group from all around the globe are expanding the field not just to increase the understanding of biological phenomena, but also explore the potential use of bioelectronics in artificial synaptic systems, health care and interfaces for machines (BERGGREN; RICHTER-DAHLFORS, 2007; MALLIARAS, 2013; RIVNAY; OWENS; MALLIARAS, 2014; BETTINGER, 2015; SCHWEICHER et al., 2020). This new era for bioelectronics was coined as *Organic Bioelectronics* by Magnus Berggren and Agneta Richter-Dahlfors in their critical review of 2007 (BERGGREN; RICHTER-DAHLFORS, 2007).

The bioelectronic field demonstrates tremendous promise and hype for technological applications and is continuously present in popular media as in TV series and Hollywood films. Take as an example Doctor Who, famous British science fiction since 1963 from BBC ("BBC One - Doctor Who",). The show follows the main character's adventures, "the Doctor", across time and space using only his versatile sonic screwdriver. This device can diagnose any disease or substance in the human body or environment. During his quests, he often finds events involving bionic humans, humans whose advanced technology allowed cybernetics enhancements interface as an integral part of the body, and even complete transference of human conscience into inorganic machines ("BBC One - Doctor Who",). Even if in the real world we are still technologically far behind the show, significant progress has been made in the areas of biosensing and drug-delivery (HOLZINGER; GOFF; COSNIER, 2014; MEHROTRA, 2016; HOSSEN et al., 2019; RAZA et al., 2019), electronic skin (LEE; LEE, 2019; SOMEYA; AMAGAI, 2019), artificial sensory systems and peripheral nerve interfaces (BETTINGER, 2018; JUNG et al., 2019), prosthetic limbs and robotic exoskeletons suits (FRITZ; PATZER; GALEN, 2017; GORGEY, 2018), and neural interfaces (MOULTON et al.,

2012; LAGO; CESTER, 2017; RIVNAY et al., 2017; LEE; LEE, 2019). Additionally, pacemakers and cochlear implants can be viewed as key applications already widely spread in the consumer market (MALLIARAS, 2013; RIVNAY; OWENS; MALLIARAS, 2014; BETTINGER, 2015). Such technological advances have also increased the clinical interest in search of Parkinson's and chronic pain therapies and alternative approaches for controlling epileptic seizures using electrical stimulation of nerve cells and neurons to modulate their behavior through implanted microelectrode arrays (MOULTON et al., 2012).

Most of these application combines organic and optoelectronic devices with the biological world. The interaction between these two worlds can happen in two directions (schematically illustrated in Figure 1): from biology to organic electronics, where a signal is obtained in an organic device from a biological process or reaction; and from organic electronics to biology, where the organic device trigs biological process or biochemical reaction (OWENS; MALLIARAS, 2010; MOULTON et al., 2012; SIMON et al., 2016). This means that a fundamental task for organic bioelectronics is to interface organic devices and biological systems such that there is efficient transduction of the ionic/protonic signals in biological media to the electronic signals to a readout computer and vice versa (OWENS; MALLIARAS, 2010; RIVNAY et al., 2013; RIVNAY; OWENS; MALLIARAS, 2014; MEREDITH et al., 2016; BRAENDLEIN et al., 2017; INAL; MALLIARAS; RIVNAY, 2017; INAL et al., 2018).

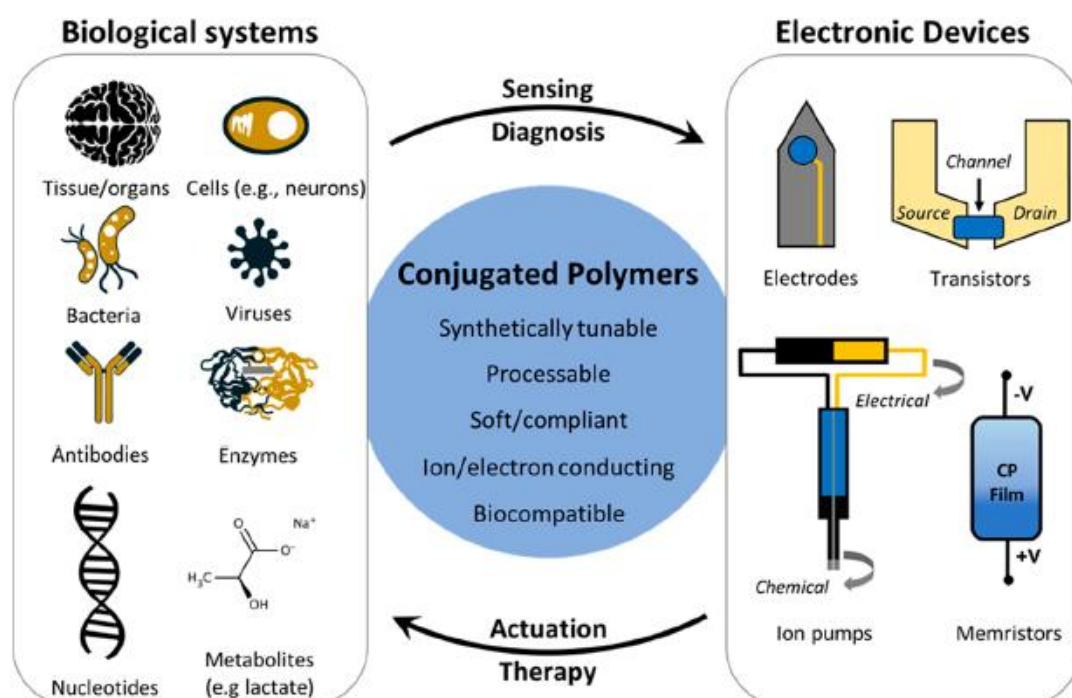


Figure 1. Organic bioelectronic scope. Biological moieties can be interfaced with organic electronic devices for electroceuticals purposes. Reprinted with permission from (INAL et al., 2018). Copyright© 2018, American Chemical Society.

However, transduction is not simple to be achieved because both ions and electrons have different chemical and physical properties (AMDURSKY; GŁOWACKI; MEREDITH, 2019). For instance, an ionic charge is usually treated with classical physics, while electrons and holes are quantum mechanical entities. Also, ions are slow charge carriers (mobilities less than 10^{-2} cm²/Vs) that can move through a medium by the center of mass diffusion; on the other hand, electrons can move faster than ions and their motions are strongly affected by local structure and disorder (AMDURSKY; GŁOWACKI; MEREDITH, 2019). The trapping properties are different as well: an electronic trap usually is a localized state within the energy band gap whereas for ionic entities trapping occurs chemically via an inter-or-intramolecular trap (MEREDITH et al., 2016).

One way to reach the transduction is fabricating devices that can amplify ionic signals by incorporating materials that can sustain both electronic and protonic/ionic signals, i.e. hybrid or mixed ionic/electronic conductors. Rolandi's group provided one example in 2011, where the first bioprotonic field-effect transistor was demonstrated by using hydrogenated palladium as an electrode (ZHONG et al., 2011). The hydrogenated palladium electrode was able to take an electronic input and through electrochemical means inject a proton into the device. In a more recent example, Gorodetsky's group showed, through humidity-dependent electrical measurements, that a high carboxylate protein may form hydrophilic water channels that can serve as effective conduits for proton transport (ORDINARIO et al., 2014).

As in any emergent research field, the critical task is the correct selection of proper material. In organic bioelectronics, usually there is the desire for features such as mixed electron-ionic charge transport, mechanical flexibility and elasticity, biodegradability, biocompatibility with ability to cell adhesion and cell proliferation (BERGGREN; RICHTER-DAHLFORS, 2007; OWENS; MALLIARAS, 2010; RIVNAY; OWENS; MALLIARAS, 2014). Today, an omnipresent in the bioelectronic field is the commercially available poly(3,4-ethylene dioxythiophene) doped with poly(styrene sulfonate), i.e., PEDOT:PSS. This bicomponent macromolecule can fulfill most of these properties because it is chemically stable and biocompatible (BERGGREN; RICHTER-DAHLFORS, 2007); and the PSS dopant not only increase the conductivity of the system and assist in the ion transport but also allow better dispersion in water of conducting part PEDOT (GROENENDAAL et al., 2003; BERGGREN; RICHTER-DAHLFORS, 2007; STAVRINIDOU et al., 2013; RIVNAY et al., 2016). Polyaniline, proteins and polysaccharides are materials that can also be found in this field. For instance, polyaniline (PANI) is garnering attention (especially for tissue engineering) due to its

good biocompatibility, superior stability in electrolytes and high electrical conductivity (HUANG; HUMPHREY; MACDIARMID, 1986; LIAO et al., 2015). The downside is that to sustain a high conductivity, there is the need to keep it in its protonated state, which implies that the charge transport would be significantly degraded in physiological environments (HUANG; HUMPHREY; MACDIARMID, 1986; FOCKE; WNEK; WEI, 1987). To improve PANI biocompatibility and the interface between devices and the biological systems, researchers usually functionalize the material with selected dopants, nanostructured materials and biocompatible elements (HUANG et al., 2003; LIAO et al., 2015). On the other hand, the mechanical flexibility of protein, and specially fibers, is of great importance for developing smart e-textiles (LIAO et al., 2015) and high-performance flexible natural-cotton-fiber-based organic transistors have already been fabricated in physiological conditions (TARABELLA et al., 2012). However, as active materials, they have been barely touched (AMDURSKY; GŁOWACKI; MEREDITH, 2019).

Another promising candidate that can fulfill all these requirements and will be the focus of this thesis is the natural pigment melanin.

1.2 INTRODUCTION TO MELANIN

Melanin can be considered the primary pigment in the biosphere and it is widespread in humans, animals, plants and microorganisms. Melanin is present in the defense mechanism of some cephalopods (PROTA, 1992; DERBY, 2014). It is also present in our daily life, for instance, in black spots on moldy food or surfaces, in dark parts of banana peels and in the browning of an apple (PLONKA; GRABACKA, 2006). It is also responsible for the gray-blue-green-yellow-hazel-brown eye color and human skin complexations (JABLONSKI; CHAPLIN, 2010; D'ISCHIA et al., 2013, 2015; DORGALELEH et al., 2020).

In humans, this pigment is produced mainly in melanocyte cells, known as melanosomes, and then deposited throughout the organism within the melanocyte or in the epidermis, hair, eyes, as well as in several internal parts of the body, summarized in Figure 2 (DUBEY; ROULIN, 2014). Nevertheless, melanin is not present only in today's fauna and flora. Recent studies have found melanosomes organelles in a three million-year-old fossil fur from mammal species *Apodemus atavus* (MANNING et al., 2019), in a 54 million-year-old fossilized eye from crane-flies (LINDGREN et al., 2019), in a 110 million-year-old fossil found in Brazil in Ceará region, from a pterosaur *Tupandactylus imperator* (PINHEIRO et al., 2019) and also in a dinosaur fossil feathers from the late Jurassic, 145-161 million years ago (LI et al., 2010).

Localization and function of melanins in vertebrates

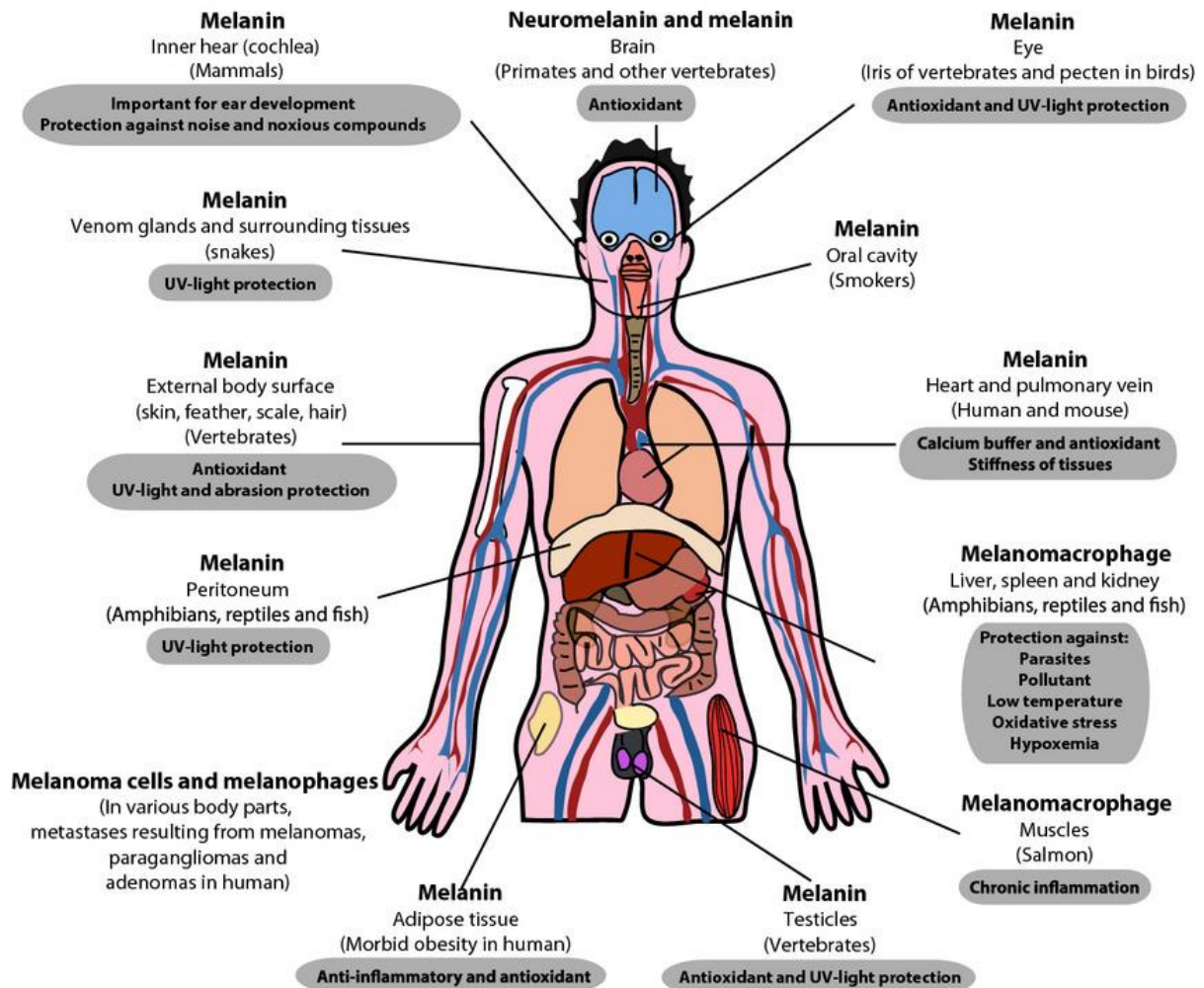
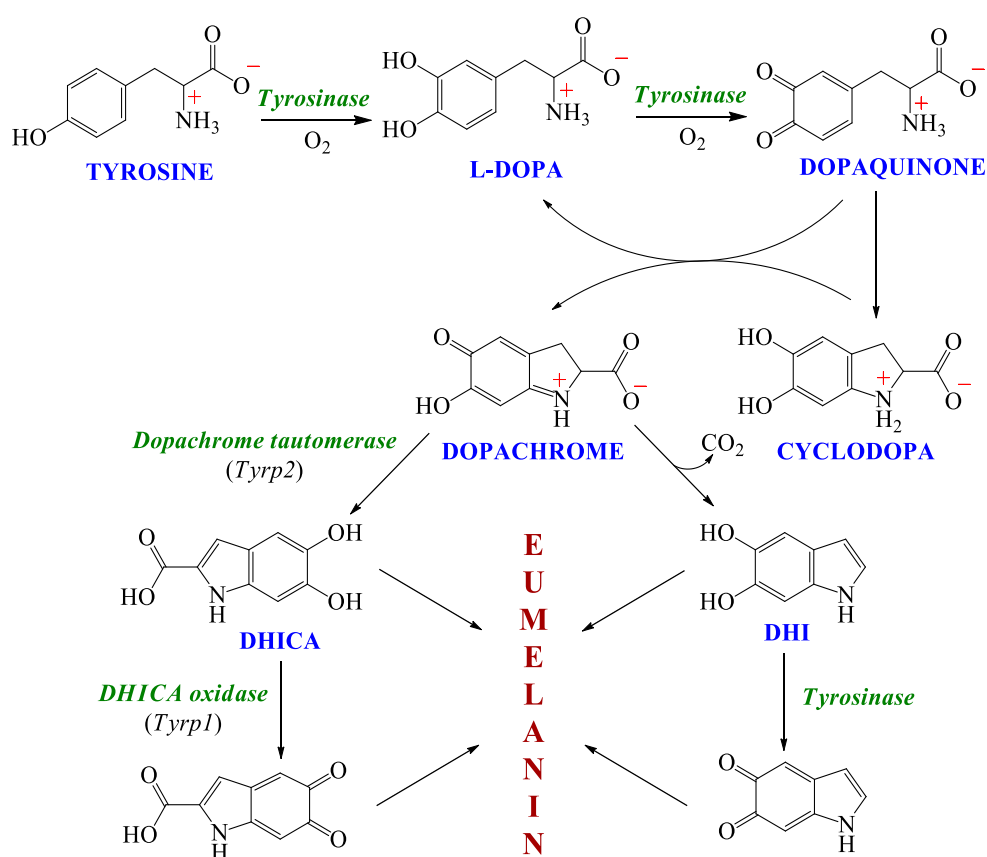


Figure 2. Melanin's function and localization in vertebrates. Reprinted with permission from (DUBEY; ROULIN, 2014). Copyright© 2014, John Wiley & Sons A/S.

The denomination “melanin” derives from the Greek μέλας that means black/dark. It is a generic term introduced by Aristotle denoting pigments of various structures whose origin can be obtained through the oxidation and polymerization of tyrosinase in animals or of phenolic compounds in lower organisms (BLOIS, 1978; D'ISCHIA et al., 2013, 2015). However, unlike many other natural pigments, melanin does not have a unique and well-defined structure. Hence, it was proposed a classification based on its chemical structures and precursor.

The most common and abundant group is eumelanin. This pigment class has a brown-black coloration and is formed predominantly from the monomers 5,6-dihydroxyindole (DHI) and 5,6-dihydroxy-indole-2-carboxylic acid (DHICA). *In vivo*, eumelanin synthesis occurs in melanocytes being regulated by the melanocortin-1 receptor (D'ISCHIA et al., 2015). The beginning of the synthesis is the hydroxyalkylation of tyrosine to form 3,4-dihydroxyphenyl-L-alanine (L-DOPA) followed by the oxidation of L-DOPA into

dopamine, both steps in the presence of the tyrosinase enzyme and oxygen. Dopamine is a highly reactive intermediate capable of spontaneously cyclize to cyclodopa that can rearrange through redox processes to generate the dopachrome. Afterward, the presence of the enzyme tyrosinase and its derivatives encoded by *Tyrp1* and *Tyrp2* genes rearrange the dopachrome to forming the DHI or DHICA structures (PROTA, 1992; MUNOZ-MUNOZ et al., 2009; PANZELLA et al., 2013; D'ISCHIA et al., 2015). Scheme 1 display a brief overview of this process. It is worth point out that this is a radical synthesis and all the structures mentioned can and will be present in the final eumelanin structure.



Scheme 1. Eumelanogenesis pathway. Adapted with permission from (BRONZE-UHLE et al., 2016). Copyright© 2016, Society of Chemical Industry.

The second group is known as pheomelanin. It has a red-yellow color and is found mainly in redheaded individuals (ITO; WAKAMATSU, 2003). The pheomelanin synthetic process is similar to eumelanin until the formation of dopaquinone. At this stage, the L-cysteine amino acid is incorporated into the synthesis to form the monomers of benzothiazine and benzothiazole, units that, in addition to nitrogen, contain sulfur in its backbone structure (ITO, 2003; SOLANO, 2014).

Another important group of melanin can be found in the brainstem of specific populations of catecholaminergic neurons. This group is called neuromelanin. It is a dark and insoluble particle composed of a combination of the previous groups being eumelanin in the outer shell and pheomelanin in the core (BUSH et al., 2006; ZUCCA et al., 2014; MENTER, 2016). Curiously, humans exhibit the highest amount of neuromelanin in all primates and it is wholly absent in many other species (FEDOROW et al., 2005).

The last group of this class of pigment is named allomelanin, i.e., “other”-melanin, or pyromelanins. This group is the least studied and can be found in insects, plants and microorganisms like fungi (PLONKA; GRABACKA, 2006; SOLANO, 2014). Unlike eumelanin and pheomelanin which have a common origin, allomelanin can be obtained from the oxidation/polymerization of phenolic substrates such as catechols, 1,8-dihydroxynaphthalene, 4-hydroxyphenylacetic acids, γ -glutaminy-4-hydroxybenzene and 2,5-dihydroxyphenyl-acetate (homogentisate). In a similar manner to eumelanin and pheomelanin in the human body, allomelanin is also involved in a series of function related to pigmentation, wound healing, camouflage and defense reactions against pathogens, heavy metals, high concentrations of salts, UV radiation and extreme environment conditions (GESSLER; EGOROVA; BELOZERSKAYA, 2014; VARGA et al., 2016; CORDERO; CASADEVALL, 2017; BAREK et al., 2018). An unexpected consequence of the last roles has led to the intriguing hypothesis that fungal melanin could transduce radiation into metabolic energy. Such a proposition was raised after the discovery in Chernobyl’s reactors that certain melanized fungi could respond to the exposure of the ionizing radiation with enhanced growth (DADACHOVA; CASADEVALL, 2008). Further along, allomelanin can contribute to virulence in pathogenic fungi, in which it promotes the tissue invasion and deactivated the plant defense system (BELTRÁN-GARCÍA et al., 2014).

Among these subgroups, eumelanin is considered the archetype of melanin and the terms “eumelanin” and “melanin” are usually used interchangeably in the literature. Thus, since eumelanin is the only focus of this thesis, it will be hereafter referred to as simply *melanin*.

1.2.1 Physical-chemical properties and biological functions

Biologists, chemists, physicists and materials scientists have shown significant interest in the melanin pigment because it can be found throughout the biosphere with exotic physical and chemical properties. Several physical and chemical techniques have been applied to the study of its properties. Surprisingly, they are not fully understood until now because of the lack of precisely-defined chemical and physical characteristics, implying in energetic disorder and the

highly insoluble nature of the pigment. These features make it a laborious task to obtain any possible structure-property correlation (PROTA, 1992, 2000; MEREDITH; SARNA, 2006; D'ISCHIA et al., 2009, 2015). Hence, there is some speculation towards its implications for biological function.

One of the fundamental properties of melanin is the excellent ability to chelate and also to bind with metal ions (LYTTKENS et al., 1979; D'AMATO; LIPMAN; SNYDER, 1986; LARSSON; LARSSON; TJÄLVE, 1988; HONG; SIMON, 2007; KARLSSON; LINDQUIST, 2016). This ability has a potential benefit since it could allow drugs to remain in the body for longer and clean the environment from heavy metals pollution. However, this ability may also have an undesirable effect. For instance, the metal chelation ability has been correlated to neurological diseases like Alzheimer's and Parkinson's (MANN; YATES, 1983; D'AMATO; LIPMAN; SNYDER, 1986; LINDQUIST; LARSSON; LYDÉN-SOKIKIWSKI, 1987; ZUCCA et al., 2004; LIU; SIMON, 2005; HONG; SIMON, 2007; PAL et al., 2015).

Additionally, melanin has an unusual optical absorbance without any distinct peak in the spectra, behaving like a broadband, monotonic exponential decaying function (WOLBARSH; WALSH; GEORGE, 1981; TRAN; POWELL; MEREDITH, 2006), see Figure 29 in Chapter IV. Moreover, unlike most chromophores, it shows very low fluorescence with the ability to dissipate more than 99.9 % of the UV and visible absorbed radiation non-radiatively (MEREDITH; RIESZ, 2004). Both of these properties may be related to the photoprotection effect. When melanin absorbs the light, the internal conversion dissipates the optical light into heat and molecular vibration. Thus, fewer high-energy photons will induce DNA damage to the surrounding tissues (WOLBARSH; WALSH; GEORGE, 1981; KOLLIAS et al., 1991; MEREDITH et al., 2006). In fact, it has been shown that high levels of natural skin pigmentation present less susceptibility to the harmful effects of UV radiation, while at the opposite extreme, individuals with albinism will be more sensitive to light (BRENNER; HEARING, 2008; MONTOLIU et al., 2014).

The role melanin plays as a photosensitizer, on the other side, should be looked at with care since it has also been involved in photodamage. Some studies point to a universal relation between melanin and melanoma, a type of skin cancer formed in the epidermal melanocytes (KOCH; CHEDEKEL, 1986; GILCHREST et al., 1999; NOONAN et al., 2012). Furthermore, it has been demonstrated that when melanin is exposed to UV-visible light, there is the formation of reactive oxygen species (ROS) that could cause DNA damage (FELIX et al., 1978a; SARNA et al., 1980; KOCH; CHEDEKEL, 1986, 1987).

Besides the formation of such ROS radicals, melanin also presents paramagnetic species intrinsically related to its structure. These radicals are persistent and stable even under harsh conditions in both solution and solid-state (COPE; SEVER; POLIS, 1963; BLOIS; ZAHLAN; MALING, 1964). Also, melanin's free-radical may feature prominently as either a charge carrier or at least be an indirect spin-label for its charge transport (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015; MOSTERT et al., 2018).

The study of melanin's electrical and photophysical properties goes back to the '60s being one of the earliest bio-organic materials to have been studied experimentally and theoretically (LONGUET-HIGGINS, 1960; PULLMAN; PULLMAN, 1961; POWELL; ROSENBERG, 1970; MCGINNESS, 1972). It was proposed that melanin behave like an amorphous semiconductor with a bi-stable reversible switching (POWELL; ROSENBERG, 1970; MCGINNESS; CORRY; PROCTOR, 1974; CULP; ECKELS; SIDLES, 1975; FILATOV; MCGINNESS; CORRY, 1976; JASTRZEBSKA et al., 1995). This switch would only happen when the melanin was hydrated, which was an odd feature to the consistent amorphous semiconductor model (MOSTERT et al., 2010), however not completely unsupported (REALI; SAINI; SANTATO, 2021). Nonetheless, the apparent switching ability may be necessary for disorders such as Schizophrenia, deafness and Parkinson's (MCGINNESS; CORRY; PROCTOR, 1974), but it also could be involved in eye disorders and visual process (SEVER; COPE; POLIS, 1962; COPE; SEVER; POLIS, 1963; SARNA, 1992).

1.2.2 Technology issues and synthetic derivatives

Melanin and melanin-like materials are a versatile class of compounds with many technological applications due to the materials' physical and chemical properties combined with their inherent biocompatibility. Hence, potential application for the melanin family covers several fields, including organic electronics and bioelectronics, biomedicine and cosmetics, as summarized in Table 1. Also, glasses with infused melanin to the lens are already available commercially (TRUEBLUE,). However, for a long time, melanin was considered a difficult material to work with due to the difficulty in the extraction process from its natural sources without the interference of proteins impurities and possible structural degradation effects, but most of all its high insolubility (BLOIS, 1978; PROTA, 1992).

Table 1. Examples of different technological applications with melanin and melanin-like materials.

Application	Melanin	Reference
Anti-inflammatory	Polydopamine	(WANG et al., 2020)
Artificial muscles	Polydopamine	(TIAN et al., 2018)
Biomimetic interfaces	Eumelanin	(WÜNSCHE et al., 2013a)
	Sigma-melanin	(AMBRICO et al., 2014)
	DHI-eumelanin	(PEZZELLA et al., 2015)
	Polycysteinyldopamine	(VECCHIA et al., 2015)
Bioremediation	Allomelanin	(CORDERO; CASADEVALL, 2017; CORDERO; VIJ; CASADEVALL, 2017)
Drug release	Sepia-melanin	(ARAÚJO et al., 2014)
	Sigma-melanin with polyethylene glycol chains	(ZHANG et al., 2015)
Electrochemical biosensor	Polydopamine	(WANG et al., 2014)
Electronic ink	Polydopamine	(CHANG et al., 2017)
Humidity sensor	Polydopamine	(WU; WEE; HONG, 2015)
Fertilizer	Not specified	(HERRERA, 2019)
Ion-to-electron transduction & electrochemical transistors	Eumelanin	(SHELIAKINA; MOSTERT; MEREDITH, 2018a)
	Copper-eumelanin	(MOSTERT et al., 2020)
Memory device	Sigma-melanin	(AMBRICO et al., 2010, 2012)
	Sigma and Sepia and Sulfonated-melanin	(DI MAURO et al., 2016)
Packaging	Polydopamine	(LIU et al., 2019)
	Sepia and DHI and DHICA-melanin	(DI MAURO et al., 2019)
Pigments	Polydopamine	(XIAO et al., 2017)
	Sepia and DHI and DHICA-melanin	(DI MAURO et al., 2019)
Photocatalysis	DHI-melanin	(MIGLIACCIO et al., 2018)
	Polydopamine	(LEE et al., 2018)
Photothermal therapy	Polydopamine	(LIU et al., 2013)
	Polydopamine	(WANG et al., 2016a)
	Sepia-melanin and polydopamine	(JIANG et al., 2017)

Table 1. Continuation.

Application	Melanin	Reference
Photovoltaic	Eumelanin	(MEREDITH et al., 2005)
	Sepia-melanin	(MATSUURA et al., 2012)
	Sepia-melanin	(LEE et al., 2013)
	DHI-melanin	(ANTIDORMI et al., 2017)
	DHI-melanin	(PINNA et al., 2017)
Radioprotection	Eumelanin and feomelanin	(SCHWEITZER et al., 2010)
	Allomelanin	(CORDERO, 2017; CORDERO; CASADEVALL, 2017)
Piezoelectric sensor	Tea-melanin	(HUANG et al., 2007)
pH sensor	Eumelanin	(PIACENTI-SILVA et al., 2014a)
	Sulfonated-melanin	(TEHRANI et al., 2020)
Shape-memory	Polydopamine	(CHEN et al., 2018)
Storage	Eumelanin/vanadium pentoxide hybrid material	(OLIVEIRA et al., 2000)
	Sepia and DHICA-melanin and eumelanin	(KIN et al., 2013)
	Polydopamine	(BIE et al., 2016)
	Sigma-melanin	(KUMAR et al., 2016)
	DHI-melanin and eumelanin	(XU et al., 2019)
Tissue engineering	Sigma-melanin	(BETTINGER et al., 2009)
	Polydopamine	(KWON et al., 2016)
	Sepia-melanin	(SRISUK et al., 2018)
UV-shielding	Sepia-melanin	(WANG et al., 2016b)
	Eumelanin and oxygenated-melanin	(ALBANO et al., 2019)

The low solubility of melanin is a matter of considerable technological relevance since its use in optoelectronic and bioelectronic devices depends on the deposition of homogeneous layers of the material (AMBRICO et al., 2015; D'ISCHIA et al., 2015). Currently, several techniques have been used to overcome this barrier: electrodeposition (DÍAZ et al., 2005; KIM et al., 2011; KLOSTERMAN; RILEY; BETTINGER, 2015), electro-spray (ABBAS et al., 2009), matrix-assisted pulsed laser deposition (BLOISI et al., 2011), solid-state polymerization (PEZZELLA et al., 2015), layer-by-layer (GUIN et al., 2015), self-deposition by an enzymatic process (STRUBE; BÜNGELER; BREMSER, 2015), evaporation (XIAO et al., 2015) and screen-print (GURME et al., 2018).

A simpler alternative would be through techniques such as spin-coating or drop-casting where concentrated aqueous sodium hydroxide or ammonia solutions are used (DA SILVA et al., 2004; BOTHMA et al., 2008; WÜNSCHE et al., 2013b). However, it is essential to consider that the apparent solubility caused by this procedure may be related to the dissolution of fragments of the structure with a limited degree of polymerization or even the degradation effects caused by the oxidative fission of the indole ring (D'ISCHIA et al., 2013; AMBRICO et al., 2015). Currently, it is possible to commercially obtain soluble melanin samples synthesized from tyrosine with hydrogen peroxide. The commercial melanin can be dispersed in a slightly alkaline aqueous solution or dimethylsulfoxide (DMSO); however, such material appears to be quite different from natural and synthetic melanin since structural analyses suggest the absence of the basic melanin indole units (D'ISCHIA et al., 2013; AMBRICO et al., 2015).

Considering that the solubility depends on the polymer nature, i.e., monomer composition, a promising technological pathway is to slightly modify the structure or aggregation state of the melanin macromolecule to increase its solubility, while the others physicochemical properties are kept as much as possible unchanged. In this way, several research groups are working on this issue and different functional groups such as sulfonated (DEZIDÉRIO et al., 2004; PIACENTI-SILVA et al., 2014b; PAULIN et al., 2018) benzyl and octyl ester (LAWRIE; MEREDITH; MCGEARY, 2008), *S-galactosyl* (PEZZELLA et al., 2009), triethylene glycol (CICCO et al., 2015) and carboxylic acids (BRONZE-UHLE et al., 2016) have been used as a means to obtain soluble melanin.

One of the first soluble derivatives, and, in fact, probably the first melanin thin-film reported in the literature, was proposed by Dezydério and co-workers back in 2004 (DEZIDÉRIO et al., 2004). In this work, the route used to synthesize melanin was the oxidation of 3,4-dihydroxy-L-phenylalanine (L-DOPA) and DMSO by benzoyl peroxide. The DMSO oxidation was a crucial step because it will be responsible for the formation of the methanesulfonic anhydride that will functionalize the phenolic hydroxyls and nitrogen atom of the monomers of DHI and DHICA with the sulfonated groups (BRONZE-UHLE et al., 2013), Figure 3. The sulfonated groups allow the production of more homogeneous melanin, with higher thermal stability (DEZIDÉRIO et al., 2004) and also solubility in DMSO, dimethylformamide and N-methyl-2-pyrrolidone (PAULIN, 2016; PAULIN et al., 2018), which, as mentioned before, enables the production of homogeneous thin films with excellent adhesion even on hydrophobic surfaces (DA SILVA et al., 2004; DEZIDÉRIO et al., 2004;

WÜNSCHE et al., 2013b; ALBANO et al., 2016). Also, these derivatives are biocompatible (PIACENTI-SILVA et al., 2016) with the ability to support proton transport (ALBANO et al., 2016). Hence, these properties make sulfonated melanin, i.e., melanin synthesized in DMSO, an attractive candidate for bioelectronics applications.

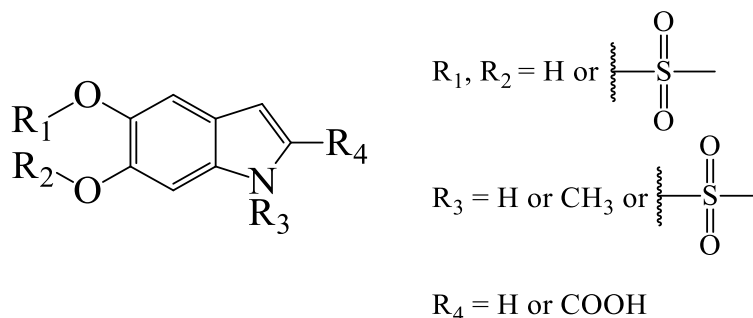


Figure 3. Proposed monomeric unit of sulfonated melanin.

Although the melanin synthesized by Dezidério *et al.* has good technological potential, its synthesis is relatively slow (57 days). Since the conditions under which the reactions occur are critical and changes in the synthetic procedure from the same substrate may present variations in the polymer and macromolecular structure (D'ISCHIA et al., 2013), two different strategies were proposed in an attempt to optimize the reaction rate of this derivative. The first approach evaluated the effect of different temperatures in the traditional route proposed by Dezidério *et al.*. In this case, as the temperature increases, the synthetic mechanism accelerates, reducing the reaction time to 11 days when heated to 100 °C (PIACENTI-SILVA et al., 2014b). In addition to increasing the synthesis speed, the increase in temperature also decreases the number of carboxylic groups from the indole structures given rise to a final polymer with a higher amount of DHI structures (PIACENTI-SILVA et al., 2014b).

The second strategy studied the synthesis's oxidizing agent, i.e., molecular oxygen replaced the benzoyl peroxide. The molecular oxygen is considered an ideal oxidant since it is cheap, abundant and non-toxic with high academic and industrial interest (RONSEIN et al., 2006). In this alternative synthetic route, the reaction rate increases even more when compared to the traditional procedure. For example, it can be decreased to only three days when 8 atm of O₂ pressure is used (PAULIN, 2016; PAULIN et al., 2018). However, the synthetic derivative presented two significant structural changes. The higher the pressure, the higher the number of carboxyl and carboxylate groups than the one synthesized through the traditional route in DMSO. The increase of such groups could be linked to a higher amount of DHICA structures or by-products from the indole ring's degradation (NAPOLITANO et al., 1995; GALVÁN et

al., 2018). Nevertheless, due to *indirect* structural characterization, the first option seems to be the case (BRONZE-UHLE et al., 2016; PAULIN et al., 2018). However, it is important to stress that the increase of the structure oxidation does not follow a linear behavior with the rise of the oxygen pressure; that is, a higher amount of carboxylic groups is obtained for the sample synthesized with 4 atm of O₂ instead of 8 atm (PAULIN, 2016; PAULIN et al., 2018), probably due to changes in the kinetic and thermodynamic process of the reaction, that are not well understood yet.

The second structural change is related to the polymerization process of the sulfonated monomers. In the traditional (and heated) synthetic procedure, the polymerization may take place at the indole structure's carbon atoms, Figure 4(a). However, in the presence of molecular oxygen, such a route does not seem to be possible. Thus, we speculated that it might happen through hydroxyl groups from indole and carboxyl structures, Figure 4(b) (PAULIN et al., 2018).

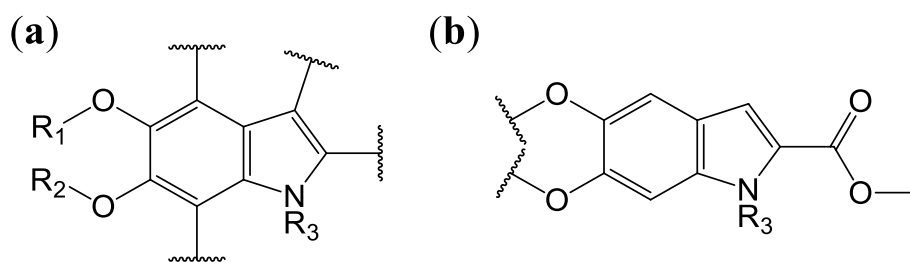


Figure 4. Possible available positions for polymerization of melanin synthesized in DMSO from (a) traditional and (b) molecular oxygen pressure synthetic approaches.

Although exotic functional groups' presence is not relevant from a technological point of view, the structural question is of fundamental importance when trying to understand biological samples' structure-property relationship. Considering that much of the knowledge gained about the structure and functionality of melanin is derived from studies of synthetic materials, it would be interesting to obtain a synthetic derivative whose system is similar to a natural one.

Traditionally, melanin is synthesized through the auto-oxidation of L-DOPA in an alkaline aqueous solution with the bubbling of atmospheric air (D'ISCHIA et al., 2013). In this process, the melanin obtained is insoluble, with high chemical heterogeneity and a low amount of DHICA monomer. However, natural melanin usually presents a higher amount of DHICA structures due to the tyrosinase enzyme presence widely spared in natural melanogenesis (MUNOZ-MUNOZ et al., 2009; PANZELLA et al., 2013). Therefore, increase the number of carboxylic groups in a synthetic derivative could result in a material more likely to the natural

one. Combining the traditional synthesis with the molecular oxygen pressure approach can achieve this condition (BRONZE-UHLE et al., 2016; PAULIN, 2016). In this case, rather than bubbling air during synthesis, the molecular oxygen pressure was used to supply the oxygen during melanogenesis. Bronze-Uhle *et al.* proposed that the synthetic media's oxygen pressure could act like tyrosinase enzyme in the natural environment (BRONZE-UHLE et al., 2016).

1.3 MOTIVATION AND GENERAL CONSIDERATIONS

Despite the abundance of melanin in nature, details regarding its structure, properties and functions are not well established yet. In this way, the technological application of melanin in stable organic (bio)electronic devices with excellent performance accentuates the need to improve the understanding of its properties. Therefore, the work described in this thesis seeks to deepen the study of the structure and optoelectronic properties of synthetic melanin and applied them in organic devices. With this aim:

Chapter II Methodology and Characterization Techniques describes the synthetic methodology of the melanin derivatives with a brief overview of the techniques used.

Chapter III Structural composition the melanin derivatives structures are discussed for pellets and thin-films using X-ray photoelectron spectroscopy.

Chapter IV Optical Characteristics of Melanin Derivatives presents the optical properties (photoluminescence, UV-Vis-NIR and ellipsometry spectroscopy) in solution and thin-films.

Chapter V Free Radical Nature of Melanin and Melanin Derivatives discuss the nature of the melanins free-radical with electron paramagnetic resonance spectroscopy, spectral simulations and electronic structure calculations.

Chapter VI Charge Transport Properties also evaluated the melanin charge transport properties using DC and electrochemical impedance spectroscopy under hydration control. The proton dynamic of standard and sulfonated melanin derivatives is also compared using quasi-elastic neutron scattering.

Chapter VII Melanin-Based Device sulfonated melanin was used as active material in a pH sensor.

Chapter VIII General Conclusions a review of the major conclusions is drawn and perspectives on future directions are given.

CHAPTER II

METHODOLOGY AND CHARACTERIZATION TECHNIQUES

2.1 METHODOLOGY

2.1.1 Nomenclature and general information of the synthetic melanin derivatives

The present study aimed to characterize a series of different soluble melanin derivatives and it was used for comparison the standard and insoluble melanin (referred here as NFMel-I).

Two water-soluble derivatives were synthesized in water following a similar procedure to the standard one (NFMel-S) or under oxygen pressure (NFMel-6P). These samples have identical structures but with different oxidation states, being higher for NFMel-6P compared to NFMel-S and NFMel-I. NFMel-I, NFMel-S and NFMel-6P will also be called “non-functionalized melanin” and “NF-Melanin” in the following chapters.

Besides NF-Melanin, this project also evaluated four soluble sulfonated melanin derivatives. These samples are soluble in DMSO, dimethylformamide (DMF) and N-methyl-2-pyrrolidone (NMP), with the basic monomeric structure like the one present in Figure 3. SMel is considered the traditional sulfonated melanin derivative and it was synthesized at room temperature. However, if the SMel synthetic procedure were heated in a silicone bath at 100 °C, the new sample will be named SMel-T. The synthetic route for sulfonated melanin also follows an alternative process using 4 (SMel-4P) or 8 (SMel-8P) atm of oxygen pressure. SMel and SMel-T should have a similar polymerization structure (Figure 4(a)) that is different from SMel-4P and SMel-8P (Figure 4(b)). Similar to NF-Melanin, these four samples also show different oxidation states, following $\text{SMel-T} < \text{SMel} < \text{SMel-8P} < \text{SMel-4P}$. These samples will also be called as “functionalized melanin”, “sulfonated melanin” and “S-Melanin”.

The most relevant details about all these samples are summarized in Table 2.

2.1.2 Chemical synthesis

All commercially available chemicals and reagents were used without further purification.

NFMel-I: Standard and insoluble non-functionalized melanin

5 g of 3,4-dihydroxy-phenyl-DL-alanine (DL-DOPA; Sigma-Aldrich, $\geq 98\%$) were dissolved in 2 L of deionized water. Ammonia (NH_3 ; Sigma-Aldrich, 28 %) was used to adjust the mixture to pH 8. The solution was kept under constant stirring and with air bubbling for three days. The pH was measured continuously using litmus paper and, when necessary, a drop of NH_3 was added to maintain the alkaline conditions. After three days, concentrated

hydrochloric acid (Sigma-Aldrich, 25%) was used to adjust to pH 2 to precipitate the melanin. The aggregated solution was filtered, washed several times using deionized water and dried overnight under ambient conditions to yield a black powder.

Table 2. Information regarding the melanin derivatives.

Group	Sample	Polymerization	Functionalization	Solubility
NF-Melanin [†]	NFMel-I	Carbon atoms (Figure 4(a))	None	Insoluble
	NFMel-S	Carbon atoms (Figure 4(a))	None	H ₂ O
	NFMel-6P	Carbon atoms (Figure 4(a))	None	H ₂ O
S-Melanin [‡]	SMel	Carbon atoms (Figure 4(a))	SO ₂ CH ₃ group	DMSO, DMF and NMP
	SMel-T	Carbon atoms (Figure 4(a))	SO ₂ CH ₃ group	DMSO, DMF and NMP
	SMel-4P	Oxygen atoms (Figure 4(b))	SO ₂ CH ₃ group	DMSO, DMF and NMP
	SMel-8P	Oxygen atoms (Figure 4(b))	SO ₂ CH ₃ group	DMSO, DMF and NMP

[†] Non-functionalized melanin

[‡] Sulfonated melanin

NFMel-S: water-soluble non-functionalized melanin

1.0 g of DL-DOPA were dissolved in 200 mL of MiliQ water (18 MΩ cm). The mixture's pH was adjusted to be between 8 e 10 by the addition of 1.4 mL of ammonium hydroxide (NH₄OH; Synth, 28-30%). The NH₄OH was chosen to avoid the continuous need to check the solution pH. The solution was kept stirring at room temperature (27 °C) and oxygenated using an air pump for three days. For extraction and purification, a 3500 MWCO dialysis membrane was used with MiliQ water as a dialysate medium, which was changed every day until no further color change could be observed (approximately four to six days). Finally, drying the aggregated solution in an oven at 90 °C for two days yielded NFMel-S black powder.

NFMel-6P: Oxygenated water-soluble non-functionalized melanin

The NFMel-6P synthetic route follows the same NFMel-S process; however, instead of using air bubbles in the solution, the mixture of 0.3 g DL-DOPA dissolved in 60 mL of MiliQ water (18 M Ω cm) and 400 μ L of NH₄OH was placed in a 150 mL stainless steel reactor. The synthesis's oxygenation happens by increasing the internal pressure to 6 atm of industrial oxygen gas (O₂). The solution was stirred for 6 hours. NFMel-6P was extracted and purified using a 3500 MWCO dialysis membrane and MiliQ water as dialysate medium, which was also changed every day until no further color change could be observed. The aggregated solution was dried in an oven at 90 °C for two days.

SMel: Standard and soluble functionalized melanin

In 200 mL of DMSO (PA, Vetec, 99.9%), 1.50 g of DL-DOPA and 0.93 g of benzoyl peroxide (Vetec, 75.0-80.0 %) were dissolved in a flat bottom flask. This mixture was stirred under magnetic for 57 days at room temperature (~ 27 °C). For purification, the reaction solution's temperature was increased to 140 °C to concentrate it to ¼ of the initial volume. Once it cooled down to room temperature, 150 mL of acetonitrile (Synth, 99.5%) was added to the concentrated solution. The new solution was allowed to stand for two days to separate SMel from synthetic by-products. After the resting period, SMel was extracted by centrifugation at a speed of 2500 rpm for 15 minutes and the precipitate was dried in the oven at 90 °C for two days.

SMel-T: Heated and soluble functionalized melanin

SMel-T was synthesized using a mixture of 1.50 g of DL-DOPA and 0.93 g of benzoyl peroxide dissolved in 200 mL of DMSO in a round bottom flask. The mixture was kept stirring in a temperature-controlled silicone bath at 100 °C for eleven days in reflux. A sequence of solution concentration to ¼ of the initial volume (at 140 °C), dissolution in 150 mL of acetonitrile, centrifugation (2500 rpm for 15 minutes) and drying in an oven (at 90 °C) was used for extraction and purification of the sample.

SMel-4P: 4 atm of O₂ pressure and soluble functionalized melanin

0.45 g of DL-DOPA was dissolved in 60 mL of DMSO and placed in a 150 mL stainless steel reactor whose internal pressure was increased to 4 atm with the introduction of O₂ gas and kept under magnetic stirring for six days. A sequence of solution concentration to ¼ of the

initial volume (at 140 °C), dissolution in 45 mL of acetonitrile, centrifugation (2500 rpm for 15 minutes) and drying in an oven (at 90 °C) was used for extraction and purification of the sample.

SMel-8P: 8 atm of O₂ pressure and soluble functionalized melanin

In a 150 mL stainless steel reactor, 0.45 g of DL-DOPA was mixed with 60 mL of DMSO. The reactor's internal pressure was increased to 8 atm with O₂ gas and kept under magnetic stirring for three days. A sequence of solution concentration to ¼ of the initial volume (at 140 °C), dissolution in acetonitrile (45 mL), centrifugation (2500 rpm for 15 minutes) and drying in an oven (at 90 °C) was used for extraction and purification of the sample.

2.1.3 Setup for hydration control

The measurements carried out with controlled hydration were performed using a specific module for each characterization (details about each of these modules will be presented in the appropriate section) coupled to a steam delivery system, Figure 5. In this system, the measurements were performed as a function of the relative pressure, i.e., the pressure corresponding to a specific relative humidity. The desired relative water vapor pressure, verified by a calibrated BOC-Edwards GK series (0-50 mbar) gauge, is obtained by opening/closing the valve (V_R) of the solvent reservoir (R_S). Note that two conditions must be obtained for this to happen: i) the system must be in a vacuum (obtained with a rotary vacuum pump); and, ii) the use of degassed solvent. The second condition is necessary to ensure that the pressure arises exclusively from the solvent vapor and was obtained by doing three freeze–pump–thaw cycles with liquid nitrogen. Once a relative pressure was obtained, the system was allowed to get in equilibrium, between 20 minutes and one hour, before performing the measurement.

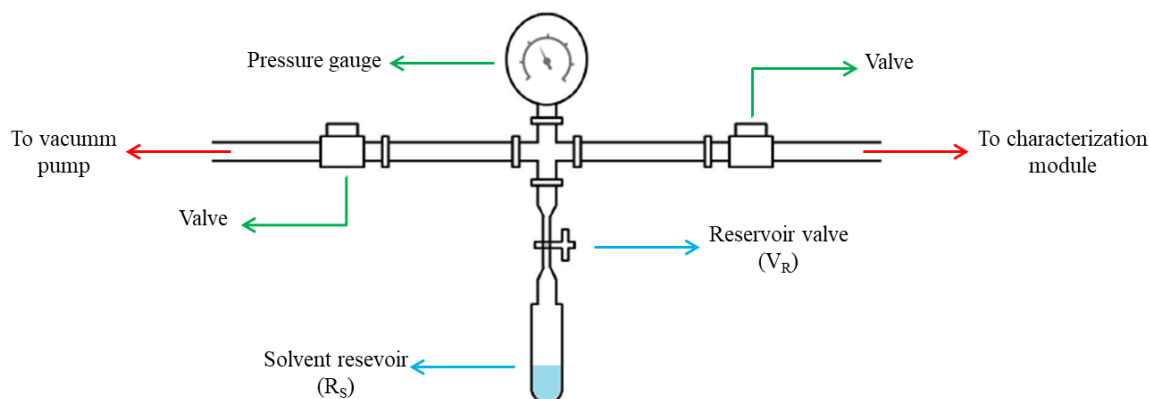


Figure 5. Experimental setup of the steam delivery system.

2.2 CHARACTERIZATION TECHNIQUES

2.2.1 X-ray photoelectron spectroscopy

X-Ray Photoelectron Spectroscopy (XPS) is a technique based on the physical principle known as the Photoelectric Effect and it can provide the elemental identification and chemical composition of a sample. Simplistically, an atom of the sample surface layer absorbs X-ray radiation giving rise to an excited state. This atom in an excited state will emit a photoelectron to relax to the ground state. Thus, from the analysis of the intensity of the photoelectrons as a function of their respective kinetic energy, it is possible to determine the binding energy of the photoelectric peaks and to directly characterize the elemental composition, chemical state and atom coordination on the surface of a sample (MOULDER et al., 1992; RIBEIRO et al., 2003; SKOOG; HOLLER; CROUCH, 2007; FADLEY, 2010). However, a complete analysis of the bulk of the system may not be described with high reliability. Thus, an alternative to obtain chemical information on a micro scale from this technique is to analyze the chemical composition on a depth scale, by combining the traditional XPS analyses with a surface corrosion sequence with ions or clusters of inert atoms (SKOOG; HOLLER; CROUCH, 2007; FADLEY, 2010).

2.2.2 UV-Vis-NIR spectroscopy

UV-Vis-NIR spectroscopy is used to measure the absorbance, transmittance and reflectance properties of substances in solution or solid-state from predetermined ultra-violet (UV), visible (Vis) and near infra-red (NIR) electromagnetic radiation wavelengths. Basically, the technique consists of monochromatic radiation of initial intensity I_0 focusing on a medium that can absorb and/or reflect the beam, which will decrease its intensity to a transmitted intensity T_I . However, if the medium is not transparent or homogeneous, the radiation will be spread through the system, reducing its intensity (CUSTODIO; KUBOTA; ANDRADE, 2000; VOGEL; MENDHAM, 2000). All these phenomena are related to each other by a linear expression described by Eq. 1,

$$I_0 = R_I + S + A + T_I \quad (1)$$

where R_I represents the intensity of the reflected beam, S the intensity of the scattered part and A the amount of radiation absorbed by the medium. However, the light absorption

characterizations are usually performed with a homogeneous media, which makes the scattered radiation negligible.

The intensity of the energy absorbed is an intrinsic characteristic of the materials and is dependent on a specific frequency, thickness and concentration. This property can be described by the absorbance (CONSTANTINO, 2006; SKOOG et al., 2006). The Lambert-Beer Law, Eq. 2, describes the absorbance A as,

$$A(\lambda) = -\ln\left(\frac{T_I}{I_0}\right) = \varepsilon(\lambda) c L \quad (2)$$

where c is the concentration of the solution, L the optical path size and ε is an optical constant called the absorption coefficient or extinction coefficient that depends on the wavelength (λ) of the electromagnetic radiation incident on the sample and the material to be studied (CONSTANTINO, 2006; SKOOG et al., 2006). Therefore, the Lambert-Beer Law can be applied to low concentration solutions to obtain $\varepsilon(\lambda)$ experimentally (CONSTANTINO, 2006).

Considering that the optical path is the thickness of the optical cuvette, usually 1 cm, plotting the absorbance as a function of the solution's concentration should give a straight line, Figure 6, whose angular coefficient will be the absorption coefficient.

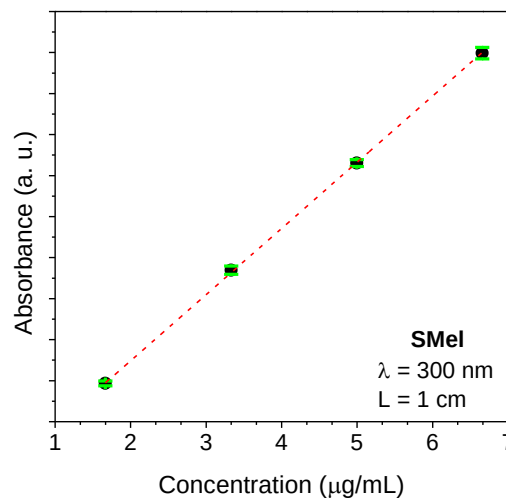


Figure 6. Absorbance as a function of concentration for SMel in DMSO.

2.2.3 Ellipsometry

Ellipsometry is a technique used to measure changes in the polarization state of a polarized vector wave interacting with a material (AZZAM; BASHARA, 1977; FUJIWARA,

2007), usually light waves. It can obtain optical information of thin-film, surface, interface, and multi-layered structures through the light's transmission or reflectance. This measurement analyzes the changes in the light's polarization instead of intensity changes in UV-Vis spectroscopy (FUJIWARA, 2007).

By changing the wavelength of light at a particular incidence angle, it is possible to measure the values of the amplitude ratio, Ψ , and phase difference, Δ , between p- and s-polarized light waves (AZZAM; BASHARA, 1977; FUJIWARA, 2007). Thus, the data analysis necessarily requires the use of an optical model for the extraction of physical constants such as refractive index n , extinction coefficient k and thickness of the sample.

2.2.4 Photoluminescence

Photoluminescence is a non-destructive method used to explore the material's electronic structure. When electromagnetic radiation is absorbed in a process called photo-excitation, the excess energy causes the electrons to move to higher energy state. When the electron returns to its equilibrium state, it releases the accumulated energy through radiative (light emission) or non-radiative (internal and external energy conversion or vibrational relaxation) processes. In this way, the emitted light's energy is directly related to the difference of the energy levels between two electronic states involved in the transition from the excited state to the equilibrium state (LAKOWICZ, 2006a; SKOOG et al., 2006).

The quantum-yield $\phi(\lambda)$ is an intrinsic property of each molecule and is, therefore, a useful parameter for comparing the intensity of photoluminescence between different samples. It is defined as the ratio between the number of photons emitted and the number of photons absorbed at a given excitation wavelength (LAKOWICZ, 2006a; SKOOG et al., 2006). Eq. 3 allows the calculation of the quantum-yield (MEREDITH; RIESZ, 2004)

$$\phi_M(\lambda) = \phi_S \left(\frac{\alpha_M}{\alpha_S} \right) \left(\frac{n_M}{n_S} \right)^2 \quad (3)$$

where α is the graph coefficient obtained from the area of the sample emission spectrum as a function of the absorbance and n represents the solvent refractive index. Subscript S represents the standard sample with a known quantum yield, while M represents, in our case, the melanin sample.

2.2.5 Electron paramagnetic resonance

2.2.5.1 Overview

Electronic paramagnetic resonance (EPR) is a spectroscopy technique used in systems with paramagnetic centers such as free radicals, ionic radicals and molecules in triplet states. It can be used to explore the chemical structure of these species or their interactions with the chemical environment and the dynamics of processes in which they may be involved (POOLE, 1982; YADAV, 2005; WEIL; BOLTON, 2007; BRUSTOLON, 2009).

EPR is based on the absorption of electromagnetic radiation, usually in the microwave frequency region, by a paramagnetic sample in the presence of an external magnetic field. The presence of the external magnetic field is a necessary condition for the occurrence of the Zeeman Effect that will promote the splitting of paramagnetic levels for the different spin states of the sample (Figure 7(a)); in other words, the magnetic field enables the breaking of the degeneracy of the spin states.

If the microwave frequency is kept fixed while the magnetic field is varied, the microwave's absorption will only occur when the energy $h\nu$ of the radiation coincides with the energy difference between the spin states (POOLE, 1983; WEIL; BOLTON, 2007; BRUSTOLON, 2009). Mathematically, Eq. 4 describes the absorption and it is the fundamental equation of EPR spectroscopy.

$$h\nu = E_{\alpha} - E_{\beta} = g |\mu_B| H_0 \quad (4)$$

where h corresponds to the Planck constant, ν is the resonance frequency, E_{α} and E_{β} are the spin energies at high and low energy levels, respectively; g , denoted by g-factor or g-value, is a dimensionless constant that determines the magnetic dipole moment, μ_B is an atomic unit of the magnetic moment called *Bohr magneton* and H_0 is the magnetic resonant field.

It is interesting to note that the spin of the electron may have its resonance condition altered in the presence of a nuclear spin that provides an additional magnetic field to the system due to its magnetic moment. The presence of this new magnetic field will create additional energy states, once forbidden, giving rise to spectra whose resonance lines can be divided into several components, being in general equivalent to $2I+1$ for interaction with a spin nuclear I (POOLE, 1983; WEIL; BOLTON, 2007; BRUSTOLON, 2009). The EPR spectral lines' width gives the degree of interaction between the unpaired electron and its perturbing nucleus. Such a process is known as hyperfine interaction.

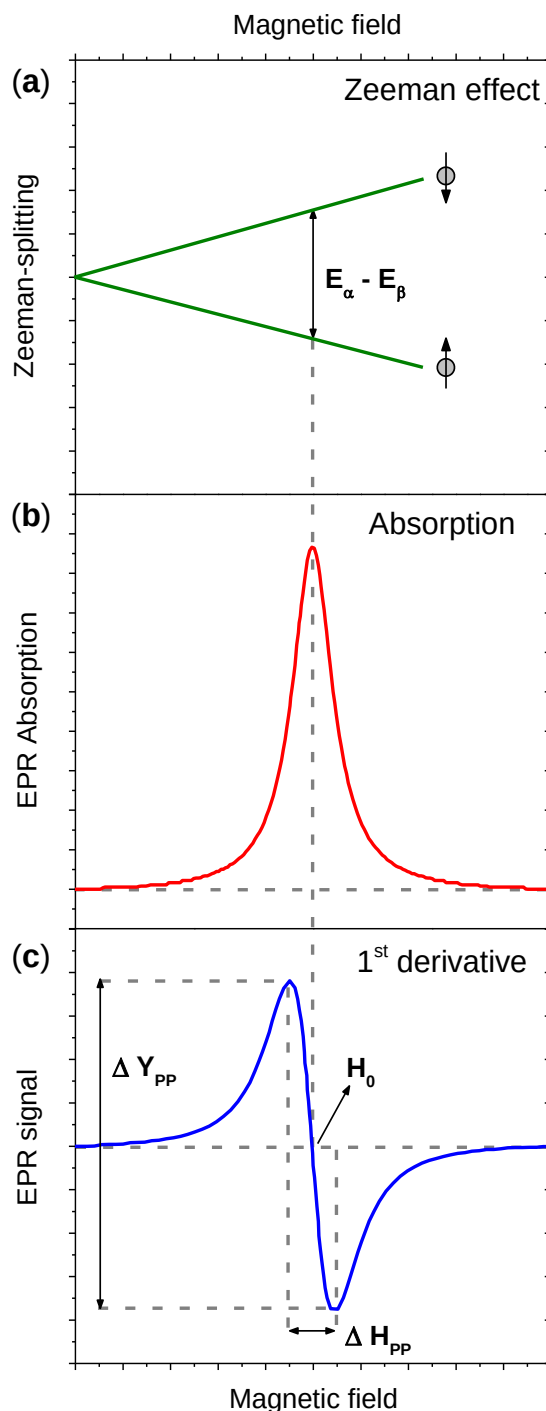


Figure 7. (a) Energy-level scheme of a simple $\frac{1}{2}$ spin system as a function of the magnetic field. (b) Microwave absorption of this system. (c) The derivative of the absorption spectrum (b), which corresponds to the typical EPR signal obtained experimentally.

Significant spin-orbit interactions are present in systems with heavy nuclei or crystals where the atoms have fixed orientations. This interaction will result in a g -value with an anisotropic character; that is, the system will undergo different interactions of the magnetic field depending on the molecule's orientation axis, providing different g -value values for each molecular axis, i.e., $g_x \neq g_y \neq g_z$. However, in many organic materials, paramagnetic systems

have little spin-orbit interaction such that the value of the effective g-value is independent of the magnetic field direction. In this case, when the effective g-value is identical across all axes of the sample, $g_x = g_y = g_z$, it is said to be isotropic (POOLE, 1983; WEIL; BOLTON, 2007; BRUSTOLON, 2009).

As mentioned above, by varying the magnetic field intensity during data collection, the microwaves' absorption is obtained. Experimentally, this absorption results in a Gaussian-like line form shown in Figure 7(b). However, since the measurement is carried out with a modulated magnetic field and a lock-in detection, the signal obtained corresponds to the first derivative of the absorption, Figure 7(c) (POOLE, 1983; WEIL; BOLTON, 2007; BRUSTOLON, 2009). In this way, from the traditional EPR signal presented in Figure 7(c) it is possible to find the main experimental parameters of this technique, which are: i) signal shape, ii) the number of peaks, iii) position of the resonant magnetic field H_0 , iv) the linewidth ΔH_{pp} and; v) the peak-to-peak signal amplitude ΔY_{pp} .

In addition to the experimental parameters, another useful parameter is the spin density that serves to quantify the concentration of spin in a sample. For determination of the spin density, the signal from the studied sample is compared to the standard sample signal, usually of amorphous silicon, containing a known spin number. In this way, the spin density (ρ_N) can be estimated using Eq. 5 where it is considered the area A_{RC} under the resonance curve, a constant C_E specific to each equipment, the temperature T , the modulation amplitude AM and the applied microwave power P (POOLE, 1983; WEIL; BOLTON, 2007; BRUSTOLON, 2009).

$$\rho_N = A_{RC} \frac{C_E T}{AM \sqrt{P}} \quad (5)$$

For a more detailed interpretation of the EPR signals, numerical simulations can be used. Among the various computational tools available, EasySpin package (STOLL; SCHWEIGER, 2006) written in MATLAB® (*MathWorks, Inc*) has been gaining much attention because it is freely available on the internet (STOLL, 2018). This computational package has several high-level functions that separate the resonance lines (g-value, linewidth and intensity) of the paramagnetic species present in the studied systems and thus identify their spectroscopic characteristics. Not only that, it is possible to simulate EPR spectra with isotropic and/or anisotropic g-value, different line forms and linewidths, hyperfine interactions and an arbitrary number of electronic and nuclear spins of samples in several physical states (powders, crystals

or liquids) and experimental conditions (continuous or pulsed wave, low or high temperature) (STOLL; SCHWEIGER, 2006).

2.2.5.2 High-Field/High-frequency EPR

For disordered materials, standard X-band EPR spectra has limited resolution and the broad lines hides information on molecular orientation and magnetic parameters (MÖBIUS; LUBITZ; SAVITSKY, 2013). Hence, the use of higher microwave frequencies and higher magnetic fields has important features:

- i) *enhanced spectral or g-value resolution*: g-values are directly proportional to the EPR spectrometer microwave frequency. Hence, a higher external Zeeman field will be able to increase the resolution of the g-values in relation to the hyperfine coupling (Figure 8), since it can separate the field-dependent spin interactions from the field-independent ones.

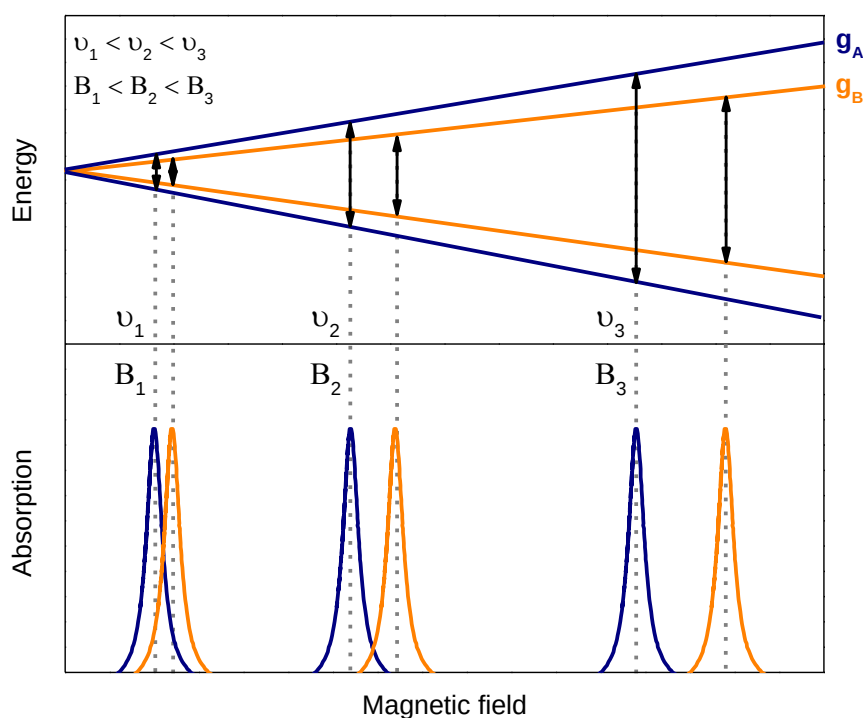


Figure 8. Enhanced spectral resolution by HFEPR of a two spins system with isotropic lineshapes and similar g-values. At ν_1/B_1 the two EPR lines are almost completely overlapping; as ν_i/B_i increases, the two lines gets more separated.

- ii) *enhanced orientational selectivity in disordered samples*: materials with different spin-orbit can yield different response with respect to the Zeeman-field. Hence, as the external magnetic field is increased, a specific orientation relative

to high field is obtained, which enables differentiate the g-tensor anisotropy (Figure 9), i.e., the orientation of their main axes within the g-matrix system.

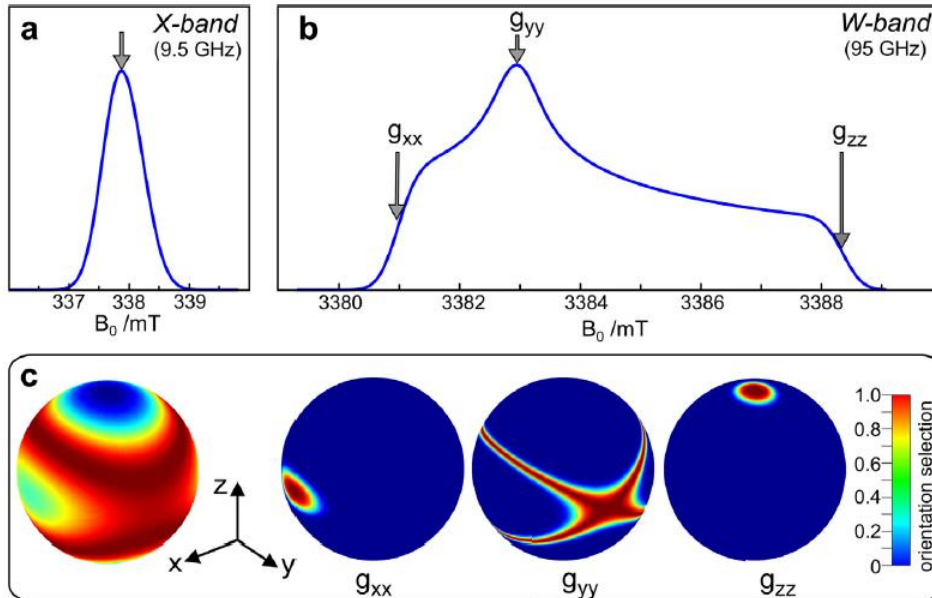


Figure 9. Enhanced orientational selectivity by HFEPR. Reprinted with permission from from (MÖBIUS; LUBITZ; SAVITSKY, 2013). Copyright© 2013 Elsevier B.V.

- iii) *enhanced detection sensitivity for restricted-volume materials;*
- iv) *enhanced sensitivity for probing fast motional dynamics;*
- v) *enhanced low-temperature electron-spin polarization.*

For more details, please, see (BENNATI; PRISNER, 2005; MÖBIUS; SAVITSKY, 2008; MÖBIUS; LUBITZ; SAVITSKY, 2013; ROESSLER; SALVADORI, 2018).

2.2.6 DC conductivity measurements

Direct current (DC) conductivity measurement is the most straightforward and essential step for materials' electrical characterization. In a DC conductivity measurement, the rate at which all movable charges flow in a material is measured as a function of a potential difference between the electrodes. It is commonly measured as current as a function of voltage (J vs V) and can give valuable insight into the nature of electrical conduction through a material or electronic device.

2.2.7 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is a versatile and powerful technique used to investigate the carrier's dynamic processes, in bulk material or its interface with other

systems (BISQUERT et al., 1998, 1999; BISQUERT; COMPTE, 2001; BISQUERT; GARCIA-BELMONTE; PITARCH, 2003; LVOVICH, 2012; LASIA, 2014).

The EIS general approach is to apply an oscillating electrical stimulus to the electrodes and observe the response; it consists of measuring an AC electric current at an angular frequency when an AC voltage is applied to the system or vice versa (LVOVICH, 2012; LASIA, 2014). Typically, the impedance is measured by sweeping the angular frequency over a range of Hz (or mHz) values up to MHz while maintaining the constant amplitude of the voltage or current.

System parameters such as resistances, impedances and frequency-dependent responses are represented using the Bode and Nyquist plots, Figure 10. While Bode shows the impedance $Z(\nu)$ and angle of phase $\theta(\nu)$ as a function of frequency (ν), Nyquist represents the real $Z'(\nu)$ and imaginary $Z''(\nu)$ components in a complex plane. The latter's advantage is the possibility of obtaining electrochemical parameters such as solution resistance, contact resistance, electrode bias resistance, and double-layer capacitance (LVOVICH, 2012; LASIA, 2014).

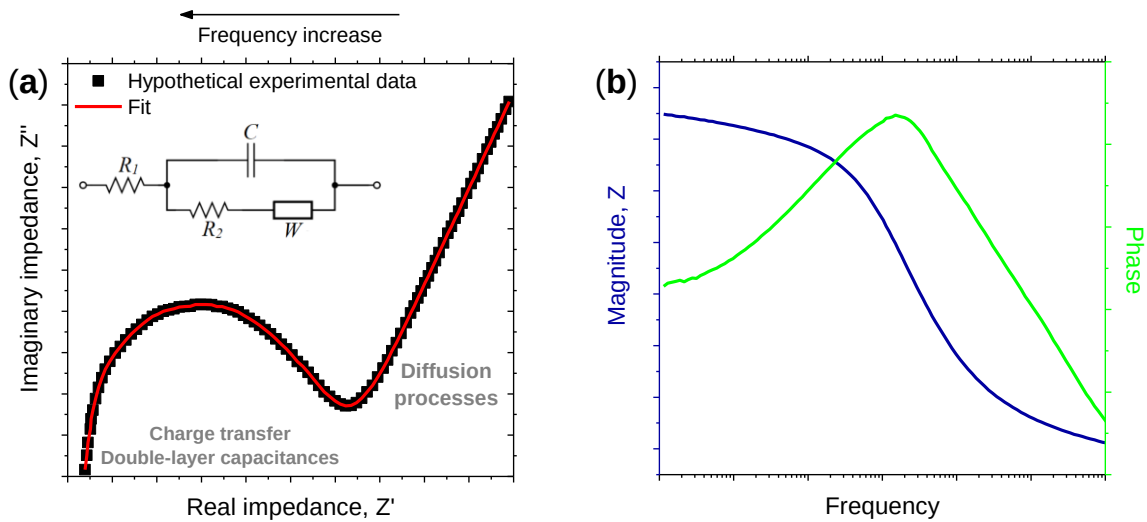


Figure 10. A typical example of (a) Nyquist and (b) Bode plots. The equivalent circuit inserted in the Nyquist plot represents the model used to fit the data.

For data analysis, the Nyquist plot is modeled in an equivalent circuit to study the system's specific physical model (LVOVICH, 2012; LASIA, 2014). Figure 10(a) displays a simple example of a Randles circuit. The equivalent circuit of such a system must contain at least four different parameters: a resistor (R_1) connected in parallel with a capacitor (C) and another resistor (R_2) in series with a Warburg element (W). The semicircle is a feature that appears due to the resistor and capacitor in the equivalent circuit to represent the occurrence of charge

transfer and its diameter is equal to the polarization resistance. Additionally, the tail of the graph represents diffusion processes and is related to the Warburg element. Also, the intersection of the high-frequency region in the real axis of the plot is used to obtain the solution's resistance.

2.2.8 Adsorption isotherm

Whenever a solid is exposed to a liquid or gas, molecules, atoms or ions increases the density of an interface (ROUQUEROL et al., 2014; THOMMES et al., 2015). This phenomenon is called adsorption and can be considered an interaction between the adsorbent (solid material on which adsorption occurs) and an adsorptive (adsorbable substance in the fluid phase). For a gas/solid system, as in the present case, it will happen outside the solid structure and in the vicinity of the solid surface (ROUQUEROL et al., 2014; THOMMES et al., 2015). There are two kinds of forces that dominate adsorption: chemisorption and physisorption (physical adsorption). The essential difference between these two types is that in the first case, there is some chemical bonding involved to the reactive parts of the surface to the formation of new compounds and it is confined to a monolayer, whereas the last case is a more general phenomenon with a relatively low degree of specificity and, at high relative pressure such as saturation pressure, the formation of multilayers of adsorbable at the solid/fluid interface (ROUQUEROL et al., 2014; THOMMES et al., 2015).

At a given fixed temperature T , the relation between the amount of gas adsorbed (n^a , in mol) and the solid mass (m^s , in gram) is dependent on the equilibrium pressure (p) and the nature of interaction if the gas-solid system following the relation present at Eq. 6

$$\frac{n^a}{m^s} = f\left(\frac{p}{p^o}\right)_{T, \text{ system}} \quad (6)$$

where p^o is the saturation pressure.

Eq. 6 represents the adsorption isotherm and is the adsorbate retention capacity, i.e., the relationship between the amount of adsorbed by a unit of solid mass and the relative pressure at a specific temperature. Thus, the isotherm shape is the first tool to understand the interaction between the solid chemical structure and the fluid. Brunauer and co-works (BRUNAUER et al., 1940) classified the isotherms according to process and shape and established five different types. However, nowadays, the International Union of Pure and Applied Chemistry (IUPAC) updated its classification to eight different isotherms (THOMMES et al., 2015).

2.2.9 Quasi-elastic neutron scattering

Quasi-elastic neutron scattering (QENS) is a powerful technique used to study the dynamic processes of atoms or ions within soft matter, including complex biological systems, covering length scales around inter and intramolecular distances, about 1-30 Å, and time scales in the range of 10^{-13} - 10^{-9} s (JOBIC; THEODOROU, 2007; SAKAI; ARBE, 2009; CAVAYE, 2019). Therefore, it is suitable to study dynamic processes that can be important in developing an organic device, such as ionic diffusion and polymer relaxation. Additionally, QENS can be viewed as a complementary characterization for atomic/molecular diffusion to dynamic light scattering, X-ray photon correlation spectroscopy, nuclear magnetic resonance, tracer diffusion, and dielectric spectroscopy because none of this technique can provide detailed information about transport mechanisms in the condensed materials (SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; SAKAI, 2013; TYAGI; CHATHOTH, 2016).

In QENS, the length scale of the motions is given by the scattering vector $Q = 4 \pi \sin(\theta_{NS}) / \lambda_N$. Q is the momentum transfer of neutron ¹, θ_{NS} is the scattering angle and λ_N is the neutron wavelength (EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016). In most cases, the instrument measures the scattering over a range of angles that corresponds to $0.1 \text{ \AA}^{-1} < Q < 3.0 \text{ \AA}^{-1}$, i.e., 2 Å - 60 Å in real space, well suited for local atomic and molecular motions characterization. Additionally, the energy of the neutron is in the order of a few meV, being compatible with the activation energy of solid-state excitations, molecular relaxations and dynamic process in soft materials (EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016). Such characteristic implies that when a neutron gain or loses energy, the exchange energy between the neutron and the sample will be a significant fraction of the initial neutron energy, which easy to measure with accuracy. Besides this technique typically does not alter or destroy the samples during or after the experiment, an essential characteristic for biological samples, due to its low energy, compared to X-rays, for instance (TYAGI; CHATHOTH, 2016).

In neutron scattering experiments, the sample is the target of a narrow and well-defined energy (or wavelength) distribution of a cold beam of neutrons. The strong nuclear interaction between the beam and the sample atoms will scatter the neutrons in various directions, changing

¹ It is important to stress that, even though Q is usually referred as momentum transfer, it rigorous corresponds to a wave vector transfer (JOBIC; THEODOROU, 2007).

its energy distribution compared to the initial one. If the time scale of the motions is too slow to the time range of the instrument, the neutrons will scatter elastically with no exchange of energy with the sample; on the other hand, if the time scale of the motions matches instrument resolution, strong interactions are expected (SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016). In this case, the interactions could result in inelastic scattering that is represented as a shift in the scattered peak from the incident energy; or the scattering could appear as a broadening of the incident energy distribution immediately surrounding the elastic peak, Figure 11, referred to as QENS (JOBIC; THEODOROU, 2007; SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016).

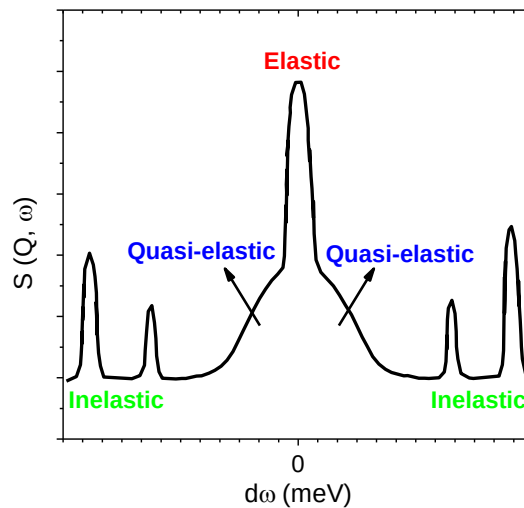


Figure 11. Schematic of a scattering function $S(Q, \omega)$ as a function of energy exchanged $d\omega$ with the representation of elastic, quasi-elastic and inelastic peaks. Inspired by (SAKAI, 2013).

The scattering intensity in QENS experiments is measured as a function of the scattering angle 2θ and the exchange energy $d\omega$ between neutrons and the sample (SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016). Also, it is a combination of incoherent (correlations between the positions of the same atom at different times, i.e., self-motion) and coherent (correlations between the positions of different atoms at different times, i.e., collective motion) contributions that are weighted by the corresponding scattering cross-sections (JOBIC; THEODOROU, 2007; SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-

SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016). The cross-sections of the neutron scattered depend only on the interaction between the neutron and the nuclei.

The intensity of scattered neutrons can be expressed as the double differential cross-section, Eq. 7,

$$\frac{d^2\sigma_{CS}}{d\Omega d\omega} = \frac{k_f}{k_i} \left[\frac{\sigma_{Coh}}{4\pi} S_{Coh}(Q, \omega) + \frac{\sigma_{Inc}}{4\pi} S_{Inc}(Q, \omega) \right] \quad (7)$$

with σ is the scattering cross-section, $d\Omega$ is the solid angle, $d\omega$ is the energy exchange, k_f is the wavenumber of the scattered neutrons and k_i is the wavenumber of the incident neutrons. The subscripts *Coh* and *Inc* indicate coherent and incoherent, respectively. $S(Q, \omega)$ is called the scattering function, or dynamical structure factor, and it contains the dynamic processes information (in Q and ω space) on the sample within the resolution of the experiment (EMBS; JURANYI; HEMPELMANN, 2010; SAKAI; ALBA-SIMIONESCO; CHEN, 2012; TYAGI; CHATHOTH, 2016).

Considering that the hydrogen cross-section is much higher than other elements, Table 3, the scatter intensity signal of protonates samples will be dominated by the incoherent contribution from hydrogens in the sample. Another valuable piece of information in Table 3 is that the cross-section of hydrogen (^1H) and deuterium (^2H or D) is very different. Thus, the substitution of ^1H for ^2H will massively diminish the incoherently scattered intensity enabling the study of isolated dynamics to a particular molecular group or component in a multi-component system by “labeling” a subsystem (SAKAI; ARBE, 2009; EMBS; JURANYI; HEMPELMANN, 2010; TYAGI; CHATHOTH, 2016)

Table 3. Neutron scattering cross-section of elements present in NF-Melanin and S-Melanin.

Element	Atomic number	σ_{coh} (barn)	σ_{inc} (barn)	σ_{Total} (barn)
H	1	1.76	80.27	82.03
	2	5.59	2.05	7.64
C	12	5.56	0.00	5.56
N	14	11.03	0.50	11.53
O	16	4.23	0.00	4.23
S	32	0.99	0.00	0.989

Fonte: (SEARS, 1992)

CHAPTER III

STRUCTURAL COMPOSITION

3.1 INTRODUCTION

Although there is an extensive collection of studies in the literature seeking to determine the physicochemical properties of melanin, its characterization is still considered a challenging task due, at least in part, to the difficulty in accurately studying the structure of this macromolecule (MEREDITH et al., 2006; MEREDITH; SARNA, 2006; D'ISCHIA et al., 2015). However, it is accepted that melanin is a heterogeneous macromolecule arising mainly from the polymerization of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and their various redox forms ortho-hydroquinone (HQ), *o*-semiquinone (SQ*), *o*-(indole)quinone (IQ) and *o*-quinone(imine) (QI) (CHEDEKEL; AHENE; ZEISE, 1992; MEREDITH; SARNA, 2006; D'ISCHIA et al., 2013; PANZELLA et al., 2013). Furthermore, it can also contain, in less concentration, uncyclized and nitrogen-protonated (Ndef⁺) units that are residues of synthetic intermediates and precursors, Scheme 1, and degradation products from the indole ring fission such as pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA) (CÁNOVAS et al., 1982; CHEDEKEL; AHENE; ZEISE, 1992; D'ISCHIA et al., 2013). All of these structures, presented in Figure 12, with the inherent ability to form multiple bonds, account for the melanin chemical heterogeneity. It is necessary to clarify that the presence of Ndef⁺ structure may seem unusual, however it is strongly supported by the necessity of fitting N1s XPS spectra of a series of melanin and melanin-like materials with polaronic species (CLARK et al., 1990; BERNSMANN et al., 2009; BRONZE-UHLE et al., 2016) and with surface enhanced raman scattering and infrared spectroscopy (CENTENO; SHAMIR, 2008).

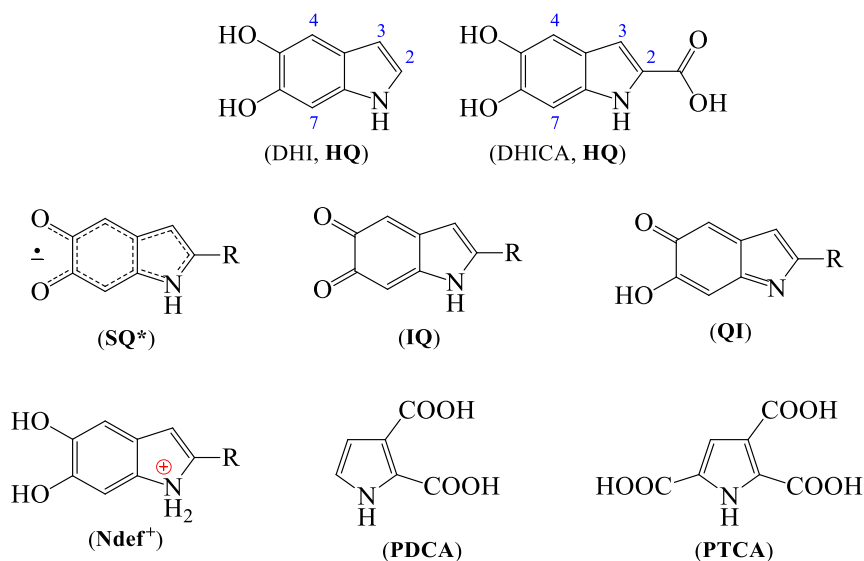


Figure 12. Building blocks of melanin. R = H (for DHI-like species) or COOH (for DHICA-like species).

Remarkably, despite its abundance in nature, as a result of the complexity of melanin and the difficulty in solution or crystallographic structural studies through current biochemical and biophysical techniques, little is known on the macromolecular and supramolecular level of the melanin structure (PROTA, 1992; MEREDITH et al., 2006; MEREDITH; SARNA, 2006; D'ISCHIA et al., 2015).

Initially, based on early chemical studies, it was speculated that melanin was composed of linear indole molecules and/or sheets of indole polymers, with no inherent limit to the sizes of these segments in analogy to graphite (MEREDITH; SARNA, 2006). Nevertheless, from these assumptions, it would be expected that melanin would have a crystalline structure with a lattice spacing of the indolequinone, which is not the case. Melanin seems to have a length scale of order 3-4 Å, usually associated with π - π interactions of an aromatic compound and consistent with π - π stacking (THATHACHARI; BLOIS, 1969; ZAJAC et al., 1994; CHEUN, 2004; SIMON; ITO, 2004). Additionally, melanin presented a higher degree of structural fragmentation, which led to the rejection of this model (CLEMO; DUXBURY; SWAN, 1952; NICOLAUS; PIATTELLI, 1962). Hence, the extended heteropolymer model came to light to update such a paradigm.

The heteropolymer model, proposed by Marsden S. Blois in 1978, declares that melanin should consist of several monomeric building blocks, as mentioned earlier, incorporated into the secondary structure during melanogenesis, and, thus, lead to a more heterogeneous and irregular system (BLOIS, 1978). In this case, the monomers should be linked through different kinds of covalent bonds, yielding a highly cross-linked polymer with large sheets in extent, Figure 13. Considering the multiple available bonding positions of DHI and DHICA structures, it has been proposed that the coupling between different monomers can occur at the 2, 3, 4, and 7 carbon positions, indicated in Figure 12, and the 4-4 is the preferential one (MEREDITH; SARNA, 2006). Such a variety of coupling positions can result in mainly planar oligomers with different structures and sizes (D'ISCHIA et al., 2009). Additionally, in DHICA monomers, due to the presence of the carboxyl group at position 2 there is a decrease in the potential coupling sites leading to the formation of more linear structures with a twist at dihedral angles between the rings to minimize the electrostatic interactions (D'ISCHIA et al., 2009; PANZELLA et al., 2013).

The heteropolymer model also predicts two fundamental physicochemical properties of melanin. In this model, melanin should have an optical absorption resulted from the sum of different chromophores, which can give rises to its black appearance (BLOIS, 1978); an

anticipation of the chemical disorder model demonstrated almost 30 years later. Also, melanin should entrap free radicals produced during melanogenesis in its structure, where they would be permanently stabilized (BLOIS, 1978).

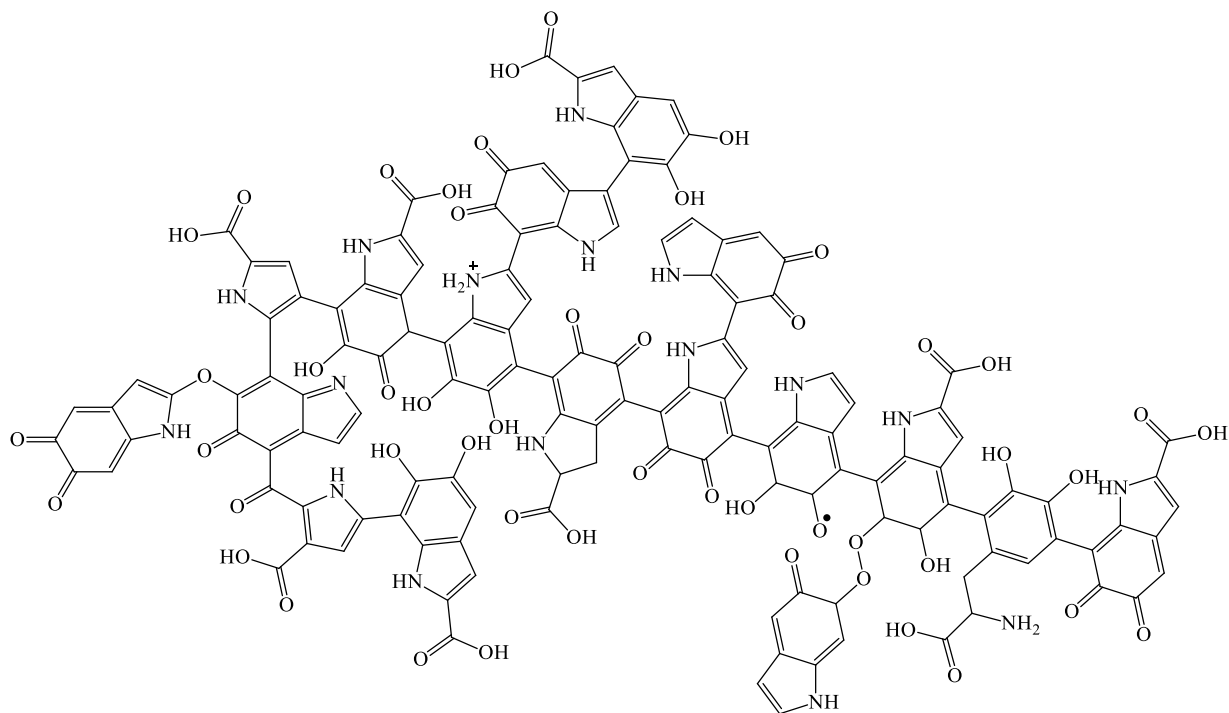


Figure 13. An example of the secondary structure following the heteropolymer model point of view. Inspired by (BLOIS, 1978; PROTA, 1992).

This heteropolymer model was accepted for a long time because the large sheets could explain the melanin's chemical insolubility and describe, at least in parts, its optoelectronic properties (MEREDITH; SARNA, 2006). However, it failed to explain the real space images of melanin structure. Measurements of transmission electron microscopy (ZAJAC et al., 1994; WATT; BOTHMA; MEREDITH, 2009; CHEN et al., 2013), X-ray diffraction (CHENG et al., 1994; CHENG; MOSS; EISNER, 1994; DA SILVA et al., 2004), X-ray scattering (GALLAS et al., 1999) and atomic force microscope (CLANCY; SIMON, 2001) indicates that melanin forms stacked structures, Figure 14. In this new point of view, planar sheets stack via non-covalent bonding (supramolecular assembly) mainly from π - π interaction with a spacing of 3.3-4.0 Å, in a graphite-like manner. These planar sheets should be composed of 4-8 monomers and have dimensions in height and length of approximately 10-20 and 15-20 Å, respectively (KAXIRAS et al., 2006; MEREDITH; SARNA, 2006; D'ISCHIA et al., 2009). However, the possible formation of the extended heteropolymer model during the synthesis process cannot be completely ruled out (MEREDITH; SARNA, 2006).

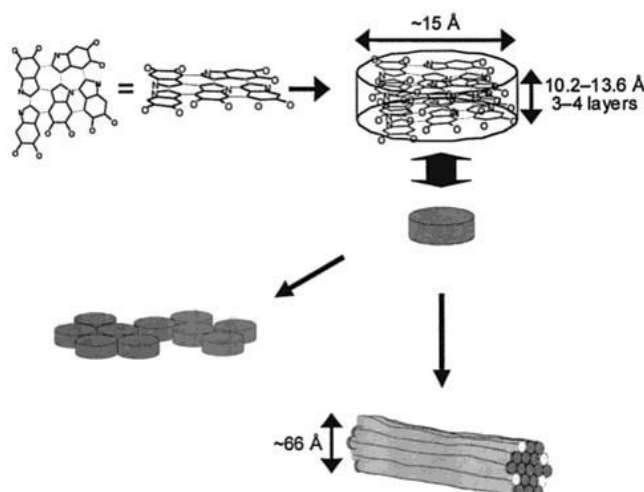


Figure 14. Stacked oligomer model. Reprinted with permission from (CLANCY; SIMON, 2001). Copyright© 2001, American Chemical Society.

The discussion above shows that melanin is a compound with various monomers that results in significant chemical diversity and heterogeneity in a specific sample. In fact, there is a truism in melanin biophysics that “no two melanin particles can ever be made the same” (PROTA, 1992). Consequently, the ratio between carboxylate and non-carboxylate monomers and between the proportion of reduced, oxidized and semi-reduced/semi-oxidized forms will actively be dependent on precursors and reaction conditions. For instance, while the synthetic pigment generally has about 90% of decarboxylated monomers (DHI), *in vivo* melanin obtained from *Cephalopoda* marine mollusks, i.e., sepia-melanin, has a DHICA/DHI ratio higher than 50% (PANZELLA et al., 2013).

The difference in the DHI and DHICA monomers ratio is an essential feature in melanin because it affects its physicochemical and biological properties (PANZELLA et al., 2013; PIACENTI-SILVA et al., 2014b; PAULIN et al., 2018). Not only that, but the degree of protonation is also an essential characteristic since, as shown in Figure 12, melanin contains amine, carboxyl, and phenolic hydroxyl groups that can act as electronic e proton donors or acceptors, a fundamental property for bioelectronics applications. Figure 15 presents an overall view of how the coupling mode of the indole units can affect the aggregation at the supramolecular level, the extent of electron delocalization and paramagnetic and absorption properties.

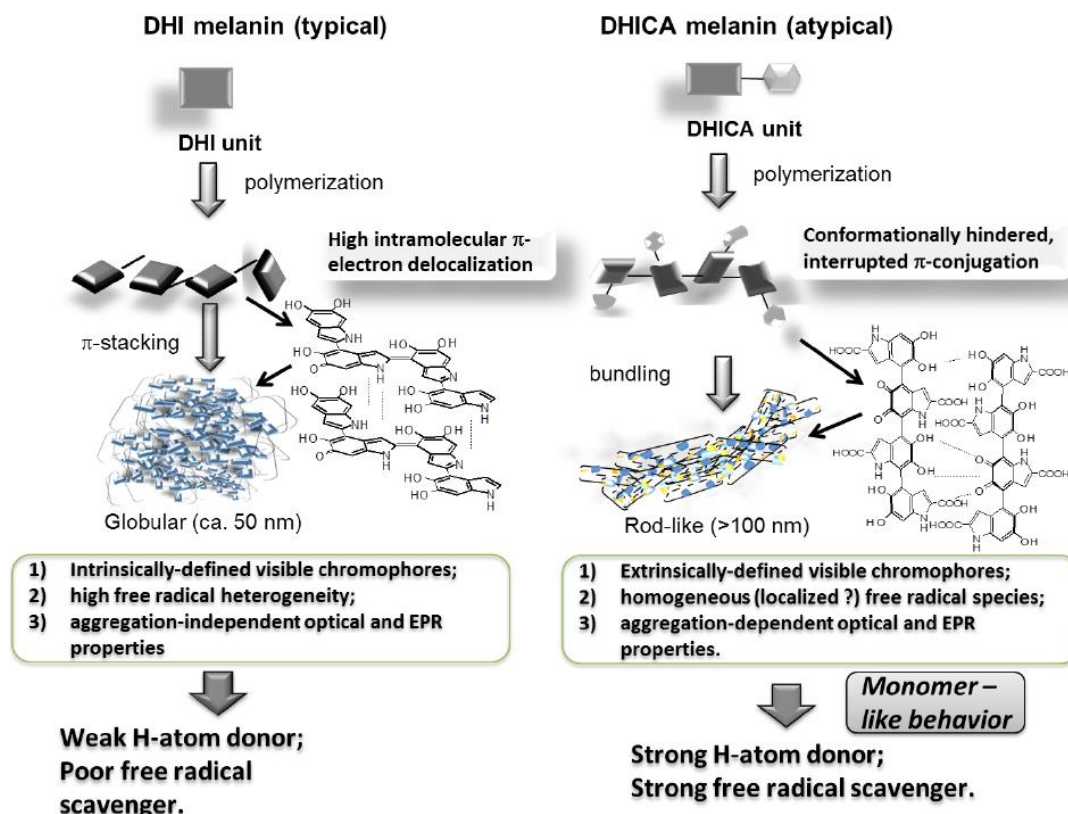


Figure 15. Main differences between the structure-property relationships of synthetic DHI-rich melanin and DHICA-rich Melanin. Reprinted with permission from (D'ISCHIA et al., 2015). Copyright© 2015, John Wiley & Sons A/S.

In this chapter, considering the chemical diversity of melanin, the chemical information of several synthetic melanins will be evaluated and compared. The differences between pressed powder samples, which can be considered as pure melanins, and those spin-coated for technological applications were studied. These latter films are usually spin-coated out of a solvent and there is some question as to whether some chemical modification is occurring during this intermediate fabrication step (WÜNSCHE et al., 2013b).

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Sample preparation

Melanin was analyzed in two different formats: pellet and thin-film. For the pellets, approximately 150 mg of each melanin derivative was crushed to a fine powder and pressed into 13 mm of diameter and 1 mm of thickness using a standard pellet press under 10 tons of pressure for 5 minutes. Afterward, for XPS analysis, the pellets were broken into small pieces with around 0.3 cm².

Melanin thin-films were deposited into glass slides (25 x 25 mm) that were initially cleaned with a soap solution (Alcanox®), rinsed in water, ultra-sonicated in acetone (15 min)

and 2-propanol (15 min). Prior to deposition, the glass was dried with nitrogen flow and treated with UV-ozone (20 min). The films were prepared by two steps spin-coating (1000 rpm for 60 s and 4000 rpm for 30 s) with 30 mg/mL solution. Due to the insolubility of NFMel-I, thin-films are usually made by dispersing the sample in a mixture of distilled water and ammonia (1 H₂O:2 NH₃ %v) (BOTHMA et al., 2008). Hence, 30 mg of NFMel-I were dispersed in 1 mL of such mixture, stirred for 1 hour at 50 °C, ultra-sonicated for another 1 hour, and filtered with a 0.45 µm Hydrophobic PTFE filter (Cole-Parmer). Additionally, as NFMel-S and NFMel-6P are water-soluble samples, a less concentrated ammonia solution was used to decrease the possibility of any structural alterations (WÜNSCHE et al., 2013b; CLULOW et al., 2017). In this case, 30 mg of NFMel-S and NFMel-6P was diluted in 1 mL of a mixture of deionized water and ammonia (3 H₂O:2 NH₃ %v) or 1 mL of deionized water only (1 H₂O:0 NH₃ %v) before following the same procedure from before. For sulfonated melanin derivatives (synthesized in DMSO), 30 mg of the sample were dispersed in 1 mL of anhydrous DMSO (Sigma-Aldrich, ≥ 99.9%), or anhydrous N,N-dimethylformamide (DMF; Sigma-Aldrich, ≥ 99.8%), or anhydrous 1-Methyl-2-pyrrolidone (NMP; Sigma-Aldrich, ≥ 99.5%) stirred for 1 hour at 50 °C and filtered with a 0.45 µm Hydrophobic PTFE filter (Cole-Parmer).

3.2.2 XPS Measurements

The XPS was performed on a Kratos Axis Supra using a 225 W AlK α A-rays with an emission current of 15 mA and equipped with a monochromator. The X-rays typically illuminates a 300 x 700 µm area, with sampling depth being limited by the mean free path of an electron in this energy range to < 10 nm. High-resolution experiments were performed with a pass energy of 40 eV, with a hybrid lens setting, 0.1 eV step size, 1 s dwell time, and up to four sweeps depending on the signal/noise ratio. For depth profiles, XPS spectra were collected after etching by a rastered (3 x 3 mm) Ar₅₀₀⁺ cluster beam for between 0-600 s. At first, we have evaluated the stability of the melanin sample using the depth-profile analysis in NFMel-I and SMel with 5 and 10 kV. By integrating the carbon peak areas, Figure 16 shows an increase in the atomic% for both pellets at 10 kV acceleration voltage, which can be understood as the formation of graphite-like structures. Thus, the result indicates that the samples are unstable at such etching energy. Hence, all the measurements were performed with 5 kV to avoid possible melanin structure degradation. During depth profiling, the excess argon caused pressure in the sample analysis chamber to rise to 3.0 x 10⁻⁷ Torr from the instrument base pressure of 4.4 x 10⁻⁹ Torr. The integral charge neutralizer was used to limit the build-up of any surface charge.

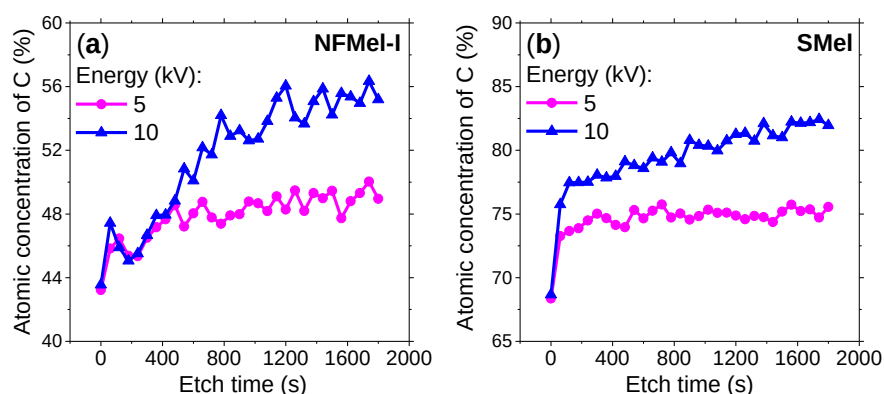


Figure 16. Atomic concentration quantification of carbon C1s in depth of (a) NFMel-I and (b) SMel pellets.

The analysis was performed in CasaXPS (2.3.17dev6.4k) using the Kratos sensitivity factor library. A Shirley background was used, and mixed Gaussian-Lorentzian peaks “GL(30)” fitted to the raw data. After fitting, the x-axis of all spectra was calibrated to the C-C component in the C1s fit.

3.3 RESULTS AND DISCUSSION ²

As mentioned in section 3.1, the structural disorder is well known, and the fact that the stacked oligomeric structure leads to melanin’s well-known insolubility has made its chemical analysis (e.g., simple elemental analysis) a notorious problem (MEREDITH; SARNA, 2006).

One way researchers have dealt with their chemical analysis is to use x-ray photoelectron spectroscopy (XPS) to determine the basic atomic ratios of their samples (CLARK et al., 1990; HONG; SIMON, 2005; ABBAS et al., 2009; BERNSMANN et al., 2009; WÜNSCHE et al., 2013b; ZANGMEISTER; MORRIS; TARLOV, 2013). The ease of use and the ability to skip the use of solvents and bypass the insolubility problem is a significant benefit. Furthermore, melanin is highly hygroscopic (MOSTERT et al., 2010; RIENECKER et al., 2015; CLULOW et al., 2017). Hence using XPS, a technique done under vacuum, will have the bonus of removing the substantial contribution of physisorbed water, which can skew an elemental analysis.

However, XPS is a surface technique, and the question always remains as to whether the spectra observed is an accurate indication of the bulk of the material. To address this primary issue and investigate whether the use of XPS in recent literature has been legitimate, it was

² The results presented in this section were published in Paper V. Reproducible with permission.

investigated the XPS depth-profile of melanin and melanin derivatives to see whether there is indeed a difference between the surface and the bulk composition.

Synthetic Non-Functionalized Insoluble Melanin Powder

Our study begins with the XPS spectra of the pellet sample labeled NFMel-I, a sample made with the standard procedure and accepted as the synthetic version of melanin (D'ISCHIA et al., 2013), which enables us to use it as reference to compare. From the surface high-resolution scans (i.e., time = 0 seconds, Figure 17), we estimate the atomic content for carbon (C1s), oxygen (O1s) and nitrogen (N1s) and compare it to the values we would expect for a non-functionalized poly-DHI (NF-DHI) and non-functionalized poly-DHICA (NF-DHICA) systems. In principle, the synthetic melanins should roughly be in between the aforementioned polyindoquinone systems. The atomic percentages and ratios are shown in Table 4 (first three rows) for the pellet surface. The data in Table 4 were renormalized to exclude the pellets contaminants of sulfur (S2p), calcium (Ca2p) and silicon (Si2p) (see Table A1, in Appendix A, for each atomic composition) from syntheses solvents impurities and sample handling.

Table 4. The atomic composition (%) and atomic ratios determined from pressed powder pellets of standard synthetic melanin samples.

Samples	C1s	O1s	N1s	C/N	O/N	C/O
NF-DHI (Theoretical)	72.7	18.2	9.1	8	2	4
NF-DHICA (Theoretical)	64.3	29.6	7.1	9	4	2.2
NFMel-I (Pellet surface)	69.1	22.6	8.2	8.4	2.7	3.1
NFMel-I (Pellet bulk)	69.7	19.2	11.1	6.3	1.7	3.6

By comparing the C/O/N contents, it suggests that these samples are a combination of DHI and DHICA (in fewer amount) monomers. Hence, at the moment, the surface scan result does appear to validate the XPS method for elemental analysis. However, as indicated in the introduction, the surface may still not represent the bulk. To this end, we inspect the resulting depth profile spectra (Figure 17(a-c), time > 0 s).

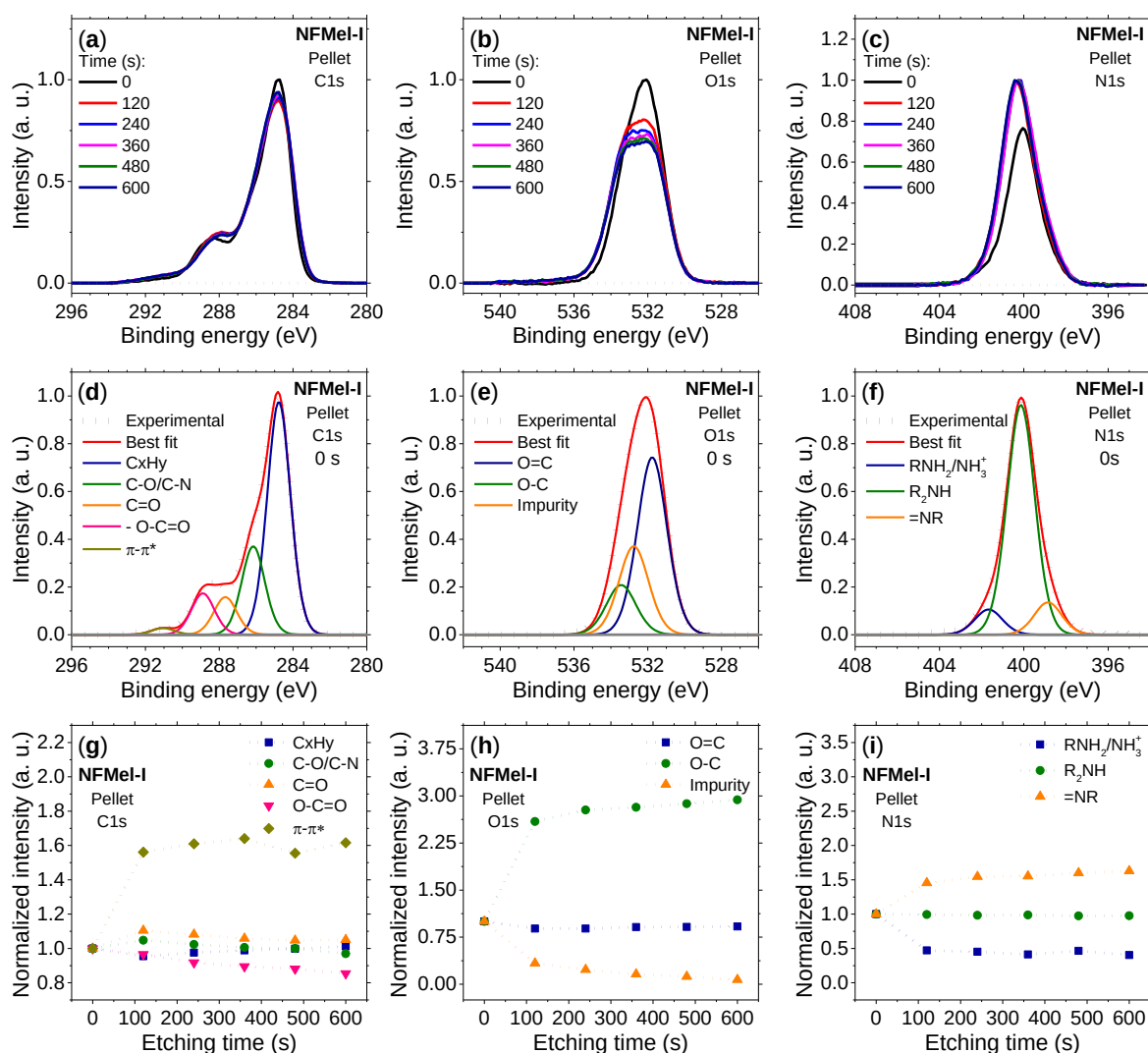


Figure 17. (a-c) High-resolution XPS depth profiling spectra of NFMel-I pellet. The legends indicate the time since start of depth profiling. 0 seconds is equivalent to a standard surface scan, whereas 600 s (after five etching procedure with 5 kV energy during 120 s each) is considered the pellet bulk. (d-f) High-resolution XPS spectra simulation for a surface pellet of NFMel-I. The same modeling was applied to the depth profile spectra. (g-i) Functional group distribution as a function of etching time normalized by the surface intensity.

The carbon spectra, Figure 17(a), shows minimal changes with etching, which demonstrate that the underlying carbon chemistry is unchanged between the surface and the bulk. However, one may see that the intensity of O1s spectra, Figure 17(b), decreases of about 9.4%, in area, while N1s, Figure 17(c), increase about 38.8%. The difference in the oxygen spectra could be understood as the removal of water trapped within the macromolecule or the removal of surface impurities. Given that the reported binding energies for H₂O can range from ~533 – 535 eV with outliers to 538 eV (see example in references (WAGNER; ZATKO;

RAYMOND, 1980; LUNDHOLM et al., 1986; HERMAN et al., 2003)), the removal of water is an unlikely reason. Hence, we ascribe the change to the removal of oxygen impurities.

Regarding the nitrogen behavior, the binding energy is commensurate with that of indole nitrogen, as has been observed for melanin and melanin-like materials (CLARK et al., 1990; HONG; SIMON, 2005; ZANGMEISTER; MORRIS; TARLOV, 2013). Therefore, we suggest that the indole nitrogen on the surface is a slightly different chemical species to that which is seen in the bulk, but both are indoles. In fact, the depth profile spectra in Figure 17(c) show a subtle shift to higher binding energy (+ 0.2 eV), which could be understood as changes in the nitrogen atom local charge. Such variation suggests a lower electron density, probably due to the possibility of a more systematic opportunity for non-covalent interactions (like hydrogen bonding) given by a packed structure (KERBER et al., 1996).

With the above, we calculated the atomic percentages and ratios of the pellet bulk (i.e., with 600 s of etching) and the results are shown in Table 4 (last row). The data indicate that the bulk is well within the expected values for a melanin sample, barring the nitrogen content only slightly outside the expected range. Therefore, the depth profile data for the bulk confirms that the surface and the bulk chemical composition of the standard melanin are similar enough to that of a surface scan, which validates and confirms previous elemental analysis done via XPS.

However, to make the above even more useful for any future work, we now turn to some peaks modeling. A representative peak simulation of the high-resolution XPS spectra of C1s, O1s and N1s regions at the pellet surface is shown in Figure 17(d-f). The assigned chemical groups with their respective energies are listed in Table A2 and are compatible with those from the literature (CLARK et al., 1990; HONG; SIMON, 2005; BERNSMANN et al., 2009; ZANGMEISTER; MORRIS; TARLOV, 2013; BRONZE-UHLE et al., 2016). The same model was a good representative of these three regions for all depth profiled spectra. The only difference was the atomic composition intensity of each peak shown in Figure 17(g-i).

There are five carbon species assigned to C_xH_y, C-O/C-N, C=O, O-C=O, and π - π^* to fit the C1s region reasonably. The intensity of the primary peaks attributed to C_xH_y, C-O/C-N and C=O species seems not to suffer significant variation with etching time, which indicates that the basic melanin indole structure remains the same from the surface to the bulk. On the other hand, a slight decrease in intensity was observed for O-C=O species, whereas π - π^* satellite showed a significant increase with etching time. The latter can indicate higher strength of extended delocalized electrons from aromatic carbon species in bulk, probably related to the π - π packing in the melanins (HUNTER; SANDERS, 1990; WATT; BOTHMA; MEREDITH,

2009). Moreover, Figure 12 shows that the basic monomers of melanin have quinone oxygen/ketones, hydroxy and carboxyl groups (for DHICA species only). Hence, our attributions of two peaks assigned to O=C and O–C species for the fit of the O1s region are in good agreement with the expected chemical composition. However, these species seem to be consistent across the entire sample with the change in intensities related to the removal of surface impurities, as discussed above. Note that the ratio of ketone/hydroxyl indole groups will not necessarily be one to one due to the structural disorder and inability to precisely control the different monomeric species' oxidation states that build up the samples. The N1s regions were fitted with three different indole nitrogen species. Based on the melanin chemical structure, the main species (R_2-NH) is understood as the pyrrole-like nitrogen from the indole structure, while the imine ($=NR$) functionality as the tautomer quinone imine (QI in Figure 12) and potential non-cyclized structures and polaron species (RNH_2/NH_3^+). Interestingly, in the N1s region, R_2-NH species do not vary with the etching time, which corroborates the above discussion that the basic melanin is not changed from the surface to the bulk.

Synthetic Non-Functionalized Insoluble Melanin Thin-film

As mentioned previously, melanin thin films are an important morphology for device applications and should be investigated. Thin-films for standard synthetic melanins are usually obtained using an ammonia solution to dissolve the initial powder and improve its adhesion. Still, it can also increase the sample's nitrogen content (WÜNSCHE et al., 2013b; CLULOW et al., 2017). Hence, we would like to evaluate if ammonia would significantly affect the chemical information on the melanin sample, i.e., the stability of the indole ring structure and oxidation state. The corresponding atomic percentages and ratios are given in Table 5 and the resultant XPS spectra can be seen in Figure 18(a-c). Again, the results presented in Table 5 were renormalized to exclude sulfur (S2p), calcium (Ca2p), silicon (Si2p) and sodium (Na1s) impurities (see Table A3).

Table 5. The atomic composition (%) and atomic ratios determined from the spin-coated thin-films of standard synthetic melanin samples.

Samples	C1s	O1s	N1s	C/N	O/N	C/O
NFMel-I (H ₂ O+NH ₃ film surface)	61.2	26.8	12.0	5.1	2.2	2.3
NFMel-I (H ₂ O+NH ₃ film bulk)	62.9	22.7	14.3	4.4	1.6	2.8

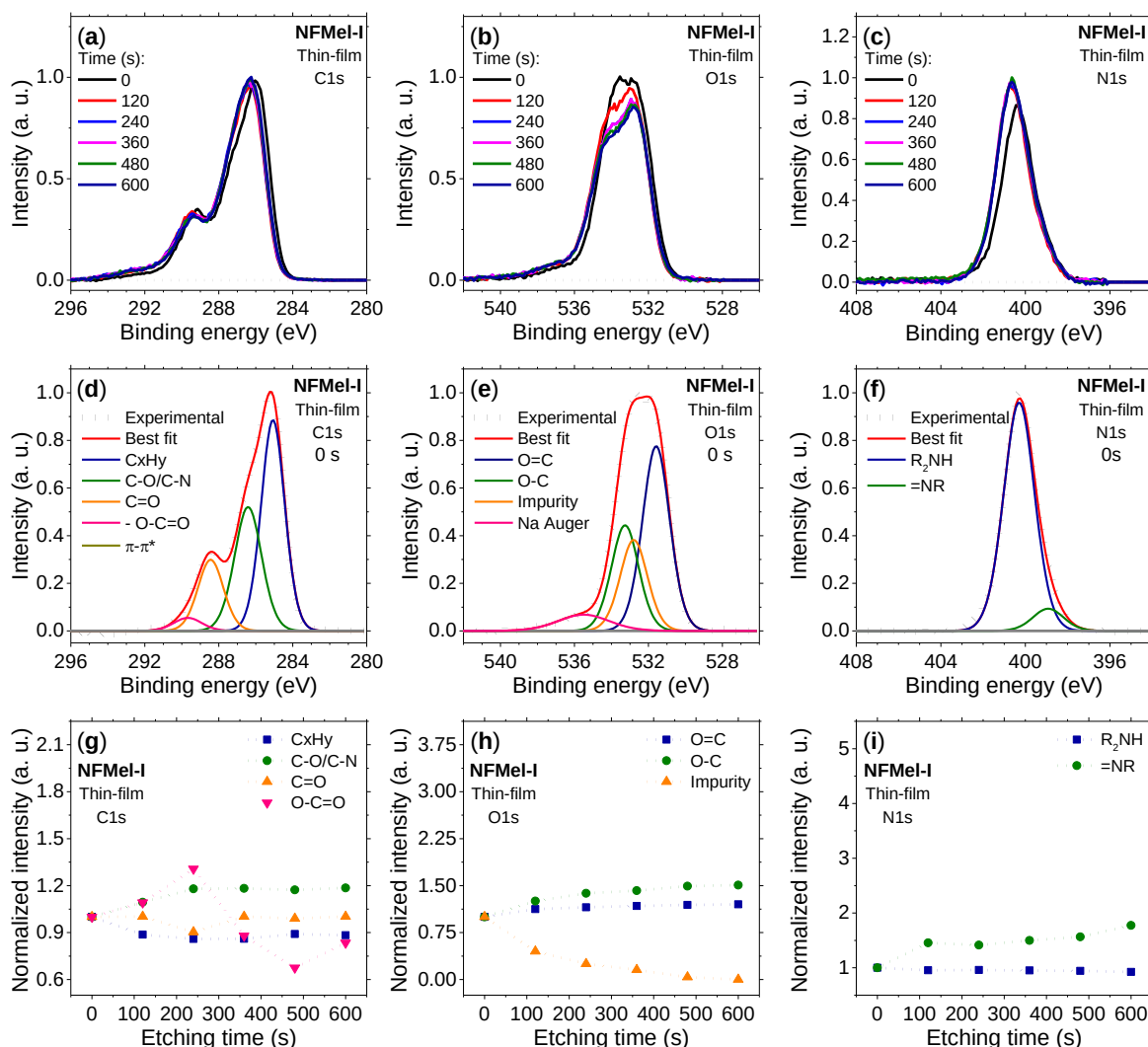


Figure 18. (a-c) C1s, O1s and N1s high-resolution XPS depth profiling for NFMel-I thin-film from standard water and ammonia procedure ($\text{H}_2\text{O}+\text{NH}_3$). Also indicated in the legends is the time since the start of depth profiling. 0 seconds, in this case, is equivalent to a standard surface scan. 600 s (equal to 5 different etching with 5 kV energy during 120 s each) represents the thin-film bulk. (d-f) High-resolution XPS spectra simulation of C1s, O1s and N1s regions for a surface thin-film of NFMel-I prepared using standard ammonia procedure ($\text{H}_2\text{O}+\text{NH}_3$). The same modeling was applied to the depth profile spectra. (g-i) Functional group distribution as a function of etching time normalized by the surface intensity.

The films prepared with the traditional ammonia solutions showed a few divergences to the theoretical values for the surface scans. It is clear that ammonia increases the amount of nitrogen in the film, as shown in a previous study (WÜNSCHE et al., 2013b). As a result, the percentage of carbon and oxygen values is also changed. Such alterations could indicate an adjustment in the melanin stacked oligomer particles' surface exposed to the high oxidative environment. When we turn to the bulk result (e.g., 600 seconds), we see some changes in carbon peaks, some in the oxygens, but in either case, not nearly as much as was observed for

the pellets. It appears that the thin-film processing removed the oxygen impurity, or the pellet press imparted a contaminant, or the films are less conducive to adsorb pollutants from the environment. Again, the nitrogen peak shows a distinct difference, with a shift to higher energy. Furthermore, we see similar behavior for the other thin-film samples, which indicates that the solution process leads to some chemical redistribution by altering the π - π staking strength or changing some of the indole structure's underlying chemistry. However, to gain a deeper understanding of the chemistry involved, especially the nitrogen, we undertook modeling the depth profile results shown in Figure 18(d-i).

The overall depth profile behavior of the thin-films modeling is in accordance with the results from the pellets, nonetheless, with some divergences. Similar to pellet data, C_xH_y, C-O/C-N and C=O contributions do not suffer significant differences with etching. Still, the O-C=O varies differently, probably due to the oxidative environment caused by ammonia. Even though we have considered π - π^* satellite in the simulations, their intensities were negligible compared to the other peaks. This could be related to possible oligomeric sheets' destacking effect caused by water or alkaline solution (HUNTER; SANDERS, 1990; MOSTERT et al., 2013; JU et al., 2016). Also, it is possible to see that the change in intensity of O-C (Figure 18(h)) and =NR (Figure 18(i)) are smaller than what was seen in powder, which could be related to fewer impurities adsorbed in the film as mentioned before. The main difference was the presence of a broad peak at 536.0 ± 0.5 eV that can be attributed to sodium Auger electrons originated from the glass substrates (SAHA et al., 1996) and the absence of the peak related to non-cyclized structures. In any case, it does appear that for the films, the surface and the bulk profiles are much closer to one another than the pellet samples. Therefore, thin-film characterization of the surface scan is an even better approximation of the chemical composition, demonstrating the potential for elemental composition characterization of melanin by XPS.

Synthetic Non-Functionalized Soluble Melanin Samples

In order to evaluate if the above discussion could be applied to other samples, we have considered different soluble, non-functionalized melanin derivatives and soluble sulfonated melanin derivatives (which we will turn to in a little while).

The two water-soluble non-functionalized melanin derivatives chosen to be studied here were obtained by slightly changing each sample's synthetic parameters. These samples have similar structures to the standard one discussed above. Still, they tend to show a higher amount

of carboxylic acid content or, alternatively, an increase in its oxidation state (BRONZE-UHLE et al., 2016). Our study begins with the estimation of the atomic content for carbon (C1s), oxygen (O1s) and nitrogen (N1s) and their ratios for the pellet and thin-film systems. However, we will first focus on the pellet morphology data, as presented in Table 6, which shows the atomic percentages and ratios after excluding the of sulfur (S2p), calcium (Ca2p) and silicon (Si2p) impurities (see Table A1, in Appendix A).

Table 6. The atomic composition (%) and atomic ratios determined from pressed powder pellets of soluble non-functionalized melanin samples.

Samples	C1s	O1s	N1s	C/N	O/N	C/O
NF-DHI (Theoretical)	72.7	18.2	9.1	8	2	4
NF-DHICA (Theoretical)	64.3	29.6	7.1	9	4	2.2
NFMel-S (Pellet surface)	67.6	23.3	9.2	7.4	2.5	2.9
NFMel-S (Pellet bulk)	71.2	17.7	11.1	6.4	1.6	4.0
NFMel-6P (Pellet surface)	65.9	24.5	9.6	6.9	2.5	2.7
NFMel-6P (Pellet bulk)	68.4	20.2	11.4	6.0	1.8	3.4

The XPS C/O/N content of NFMel-S and NFMel-6P present in Table 6 is compatible with the one found for NFMel-I, indicating that these samples are also a combination of DHI and DHICA monomers. To deepen our analysis, we also evaluate the high-resolution depth profiling spectra of NFMel-S and NFMel-6P and they are displayed in Figure 19.

Comparing both the surface (0 s) and the bulk (600 s) of each material, in the same way we did above for NFMel-I, one can also see a decrease in the O1s spectrum area (of about 22.7% for NFMel-S and 21.9 % for NFMel-6P) and an increase in the N1s spectrum area (of 23.9 % for NFMel-S and 12.8 % for NFMel-6P) after the etching. Another feature of N1s spectra is that there is also a slight shift towards higher energy (+ 0.2 eV for NFMel-S and NFMel-6P)

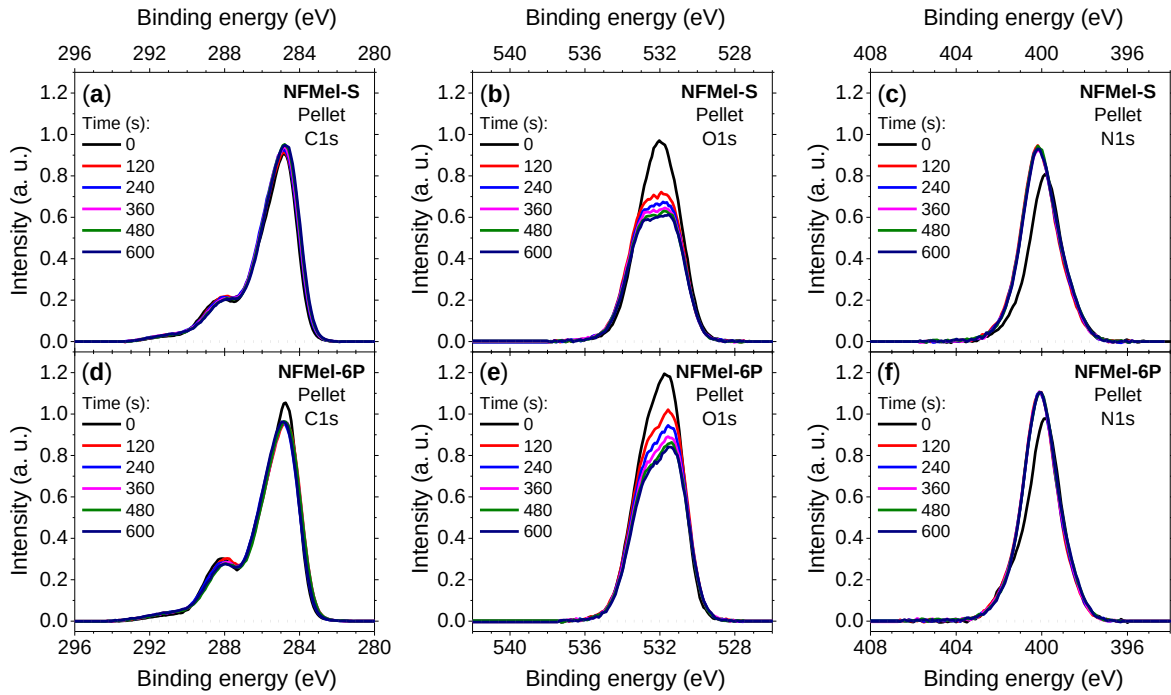


Figure 19. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P pellet (see legend). In the legends, 0 s corresponds to the equivalent of a standard surface scan and 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).

The modeling analysis of these spectra was identical to the NFMel-I for all the C1s, O1s and N1s regions (Figure 17(d-f)). The only difference was the atomic composition intensity of each peak that is shown in Figure 20. The variation of each peak assignment has the same trend as the ones in Figure 17(g-i) for NFMel-I, indicating that the different melanin samples have the same etching behavior. Therefore, such a result implies, again, that a surface scan can be an excellent alternative to obtain the elemental composition of the system with a fair approximation to all modeling peaks for all kinds of “standard melanin”.

We now tackle the thin-film morphologies of NFMel-S and NFMel-6P samples. Initially, our focus was on the atomic composition of the films prepared with water and ammonia mixtures. The results present in Table 7 show the renormalized values to exclude possible film contaminants (see in Table A3, in Appendix A, the composition of sulfur S2p, calcium Ca2p, silicon Si2p and sodium Na1s).

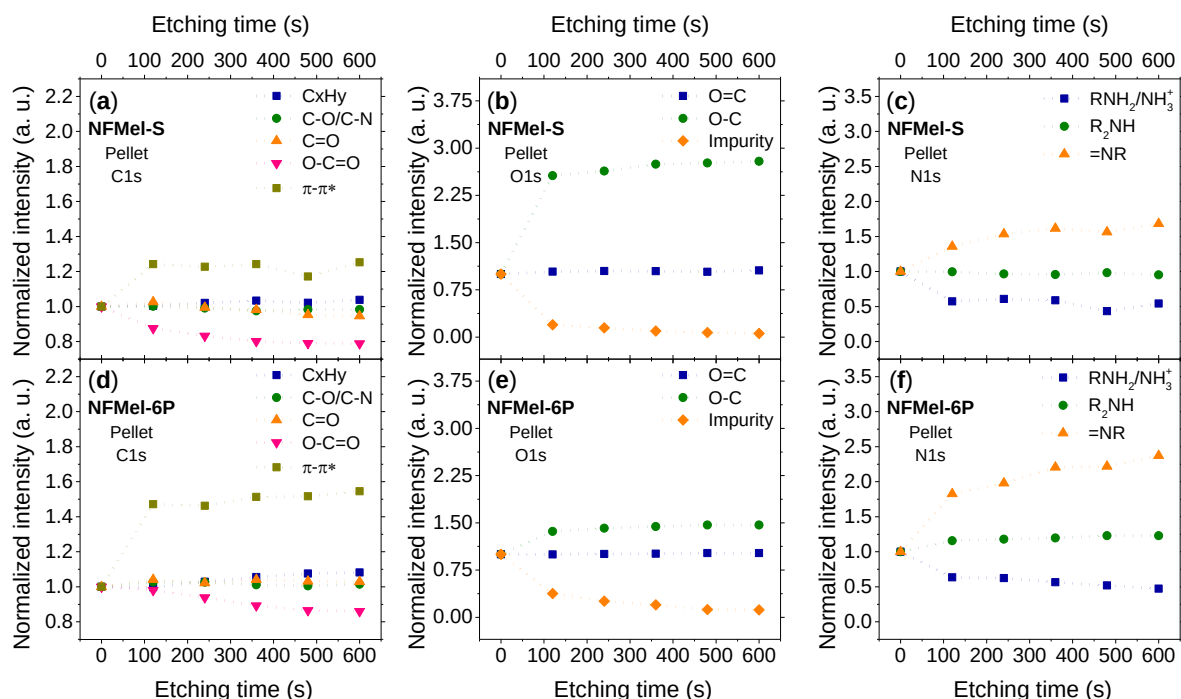


Figure 20. XPS functional groups distribution for NFMel-S and NFMel-6P pellets as a function of etching time. The surface intensity normalized the intensity of each group.

Table 7. The atomic composition (%) and atomic ratios determined from spin-coated thin-films of soluble non-functionalized melanin samples from H₂O+NH₃ solutions.

Samples	C1s	O1s	N1s	C/N	O/N	C/O
NFMel-S (H ₂ O+NH ₃ film surface)	62.3	25.2	12.5	5.0	2.0	2.5
NFMel-S (H ₂ O+NH ₃ film bulk)	60.7	23.3	16.0	3.8	1.5	2.6
NFMel-6P (H ₂ O+NH ₃ film surface)	57.9	29.4	12.7	4.6	2.3	2.0
NFMel-6P (H ₂ O+NH ₃ film bulk)	56.3	27.8	15.9	3.5	1.8	2.0

Once more, the trend we observe for the soluble samples is compatible with NFMel-I. There is an increase in the nitrogen content of the film with a slight decrease in carbon and oxygen content. As we mentioned before, such variation can be related to the remolding of the oligomer particles' surface. However, what caught the most attention was the higher variation of the atomic composition of NFMel-6P in comparison to NFMel-S (that is more or less the same as NFMel-I), which could imply that such a sample is more unstable under a high oxidative environment caused by the ammonia. Therefore, to evaluate the possible effect of the ammonia solution in these samples and understand the chemistry involved, we combine the

high-resolution spectra analysis, shown in Figure 21, with their spectra simulation. The same number of peaks as used for NFMel-I (Figure 17) was necessary for NFMel-S and NFMel-6P, but with differences in intensity shown in Figure 22.

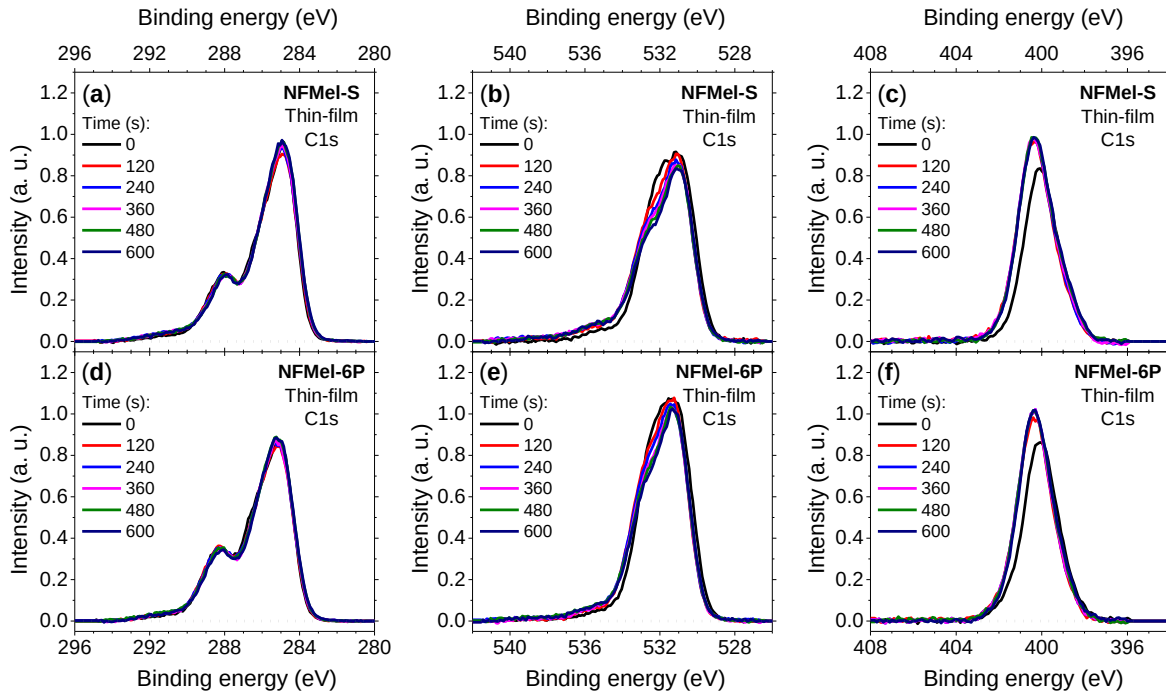


Figure 21. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P thin-films (see legend) following the standard water and ammonia procedure. Time equal to 0 s corresponds to the equivalent of a standard surface scan, whereas 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).

The result presented in Figure 21 and Figure 22 indicates no significant differences between the surface and the bulk of each thin-film samples. The main differences are related to O-C=O and =NR peaks of NFMel-6P that changes with the fabrication procedure or even with etching. Such variation could be a consequence of the higher oxidation state of NFMel-6P that lead to smaller particles (compared to NFMel-S) (BRONZE-UHLE et al., 2016) that are unable to withstand the deposition process and the etching step, or it could be related to the higher amount of oxygen impurity as we can see in Figure 22(b and e).

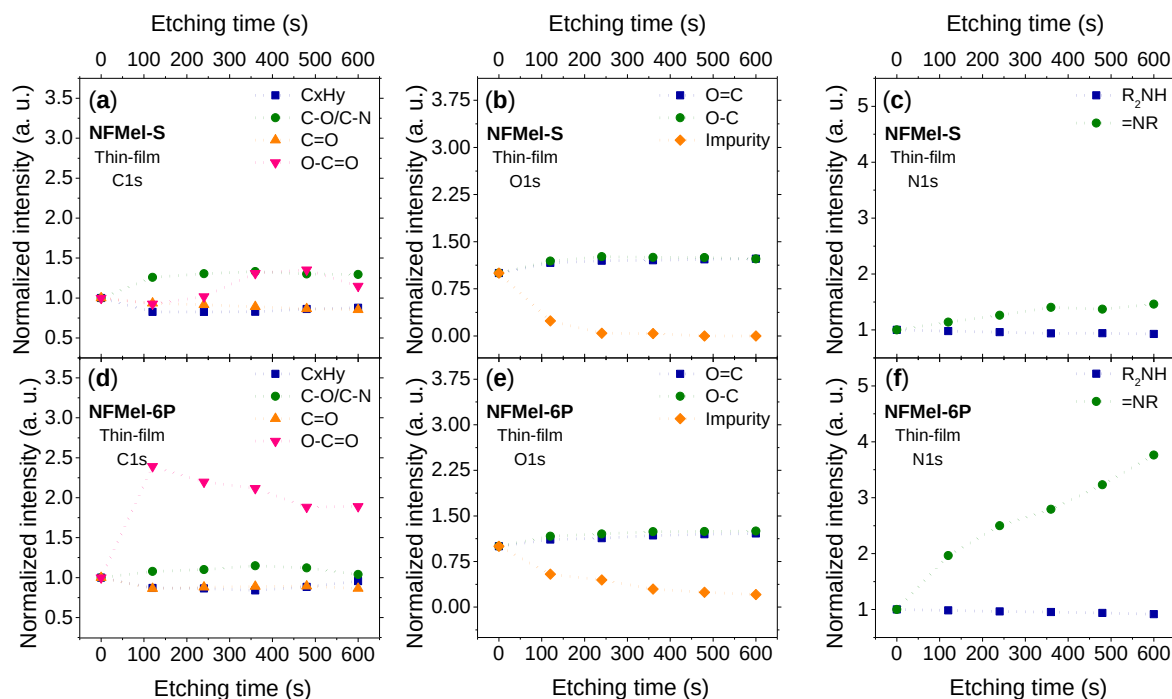


Figure 22. XPS functional groups distribution for NFMel-S and NFMel-6P thin-film from water&ammonia procedure as a function of etching time. The surface intensity normalized the intensity of each group.

Now, we turn to NFMel-S and NFMel-6P thin-film prepared using only water. The atomic percentages and ratios of each sample are given in Table 8 with their resultant depth profile XPS spectra in Figure 23. Table 8 shows the renormalized data to exclude calcium (Ca2p), sulfur (S2p), silicon (Si2p) and sodium (Na1s) impurities (see Table A3, in Appendix A).

Table 8. The atomic composition (%) and atomic ratios determined from spin-coated thin-films of soluble non-functionalized melanin samples from H₂O solutions.

Samples	C1s	O1s	N1s	C/N	O/N	C/O
NFMel-S (H ₂ O film surface)	64.0	25.5	10.5	6.1	2.4	2.5
NFMel-S (H ₂ O film bulk)	60.8	25.7	13.5	4.5	1.9	2.4
NFMel-6P (H ₂ O film surface)	61.1	27.9	11.0	5.5	2.5	2.2
NFMel-6P (H ₂ O film bulk)	60.7	26.4	12.9	4.7	2.0	2.3

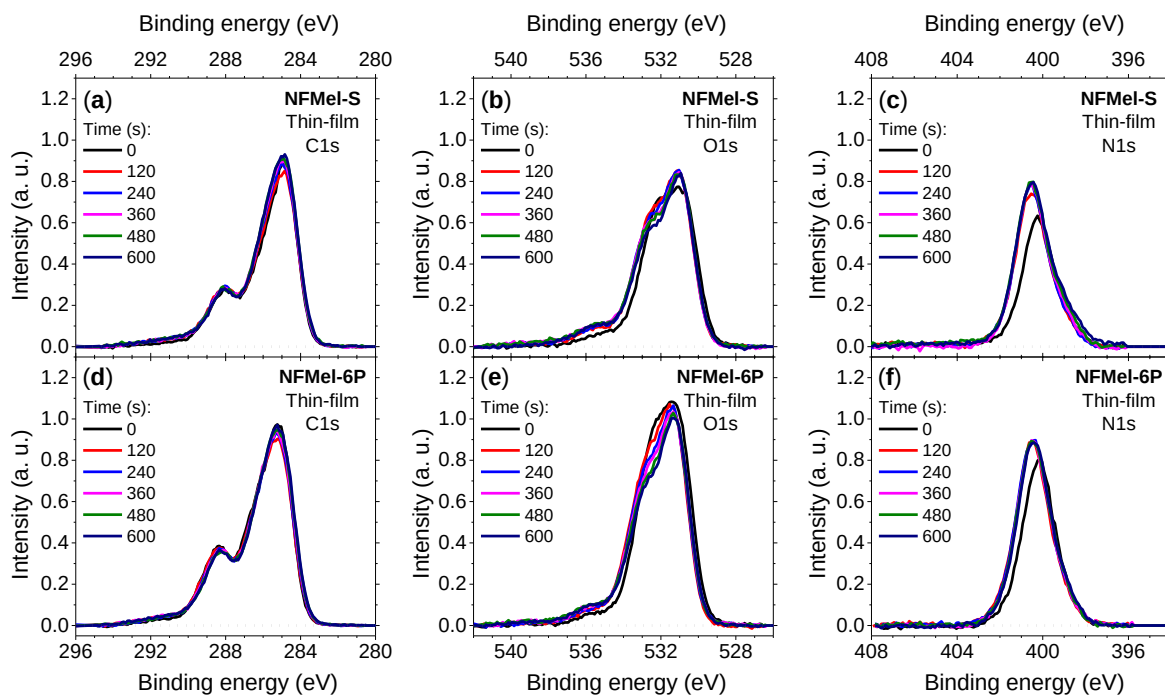


Figure 23. High-resolution (a, d) C1s, (b, e) O1s, and (c, f) N1s XPS depth profiling for NFMel-S and NFMel-6P thin-films (see legend) using the water-only procedure. Time equal to 0 s corresponds to the equivalent of a standard surface scan, whereas 600 s to the system bulk (after 5 different etching with 5 kV energy during 120 s each).

Initially, we had expected that the estimation of the elemental analysis of the H₂O thin-films would be similar to the pellets data (Table 6) because no high oxidation environment was used to prepare the films. However, comparing the data from Table 8 to Table 6 & 7, it appears that the values are within both pellet and H₂O+NH₃ thin-film results. These values may reflect simply sample to sample variation due to the chemical heterogeneity of melanins or that the overall deposition procedure is somewhat affecting the macrostructure organization of the melanin samples. In any case, the atomic values are consistent for the surface and depth profile of the bulk, corroborating that a basic surface XPS scan essentially captures the bulk elemental composition for these materials.

When it comes to the details of the underlying chemistry, the actual XPS profiles in Figure 24, compared to previous data above (Figure 20 and Figure 22), show some differences. For instance, the intensity of the surface C1s peaks change as we go deeper into the sample, but the differences are consistent with all the etching. Such behavior could indicate that the surface chemistry was altered or using water during deposition the surface is more affected by the impurities, which could imply that the ammonia could assist the cleaning of the samples.

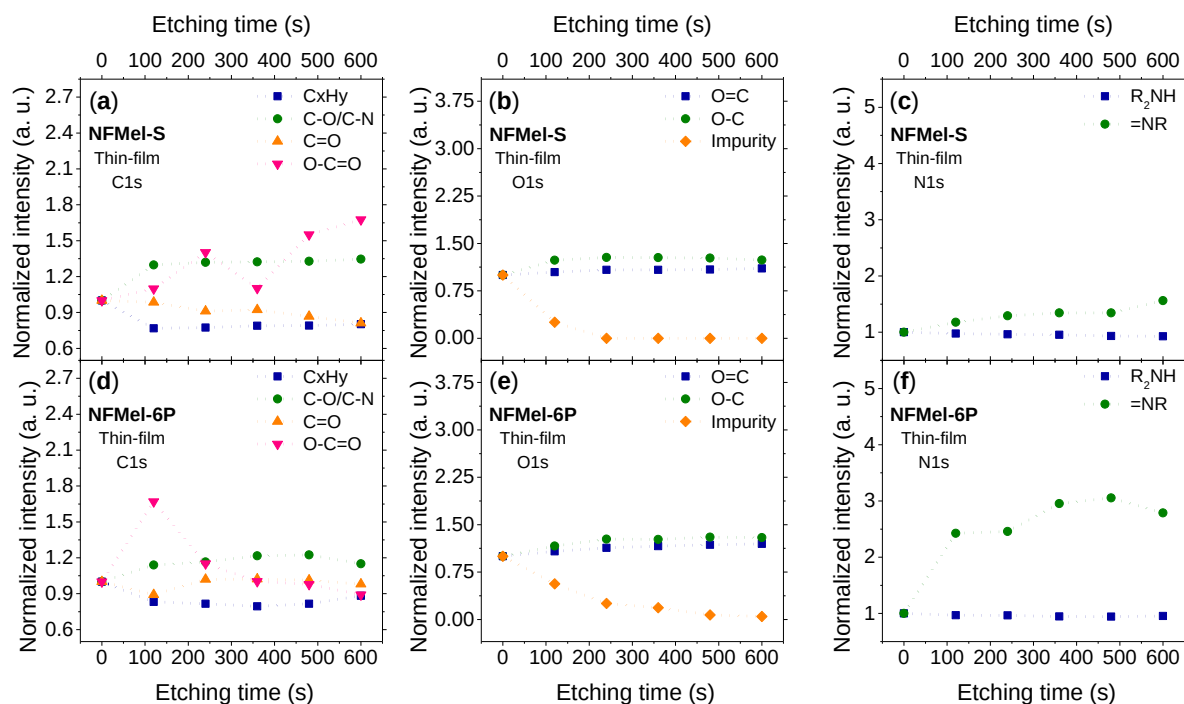


Figure 24. XPS functional groups distribution for NFMel-S and NFMel-6P thin-film from water procedure as a function of etching time. The surface intensity normalized the intensity of each group.

Another interesting result is that the comparing the O-C=O and =NR peaks of NFMel-6P from ammonia solution (Figure 22(d and f)) with only water (Figure 24(d and f)), it is possible to observe that there is no significant alteration in its intensity, implying that the ammonia and the deposition process can influence the chemistry of, at least, NFMel-6P.

In short, NFMel-S and NFMel-6P behave similarly to NFMel-I in all aspects. Therefore, these results corroborate our initial proposal that the chemical composition of both pellets and thin-films (spun out of $\text{H}_2\text{O}+\text{NH}_3$ or just H_2O) surface can be a good approximation of the bulk a particular melanin sample.

Powdered Synthetic Sulfonated Melanin Samples

We now turn our attention to the investigation of other synthetic melanin systems with a more complex structure to see whether the trends observed above continue. The data presented below are on sulfonated melanin that incorporates sulfonated structures (Figure 3) in the basic DHI and DHICA monomer moieties.

The C1s, O1s, N1s, and S2p atomic ratios were obtained for the sulfonated samples and are shown in Table 9. Note that such values do not take into account calcium (Ca2p) and silicon (Si2p) impurities (see Table A4, in Appendix A). For comparison, we also show the theoretical

values expected for sulfonated-DHI (S-DHI) and sulfonated-DHICA (S-DHICA) monomers considering the presence of only one sulfonated group attached in any of the possible positions (Figure 3).

Table 9. The atomic composition (%) and atomic ratios determined from pressed powder pellets of sulfonated melanin samples.

Samples	C1s	O1s	N1s	S2p	C/N	O/N	S/N	C/O
S-DHI (Theoretical)	60.1	26.7	6.7	6.7	9	4	1	2.25
S-DHICA (Theoretical)	55.6	33.3	5.6	5.6	10	6	1	1.7
SMel (Pellet surface)	72.3	22.4	3.5	1.8	20.7	6.4	0.5	3.2
SMel (Pellet bulk)	76.1	17.9	4.3	1.7	17.6	4.2	0.4	4.2
SMel-T (Pellet surface)	69.6	23.2	4.4	2.8	15.9	5.3	0.6	3.0
SMel-T (Pellet bulk)	75.4	16.9	5.2	2.5	14.4	3.2	0.5	4.5
SMel-4P (Pellet surface)	66.1	25.0	5.6	3.4	11.8	4.5	0.6	2.6
SMel-4P (Pellet bulk)	69.7	20.1	7.0	3.2	9.9	2.9	0.5	3.5
SMel-8P (Pellet surface)	67.0	24.1	5.8	3.0	11.5	4.1	0.5	2.8
SMel-8P (Pellet bulk)	70.1	20.1	6.8	2.9	10.3	2.9	0.4	3.5

Based on the elemental analysis shown in Table 9, one may see that the C/O/N/S amount is different from the theoretical considering the proposed sulfonated structure. However, they are compatible with the non-functionalized monomers (see Table 4 to Table 6), suggesting that not all monomer moieties have sulfonated groups attached to them as confirmed by the ~0.5 atomic ratio S/N. The low amount of nitrogen for SMel and SMel-T, which is probably related to the chemical heterogeneity discussed earlier, also catches the attention. However, we cannot completely rule out the possibility that other chemical entities have replaced some of the nitrogen from DL-DOPA before intramolecular cyclization or that the sulfur group could potentially de-stabilize the DL-DOPA nitrogen and remove it from the melanin precursor. In-depth analysis with ^{15}N , ^{33}S and ^1H - ^{13}C nuclear magnetic resonance technique should assist a more precise structural elucidation; however, they are not in the scope of the present study.

As we aim to evaluate if the surface analysis of the sulfonated melanin samples is a good representation of the bulk, we also inspected the depth profile of these samples. Figure 25 shows the C1s, O1s, N1s, and S2p spectra for SMel pellets. The same behavior was found for the other three samples, and their spectra are shown in Figure A1-A3, in Appendix A.

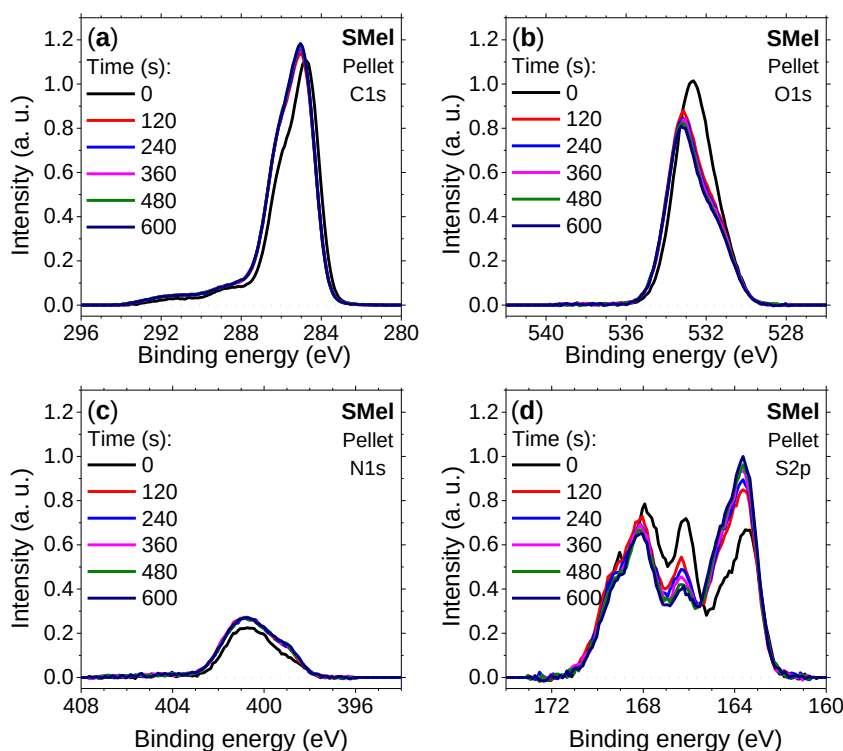


Figure 25. High-resolution (a) C1s, (b) O1s, (c) N1s, and (d) S2p XPS depth profiling of SMel pellet.

By comparing Figure 25 with the non-functionalized melanin pellet spectra in Figure 17 and Figure 19(a-c), it is possible to observe that the overall high-resolution depth profile behaves in the same manner with a decrease in the O1s spectrum area of 15.3 % and an increase of 31.8 % for N1s. The main differences are related to a higher intensity of S2p spectra (with 1.4% variation between surface and bulk) and the slight increase (about 11.3 %) of the C1s spectrum area, which can be attributed to the sulfonated groups attached to the melanin indole ring. Another divergence is the absence of a significant shift of the N1s peak position with etching, in agreement with an earlier preposition that sulfonated melanin has a less densely packed structure (PAULIN et al., 2018).

Here, we also investigate the chemistry involved in this sample using depth-profile modeling, see Figure 26. The assigned chemical groups with their respective energies are listed

in Table A5, in Appendix A. Again, in this case, the same model was applied to the other sulfonated samples. The result is shown in Figure A4, in Appendix A.

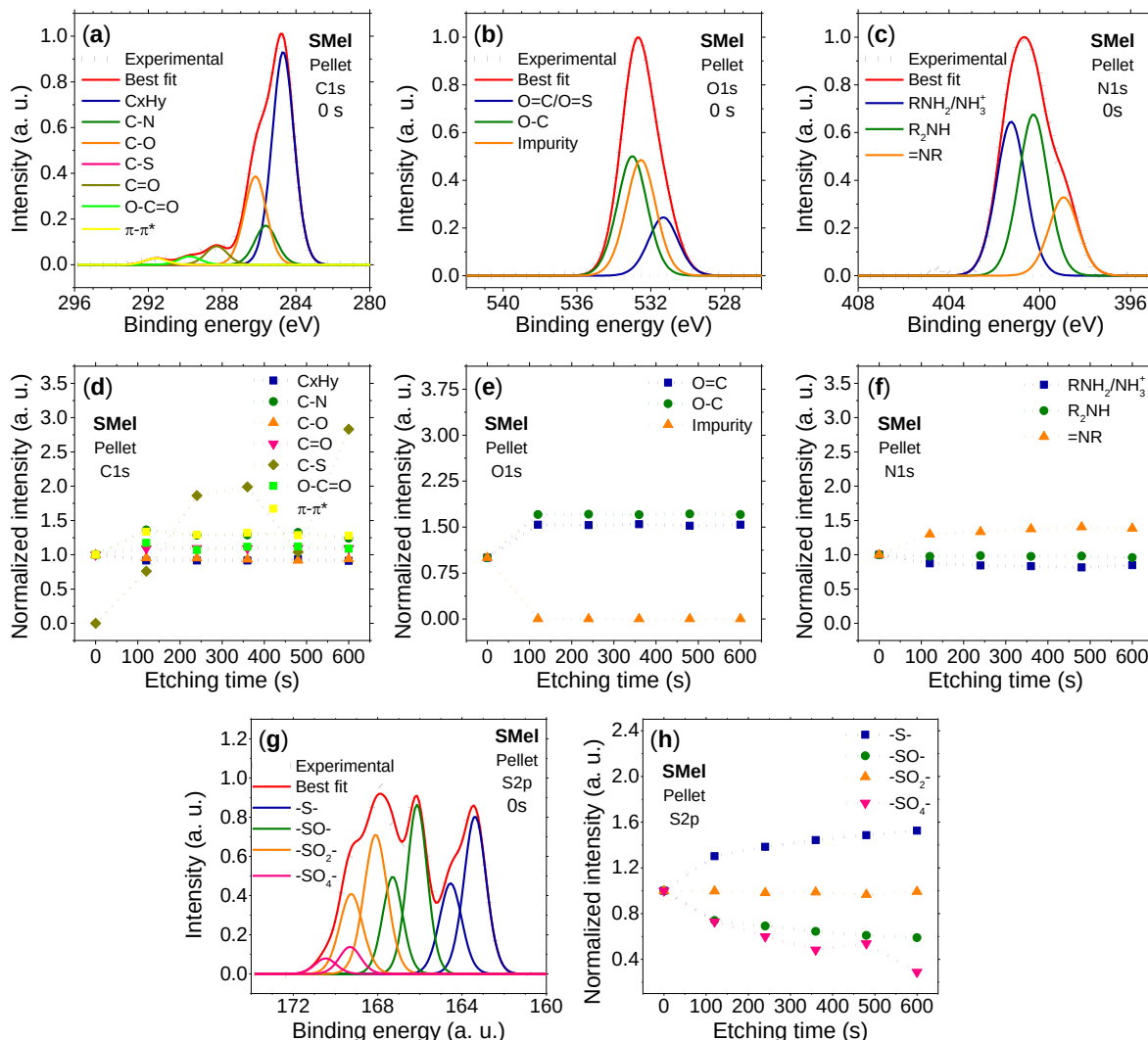


Figure 26. (a, b, c, g) C1s, O1s, N1s and S2p high-resolution XPS spectra simulation for the SMel pellet surface. The same modeling was applied to the depth profile spectra. For S2p high-resolution spectra, high intensity and low binding energy refer to S2p3/2, whereas the opposite to S2p1/2. (d, e, f, h) Functional group distribution as a function of etching time normalized by the surface intensity.

For sulfonated melanin, seven carbon species (CxHy, C-N, C-O, C-S, C=O, O-C=O and $\pi-\pi^*$) were considered to fit the C1s region. As shown in Figure 26(d), the peaks attributed to the basic melanin structure do not vary with etching, indicating that the same system is obtained for both surface and bulk. The only variation was related to the C-S peak associated with the sulfonated groups and DMSO trapped in between melanin layers. In the same way, the oxygen peak was deconvoluted to three components related to O-C and O=C/O=S bonds from ketones,

hydroxyl, carboxyl and sulfonated groups and oxygen impurities absorbed at the surface. N1s region was fitted considering three different nitrogen species: R₂-NH as the main specie related to indole structure, =NR related to imine group and RNH₂/NH₃⁺ from non-cyclized forms. The proposed model is compatible with non-functionalized melanin, which could point to similar systems.

Additionally, note that both O1s and N1s also do not suffer significant variations with etching, indicating high stability of the sulfonated melanin macrostructure. The S2p spectrum shows three significant contributions from -S-, -SO- and -SO₂- that are associated with the sulfonated groups and synthetic DMSO, whereas another component of low intensity and high binding energy was ascribed to -SO₄- from synthetic by-product contamination (PAULIN et al., 2018). Figure 26(h) indicates that there is no high deviation between the surface and bulk of sulfonated melanin. The small variations could be understood with the removal of synthetic impurities in the samples. Therefore, the result here agrees with our previous discussion that we can use a surface analysis to characterize the bulk of sulfonated melanin derivative.

Thin-Film Synthetic Sulfonated Melanin Samples

Thin-films of SMel are usually made from DMSO solution (DA SILVA et al., 2004; DEZIDÉRIO et al., 2004; LORITE et al., 2006; WÜNSCHE et al., 2013b; ALBANO et al., 2016; PIACENTI-SILVA et al., 2016; TEHRANI et al., 2020); however, recently, it has been shown that sulfonated melanin samples are also soluble in solvents like DMF and NMP (PAULIN et al., 2018). Thus, we intend to evaluate each derivative's interaction with these different solvents and verify if the surface of the thin-film is still compatible with its bulk. Hence, Table 10 presents the elemental analysis obtained at each film's surface and Figure 27 their depth profile. In the present case, Table 10 also excludes the impurities of calcium (Ca2p), silicon (Si2p) and sodium (Na1s), see Table A6, in Appendix A.

The values displayed in Table 10 are in agreement with the pellet analysis (Table 9), indicating that pieces of information about the basic melanin structure, i.e., aromatic carbon structure, is not lost, as evidenced by the C1s, O1s, and N1s high-resolution spectra in Figure 27. However, S2p spectra do show small changes. Such variations can not necessarily be related to a fundamental alteration in the melanin structure; instead, it could only be a response of higher concentration of the solvent trapped in between the melanin particles or to some modifications in the sulfonated group due to instability of this group (BRONZE-UHLE et al., 2013). In fact, when analyzing the sample's chemistry information, as evidenced in Figure 28

and Figures A5-A7, in Appendix A, we cannot see higher variations when comparing the surface and the bulk. The more considerable variation observed with NMP solution (Figure 27, Figure 28 and Figures A1-A7, in Appendix A) could only be an indication that the films are not thick enough or not homogeneous so that the glass substrate is contributing to the signal, as evidenced by the higher proportion of impurities in Table A6, in Appendix A. Additionally, note that the thin-films fabrication process of sulfonated melanin also shows the ability to clean the sample, as there was no need to use the peak assigned to synthesize impurities ($-\text{SO}_4^-$) fit the S2p high-resolution spectra.

Table 10. The atomic composition (%) and atomic ratios determined from the surface of spin-coated thin films of sulfonated melanin samples.

Samples	Solvents	C1s	O1s	N1s	S2p	C/N	O/N	S/N	C/O
S-DHI (Theoretical)	#	60.1	26.7	6.7	6.7	9	4	1	2.25
S-DHICA (Theoretical)	#	55.6	33.3	5.6	5.6	10	6	1	1.7
SMel	DMSO	66.1	29.4	3.3	1.1	19.9	8.8	0.3	2.2
	DMF	74.1	22.1	2.9	0.9	25.2	7.5	0.3	3.4
	NMP	61.5	34.0	3.5	1.1	17.6	9.7	0.3	1.8
SMel-T	DMSO	64.7	29.9	3.9	1.6	16.7	7.7	0.4	2.2
	DMF	67.0	26.8	4.3	1.9	15.5	6.2	0.4	2.5
	NMP	60.4	33.4	4.6	1.7	13.2	7.3	0.4	1.8
SMel-4P	DMSO	56.4	34.8	6.9	1.8	8.2	5.0	0.3	1.6
	DMF	66.5	27.3	4.5	1.7	14.8	6.1	0.4	2.4
	NMP	56.5	36.1	5.6	1.8	10.2	6.5	0.3	1.6
SMel-8P	DMSO	57.4	35.1	6.4	1.0	9.0	5.5	0.2	1.6
	DMF	67.7	26.3	4.4	1.6	15.4	6.0	0.4	2.6
	NMP	58.1	33.9	6.1	1.9	9.5	5.5	0.3	1.7

Table 11. The atomic composition (%) and atomic ratios determined from the bulk of spin-coated thin films of sulfonated melanin samples.

Samples	Solvents	C1s	O1s	N1s	S2p	C/N	O/N	S/N	C/O
S-DHI (Theoretical)	#	60.1	26.7	6.7	6.7	9	4	1	2.25
S-DHICA (Theoretical)	#	55.6	33.3	5.6	5.6	10	6	1	1.7
SMel	DMSO	67.8	27.1	4.2	0.9	16.3	6.5	0.2	2.5
	DMF	72.4	22.4	4.3	1.0	17.0	5.2	0.2	3.2
	NMP	8.4	90.9	0.6	0.2	13.5	147.2	0.3	0.1
SMel-T	DMSO	58.0	35.8	4.6	1.6	12.5	7.7	0.3	1.6
	DMF	71.3	21.4	5.4	1.9	13.1	3.9	0.3	3.3
	NMP	62.7	30.3	5.4	1.6	11.7	5.6	0.3	2.1
SMel-4P	DMSO	53.0	38.0	7.5	1.6	7.1	5.1	0.2	1.4
	DMF	74.4	18.9	5.0	1.7	14.7	3.7	0.3	3.9
	NMP	7.5	90.8	1.2	0.4	6.3	75.7	0.3	0.1
SMel-8P	DMSO	55.7	36.5	6.9	0.9	8.1	5.3	0.1	1.5
	DMF	71.3	22.1	4.8	14.7	4.6	4.6	0.4	3.2
	NMP	12.5	85.8	1.6	0.1	7.9	54.3	0.1	0.1

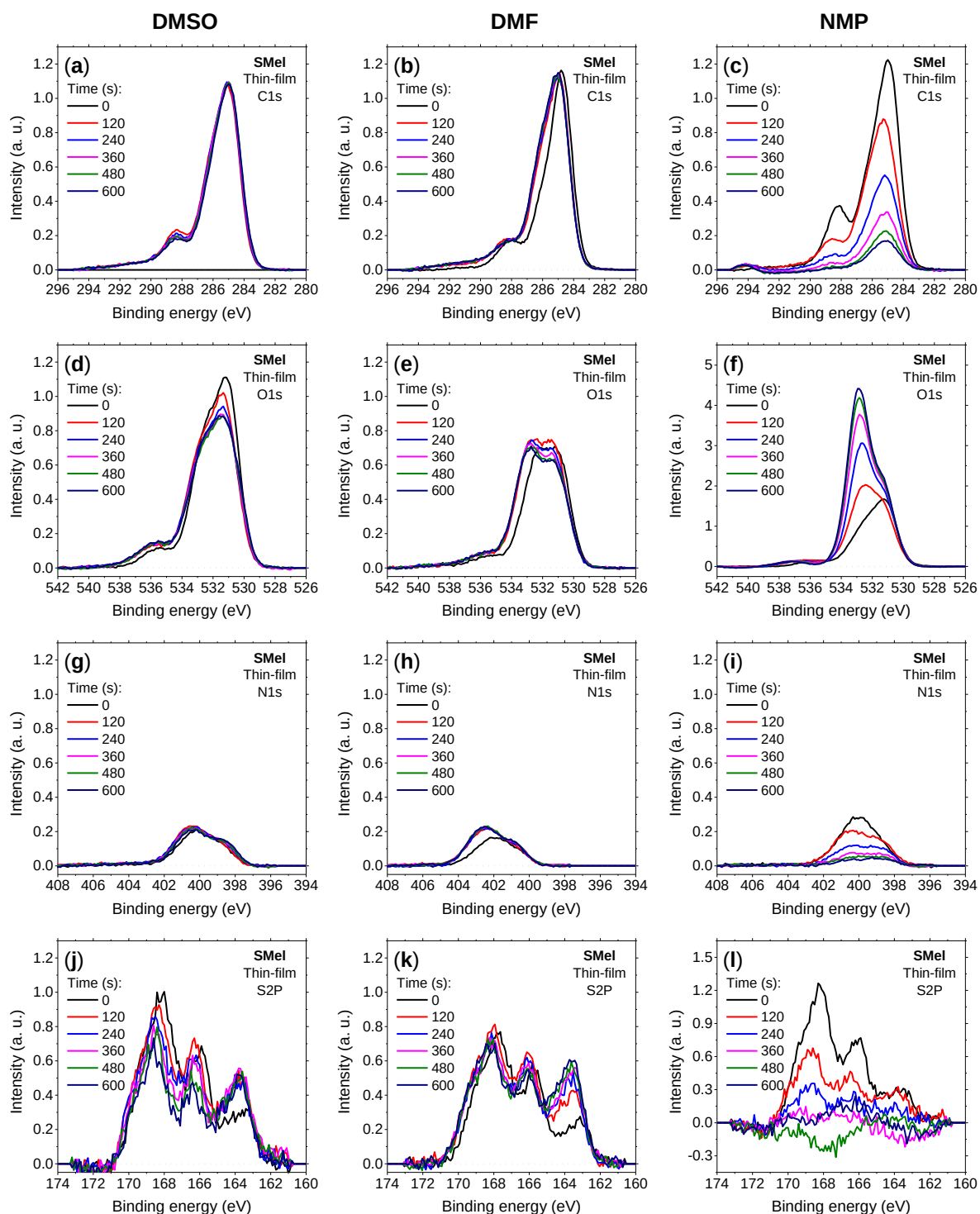


Figure 27. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel thin-film from DMSO (left column), DMF (middle column) and NMP (right column) solutions.

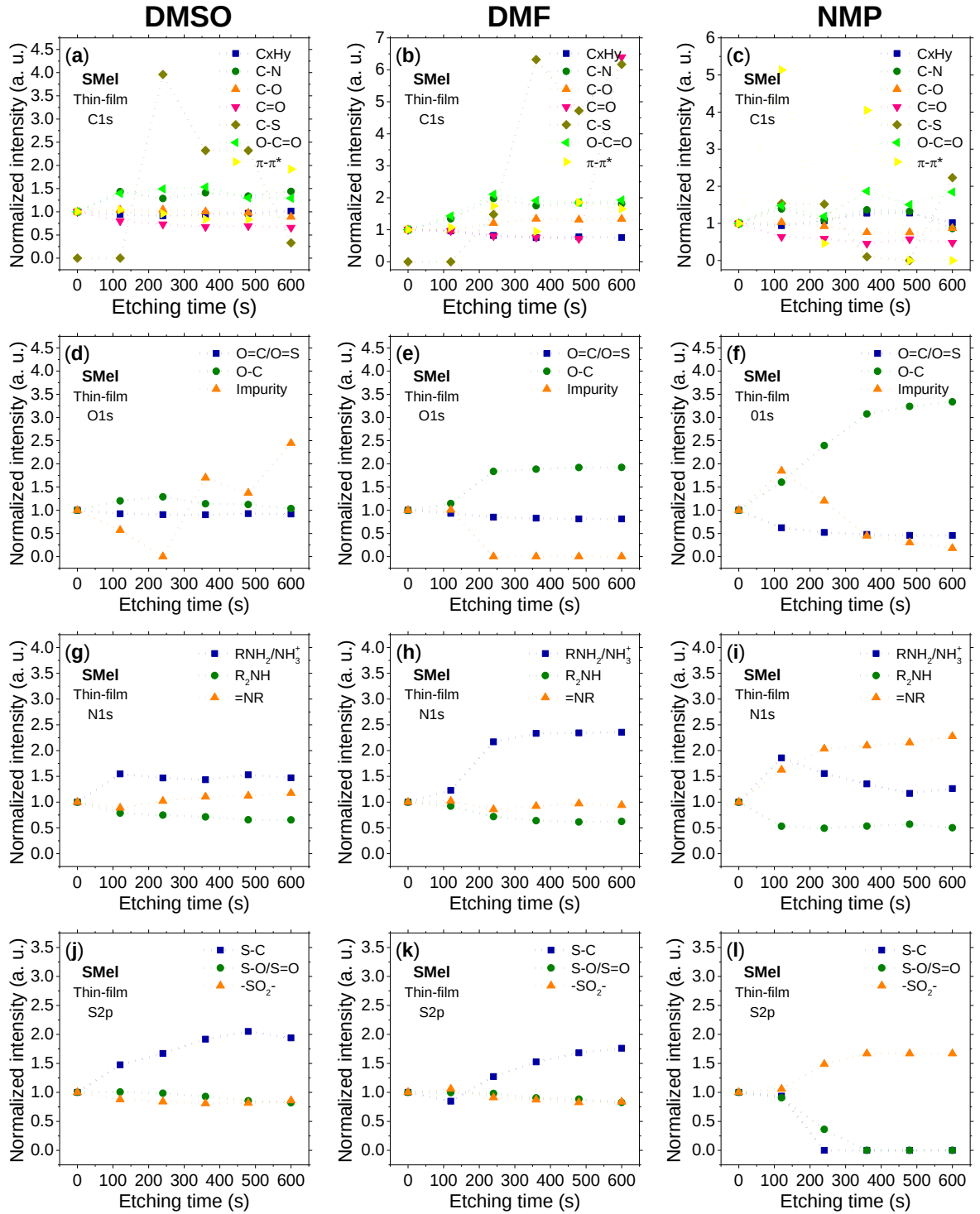


Figure 28. XPS functional groups distribution for SMel thin-film from DMSO (left column), DMF (middle column), and NMP (right column) solutions as a function of etching time. The surface intensity normalized the intensity of each group.

Therefore, based on what we have shown in this section, we have demonstrated that the film deposition process, regardless of which solvent is used, does not change melanin's basic structure. In addition, no significant variation was observed when comparing the surface and

bulk of the sample, corroborating our proposal that a surface XPS scan can be used to obtain the elemental composition of any melanin derivative (with non-metallic atoms) because the surface seems to be a good representation of the bulk structure.

3.4 CONCLUSION

A couple of basic rules can be applied to the study of melanins that do not include metallic atoms from the work presented here. The first is that a surface scan will essentially give the same atomic ratio as the bulk and, therefore, XPS is an excellent tool for elemental analysis. The second rule is that, for standard melanins, there are slight differences in the chemistry of the bulk and the surface. The final rule is for melanin thin-films and all morphologies of the other melanins (soluble and sulfonated), the atomic chemistry of the surface is representative of the bulk as well. Overall, the data shows that the use of XPS as an elemental analysis tool has been placed on a firmer footing and should be part of the basic characterization of the melanin systems.

CHAPTER IV

OPTICAL CHARACTERISTICS OF MELANIN DERIVATIVES

4.1 INTRODUCTION

One of the most unusual characteristics of melanin is its strong featureless broadband optical absorption exponentially decreasing with the wavelength from the ultra-violet to visible and near infra-red spectral regions (Figure 29) that give its black/dark appearance (STARK *et al.*, 2003; MEREDITH *et al.*, 2006; MEREDITH; SARNA, 2006; TRAN; POWELL; MEREDITH, 2006). This behavior differs from a typical organic molecule, which usually presents a peaked profile correspondent to an electronic and vibrational excitation between the ground and excited states (WOLBARSHT; WALSH; GEORGE, 1981), and appears more to an inorganic material (MEREDITH; SARNA, 2006). Also, this feature can often be an indication of an extended π -conjugation structure that has been used to support the amorphous semiconductor property of melanin (MCGINNESS, 1972).

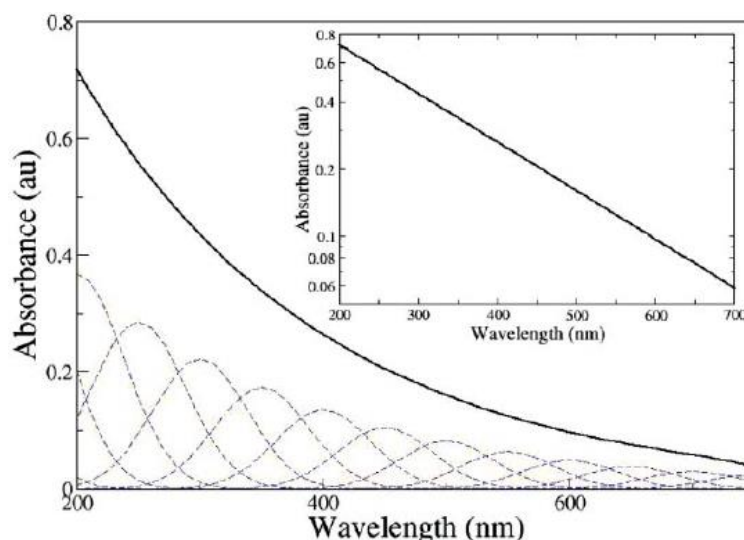


Figure 29. Monoatomic profile of melanin absorption. The solid line represents the simulated absorbance spectrum, while dashed lines are the individual absorbances of several different chromophores. Reproduced from (MEREDITH *et al.*, 2006) with permission from The Royal Society of Chemistry.

Considering that natural and standard synthetic melanin is highly insoluble and can only obtain a fine colloidal suspension in solution, many authors have assumed that light scattering events could play a significant role in the absorbance properties rather than electronic ones (WOLBARSHT; WALSH; GEORGE, 1981). However, Riesz *et al.* conducted optical studies in dilute solutions of melanin with different optical geometry measurements and demonstrated that less than 6 % of the total optical attenuation across UV and visible spectrum contributes to scattering (RIESZ; GILMORE; MEREDITH, 2006). This result implied that most of the optical absorption is due to electronic absorption.

To describe the absorbance, Tran *et al.* proposed that a chemical disorder model could explain the optical properties (TRAN; POWELL; MEREDITH, 2006). In this model, the absorption spectrum is an overlap of the absorptions of the different melanin monomers. Since melanin is a highly disordered system, as mentioned in CHAPTER III, each of the chemical species present in the structure will absorb light in a different energy/wavelength region so that the sum of all absorbances will cause the formation of its monoatomic spectrum. The experimental absorbance was reproducible by a computational simulation based on a linear combination of eleven different Gaussians, Figure 29 (MEREDITH *et al.*, 2006). Later, Pezzella and co-workers proposed that the coexistence of oxidized and reduced monomers also contributes to the broadband absorption (PEZZELLA *et al.*, 2009), which strongly supported this model.

Another evidence from electronic excitations from different monomers arises from the photoluminescence (PL) spectra. Figure 30 displays the PL spectra for excitation energy starting at 360 nm and ending at 380 nm with 5 nm increments. From these spectra, it is possible to see that the positions, widths and relative intensities of the emission change with excitation wavelength (energy), an indication that distinct chemical species with different fundamental highest occupied molecular orbital (HOMO)-lowest unoccupied molecular orbital (LUMO) gaps are excited at each excitation energy (MEREDITH; RIESZ, 2004; PERNA *et al.*, 2009; LIN *et al.*, 2015).

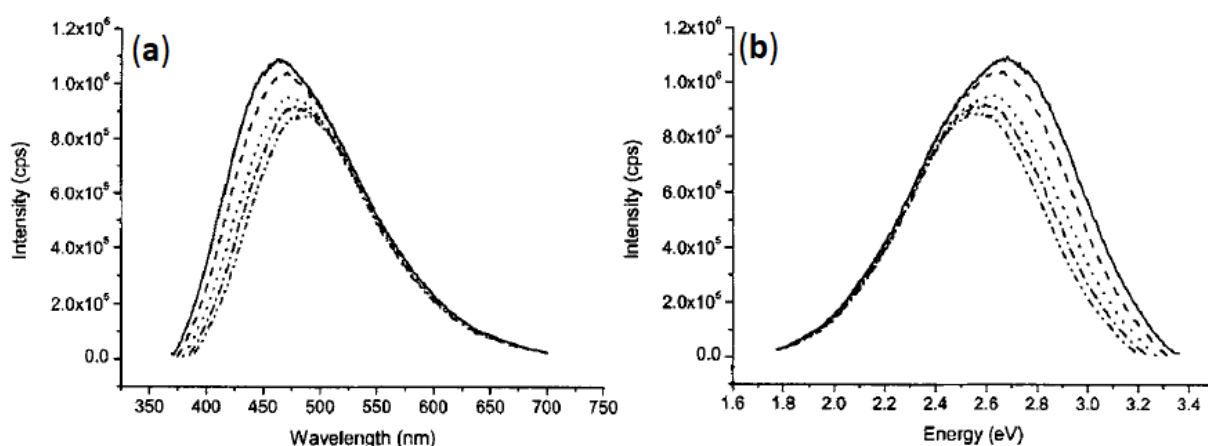


Figure 30. The photo-luminescence spectra for eumelanin plotted as a function of (a) wavelength or (b) energy of light for five excitation energy: from 360 nm (solid line) and in 5 nm increments to 380 nm (inner dashed line). Reprinted with permission from (MEREDITH; RIESZ, 2004). Copyright© 2004, John Wiley & Sons, Inc.

It is worth noting that even with a broad absorption, the radiative quantum yield is minimal, and more than 99 % of the optical energy absorbed by the melanin will be converted

into heat via nonradiative relaxation pathways (MEREDITH; RIESZ, 2004; MEREDITH et al., 2006).

However, the superposition principle proposed by the chemical disorder model did not satisfactorily explain the monotonic increase of the absorption spectrum towards higher energy. A more recent study showed that such a fundamental optical feature of melanin could be a consequence of a *geometric disorder* (CHEN et al., 2014). In this case, instead of the chemical heterogeneity from different monomers, the broadband and smooth shape of the absorption is a consequence of randomly oriented stacked melanin sheets with different sizes. Such heterogeneous aggregation gives rise to peculiar excitonic interactions among each oligomer, which would contribute to an enhancement of the absorption (CHEN et al., 2014).

This chapter evaluates the optical properties of different melanin derivatives. In detail, two main studies will be performed: i) the absorption and photoluminescence properties of sulfonated derivatives in solution will be discussed, and; ii) thin-films will be analyzed through transmission, reflectance and ellipsometry.

4.2 OPTICAL PROPERTIES OF S-MELANIN IN SOLUTION

4.2.1 Methodology

4.2.1.1 Absorption and photoluminescence characterization

To obtain SMel, SMel-4P and SMel-8P absorption coefficient, each sample's DMSO stock solutions were initially prepared with 5 mg/mL concentration and stirred for one hour to improve the sample's dilution/dispersibility. These stock solutions were then further diluted in concentrations ranging from 1 to 7 $\mu\text{g/mL}$. Beer-Lambert Law, Eq. 2, was used to calculate the molar absorption coefficient.

PL measurements were performed at different excitation wavelengths (λ_{Ex}) using 17 $\mu\text{g/mL}$ solutions. Also, quantum yield efficiency (ϕ) was calculated following Eq. 3. For this, fluorescein in 0.1 M NaOH solution ranging from 0.16 to 0.50 $\mu\text{g/mL}$ ($\lambda_{\text{Ex}} = 490 \text{ nm}$, $\phi_s = 0.92$ and $n_s = 1.33$) was used as the standard. For melanin samples, $\lambda_{\text{Ex}} = 425 \text{ nm}$ and $n_M = 1.48$ was considered.

The absorbance and PL measurements were made in triplicate in Shimadzu UVmini-1240 and Varian Cary Eclipse, respectively, with a quartz cuvette of 1 cm of optical length.

4.2.2 Results and Discussion³

The optical properties of melanin synthesized in DMSO with O₂ pressure (SMel-4P and SMel-8P) were compared with the traditional melanin synthesized in DMSO (SMel). Figure 31 shows the molar absorption coefficient of the different samples and, as one would expect for melanin, the absorption decreases as the wavelength increases. However, even though the optical characteristic is similar to those in the literature, small changes indicate structural differences (PELES; SIMON, 2011; PIACENTI-SILVA et al., 2014b). Note that for higher energies, SMel has the lowest absorption coefficient compared with the samples synthesized under O₂ pressure.

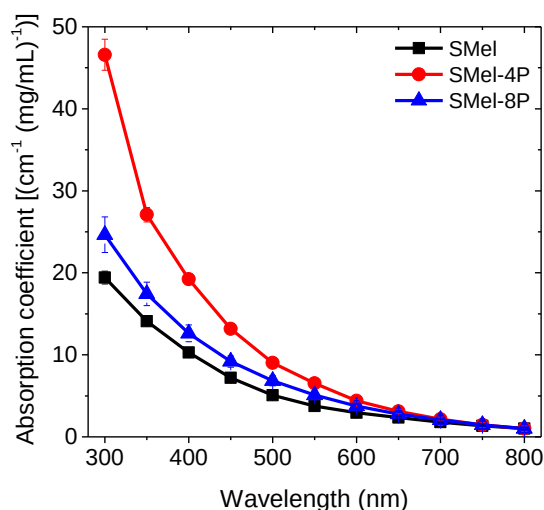


Figure 31. Molar absorption coefficient as a function of wavelength for SMel, SMel-4P e SMel-8P.

The increase in the number of decarboxylated-sulfonated oligomers (that is, DHI) decreases the absorption (PIACENTI-SILVA et al., 2014b). Thus, a lower absorption coefficient can be associated with a higher DHI/DHICA ratio. Therefore, from Figure 31, SMel-4P has a higher concentration of carboxylate structures, followed by SMel-8P and SMel. XPS (in powder), FTIR and Nuclear Magnetic Resonance measurements corroborate this assumption. See references (PAULIN, 2016; PAULIN et al., 2018) for structural characterizations.

The emission characteristics of these samples were also studied. Initially, the emission spectrum for different excitation energies was verified and the result is present in Figure 32. As

³ The results presented in this section were published in Paper I. Reproducible with permission.

can be seen, when excited at small wavelengths, the PL spectrum is composed of a broad signal, however as the excitation wavelength increases, the linewidth decreases and the spectral position undergoes a red-shift. This behavior suggests that the structures responsible for the emission are large oligomers composed of different monomers (MEREDITH; RIESZ, 2004; PERNA et al., 2009; LIN et al., 2015).

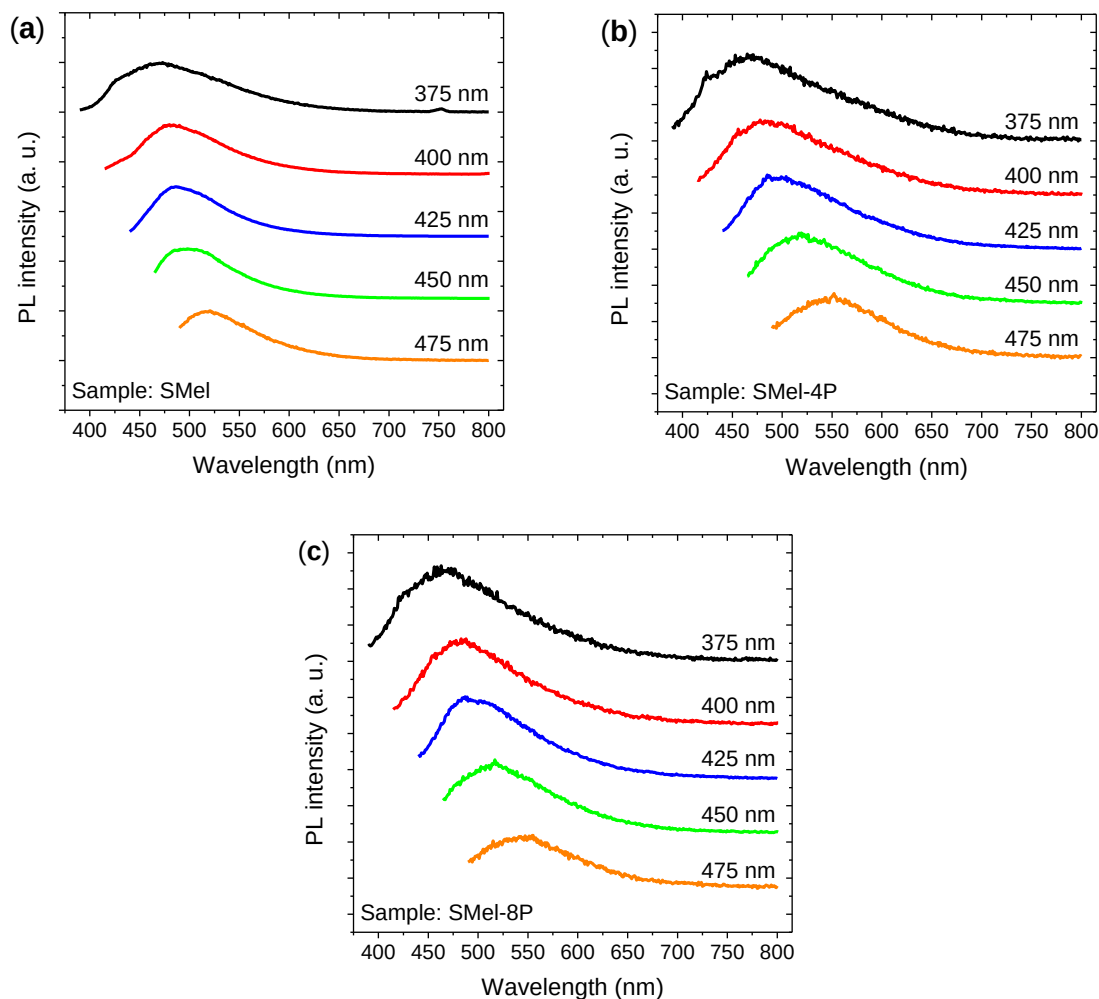


Figure 32. PL spectra at different excitation wavelengths at room temperature.

From the analysis of the different excitations, it was observed that the PL spectrum is characterized by a maximum emission intensity at 487 nm for SMel when excited at 425 nm, see Figure 33. At this excitation, the PL quantum yields was calculated for SMel to be $(2.12 \pm 0.02) \%$, SMel-4P $(0.22 \pm 0.01) \%$ and SMel-8P $(0.19 \pm 0.02) \%$. These results are one or two orders of magnitude greater than those reported in the literature for non-functionalized synthetic melanin (MEREDITH & RIESZ, 2004).

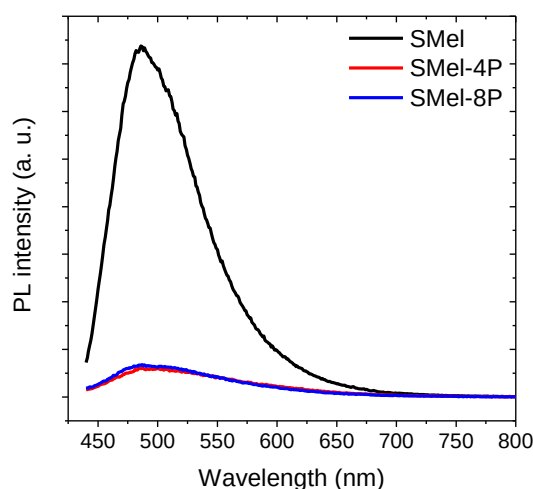


Figure 33. SMel, SMel-4P and SMel-8P PL spectra at $\lambda_{\text{Ex}} = 425$ nm.

As mentioned in the introduction (section 4.1), one of the melanin's characteristic properties is its low photoluminescence, typically from photoexcited electronic states decay through non-radiative decay, with PL yields lower than 0.07 % (MEREDITH; RIESZ, 2004). Nevertheless, in the case of sulfonate-melanin analogues, it is higher. Several PL quenching mechanisms propose that the non-radiative processes occur due to proton transfer (OLSEN et al., 2007; GAUDEN et al., 2009; HUIJSER; PEZZELLA; SUNDSTRÖM, 2011; CORANI et al., 2014), so the presence of sulfonated groups decreases the protonation in the catechol structure and increases, as a consequence, the radiative emission efficiency of the molecule. Besides, PL quenching is also induced by π - π stacking (LIN et al., 2015), which is diminished in S-Melanin due to sterical hindrance of the functional group (PAULIN et al., 2018). On the other hand, it is well established that carboxylic acid can quench the fluorescence of indole structures (LAKOWICZ, 2006b) and it has also been proposed that the intensity of photoluminescence of melanin is proportional to the conjugation of the structural backbone (DAVIDENKO et al., 2005). Thus, based on the results discussed above, the breakdown of the conjugation and the higher DHICA/DHI ratio of the samples synthesized with oxygen pressure may be the cause of the decrease in PL quantum efficiency compared to SMel.

The role of sulfonated groups and protonation can be tested by changing the concentration of OH^- and H^+ ions in solution. For that matter, a concentrated solution of ammonium hydroxide (28%) and glacial acetic acid (CH_3COOH) ranging from 0 to 50 μL was added to the 17 $\mu\text{g/mL}$ SMel solution. When a small amount of NH_4OH was added to the solution, the PL intensity decreases exponentially; however, when CH_3COOH was added it decreases linearly, Figure 34. This result can be understood, in the first case, as the removal of the sulfonate groups from the

macromolecule (BRONZE-UHLE et al., 2013) and in the latter to the quenching effect due to polymer protonation (OLSEN et al., 2007; GAUDEN et al., 2009; HUIJSER; PEZZELLA; SUNDSTRÖM, 2011; CORANI et al., 2014).

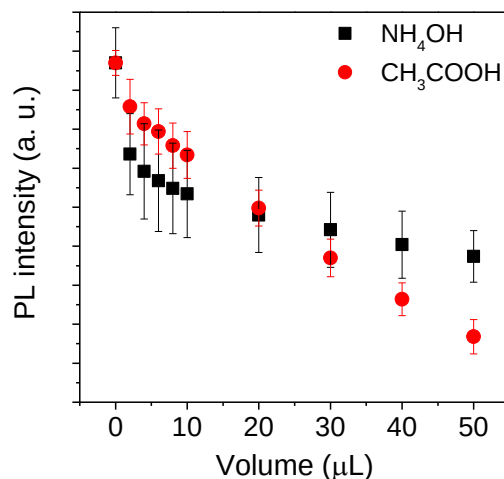


Figure 34. NH₄OH and CH₃COOH effect on the PL intensity at 487 nm with excitation at 425 nm of SMel.

4.3 OPTICAL PROPERTIES OF MELANIN DERIVATIVES IN THIN-FILMS

4.3.1 Methodology

4.3.1.1 UV-Vis and ellipsometry characterization

The same solutions from XPS measurements (CHAPTER III, Section 3.2.1) were used to make the thin-films. The difference was the spin-coating procedure. It was a two-step procedure: i) 5 s at 500 rpm, and; ii) 60 s at 2000 rpm.

Transmission and reflectance spectra were obtained on standard glass slides using a 150 mm diameter integrating sphere coupled to a Lambda 1050 UV-Vis-NIR spectrometer (Perkin-Elmer).

Ellipsometry measurements were taken on a silicon wafer with a J. A. Woollam M-2000 spectroscopic ellipsometer equipped with a xenon lamp source over a wavelength range between 370–1700 nm. Simulation analysis of the Variable Angle Spectroscopic Ellipsometry (VASE) experiment in 1 nm steps with incidence angles of 65°, 70° and 75° relative to the surface normal was used to determine the optical constants using the CompleteEASE software (v. 5.23).

4.3.1.2 Electronic structure calculations

Electronic structure calculations were conducted for NF and S-Melanin's monomeric structure, illustrated in Figure 35, to better understand the refractive index values.

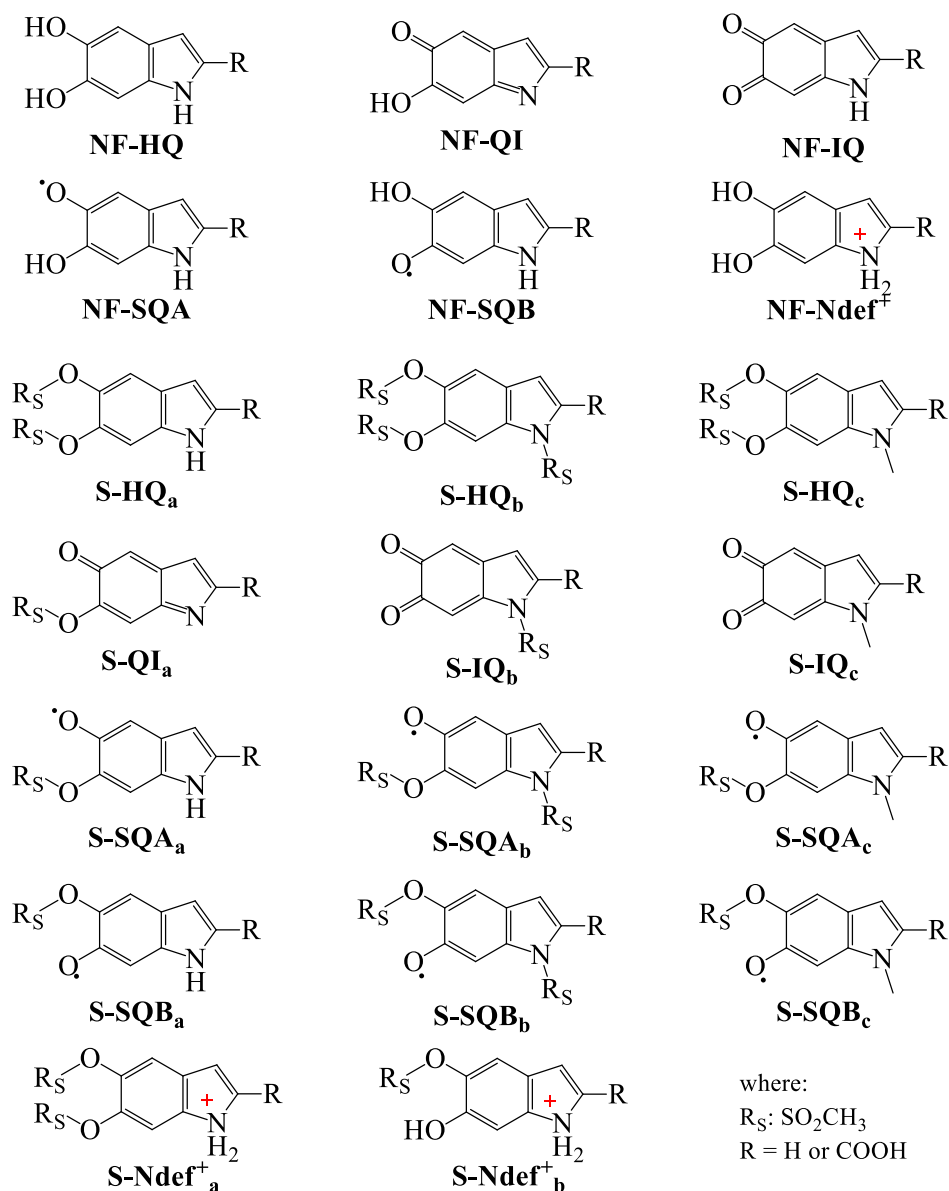


Figure 35. NF and S-Melanin units considered in DFT calculations.

The most stable geometry for the calculations for NF-Melanin was reported in (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015) were considered. However, to better evaluate the S-structural features of the compounds, 50 distinct conformers were designed for each monomeric structure via molecular dynamic (MD) calculations at high temperature. For this purpose, the monomeric forms were placed in contact with a thermal reservoir at 1000 K and allow to evolve (during 500 ps); a distinct conformer structure was stored for every 10 ps

for subsequent geometry optimization (BATAGIN-NETO et al., 2013; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). All the obtained conformers were pre-optimized in a Hartree-Fock (HF) approach using the PM6 semiempirical approximation as implemented in MOPAC2016 computational package (STEWART, 1990, 2018). The structures were then fully optimized *in vacuo* in the framework of the density functional theory (DFT) using the B3LYP (VOSKO; WILK; NUSAIR, 1980; LEE; YANG; PARR, 1988; BECKE, 1993; STEPHENS et al., 1994) hybrid exchange-correlation functional and 6-31G basis set on all the atoms. The most stable neutral structures were selected and fully optimized in a DFT/B3LYP/6-31G(d,p) approach. These structures were used for subsequent evaluations. DFT-based geometry optimizations were carried out with the aid of GAMESS (DFT/B3LYP/6-31G approach for pre-optimization for all the conformers) and Gaussian 09 (DFT/B3LYP/6-31G(d,p) for the most stable structures) computational packages (SCHMIDT et al., 1993; FRISCH et al., 2009).

The refractive indices of the molecules were theoretically predicted from the macroscopic optical susceptibilities, χ (HAICHUAN et al., 2014):

$$n = \sqrt{1 + 4 \cdot \pi \cdot \chi} \quad (8)$$

where,

$$\chi = \frac{N \alpha'}{1 - \left(\frac{4\pi}{3}\right) N \alpha'} \quad (9)$$

$$N = \frac{\Pi \cdot N_A \cdot \rho_m}{M} \quad (10)$$

$$\alpha' = \frac{\alpha_{iso}}{4\pi\epsilon_0} \quad (11)$$

$$\alpha_{iso} = \frac{1}{3}(\alpha_{xx} + \alpha_{yy} + \alpha_{zz}) \quad (12)$$

N is the density of molecules, α' represents the volume of polarization, Π is coefficient of 10^6 , N_A is the Avogadro's constant, ρ_m the mass density, M the molar mass and ϵ_0 the vacuum permittivity. α_{iso} represents the polarization constant, which was estimated via DFT/B3LYP/6-31G(d) calculations.

4.3.2 Results and Discussion⁴

A comparative characteristic study was undertaken for both NF and S-Melanin derivatives to provide any valuable information about the optical behavior and electronic structure.

The transmittance and reflectance behaviors of each melanin derivative are displayed in Figure 36 (and Figure B1&B2 in Appendix B). In most cases, it is possible to divide the transmittance spectra into three different regions, being two of them dominated by the glass substrate ($\lambda_{\text{Glass}} < 350 \text{ nm}$ and $\lambda_{\text{Glass}} > 850 \text{ nm}$), and the other one dominated by the melanin thin layer ($350 \geq \lambda_{\text{Melanin}} \leq 850 \text{ nm}$). As shown in Figure 36(a, b), the melanin transmittance spectra show an exponential decay in the visible region with a sudden drop at high energies. Such behavior is related to a high absorbance in the ultraviolet and visible regions, as shown later. Additionally, the NF-Melanin films displayed high transmission at 500 nm, around 75.6% from alkaline solution ($\text{H}_2\text{O}+\text{NH}_3$) and 83.5% from a neutral solution (distilled water); whereas for S-Melanin 82.8% from DMSO, 77.1% from DMF and 84.6% from NMP solutions. Furthermore, reflectance lower than 15% was obtained for all samples Figure 36(c, d). The intensity differences for both transmittance and reflectance are probably related to different film thicknesses.

Considering the negligible optical scattering of the films (see Figure B3), the absorption (A) was calculated from the simple relation involving the transmittance (T) and reflectance (R): $A = 1 - T - R$. The values normalized by the film thickness (estimated from the optical transmission spectra (KERREMANS et al., 2020)) is shown in Figure 37 and Figure B4. As can be seen, there is no essential change in the qualitative behavior between both sample groups. The absorbance spectra exhibit a featureless decaying response as a function of increasing wavelength. However, it is possible to note that there are differences in intensity, indicating that in sulfonated melanin derivatives, the light-absorbing chemical moieties are present in smaller quantities than non-functionalized ones.

⁴ The results presented in this section is part of Paper VI, in preparation.

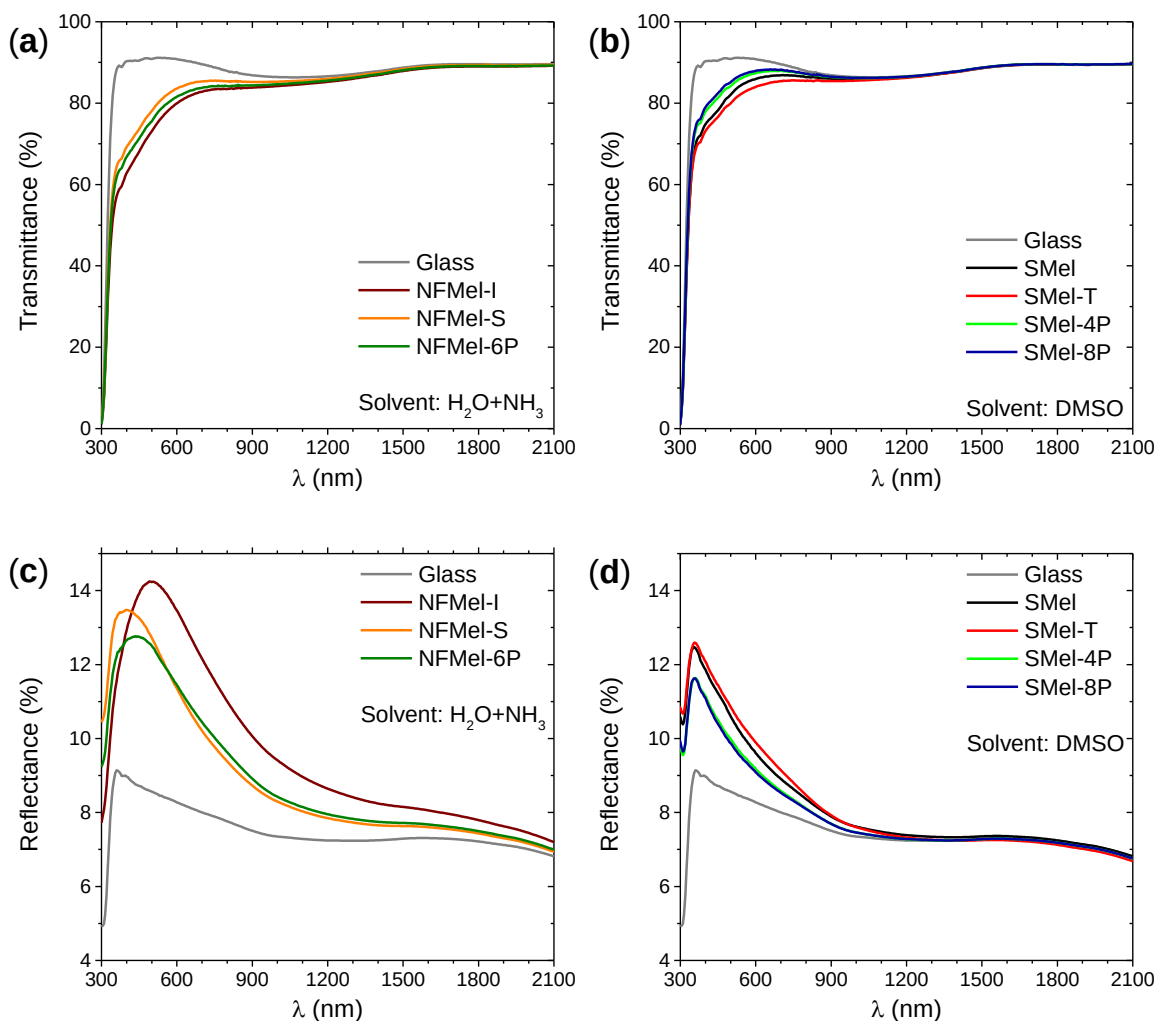


Figure 36. Transmittance (a, b) and reflectance (c, d) spectra of NF-Melanin (a, c) and S-Melanin (b, d). Similar features can be observed for other solvents.

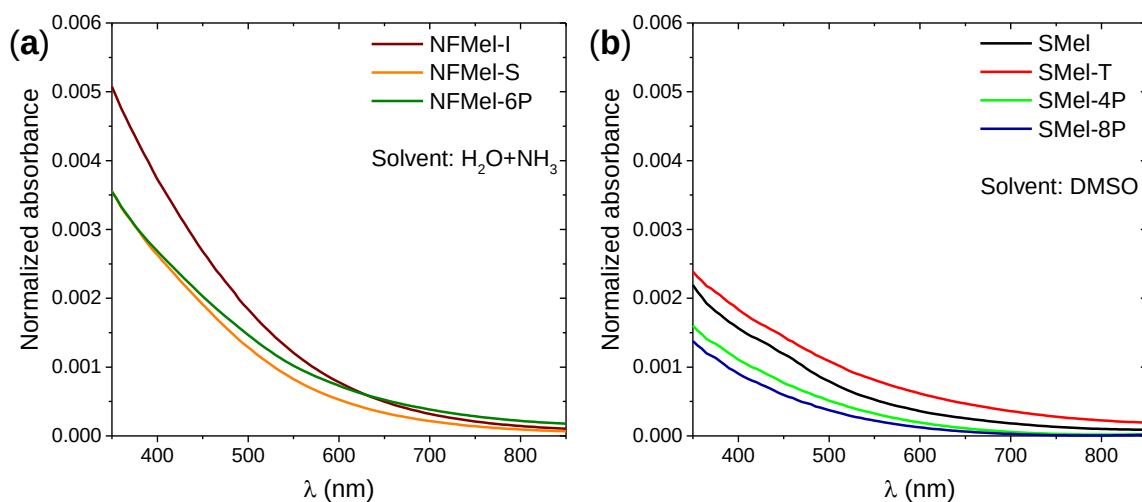


Figure 37. Calculated absorbance spectra normalized by the film thickness of NF-Melanin and S-Melanin. Similar behavior was found for other samples (see Figure B3).

Usually, melanin chromophores can be defined by the conjugation length of absorbing species and different oxidized and reduced chromophores units (PEZZELLA et al., 2009; TUNA et al., 2016). Therefore, the optical absorption differences can reflect the differences in oligomeric structures due to the presence of the sulfonated groups. As a rule, higher molecular weight oligomers should present higher absorption compared to oligomers with approximately 1000 amu, which indicates that non-functionalized derivatives have higher molecular weight than sulfonated ones (NOFSINGER; FOREST; SIMON, 1999; TUNA et al., 2016). Besides, melanins tend to aggregate, reduce solubility and increase light absorption and dispersion (NOFSINGER; WEINERT; SIMON, 2002). Hence, S-Melanin's lower absorption and better solubility compared to NF-Melanin could be partially explained if one considers that the first is composed of smaller structures with a low degree of aggregation, probably due to the sterical hindrance of the sulfonated group. Also, it is proposed that the functionalization occurs at the hydroxyl of the indole monomeric structure (BRONZE-UHLE et al., 2013), which could, by itself, perturb the interactions between the oxidized and reduced moieties in the polymer chain affecting its optical response.

As mentioned earlier (section 1.2.2), melanins are being used as material for optoelectronic applications, which means that another important property to study are its optical constants, i.e., extinction coefficient (k) and refractive index (n). Figure 38 shows the variable angle spectroscopic ellipsometry (VASE) spectra. A three-layer model was employed to fit the ellipsometry parameters $\Delta(\lambda)$ (delta) and $\Psi(\lambda)$ (psi): i) polycrystalline silicon as substrate, ii) silicon oxide, and iii) a homogeneous synthetic melanin coating. Considering that melanin is regarded as a high-absorbing and amorphous material, a multi oscillator Tauc-Lorentz dispersion model was used to determine its optical properties (LI et al., 2020). We have also tried to use single-layer Bruggeman effective medium approximation to account for any additional roughness of the melanin layer; however, no improvement in the fit was obtained. Therefore, to avoid over parameterization, we have constrained our fit with only three proposed layers.

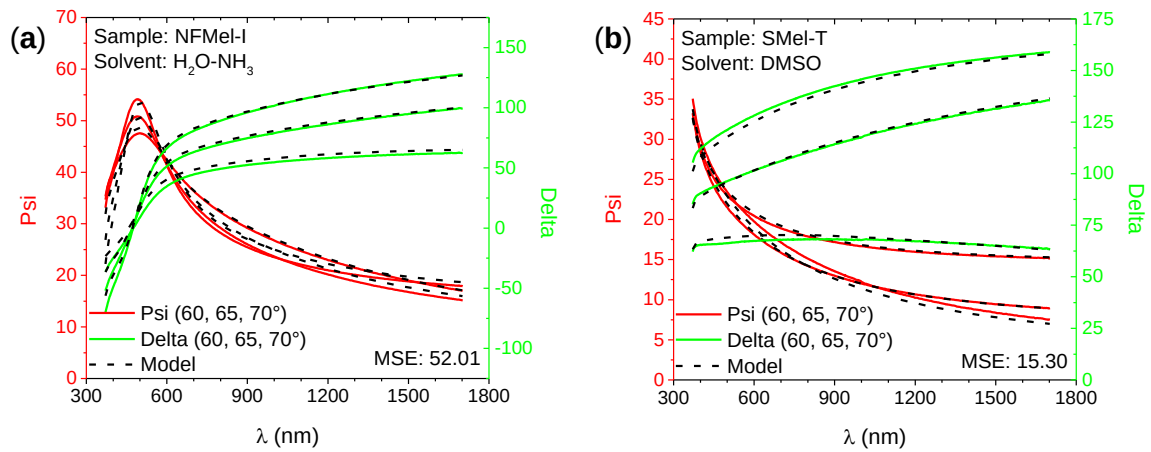


Figure 38. VASE spectra of (a) NFMel-I in H₂O+NH₃ and (b) SMel-T in DMSO.

The thin-films optical constant is shown in Figure 39. As can be seen, the extinction coefficient is compatible with melanin's absorption behavior, showing an intensity decay with the wavelength, and it is in line with solution measurements (PIACENTI-SILVA et al., 2014b; PAULIN et al., 2018). The refractive index of NF and S-Melanin increases towards higher energies. Even though $n(\lambda)$ displays a similar form, the spectra have shown different magnitude with no observed pattern (Figure 39). To obtain the refraction index of each melanin derivative, $n(\lambda)$ was fit to the Cauchy equation, $n(\lambda) = n_{Cauch} + \xi_{Cauch} \lambda^{-2}$, where ξ_{Cauch} is a proportionality factor (STAVENGA et al., 2015; KERREMANS et al., 2020). The n_{Cauch} (for $\lambda \rightarrow \infty$) of all samples is summarized in Figure 40.

The low variance of the refractive index between the different melanin derivatives was unexpected due to the sulfur atom's well-known ability to increase n of many other materials (LIU; UEDA, 2009; KLEINE et al., 2020). It is known that high packing density materials can have high- n values (LIU; UEDA, 2009; HIGASHIHARA; UEDA, 2015), which implies that NF-melanin can intrinsically be a material with high- n . On this basis, S-melanin's lower packing structure (PAULIN et al., 2018) could decrease its refractive index. Accordingly, the combination of low packing density and the presence of the sulfur atom of S-Melanin should be responsible for the similarity in the refractive index of both groups of samples.

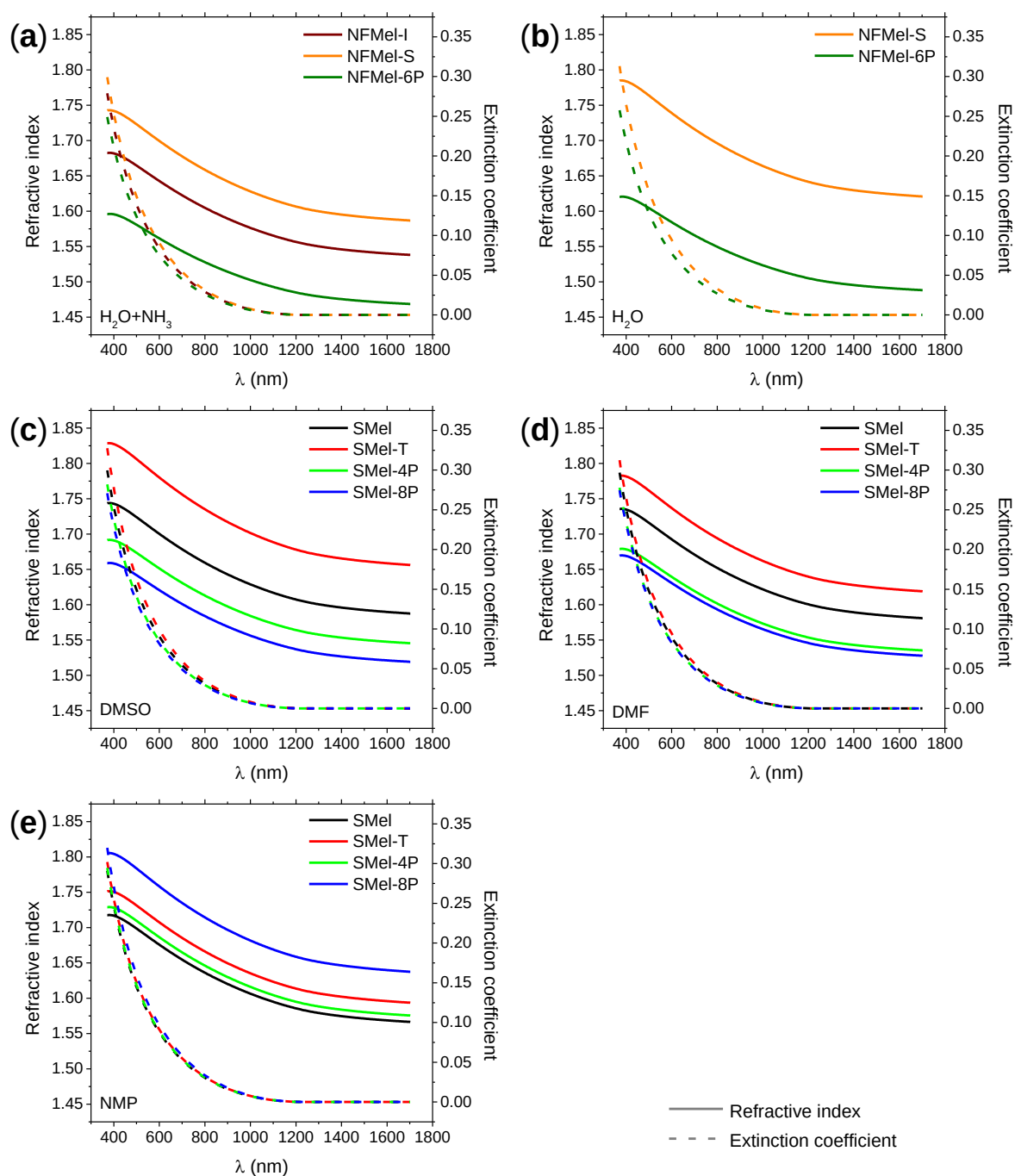


Figure 39. Optical constants of all NF and S-Melanin derivatives for the different films (see legend). The solid line represents the refractive index and the dash line the extinction coefficient.

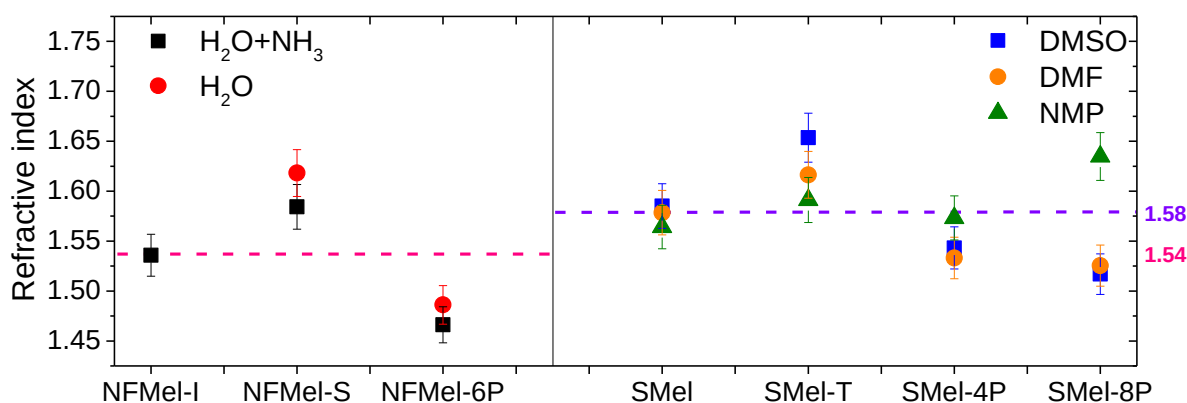


Figure 40. Refractive index values, following $n(\lambda) = n_{Cauch} + \xi_{Cauch} \lambda^{-2}$ with $\lambda \rightarrow \infty$, for NF- and S-Melanin. The left side legend indicates the refractive index scale; whereas the right side, the average value of the refractive index for NF-melanin (1.54 ± 0.06) and S-melanin (1.58 ± 0.04), represented by the pink- and violet-colored dashed lines.

Figure 41 displays the theoretical calculation performed to get further insights regarding the refractive index values for each possible monomeric species (see Figure 35). As can be noticed, the n values of the different monomeric species do not show significant variations, being compatible with the estimated experimental values; however, S-units tends to be slightly higher. Therefore, the theoretical calculations indicate that the similarities between NF and S-melanin could also be explained, in the case of the latter, by the heterogeneous system composed of both NF and S moieties (as discussed in section 3.3).

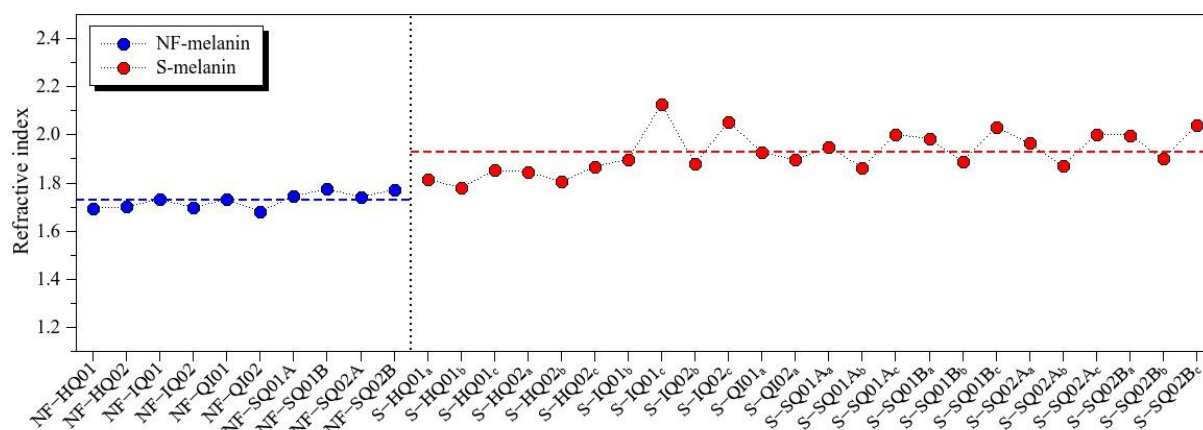


Figure 41. Theoretical refractive indexes of melanin units. The dashed lines represent the average values: 1.73 and 1.93 for NF and S-units, respectively. **01** number refers to DHI units, whereas **02** to DHICA units (details in Figure 35).

Another important parameter for optical applications is the optical dispersion of a material in the visible region (LIU; UEDA, 2009; HIGASHIHARA; UEDA, 2015). The optical

dispersion is evaluated using Abbe's number (ν_D) (LIU et al., 2007; LIU; UEDA, 2009; HIGASHIHARA; UEDA, 2015) and it is determined by $\nu_D = (n_G - 1) \cdot (n_B - n_R)^{-1}$, where n_B , n_G and n_R are refractive indices of the material at the wavelengths of the three primary colors (blue at 486 nm, green at 589 nm, and red at 656 nm). Both NF and S-Melanin derivatives displayed a low Abbe's number (17.86 ± 0.14 , see Table B2), implying that the melanin films will present higher dispersion and also higher chromatic aberration when compared to others standard materials as poly-methyl-methacrylate ($\nu_D = 57$) and polystyrene ($\nu_D = 30$) (LIU et al., 2007), which potentially limits their use for photonic devices. However, note that for applications where high absorption material is needed, melanin would still be a good candidate.

4.4 CONCLUSION

In this chapter, the optical properties in solution and thin-films deposited by spin-coating were investigated. The results show that sulfonated melanin's optical characteristics behave similarly to non-functionalized ones; however, two points should be raised. i) in solution, sulfonated melanin has higher photoluminescence and higher quantum-yield; ii) the thin-film refractive index was 1.54 ± 0.06 for NF-Melanin and 1.58 ± 0.04 for S-Melanin. Such differences were discussed based on their structures. The thin-films also showed a low Abbe's number (17.86 ± 0.14), indicating that the materials could have higher dispersion and chromatic aberration.

CHAPTER V

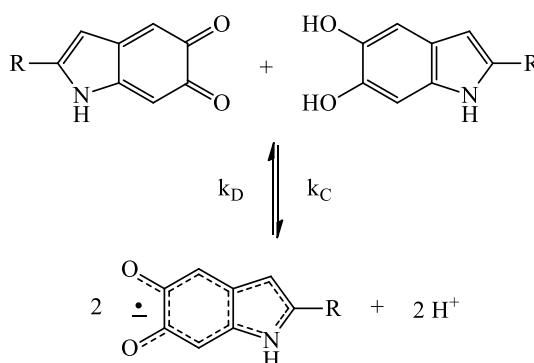
FREE-RADICAL NATURE OF MELANIN AND MELANIN DERIVATIVES

5.1 INTRODUCTION

Another unusual feature of melanin is the presence of a persistent paramagnetic signal even under extreme experimental conditions (SEALY et al., 1980), which can easily be detectable with continuous-wave (CW) electron paramagnetic resonance (EPR). In fact, melanin was one of the first biological material characterized by this technique back in the '50s (COMMONER; TOWNSEND; PAKE, 1954).

Although there are many studies on melanin's paramagnetic properties, there is no consensus on the nature of the paramagnetic species existing in these systems. However, it is highly accepted the existence of at least two different free-radical species, being one of them produced during the growth of the oligomers and aggregates macrostructure in melanin synthesis; and the other one is viewed as a molecular probe associated to melanin monomer units and the redox state of its functional groups (SEALY et al., 1980; SARNA, 1992; SARNA; PLONKA, 2005; SARNA; SWARTZ, 2006). These two paramagnetic centers can be considered intrinsic and extrinsic free-radicals.

It is believed that the comproportionation equilibrium reaction, Scheme 2, governs the concentration of the extrinsic free-radicals in melanin. An early study shown that the concentration of melanin's free-radicals could be one SQ* to 1000 fully reduced (HQ) and fully oxidized (IQ) units (FRONCISZ; SARNA; HYDE, 1980). Evidence of such equilibrium was found in a series of chemical studies and played a significant role in the development of the current understanding of some basic physicochemical properties of melanin (SARNA; PLONKA, 2005; MEREDITH; SARNA, 2006; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015; MOSTERT et al., 2018). Given these functional groups' intramolecular equilibria, melanin is considered a dynamic system (SARNA; PLONKA, 2005; SARNA; SWARTZ, 2006).



Scheme 2. The comproportionation equilibrium reaction. Quinone and hydroquinone moieties interact together to obtain an intermediate redox state like the negatively charged semiquinones and protons.

Even though no physicochemical procedure could irreversibly quench the EPR signal without degradation of the basic melanin structure, they are by no means stable. In reality, it was proven that the intensity of the extrinsic paramagnetic center can be sensitive to many external factors such as metallic centers (FELIX et al., 1978b; FRONCISZ; SARNA; HYDE, 1980; NAJDER-KOZDROWSKA et al., 2009), temperature (FELIX; HYDE; SEALY, 1979; CHIO; HYDE; SEALY, 1980), pH (CHIO; HYDE; SEALY, 1982; MOSTERT et al., 2018), light (SARNA; SEALY, 1984), presence of the oxidizing and reducing agents (DUNFORD et al., 1995; RESZKA; MATUSZAK; CHIGNELL, 1998) and hydration (MOSTERT et al., 2013). Thus, any agent capable of modifying the ratio between the comproportionation rate constant k_C and the disproportionation ratio constant k_D will affect the EPR signal intensity and, consequently, the radical content.

It is worth noting that, since the intrinsic radicals are inaccessible to extraneous reactive agents, trapped within melanin macrostructure, such species should present low chemical reactivity and can be considered more “stable” than its counterpart. In this way, the intrinsic radicals are usually reported as a spin-label on the melanin molecular state (SEALY et al., 1980; SARNA; SWARTZ, 2006).

Mostert *et al.* defined the intrinsic radical as carbon-centered radicals (CCR), i.e., spin density located in carbon atoms, and the extrinsic one as semiquinone-free radicals (SFR), i.e., spin density located in oxygen atoms (MOSTERT et al., 2013). Today, g-values and line shape can be used to distinguish these species (BLOIS; ZAHLAN; MALING, 1964; CHIO; HYDE; SEALY, 1980, 1982; MOSTERT et al., 2013; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015).

Based on the brief discussion above, one may expect that CCRs, protected from the environment in internal regions of the melanin structure, govern the EPR signal in acidic and neutral samples (dry powder or solutions/suspensions) and present a weak dependence on temperature and pH. On the other hand, SFRs should dominate at alkaline samples/solutions, showing a strong dependence on pH (especially the signal intensity). Moreover, these paramagnetic species have similar g-values around 2.0030 for CCR and 2.0050 for SFR (MOSTERT et al., 2013; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). However, it should be stressed that, even if the structural nature of SFR is highly accepted to arise from semiquinone species, the nature of CCR is still poorly understood.

Additionally, only a few reports in the literature attempt to solve the melanin EPR signs, and these are still inconclusive. For instance, simulation analysis of Q-band ($\nu = 35$ GHz) EPR

spectra recorded over a range of pH for different melanins showed the presence of two paramagnetic species (GRADY; BORG, 1968). Grady and Borg speculate that such species should arise from anionic and neutral radicals with different g-values. Later, this analysis was further refined using computer simulation analysis. The authors proposed the existence of four different species that could be related to cation radicals with extended delocalization of their unpaired spins and anion o-semiquinone radicals with relatively localized unpaired electrons (PASENKIEWICZ-GIERULA; SEALY, 1986).

More recently, to correlate melanin structures and the paramagnetic centers responsible for EPR spectra, DFT calculation of g-values and hyperfine coupling constant was carried out (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). Batagin-Neto and co-workers showed that the signals generically described as SFR could be associated with fully and semi-oxidized monomers (IQ-anion and SQ* radicals), whereas CCRs to hydroquinone (HQ) and nitrogen protonated defects (Ndef), Figure 12.

In this chapter, some EPR spectra found in the literature were subject to simulation analysis to give some insight into the structural nature of CCR species. Also, X-band EPR spectra of sulfonated melanin derivatives were measured under hydration control. Lastly, the anisotropy of NF and S-melanin was analyzed using HFEPR, probably for the first time to the best of our knowledge.

5.2 THE NATURE OF INTRINSIC AND EXTRINSIC FREE-RADICAL OF NF-MELANIN

5.2.1 Methodology

5.2.1.1 Data collection and spectra simulation

The EPR spectra were extracted from the work of Chio *et al.* (CHIO; HYDE; SEALY, 1982), Mostert *et al.* (MOSTERT et al., 2013) and Panzella *et al.* (PANZELLA et al., 2013) employing the software WebPlotDigitizer (ROHATGI, 2018).

To gain more insights on the EPR data, simulation of the EPR spectra were done using EasySpin (v. 5.2.13) implemented in the MATLAB® (MathWorks, Inc) software package. Also, the samples were considered to have isotropic broadening signals and linewidths described by Voigtian line shapes function, that is, a convolution of a Gaussian and a Lorentzian. Pepper and garlic routines were employed in the fitting procedure for powders and solutions, respectively (STOLL; SCHWEIGER, 2006). OriginPro 9.0 (OriginLab Corp., MA, USA) computational

package was used for interpolation and spin concentration determination. In order to reduce the number of variables involved in the fittings and then turn it feasible, we have tried to keep as fixed as possible the parameters associated with signals line shapes and widths during the fittings.

5.2.1.2 Density functional theory calculations for NF-Melanin

Electronic structure calculations were carried out to estimate the molecular energy levels of non-functionalized melanin subunits. For this purpose, 50 random structures were constructed for each monomer from the rotation of OH and COOH groups or molecular dynamics calculations at high temperatures (1000 K), following the methodology presented in literature (BATAGIN-NETO *et al.*, 2013; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). The geometries were then pre-optimized via the PM6 semi-empirical method (STEWART, 2007) using a Hartree–Fock approach, and then fully optimized using density functional theory (DFT), with Becke’s LYP (B3LYP) exchange–correlation functional and the 6-31G basis set. The most stable structures were then identified and their Kohn-Sham orbital energies extracted with the aid of Gabedit computational package (ALLOUCHE, 2012). Pre-optimizations were carried out with the aid of MOPAC2012 computational package (STEWART, 1990) and DFT calculations (full optimizations and properties) were performed with the aid of GAMESS computer package (SCHMIDT *et al.*, 1993).

5.2.2 Results and Discussion⁵

The EPR spectra of natural and synthetic melanin under different pH conditions reported by Chio *et al.* (CHIO; HYDE; SEALY, 1982) were used as a starting point. At first, only two resonant components were employed to fit the spectra, with no success. For most of the cases, a good fit was observed in the central region of the spectra with large deviations elsewhere. In particular, with only two components, it was not possible to adjust the typical asymmetries observed in the spectra (see Figure C1 at Appendix C.1). These difficulties were overcome by considering a third component.

Figure 42(a, b and c) illustrate the results obtained for the experimental data reported by Chio *et al.* (CHIO; HYDE; SEALY, 1982) at distinct pH (1.6, 6.9 and 12.3, respectively). The experimental, simulated and the individual signals of the three components are presented in black, red, and blue-pink-green lines, respectively. It is important to stress that despite

⁵ The results presented in this section were published in Article II. Reproducible with permission.

employing three distinct resonant lines to fit the spectra presented in Figure 42, the g -values and linewidths (ΔH_{PP}) were kept fixed for each component, the only free parameter was the intensity of the components. As can be seen, a reasonable adjustment is obtained ($RMSD < 0.040$), which suggests that at least three populations of distinct paramagnetic centers are present in these samples. The changes in intensity presented in Figure 42(d) are partially explained by the comproportionation reaction (Scheme 2). Note that components **NF-A** and **NF-C** follow strictly the trend imposed by Scheme 2, while component **NF-B** has no explicit dependence.

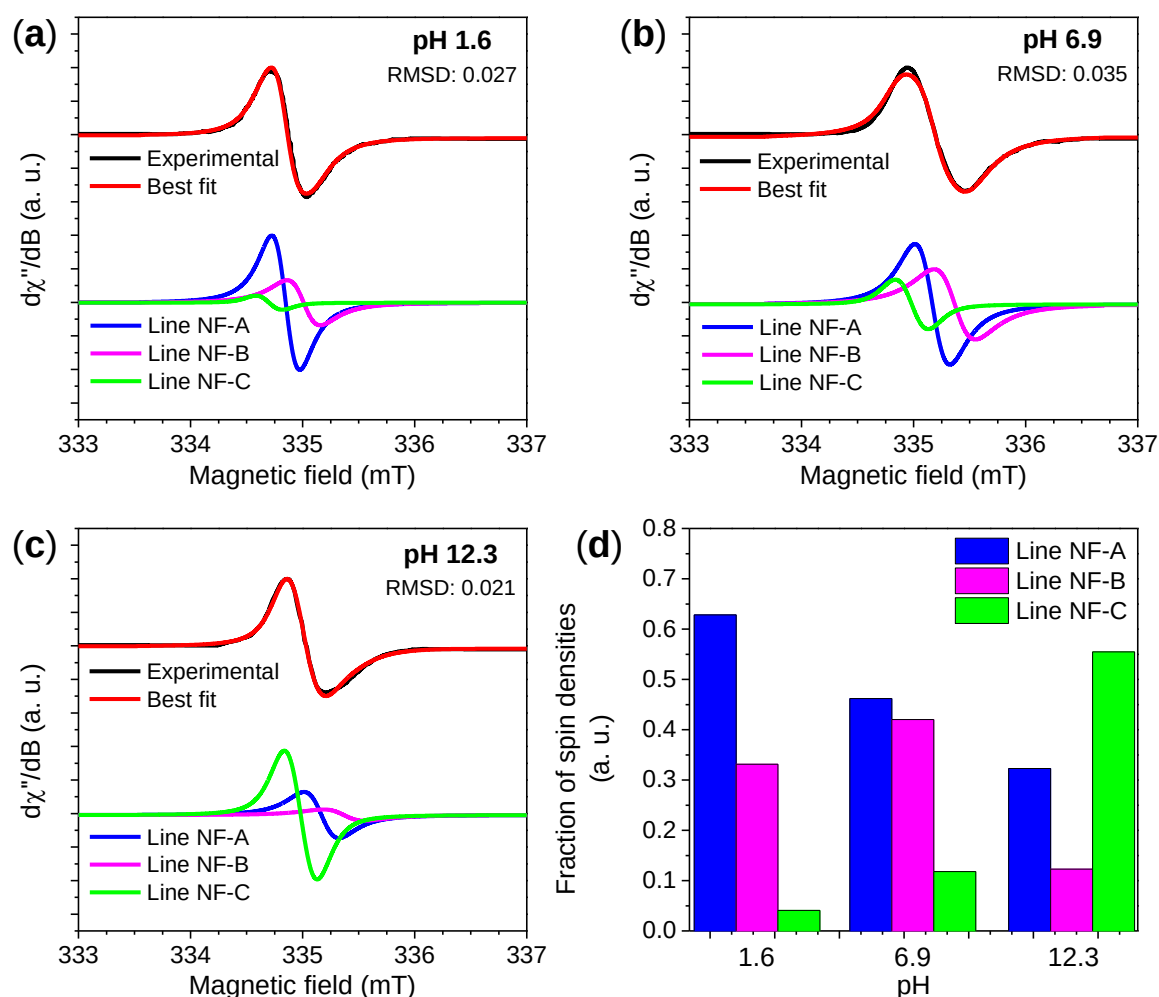


Figure 42. Melanin X-band EPR spectrum simulation at pH (a) 1.6, (b) 6.9 and (c) 12.3. (d) Estimation of the spin densities of each component of the melanin EPR line for the different pH. The black line represents the experimental spectra while the red is the simulated one. Blue, pink and green lines and bars represent the three components used in the fit. Experimental data reprinted with permission from Chio *et al.* (CHIO; HYDE; SEALY, 1982) Copyright © 1982 Published by Elsevier Inc.

Table 12 shows the spectroscopic parameters estimated for each component of the simulated spectra.

Table 12. Spectral parameters of the simulated signals of NF-Melanin.

Lines	g-Values	ΔH_{PP} (mT)	Assignment
NF-A	2.0038	0.380	CCR
NF-B	2.0026	0.396	CCR
NF-C	2.0049	0.350	SFR

In general, there is a consensus in the literature that CCR species dominate the EPR spectra of melanin suspensions at low pH, while SFR at higher pH, explained by changes induced by OH^- and/or H_2O species in the balance of Scheme 2. From Figure 42 and Table 12 notice that component **NF-C** presents the highest g-value and lowest linewidth, compatible with SFR (MOSTERT et al., 2013, 2018; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). On the other hand, components **NF-A** and **NF-B** are dominant at low pH, which indicates that both refer to CCR (MEREDITH; SARNA, 2006; MOSTERT et al., 2013). Notice that the ΔH_{PP} of CCR and SFR components are similar, which suggest that the larger linewidths typically ascribed to CCRs species in the literature (CHIO; HYDE; SEALY, 1982) could be linked to the superposition of two signals with slight different g-values.

As a matter of fact, as shown before, the existence of more than two paramagnetic species in melanin have already been proposed using Q-band EPR (GRADY; BORG, 1968; PASENKIEWICZ-GIERULA; SEALY, 1986). However, the great challenge is understanding their origin. In a previous work of our group, it has been demonstrated through DFT calculations that charged and radical species of melanin units (Figure 12) have different EPR signatures (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). In particular, it was shown that signals with higher g-values and relative low linewidths (*i.e.* smaller hyperfine constants) associated with SFR centers, could be ascribed to IQ^- and SQ^* species, compatible with the literature (SEALY et al., 1982; MEREDITH; SARNA, 2006; MOSTERT et al., 2013). On the other hand, based on the calculated g-values, the CCR signals should be ascribed to several species such as neutral N-def, QH_2 -anion, QH_2 -cation and QI -cation, however, the proposition that CCRs should present larger unsolved hyperfines, associated with the high ionization potentials of the structures, N-def and QH_2 -anion was proposed as the most likely species. Nonetheless, the mechanism associated with the formation of such species into melanin macromolecule is not easy to understand, mainly QH_2 -anion.

Indeed, as evidenced in Figure 43, the LUMO levels of the QH₂ are quite high in comparison to other subunits, so that QH₂ are not supposed to act as electron traps in melanin. In this context, a relevant mechanism that was not considered in the previous work is the role of N-def⁺ structures as electron acceptors. From a naive analysis of Figure 43, it is possible to see that since N-def⁺ is present in the macromolecular structure of melanins (protected from the environment) it acts as strong electron acceptors, mainly from QH₂ species, leading to the formation of the paramagnetic species N-def (neutral) and QH₂-cation in the system, whose EPR parameters are compatible with the CCR signal (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). Additionally, the stabilization of free radicals through electron trapping at deep defect states has already been proposed by other group using Hückel theory for band structures (GALVÃO; CALDAS, 1988).

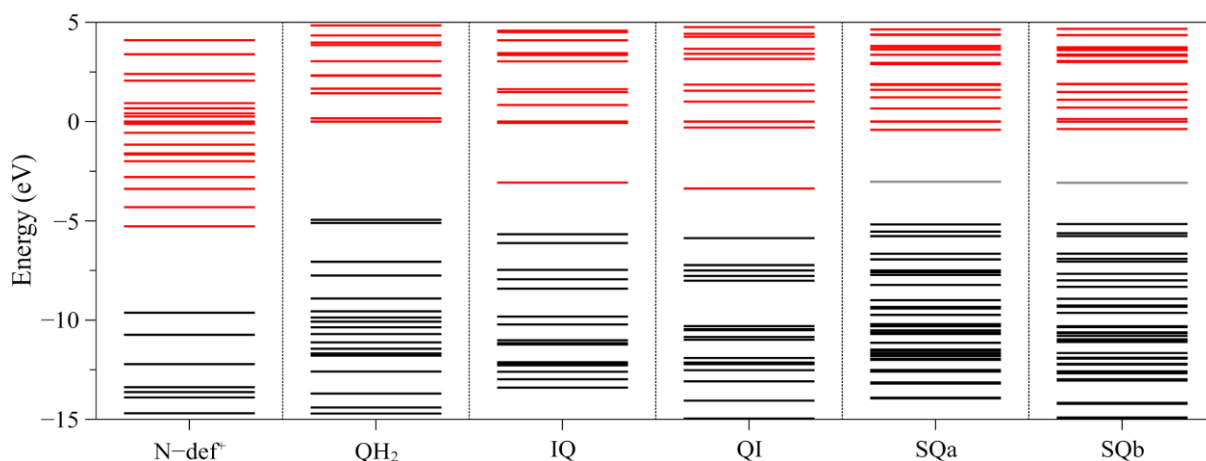


Figure 43. Energy levels around the frontier orbitals of melanin subunits. The color represents occupation: occupied orbitals (black), unoccupied orbitals (red), and semi-occupied orbitals (grey).

This result is consistent with the weak dependence of CCR spin densities on pH. Since the N-def⁺ structures can be considered a kind of QH₂ species with an H⁺ ion adsorbed on the nitrogen (that is trapped between the melanin layers), they are supposed to stabilize in the bulk of the macromolecule, protected from changes in the environment. This result is also compatible with the destacking mechanism proposed by Mostert *et al.*, that explains the weak consumption of CCR centers induced by melanin hydration that induces destacking of the melanin layers and exposure of such species (MOSTERT *et al.*, 2013).

Another relevant feature from DFT is the prediction of a higher g-values and lower effective hyperfines for DHICA compared to DHI. This result suggests that signals **NF-A** and

NF-B can be associated with DHICA and DHI species, respectively, and consequently that EPR could be used to measure the DHICA/DHI ratio in melanin qualitatively.

To reinforce our proposal of three components composing melanin EPR signal, we have considered another work found in the literature that used hydration-controlled measurements on acidic, neutral and alkaline solid-state melanin samples. Mostert *et al.* (MOSTERT *et al.*, 2013) showed that the EPR signal is dominated by CCR species, presenting small contributions of SFRs in alkaline samples. Figure 44 presents the best fits obtained from the experimental data considering three components. In these simulations, the spectroscopic parameters used are close to those in Figure 42.

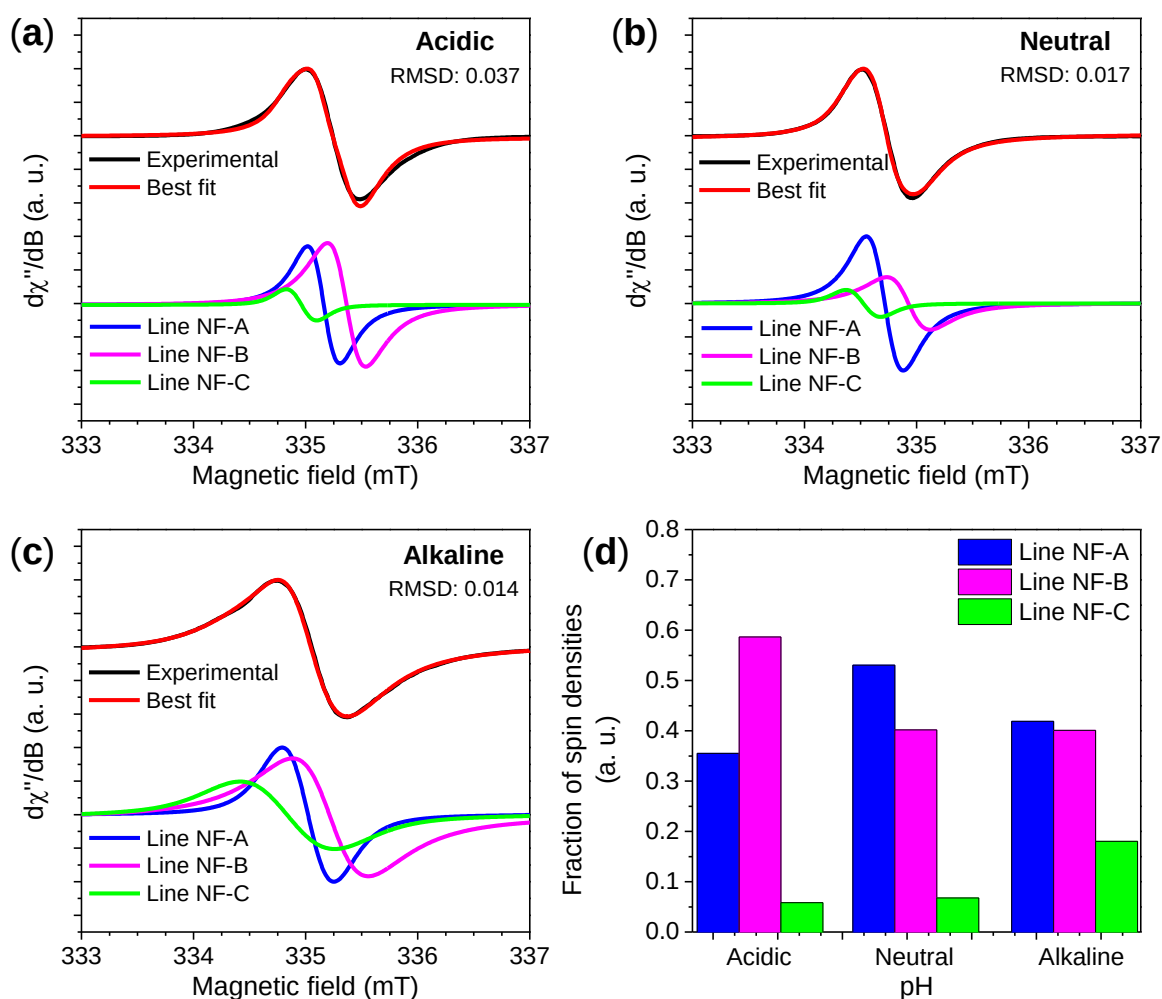


Figure 44. Melanin X-band EPR spectra simulation obtained from (a) acidic, (b) neutral and (c) alkaline solid-state samples in hydration-controlled conditions. (d) Spin densities estimation for each component of the different samples. Experimental data reprinted with permission from Mostert *et al.* (MOSTERT *et al.*, 2013). Copyright © 2013, American Chemical Society.

As can be seen, good fits were obtained. As in Figure 42, three components are necessary to fit the spectra (see Figure C2, at Appendix C.1, for fits based on two components). For the best fit the same *g*-values and linewidths were used for acidic and neutral samples, however for alkaline samples, although the *g*-values remained the same, the linewidths used were broader; 0.558, 0.672 and 1.000 for components **NF-A**, **NF-B** and **NF-C**, respectively. These deviations are assigned to the experimental procedure used to increase the sample pH, that may induce changes in the CCR species (MOSTERT *et al.*, 2013, 2018) and also induce degradation.

For solid samples, the spin densities of signals **NF-A** and **NF-B** are higher than that of signal **NF-C** independent of the pH, which was indeed expected, since a higher proportion of CCR species is commonly observed in solid-state samples. In particular, note that signals **NF-A** and **NF-B** have different dependences on pH when compared to solutions. In fact, Mostert and co-authors noticed a decrease in the CCR signal intensity after alkalization/hydration of the samples. Our results suggest that CCRB species (CCR associated with signal **NF-B**) are initially more affected by hydration than CCRA ones (CCR associated with signal **NF-A**). An opposite effect is observed for alkaline samples, leading to an equilibration between CCRA and CCRB populations.

Considering a possible association of CCRA and CCRB with DHICA and DHI paramagnetic species, such effects could be understood based on the destacking mechanisms proposed by Mostert *et al.* (MOSTERT *et al.*, 2013). In this context, the water molecules could act as a destabilizing agent of the stacked substructures of melanin, facilitating the destacking and exposing the internal and previously protected CCR species to the environment. As a matter of fact, due to the typical connections between adjacent units (MEREDITH; SARNA, 2006; MOSTERT *et al.*, 2013; PANZELLA *et al.*, 2013) this effect should be more pronounced in the DHI-based substructures than in the twisted DHICA, resulting in an apparent stabilization of CCRA in wet samples. On the other hand, after the alkalization of the samples, additional structural changes are observed as already identified in the parameters coming from the fittings, probably leading to the formation of additional centers or modified structures. Note that this result is in agreement with the previously discussed mechanism of CCR formation.

In addition, given the number of new routes that have been proposed to synthesize various melanin derivatives with slight differences in chemical structures, it is also interesting to evaluate if the same EPR components can still be employed for the fitting. Thus, we have carried out solid-state X-band EPR measurements of DHI and DHICA-based melanin from the data reported by Panzella *et al.* (PANZELLA *et al.*, 2013). The different proportion of DHICA

over DHI structures in a melanin sample particularly relevant since the biological, structural, optical and even paramagnetic/free radical properties are generally governed by this ratio (MEREDITH; SARNA, 2006; PANZELLA et al., 2013; D'ISCHIA et al., 2015). In Panzella work, DHI and DHICA-melanin were obtained through L-DOPA oxidation by potassium hydrogen carbonate and potassium ferricyanide with sodium dithionite or sodium metabisulphite (for DHI or DHICA, respectively) (EDGE et al., 2006; PANZELLA et al., 2013). Figure 45 illustrates the three components-based fits for DHI- and DHICA-Melanin (see Figure C3, in Appendix C.1, for the simulations with only 1 or 2 components).

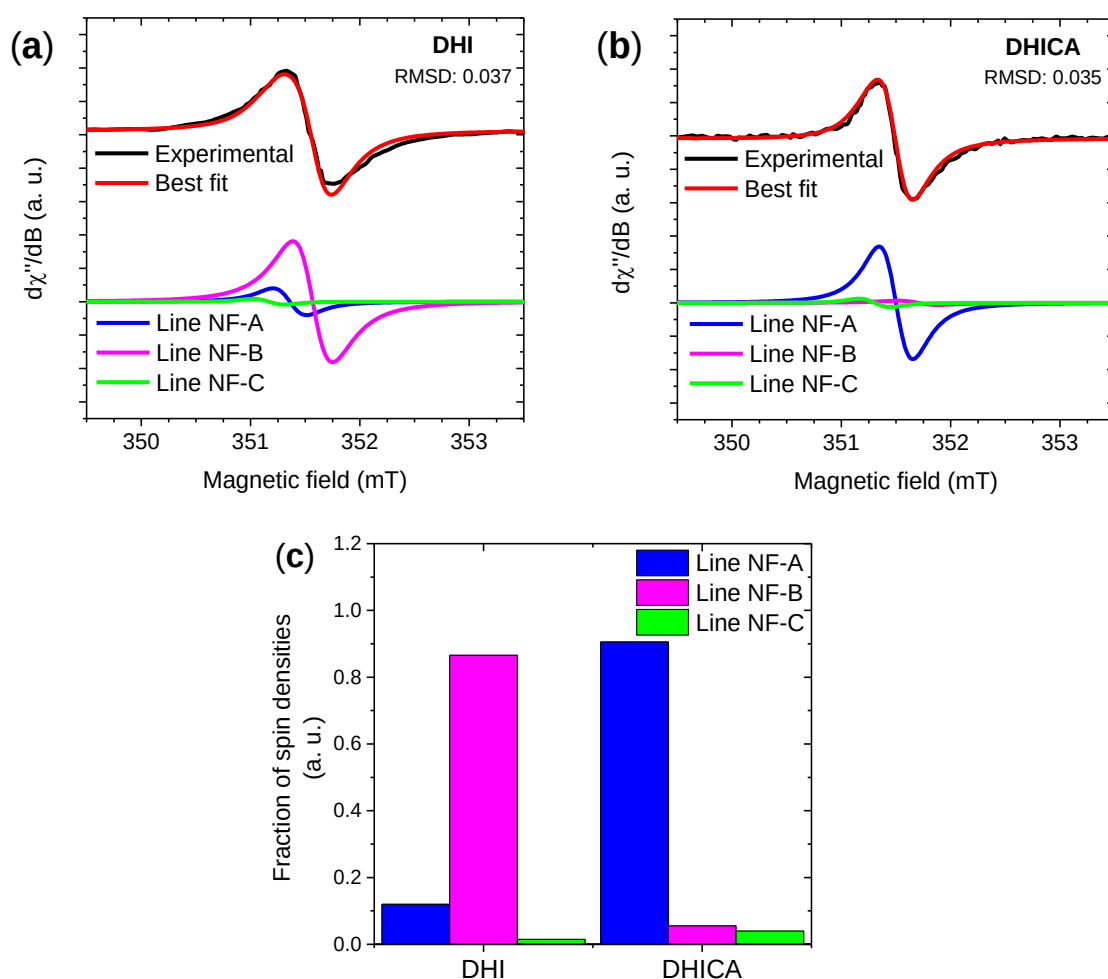


Figure 45. Melanin X-band EPR spectra simulation obtained from (a) DHI and (b) DHICA solid-state samples. (c) Estimation of the spin densities of each component of the EPR line for the different samples. Experimental data reprinted with permission from (PANZELLA et al., 2013) Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

As can be seen in Figure 45, the EPR spectra are well fitted by three components, in which again the CCRB is assigned to DHI and CCRA to DHICA-rich samples, with a clear

predominance of one component which re-enforces our assignment. In addition, notice the low contribution from SFR species (component **NF-C**), in agreement with what is proposed by Panzella *et al.* (PANZELLA *et al.*, 2013).

In summary, from the above presented results, it is possible to identify the presence of three common paramagnetic species in distinct melanin samples. In particular, it is possible to associate two distinct species to the widely reported CCR centers which can be assigned to DHI and DHICA paramagnetic moieties. The presence of these centers is responsible for the typical asymmetric lineshape obtained from melanin. Based on the g-values and linewidths of the signals and energy levels alignments of the melanin units, it was possible to associate the CCR signals with QH₂-cation and N-Def species.

5.3 X-BAND CW-EPR OF S-MELANIN UNDER HYDRATION CONTROL

5.3.1 Methodology

5.3.1.1 Sample preparation

Approximately 150 mg of each NF- and S-Melanin derivative (CHAPTER II, Section 2.1.2) was crushed to a fine powder and pressed into a pellet with roughly 13 mm of diameter and 1 mm of thickness using a standard pellet press under 10 tons of pressure for 5 minutes. Since the standard EPR quartz tube had an inner diameter of 3 mm, it was necessary to break the resulting pellet into a small piece suitable to fit inside.

5.3.1.2 X-band CW-EPR under hydration-control measurements

For the hydration-control, the quartz tube was connected to a specially made glass funnel and then to a KF16 stainless steel vacuum tubing with a BOC-Edwards glass to KF16 converter. This system was attached to the stem delivery system (CHAPTER II, section 2.1.3). Once the attachment was completed, the rotary pump was torn on to evacuate the line from atmospheric fluids for one hour. After sufficient vacuum ($< 10^{-3}$ mbar), the line was isolated from the pump and the quartz tube placed within the sample cavity of the spectrometer. Due to the number of samples and the limited time to the equipment, only two hydration data points were taken: dry (at vacuum condition) and 19.0 mbar (~ 80.4 % humidity).

The CW X-band EPR spectra were recorded using a Bruker EMX Micro X, CW-EPR spectrometer equipped with a high-power cavity/high-sensitivity ER4119HS resonator. The spectra were taken at a modulation frequency of 100 kHz, modulation amplitude of 0.025 mT

with a scan width of 6 mT and microwave powers of 0.03 or 10.00 mW. 2,2-diphenyl-1-picrylhydrazyl (DPPH) with $g = 2.0036$ for g -value calibration.

It is important to note here that the diode current, a proxy for the quality factor Q , of the EPR cavity did not change after the addition of water vapor into the system. Such behavior indicates that the variation of the signal intensity is not an experimental artifact caused by dielectric losses (DALAL; EATON; EATON, 1981), but rather change in the spin concentration.

5.3.1.3 Spectral simulation

The EPR spectra were analyzed in a similar manner to previously section 5.2.1.1 (above). EasySpin, v.5.2.13, (STOLL; SCHWEIGER, 2006) was used with the pepper routine, using pseudo-Voigtian ($\sim 65\%$ Lorentzian character) function and isotropic broadening. The proposed pseudo-Voigt lineshape is a requirement for a complete analysis of the EPR signal asymmetry (GRADY; BORG, 1968).

To reduce the number of variables used in the fittings, the parameters associated with the g -values, linewidths and lineshapes were kept fixed during the fitting procedure. However, it should be noted that, in the initial simulations, we have considered different amounts of resonant lines and the spectral parameters converged to values close to those discussed below.

OriginPro 9.0 was used to doubly integrate the first derivative EPR spectra to determine spin concentrations. In this work, we confine our analysis to low microwave power spectra only to avoid any possible saturated effects originating from a higher microwave intensity.

5.3.1.4 Density function theory calculations for S-Melanin

To better understand the origin of the EPR signal in S-Melanins, electronic structure calculations were conducted for charged and radicalar species of sulfonated DHI and DHICA units, shown in Figure 35.

Initially, the optimization of neutral structural was performed similarly to section 4.3.1.2 and then it was used as starting point to optimize the charged units using the same computational DFT/B3LYP/6-31G(d,p) approach (Gaussian 09). For the evaluation of paramagnetic features of S-Ndef⁺, it was considered their neutralized structures (i.e., S-Ndef=S-Ndef⁺+e⁻).

The g -values (g_{iso}) were obtained from the average of the principal values of the $g^T g$ matrix (g_{xx} , g_{yy} and g_{zz} , square roots of the eigenvalues of $g^T g$) (WEIL; BOLTON, 2007) estimated with the aid of Orca 3.0.3 computational package (NEESE, 2012), using the DFT/B3LYP/6-31G(d,p) approach.

The energy of the frontier levels of the building blocks (the highest occupied molecular orbital, E_{HOMO} ; and the lowest unoccupied molecular orbital, E_{LUMO}) were estimated from the ionization potentials (IP) and electron affinities (EA) as follows:

$$E_{HOMO} = -IP = -[E_T(M^+) - E_T(M)] \quad (13)$$

$$E_{LUMO} = -EA = -[E_T(M) - E_T(M^-)] \quad (14)$$

where $E_T(M^+)$, $E_T(M)$ and $E_T(M^-)$ represent the total energy of the molecule M at distinct charge configurations (cation, neutral and anion), considering a same geometry (optimized for M at neutral configuration).

The potentialities of charge transfer between S and NF-eumelanin building blocks were estimated via donor-acceptor map (DAM) based on molecule's philicities (ω^+ and ω^-):

$$\omega_M^- = \frac{(3 IP + EA)^2}{16 (IP - EA)} \quad (15)$$

$$\omega_M^+ = \frac{(IP + 3 EA)^2}{16 (IP - EA)} \quad (16)$$

and relative electron-accepting (R_a) and electron-donating (R_d) powers, given by (GÁZQUEZ; CEDILLO; VELA, 2007; MARTÍNEZ et al., 2008; HERNANDEZ; TENORIO, 2018):

$$R_a = \frac{\omega_M^+}{\omega_{Fluorine}^+} \quad (17)$$

$$R_d = \frac{\omega_M^-}{\omega_{Sodium}^-} \quad (18)$$

where ω_M^- and ω_M^+ represent the philicity powers of the molecule M, and $\omega_{Fluorine}^+$ and ω_{Sodium}^- the powers associated with fluorine and sodium atoms (estimated from experimental IP and EA values), that are considered electron acceptor and electron donating model systems (MARTÍNEZ et al., 2008).

Low (higher) values of ω^- (ω^+) imply a greater capacity for donating (accepting) electrons. In this sense, R_a and R_d descriptors indicate the charge transfer potentiality of M in relation to fluorine and sodium atoms. $R_a > 1$ and $R_d < 1$ indicate that M is a more effective electron acceptor and electron donor than the model systems.

Additionally, the net spin populations over the structure of S-units was computed via Mulliken population analysis in a DFT/B3LYP/6-31G(d,p) approach (Orca computational

software). Red and blue colors represent positive and negative relative spin densities values, respectively (a red-white-blue scale is followed).

5.3.2 Results and Discussion⁶

Considering that the hydration level is a key parameter for the paramagnetic, electronic characterization of melanin, we systematically studied the free radical EPR signal of S-melanin derivatives at different hydration levels (dry, 0 % RH, and wet, 80 % RH). As displayed in Figure 46 (solid lines, 0.03 mW), no significant spectral feature or intensity differences were observed between the hydration levels (also see Figure C4 in Appendix C.2). The asymmetric spectra obtained showed an isotropic g value ranging from 2.0044 to 2.0047 (Figure C5 in Appendix C.2).

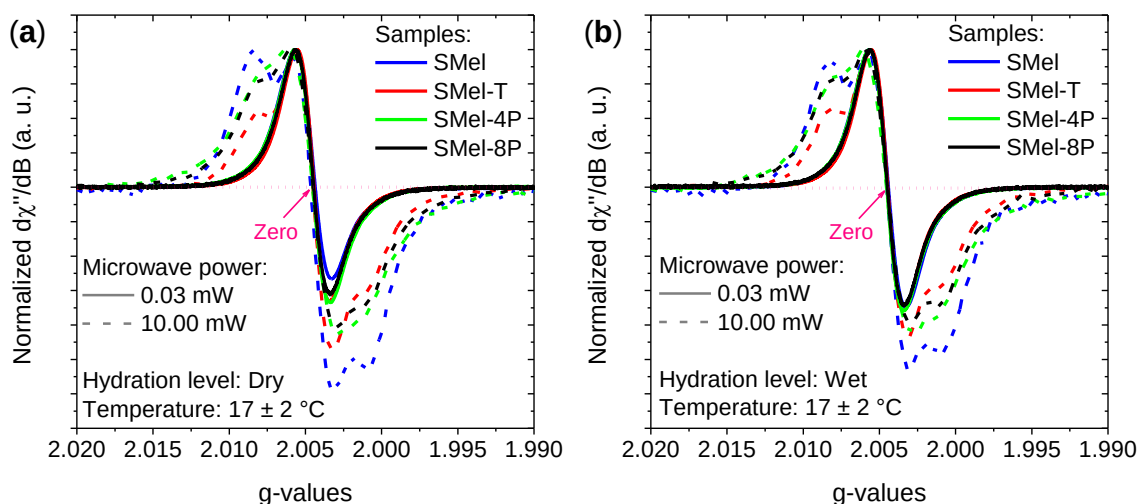


Figure 46. CW X-band EPR spectra normalized to the positive peak for the four S-melanin under (a) dry (0% RH) or (b) wet (80% RH) hydration conditions, at low and high microwave power (see legend).

It is known that the EPR spectra of melanins can change dramatically with the application of higher microwave powers. As a matter of fact, the resultant EPR spectra recorded at 10.00 mW (dashed lines in Figure 46) show that the EPR spectra exhibit new features from broader lines, which are not present at the low microwave power. Such behavior could be related to distorted lineshapes as a result of saturation transfer phenomena between different components that may be present in the system (POOLE, 1983; WEIL; BOLTON, 2007). However, it has

⁶ The results presented in this section were published in Article IV. Reproducible with permission.

also been shown that changing the nature of the paramagnetic species, due to changing the pH, can modify the EPR spectrum in the same way as saturation effects (MOSTERT et al., 2013, 2018; RIENECKER et al., 2015). Hence, an alternative possibility to the high microwave power behavior suggests that the paramagnetic system of S-melanin could be composed of at least two different radical populations with similar g -values that have slightly different spin-lattice relaxation rates, in a similar manner to NF-melanin (see Figure C6, in Appendix C.2, for comparison).

Another interesting feature in Figure 46 is the change in lineshape; that is, the signal asymmetry around the maximum absorption (or when the derivative lines cross zero). Such behavior would be an indicator that the different lines that result in the EPR signal (i.e., different paramagnetic species) do not have the same g -value, or, the species have a different anisotropic g .

To gain more insights into the nature of the paramagnetic properties, the low microwave power spectra were analyzed using simulations with EasySpin (STOLL; SCHWEIGER, 2006). It should be stressed that even though the EPR signal may be anisotropic in nature, we restricted our analysis to consider only isotropic g -values to not over parameterize the problem. This assumption provides an excellent fit. However, if some species have a g anisotropy, a complete analysis will only be possible at HFEPR spectroscopy (MÖBIUS; SAVITSKY, 2008), which will be explored in section 5.4.2.

Initially, our simulations considered the existence of one or two resonant lines. However, the fits were poor. Therefore, a third component was introduced. Figure 47 shows experimental spectra (black), the simulated fitted line (red) and the three simulated components (blue, pink and green) for SMel-T, which is used as an example (see Figure C8-C10, in Appendix C.2, for the other samples). The g -values and linewidths obtained for the three different lines in the dry samples were kept fixed and used for the wet spectra simulations (Figure C7 and C8-C10, in Appendix C.2, for the other samples) to facilitate the data analysis. The only free variables were the intensities of the signals, which were transformed into an estimated spin concentration of each species (Figure 48). Reasonable fits with a root-mean-square deviation (RMSD) below 0.020 were obtained (compared to 0.060 for one or 0.050 for two species). Hence, given the modelling parameters, the simulations potentially indicate the presence of at least three paramagnetic centers in S-melanin.

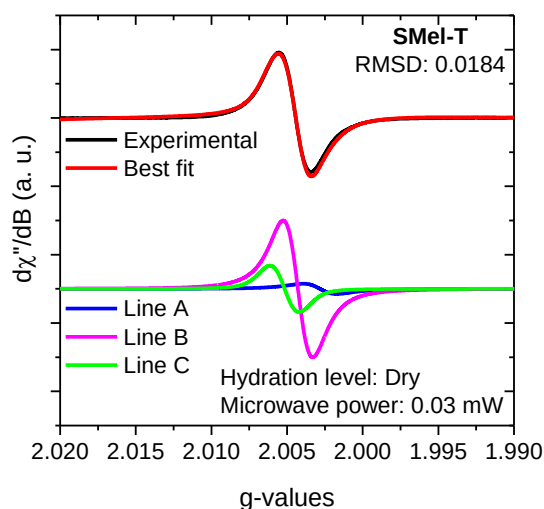


Figure 47. SMel-T CW X-band EPR spectra simulations at a microwave power of 0.03 mW under dry condition (0% RH). Black and red lines represent the experimental and simulated spectra, while blue, pink and green the three components used in the fitting. SMel-T simulations for wet spectra are shown in Figure C7, whereas for the other samples, please see Figures C8-C10, in Appendix C.2. The key differences in the parameters are shown in Figure 48.

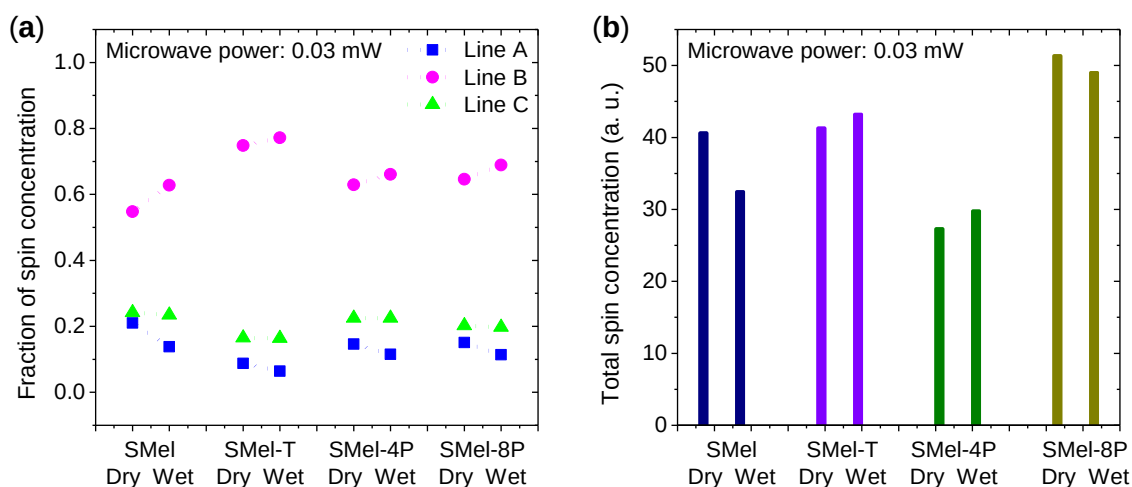


Figure 48. Spin concentration for each component of S-melanin at a microwave power of 0.03 mW. The uncertainty bars (propagated from the difference between modelled and experimental data) are smaller than the data point resolution.

To understand the data in Figure 48, we refer to Table 13 where the estimated and fixed spectroscopic parameters for each component are shown.

Based on the spectroscopic parameters in Table 13, it is possible to see that the g-value of components **S-A**, **S-B** and **S-C** are compatible with NF-melanin (Figure C11, in Appendix C.2) and they are within experimental variations to previous simulations (PAULIN; BATAGIN-NETO; GRAEFF, 2019). The similarity is an indication that the S-melanin

paramagnetic response could be consistent with NF-melanin. In fact, given the relative concentration of sulphur to nitrogen atoms, as estimated via XPS reported in section 3.3 and elsewhere (PAULIN et al., 2018), NF-melanin moieties should be present within the polymer.

Table 13. The spectral parameters of the simulated signals for all four S-melanin samples under both dry and wet conditions.

Resonant lines	<i>g</i> -Values	ΔH_{PP} (mT)	Assignment
S-A	2.0028	0.456	CCR
S-B	2.0043	0.421	SFR
S-C	2.0052	0.392	SFR

Figure 48 indicates that the paramagnetic species exhibited a hydration dependence, which is in agreement with NF-melanin data, but far more subdued in intensity (compare with Figures C12-C13, in Appendix C.2). Usually, the NF-melanin free-radical system is discussed in terms of a supposed carbon-centered radical (CCR) and semiquinone free-radical (SFR). It was proposed that CCR has *g* values below 2.004 and it is located within the stacked oligomeric sheets. In contrast, the SFR is situated at the exterior of the macro-structure, in close contact with the environment, with *g* higher than 2.004 (MOSTERT et al., 2013; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). An interesting feature about these two groups is that experiments carried out with hydration-controlled atmosphere have shown that water and OH⁻ ions can quench the CCR signals generating SFR due to destacking of NF-oligomers (MOSTERT et al., 2013). Hence, considering that the paramagnetic centers of NF-melanin are integrally involved in its electrochemical processes, it should follow that similar free-radicals are present in S-melanin because their electrochemical properties seem to be virtually the same, with the presence of active moieties capable of undergo oxidation and reduction processes (ALBANO et al., 2016; TEHRANI et al., 2020). Therefore, after the addition of water in the system, the intensity of component **S-A** decreases and component **S-B** increases (Figure 48a), which could potentially indicate that a CCR species is destroyed to form SFR.

On the other hand, component **S-C** has lower (to none) hydration effects on the spin concentration. In NF-melanin, component **S-C** has a smaller linewidth compared to components **S-A** and **S-B**, which has been proposed to be an indication of an SFR (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). The same trend is observed for S-melanin, i.e., the **S-C**

component linewidth is smaller than the **S-A** and **S-B** component line widths. As such, the same inference can be drawn, that the **S-C** component in S-melanin is indicative of an SFR species.

These observations could potentially mean an absence of surface interactions between S and NF-moieties and water. Considering that S-melanin may be more hydrophobic, due to the presence of additional methyl groups attached to the sulfonates, and its solubility in DMSO, it is likely that the macromolecule that was formed under the synthesis conditions have led to S-structure sitting on the surface of the particle. Consequently, S-moieties could i) be not affected by hydration (due to the mentioned hydrophobic properties); and, ii) act as effective spacers between reduced and oxidized NF-units to prevent the comproportionation reaction of NF-monomers (Scheme 2) with the hydrating environment.

Figure 48b also shows that the hydration sensitivity of SMel, regarding the spin concentration, is higher than the other sulfonated samples (in other words, there is a variation in spin concentration for SMel of 20% with hydration, whereas for the others samples ~ 6%), which could point towards different macro-structure sizes. If one assumes that SMel has smaller particles compared to SMel-T (as shown by TEM, see Figure C14, in Appendix C.2), it would be expected that the water molecules can easily penetrate through its macro-structure. Hence, following a potential destacking mechanism, the presence of water between the melanin's oligomeric sheets would diminish π - π stacking strength, exposing the CCR that was trapped within the macrostructure. However, a natural question is whether the paramagnetic centers in S-melanin are indeed NF-melanin moieties, somehow trapped and isolated even after water addition, or whether the lineshapes can potentially be ascribed to concomitant S-melanin radicals with similar EPR response.

We also note that from the results presented above indicates that the different polymerization processes, through carbon atoms in the case of SMel and SMel-T, Figure 4a, and through oxygen atoms for SMel-4P and SMel-8P, Figure 4b, (PAULIN et al., 2018), of S-melanin did not affect its paramagnetic properties and the EPR signal can be fitted with the same components. Consequently, the monomeric nature of these samples seems to be more important than the secondary structure, in accordance with a previous theoretical study (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015).

To investigate the above concerns and investigate if one could tell the difference between the two potential scenarios in more detail, we turn to computational modelling. As suggested above, the monomeric structures seem to play a significant role in the EPR signal. Therefore, to evaluate which paramagnetic center could be associated with the three simulated species,

methods) leads to a g -value of 2.0059, which is slightly higher than the signals **S-C** presented in Table 13 and are compatible with those reported elsewhere (GEOFFROY; LUCKEN, 1971).

The g -value of S-units can be divided in three main groups, which are associated with $g < 2.0040$ and $g > 2.0040$ paramagnetic centres:

- **$g < 2.0040$:** S-HQ02 (anion), S-HQ01 (cation), S-HQ02 (cation), S-QI01 (cation); S-QI02 (cation); S-Ndef01 and S-Ndef02.
- **$g > 2.0040$:** S-IQ01 (anion), S-IQ02 (anion), S-QI01 (anion), S-QI02 (anion), S-IQ01 (cation), S-IQ02(cation), S-SQA01; S-SQB01; S-SQ02A and S-SQ02B.
- **$g \sim 2.0059$ (interlayer sulfonated groups):** S-HQ01 (anion).

These subdivisions are compatible with those reported for NF-melanins (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015), with sulfonated and non-functionalized units being classified in the same category. The only exceptions are the degraded S-HQ01-based structures, mentioned before. Such a result indicates that S-melanin units share similar paramagnetic variables to NF-melanins units. In particular, the results suggest that the resonant line **S-A** could be related to low g -value structures like S-HQ02 (anion), S-HQ (cation) and S-Ndef, while line **S-B** can be ascribed to S-QI (anion) species with intermediate g -values. S-SQ (radicals) and S-IQ (anion) species are candidates for line **S-C**.

To better interpret the electrochemistry associated with the formation of paramagnetic centers in S-melanins, the relative alignment between the frontier energy levels (HOMO and LUMO, Figure 50) and the electron donation/acceptance powers of the S-units were estimated and compared with NF-monomers (Figure 51).

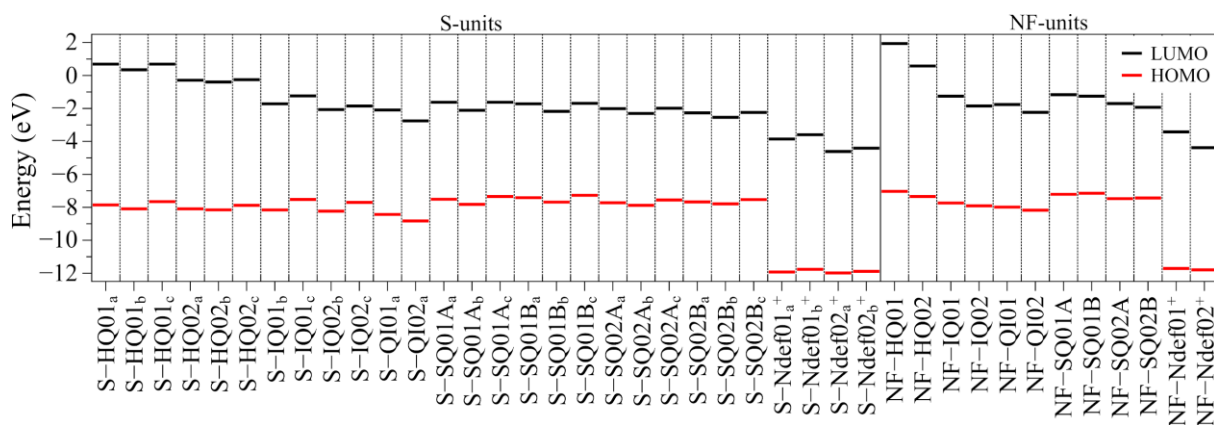


Figure 50. Frontier energy levels of neutral S- and NF-melanin building blocks.

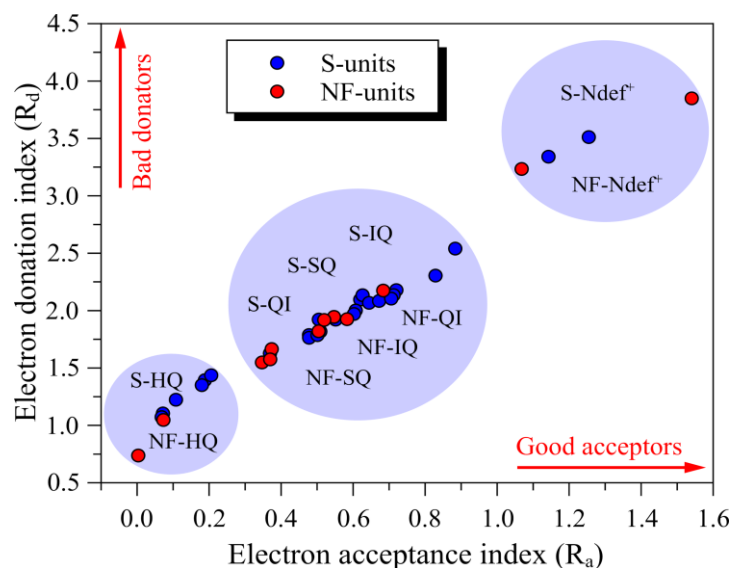


Figure 51. DAM of S and NF-melanin monomers.

S and NF-units present similar electronic features. A detailed analysis exhibit that NF-monomers show a slightly higher tendency to donate electrons than their S-counterparts. In contrast, the opposite behavior is obtained for S-monomers, i.e., S-monomers tends to accept electrons more easily. However, these differences appear to be slight and may indicate similar electrochemistry, which may explain the similarity between S-melanin and NF-melanin electrochemical data (ALBANO et al., 2016; TEHRANI et al., 2020). A relevant feature to note is the definition of Ndef⁺ as effective electron acceptors, which indicate the plausibility of the formation of Ndef-based paramagnetic species in the system.

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As mentioned above, we anticipate that a number of the molecular units present in S-melanin do not contain sulfonate groups. Hence, the formation of paramagnetic centers in S-melanins could include charge transfer and/or trapping between S and NF-monomers, as well as to the surrounding environment, leading to the formation of anionic and cationic structures

that can contribute to the EPR signal. The similarity between the frontier levels of NF and S-units and the presence of trapping states associated with Ndef⁺ is compatible with this hypothesis.

The following could be the case: i) the resonant line **S-A** can be attributed to the neutralized S-Ndef species that act as electron traps in the systems (as well as other cationic species generated in the process); ii) the resonant line **S-B** can be ascribed to S-QI (anion) which is reinforced by the low LUMO levels of these structures; iii) the resonant line **S-C** is mainly associated with S-SQ, S-IQ (anion) and S-IQ (cation) species. The proposed structures are consistent with the previous discussion regarding the water effect on the spin concentration. For example, the disruption of the duplet configuration of S-Ndef centers ($\text{S-Ndef} + \text{H}_2\text{O} = \text{S-HQ} + \text{H}_3\text{O}^+ + \text{e}^-$) decreases component **S-A** after hydration (Figure 48a). With the rearranging of the neutral S-Ndef center, there will be a change in the electronic charge experienced in the system, and, consequently, the extra electron would be donated to S-QI units originating anionic S-QI (component **S-B**) or to S-HQ units originating anionic S-HQ and $\bullet\text{SO}_2\text{CH}_3$ radical. Considering the simulated parameters (Table 13) and the energy levels of the monomeric structures (Figure 50), the first case seems to be the most probable one. Note that a possible transformation of component **S-A** into component **S-B**, while maintaining component **S-C** unchanged, could result in the apparent lack of hydration effect shown in Figure C5, in Appendix C.2.

The electronic conductivity behavior of NF-melanin is usually viewed with the presence of NF-Ndef being able to trap electrons and/or holes. At the same time, the formation of plausible NF-IQ, NF-QI and NF-SQ species can be a source of electron and proton (MOSTERT et al., 2012a; BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015; ALVES et al., 2020). Therefore, the similarities between the free-radical nature and frontier energy levels of NF and S-melanin would point to similar electronic properties. However, it is possible to speculate that S-melanin should present lower protonic current and less sensitivity to hydration due to the presence of S-groups (as will be shown in section 6.2.2).

5.4 HIGH-FIELD/HIGH-FREQUENCY EPR SPECTROSCOPY FOR NF AND S-MELANIN

5.4.1 Methodology

5.4.1.1 EPR measurement

Continuous-wave (CW) HFEPR measurements were performed with a Bruker Elexsys 780 EPR quasi-optical spectrometer measuring simultaneously the induction and absorption spectra. The spectra were acquired with quadrature detection at 263 GHz microwave frequency with 10 kHz modulation frequency, 5 G of modulation amplitude, 600 G of sweep width and 0.1, 1.0 and 10.00 mW microwave powers. Trapped atomic nitrogen in the endohedral fullerene (N@C_{60} , $g_{\text{iso}} = 2.0020$ (WITTMANN et al., 2018)) was used as reference for g -value determination.

5.4.1.2 Data analysis and DFT calculations

The HFEPR spectra were processed by baseline and phase correction, which were obtained by adjusting the admixture of a linear combination between the absorption signal with its Hilbert transform until the spectrum integrated displayed a zero base line (EARLE; BUDIL; FREED, 1993). Details in section C4 (Appendix C.) The spectra were then analyzed using EasySpin (v.5.2.13) (STOLL; SCHWEIGER, 2006) with the pepper routine with gaussian function and anisotropic broadening. To understand the EPR signal anisotropy, DFT calculations was performed similarly to section 5.3.1.4.

5.4.2 Results and Discussion⁷

As mentioned earlier, most of the characterization of melanin's free-radical species was performed using X-band and more detailed information cannot be obtained due to the absence of resolved hyperfine coupling and the limited g resolution. Hence, for a complete hyperfine structure and g anisotropy analysis, HFEPR spectroscopy needs to be used. In Figure 52, we present the 263 GHz HFEPR absorption signal of NF and S-melanin at different microwave powers. All spectra show an asymmetric signal (Figure C19, in Appendix C) with significant anisotropy, distinct rhombic character and no clear resolved hyperfine interaction. Such features are different from the corresponding X-band spectra (see sections 5.2, 5.3 and Figure C20).

⁷ The results presented in this section is part of Paper VII, in preparation.

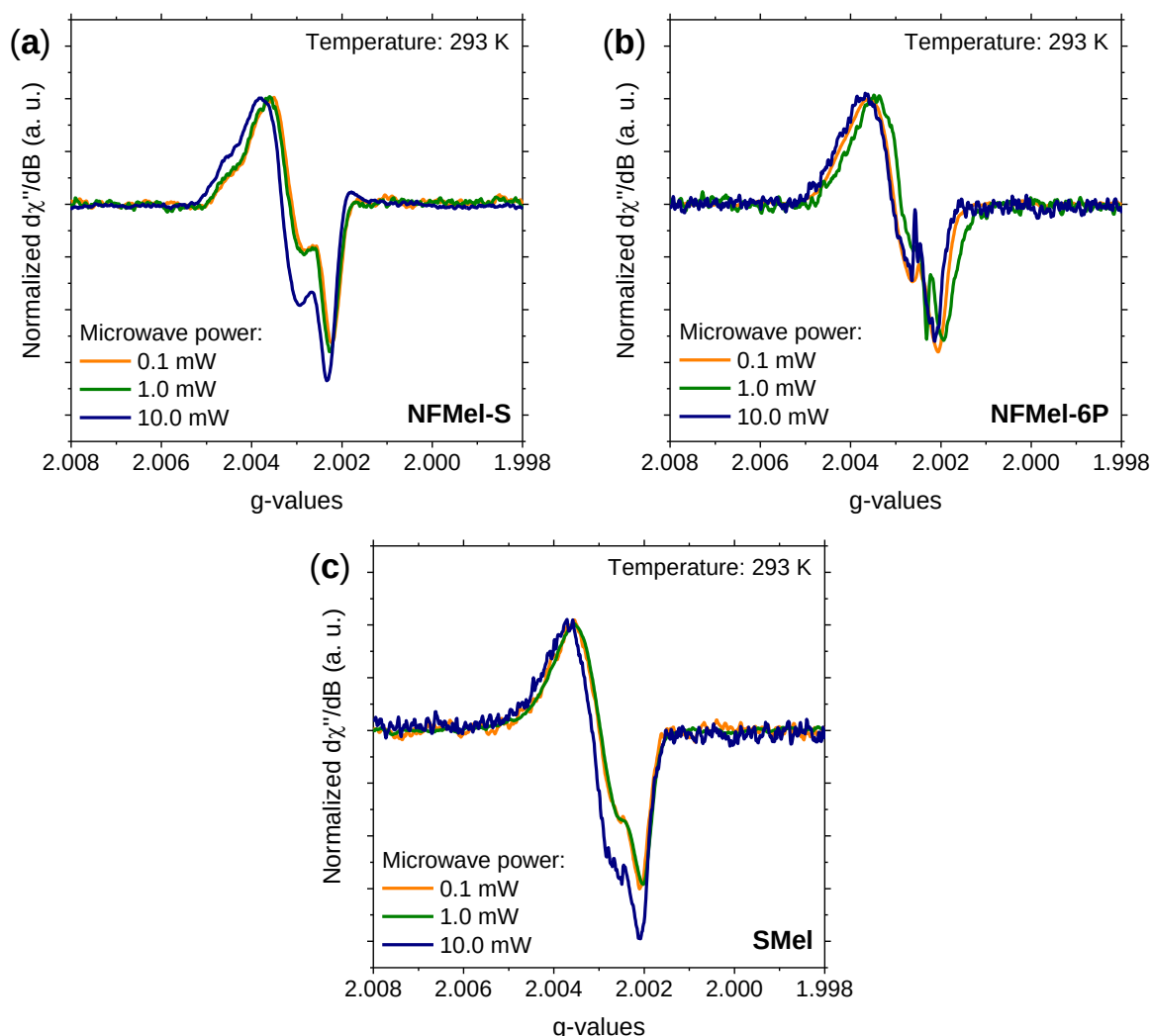


Figure 52. Normalized 263 GHz EPR absorption spectra at 293 K and different microwave power for (a) NFMel-S, (b) NFMel-6P and (c) SMel.

The distortion of the melanin EPR spectra at higher microwave power can be an indication that the paramagnetic system is composed of different species with similar g -values and different spin-lattice relaxation rates as a result of saturation (MOSTERT et al., 2013, 2018; RIENECKER et al., 2015; PAULIN et al., 2020).

The melanin free-radical system was evaluated at low microwave power with EasySpin (STOLL; SCHWEIGER, 2006). We first tested the hypothesis that the spectra are comprised of isotropic broadened lines (Figure C21), anisotropic broadened with axial symmetry (Figure C22), or rhombic symmetry line (Figure C23) with no success. The spectra were satisfactorily fitted assuming anisotropic broadened lines with rhombic symmetry and g -strain (Figure C24). These results are different from earlier observations on Q-band EPR (PASENKIEWICZ-GIERULA; SEALY, 1986) due to the better resolution of 263 GHz spectroscopy. Note that the

rhombic symmetry is commonly a good representation of indole materials (TIPIKIN et al., 2016; DAVIS et al., 2018). Additionally, we also evaluated the presence of multiples resonant lines (RL) in the system. We tested one, two and three RL. The fit was good in all cases; however, when three RL are used, two of them have spectral features so similar that we could be just overparameterizing the analysis (Table C3). Therefore, we disregard such a possibility. On the other hand, with only one RL, we could not reproduce the shoulder at high g (Figure C24a). Hence, we assumed that the spectra are composed of at least two different RL. To further test this idea, we turned to the saturation phenomena mentioned above. The idea is that if the fitting lines do work at high microwave power spectra by changing only their intensity, our attribution is correct. In figures C25-C27, we show that at 10 mW we cannot fit the spectra with one RL, whereas in the case of two RL the fit is good and there is a relative variation in the spin density of each component (Table C4).

Figure 53 shows the HFEPR spectra and their respective simulation considering two paramagnetic species and rhombic symmetry for NFMel-S, NFMel-6P and SMel. Moreover, Table 14 shows the spectral parameters estimated from the fit in Figure 53. We also estimate the anisotropy of the g -tensor (Δg), given by the difference between g_{xx} and g_{zz} , which present greater precision than the absolute g -values (FUCHS et al., 2003) and the spectrum rhombicity, calculated from $[(g_{xx} - g_{yy}).(g_{xx} - g_{zz})^{-1}]$ (DAVIS et al., 2018).

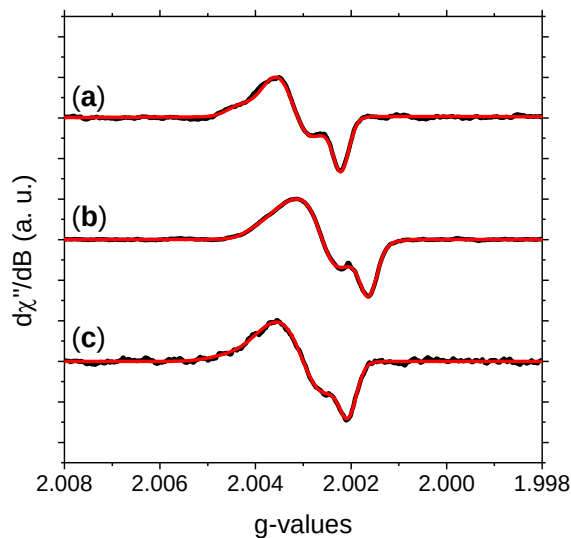


Figure 53. Experimental HFEPR spectra (black line) and their simulation (red line) for (a) NFMel-S, (b) NFMel-6P and (c) SMel at 0.1 mW microwave power. Simulation parameters are displayed in Table 14.

Table 14. Experimentally Determined g-Values for Melanin Derivatives.

	<i>NFMel-S</i>		<i>NFMel-6P</i>		<i>SMel</i>	
Component	A-I	A-II	B-I	B-II	C-I	C-II
g_{xx}	2.0043	2.0037	2.0042	2.0038	2.0046	2.0038
g_{yy}	2.0030	2.0032	2.0032	2.0031	2.0033	2.0030
g_{zz}	2.0023	2.0022	2.0021	2.0021	2.0024	2.0021
$\sigma_{xx} (10^{-4})$	5.10	5.71	5.00	9.58	6.98	8.61
$\sigma_{yy} (10^{-4})$	9.99	4.83	8.77	6.00	2.27	6.54
$\sigma_{zz} (10^{-4})$	0.02	0.01	5.46	2.32	2.03	1.60
g_{iso}	2.0032	2.0030	2.0032	2.0030	2.0034	2.0030
$\Delta g (10^5)$	200	150	210	170	220	170
<i>Rhombicity</i>	0.64	0.33	0.48	0.41	0.59	0.47

We first take a look at the simulation analysis of the NFMel-S (columns 2 and 3). Our results indicate this sample has an HFEPR with a low isotropic g -value ($g_{iso} < 2.0035$) and g -anisotropies (Δg) of 200 for specie **A-I** and 150 for **A-II**. Such values indicate i) the presence of CCR paramagnetic species, and; ii) the principal g -values of **A-I** can be fully resolved at the magnetic fields used for this study, whereas for **A-II** are not resolved, i.e., there is an overlapping of g_{xx} and g_{yy} .

For a π radical, the spectra's high-field edge (g_{zz}) has low spin-orbit coupling and is usually oriented perpendicular to the aromatic system (STONE, 1963a, 1963b). In contrast, the in-plane orientations correspondent to g_{xx} and g_{yy} exhibit large shifts due to spin-orbit coupling originated from the radical structure and environment (STONE, 1963a, 1963b). The g -strain, the full width at half maximum of the Gaussian distributions of the main g -values, demonstrates low directional dependence at g_{zz} and moderate to high at g_{xx} and g_{yy} . Such behavior would indicate that, although the species may be similar, they should present some *local* structural variation.

The g -values of NFMel-6P behaves similarly to NFMel-S, but with small variations that are within experimental erros. The significant difference is an increased directional dependence. This result indicates that NFMel-S and NFMel-6P have similar free-radical species, as we have speculated in an earlier study (PAULIN; BATAGIN-NETO; GRAEFF, 2019; PAULIN et al., 2020). However, the different oxidation states of these samples and the different macrostructure are probably responsible for the changes.

To help with the interpretation, we analyze the g anisotropy obtained from the density functional theory of each anionic, cationic and radical structure summarized in Figure 54.

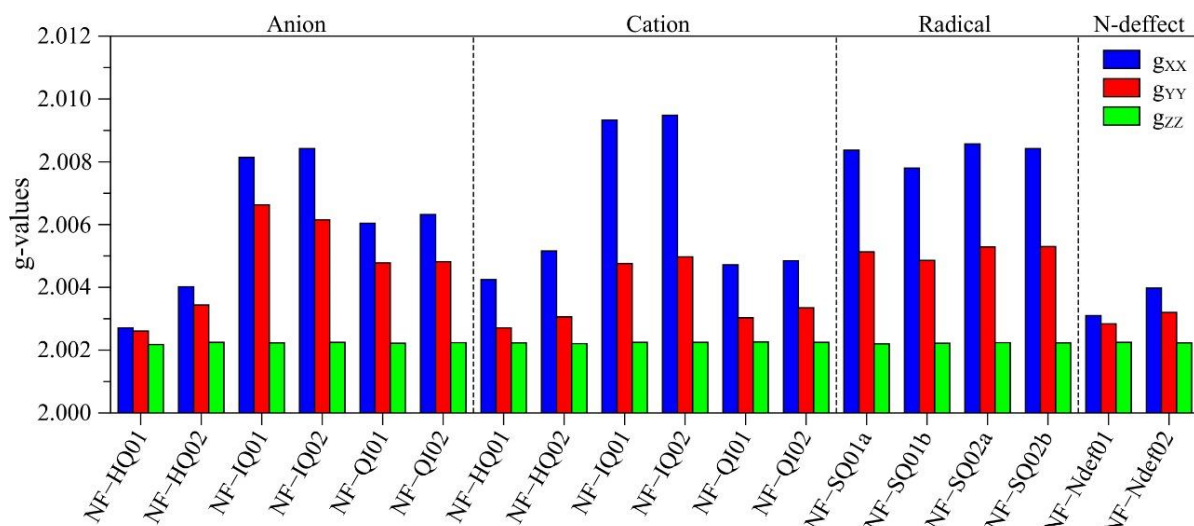


Figure 54. Different g -values obtained for anionic, cationic and radical NF-melanin units. **01** and **02** refer to NF-DHI and NF-DHICA species, respectively.

Our group's earlier study showed that CCR species associated with $g_{iso} < 2.0040$ would be compatible with anion and cation NF-HQ, cation NF-QI and neutral NF-Ndef⁺ (NF-Ndef); whereas SFR, $g_{iso} > 2.0040$, with anion NF-IQ and radicals NF-SQ (BATAGIN-NETO; BRONZE-UHLE; GRAEFF, 2015). To gain insight into each radical anisotropy, we convert the theoretical g -values into Δg and rhombicity and compare them in Figure 55a. From Figure 55a, it is possible to see that the species attributed to CCR has $\Delta g < 300$, whereas for SFR $\Delta g > 300$ with no apparent order for rhombicity. Additionally, based on the spectroscopic values in Table 14, the radical **A-I** can be assigned to cationic NF-QI and **A-II** to neutral NF-Ndef01 and anionic NF-HQ02. On the other hand, species **B-I** and **B-II** seem to be related predominantly to species NF-Ndef02 (neutral).

The isotropic g below 2.0035 and the low Δg in Table 14 indicate that the HFEPR signal could be ascribed to only CCR species. Some studies suggest that semiquinone species should present a g_{xx} higher than 2.0055 (Figure 54 and references (BÄHRLE et al., 2015; TIPIKIN et al., 2016)) at the low-field edge of the HFEPR absorption spectra. However, we do not see any g tensor component in Figure 52.

The possible absence of SFR species on melanin is an odd feature at first. However, our result does not imply the NF-SQ does not exist in melanin; it could mean that the experimental condition of the measurement favors the presence of only CCR species. As a matter of fact, we have shown that melanin pellets result in a higher amount of CCR species (PAULIN;

BATAGIN-NETO; GRAEFF, 2019). On top of that, based on the synthetic procedure's purification process, we also expect that our samples are at a neutral level, which also points towards low SFR content (PAULIN; BATAGIN-NETO; GRAEFF, 2019). Moreover, there is the possibility that organic radical with close g (as melanin does have, Figure 54) require even higher fields than 263 GHz to fully resolved all species in the system (EATON; EATON, 1999). Another possibility is that the measured signal is already saturated or at the vicinity of saturation at 0.1 mW (Figure C28). In such case, the proper experimental approach would be to measure the HFEPR spectra at even low microwave power, however the measurements were taken at the lower limit of the spectrometer, which hinders further analysis.

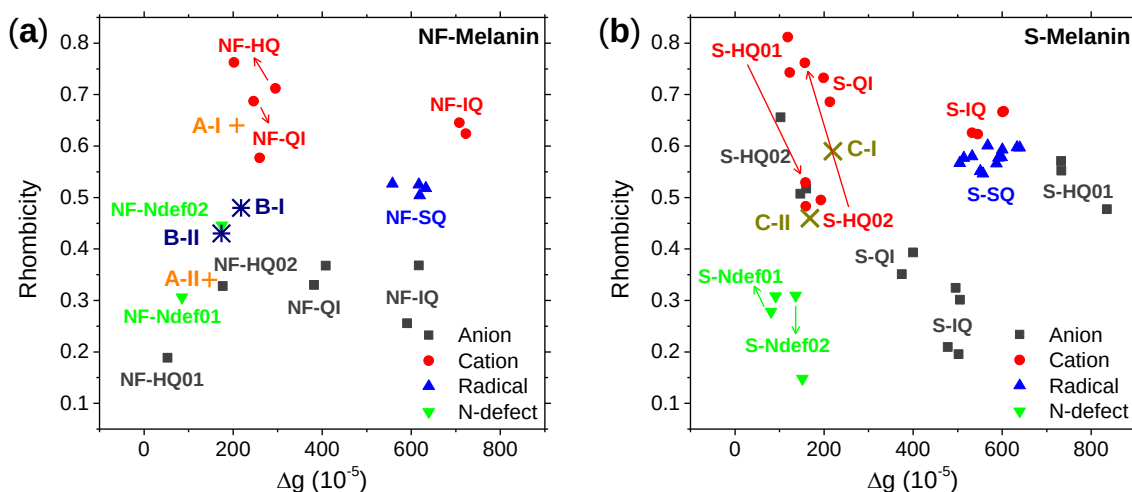


Figure 55. The plot of the rhombicity as a function of g -value anisotropy for the different (a) NF-melanin and (b) S-melanin radical species. The theoretical radicals are represented by the square, circle and triangle symbols; the crosses are each of the experimental radicals. A purely isotropic signal would have $\Delta g = 0$ and a completely axial symmetry signal would be obtained with rhombicity equal to 1.0 or 0.0.

Now, we turn to sulfonated melanin, i.e., SMel. Based on Table 14, it is possible to observe that the spectroscopic features of SMel are similar to non-functionalized melanin, with low isotropic g -value ($g_{iso} < 2.0035$) and Δg of 220 for specie **C-I** and 170 for **C-II**. However, different from NF-melanin that has higher directional dependence at g_{xx} and g_{yy} , the **C-I** component of SMel displayed a low dependence at g_{yy} , probably due to the sulfonated groups. In previous work, we have shown that the free-radical nature of SMel is compatible with NF-melanin (PAULIN et al., 2020). Hence, the difference in the spectroscopy values may suggest that there are both radicals in S-melanin, that is, sulfonated and non-functionalized radicals.

The g anisotropy of anionic, cationic and radical S-units was also obtained from DFT and they are displayed in Figure 56. Similarly to the NF-melanin, the same g_{iso} category can be applied for S-melanin (PAULIN et al., 2020), with the difference that S-HQ01 has an isotropic g higher than 2.0040. Such effect was assigned to a defect from the sulfonated species (PAULIN et al., 2020). Also, each radical g component Figure 55b follows a similar order of NF-melanin. A close analysis of Figure 55b indicates that species **C-I** is close related to cationic NF-QI, whereas for **C-II** anionic to S-HQ-02(a, b) cationic S-HQ01b and S-HQ02(a, c) and neutral NF-Ndef02.

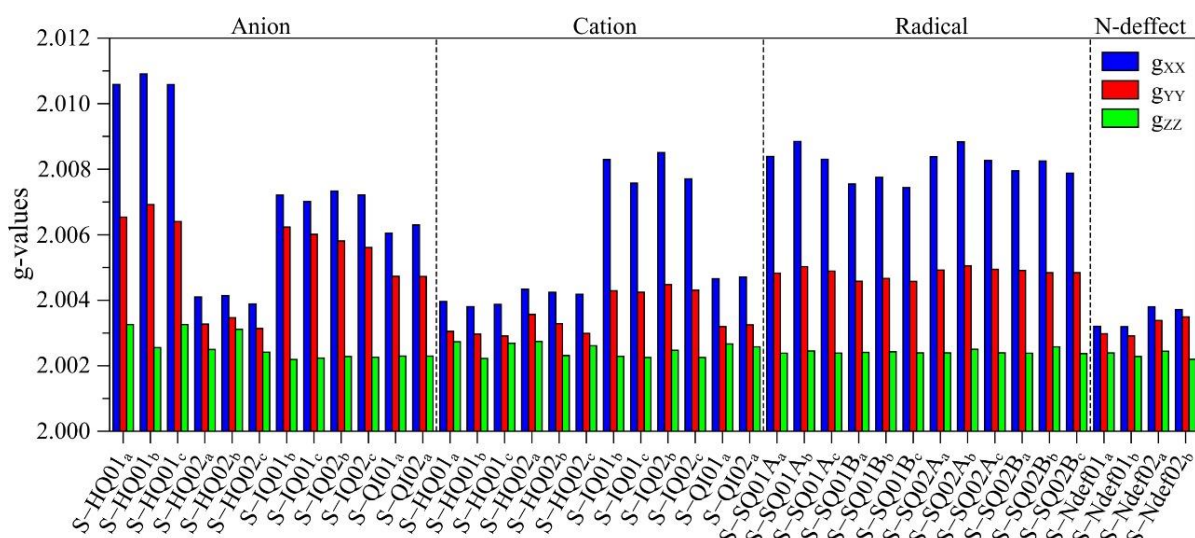


Figure 56. Anisotropic g -values of anionic, cationic and radical S-melanin monomers. **01** refers to S-DHI and **02** to S-DHICA species.

Both groups' spectroscopic similarities suggest that SMel can have an equivalent paramagnetic system to NFMel-S and NFMel-6P, consistent with previous X-band analysis (PAULIN; BATAGIN-NETO; GRAEFF, 2019; PAULIN et al., 2020). Hence, more sophisticated pH-dependent EPR studies on multifrequency continuous-wave/pulsed EPR and pulsed ENDOR experiments will be necessary to understand the melanin electronic structure fully.

5.5 CONCLUSION

In this chapter, it was possible to identify and discuss the nature of paramagnetic species found in different melanin samples. Our results suggest that the EPR spectra of non-functionalized melanin samples are generically composed of three distinct components: two of them related to carbon-centered radicals (CCR) species and the other to semiquinone free radicals (SFR). Also, analysis of the EPR parameters and energy levels alignments of its

building block subunits suggests that the CCR formation is associated with N-def species in the bulk of melanin macrostructure. The results from samples with distinct DHI/DHICA ratios suggest that CCR components can be related to DHICA and DHI species.

On the other hand, we have combined hydration-controlled, solid-state X-band CW EPR spectroscopy and electronic structure calculations to study the nature of paramagnetic species of S-melanin derivatives. Our results show that the S-melanin EPR signal appears similar to NF-melanin. Simulation analyses of the EPR signals suggest that the S-melanin signal comprises three different resonant lines that are compatible with NF-melanin. Intriguingly, the theoretical calculations indicate that the EPR signal could arise from distinct S-units, in particular S-Ndef, S-QI (anion) and S-SQ (radical)/S-IQ (anion). Based on S-melanin's free-radical properties, we have speculated some potential positions for sulfonated groups binding on its oligomeric structure and possible charge transport behavior.

Finally, using HFEPR we have shown that CCR species has low g-anisotropy, whereas SFR higher. Additionally, S-melanin presents a similar behavior to NF-Melanin even at high-fields, which corroborate our X-band analysis.

CHAPTER VI

CHARGE TRANSPORT PROPERTIES

6.1 INTRODUCTION

The charge transport study in melanin began in the early '60s with Longuet-Higgins (LONGUET-HIGGINS, 1960) and Pullman and Pullman (PULLMAN; PULLMAN, 1961) suggestions that, theoretically, the electronic delocalization of the melanin indole structure could allow semiconductivity. A few years later, the first electrical and photoconductivity observations started to emerge, including water's importance in the electrical properties (ROSENBERG; POSTOW, 1969; POWELL; ROSENBERG, 1970).

However, it was just with the two pioneering works of McGinness (MCGINNESS, 1972; MCGINNESS; CORRY; PROCTOR, 1974) that the electronic potential of melanin started to show up. McGinness *et al.* demonstrated that a moisturized melanin sample sandwiched between two gold-leaf electrodes had a reversible switching behavior; particularly, it could be seen as an excitation of electronic charge carrier from a low mobility state to a high mobility state with the application of moderate electric fields. Hence, melanin was established as an amorphous organic semiconductor.

The reversible switching behavior was explained following a dielectric theory. This theory modifies the Arrhenius temperature dependence of the conductivity, usually used to describe semiconductivity, to account for the water effect in the material, Eq. 19, and does not imply any specific conduction mechanism or type of charge carrier (ROSENBERG; POSTOW, 1969).

$$\sigma(T, \kappa_{Wet}) = \sigma_0 \exp\left(-\frac{E_{Dry}}{2 k_B T}\right) \exp\left[\frac{e^2}{2 k_B T R} \left(\frac{1}{\kappa_{Dry}} - \frac{1}{\kappa_{Wet}}\right)\right] \quad (19)$$

where, σ_0 is the dry conductivity in the infinite temperature limit, T is the temperature, R is the screening length of water, k_B is Boltzmann's constant, E_{Dry} and κ_{Dry} are, respectively, the energy gap and the dielectric constant of dry melanin and κ_{Wet} is the dielectric constant of wet melanin. The idea is that the presence of water is capable of changing the local dielectric constant of organic semiconductor systems; in other words, the presence of water in the system increases the average dielectric constant, which results in a reduction in the energy gap, allowing the resistive switching behavior to emerge at low voltages (ROSENBERG; POSTOW, 1969; MEREDITH; SARNA, 2006).

In the following decades, the amorphous semiconductor model dominated the interpretation of most, if not all, electrical studies on melanin (POWELL; ROSENBERG, 1970;

MCGINNESS; CORRY; PROCTOR, 1974; CULP; ECKELS; SIDLES, 1975; FILATOV; MCGINNESS; CORRY, 1976; BARALDI et al., 1979; BRIDELLI; CAPELLETTI; CRIPPA, 1981; OSAK et al., 1989, 1995; JASTRZEBSKA et al., 1995, 2002; JASTRZEBSKA; JUSSILA; ISOTALO, 1998; JASTRZEBSKA; KOCOT; TAJBER, 2002; CAPOZZI et al., 2006; GONÇALVES; FILHO; GRAEFF, 2006; ABBAS et al., 2009; LIGONZO et al., 2009). The most notable feature within the massive amount of work and a significant influence on the electrical properties was the hydration dependent conductivity. Such hydration dependence is quite impressive since the conductivity changes up to 8 orders of magnitude over the humidity range, Figure 57.

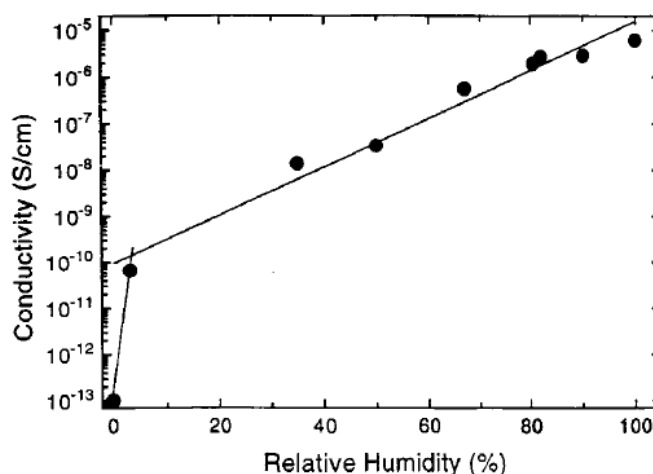


Figure 57. Standard melanin conductivity, in pellet, as a function of relative humidity. Reprinted with permission from (JASTRZEBSKA et al., 1995). Copyright© 1996, Taylor & Francis.

Even with the recognition of the importance of hydration on melanin's electrical properties, many different authors searching for its dominant charge carrier lacked in performing such a control experiment, which resulted in several inconsistent results. However, a coulometric study under hydration-control determined that melanin seems to conduct about 65 % protons and 35 % electrons in the wet state (POWELL; ROSENBERG, 1970). In that time, the split of the water molecule in the electrode to increase the number of proton carriers was a possible explanation for this result (POWELL; ROSENBERG, 1970; GLASSER, 1975). Another attempt at determining the charge carriers used a combination of Child's law behavior in IV measurements with AC and dielectric spectroscopy data. This set of information has interpreted the dominant charge transport mechanism of melanin due to the hopping of small polarons (OSAK et al., 1989; JASTRZEBSKA; JUSSILA; ISOTALO, 1998; JASTRZEBSKA et al., 2002).

While most of the studies since the late '60s focused only on the electronic nature of melanin conductivity, it was just in 2006 with the work of Gonçalves *et al.* that the charge transport started to shift towards a more complex mechanism to account for the role of protons in the electrical properties of melanin (GONÇALVES; FILHO; GRAEFF, 2006). By joining temperature-dependent conductivity, infrared spectroscopy and EPR measurements, the authors considered that protons could be the dominant type of charge carrier on melanin, similarly to the pH-dependent electron conduction of polyaniline, where the protons can modulate the density of mobile electrons on the backbone structure.

A few years later, a more precise and controlled hydration study performed by Mostert and collaborators re-evaluated the proton mechanism of Gonçalves *et al.* and provided new insights into the proton's role in the charge transport of melanin. They showed that the melanin conductivity was incompatible with the amorphous semiconductor behavior and the modified dielectric model of the early '70s (MOSTERT *et al.*, 2012a, 2012b). They proposed that the increase of conductivity with hydration was not due to dielectric changes but rather an increase in both proton and electron concentrations. In this new point of view, upon adsorption of water, there is the formation of free-carriers, i.e., ions (protons, H^+) and extrinsic free-radicals (electrons, in the form of semiquinone radicals) into the hydrating matrix following the comproportionation equilibrium reaction, Scheme 2, a similar effect of the pH in EPR measurements (MOSTERT *et al.*, 2012a). The authors termed this phenomenon “water-induced chemical self-doping” and advocated melanin as a mixed ionic-electronic conductor rather than an amorphous semiconductor to explain its exotic electrical property.

This hybrid conductivity model of electrons and protons is supported by a series of EPR experiments as a function of the light intensity (COPE; SEVER; POLIS, 1963), metallic ions (FELIX *et al.*, 1978b), temperature (CHIO; HYDE; SEALY, 1980) and pH (CHIO; HYDE; SEALY, 1982); and also by transient current analysis, current-voltage and electrochemical impedance spectroscopy (WÜNSCHE *et al.*, 2013b, 2015; DI MAURO *et al.*, 2016). Not only that, the hybrid conductivity became a generic model for other materials with ionizable groups such as polysaccharides and proteins (ZHONG *et al.*, 2011; ORDINARY *et al.*, 2014).

In the last couple of years, the mixed driving behavior of melanin conductivity is starting to be questioned again. Using light-induced paramagnetic resonance analyses and impedance/dielectric spectroscopy, the same group shown that wet melanins might be exclusively a proton-conducting matrix system with no electronic components (MOSTERT *et al.*, 2018; SHELIAKINA; MOSTERT; MEREDITH, 2018b). The development of a solid-state

organic electrochemical transistor capable of converting proton to electronics currents exemplified this hypothesis since a melanin film used as a solid electrolyte could directly inject protons into the *p*-type semiconductor channel of the device through a volumetric mechanism (SHELIAKINA; MOSTERT; MEREDITH, 2018a). On the other hand, it has recently been shown that dry Sepia-melanin (i.e., natural melanin) pellets between copper and stainless-steel electrodes showed a quasi-linear and metallic-like electrical response (REALI et al., 2020; REALI; SAINI; SANTATO, 2021). The absence of metallic filaments bridging the electrodes and phase transformation (amorphous to graphitic carbon) indicates that the electronic response of Sepia-melanin's dry pellets would be predominantly electronic.

Based on the above ideas, Santato's group has proposed a new perspective on semiconductivity's classical concept towards protonic transport on bio-sourced materials. In this case, the charge carrier type of biomaterials is not the determinant for the semiconducting properties but rather their extended systems of energy bands (REALI; SAINI; SANTATO, 2021). From this new semiconductivity point of view, melanin would feature both electronic and protonic charge carries, with a switch from predominant electronic to predominant protonic conduction depending on the hydration. Additionally, they claim that the highly hydrated melanin conductivity properties can be explained by a doped protonic semiconductor model (REALI; SAINI; SANTATO, 2021).

Regardless of which mechanisms is dominant, protons' motions in synthetic melanin are an open and important question. The primary evidence so far comes from Mostert *et al.*, who got a muon hopping rate of $0.3 \mu\text{s}^{-1}$ (MOSTERT et al., 2012a). The muon hopping can be viewed as an approximation for the proton movement in melanin. The value obtained is compatible with the diffusion mobilities mediated by an incoherent proton hopping mechanism such as the Grötthuss mechanism, Figure 58 (GLASSER, 1975; AMDURSKY; GŁOWACKI; MEREDITH, 2019). Suppose one considered the presence of a massive positive charge carrier in this mechanism, it would be comprehensible that in the earliest studies, a *p*-type small polaron carrier were considered. However, such evidence does not exclude the possibility of a proton moving through a center of mass diffusion or “vehicle mechanism” (GLASSER, 1975).

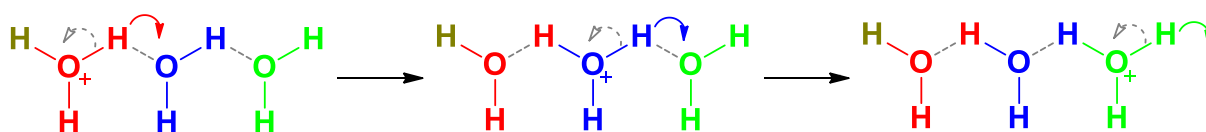


Figure 58. Anionic charge hopping through a hydrogen-bonded network following the Grötthuss mechanism. Dashed grey arrow indicates the electron pathway, whereas the solid-colored arrow the hydrogen pathway.

In this regard, it is not the purpose of this chapter to set a stone on melanin's charge transport mechanism. Instead, in the first part of this chapter, DC and AC measurements were used to verify the effect of different thin-film processes on NF-melanin's charge transport and provide a more detailed study on the S-melanin charge transport for the first time. This chapter finalizes with preliminary results on the proton dynamic in the melanin system using neutron scattering.

6.2 COMPARISON OF NF AND S-MELANIN CHARGE TRANSPORT

6.2.1 Methodology

6.2.1.1 Device fabrication

Melanin samples were synthesized as described in CHAPTER II, Section 2.1.2.

Glass slides (10 mm x 10 mm) were cleaned with Alcanox[®], rinsed in water, ultra-sonicated in acetone (15 min), 2-propanol (another 15 min) and dried under a flow of nitrogen. Then, 50 nm of a gold interdigitated electrode with channel length and width of 100 μ m and 11.2 mm, respectively, were thermally evaporated. The electrodes were finally treated with UV-Ozone (5 min) and, soon after, melanin thin-film was spin-cast at 500 rpm for 5 s followed by 1500 rpm for 60 s. For these films, melanin solutions were prepared as described in CHAPTER III, Section 3.2.1. At least six different pairs of electrodes from 2 different substrates were used.

6.2.1.2 Electrical measurements

Humidity dependent measurements were carried out using a JANIS Research Cryogen Free Micromanipulated probe station attached to the steam delivery system (CHAPTER II, Section 2.1.3). The JANIS chamber was initially pumped down to vacuum overnight using a rotary pump to ensure that all samples were completely dried. Four water vapor pressures were used as described in the steam delivery system section: 0.0, 10.0, 20.0 and 24.9 mbar. All these pressure data were normalized to a relative pressure value, using a water saturation pressure of 24.9 mbar at room temperature (21.5 ± 0.1 °C).

The current-voltage (JV) data were measured using a Keithley 2450. The voltage was swept forward and backward from -1 to 1 V to prevent electrochemical water splitting into hydrogen and oxygen, which occurs at a potential difference of 1.2 V, and reduced additional systematic uncertainty in the measurement. Electrochemical impedance spectroscopy was taken

sweeping through a frequency range from 10^{-2} Hz to 10^4 Hz only at saturation vapor pressure with a two-electrode cell and Metrohm Autolab PGSTAT302N with FRA32M module.

6.2.2 Results and discussion⁸

DC and AC measurements were done to study the several soluble melanin derivatives' charge properties and compare them to the standard melanin (NFMel-I). Figure 59 presents the current vs. voltage curves as a function of hydration level for NFMel-I in $\text{H}_2\text{O-NH}_3$ and SMel in DMSO. The behavior observed for these two samples is an excellent representative for the other non-functionalized (Figure D1 & D2, in Appendix D) and sulfonated (Figure D3) derivatives. Besides, due to the best results current intensity (Figure D4) at room atmosphere using DMSO as a solvent, we have focused only on it for our analysis.

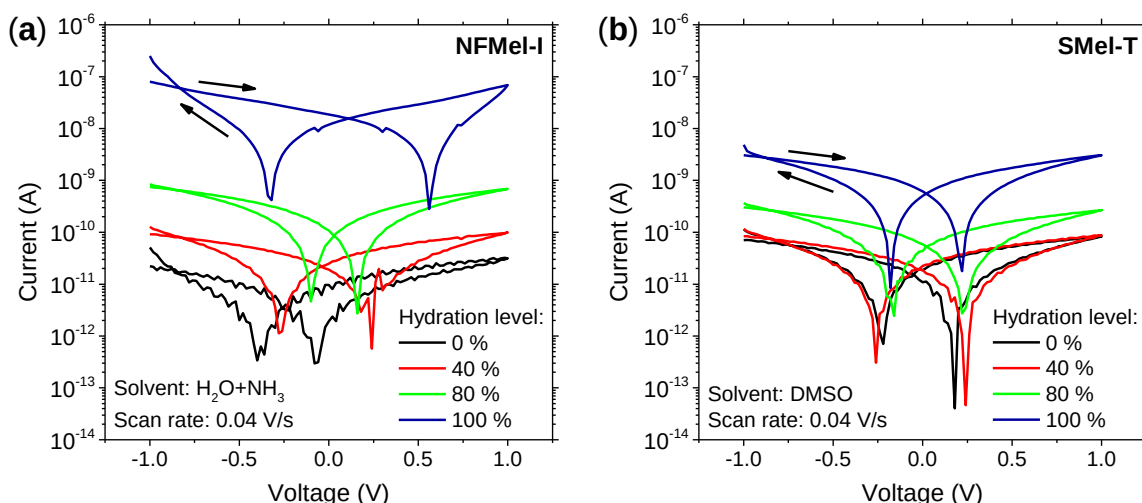


Figure 59. Current as a function of voltage for (a) NFMel-I in $\text{H}_2\text{O-NH}_3$ and (b) SMel-T in DMSO at different hydration levels. Each IV curve is an average of at least six different pairs of gold interdigitated electrodes. Similar behavior was found for other samples.

From Figure 59, it is clear that for both groups of samples, the current increases as a function of hydration, which could be understood as a rise in charge carriers, protonic and electronic, due to the shifting of the comproportionation reaction (Scheme 2). Figure 59 also shows a hysteresis between forward and backward sweeps linked to the protonic species' capacitive effects due to their slow motion and inability to be extracted at the gold electrode. However, one may see that S-Melanin has a lower sensitivity towards hydration, which would result from the lower interaction of its macrostructure with water molecule (section 5.3.2).

⁸ The results presented in this section is part of Paper VI, in preparation.

With this IV data, it was possible to get the conductivity as a function of hydration, and the result can be seen in Figure 60. This type of conductivity plot ranging from the entire hydration level for water is the first conductivity isotherm for the soluble derivatives used in this study.

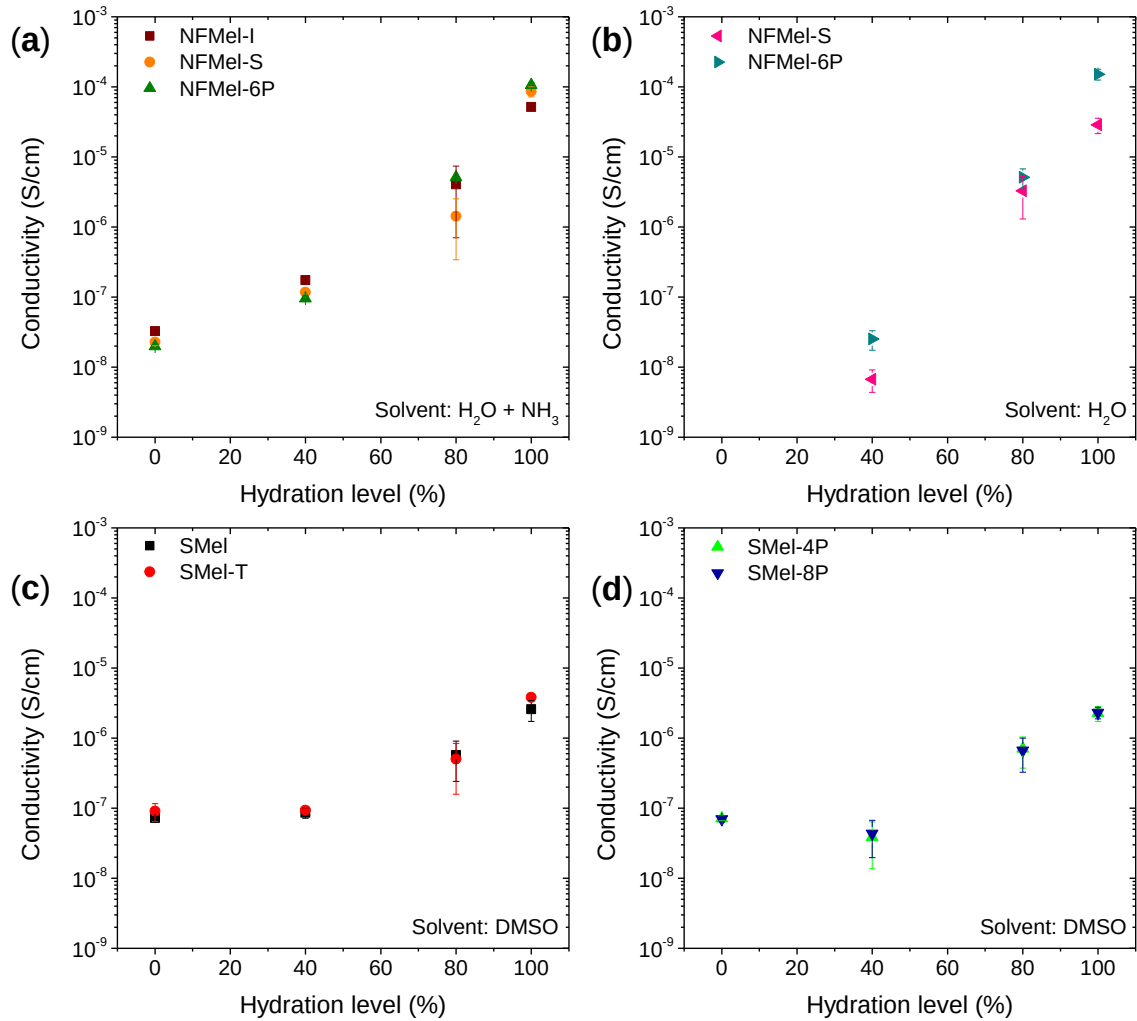


Figure 60. (a, b) NF-Melanin and (c, d) S-Melanin conductivity as a function of hydration. The conductivity was calculated through the inverse of the sample resistivity normalized by the electrode geometry.

Figure 60(a, b) shows, for the films prepared with $H_2O + NH_3$ solution, a variation of 10^{-8} to 10^{-4} S/cm ranging from 0 to 100% humidity. On the other hand, from 40 to 100% humidity, only distilled water resulted in a variation of 10^{-9} to 10^{-4} S/cm. Note here that we could not calculate these samples' dry conductivity due to a high capacitive effect, i.e., the current vs. voltage curve at 0% humidity had a box-like shape. The increase of the conductivity with hydration demonstrates that the ionic component may have a significant role in its charge

transport. Our result suggests that the ammonia's oxidation environment could assist in the deposition process and induce some change in the samples' aromaticity and improve their water sensibility. One possibility would be that the ammonia de-stack NF-Melanin sheets, resulting in a higher surface area for melanin and water interaction. A similar assumption could also be verified by a slight increase in the conductivity of NFMel-6P compared to NFMel-S in both cases. It is known that NFMel-6P is composed of smaller particles (BRONZE-UHLE et al., 2016), which could lead to easy pathways for the water molecules to permeate through its macrostructure.

On the other hand, it was found that S-Melanin's conductivity varies from 10^{-8} to 10^{-6} S/cm in the whole hydration level, Figure 60(c, d). The lower hysteresis, Figure 59, and the lower conductivity, Figure 60, of S-Melanin derivatives could be partially explained by the lower S-macrostructure interaction with water (section 5.3.2). The lower interaction with water content for the same hydration level would tend to indicate less ability to solvate protons than NF-Melanin. So, if there are fewer protons available due to the presence of the sulfonate groups at the indole ring's hydroxyl group, and less water means less ability to solvate, these together will undoubtedly give a lower conductivity. Another interesting feature comparing both groups of samples at 100% hydration is that S-Melanin still shows an ohmic-like behavior, which is not the case of NF-Melanin. The non-ideal current vs. voltage characteristic can be considered a signature of protonic (and ionic) transport (GLASSER, 1975; HUGGINS, 2002), which corroborates our assumption that NF-Melanin has a higher concentration of movable ions than its S-counterpart. A meticulous investigation of the current vs. voltage curve symmetry could be possible with the Maxwell-Wagner model (IWAMOTO, 2015), allowing us to also refine the conductivity values found in Figure 60. This study will be done in the near future.

It is worth noting that it is impossible to distinguish between electronic and ionic contributions with DC measurements and cannot provide a complete insight into the melanin derivatives charge transport. Therefore, in an attempt to investigate the charge transport dynamics of NF and S-melanin, we have conducted electrochemical impedance spectroscopy (EIS) studies at 100% hydration level. Figure 61 displays the NFMel-I and SMel-T EIS spectra as examples. Similar behavior was obtained for the other samples (see Figure D5-D7). The imaginary vs. real components of the complex impedance exhibited a semicircle coupled to an upward nearly-linear line (or tail) at lower frequencies. Hence, we have modeled our data with a Randles circuit. In the insert of Figure 61(a), a schematic of the Randles circuit is presented: R1 is a resistor for the contact resistance, a second resistor R2 represents the ionic flow with

the Warburg element W capturing the ionic/electrode effects. The CPE, or constant phase element, captures the capacitive effects of the rest of the material (BISQUERT et al., 1998; HUGGINS, 2002).

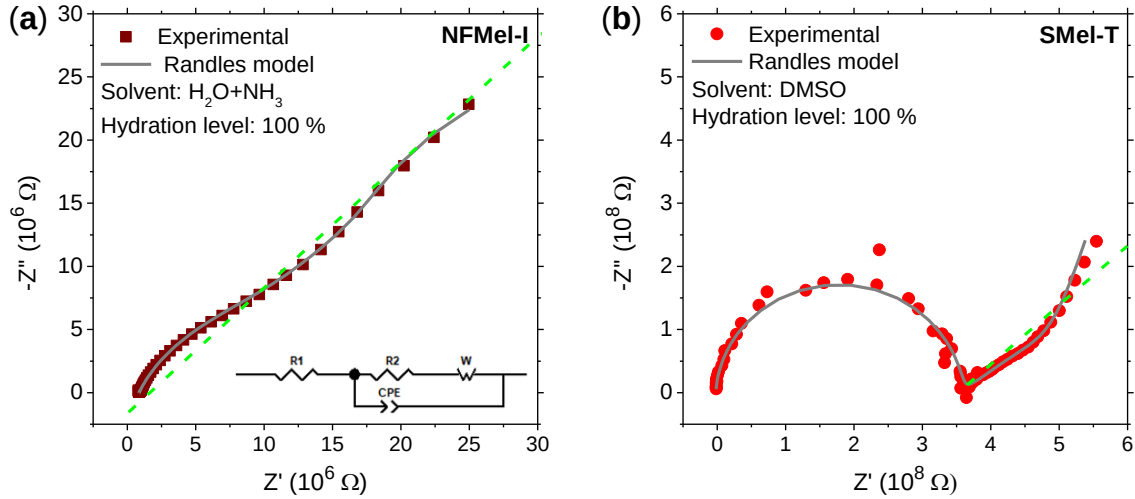


Figure 61. Nyquist plots from EIS measurements on (a) NFMel-I and (b) SMel-T at 100% hydration level. Scatter colored plots represent experimental data, while continuous grey lines the fit using the Randles circuit as a model. Scatter colored plots represent experimental data, while straight gray lines the fit using the Randles circuit (insert in part a) as a model. The dashed green line is set at 45° .

The complex impedance of CPE (Z_{CPE}) can be defined by $Z_{CPE} = [Q \cdot (j \omega)^\eta]^{-1}$, where j is the imaginary unity ($\sqrt{-1}$) and Q and η are fitting parameters, with $0 \leq \eta \leq 1$ (BISQUERT et al., 1998). For $\eta = 0$, CPE acts as pure resistor; and for $\eta = 1$ as a pure capacitor. Based on our modeling, we have found $\eta = 0.78 \pm 0.08$ for NF-melanin and $\eta = 0.97 \pm 0.02$ for S-melanin, implying that the CPE acts as a capacitor. This difference could be an indication of the different transport mechanisms in the AC regime. NF-melanin's Nyquist plot is almost a straight line with $\sim 45^\circ$ slope over most of the frequency range, whereas, for S-melanin, there is a clear separation of a semicircle at high frequencies and the Warburg tail at low frequencies.

For a better comparison of the charge transport mechanism, we analyze the impedance modulus and phase angle as a function of frequency in Figure 62 for NF-Melanin and Figure 63 for sulfonated derivatives.

Figure 62(a, b) shows, in the high-frequency region ($f > 100$ Hz), the impedance response of all samples is governed by a frequency-independent electronic process, which is compatible with the low phase angle in Figure 62(c, d) (KWON et al., 2016); however, at the low-frequency domain ($f < 10$ Hz), the ionic species diffusion takes place and control the system response.

The phase angle variation at the low frequency can suggest capacitive double layer formation with a significant impact on redox reactions (KWON et al., 2016).

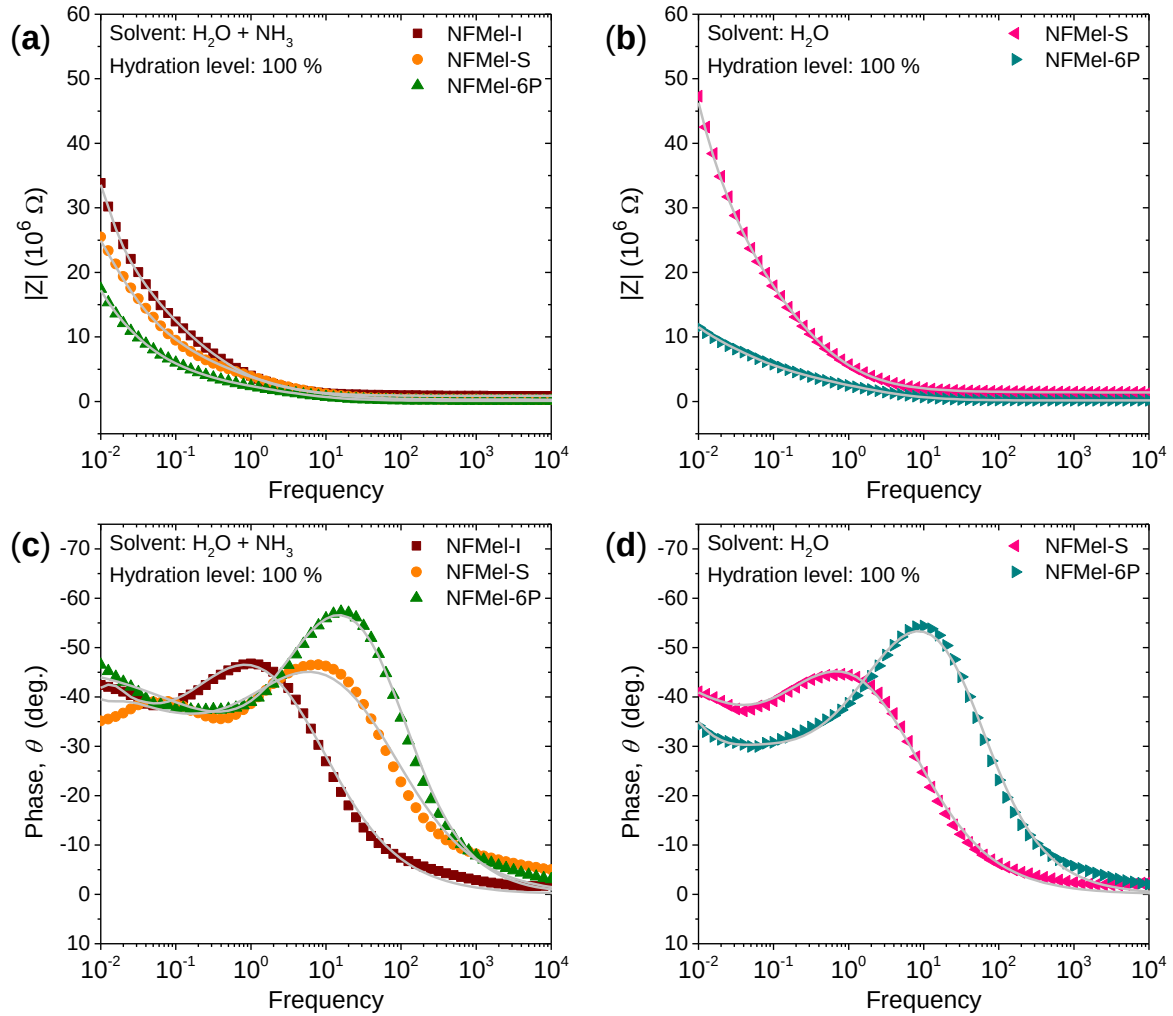


Figure 62. (a, b) Modulus and (c, d) phase angle as a function of frequency for NF-Melania films prepared using (a, c) water&ammonia and (b, d) water solution. Scatter colored plots represent experimental data, while continuous light grey lines the theoretical fit using a Randles circuit as a model.

The overall similarities between the three NF-samples indicate that film deposition's different methodologies provide only subtle changes to the charge transport. Most of all, the ionic component could be a consequence of small alterations in their macrostructures. When one compares the three samples in Figure 62(a, b), it is possible to observe a slight decrease in the NFMel-6P impedance samples at the low-frequency domain. Considering that in the low-frequency region, ions dominate impedance, it is possible to speculate that the small impedance would be the consequence of an increase in the ionic conductivity, as expected for a higher

concentration of COOH in such sample (ORDINARIO et al., 2014; BRONZE-UHLE et al., 2016). If we estimate the ionic conductivity of the semicircle diameter of the Nyquist plot (SOBOLEVA et al., 2008; WÜNSCHE et al., 2015), it is obtained values of 7.6, 16 and 30 $\text{mS}\cdot\text{cm}^{-1}$ for NFMel-I, NFMel-S and NFMel-6P, respectively, in $\text{H}_2\text{O}+\text{NH}_3$ and 4.7 $\text{mS}\cdot\text{cm}^{-1}$ for NFMel-S and 31 $\text{mS}\cdot\text{cm}^{-1}$ NFMel-6P in only H_2O . These ionic conductivities are compatible with our previous discussion and the values reported in the literature for NFMel-I (WÜNSCHE et al., 2015; SHELIAKINA; MOSTERT; MEREDITH, 2018b) and other proton conducting materials (SOBOLEVA et al., 2008; ORDINARIO et al., 2014).

Additionally, note that there are no significant differences in the impedance of NFMel-6P when comparing the solution using an oxidizing (as in $\text{H}_2\text{O}+\text{NH}_3$ solvent) or neutral (deionized H_2O only) environment; however, it increases for NFMel-S. Such an increase of NFMel-S could be compatible with a large structure with a lower active surface and diminished interaction with the water vapor. Hence, ammonia's use assists the adherence of NF-melanin on the substrate surface and induces small changes in its macrostructure that allow an increase in the ionic conductivity. Considering that NFMel-6P has smaller particles than NFMel-S (BRONZE-UHLE et al., 2016), such structural variation could not be higher enough to induce some significant charge alterations. Most importantly, section 3.3 indicates no significant structural changes were observed, which ensures that we are studying melanin's electrical properties.

By contrast, if one compares Figure 62 and Figure 63, the differences between NF and S-melanin's charge transport behavior are evident. In the low-frequency domain of S-melanin, it is possible to see a subtle decrease in the impedance modulus until it reaches $f > 100$ Hz, see Figure 63(a, b), when the impedance drops. This result could indicate that S-melanin has movable ions in fewer amounts (as discussed above) and lower ionic conductivity. Indeed, these samples estimated ionic conductivity was 0.2, 0.4, 0.2 and 0.1 $\text{mS}\cdot\text{cm}^{-1}$ for SMel, SMel-T, SMel-4P and SMel-8P, respectively. However, the change in the impedance modulus at high-frequency could imply a preference for electronic charge transport. Note that the variation of impedance is more visible for samples synthesized with oxygen (SMel-4P and SMel-8P), suggesting that the different polymerization structures from oxygen atoms result in a higher impedance, probably from the break of conjugation (PAULIN et al., 2018). The angle phase, Figure 63(c, d), also supports the speculated mechanism since the variation in the high-frequency domain is more significant than in the low-frequency region.

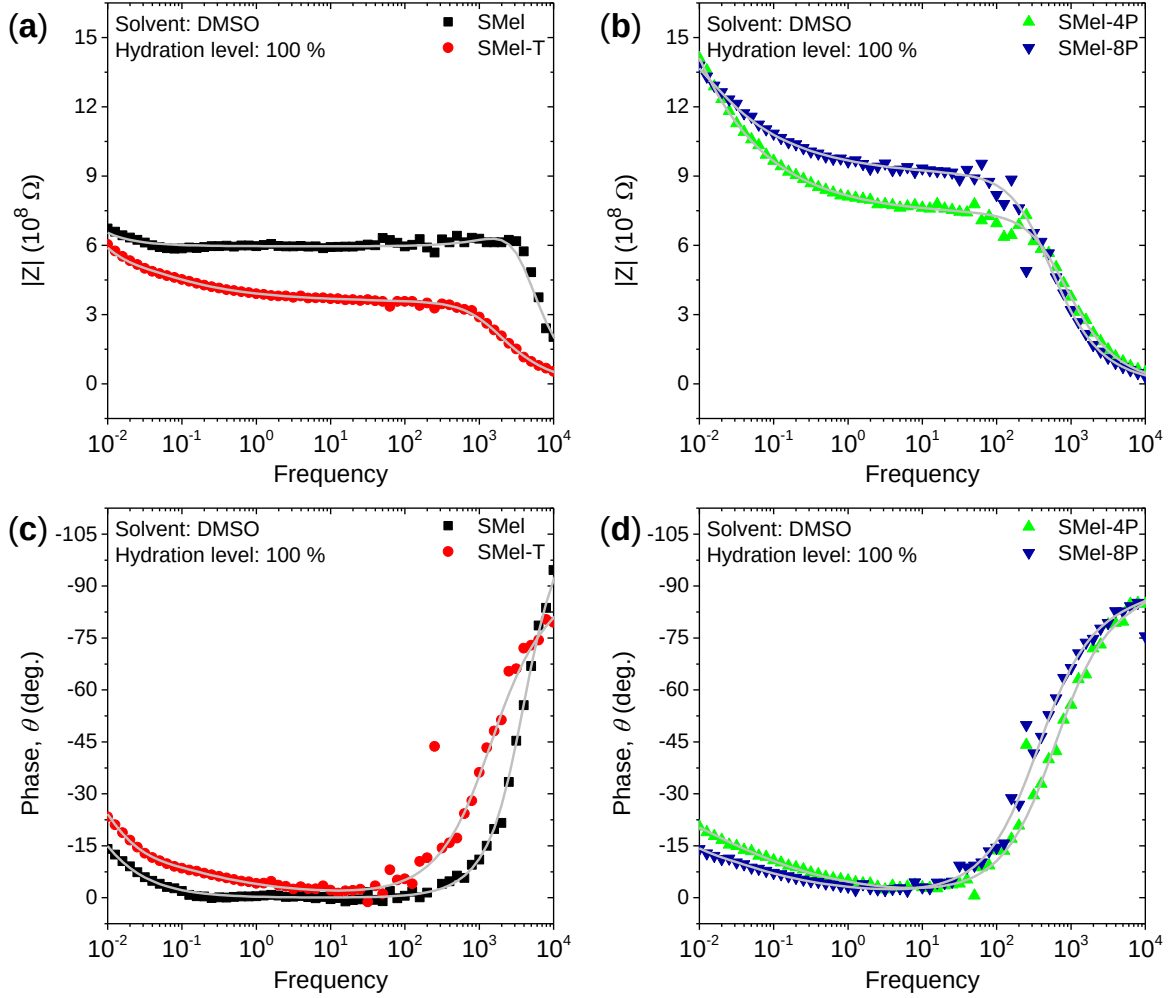


Figure 63. (a, b) Modulus and (c, d) phase angle as a function of frequency for S-Melanin films prepared using DMSO solution. Scatter colored plots represent experimental data, while continuous light grey lines the theoretical fit using a Randles circuit as a model.

To get further information, we have also estimated the AC conductivity of the samples ($\sigma(\nu) = d \cdot (A \cdot Z')^{-1}$, where d is the thickness and A is the electrode area). As can be seen in Figure 64, NF-melanin and S-melanin have opposite AC conductivity behavior. For instance, NF-melanin is dominated by a frequency-dependent conductivity followed by a frequency-independent plateau formation. The frequency-dependent conductivity at the low-frequency region is ascribed as a polarization effect at the electrode-sample interface, whereas the high-frequency domain is dispersive (GREENHOE et al., 2016; AMBRICO et al., 2017).

The conductivity σ behavior as a function of the frequency is usually described by the Jonsher's power equation $\sigma = \sigma_{DC} + A \omega^S$ with σ_{DC} being a non-dependence contribution of AC signal, S value ranging from $0 < S < 1$ and A is associated with the polarization strength (JONSCHER, 1981). However, our analysis considering this single power function was not

successful. For future investigation, we believe that a multi-power function ($\sigma = \sigma_{DC} + \sum A_i \omega_i^{S_i}$) expressed by the Jump Relaxation Model (ELLIOTT, 1987; FUNKE, 1993) could be a better approach to be considered.

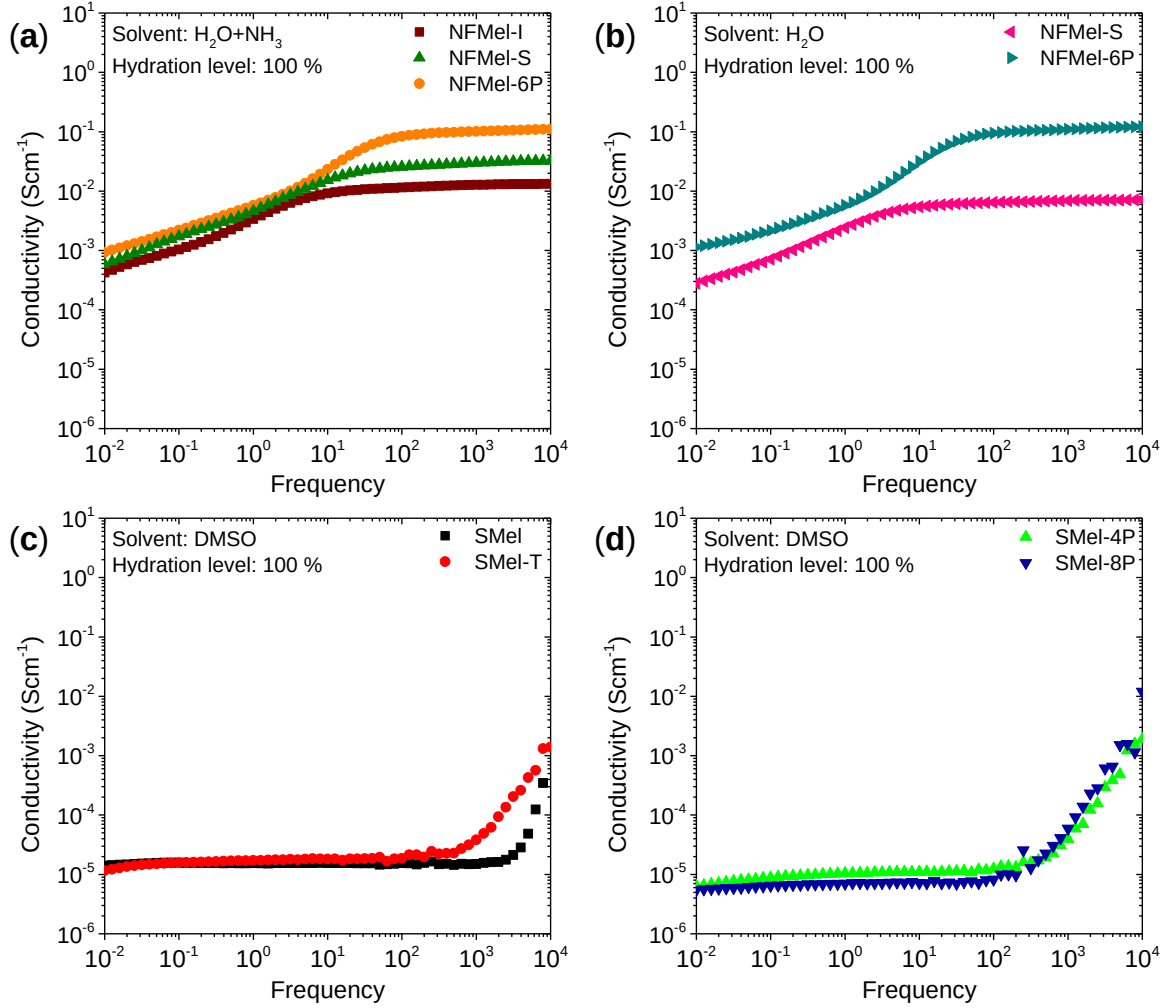


Figure 64. (a, b) NF-melanin and (c, d) S-melanin frequency dependency AC conductivity at 100% hydration level.

On the other hand, it has recently been proposed that melanin's charge carrier can “switch” from electrons to protons depending on the hydration (REALI; SAINI; SANTATO, 2021). As NF-melanin can be viewed as a proton conductivity material at a high humidity level, we could ascribe the polarization effect in the low-frequency domain to the capacitance effect of ions at the electrode interface, indicating that it would be highly doped at saturation conduction. However, as discussed earlier, S-melanin has low dependence on hydration, implying that doping should be different at the same experimental condition. Therefore, the absence of a significant polarization effect could indicate lower protonic conductivity, even at 100 %

hydration, as mentioned before. Accordingly, it is possible to speculate that the sulfonate group's presence can potentially change the dominant charge carrier of the sample from protons to electrons.

Considering that the DMSO boiling point is about 190 °C, it is unlikely that all of the solvent molecule was removed with the vacuum step. Therefore, another possibility would be that the DMSO residues compete with the water, hindering the hydration effect on S-melanin, i.e., the DMSO block the available position for water solvation. However, more studies are necessary to fully understand the effect of the functional group and the DMSO on the S-melanin electronic properties.

6.3 QUASI-ELASTIC NEUTRON SCATTERING FOR PROBING PROTON DIFFUSION

6.3.1 Methodology

6.3.1.1 Adsorption isotherm

To establish the water adsorption behavior of NFMel-I and SMel-T (synthesized as described in CHAPTER II, Section 2.1.2), a previously published method was followed (MOSTERT et al., 2010). In brief, the steam delivery system (CHAPTER II, Section 2.1.3) was attached to a vacuum microbalance (CI Electronics Ltd.). To record the data, the microbalance was connected to an external CI STABAL control unit where electronic signals were processed into a mass value. The samples were pumped down over the weekend to obtain the dry mass.

The desired H₂O pressure was admitted into the system for the adsorption measurements, and the reservoir was closed. Considering that melanin is exceptionally hygroscopic, a substantial drop in the pressure was observed as the reservoir was sealed. Hence, solvent steam was allowed into the system until the pressure was relatively constant. Once a continuous desired pressure was obtained, the mass of the sample was monitored overnight. The following data points were obtained in the same manner, however, at higher relative pressures. The experiments were conducted at room temperature (21.5 ± 0.1 °C). The weight was then converted to the amount of water absorbed per gram of sample, allowing a direct comparison between NFMel-I and SMel-T. The result is presented in Figure 65 and it indicates that the water adsorption isotherm of NFMel-I appears to be more like a 1-Type isotherm (one-layer adsorption or chemisorption), whereas for SMel-T it resembles more a classical 2-Type isotherm (infinite layers adsorption or physisorbed system) (MOSTERT et al., 2010). Such

difference suggests that sulfonated-melanin have different hydration properties compared to non-functionalized ones.

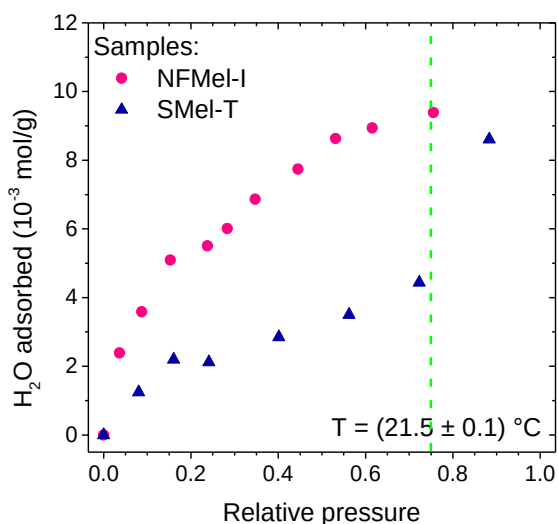


Figure 65. Equilibrium adsorption isotherm for water adsorption of NFMel-I and SMel-T powder. The dashed green line indicates the humidity level of 75%.

6.3.1.2 Sample preparations

For NFMel-I, two different experimental conditions were used in this study. In both cases, the sample was initially dried in a vacuum oven at 50 °C for 05 hours and then loaded to the steam delivery system (section CHAPTER II, Section 2.1.3), which was pumped down overnight to ensure that there was no adsorbed water in the system. Afterward, NFMel-I was transferred to a dried box (Dry NFMel-I) or kept in the system and the pressure was increased to 19 mbar with H₂O steam to ensure that 17.5 moles of water be present in the sample (Wet NFMel-I). In the last case, the sample was then transferred to a wet box (NaCl saturated solution, 75% relative humidity). Approximately 500 mg of each sample was placed in an aluminum sachet and sealed into a cylindrical aluminum sample can that had an annular sample space of 0.5 mm-thick. The same procedure of Wet NFMel-I was performed for SMel-T to understand the QENS behavior of S-melanin.

6.3.1.3 QENS measurements

QENS data were acquired on the backscattering IRIS spectrometer at the neutron spallation ISIS Pulsed Neutron and Muon Source of the Rutherford Appleton Laboratory in the United Kingdom (CARLILE; ADAMS, 1992). The measurements were performed using the

PG002 reflection of the pyrolytic graphite analyzer bank that affords a resolution of 17.5 μeV , a dynamical range from -0.5 to 0.5 meV and a Q-range from 0.442 to 1.854 $\text{\AA}^{-1}$. A vanadium sample, a purely incoherent elastic scatterer, was measured to define the instrument resolution and correct it for detector efficiency.

The sample can is equipped with bottom and top-mounted copper blocks containing thermocouples and thermal resistance heaters to control the cell temperature during neutron data collection with a closed-cycle refrigerator. Once installed, the sample was cooled to base temperature (~ 5 K), a high signal-to-noise ratio QENS spectrum was collected to provide a resolution function for the instrument for use in later data fitting analysis. The sample was subsequently heated and 30 μA QENS spectra were taken at 10 K intervals from the base temperature to 325 K and the measurement time at each temperature was approximately 10 min. For 250, 275, 300 and 325 K, 750 μA QENS spectra were taken (~ 4 hours) to improve the signal-to-noise ratio at these temperatures for further analysis. All data analysis was performed using the Mantid software (ARNOLD et al., 2014).

6.3.2 Results and Discussion⁹

QENS is a powerful technique that can be used to study the dynamics of atoms or ions within a sample (JOBIC; THEODOROU, 2007; CAVAYE, 2019). This technique is even more interesting for melanin due to the presence of a high amount of hydrogen atoms in its structure, which can yield a strong signal for their motions due to the large incoherent neutron scattering power of this atom. Hence, the technique offers the ability to probe the melanin ionic motion. Figure 66 shows the QENS spectra for NFMel-I and SMel-T.

In overly simplistic terms, there is no motion at absolute zero, and all neutron scattering is therefore elastic. As temperature increases, atoms begin to move according to the energy available. The scattering of an incident neutron caused by atoms and/or ions translational motions results in a broadening of the elastic peak, lessening its intensity and increases the background signal intensity. Hence, quasi-elastic scattering looks at a very narrow energy range around the elastic peak, i.e., the spectrum immediately surrounding the zero-energy peak. The decrease in the elastic peak with increased movement caused by increased temperature (see Figure D9 for a zoom-in at $\omega = 0$ meV) can either relate to transitions outside the energy range (rotations and vibrations) or through scattering from moving particles, which essentially doppler shift the energy of the neutrons.

⁹ The results presented in this section is part of Paper VIII, in preparation.

The result presented in Figure 66a for dried NFMel-I suggests no significant motion in the sample. The small broadening could be related to vibration and/or rotation of hydrogen atoms in the melanin backbone structure. However, wet samples, Figure 66(b, c), clearly show an increase in the broadening of the scattering function $S(Q, \omega)$, indicating faster motions.

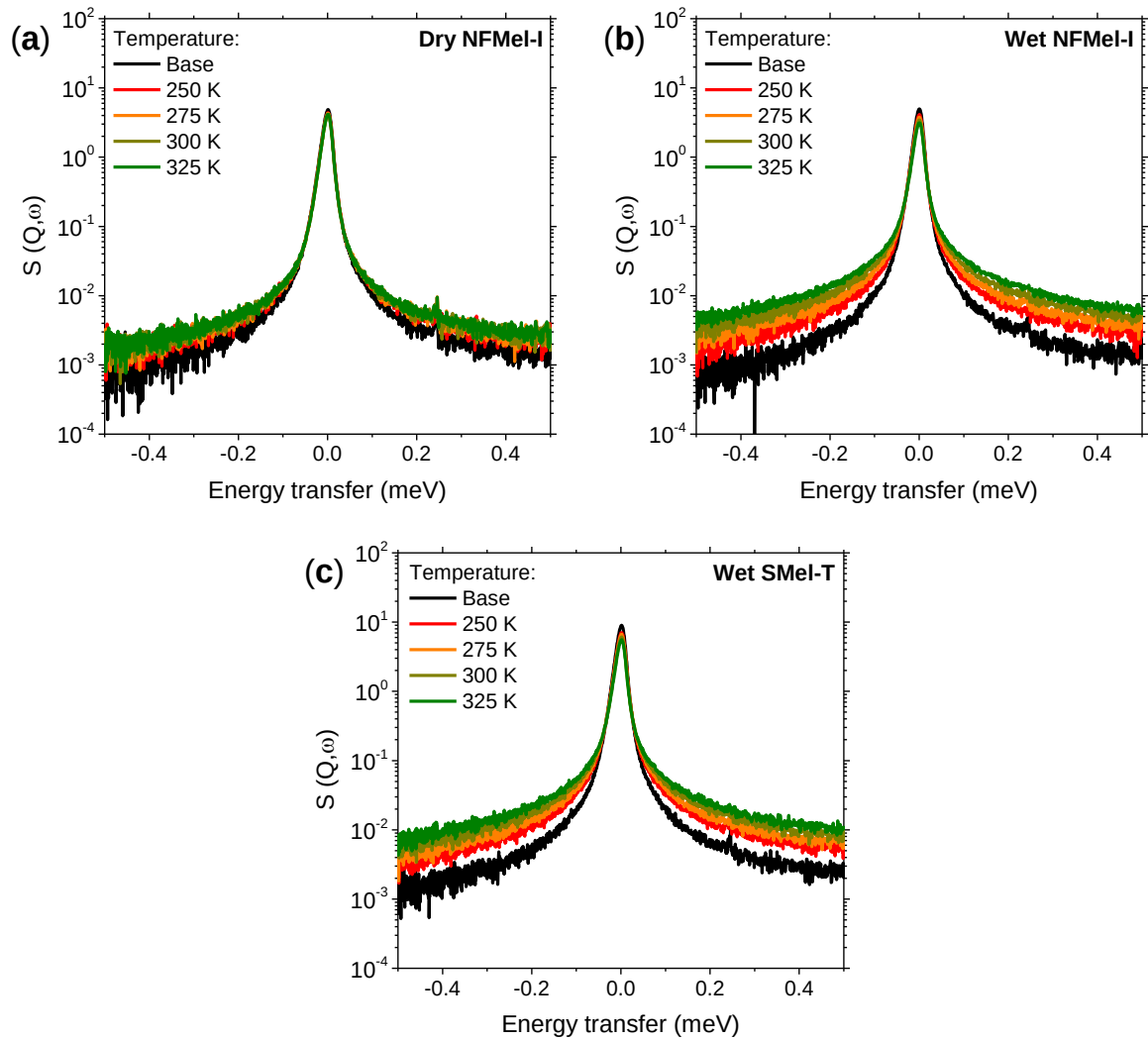


Figure 66. QENS spectra for (a) dry NFMel-I, (b) wet NFMel-I and (c) wet SMel-T at different temperatures.

The total scattering intensity across all (Q, ω) space should be constant, but the elastic fraction and the inelastic fraction in the energy range observed will change. Therefore, to get further insights on the NF and S-melanin motions, the elastic and inelastic windows with Q summed (Figure 67) were obtained. The analysis starts with calculating the area under the quasi-elastic peak measured by summing all detectors (all Q) and integrating between -0.0175

and +0.0175 meV. This interval is considered an approximation of the instrument resolution and, therefore, it is assumed to be the elastic intensity.

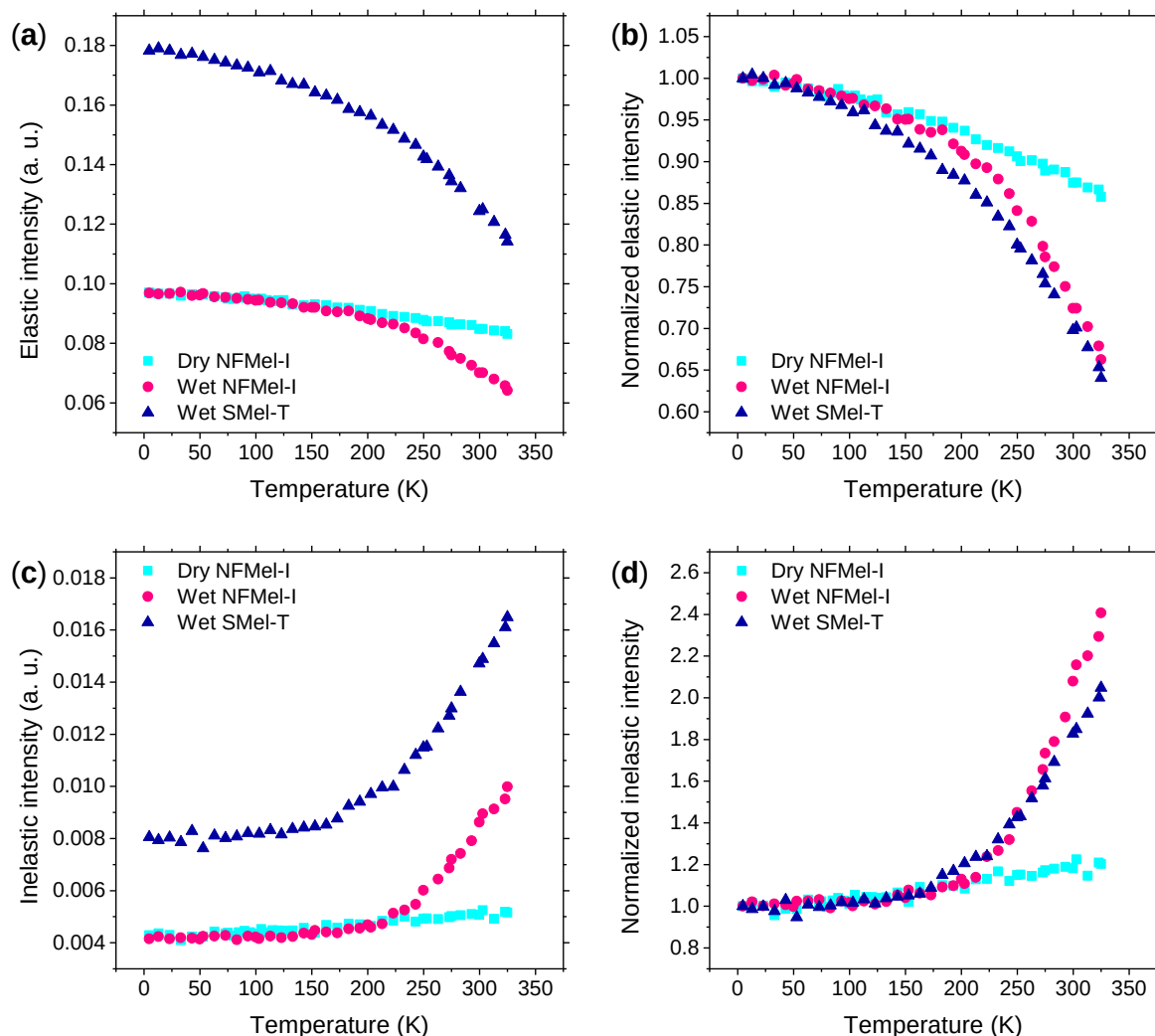


Figure 67. (a, b) Elastic and (c, d) inelastic window scans (Q-summed) for NF and S-melanin derivatives.

The elastic window's absolute intensity at low temperature indicates the relative scattering strength affected by the sample shape, mass and composition. Hence, Figure 67a show that Dry and Wet NFMel-I have similar scattering strengths that may indicate an equal amount of hydrogen is present in a similar sample geometry and mass. Alternatively, there may be changes in the sample that cancel co-incidentally. On the other hand, Wet SMel-T has the highest absolute intensity, which could be explained by replacing the indole hydroxyl hydrogen with the sulfonated group (Figure 3) or an increase in the water in the system. The high amount of hydrogen atoms in both cases should undoubtedly increase the intensity of the scattering.

However, the water adsorption isotherm of SMel-T indicates, for a given relative hydration, that the amount of water molecule adsorbed should be small than NFMel-I (Figure 65). Hence, the increase in the scattering intensity is related to the presence of the sulfonated group.

Figure 67b displays the elastic window normalized by the lowest measured temperature to consider any possible mass variation or scattering strength and ensure that the samples have equal scattering intensity to simplify the comparison. As the temperature increases, the samples' motion increases, resulting in a decrease in the elastic intensity. Unless there are phase changes, the decrease should be linear. Additionally, if the motion behavior is similar, the samples should have the same gradient. Dry NFMel-I displays a shallow gradient, whereas Wet NFMel-I and Wet SMel-T both show significant intensity decreases, indicating a greater proportion of mobile hydrogen in wet samples than when compared to the dry one. Moreover, there are more subtle differences in the gradients between samples (that is, a break in the linearity) that could show different kinetics. For instance, Dry NFMel-I shows only one phase change at around 120 K; and the wet samples two, being one at 117 K and the other at 233 K. Such result is compatible with previously broadband dielectric spectroscopy (MOTOVILOV et al., 2019).

In a similar method to the elastic window, a representation of the inelastic area under the quasi-elastic peak was obtained by summing all detectors (all Q) and integrating between 0.025 and 0.5 meV. The inelastic window's absolute intensity at low temperature in Figure 67c shows that Wet SMel-T has the highest intensity, possibly from coherent diffraction leading to increased background. The other samples show similar inelastic intensity at base temperature.

The variation of the normalized intensity of the inelastic window in Figure 67d with temperature appears more illuminating than the elastic, as the variation between the Wet NFMel-I and Wet SMel-T is more pronounced. The Dry NFMel-I shows little movement, and the addition of water causes more H-motion. However, the most important information is related to the increase in the inelastic intensity for temperatures higher than 270 K. At such temperatures, Figure 67d indicates that Wet NFMel-I has a strong proton motion when compared to Wet SMel-T. Such a result is in line with the lower sensitivity of free-radicals centers towards hydration observed with EPR (section 5.3.2) and the ionic current of sulfonated melanin and DC & AC measurements (section 6.2.2).

6.4 CONCLUSION

The charge transport of different melanin derivatives was evaluated using AC and DC measurements. The results in this chapter show no drastic changes in NF-Melanin samples'

electrical behavior using an alkaline solution (or not) to deposit the films. Additionally, S-Melanin's electrical conductivity proved to be hydration dependent, i.e., it increases with the water content, but with less variation than the NF counterpart. Such a result indicates that the S-Melanin has active sites for ions motion even with the functional groups' presence. An analysis of impedance measurements demonstrates that the sulfonated group decreases the S-melanin's ionic conductivity, but it may favor electronic currents at high hydration.

At the end of this chapter, it was shown that the QENS behavior of SMel-T is very similar to NFMel-I, which indicates that both samples could sustain the same type of motion. However, in line with DC and AC measurements, QENS also shows that SMel-T has a lower ionic/protonic activity at temperatures higher than 270K.

All these findings can be understood based on the structural differences and they are an essential step towards the development of bioelectronic devices based on melanin, especially in the case of sulfonated derivatives.

CHAPTER VII

MELANIN-BASED DEVICE

7.1 INTRODUCTION

The evaluation of pH is a field of enormous interest in both industrial and academic setting regarding, among other applications, medical diagnostics since biological processes can be driven by its alterations (MCKINLEY, 2008; GUINOVART et al., 2014; BANDODKAR; JIA; WANG, 2015; KHAN; ANSARI; ALI, 2015; KHAN et al., 2017; CICHOSZ; MASEK; ZABORSKI, 2018; NAG; MITRA; MUKHOPADHYAY, 2018; QIN et al., 2019). A good example is skin wound repair. The healing process is considered a dynamic process that has distinct stages, illustrated in Figure 68(a-c), that can be referenced as inflammation (to prevent fluids losses and infections and removal of dead and dying tissues), new tissue formation (cellular proliferation and migration of different cell types) and remodeling (a decline of all of the processes activated after injury until its termination) (GURTNER et al., 2008). Even though the healthy skin usually has a pH of 4-6, it can vary according to time during the healing process, as present in Figure 68(d and e). Hence, it is essential to evaluate a wound's pH since the tissue could present bacterial infections in alkaline environments (GURTNER et al., 2008; QIN et al., 2019). Therefore, a biosensor capable of sensing such pH variations would be an exciting candidate for clinical application.

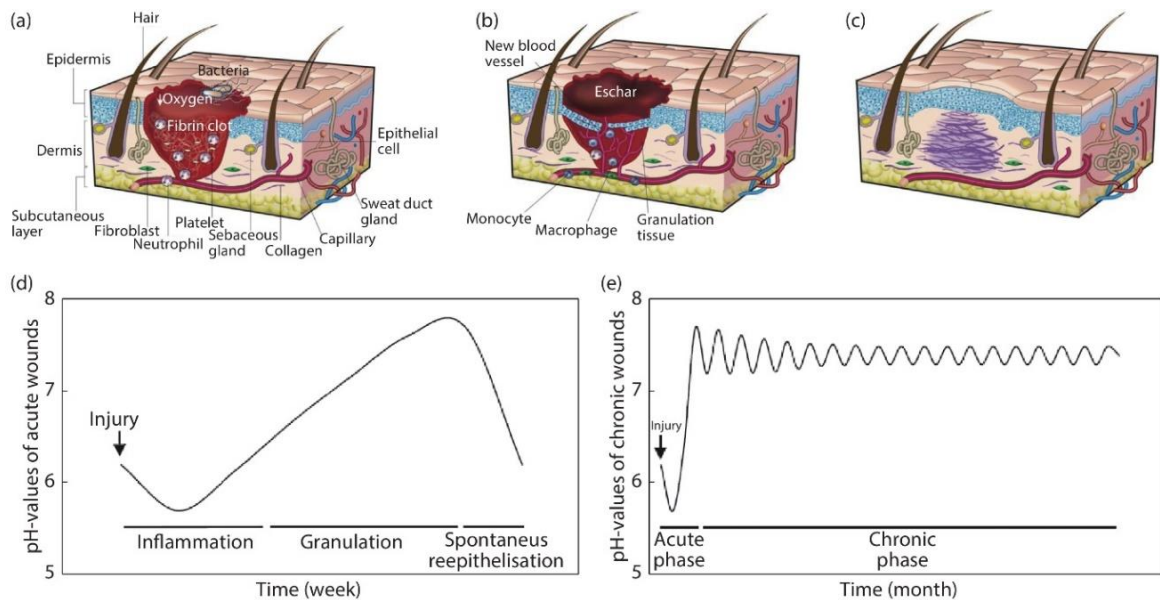


Figure 68. Steps of the healing process on skin wound: (a) inflammation, (b) new tissue formation, and (c) remodeling. Alteration of pH as a function of time in (d) acute and (e) chronic wounds. (a-c) Reprinted with permission from (GURTNER et al., 2008). Copyright© 2008, Springer Nature. (d, e) Reprinted with permission from (SCHNEIDER et al., 2007). Copyright© 2007, Springer Nature.

A pH sensor can be assembled using an ion-sensitive layer with many materials (inorganic and organic). However, a great interest is to obtain a high sensitivity device using biomolecules. Recently, a pH sensor device was developed using standard melanin in an extended gate field-effect transistors (EGFET) with a higher sensitivity to pH than indium tin oxide (PIACENTISILVA et al., 2014a). However, it is known that standard melanin thin-films can be dissolved in slight alkaline aqueous solutions, which could degrade the device if direct contact to the system is necessary.

Therefore, a melanin derivative with device quality thin-films and stability in alkaline solution would be ideal for pH sensing and sulfonated melanin derivatives can fulfill all these requirements. As mentioned before, sulfonated melanin can be spin-coated in both hydrophilic and hydrophobic surfaces, which means that these samples can be deposited on substrates with minimal preparation; and it is stable in aqueous solutions of differing pH, including alkaline solutions (ALBANO et al., 2016). Based on this context, in this chapter, the interest is to investigate the ability of sulfonated melanin, in special SMel, to act in a sensitivity layer in upon a newly developed (at Swansea University) carbon/graphene screen-printed electrode and investigate a potential mechanism of operation for the sulfonated melanin pH sensor. The use of carbon/graphene as a base electrode material is because graphene is a relatively common material in (bio)organic electronics presenting high carrier concentration with high mobility, biocompatibility, mechanical reliability and electrochemical stability (GEIM, 2009). Another attractive of such a device is the low cost and simplicity of the screen-printing technique that could easily be extended to industrial scales (HART; WRING, 1997).

7.2 EXPERIMENTAL PROCEDURE

7.2.1 Device assembly

The electrodes of the pH sensor were made using an R29 series screen-printer (Reprint, UK) under ambient conditions. In a flexible substrate of polyethylene terephthalate (PET) with a thickness of 175 μm (HIFI Melinex 339), a conductive silver (SunTronic ref: AST6025) as a reference electrode was printed, as well as carbon as an auxiliary electrode and carbon/graphene as a working electrode, in the geometry displayed in Figure 69. To protect the electrode, an insulating ink (Gwent, D214011D5) was applied in part of the sensor. The pH sensor was completed by depositing SMel (synthesized as described in CHAPTER II, section 2.1.2) by two

steps spin-coating (1000 rpm for 60 and 4000 rpm for 30s) on top of the working electrode. More details about the electrodes fabrication can be viewed in (TEHRANI et al., 2020).

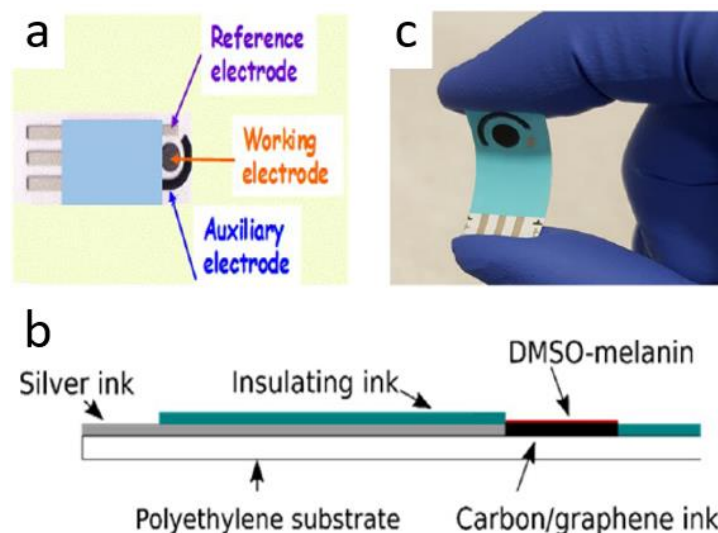


Figure 69. Screen-printed electrodes from (a) top and (b) cross-section point of view. (c) Photograph of the flexible sensor. Reprinted with permission from (TEHRANI et al., 2020). Copyright© 2020, IOP Publishing.

7.2.2 Characterization Techniques

X-ray photoelectron spectroscopy (XPS) measurements were taken with a Kratos Axis Supra XPS system with a monochromatic Al K α X-Ray source, with an emission current of 15 mA. Electrical measurements were performed using a 4-probe “EverBeing probe station” with a 2636B Keithley Unit.

The electrochemical analysis was performed on four different samples using the advanced potentiostat (PGSTAT-302 from Autolab, Metrohm Autolab, Runcorn, UK) with the scanning voltage in the range of 0.0 V to 0.8 V. Standard 3-electrode system was used for the electrochemical evaluation, where printed graphene/melanin was used as the working electrode, Ag/AgCl as the reference electrode and printed carbon as the counter electrode. An electrolyte of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in solution phosphate buffer saline (PBS) solution (pH = 7.4). pH test and analysis of sensor were performed using the Ana-Pot from Zimmer and Peacock ltd in the current range of 100 nA for 600 s at different buffer solutions. pH 4, pH 7 and pH 10 buffer solutions were obtained from Hanna Instruments and the intermediate pH levels were produced by mixing these stocks together. pH 2 and pH 3 were created by adding hydrochloric acid to the pH 4 buffer, while pH 11 and pH 12 by adding sodium hydroxide to the pH 10 buffer solution. To account for any heterogeneity in the film deposition, five different blank and SMel coated electrodes were tested.

7.3 RESULTS AND DISCUSSION¹⁰

7.3.1 Characterization of melanin-coated pH sensor

The presence of SMel in the carbon/graphene electrode was obtained by N 1s high-resolution XPS spectrum and IV measurements. The initial chemical composition of the SMel coated electrode surface is depicted in Figure 70 (black line). For contrast, XPS measurements were also taken on spin-coated SMel on PET substrate (Figure 70, red line) and uncoated carbon/graphene electrode (Figure 70, blue line). In these spectra, one may see that melanin has only one broad nitrogen-peak ranging from 397 to 403 eV that is related to nitrogen of the indole structure, Figure 3 and Figure 12, in agreement with a previous study (PAULIN et al., 2018); while carbon/graphene ink also has only one nitrogen peak at around 408.2 eV from nitrate compounds. Moreover, when the carbon/graphene ink is coated with a SMel layer both nitrogen peaks can be observed, suggesting that the melanin sample is present at the working electrode's surface. Another evidence arises from the small shift towards lower binding energies found for the carbon/graphene ink nitrogen-peak that is probably related to some structural and electronic interaction between SMel and graphene (GARGIULO et al., 2015). *J* vs. *V* measurements also show the presence of SMel across the screen-printed electrode, since the resistivity of the coated electrode increased from $2.61 \pm 0.105 \, \Omega$ to $8.42 \pm 0.988 \, \Omega$ (TEHRANI et al., 2020).

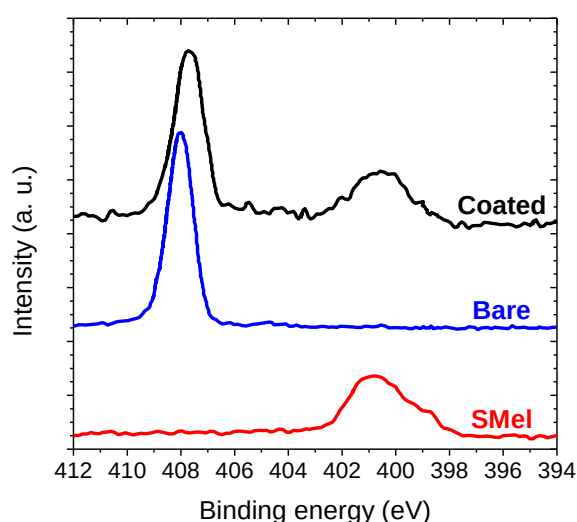


Figure 70. (a) High-resolution N 1s XPS spectra for SMel coated electrode (Black), carbon/graphene ink on a bare electrode (Blue) and SMel (Red). The nitrogen peaks around 401 on the coated electrode indicate that melanin is present at the surface of the sensor working electrode.

¹⁰ The results presented in this section is part of Paper III.

In order to evaluate the capability of SMel detect changes in pH and the stability of the coated electrode in real-time, the potential of the sensor was measured continuously in solution across a wide range of pH (from 2 to 12, with an increase of 1 unit per steps) by open-circuit potentiometry over 10 minutes periods exposing it to standards pH references. As shown in Figure 71(a), there are evident changes in potential at every pH change. The slope of the mean voltage measured with each solution from this data indicate that under these conditions, the sensor has a sensitivity of 35.25 ± 0.59 mV/pH.

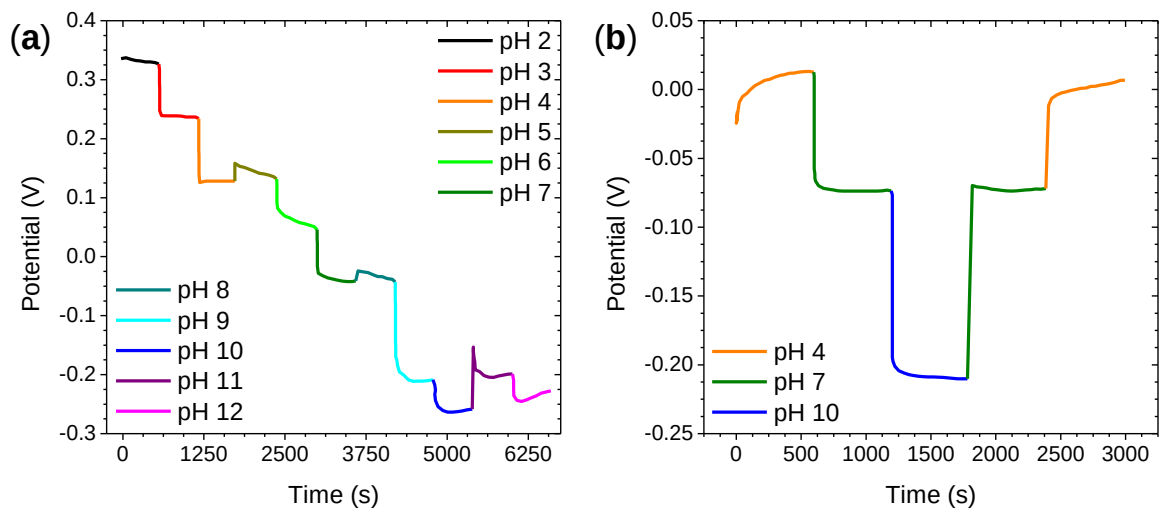


Figure 71. (a) pH sensing performed of the pH sensor in a wide range of pH, from 2 to 12. (b) The ability of the SMel coated electrode to recover following exposure to high pH solution.

Another critical test is to check the hysteresis of the electrode behavior. In this case, a similar analysis as before was performed for buffer solutions of pH 4, pH 7 and pH 10 followed again by pH 7 and pH 4, as displayed in Figure 71b. In this case, the sensitivity only lost 2.3% in the recovery step, suggesting that the sensor remains sensitive to pH changes following exposure to high and low pH levels and implies that the pH sensor is robust/stable and can keep its efficiency for multiple uses.

Considering that the aim is to obtain a bioelectronic and potential sensor for biomedical applications, it would be interesting to test this device's sensitivity under physiological conditions, between pH 5 and pH 8, as shown in Figure 68(d and e) for instance. The pH sensor sensitivity under physiological conditions is displayed in Figure 72 and, as can be seen, SMel coated electrode has a sensitivity of 62.00 ± 7.00 mV/pH, which is an increase of five times compared to the uncoated one (12.65 ± 0.02 mV/pH), with better linearity and better reproducibility.

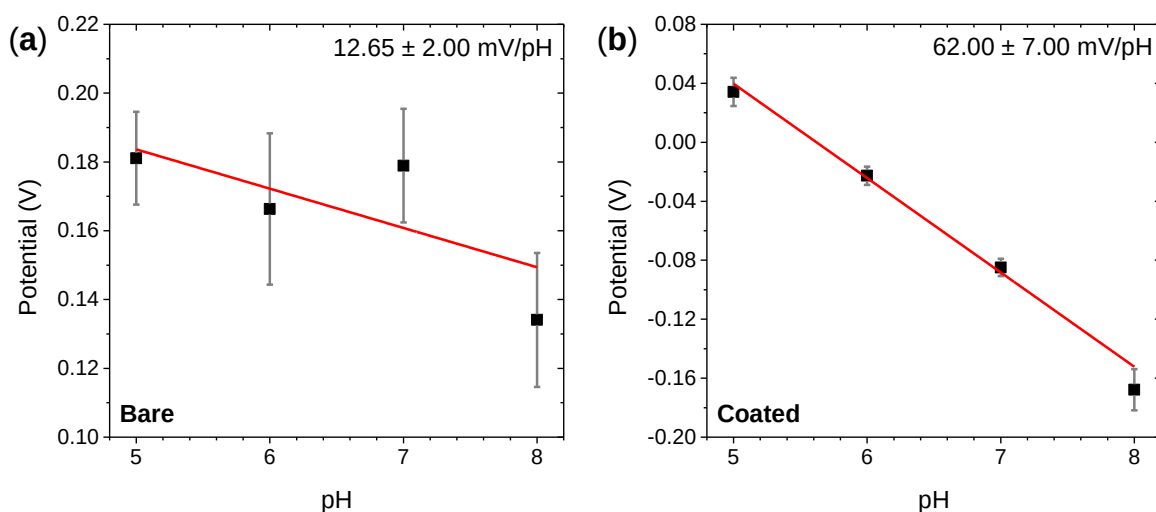


Figure 72. The sensitivity of (a) bare and (b) SMel coated electrodes under physiological conditions.

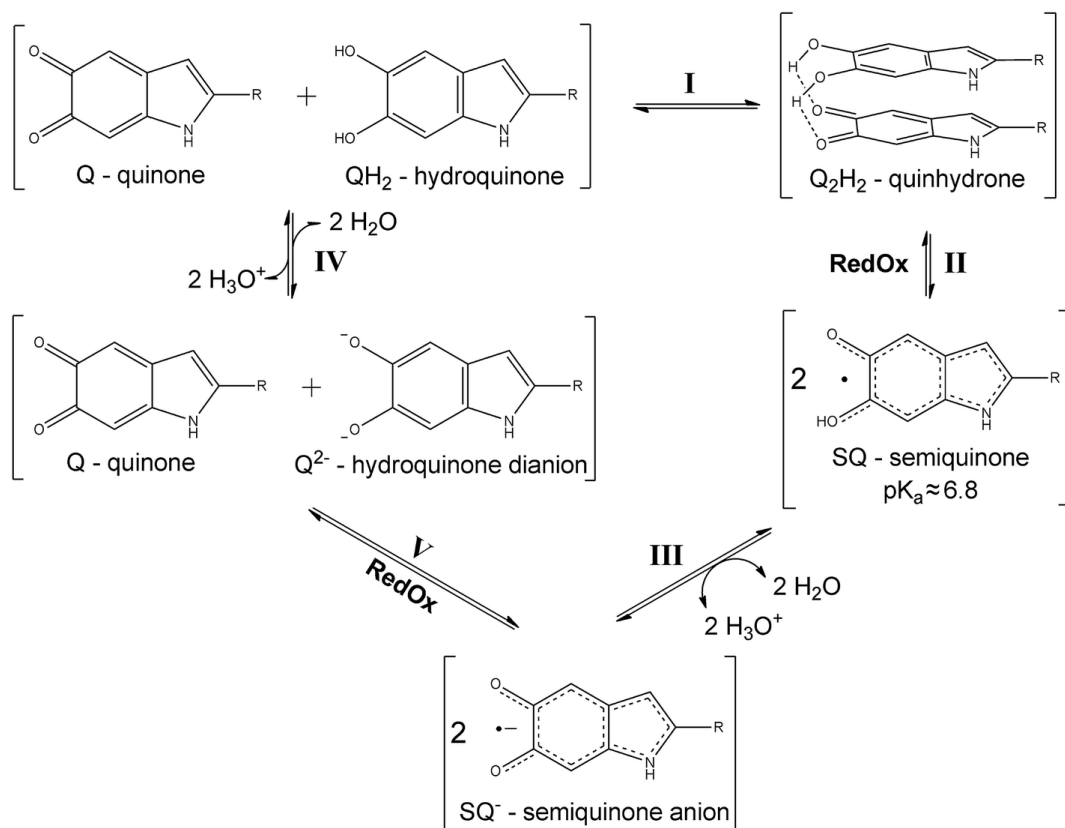
In light of the biomedical applications, the sensor's effectiveness in measuring the pH of real (i.e., non-reference buffer) samples was assessed. In a first test, the sensor's stability for a common clinical sample type as blood plasma was sought. In such a case, the sensor's surface was kept in contact with the blood plasma (obtained from healthy volunteers) for 10 minutes, rinsed with distilled deionized water and then used to measure buffer solution of pH 7.2, pH 7.3 and pH 7.4. After the interaction with the blood plasma, the sensor exhibit sensitivity to the different pH, indicating that the proposed device is robust enough to be considered a candidate in biomedical device development (TEHRANI et al., 2020).

Following the above idea, the sensor operability was determined by measuring the pH of different culturing media types (lysogeny broth, brain heart infusion and nutrient broth) (WHELAN et al.,). Under these various media, the proposed pH sensor shows a sensitivity of 60.14 ± 2.55 mV/pH. Additionally, the sensor was also able to precisely obtain the solutions' pH with a variation of only 2.9% compared to an industrial standard glass electrode pH-meter. Therefore, the graphene-melanin sensor can accurately measure the pH of different culturing media, even in the presence of bacterial growth.

7.3.2 Mechanism of action

It is known that melanin is a very dynamic system since it belongs to the highly reactive *o*-quinone chemical compounds (ITO, 2003) with interconversion between its various oxidative states, as shown in Scheme 3. Hence, melanin can be considered an attractive material for electrochemical sensing. Additionally, it has been reported sensitivity as a function of pH of

50 mV/pH experimentally (HORAK; WEEKS, 1993; PIACENTI-SILVA et al., 2014a) and 64 mV/pH theoretically (MOSTERT, 2021) for the standard material.



Scheme 3. Redox transformations of hydroquinones blocks within melanin in solid-state (pathway I and II) and aqueous environment (pathway III, IV and V). Reprinted with permission from (MOTOVILOV et al., 2019). Copyright© 2019, Royal Society of Chemistry.

The pH sensitivity of SMel is in line with the previously reported values, which is unexpected since it contains additional sulfonated moieties attached to the hydroxyl groups of the indole ring (Figure 3) that could be a limiting factor to the electrochemical reaction.

In previous work, an extended gate field effect transistor (EGFET) was developed using standard melanin. It was proposed that the pK_as of the various constituents are responsible for the pH sensing effect (PIACENTI-SILVA et al., 2014). It has also been shown that the pH/E behavior is due to the reduction potentials of the one-electron redox reactions for the quinone/semiquinone and semiquinone/hydroquinone pairs (MOSTERT, 2019). Both these reactions together yield the comproportionation reaction, Scheme 2 and Scheme 3 (pathways IV and V), and yield a sensitivity of around 64 mV/pH, in line with our observations (62 ± 7 mV/pH). Therefore, one may speculate that both the pK_as and the redox potentials' positions drive the redox chemistry and ultimately make the device developed a sensitive pH sensor.

Based on the discussion above, it seems that sulfonated melanin acts electrochemically as the standard melanin, i.e., the comproportionation reaction is still an active pathway. As discussed in the previous chapter, not all the hydroxyl groups of the indole ring will be sulfonated. Thus, there are still moieties that can undergo the standard melanin electrochemistry, implying that sulfonated groups may only be spectators to this process. Consequently, it appears that S-Melanin has a similar sensitivity to standard samples as a result of analogous redox chemistry, but with the additional advantages of material processability and stability. The downside would be fewer standard melanin moieties, which would decrease the total current of the device. However, since the potential is what matters, this is not a significant problem and further device engineering should enhance the device's accuracy.

Moreover, it should be noted that sulfonated melanin acted in an ion to electron transducing device; with high sensitivity, the sensor took an ionic activity (pH) and transduced it into an electronic signal.

7.4 CONCLUSION

In this section, sulfonated melanin (i.e., SMel) was applied as a sensitivity layer in a carbon/graphene screen-printed electrode. By using SMel to the surface of the working electrode, it seems that the electrochemical characteristics of the electrode are altered in such a way that causes the sensor to become sensitive to a broad pH range, yielding a sensitivity of 62 mV/pH at physiological conditions and about 60 mV/pH at different culturing media. Additionally, the electrode composed of SMel and carbon/graphene ink acted as an ion to electron transducing element; it took an ionic activity (pH) and transduced it into an electronic signal.

CHAPTER VIII

GENERAL CONCLUSIONS

8.1 CONCLUSIONS

Organic bioelectronics is an emerging research field in which the main challenge lies at the interface of organic electronic devices and biological systems. The natural pigment melanin presents great potential among many potential materials due to its charge transport properties and inherent biocompatibility. Therefore, the main goal of the work presented in this thesis was to characterize synthetic melanin and melanin derivatives and apply them to bioelectronic device. Additionally, a thorough review of melanin properties was given to familiarize the readers about its (somewhat exotic) properties at the beginning of each chapter.

A first step in developing a bioelectronic device is to understand the primary characteristic of the active material; here, soluble non-functionalized and soluble sulfonated melanin derivatives. To this end, all the samples were initially structurally and optically characterized using depth-profile XPS, UV-Vis-NIR and ellipsometry spectroscopy. CHAPTER III showed that the sulfonate group of the functionalized samples does not interfere in the basic indole structure of melanin. Also, it was also possible to show that a surface analysis can represent a more in-depth thin-film layer. Besides, CHAPTER IV indicated that both groups of samples have the same optical behavior. However, a significant difference was that the solution quantum-yield efficiency of sulfonated melanin was up to two orders of magnitude higher than non-functionalized ones. Our films displayed high transmittance, high- n values (1.54 ± 0.06 for NF-Melanin and 1.58 ± 0.04 for S-Melanin) and low Abbe's number (17.86 ± 0.14). The similarities in spectra and values comparing NF and S-Melanin were discussed based on the structural differences.

If the stable free-radicals presented in melanin structure may feature prominently as either a charge carrier or a spin-label for charge transport, it is essential to understand its nature. Thus, in the first part of CHAPTER V, it was proposed that N_{def}^+ structures of DHI and DHICA monomers could be responsible for the origin of CCR free-radical using simulation analysis of several EPR spectra found in the literature. Secondly, the solid-state X-band EPR spectra in both non-functionalized and functionalized melanin samples were compared in dry and wet conditions. The results presented here show that the S-melanin EPR signal appears similar to NF-melanin. Simulation analyses of the EPR signals suggest that the S-melanin signal comprises three different resonant lines compatible with NF-melanin. Intriguingly, the theoretical calculations indicate that the EPR signal could arise from distinct S-units, in particular S- N_{def} , S-QI (anion) and S-SQ (radical)/S-IQ (anion). HFEPR analysis also support such idea.

The charge transport was studied in CHAPTER VI with DC and electrochemical impedance spectroscopy measurements under hydration control. However, it should be pointed out that, even though the charge transport seems to be similar, lower charge/ionic density and lower hydration dependence was found for the sulfonated samples. It has been speculated that S-melanin may have a significant increase in the electronic part of the conductivity at a high hydration level when compared to standard NF-melanin. This feature was related to replacing the phenolic hydroxyl “conducting protons” with the sulfonated groups. Still, it should be noticed that sulfonated samples have better device properties since they can be deposited into any surface without previous treatments. Moreover, QENS analysis has demonstrated that the motion properties of S-Melanin are not significantly affected by the presence of sulfonated groups. Also, QENS showed that S-melanin is less sensitive towards hydration than its NF-counterpart, corroborating the EPR and DC/AC discussion.

The last subject of this thesis was to use the melanin derivative in a bioelectronic device. Thus, it was shown in CHAPTER VII that the pH sensor was able to function across a wide range of pH, with exceptional performance in physiological conditions and different culturing media. Moreover, it should be noted that the sulfonated melanin acted in an ion to electron transducing system; with high sensitivity, it took an ionic activity (pH) and transduced it into an electronic signal. This study provides the foundation for later development and optimization of the electrode's pH-sensitive properties to increase its accuracy and sensitivity further. The low cost manufactured with high sensitivity printed electrochemical pH sensor has a significant commercial interest.

In conclusion, considering the similarities between the structure and optoelectronic properties of sulfonated melanin derivatives to the standard one, it is possible to affirm undoubtedly that such samples do belong to the vast “melanin family” of compounds. The results presented in this thesis are an advance in understanding the basic soluble melanin derivatives properties. It is a foundation for designing and developing soluble melanin-based bioelectronic devices, especially regarding the sulfonated-samples.

8.2 POSSIBLE FUTURE WORKS

The results presented in this thesis have pointed some open questions for future research on melanin and some ideas will be described below.

First, it would be interesting to present some *direct* evidence of the ratio between DHI and DHICA monomers to confirm the synthetic mechanism proposed for the synthesis under

O₂ pressure and provide a more robust view on the different oxidation state discussion on the paramagnetic and electrical characterization, which could be obtained by analyzing Raman spectroscopy and quantifying PDCA and PTCA subunits of degraded structures through high-performance liquid chromatography (GALVÁN et al., 2018). Additionally, in-depth analysis with ¹⁵N, ³³S and ¹H-¹³C nuclear magnetic resonance technique should assist a more precise structural elucidation of the sulfur and indole nitrogen of S-melanin. In the line of structural characterization, a better understanding of each melanin macrostructure with microscopy analysis would also be appreciated.

Most melanin's free-radical species characterization was performed using X-band CW EPR, which cannot provide detailed information on their electronic structure. Here, we have started a more meticulously analysis; however, a temperature and pH-dependent study in multifrequency CW & pulsed EPR spectroscopy will be necessary. Pulsed electron-electron double resonance spectroscopy (PELDOR) and hyperfine-sublevel-correlation (HYSCORE) spectroscopy could also give significant outcomes on the melanin dipolar and hyperfine coupling (HARMER; MITRIKAS; SCHWEIGER, 2009; HANSON, 2010).

Next, the suggestion that S-melanin has a more dominant electronic component than NF-melanin at high hydration has not been fully explored. Hence, a better understanding of the sulfonated group and the solvent on the charge transport would be a must. Some experiments examples would be EIS under the entire hydration level to deduce the relative fraction of the charge contribution (LASIA, 2014). Another interesting experiment would be the Hall effect experiment also under hydration control to assign the polarity of the majoritarian charge carrier (MORGAN; PETHIG, 1971; MORTENSEN; MUNN; WILLIAMS, 1971). Mapping conductivity as a function of temperature under vacuum or hydration, examining other adsorbate's effect (like heavy water (SHELIAKINA; MOSTERT; MEREDITH, 2018b)) and metal (MOSTERT et al., 2020) and carbonaceous materials (GARGIULO et al., 2015) doping on the charge transport could be useful measurement candidates as well. A better comprehension of the proton conductivity and its motion could be achieved with ²H nuclear magnetic resonance and a more thoroughly neutron spectroscopy (PILI et al., 2018).

Lastly, going to a more technological point of view, the differences between NF- and S-melanin regarding charge transport and the solubility, while keeping the others properties pretty much unchanged, is the start point of the design of soluble NF- and S-melanin-base devices. Potential device architecture candidates are sensing, batteries and memory devices, field-effect transistors and electrochemical transistor. Additionally, we have shown in section 7.3.1 that

SMel-based pH sensor did not lost efficiency when interfaced with biological entities. This opens even higher exciting possibilities to investigate the devices' performance with electrically active cells (cardiomyocytes and neurons, for example), the ultimate goal of bioelectronics.

REFERENCES

- ABBAS, M. et al. Structural, electrical, electronic and optical properties of melanin films. **The European Physical Journal E**, v. 28, p. 285–291, 2009.
- ADEE, S. Original Sin. **Bioelectricity**, v. 1, p. 16–17, 2019.
- ALBANO, L. G. S. et al. Novel insights on the physicochemical properties of eumelanins and their DMSO derivatives. **Polymer International**, v. 65, p. 1315–1322, 2016.
- ALBANO, L. G. S. et al. Ultraviolet-protective thin film based on PVA–melanin/rod-coated silver nanowires and its application as a transparent capacitor. **Journal of Applied Polymer Science**, v. 136, p. 47805, 2019.
- ALLOUCHE, A. Software News and Updates Gabedit — A Graphical User Interface for Computational Chemistry Softwares. **Journal of computational chemistry**, v. 32, p. 174–182, 2012.
- ALVES, G. G. B. et al. Reactivity of eumelanin building blocks: A DFT study of monomers and dimers. **Journal of Molecular Graphics and Modelling**, v. 98, p. 107609, 2020.
Disponível em: <<https://doi.org/10.1016/j.jmkgm.2020.107609>>.
- AMBRICO, M. et al. Hysteresis-type current-voltage characteristics in Au/eumelanin/ITO/glass structure: Towards melanin based memory devices. **Organic Electronics**, v. 11, p. 1809–1814, 2010.
- AMBRICO, M. et al. Memory-like behavior as a feature of electrical signal transmission in melanin-like bio-polymers. **Applied Physics Letters**, v. 100, p. 253702- 1/253702-5, 2012.
- AMBRICO, M. et al. Melanin-like polymer layered on a nanotextured silicon surface for a hybrid biomimetic interface. **Journal of Materials Chemistry C**, v. 2, p. 573–582, 2014.
- AMBRICO, M. et al. From commercial tyrosine polymers to a tailored polydopamine platform: concepts, issues and challenges en route to melanin-based bioelectronics. **Journal of Materials Chemistry C**, v. 3, p. 6413–6423, 2015.
- AMBRICO, M. et al. Hydration-controlled anisotropic and giant permittivity in TEG-functionalized eumelanin. **Phys. Chem. Chem. Phys.**, v. 19, p. 9432–9443, 2017.
- AMDURSKY, N.; GŁOWACKI, E. D.; MEREDITH, P. Macroscale Biomolecular Electronics and Ionics. **Advanced Materials**, v. 31, p. 1802221, 2019.
- ANTIDORMI, A. et al. Assessing the Performance of Eumelanin/Si Interface for Photovoltaic Applications. **The Journal of Physical Chemistry C**, v. 121, p. 11576–11584, 2017.
- ARAÚJO, M. et al. Natural melanin: a potential pH-responsive drug release device. **International journal of pharmaceuticals**, v. 469, p. 140–5, 2014.
- ARNOLD, O. et al. Mantid - Data analysis and visualization package for neutron scattering and μ SR experiments. **Nuclear Instruments and Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors and Associated Equipment**, v. 764, p. 156–166, 2014.
- AZZAM, R. M. A.; BASHARA, N. M. **Ellipsometry and Polarized Light**. 1. ed. [s.l.] North-Holland, 1977.
- BÄHRLE, C. et al. High-Field Electron Paramagnetic Resonance and Density Functional Theory Study of Stable Organic Radicals in Lignin: Influence of the Extraction Process, Botanical Origin, and Protonation Reactions on the Radical g Tensor. **Journal of Physical Chemistry A**, v. 119, p. 6475–6482, 2015.
- BANDODKAR, A. J.; JIA, W.; WANG, J. Tattoo-Based Wearable Electrochemical Devices:

- A Review. **Electroanalysis**, v. 27, n. 3, p. 562–572, 2015.
- BARALDI, P. et al. Electrical Characteristics and Electret Behavior of Melanin. **Journal of the Electrochemical Society**, v. 126, p. 1207–1212, 1979.
- BAREK, H. et al. Insect cuticular melanins are distinctly different from those of mammalian epidermal melanins. **Pigment Cell and Melanoma Research**, v. 31, p. 384–392, 2018.
- BATAGIN-NETO, A. et al. Modelling polymers with side chains: MEH-PPV and P3HT. **Molecular Simulation**, v. 39, p. 309–321, 2013.
- BATAGIN-NETO, A.; BRONZE-UHLE, E. S.; GRAEFF, C. F. de O. Electronic structure calculations of ESR parameters of melanin units. **Physical Chemistry Chemical Physics**, v. 17, p. 7264–7274, 2015.
- BBC One - Doctor Who**. BBC, , [s.d.]. Disponível em: <<https://www.bbc.co.uk/programmes/b006q2x0>>. Acesso em: 12 out. 2019.
- BECKE, A. D. Density-functional thermochemistry. III. The role of exact exchange. **The Journal of Chemical Physics**, v. 98, p. 5648–5652, 1993.
- BELTRÁN-GARCÍA, M. J. et al. Singlet molecular oxygen generation by light-activated DHN-melanin of the fungal pathogen *Mycosphaerella fijiensis* in black sigatoka disease of bananas. **PLoS ONE**, v. 9, p. e91616, 2014.
- BENNATI, M.; PRISNER, T. F. New developments in high field electron paramagnetic resonance with applications in structural biology. **Reports on Progress in Physics**, v. 68, p. 411–448, 2005.
- BERGGREN, M.; RICHTER-DAHLFORS, A. Organic Bioelectronics. **Advanced Materials**, v. 19, p. 3201–3213, 25 set. 2007.
- BERNSMANN, F. et al. Characterization of dopamine-melanin growth on silicon oxide. **Journal of Physical Chemistry C**, v. 113, p. 8234–8242, 2009.
- BETTINGER, C. J. et al. Biocompatibility of biodegradable semiconducting melanin films for nerve tissue engineering. **Biomaterials**, v. 30, p. 3050–3057, 2009.
- BETTINGER, C. J. Materials Advances for Next-Generation Ingestible Electronic Medical Devices. **Trends in Biotechnology**, v. 33, p. 575–585, 2015.
- BETTINGER, C. J. Recent advances in materials and flexible electronics for peripheral nerve interfaces. **Bioelectronic Medicine**, v. 4, p. 1–10, 2018.
- BIE, Y. et al. Polydopamine wrapping silicon cross-linked with polyacrylic acid as high-performance anode for lithium-ion batteries. **ACS Applied Materials and Interfaces**, v. 8, p. 2899–2904, 2016.
- BISQUERT, J. et al. Impedance of constant phase element (CPE)-blocked diffusion in film electrodes. **Journal of Electroanalytical Chemistry**, v. 452, p. 229–234, 1998.
- BISQUERT, J. et al. Theoretical models for ac impedance of finite diffusion layers exhibiting low frequency dispersion. **Journal of Electroanalytical Chemistry**, v. 475, p. 152–163, 1999.
- BISQUERT, J.; COMPTE, A. Theory of the electrochemical impedance of anomalous diffusion. **Journal of Electroanalytical Chemistry**, v. 499, p. 112–120, 2001.
- BISQUERT, J.; GARCIA-BELMONTE, G.; PITARCH, Á. An explanation of anomalous diffusion patterns observed in electroactive materials by impedance methods.

ChemPhysChem, v. 4, p. 287–292, 2003.

BLOIS, M. S. The Melanins: Their Synthesis and Structure. In: SMITH, K. C. (Ed.). **Photochemical and Photobiological Reviews**. Boston, MA: Springer, 1978. p. 115–134.

BLOIS, M. S.; ZAHLAN, A. B.; MALING, J. E. Electron spin resonance studies on melanin. **Biophysical journal**, v. 4, p. 471–490, 1964.

BLOISI, F. et al. Matrix assisted pulsed laser deposition of melanin thin films. **Journal of Applied Physics**, v. 110, p. 026105-1/026105-3, 2011.

BOTHMA, J. P. et al. Device-Quality Electrically Conducting Melanin Thin Films. **Advanced Materials**, v. 20, p. 3539–3542, 2008.

BRAENDLEIN, M. et al. Voltage Amplifier Based on Organic Electrochemical Transistor. **Advanced Science**, v. 4, p. 1600247, 2017.

BRENNER, M.; HEARING, V. J. The protective role of melanin against UV damage in human skin. **Photochemistry and Photobiology**, v. 84, p. 539–549, 2008.

BRIDELLI, M.; CAPELLETTI, R.; CRIPPA, P. R. Electret state and hydrated structure of melanin. **Journal of Electroanalytical Chemistry**, v. 128, p. 555–567, 1981.

BRONZE-UHLE, E. S. et al. Synthesis and characterization of melanin in DMSO. **Journal of Molecular Structure**, v. 1047, p. 102–108, 2013.

BRONZE-UHLE, E. S. et al. Melanin synthesis under oxygen pressure. **Polymer International**, v. 65, p. 1339–1346, 2016.

BRUNAUER, S. et al. On a Theory of the van der Waals Adsorption of Gases. **Journal of the American Chemical Society**, v. 62, p. 1723–1732, 1940.

BRUSTOLON, M. **Electron Paramagnetic Resonance: A Practitioner's Toolkit**. 1. ed. [s.l.] Wiley, 2009.

BUSH, W. D. et al. The surface oxidation potential of human neuromelanin reveals a spherical architecture with a pheomelanin core and a eumelanin surface. **Proceedings of the National Academy of Sciences of the United States of America**, v. 103, n. 40, p. 14785–9, 3 out. 2006.

CÁNOVAS, F. G. et al. The role of pH in the melanin biosynthesis pathway. **Journal of Biological Chemistry**, v. 257, p. 8738–8744, 1982.

CAPOZZI, V. et al. Optical and photoelectronic properties of melanin. **Thin Solid Films**, v. 511–512, p. 362–366, 2006.

CARLILE, C. J.; ADAMS, M. A. The design of the IRIS inelastic neutron spectrometer and improvements to its analysers. **Physica B: Physics of Condensed Matter**, v. 182, p. 431–440, 1992.

CAVAYE, H. Neutron Spectroscopy: An Under-Utilised Tool for Organic Electronics Research? **Angewandte Chemie - International Edition**, v. 58, p. 9338–9346, 2019.

CENTENO, S. A.; SHAMIR, J. Surface enhanced Raman scattering (SERS) and FTIR characterization of the sepia melanin pigment used in works of art. **Journal of Molecular Structure**, v. 873, p. 149–159, 2008.

CHANG, L. et al. Synthetic Melanin E-Ink. **ACS Applied Materials and Interfaces**, v. 9, p. 16553–16560, 2017.

CHEDEKEL, M. R.; AHENE, A. B.; ZEISE, L. Melanin Standard Method: Empirical

Formula 2. **Pigment Cell Research**, v. 5, p. 240–246, 1992.

CHEN, C.-T. et al. Self-Assembly of Tetramers of 5,6-Dihydroxyindole Explains the Primary Physical Properties of Eumelanin: Experiment, Simulation, and Design. **ACS Nano**, v. 7, p. 1524–1532, 2013.

CHEN, C.-T. et al. Excitonic effects from geometric order and disorder explain broadband optical absorption in eumelanin. **Nature communications**, v. 5, p. 3859- 1/3859-10, 2014.

CHEN, T. et al. Different Geometric Information Integrated within a Single Polydopamine Pattern to Yield Dual Shape Transformations. **Macromolecular Materials and Engineering**, v. 303, p. 1800319(1–6), 2018.

CHENG, J. et al. X-Ray Characterization of Melanins - I. **Pigment cell research**, v. 7, p. 255–262, 1994.

CHENG, J.; MOSS, S. C.; EISNER, M. X-Ray Characterization of Melanins - II. **Pigment cell research**, v. 7, p. 263–273, 1994.

CHEUN, W. L. The Chemical Structure of Melanin. **Pigment Cell Research**, v. 17, p. 422–423, 2004.

CHIO, S.; HYDE, J. S.; SEALY, R. C. Temperature-Dependent Paramagnetism in Melanin Polymers. **Archives of Biochemistry and Biophysics**, v. 199, n. 1, p. 133–139, 1980.

CHIO, S.; HYDE, J. S.; SEALY, R. C. Paramagnetism in Melanins: pH Dependence. **Archives of Biochemistry and Biophysics**, v. 215, n. 1, p. 100–106, 1982.

CICCO, S. R. et al. A water-soluble eumelanin polymer with typical polyelectrolyte behaviour by triethyleneglycol N-functionalization. **Journal of Materials Chemistry C**, v. 3, p. 2810–2816, 2015.

CICHOSZ, S.; MASEK, A.; ZABORSKI, M. Polymer-based sensors: A review. **Polymer Testing**, v. 67, p. 342–348, 2018.

CLANCY, C. M. R.; SIMON, J. D. Ultrastructural Organization of Eumelanin from *Sepia officinalis* Measured by Atomic Force Microscopy. **Biochemistry**, v. 40, p. 13353–13360, 2001.

CLARK, M. B. et al. Solid-state analysis of eumelanin biopolymers by electron spectroscopy for chemical analysis. **Analytical Chemistry**, v. 62, p. 949–956, 1990.

CLEMO, G. R.; DUXBURY, F. K.; SWAN, G. A. Formation of tyrosine melanin. Part III. The use of carboxyl-labelled tyrosine and dihydroxyphenylalanine in melanin formation. **Journal of Chemical Society**, p. 3464–3468, 1952.

CLULOW, A. J. et al. The structural impact of water sorption on device-quality melanin thin films. **Soft Matter**, v. 13, p. 3954–3965, 2017.

COMMONER, B.; TOWNSEND, J.; PAKE, G. E. Free Radicals in Biological Materials. **Nature**, v. 174, p. 689–691, 1954.

CONSTANTINO, M. G. **Química orgânica - Curso básico universário (v. 3)**. [s.l: s.n.]

COPE, F. W.; SEVER, R. J.; POLIS, B. D. Reversible free radical generation in the melanin granules of the eye by visible light. **Archives of Biochemistry and Biophysics**, v. 100, p. 171–177, 1963.

CORANI, A. et al. Superior Photoprotective Motifs and Mechanisms in Eumelanins Uncovered. **Journal of the American Chemical Society**, v. 136, p. 11626–11635, 2014.

- CORDERO, R. J. B. Melanin for space travel radioprotection. **Environmental Microbiology**, v. 19, p. 2529–2532, 2017.
- CORDERO, R. J. B.; CASADEVALL, A. Functions of fungal melanin beyond virulence. **Fungal Biology Reviews**, v. 31, p. 99–112, 2017.
- CORDERO, R. J. B.; VIJ, R.; CASADEVALL, A. Microbial melanins for radioprotection and bioremediation. **Microbial Biotechnology**, v. 10, p. 1186–1190, 2017.
- CULP, C. H.; ECKELS, D. E.; SIDLES, P. H. Threshold switching in melanin. **Journal of Applied Physics**, v. 46, p. 3658–3660, 1975.
- CUSTODIO, R.; KUBOTA, L. T.; ANDRADE, J. C. De. **Lei dos Processos de Absorção da Radiação**. Disponível em: <<http://chemkeys.com/br/2000/03/18/lei-dos-processos-de-absorcao-da-radiacao/>>. Acesso em: 24 jan. 2016.
- D'AMATO, R. J.; LIPMAN, Z. P.; SNYDER, S. H. Selectivity of the Parkinsonian neurotoxin MPTP: Toxic metabolite MPP⁺ binds to neuromelanin. **Science**, v. 231, p. 987–989, 1986.
- D'ISCHIA, M. et al. Chemical and structural diversity in eumelanins: unexplored bio-optoelectronic materials. **Angewandte Chemie (International ed. in English)**, v. 48, p. 3914–3921, 2009.
- D'ISCHIA, M. et al. Melanins and melanogenesis: methods, standards, protocols. **Pigment cell & melanoma research**, v. 26, p. 616–633, 2013.
- D'ISCHIA, M. et al. Melanins and Melanogenesis: From Pigment Cells to Human Health and Technological Applications. **Pigment cell & melanoma research**, v. 28, p. 520–544, 2015.
- DA SILVA, M. I. N. et al. Synthetic melanin thin films: Structural and electrical properties. **Journal of Applied Physics**, v. 96, p. 5803–5807, 2004.
- DADACHOVA, E.; CASADEVALL, A. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. **Current Opinion in Microbiology**, v. 11, p. 525–531, 2008.
- DALAL, D. P.; EATON, S. S.; EATON, G. R. The effects of lossy solvents on quantitative EPR studies. **Journal of Magnetic Resonance (1969)**, v. 44, p. 415–428, 1981.
- DAVIDENKO, S. A. et al. Studies of Melanin Pigments of Different Origin. **Molecular Crystals and Liquid Crystals**, v. 426, p. 37–45, 2005.
- DAVIS, I. et al. High-Frequency/High-Field Electron Paramagnetic Resonance and Theoretical Studies of Tryptophan-Based Radicals. **Journal of Physical Chemistry A**, v. 122, p. 3170–3176, 2018.
- DERBY, C. D. Cephalopod ink: Production, chemistry, functions and applications. **Marine Drugs**, v. 12, p. 2700–2730, 2014.
- DEZIDÉRIO, S. N. et al. Thin films of synthetic melanin. **Journal of Non-Crystalline Solids**, v. 338–340, p. 634–638, 2004.
- DI MAURO, E. et al. Resistive switching controlled by the hydration level in thin films of the biopigment eumelanin. **Journal of Materials Chemistry C**, v. 4, p. 9544–9553, 2016.
- DI MAURO, E. et al. Smart Packaging in the Sustainability Challenge: Eumelanin as a UV-absorption Enhancer of Polymers. **IEEE Transactions on Nanotechnology**, p. 1–8, 2019.
- DÍAZ, P. et al. Electrochemical self-assembly of melanin films on gold. **Langmuir**, v. 21, p.

5924–5930, 2005.

DORGALELEH, S. et al. Molecular and biochemical mechanisms of human iris color: A comprehensive review. **Journal of Cellular Physiology**, v. 235, p. 8972–8982, 2020.

DUBEY, S.; ROULIN, A. Evolutionary and biomedical consequences of internal melanins. **Pigment Cell and Melanoma Research**, v. 27, p. 327–338, 2014.

DUNFORD, R. et al. Interaction of melanin with carbon- and oxygen-centered radicals from methanol and ethanol. **Free Radical Biology and Medicine**, v. 19, p. 735–740, 1995.

EARLE, K. A.; BUDIL, D. E.; FREED, J. H. 250-GHz EPR of nitroxides in the slow-motional regime: Models of rotational diffusion. **Journal of Physical Chemistry**, v. 97, p. 13289–13297, 1993.

EATON, G. R.; EATON, S. S. High-field and high-frequency EPR. **Applied Magnetic Resonance**, v. 16, p. 161–166, 1999.

EDGE, R. et al. Dopaquinone redox exchange with dihydroxyindole and dihydroxyindole carboxylic acid. **Pigment Cell Research**, v. 19, n. 5, p. 443–450, 2006.

ELLIOTT, S. R. A.c. conduction in amorphous chalcogenide and pnictide semiconductors. **Advances in Physics**, v. 36, p. 135–217, 1987.

EMBS, J. P.; JURANYI, F.; HEMPELMANN, R. Introduction to quasielastic neutron scattering. **Zeitschrift fur Physikalische Chemie**, v. 224, p. 5–32, 2010.

FADLEY, C. S. X-ray photoelectron spectroscopy: Progress and perspectives. **Journal of Electron Spectroscopy and Related Phenomena**, v. 178–179, p. 2–32, 2010.

FEDOROW, H. et al. Neuromelanin in human dopamine neurons: Comparison with peripheral melanins and relevance to Parkinson's disease. **Progress in Neurobiology**, v. 75, p. 109–124, 2005.

FELIX, C. C. et al. Melanin photoreactions in aerated media: electron spin resonance evidence for production of superoxide and hydrogen peroxide. **Biochemical and biophysical research communications**, v. 84, n. 2, p. 335–341, 1978a.

FELIX, C. C. et al. Interactions of melanin with metal ions. Electron spin resonance evidence for chelate complexes of metal ions with free radicals. **Journal of the American Chemical Society**, v. 100, p. 3922–3926, 1978b.

FELIX, C. C.; HYDE, J. S.; SEALY, R. C. Photoreactions of melanin: A new transient species and evidence for triplet state involvement. **Biochemical and Biophysical Research Communications**, v. 88, p. 456–461, 1979.

FILATOV, J.; MCGINNESS, J.; CORRY, P. Thermal and electronic contributions to switching in melanins. **Biopolymers**, v. 15, p. 2309–2312, 1976.

FOCKE, W. W.; WNEK, G. E.; WEI, Y. Influence of oxidation state, pH, and counterion on the conductivity of polyaniline. **Journal of Physical Chemistry**, v. 91, p. 5813–5818, 1987.

FRISCH, M. J. et al. **Gaussian 09**, 2009. .

FRITZ, H.; PATZER, D.; GALEN, S. S. Robotic exoskeletons for reengaging in everyday activities: promises, pitfalls, and opportunities. **Disability and Rehabilitation**, v. 41, p. 560–563, 2017.

FRONCISZ, W.; SARNA, T.; HYDE, J. S. Cu²⁺ Probe of Metal-Ion Binding Sites in Melanin Using Electron Paramagnetic Resonance Spectroscopy. **Archives of Biochemistry**

and Biophysics, v. 202, p. 289–303, 1980.

FUCHS, M. R. et al. The primary donor cation P⁺. in photosynthetic reaction centers of site-directed mutants of *Rhodobacter sphaeroides*: g-tensor shifts revealed by high-field EPR at 360 GHz/12.8 T. **Chemical Physics**, v. 294, p. 371–384, 2003.

FUJIWARA, H. **Spectroscopic Ellipsometry: Principles and Applications**. 1. ed. [s.l.] Wiley, 2007. v. 53

FUNKE, K. Jump relaxation in solid ionic conductors. **Progress in Solid State Chemistry**, v. 22, p. 111–195, 1993.

GALLAS, J. M. et al. Solution structure of copper ion-induced molecular aggregates of tyrosine melanin. **Biophysical journal**, v. 77, p. 1135–1142, 1999.

GALVÁN, I. et al. Raman spectroscopy quantification of eumelanin subunits in natural unaltered pigments. **Pigment Cell and Melanoma Research**, v. 31, p. 673–682, 2018.

GALVÃO, D. S.; CALDAS, M. J. Polymerization of 5,6-indolequinone: A view into the band structure of melanins. **The Journal of Chemical Physics**, v. 88, p. 4088–4091, 1988.

GARGIULO, V. et al. Supplementing π -systems: eumelanin and graphene-like integration towards highly conductive materials for the mammalian cell culture bio-interface. **Journal of Materials Chemistry B**, v. 3, p. 5070–5079, 2015.

GAUDEN, M. et al. Ultrafast excited state dynamics of 5,6-dihydroxyindole, a key eumelanin building block: nonradiative decay mechanism. **Journal of Physical Chemistry B**, v. 113, p. 12575–12580, 2009.

GÁZQUEZ, J. L.; CEDILLO, A.; VELA, A. Electrodonating and electroaccepting powers. **Journal of Physical Chemistry A**, v. 111, p. 1966–1970, 2007.

GEIM, A. K. Graphene: Status and Prospects. v. 324, n. June, p. 1530–1535, 2009.

GEOFFROY, M.; LUCKEN, E. A. C. Electron spin resonance spectra of RSO₂ radicals formed by the x radiolysis of sulfones. **The Journal of Chemical Physics**, v. 55, p. 2719–2723, 1971.

GESSELER, N. N.; EGOROVA, A. S.; BELOZERSKAYA, T. A. Melanin pigments of fungi under extreme environmental conditions (Review). **Applied Biochemistry and Microbiology**, v. 50, p. 105–113, 2014.

GILCHREST, B. A. et al. The pathogenesis of melanoma induced by ultraviolet radiation. **The New England journal of Medicine**, v. 340, p. 1341–1348, 1999.

GLASSER, L. Proton Conduction and Injection in Solids. **Chemical Reviews**, v. 75, p. 21–65, 1975.

GONÇALVES, P. J.; FILHO, O. B.; GRAEFF, C. F. O. Effects of hydrogen on the electronic properties of synthetic melanin. **Journal of Applied Physics**, v. 99, p. 104701- 1/104701-5, 2006.

GORGEY, A. S. Robotic exoskeletons: The current pros and cons. **World Journal of Orthopaedics**, v. 9, p. 112–119, 2018.

GRADY, F. J.; BORG, D. C. Electron paramagnetic resonance studies on melanins. I. The effect of pH on spectra at Q band. **Journal of the American Chemical Society**, v. 90, p. 2949–2952, 1968.

GREENHOE, B. M. et al. Universal power law behavior of the AC conductivity versus

frequency of agglomerate morphologies in conductive carbon nanotube-reinforced epoxy networks. **Journal of Polymer Science Part B: Polymer Physics**, v. 54, n. 19, p. 1918–1923, 1 out. 2016. Disponível em: <<http://doi.wiley.com/10.1002/polb.24121>>. Acesso em: 27 jan. 2018.

GROENENDAAL, L. et al. Electrochemistry of poly(3,4-alkylenedioxythiophene) derivatives. **Advanced Materials**, v. 15, p. 855–879, 2003.

GUIN, T. et al. Water-Based Melanin Multilayer Thin Films with Broadband UV Absorption. **ACS Macro Letters**, v. 4, p. 335–338, 2015.

GUINOVART, T. et al. Bandage-Based Wearable Potentiometric Sensor for Monitoring Wound pH. **Electroanalysis**, v. 26, p. 1345–1353, 2014.

GURME, S. T. et al. An Organic Bipolar Resistive Switching Memory Device Based on Natural Melanin Synthesized From *Aeromonas* sp. SNS. **Physica Status Solidi (A) Applications and Materials Science**, p. 1800550- 1/1800550-9, 2018.

GURTNER, G. C. et al. Wound repair and regeneration. **Nature**, v. 453, p. 314–321, 2008.

HAICHUAN, C. et al. Density functional theory calculation of refractive indices of the highly polar compounds. **Physics and Chemistry of Liquids**, v. 53, p. 435–442, 2014.

HANSON, G. R. Multifrequency EPR spectroscopy: A toolkit for the characterization of mono- and di-nuclear metal Ion centers in complex biological systems. In: **Structure and Function**. [s.l.] Springer Netherlands, 2010. p. 133–175.

HARMER, J.; MITRIKAS, G.; SCHWEIGER, A. Advanced Pulse EPR Methods for the Characterization of Metalloproteins. In: **High Resolution EPR. Biological Magnetic Resonance (v. 28)**. [s.l.] Springer, New York, NY, 2009. p. 13–61.

HART, J. P.; WRING, S. A. Recent developments in the design and application of screen-printed electrochemical sensors for biomedical, environmental and industrial analyses. **trends in Analytical Chemistry**, v. 16, p. 89–103, 1997.

HERMAN, G. S. et al. Experimental investigation of the interaction of water and methanol with anatase-TiO₂(101). **Journal of Physical Chemistry B**, v. 107, p. 2788–2795, 2003.

HERNANDEZ, D. A.; TENORIO, F. J. Reactivity indexes of antioxidant molecules from *Rosmarinus officinalis*. **Structural Chemistry**, v. 29, p. 741–751, 2018.

HIGASHIHARA, T.; UEDA, M. Recent progress in high refractive index polymers. **Macromolecules**, v. 48, p. 1915–1929, 2015.

HOLZINGER, M.; GOFF, A. Le; COSNIER, S. Nanomaterials for biosensing applications: A review. **Frontiers in Chemistry**, v. 2, p. 1–10, 2014.

HONG, L.; SIMON, J. D. Physical and chemical characterization of iris and choroid melanosomes isolated from newborn and mature cows. **Photochemistry and photobiology**, v. 81, p. 517–523, 2005.

HONG, L.; SIMON, J. D. Current understanding of the binding sites, capacity, affinity and biological significance of metals in melanin. **Journal of Physical Chemistry B**, v. 111, p. 7938–7947, 2007.

HORAK, V.; WEEKS, G. Poly(5,6-dihydroxyindole) melanin film electrode. **Bioorganic Chemistry**, v. 21, p. 24–33, 1993.

HOSSEN, S. et al. Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. **Journal of Advanced Research**, v. 15, p. 1–18, 2019.

- HUANG, G. S. et al. Picogram detection of metal ions by melanin-sensitized piezoelectric sensor. **Biosensors & bioelectronics**, v. 23, p. 319–25, 2007.
- HUANG, J. et al. Polyaniline nanofibers: Facile synthesis and chemical sensors. **Journal of the American Chemical Society**, v. 125, p. 314–315, 2003.
- HUANG, W. S.; HUMPHREY, B. D.; MACDIARMID, A. G. Polyaniline, a novel conducting polymer. Morphology and chemistry of its oxidation and reduction in aqueous electrolytes. **Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases**, v. 82, p. 2385–2400, 1986.
- HUGGINS, R. A. Simple method to determine electronic conductivity and ionic components of the conductors in mixed a review. **Ionics**, v. 8, p. 300–313, 2002.
- HUIJSER, A.; PEZZELLA, A.; SUNDSTRÖM, V. Functionality of epidermal melanin pigments: current knowledge on UV-dissipative mechanisms and research perspectives. **Physical chemistry chemical physics**, v. 13, p. 9119–27, 2011.
- HUNTER, C. A.; SANDERS, J. K. M. The Nature of π - π Interactions. **Journal of the American Chemical Society**, v. 112, p. 5525–5534, 1990.
- INAL, S. et al. Conjugated Polymers in Bioelectronics. **Accounts of Chemical Research**, v. 51, p. 1368–1376, 2018.
- INAL, S.; MALLIARAS, G. G.; RIVNAY, J. Benchmarking organic mixed conductors for transistors. **Nature Communications**, v. 8, p. 1767, 2017.
- ITO, S. A Chemist ' s View of Melanogenesis. **Pigment cell research**, v. 16, p. 230–236, 2003.
- ITO, S.; WAKAMATSU, K. Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. **Pigment Cell Research**, v. 16, p. 523–531, 2003.
- IWAMOTO, M. **Maxwell-Wagner Effect** Springer, Dordrecht., , 2015. . (Nota t?cnica).
- JABLONSKI, N. G.; CHAPLIN, G. Human skin pigmentation as an adaptation to UV radiation. **Proceedings of the National Academy of Sciences of the United States of America**, v. 107, p. 8962–8968, 2010.
- JASTRZEBSKA, M. et al. Dielectric studies on charge hopping in melanin polymer. **Journal of Molecular Structure**, v. 606, n. 1–3, p. 205–210, 2002.
- JASTRZEBSKA, M.; KOCOT, A.; TAJBER, L. Photoconductivity of synthetic dopa-melanin polymer. **Journal of Photochemistry and Photobiology B: Biology**, v. 66, p. 201–206, 2002.
- JASTRZEBSKA, M. M. et al. Electrical conductivity of synthetic DOPA-melanin polymer for different hydration states and temperatures. **Journal of Biomaterials Science, Polymer Edition**, v. 7, p. 577–586, 1995.
- JASTRZEBSKA, M. M.; JUSSILA, S.; ISOTALO, H. Dielectric response and a.c. conductivity of synthetic dopa-melanin polymer. **Journal of Materials Science**, v. 33, p. 4023–4028, 1998.
- JIANG, Q. et al. Red blood cell membrane-camouflaged melanin nanoparticles for enhanced photothermal therapy. **Biomaterials**, v. 143, p. 29–45, 2017.
- JOBIC, H.; THEODOROU, D. N. Quasi-elastic neutron scattering and molecular dynamics simulation as complementary techniques for studying diffusion in zeolites. **Microporous and**

Mesoporous Materials, v. 102, p. 21–50, 2007.

JONSCHER, A. K. A new understanding of the dielectric relaxation of solids. **Journal of Materials Science**, v. 16, p. 2037–2060, 1981.

JU, K. Y. et al. Clue to Understanding the Janus Behavior of Eumelanin: Investigating the Relationship between Hierarchical Assembly Structure of Eumelanin and Its Photophysical Properties. **Biomacromolecules**, v. 17, p. 2860–2872, 2016.

JUNG, Y. H. et al. Bioinspired Electronics for Artificial Sensory Systems. **Advanced Materials**, v. 31, p. 1803637, 2019.

KARLSSON, O.; LINDQUIST, N. G. Melanin and neuromelanin binding of drugs and chemicals: toxicological implications. **Archives of Toxicology**, v. 90, p. 1883–1891, 2016.

KAXIRAS, E. et al. Structural Model of Eumelanin. **Physical Review Letters**, v. 97, p. 218102, nov. 2006.

KERBER, S. J. et al. The nature of hydrogen in x-ray photoelectron spectroscopy: General patterns from hydroxides to hydrogen bonding. **Journal of Vacuum Science & Technology A: Vacuum, Surfaces, and Films**, v. 14, p. 1314–1320, 1996.

KERREMANS, R. et al. The Optical Constants of Solution-Processed Semiconductors—New Challenges with Perovskites and Non-Fullerene Acceptors. **Advanced Optical Materials**, v. 8, p. 2000319, 2020.

KHAN, M. A.; ANSARI, U.; ALI, M. N. Real-time wound management through integrated pH sensors: A review. **Sensor Review**, v. 35, p. 183–189, 2015.

KHAN, M. I. et al. A review on pH sensitive materials for sensors and detection methods. **Microsystem Technologies**, v. 23, p. 4391–4404, 2017.

KIM, I. G. et al. Electrochemical growth of synthetic melanin thin films by constant potential methods. **Electrochimica Acta**, v. 56, p. 2954–2959, 2011.

KIN, Y. J. et al. Biologically derived melanin electrodes in aqueous sodium-ion energy storage devices. **Proceedings of the National Academy of Sciences**, v. 110, p. 20912–17, 2013.

KLEINE, T. S. et al. 100th Anniversary of Macromolecular Science Viewpoint: High Refractive Index Polymers from Elemental Sulfur for Infrared Thermal Imaging and Optics. **ACS Macro Letters**, v. 9, p. 245–259, 2020.

KLOSTERMAN, L.; RILEY, J. K.; BETTINGER, C. J. Control of Heterogeneous Nucleation and Growth Kinetics of Dopamine-Melanin by Altering Substrate Chemistry. **Langmuir**, v. 31, p. 3451–3458, 2015.

KOCH, W. H.; CHEDEKEL, M. R. Photoinitiated DNA damage by melanogenic intermediates in Vitro. **Photochemistry and Photobiology**, v. 44, p. 703–710, 1986.

KOCH, W. H.; CHEDEKEL, M. R. Photogeneration of free radicals from eumelanogenic intermediates and metabolites. **Biochimica et biophysica Acta**, v. 924, p. 458–466, 1987.

KOLLIAS, N. et al. Photoprotection by melanin. **Journal of Photochemistry and Photobiology B: Biology**, v. 9, p. 135–160, 1991.

KUMAR, P. et al. Melanin-based flexible supercapacitors. **Journal of Materials Chemistry C**, v. 4, p. 9516–9525, 2016.

KWON, I. S. et al. In vitro electrochemical characterization of polydopamine melanin as a

- tissue stimulating electrode material. **Journal of Materials Chemistry B**, v. 4, p. 3031–3036, 2016.
- LAGO, N.; CESTER, A. Flexible and organic neural interfaces: A review. **Applied Sciences**, v. 7, p. 1292, 2017.
- LAKOWICZ, J. R. Introduction to Fluorescence. In: LAKOWICZ, J. R. (Ed.). **Principles of Fluorescence Spectroscopy**. 3rd. ed. [s.l.] Springer, Boston, MA, 2006a. p. 1–26.
- LAKOWICZ, J. R. Quenching of Fluorescence. In: **Principles of Fluorescence Spectroscopy**. [s.l.: s.n.]p. 277–330.
- LARSSON, P.; LARSSON, B. S.; TJÄLVE, H. Binding of aflatoxin B1 to melanin. **Food and Chemical Toxicology**, v. 4, p. 163–170, 1988.
- LASIA, A. **Electrochemical Impedance Spectroscopy and its Applications**. 1. ed. [s.l.] Springer-Verlag New York, 2014.
- LAWRIE, K. J.; MEREDITH, P.; MCGEARY, R. P. Synthesis and Polymerization Studies of Organic-Soluble Eumelanins. **Photochemistry and Photobiology**, v. 84, p. 632–638, 2008.
- LEE, C. et al. Semiconducting Synthetic Melanin-Based Organic/Inorganic Hybrid Photoanodes for Solar Water Oxidation. **ChemSusChem**, v. 11, p. 3534–3541, 2018.
- LEE, C.; YANG, W.; PARR, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. **Physical Review B**, v. 37, p. 785–789, 1988.
- LEE, J. W. et al. Dye-sensitized solar cells using purified squid ink nanoparticles coated on TiO₂ nanotubes/nanoparticles. **Journal of the Ceramic Society of Japan**, v. 121, p. 123–127, 2013.
- LEE, Y.; LEE, T. W. Organic Synapses for Neuromorphic Electronics: From Brain-Inspired Computing to Sensorimotor Nervetronics. **Accounts of Chemical Research**, v. 52, p. 964–974, 2019.
- LI, Q. et al. Plumage color patterns of an extinct dinosaur. **Science**, v. 327, p. 1369–1372, 2010.
- LI, W. et al. Characterization of broadband complex refractive index of synthetic melanin coatings and their changes after ultraviolet irradiation. **Applied Physics Letters**, v. 117, p. 203701, 2020.
- LIAO, C. et al. Flexible Organic Electronics in Biology: Materials and Devices. **Advanced Materials**, v. 27, p. 7493–7527, 2015.
- LIGONZO, T. et al. Electrical and optical properties of natural and synthetic melanin biopolymer. **Journal of Non-Crystalline Solids**, v. 355, p. 1221–1226, 2009.
- LIN, J.-H. et al. Formation of fluorescent polydopamine dots from hydroxyl radical-induced degradation of polydopamine nanoparticles. **Physical chemistry chemical physics**, v. 17, p. 15124–30, 2015.
- LINDGREN, J. et al. Fossil insect eyes shed light on trilobite optics and the arthropod pigment screen. **Nature**, v. 573, p. 122–125, 2019.
- LINDQUIST, N. G.; LARSSON, B. S.; LYDÉN-SOKIKIWSKI, A. Neuromelanin and its Possible Protective and Destructive Properties. **Pigment Cell Research**, v. 1, p. 133–136, 1987.
- LIU, J. G. et al. Highly refractive and transparent polyimides derived from 4,4'-[m-

sulfonylbis(phenylenesulfanyl)]diphthalic anhydride and various sulfur-containing aromatic diamines. **Macromolecules**, v. 40, p. 7902–7909, 2007.

LIU, J. G.; UEDA, M. High refractive index polymers: Fundamental research and practical applications. **Journal of Materials Chemistry**, v. 19, p. 8907–8919, 2009.

LIU, S. et al. Polydopamine-coated cellulose nanocrystals as an active ingredient in poly (vinyl alcohol) films towards intensifying packaging application potential. **Cellulose**, v. 7, p. 1–14, 2019.

LIU, Y. et al. Dopamine-melanin colloidal nanospheres: An efficient near-infrared photothermal therapeutic agent for in vivo cancer therapy. **Advanced Materials**, v. 25, n. 9, p. 1353–1359, 2013.

LIU, Y.; SIMON, J. D. Metal-ion interactions and the structural organization of Sepia eumelanin. **Pigment Cell Research**, v. 18, p. 42–48, 2005.

LONGUET-HIGGINS, H. C. On the origin of the free radical property of melanins. **Archives of Biochemistry and Biophysics**, v. 86, p. 231–232, 1960.

LORITE, G. S. et al. Synthetic melanin films: Assembling mechanisms, scaling behavior, and structural properties. **Journal of Applied Physics**, v. 99, p. 113511- 1/113511-6, 2006.

LUNDHOLM, M. et al. Core electron spectroscopy of water solutions. **Journal of Electron Spectroscopy and Related Phenomena**, v. 40, p. 163–180, 1986.

LVOVICH, V. F. **Impedance Spectroscopy: Applications to Electrochemical and Dielectric Phenomena**. 1. ed. [s.l.] Wiley, 2012.

LYTTKENS, L. et al. Melanin capacity to accumulate drugs in the internal ear: A study on lidocaine, bupivacaine and chlorpromazine. **Acta Oto-Laryngologica**, v. 88, p. 61–73, 1979.

MALLIARAS, G. G. Organic bioelectronics: A new era for organic electronics. **Biochimica et Biophysica Acta (BBA) - General Subjects**, v. 1830, p. 4286–4287, 2013.

MANN, D. M. A.; YATES, P. O. Possible role of neuromelanin in the pathogenesis of Parkinson's disease. **Mechanisms of Ageing and Development**, v. 21, p. 193–203, 1983.

MANNING, P. L. et al. Pheomelanin pigment remnants mapped in fossils of an extinct mammal. **Nature Communications**, v. 10, p. 2250, 2019.

MARTÍNEZ, A. et al. Donator acceptor map for carotenoids, melatonin and vitamins. **Journal of Physical Chemistry A**, v. 112, p. 9037–9042, 2008.

MATSUURA, T. et al. Utilization of size-controlled squid ink particles as enhancer for the porosity of titania electrode in dye-sensitized solar cell. **Japanese Journal of Applied Physics**, v. 51, p. 06FG07, 2012.

MCGINNESS, J.; CORRY, P.; PROCTOR, P. Amorphous semiconductor switching in melanins. **Science**, v. 183, p. 853–5, 1974.

MCGINNESS, J. E. Mobility Gaps: A Mechanism for Band Gaps in Melanins. **Science**, v. 177, p. 896–897, 1972.

MCKINLEY, B. A. ISFET and fiber optic sensor technologies: In vivo experience for critical care monitoring. **Chemical Reviews**, v. 108, p. 826–844, 2008.

MEHROTRA, P. Biosensors and their applications - A review. **Journal of Oral Biology and Craniofacial Research**, v. 6, p. 153–159, 2016.

MENTER, J. M. **Melanin from a physicochemical point of view** *Polymer International*,

2016. .

MEREDITH, P. et al. Broadband Photon-harvesting Biomolecules for Photovoltaics. In: COLLINGS, A. F.; CRITCHLEY, C. (Ed.). **Artificial Photosynthesis: From Basic Biology to Industrial Application**. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 2005. p. 37–65.

MEREDITH, P. et al. Towards structure–property–function relationships for eumelanin. **Soft Matter**, v. 2, p. 37–44, 2006.

MEREDITH, P. et al. Towards bioelectronic logic. (I. Kymissis, R. Shinar, L. Torsi, Eds.) In: SPIE Organic Photonics + Electronics, San Diego, California, United States. **Anais...** San Diego, California, United States: International Society for Optics and Photonics, 2016.

Disponível em:

<<http://proceedings.spiedigitallibrary.org/proceeding.aspx?doi=10.1117/12.2239393>>. Acesso em: 27 dez. 2017.

MEREDITH, P.; RIESZ, J. Radiative relaxation quantum yields for synthetic eumelanin. **Photochemistry and photobiology**, v. 79, p. 211–216, 2004.

MEREDITH, P.; SARNA, T. The physical and chemical properties of eumelanin. **Pigment cell research**, v. 19, p. 572–94, 2006.

MIGLIACCIO, L. et al. Aqueous photo(electro)catalysis with eumelanin thin films. **Materials Horizons**, v. 5, p. 984–990, 2018.

MÖBIUS, K.; LUBITZ, W.; SAVITSKY, A. High-field EPR on membrane proteins - Crossing the gap to NMR. **Progress in Nuclear Magnetic Resonance Spectroscopy**, v. 75, p. 1–49, 2013.

MÖBIUS, K.; SAVITSKY, A. **High-Field EPR Spectroscopy on Proteins and their Model Systems**. [s.l.] Royal Society of Chemistry, 2008.

MONTOLIU, L. et al. Increasing the complexity: new genes and new types of albinism. **Pigment cell & melanoma research**, v. 27, jan. 2014.

MORGAN, K.; PETHIG, R. D.C. Hall current measurements on organic crystals. **Journal of Materials Science**, v. 6, p. 179–182, 1971.

MORTENSEN, O. S.; MUNN, R. W.; WILLIAMS, D. F. Phenomenological theory of the hall effect in insulators. **Journal of Applied Physics**, v. 42, p. 1192–1203, 1971.

MOSTERT, A. B. et al. Gaseous adsorption in melanins: Hydrophilic biomacromolecules with high electrical conductivities. **Langmuir**, v. 26, p. 412–416, 2010.

MOSTERT, A. B. et al. Role of semiconductivity and ion transport in the electrical conduction of melanin. **Proceedings of the National Academy of Sciences of the United States of America**, v. 109, p. 8943–7, 2012a.

MOSTERT, A. B. et al. Hydration-Controlled X-Band EPR Spectroscopy: A Tool for Unravelling the Complexities of the Solid-State Free Radical in Eumelanin. **The journal of physical chemistry. B**, v. 117, p. 4965–4972, 2013.

MOSTERT, A. B. et al. The photoreactive free radical in eumelanin. **Science Advances**, v. 4, p. eaaq1293, 2018.

MOSTERT, A. B. et al. Engineering Proton Conductivity in Melanin Using Metal Doping. **Journal of Materials Chemistry B**, v. 8, p. 8050–8060, 2020.

MOSTERT, A. B. On the Free Radical Redox Chemistry of 5,6-Dihydroxyindole. **Chemical**

Physics, 2021.

MOSTERT, B. et al. On the origin of electrical conductivity in the bio-electronic material melanin. **Applied Physics Letters**, v. 100, p. 093701, 2012b.

MOTOVILOV, K. A. et al. Redox chemistry in the pigment eumelanin as a function of temperature using broadband dielectric spectroscopy. **RSC Advances**, v. 9, p. 3857–3867, 2019.

MOULDER, J. F. et al. **Handbook of X-Ray Photoelectron Spectroscopy**. 1st. ed. Minnesota, USA: Perkin-Elmer Corporation, 1992.

MOULTON, S. E. et al. Organic bionics: A new dimension in neural communications. **Advanced Functional Materials**, v. 22, p. 2003–2014, 2012.

MUNOZ-MUNOZ, J. L. et al. Generation of hydrogen peroxide in the melanin biosynthesis pathway. **Biochimica et biophysica acta**, v. 1794, p. 1017–1029, 2009.

NAG, A.; MITRA, A.; MUKHOPADHYAY, S. C. Graphene and its sensor-based applications: A review. **Sensors and Actuators, A: Physical**, v. 270, p. 177–194, 2018.

NAJDER-KOZDROWSKA, L. et al. Influence of Copper(II) Ions on Radicals in DOPA–Melanin. **Applied Magnetic Resonance**, v. 36, p. 81–88, 2009.

NAPOLITANO, A. et al. Oxidative degradation of melanins to pyrrole acids: A model study. **Tetrahedron**, v. 51, p. 5913–5920, 1995.

NEESE, F. The ORCA program system. **Wiley Interdisciplinary Reviews: Computational Molecular Science**, v. 2, p. 73–78, 2012.

NICOLAUS, R. A.; PIATTELLI, M. Structure of Melanins and Melanogenesis. **Journal of Polymer Science**, v. 58, p. 1133–1139, 1962.

NOFSINGER, J. B.; FOREST, S. E.; SIMON, J. D. Explanation for the Disparity among Absorption and Action Spectra of Eumelanin. **The Journal of Physical Chemistry B**, v. 103, p. 11428–11432, 1999.

NOFSINGER, J. B.; WEINERT, E. E.; SIMON, J. D. Establishing structure-function relationships for eumelanin. **Biopolymers**, v. 67, p. 302–305, 2002.

NOONAN, F. P. et al. Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. **Nature Communications**, v. 3, p. 884, 2012.

OLIVEIRA, H. P. et al. Synthesis, characterization and properties of a melanin-like/vanadium pentoxide hybrid compound. **Journal of Materials Chemistry**, v. 10, p. 371–375, 2000.

OLSEN, S. et al. Convergent proton-transfer photocycles violate mirror-image symmetry in a key melanin monomer. **Journal of the American Chemical Society**, v. 129, p. 6672–6673, 2007.

ORDINARIO, D. D. et al. Bulk protonic conductivity in a cephalopod structural protein. **Nature Chemistry**, v. 6, p. 596–602, 2014.

OSAK, W. et al. I-V characteristics and electrical conductivity of synthetic melanin. **Biopolymers**, v. 28, p. 1885–1890, 1989.

OSAK, W. et al. Dielectric and electric properties of synthetic melanin: the effect of europium ions. **Journal of Biological Physics**, v. 21, p. 51–65, 1995.

OWENS, R. M.; MALLIARAS, G. G. Organic Electronics at the Interface with Biology. **MRS Bulletin**, v. 35, p. 449–456, 2010.

- PAL, A. et al. Towards a unified vision of copper involvement in Alzheimer's disease: A review connecting basic, experimental, and clinical research. **Journal of Alzheimer's Disease**, v. 44, p. 343–354, 2015.
- PANZELLA, L. et al. Atypical structural and π -electron features of a melanin polymer that lead to superior free-radical-scavenging properties. **Angewandte Chemie**, v. 52, p. 12684–12687, 2013.
- PASENKIEWICZ-GIERULA, M.; SEALY, R. C. Analysis of the ESR spectrum of synthetic dopa melanin. **Biochimica et Biophysica Acta**, v. 884, p. 510–516, 1986.
- PAULIN, J. V. **Abordagem alternativa para síntese de melanina**. 2016. Universidade Estadual Paulista “Júlio de Mesquita Filho”, 2016. Disponível em: <<http://hdl.handle.net/11449/137931>>.
- PAULIN, J. V. et al. Structural and optical properties of soluble melanin analogues with enhanced photoluminescence quantum efficiency. **Polymer international**, v. 67, p. 550–556, 2018.
- PAULIN, J. V. et al. Shedding Light on the Free Radical Nature of Sulfonated Melanins. **The Journal of Physical Chemistry B**, v. 124, p. 10365–10373, 2020.
- PAULIN, J. V.; BATAGIN-NETO, A.; GRAEFF, C. F. O. Identification of common resonant lines in the EPR spectra of melanins. **The Journal of Physical Chemistry B**, v. 123, p. 1248–1255, 2019.
- PELES, D. N.; SIMON, J. D. UV-Absorption Spectra of Melanosomes Containing Varying 5,6-Dihydroxyindole and 5,6-Dihydroxyindole-2-Carboxylic acid Content. **The Journal of Physical Chemistry B**, v. 115, p. 12624–12631, 2011.
- PERNA, G. et al. Fluorescence spectroscopy of synthetic melanin in solution. **Journal of Luminescence**, v. 129, p. 44–49, 2009.
- PEZZELLA, A. et al. Disentangling eumelanin “black chromophore”: visible absorption changes as signatures of oxidation state- and aggregation-dependent dynamic interactions in a model water-soluble 5,6-dihydroxyindole polymer. **Journal of the American Chemical Society**, v. 131, p. 15270–5, 2009.
- PEZZELLA, A. et al. Stem cell-compatible eumelanin biointerface fabricated by chemically controlled solid state polymerization. **Materials Horizons**, v. 2, p. 212–220, 2015.
- PIACENTI-SILVA, M. et al. Melanin as an active layer in biosensors. **AIP Advances**, v. 4, p. 037120-1/037120-8, 2014a.
- PIACENTI-SILVA, M. et al. Temperature-enhanced synthesis of DMSO-Melanin. **Journal of Molecular Structure**, v. 1056–1057, p. 135–140, 2014b.
- PIACENTI-SILVA, M. et al. Biocompatibility investigations of synthetic melanin and melanin analogue for application in bioelectronics. **Polymer International**, v. 65, p. 1347–1354, 2016.
- PICCOLINO, M. Luigi Galvani and animal electricity: Two centuries after the foundation of electrophysiology. **Trends in Neurosciences**, v. 20, p. 443–448, 1997.
- PILI, S. et al. Enhancement of Proton Conductivity in Nonporous Metal-Organic Frameworks: The Role of Framework Proton Density and Humidity. **Chemistry of Materials**, v. 30, p. 7593–7602, 2018.
- PINHEIRO, F. L. et al. Chemical characterization of pterosaur melanin challenges color

- inferences in extinct animals. **Scientific Reports**, v. 9, p. 15947, 2019.
- PINNA, E. et al. Deciphering Molecular Mechanisms of Interface Buildup and Stability in Porous Si/Eumelanin Hybrids. **International Journal of Molecular Sciences**, v. 18, p. 1567-1/1567-20, 2017.
- PLONKA, P. M.; GRABACKA, M. Melanin synthesis in microorganisms - biotechnological and medical. **Acta Biochimica Polonica**, v. 53, p. 429–443, 2006.
- POOLE, C. P. **Electron Spin Resonance: A Comprehensive Treatise on Experimental Techniques**. 2. ed. New York: Wiley, 1982.
- POOLE, C. P. **Electron spin resonance: a comprehensive treatise on experimental techniques**. New York: John Wiley & Sons, 1983.
- POWELL, M. R.; ROSENBERG, B. The nature of the charge carriers in solvated biomacromolecules. **Bioenergetics**, v. 1, p. 493–509, 1970.
- PROTA, G. **Melanins and melanogenesis**. [s.l.] Academic Press, 1992.
- PROTA, G. Melanins, melanogenesis and melanocytes: Looking at their functional significance from the chemist's viewpoint. **Pigment Cell Research**, v. 13, p. 283–293, 2000.
- PULLMAN, A.; PULLMAN, B. The band structure of melanins. **Biochimica et Biophysica Acta**, v. 54, p. 384–385, 1961.
- QIN, M. et al. Advances in flexible and wearable pH sensors for wound healing monitoring. **Journal of Semiconductors**, v. 40, p. (in-press), 2019.
- RAZA, A. et al. “Smart” materials-based near-infrared light-responsive drug delivery systems for cancer treatment: A review. **Journal of Materials Research and Technology**, v. 8, p. 1497–1509, 2019.
- REALI, M. et al. Electronic Transport in the Biopigment Sepia Melanin. **ACS Applied Bio Materials**, v. 3, p. 5244–5252, 2020.
- REALI, M.; SAINI, P.; SANTATO, C. Electronic and protonic transport in bio-sourced materials: a new perspective on semiconductivity. **Materials Advances**, p. Advance Article, 2021.
- RESZKA, K. J.; MATUSZAK, Z.; CHIGNELL, C. F. Lactoperoxidase-Catalyzed Oxidation of Melanin by Reactive Nitrogen Species Derived From Nitrite (NO₂⁻): An EPR Study. **Free Radical Biology and Medicine**, v. 25, p. 208–216, 1998.
- RIBEIRO, E. S. et al. **Princípios Básicos de XAS e XPS**. Disponível em: <<http://chemkeys.com/br/2003/02/24/principios-basicos-de-xas-e-xps/>>. Acesso em: 31 jan. 2016.
- RIENECKER, S. B. et al. Heavy Water as a Probe of the Free Radical Nature and Electrical Conductivity of Melanin. **Journal of Physical Chemistry B**, v. 119, p. 14994–15000, 2015.
- RIESZ, J.; GILMORE, J.; MEREDITH, P. Quantitative scattering of melanin solution. **Biophysical journal**, v. 90, p. 4137–4144, 2006.
- RIVNAY, J. et al. Organic Electrochemical Transistors with Maximum Transconductance at Zero Gate Bias. **Advanced Materials**, v. 25, p. 7010–7014, 2013.
- RIVNAY, J. et al. Structural control of mixed ionic and electronic transport in conducting polymers. **Nature Communications**, v. 7, p. 1–9, 2016.
- RIVNAY, J. et al. Next-generation probes, particles, and proteins for neural interfacing.

Science Advances, v. 3, p. e1601649, 2017.

RIVNAY, J.; OWENS, R. M.; MALLIARAS, G. G. The Rise of Organic Bioelectronics. **Chemistry of Materials**, v. 26, p. 679–685, 2014.

ROESSLER, M. M.; SALVADORI, E. Principles and applications of EPR spectroscopy in the chemical sciences. **Chemical Society Reviews**, v. 47, p. 2534–2553, 2018.

ROHATGI, A. **WebPlotDigitizer (v. 4.1)**. Disponível em:
<<https://automeris.io/WebPlotDigitizer>>. Acesso em: 8 mar. 2018.

RONSEIN, G. E. et al. Oxidação de proteínas por oxigênio singlete, mecanismo de dano, estratégia para detecção e implicações biológicas. **Química Nova**, v. 29, n. 3, p. 563–568, 2006.

ROSENBERG, B.; POSTOW, E. Semiconduction in proteins and lipids - Its possible biological importance. **Annals of the New York Academy of Sciences**, v. 158, p. 158–162, 1969.

ROUQUEROL, F. et al. **Adsorption by Powders and Porous Solids: Principles, Methodology and Applications**. 2. ed. [s.l.] Academic Press, 2014.

SAHA, S. K. et al. XPS characterization of in situ prepared Ti/glass interfaces. **Surface and Interface Analysis**, v. 24, p. 113–118, 1996.

SAKAI, V. G. **Quasi-Elastic Neutron Scattering: 13th Oxford School of Neutron Scattering**. Disponível em:
<<http://www.oxfordneutronschool.org/2013/Lectures/GarciaSakai-QENS.pdf>>. Acesso em: 13 jun. 2019.

SAKAI, V. G.; ALBA-SIMIONESCO, C.; CHEN, S. H. (ed.). **Dynamics of Soft Matter: Neutron Applications**. 1. ed. [s.l.] Springer US, 2012.

SAKAI, V. G.; ARBE, A. Quasielastic neutron scattering in soft matter. **Current Opinion in Colloid and Interface Science**, v. 14, n. 6, p. 381–390, 2009.

SARNA, T. et al. Interaction of Melanin with Oxygen. **Archives of Biochemistry and Biophysics**, v. 200, p. 140–148, 1980.

SARNA, T. Properties and function of the ocular melanin - a photobiophysical view. **Journal of Photochemistry and Photobiology, B: Biology**, v. 12, p. 215–258, 1992.

SARNA, T.; PLONKA, P. M. Biophysical Studies of Melanin: Paramagnetic, Ion-Exchange and Redox Properties of Melanin Pigments and Their Photoreactivity. In: EATON, S. S.; EATON, G. R.; BERLINER, L. J. (Ed.). **Biomedical EPR, Part A: Free Radicals, Metals, Medicine, and Physiology**. 1. ed. [s.l.] Springer US, 2005. p. 125–146.

SARNA, T.; SEALY, R. C. Free radicals from eumelanins: Quantum yields and wavelength dependence. **Archives of Biochemistry and Biophysics**, v. 232, p. 574–578, 1984.

SARNA, T.; SWARTZ, H. A. The Physical Properties of Melanins. In: NORDLUND, J. J. et al. (Ed.). **The Pigmentary System: Physiology and Pathophysiology**. 2. ed. [s.l.] Blackwell Publishing, 2006. p. 191–212.

SCHMIDT, M. W. et al. General atomic and molecular electronic structure system. **Journal of Computational Chemistry**, v. 14, p. 1347–1363, 1993.

SCHNEIDER, L. A. et al. Influence of pH on wound-healing: A new perspective for wound-therapy? **Archives of Dermatological Research**, v. 298, p. 413–420, 2007.

- SCHWEICHER, G. et al. Molecular Semiconductors for Logic Operations: Dead-End or Bright Future? **Advanced Materials**, v. 32, p. 1905909, 2020.
- SCHWEITZER, A. D. et al. Melanin-covered nanoparticles for protection of bone marrow during radiation therapy of cancer. **International Journal of Radiation Oncology Biology Physics**, v. 78, p. 1494–1502, 2010.
- SEALY, R. et al. Novel free radicals in synthetic and natural pheomelanins: distinction between dopa melanins and cysteinyl-dopa melanins by ESR spectroscopy. **Proceedings of the National Academy of Sciences of the United States of America**, v. 79, p. 2885–2889, 1982.
- SEALY, R. C. et al. Structure and Reactivity of Melanins: Influence of Free Radicals and Metal Ions. In: **Free Radicals in Biology**. v. IV ed. [s.l.] Academic Press, 1980. p. 209–259.
- SEARS, V. F. Neutron scattering lengths and cross sections. **Neutron News**, v. 3, p. 26–37, 1992.
- SEVER, R. J.; COPE, F. W.; POLIS, B. D. Generation by visible light of labile free radicals in the melanin granules of the eye. **Science**, v. 137, p. 128–129, 1962.
- SHELIAKINA, M.; MOSTERT, A. B.; MEREDITH, P. An all-solid-state biocompatible ion-to-electron transducer for bioelectronics. **Materials Horizons**, v. 5, p. 256–263, 2018a.
- SHELIAKINA, M.; MOSTERT, A. B.; MEREDITH, P. Decoupling Ionic and Electronic Currents in Melanin. **Advanced Functional Materials**, v. 1805514, p. 1805514, 2018b.
- SIMON, D. T. et al. Organic Bioelectronics: Bridging the Signaling Gap between Biology and Technology. **Chemical Reviews**, v. 116, p. 13009–13041, 2016.
- SIMON, J. D.; ITO, S. The Chemical Structure of Melanin. **Pigment Cell Research**, v. 17, p. 423–424, 2004.
- SKOOG, D. A. et al. Análise Espectroquímica. In: **Fundamentos de Química Analítica**. 8. ed. [s.l.] Thomson, 2006.
- SKOOG, D. A.; HOLLER, F. J.; CROUCH, S. R. **Principles of instrumental analysis**. 6th. ed. [s.l.] David Harris, 2007.
- SOBOLEVA, T. et al. Investigation of the through-plane impedance technique for evaluation of anisotropy of proton conducting polymer membranes. **Journal of Electroanalytical Chemistry**, v. 622, p. 145–152, 2008.
- SOLANO, F. Melanins: Skin Pigments and Much More - Types, Structural Models, Biological Functions, and Formation Routes. **New Journal of Science**, v. 2014, p. 1–28, 2014.
- SOMEYA, T.; AMAGAI, M. Toward a new generation of smart skins. **Nature Biotechnology**, v. 37, p. 382–388, 2019.
- SRISUK, P. et al. Eumelanin Nanoparticle-Incorporated Polyvinyl Alcohol Nanofibrous Composite as an Electroconductive Scaffold for Skeletal Muscle Tissue Engineering. **ACS Applied Bio Materials**, v. 1, p. 1893–1905, 2018.
- STARK, K. B. et al. Spectroscopic Study and Simulation from Recent Structural Models for Eumelanin: II. Oligomers. **The Journal of Physical Chemistry B**, v. 107, p. 11558–11562, 2003.
- STAVENGA, D. G. et al. High refractive index of melanin in shiny occipital feathers of a bird of paradise. **Light: Science and Applications**, v. 4, p. e243, 2015.

STAVRINIDOU, E. et al. Direct measurement of ion mobility in a conducting polymer. **Advanced Materials**, v. 25, p. 4488–4493, 2013.

STEPHENS, P. J. et al. Ab Initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields. **Journal of Physical Chemistry**, v. 98, p. 11623–11627, 1994.

STEWART, J. J. P. MOPAC: A semiempirical molecular orbital program. **Journal of Computer-Aided Molecular Design**, v. 4, p. 1–103, 1990.

STEWART, J. J. P. Optimization of parameters for semiempirical methods V: Modification of NDDO approximations and application to 70 elements. **Journal of Molecular Modeling**, v. 13, p. 1173–1213, 2007.

STEWART, J. J. P. **MOPAC2016**. Disponível em:
<<http://openmopac.net/MOPAC2016.html>>.

STOLL, S. **EasySpin - EPR spectrum simulation**. Disponível em:
<<http://www.easyspin.org/>>.

STOLL, S.; SCHWEIGER, A. EasySpin, a comprehensive software package for spectral simulation and analysis in EPR. **Journal of Magnetic Resonance**, v. 178, p. 42–55, 2006.

STONE, A. J. Gauge invariance of the g tensor. **Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences**, v. 271, p. 424–434, 1963a.

STONE, A. J. g factors of aromatic free radicals. **Molecular Physics**, v. 6, p. 509–515, 1963b.

STRUBE, O. I.; BÜNGELER, A.; BREMSER, W. Site-Specific In Situ Synthesis of Eumelanin Nanoparticles by an Enzymatic Autodeposition-Like Process. **Biomacromolecules**, v. 16, p. 1608–1613, 2015.

TARABELLA, G. et al. A single cotton fiber organic electrochemical transistor for liquid electrolyte saline sensing. **Journal of Materials Chemistry**, v. 22, p. 23830–23834, 2012.

TEHRANI, Z. et al. Printable and Flexible Graphene pH sensors utilising Thin Film Melanin for Physiological Applications. **2D Materials**, v. 7, p. 024008, 2020.

THATHACHARI, Y. T.; BLOIS, M. S. Physical Studies on Melanins: II. X-Ray Diffraction. **Biophysical Journal**, v. 9, p. 77–89, 1969.

THOMMES, M. et al. Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report). **Pure and Applied Chemistry**, v. 87, p. 1051–1069, 2015.

TIAN, H. et al. Polydopamine-Coated Main-Chain Liquid Crystal Elastomer as Optically Driven Artificial Muscle. **ACS Applied Materials and Interfaces**, v. 10, p. 8307–8316, 2018.

TIPIKIN, D. S. et al. Possible nature of the radiation-induced signal in nails: High-field EPR, confirming chemical synthesis, and quantum chemical calculations. **Radiation Protection Dosimetry**, v. 172, p. 112–120, 2016.

TRAN, M. L.; POWELL, B. J.; MEREDITH, P. Chemical and structural disorder in eumelanins: a possible explanation for broadband absorbance. **Biophysical journal**, v. 90, p. 743–52, 2006.

TRUEBLUE. **TrueBlue Vision: Your best eye protection against harmful blue light**. Disponível em: <<https://www.truebluevision.com/>>. Acesso em: 13 nov. 2019.

- TUNA, D. et al. Onset of the Electronic Absorption Spectra of Isolated and π -Stacked Oligomers of 5,6-Dihydroxyindole: An Ab Initio Study of the Building Blocks of Eumelanin. **Journal of Physical Chemistry B**, v. 120, p. 3493–3502, 2016.
- TYAGI, M.; CHATHOTH, S. M. Quasielastic Neutron Scattering: An Advanced Technique for Studying the Relaxation Processes in Condensed Matter. In: KUMAR, C. S. S. R. (Ed.). **X-ray and Neutron Techniques for Nanomaterials Characterization**. 1. ed. [s.l.] Springer-Verlag Berlin Heidelberg, 2016. p. 761–813.
- VARGA, M. et al. Structural characterization of allomelanin from black oat. **Phytochemistry**, v. 130, p. 313–320, 2016.
- VECCHIA, N. F. Della et al. Tailoring melanins for bioelectronics: polycysteinyldopamine as an ion conducting redox-responsive polydopamine variant for pro-oxidant thin films. **Journal of Materials Chemistry C**, v. 3, p. 6525–6531, 2015.
- VOGEL, A. I.; MENDHAM, J. **Vogel's textbook of quantitative chemical analysis**. 6th. ed. [s.l.] Prentice Hall, 2000.
- VOSKO, S. H.; WILK, L.; NUSAIR, M. Accurate spin-dependent electron liquid correlation energies for local spin density calculations: a critical analysis. **Canadian Journal of Physics**, v. 58, p. 1200–1211, 1980.
- WAGNER, C. D.; ZATKO, D. A.; RAYMOND, R. H. Use of the Oxygen KLL Auger Lines in Identification of Surface Chemical States by Electron Spectroscopy for Chemical Analysis. **Analytical Chemistry**, v. 52, p. 1445–1451, 1980.
- WANG, H. B. et al. Electrochemical biosensor for simultaneous determination of guanine and adenine based on dopamine-melanin colloidal nanospheres-graphene composites. **Journal of Solid State Electrochemistry**, v. 18, p. 2435–2442, 2014.
- WANG, S. et al. Superfast and controllable microfluidic inking of anti-inflammatory melanin-like nanoparticles inspired by cephalopods. **Materials Horizons**, v. 7, p. 1573–1580, 2020.
- WANG, X. et al. Multi-responsive photothermal-chemotherapy with drug-loaded melanin-like nanoparticles for synergetic tumor ablation. **Biomaterials**, v. 81, p. 114–124, 2016a.
- WANG, Y. et al. Simultaneous Enhancements of UV-Shielding Properties and Photostability of Poly(vinyl alcohol) via Incorporation of Sepia Eumelanin. **ACS Sustainable Chemistry and Engineering**, v. 4, p. 2252–2258, 2016b.
- WATT, A. A. R.; BOTHMA, J. P.; MEREDITH, P. The supramolecular structure of melanin. **Soft Matter**, v. 5, p. 3754–3760, 2009.
- WEIL, J. A.; BOLTON, J. R. **Electron Paramagnetic Resonance: Elementary Theory and Practical Applications**. 2. ed. New York: Wiley, 2007.
- WHELAN, S. P. et al. Microbiological culturing applications for a flexible and Printable pH sensor based on graphene-melanin. **Under review**, [s.d.]
- WITTMANN, J. J. et al. High-precision measurement of the electron spin g factor of trapped atomic nitrogen in the endohedral fullerene N@C60. **Journal of Magnetic Resonance**, v. 290, p. 12–17, 2018.
- WOLBARSHT, M. L.; WALSH, A. W.; GEORGE, G. Melanin, a unique biological absorber. **Applied Optics**, v. 20, p. 2184, 1981.
- WU, T. F.; WEE, B. H.; HONG, J. D. An Ultrasensitive and Fast Moisture Sensor Based on Self-Assembled Dopamine-Melanin Thin Films. **Advanced Materials Interfaces**, v. 2, p. 1–

5, 2015.

WÜNSCHE, J. et al. In Situ Formation of Dendrites in Eumelanin Thin Films between Gold Electrodes. **Advanced Functional Materials**, v. 23, p. 5591–5598, 2013a.

WÜNSCHE, J. et al. Eumelanin thin films: solution-processing, growth, and charge transport properties. **Journal of Materials Chemistry B**, v. 1, p. 3836–3842, 2013b.

WÜNSCHE, J. et al. Protonic and Electronic Transport in Hydrated Thin Films of the Pigment Eumelanin. **Chemistry of Materials**, v. 27, p. 436–442, 2015.

XIAO, M. et al. Bio-Inspired Structural Colors Produced via Self-Assembly of Synthetic Melanin Nanoparticles. **ACS nano**, v. 9, p. 5454–5460, 2015.

XIAO, M. et al. Bioinspired bright noniridescent photonic melanin supraballs. **Science Advances**, v. 3, p. e1701151, 2017.

XU, R. et al. Melanin: A Greener Route To Enhance Energy Storage under Solar Light. **ACS Omega**, v. 4, p. 12244–12251, 2019.

YADAV, L. D. S. Electron Spin Resonance (ESR) Spectroscopy. In: **Organic Spectroscopy**. Dordrecht: Springer Netherlands, 2005. p. 224–249.

ZAJAC, G. W. et al. The fundamental unit of synthetic melanin: a verification by tunneling microscopy of X-ray scattering results. **Biochimica et Biophysica Acta**, v. 1199, p. 271–278, 1994.

ZANGMEISTER, R. A.; MORRIS, T. A.; TARLOV, M. J. Characterization of polydopamine thin films deposited at short times by autoxidation of dopamine. **Langmuir**, v. 29, p. 8619–8628, 2013.

ZHANG, R. et al. Engineering Melanin Nanoparticles as an Efficient Drug-Delivery System for Imaging-Guided Chemotherapy. **Advanced Materials**, v. 27, p. 5063–5069, 2015.

ZHONG, C. et al. A polysaccharide bioprotonic field-effect transistor. **Nature communications**, v. 2, p. 476, 2011.

ZUCCA, F. A. et al. The neuromelanin of human substantia nigra: Physiological and pathogenic aspects. **Pigment Cell Research**, v. 17, p. 610–617, 2004.

ZUCCA, F. a et al. Neuromelanin of the human substantia nigra: an update. **Neurotoxicity research**, v. 25, p. 13–23, 2014.

APPENDIX A

Table A1. The atomic composition (%) from pressed powder pellets impurities of synthetic non-functionalized melanin samples.

Samples	Surface				Bulk			
	S2p	Ca2p	Si2p	Total	S2p	Ca2p	Si2p	Total
NFMel-I	0.15	0.13	2.10	2.38	0.06	0.13	2.10	2.29
NFMel-S	0.40	0.06	2.63	3.08	0.24	0.06	2.63	2.93
NFMel-6P	0.42	0.18	0.92	1.52	0.09	0.18	0.92	1.19

Table A2. C1s, O1s and N1s photoelectron binding energies assignments for NF-Melanin. Uncertainties were calculated using the standard deviation procedure of the XPS peaks positions from all non-functionalized melanin derivatives.

Region	Functionality	Binding energy (eV)	
		Pellet	Thin-film
C1s	CxHy	285.1 ± 0.1	285.2 ± 0.2
	C-O/C-N	286.4 ± 0.1	286.4 ± 0.1
	C=O	288.1 ± 0.2	288.5 ± 0.1
	O-C=O	289.2 ± 0.1	289.7 ± 0.2
	π - π^* (shake-up)	291.4 ± 0.1	292.1 ± 0.1
O1s	O=C	531.5 ± 0.2	531.7 ± 0.1
	O-C	533.2 ± 0.2	533.4 ± 0.1
	Impurity	532.5 ± 0.2	533.0 ± 0.1
N1s	RNH ₂ /RNH ₃ ⁺	401.5 ± 0.1	#
	R ₂ NH	400.0 ± 0.1	400.5 ± 0.1
	=NR	398.7 ± 0.1	399.1 ± 0.2

Table A3. The atomic composition (%) from spin-coated thin-film impurities of synthetic non-functionalized melanin samples.

Samples	Surface					Bulk				
	S2p	Ca2p	Na1s	Si2p	Total	S2p	Ca2p	Na1s	Si2p	Total
NFMel-I (H ₂ O+NH ₃ film)	0.07	1.37	4.02	0.15	5.6	0.05	1.34	4.77	0.11	6.27
NFMel-S (H ₂ O)	0.13	1.59	6.47	0.39	8.58	0.08	1.44	9.82	0.58	11.92
NFMel-S (H ₂ O+NH ₃ film)	0.11	1.43	4.43	0.19	6.16	0.10	1.43	6.06	0.14	7.73
NFMel-6P (H ₂ O)	0.10	1.34	4.48	0.20	6.12	0.07	1.32	6.92	0.09	8.40
NFMel-6P (H ₂ O+NH ₃ film)	0.07	1.44	3.23	0.17	4.91	0.07	1.44	4.79	0.13	6.43

Table A4. The atomic composition (%) from pressed powder pellets impurities of synthetic sulfonated melanin samples.

Samples	Surface			Bulk		
	Ca2p	Si2p	Total	Ca2p	Si2p	Total
SMel	0.14	2.81	2.95	0.10	0.35	0.45
SMel-T	0.38	2.96	3.34	0.22	0.71	0.93
SMel-4P	0.34	2.67	3.01	0.21	0.43	0.64
SMel-8P	0.18	2.33	2.51	0.12	0.43	0.55

Table A5. C1s, O1s and N1s photoelectron binding energies assignments for S-Melanin. Uncertainties were calculated using the standard deviation procedure of the XPS peaks positions from all non-functionalized melanin derivatives.

Region	Functionality	Binding energy (eV)	
		Pellet	Thin-film
C1s	CxHy	284.7 ± 0.0	285.1 ± 0.1
	C-N	285.6 ± 0.0	286.0 ± 0.1
	C-O	286.2 ± 0.0	286.6 ± 0.2
	C=O	288.3 ± 0.0	288.4 ± 0.1
	C-S	285.1 ± 0.0	285.4 ± 0.1
	O-C=O	290.0 ± 0.1	289.7 ± 0.2
	π - π^* (shake-up)	291.6 ± 0.0	291.9 ± 0.2
O1s	O=C/O=S	531.4 ± 0.0	531.6 ± 0.1
	O-C	533.0 ± 0.0	533.3 ± 0.1
	Impurity	532.6 ± 0.0	532.8 ± 0.1
N1s	RNH ₂ /RNH ₃ ⁺	401.2 ± 0.0	401.3 ± 0.1
	R ₂ NH	400.2 ± 0.0	400.3 ± 0.1
	=NR	398.9 ± 0.0	398.9 ± 0.1
S2p	S-C (2p ³ / ₂)	163.2 ± 0.1	163.9 ± 0.2
	S-C (2p ¹ / ₂)	164.4 ± 0.1	165.1 ± 0.2
	S-O/S=O (2p ³ / ₂)	165.9 ± 0.1	166.5 ± 0.3
	S-O/S=O (2p ¹ / ₂)	167.0 ± 0.1	167.7 ± 0.3
	SO ₂ (2p ³ / ₂)	167.9 ± 0.1	168.8 ± 0.3
	SO ₂ (2p ¹ / ₂)	169.0 ± 0.1	170.0 ± 0.3
	SO ₄ (2p ³ / ₂)	169.0 ± 0.0	#
	SO ₄ (2p ¹ / ₂)	170.2 ± 0.0	#

Table A6. The atomic composition (%) from spin-coated thin-film impurities of synthetic sulfonated melanin samples.

Samples	Solvents	Surface				Bulk			
		Ca2p	Na1s	Si2p	Total	Ca2p	Na1s	Si2p	Total
SMel	DMSO	0.35	12.34	0.49	13.18	0.34	15.70	2.52	18.56
	DMF	1.50	6.70	0.27	8.47	1.45	7.69	0.20	9.34
	NMP	0.42	12.75	1.63	14.80	1.34	11.35	17.64	30.33
SMel-T	DMSO	1.27	10.44	2.76	14.47	1.32	13.83	5.27	20.42
	DMF	0.28	9.89	0.10	10.27	0.19	10.60	0.09	10.88
	NMP	0.49	14.79	0.43	15.71	0.48	20.55	1.83	22.86
SMel-4P	DMSO	1.23	14.27	1.19	16.69	1.28	18.52	4.34	24.14
	DMF	0.22	11.54	0.11	11.87	0.17	9.38	0.22	9.77
	NMP	0.68	12.34	2.55	15.57	0.83	10.19	19.79	30.81
SMel-8P	DMSO	1.20	13.05	0.88	15.13	1.24	16.56	4.48	22.28
	DMF	1.19	10.24	0.12	11.55	1.60	9.21	0.14	10.95
	NMP	0.41	15.53	1.25	17.19	1.01	16.04	17.74	34.79

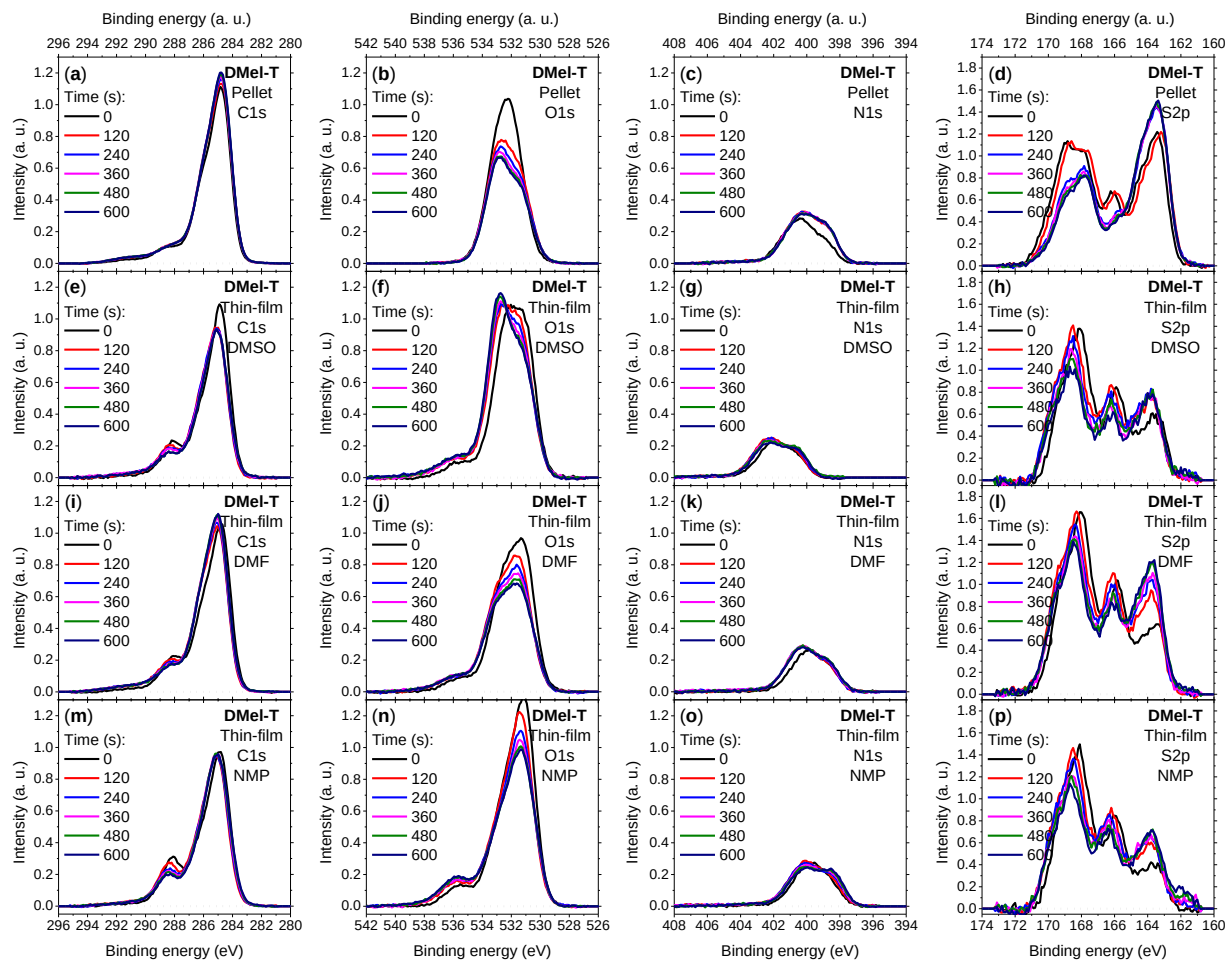


Figure A1. High-resolution (a, e, i, m) C1s, (b, f, j, n) O1s, (c, g, k, o) N1s, and (d, h, l, p) S2p XPS depth profiling spectra of SMel-T pellet (first row) and thin-film (second-to-fourth rows).

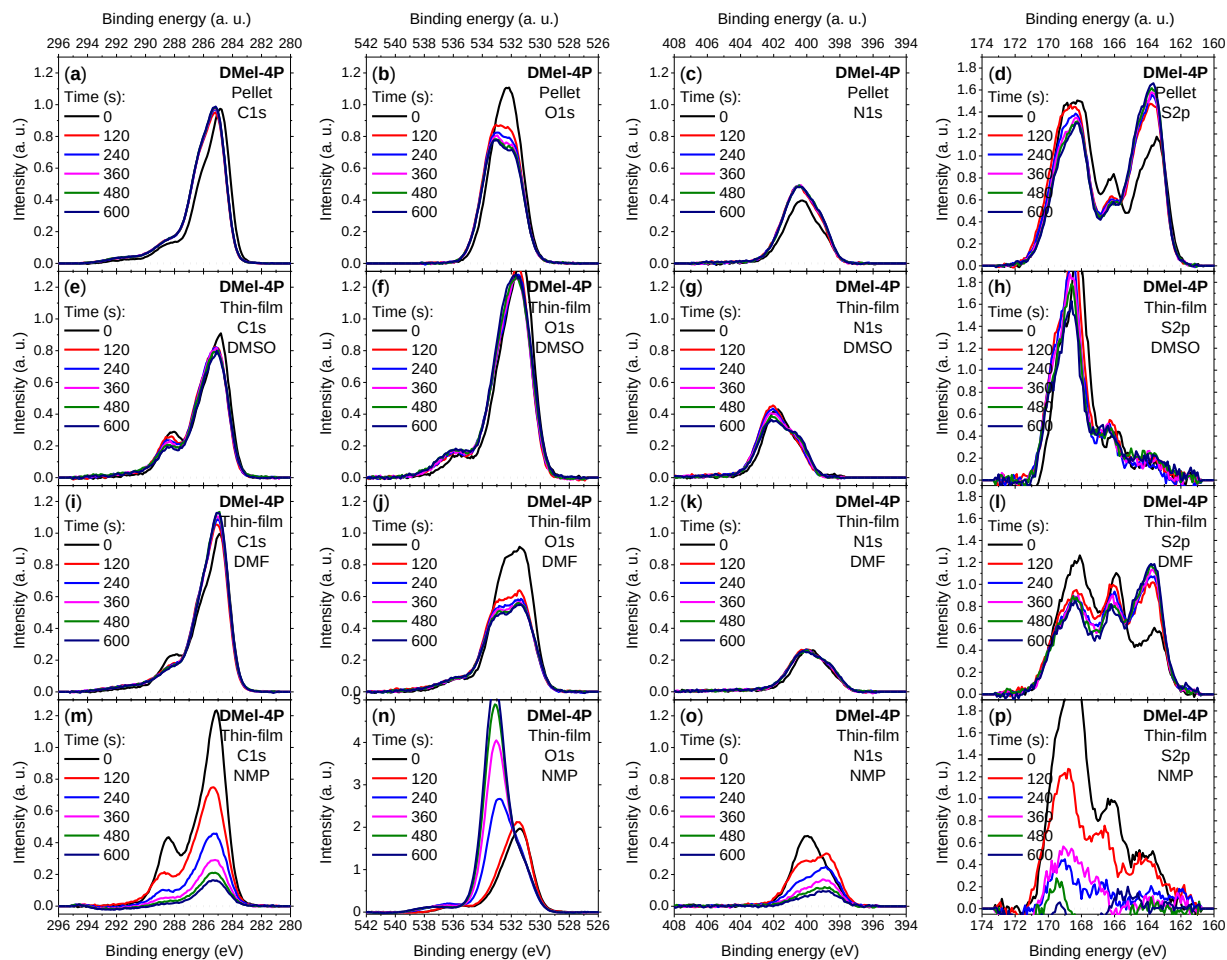


Figure A2. High-resolution (a, e, i, m) C1s, (b, f, j, n) O1s, (c, g, k, o) N1s, and (d, h, l, p) S2p XPS depth profiling spectra of SMel-4P pellet (first row) and thin-film (second-to-fourth rows).

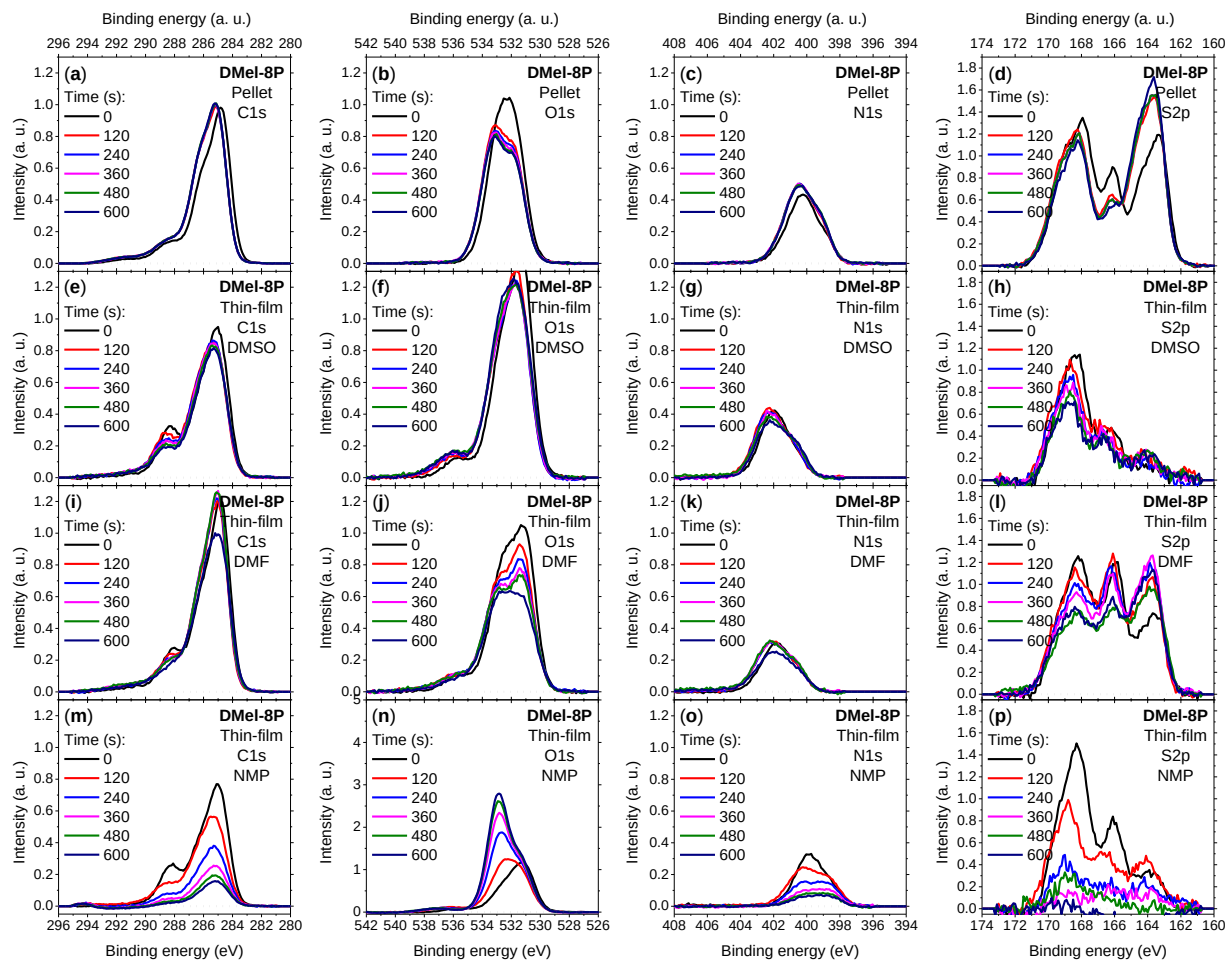


Figure A3. High-resolution (a, e, i, m) C1s, (b, f, j, n) O1s, (c, g, k, o) N1s, and (d, h, l, p) S2p XPS depth profiling spectra of SMel-8P pellet (first row) and thin-film (second-to-fourth rows).

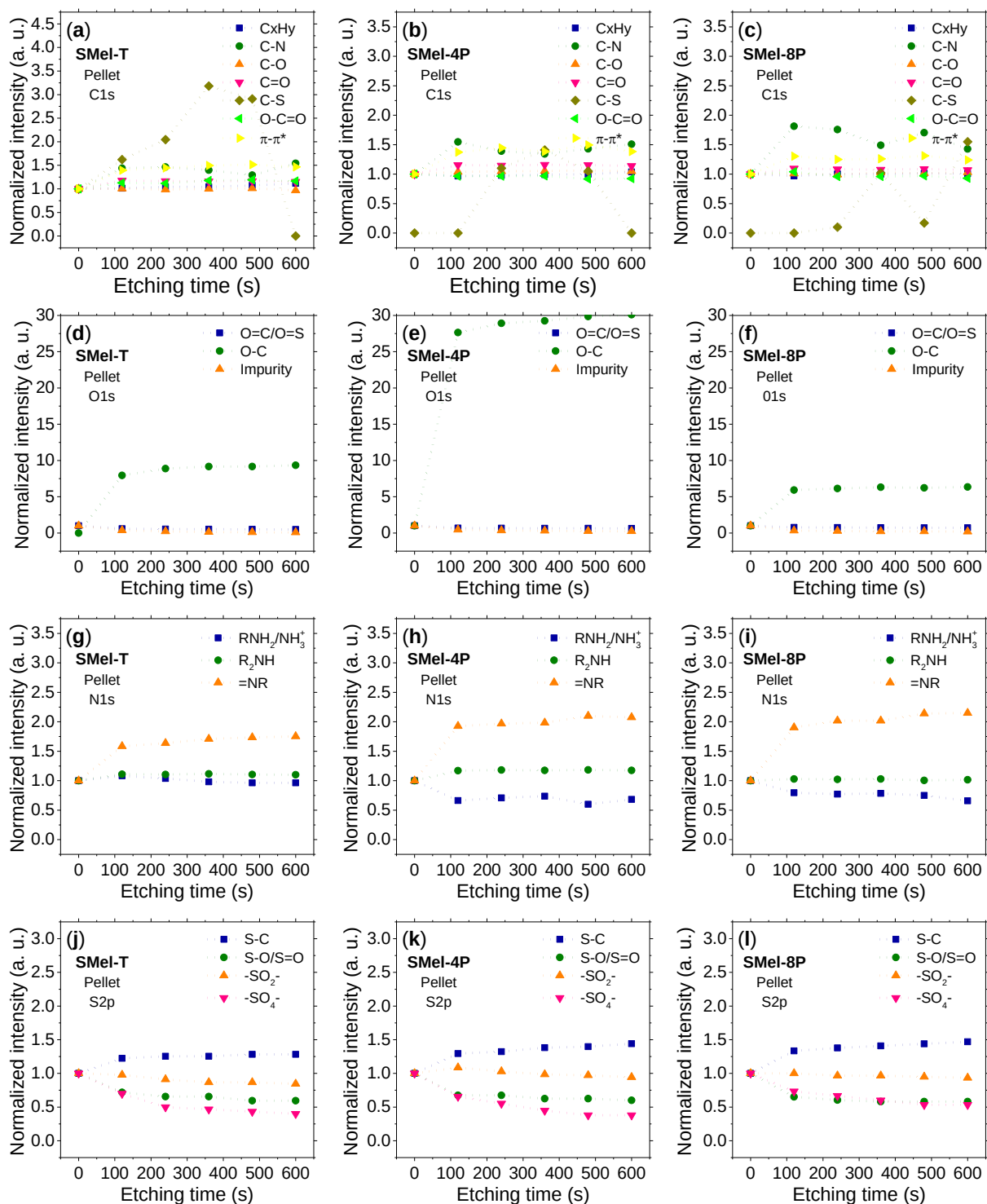


Figure A4. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel-T (left column), SMel-4P (middle column) and SMel-8P (right column) pellets.

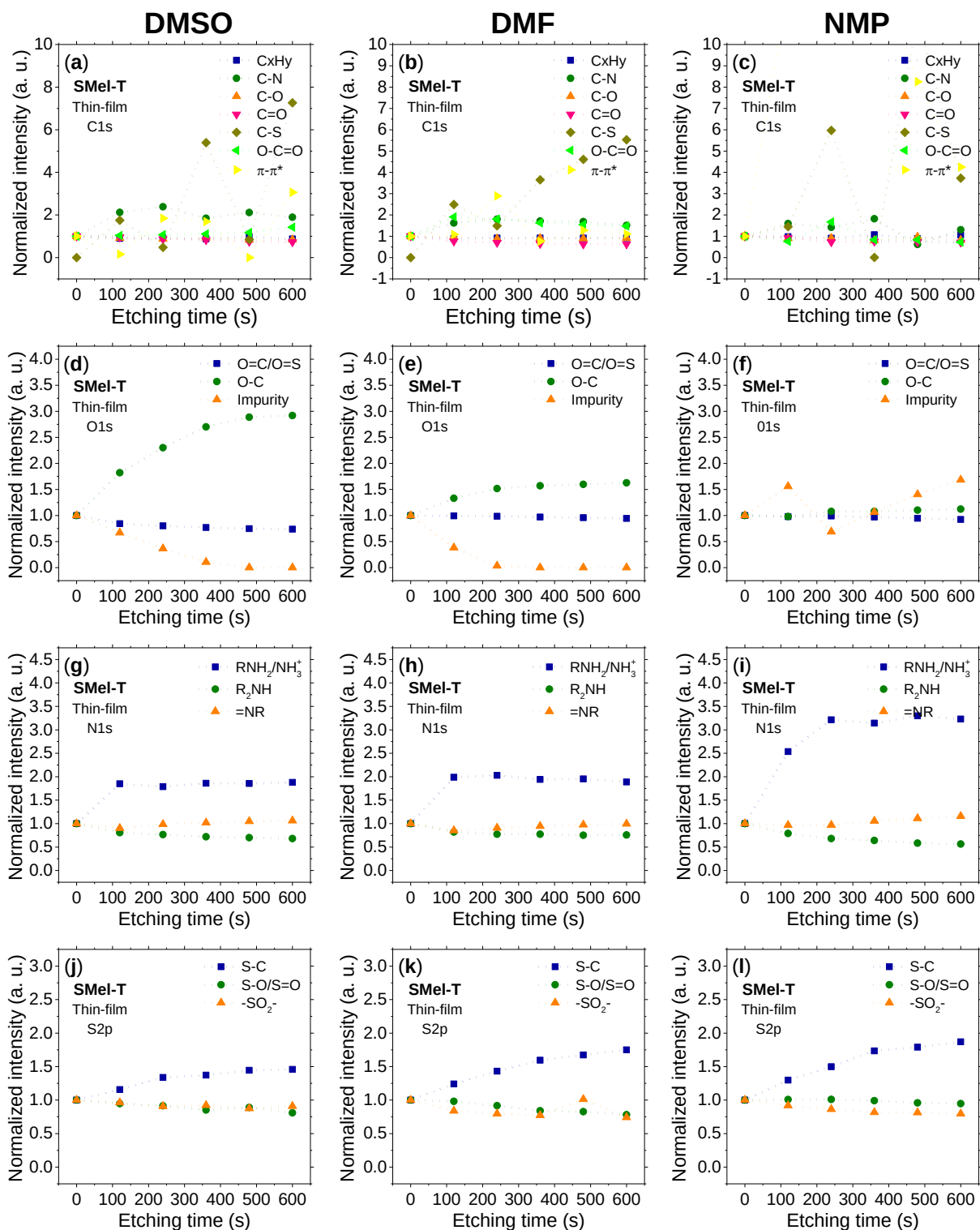


Figure A5. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel-T thin-film from DMSO (left column), DMF (middle column) and NMP (right column) solutions.

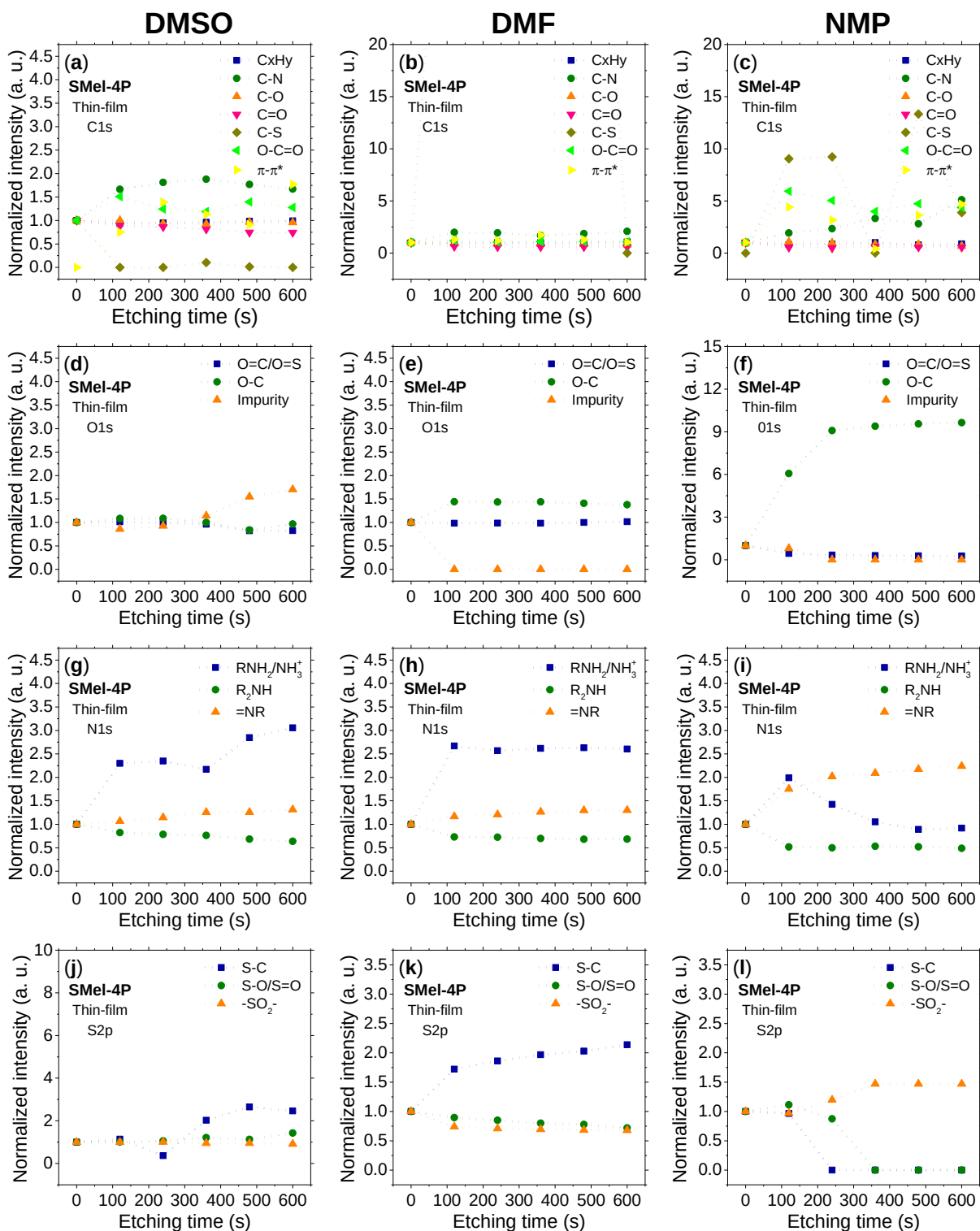


Figure A6. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel-4P thin-film from DMSO (left column), DMF (middle column) and NMP (right column) solutions.

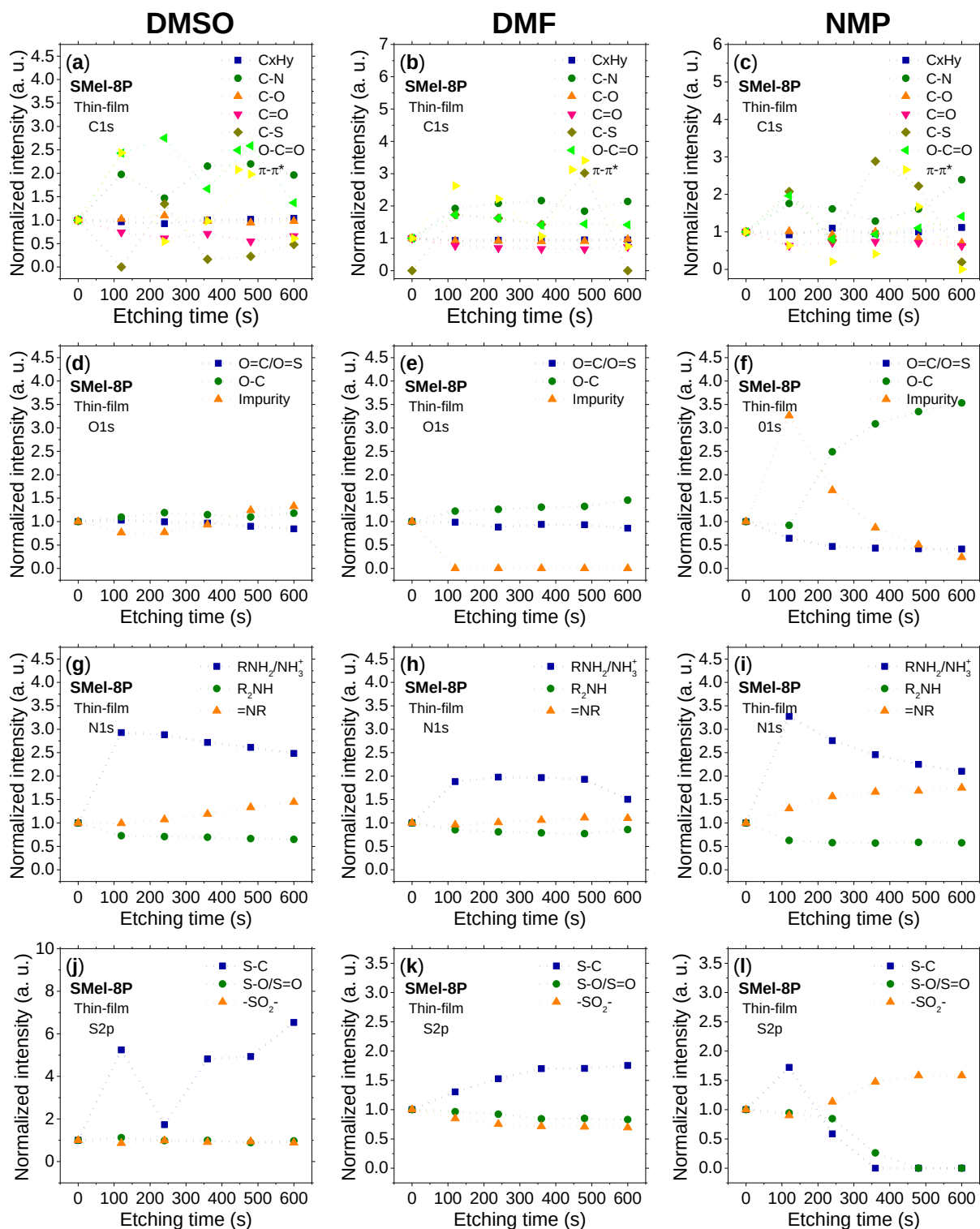


Figure A7. High-resolution (a-c) C1s, (d-f) O1s, (g-i) N1s, and (j-l) S2p XPS depth profiling of SMel-8P thin-film from DMSO (left column), DMF (middle column) and NMP (right column) solutions.

APPENDIX B

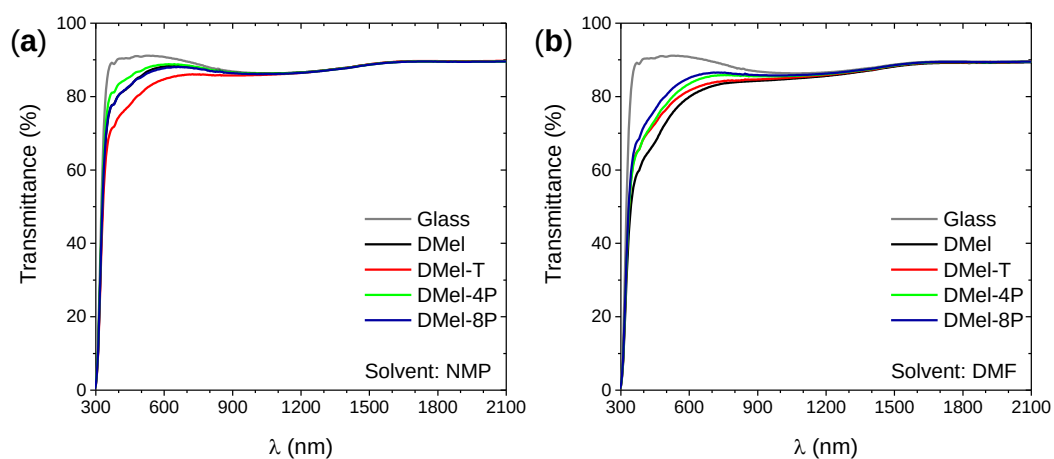


Figure B1. Transmittance spectra of S-Melanin in (a) NMP and (b) DMF solutions.

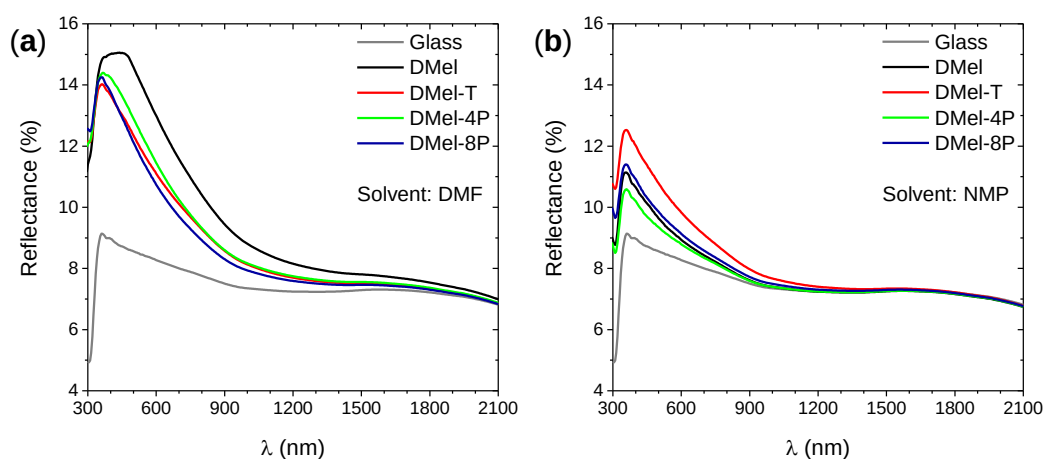


Figure B2. Reflectance spectra of S-Melanin in (a) DMF and (b) NMP solutions.

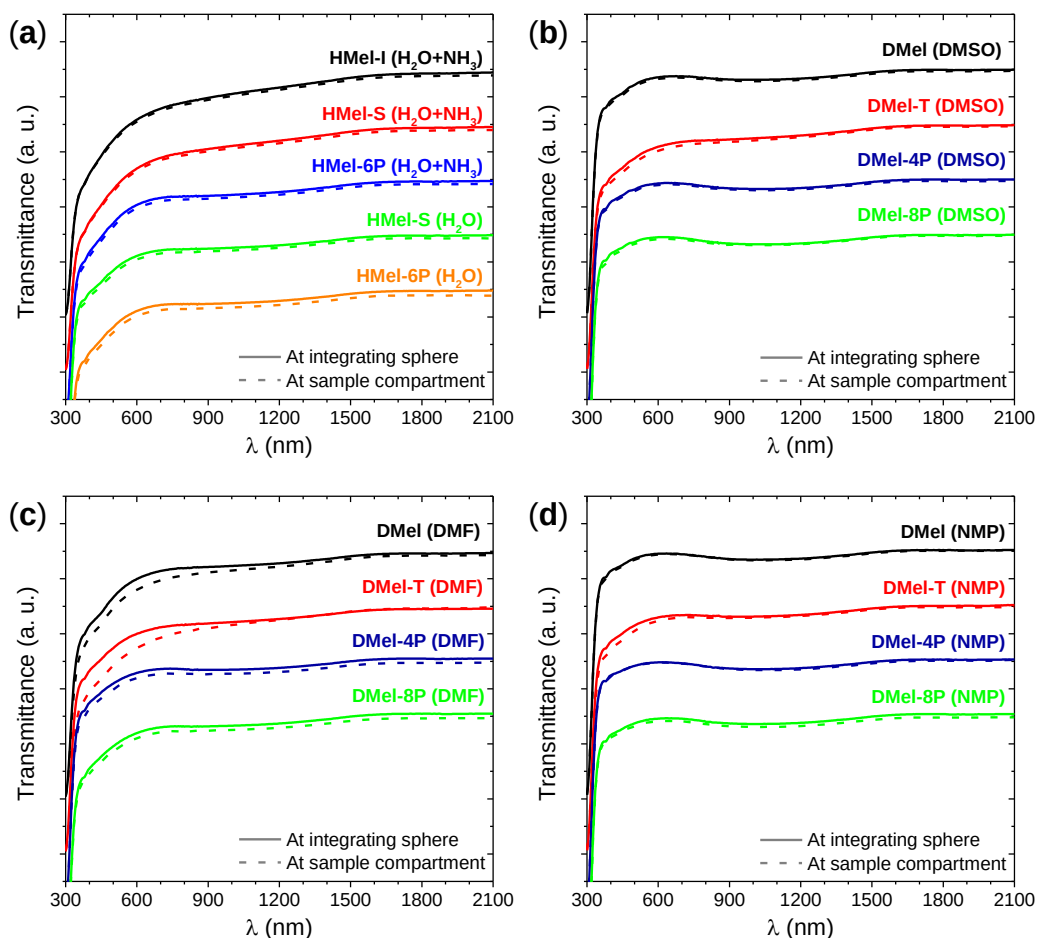


Figure B3. Transmittance spectra of melanin derivatives thin-films at different positions at the UV-Vis-NIR spectrophotometer. The continuous lines indicate the transmittance intensity at the integrating sphere, whereas the dashed lines indicate the transmittance intensity of the sample at about 30 cm from the integrating sphere. A high scatter material, would display a significant drop in its intensity. Hence, the small variation in the intensities indicates that the melanin thin-film scatter is neglectable.

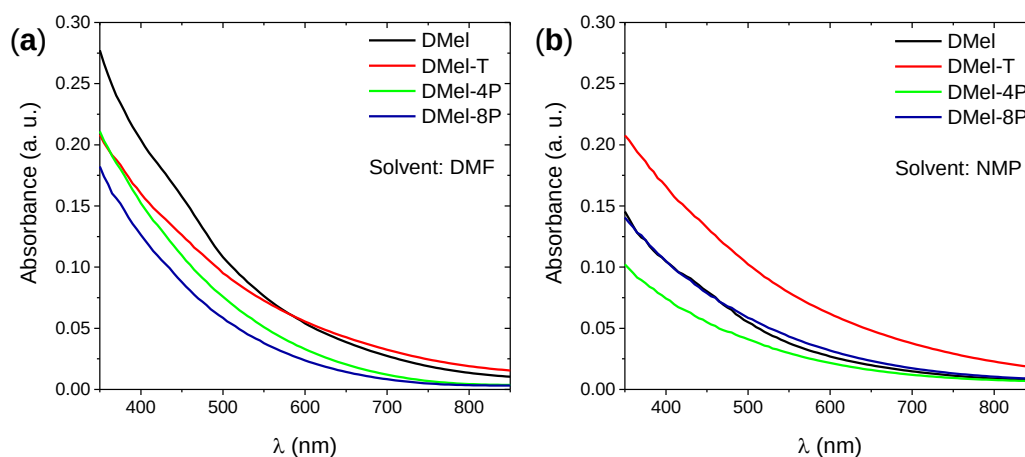


Figure B4. Absorbance spectra of S-Melanin in (a) DMF and (b) NMP solutions.

Table B1. Abbe's number for the different films.

	Abbe's number for the different films				
	H ₂ O + NH ₃	H ₂ O	DMSO	DMF	NMP
NFMel-I	17,78	#	#	#	#
NFMel-S	17,91	18,01	#	#	#
NFMel-6P	19,59	17,64	#	#	#
SMel	#	#	17,91	17,90	17,86
SMel-T	#	#	18,10	18,00	17,93
SMel-4P	#	#	17,80	17,77	17,88
SMel-8P	#	#	17,73	17,75	18,05

APPENDIX C

APENDIX C.1 – X-Band EPR supplementary material for Section 5.3.1 in the main-text

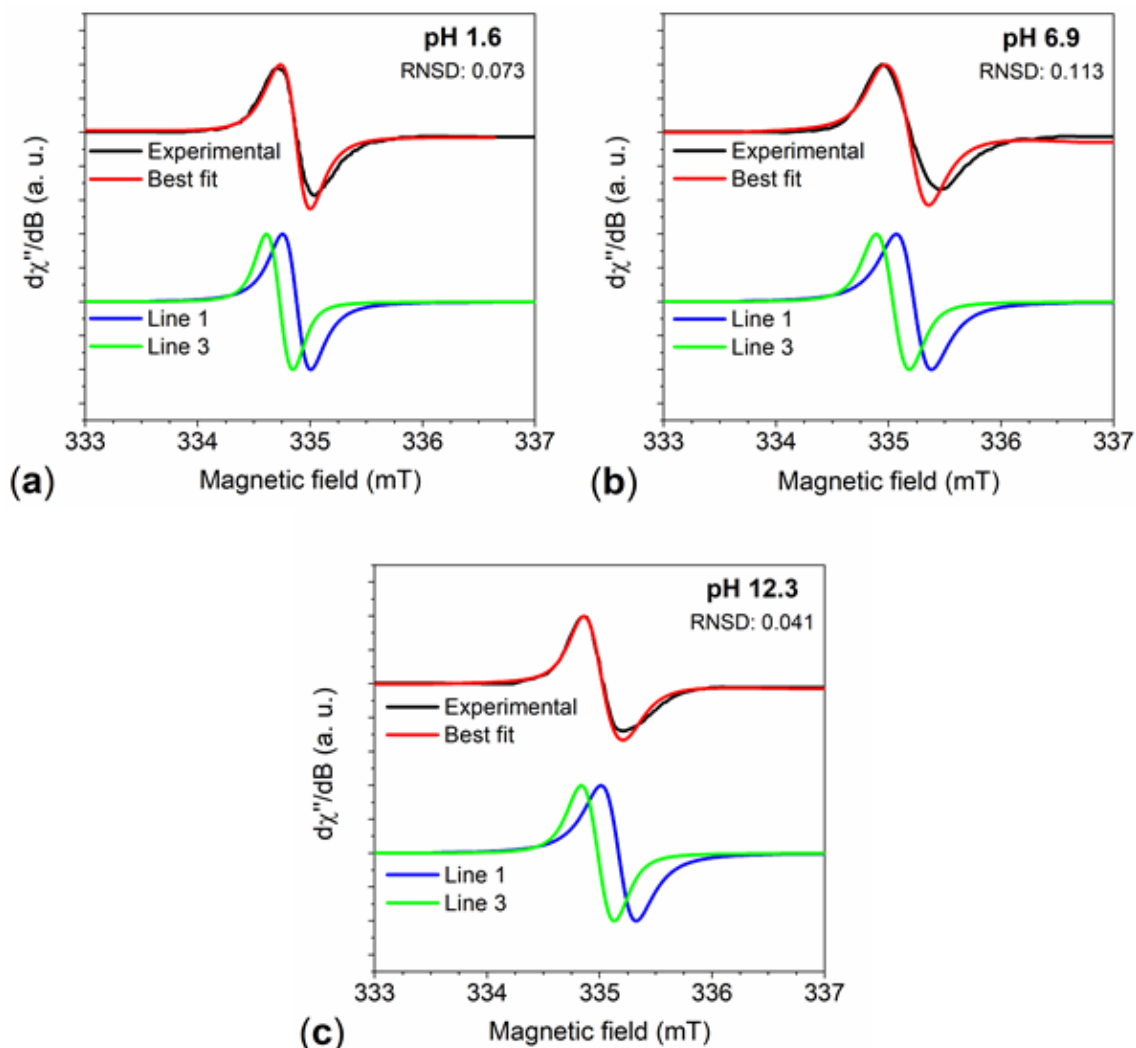


Figure C1. Melanin X-band EPR spectrum simulation at pH (a)1.6, (b) 6.9 and (c) 12.3 obtained when only two paramagnetic species are considered for fitting. Experimental data reprinted with permission from Chio *et al.* (CHIO; HYDE; SEALY, 1982). Copyright © 1982 Published by Elsevier Inc.

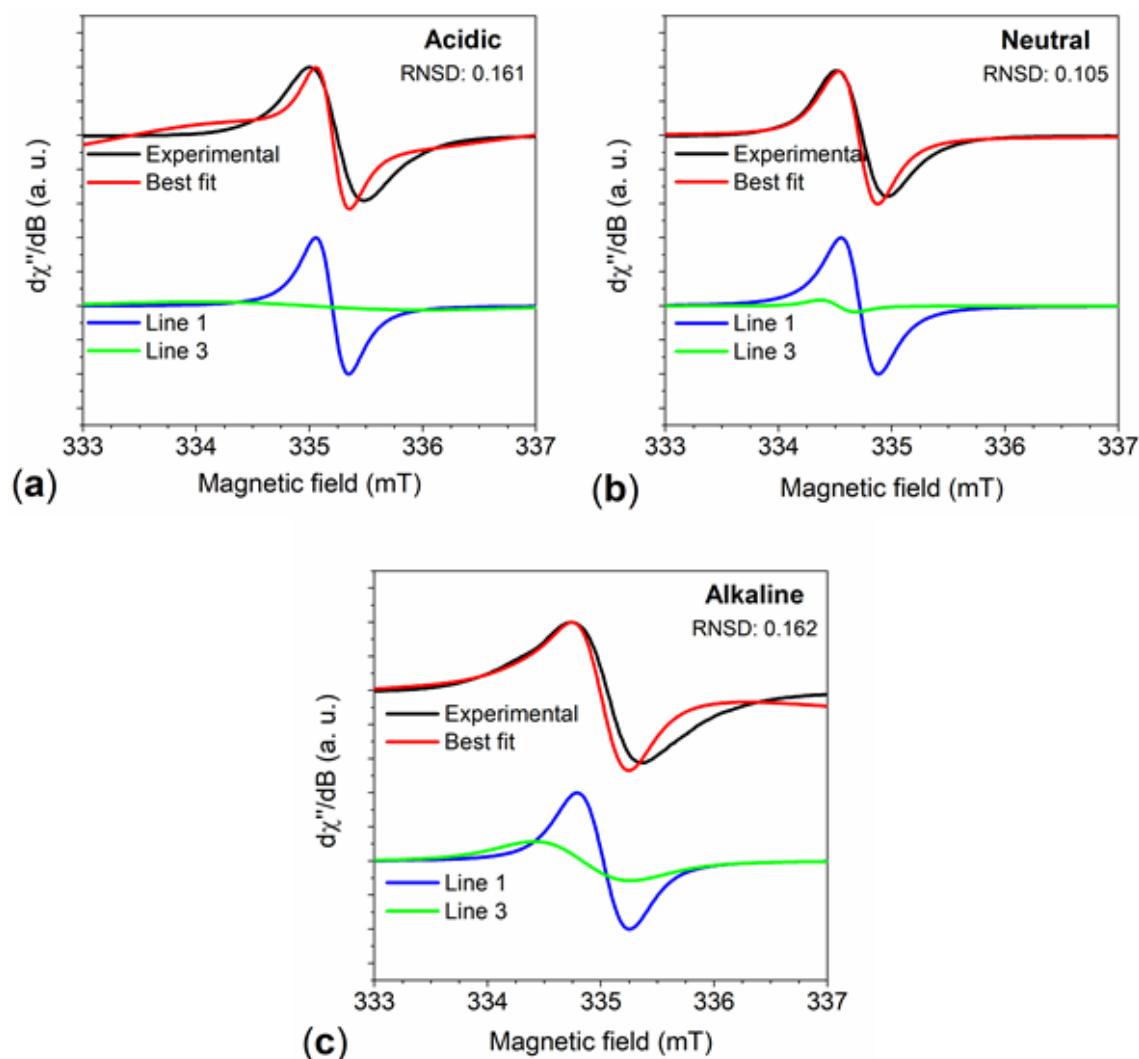


Figure C2. Melanin X-band EPR spectrum simulation from (a) acidic, (b) neutral and (c) basic solid-state samples when only two paramagnetic species are considered for fitting. Experimental data reprinted with permission from Mostert *et al.* (MOSTERT *et al.*, 2013). Copyright © 2013, American Chemical Society.

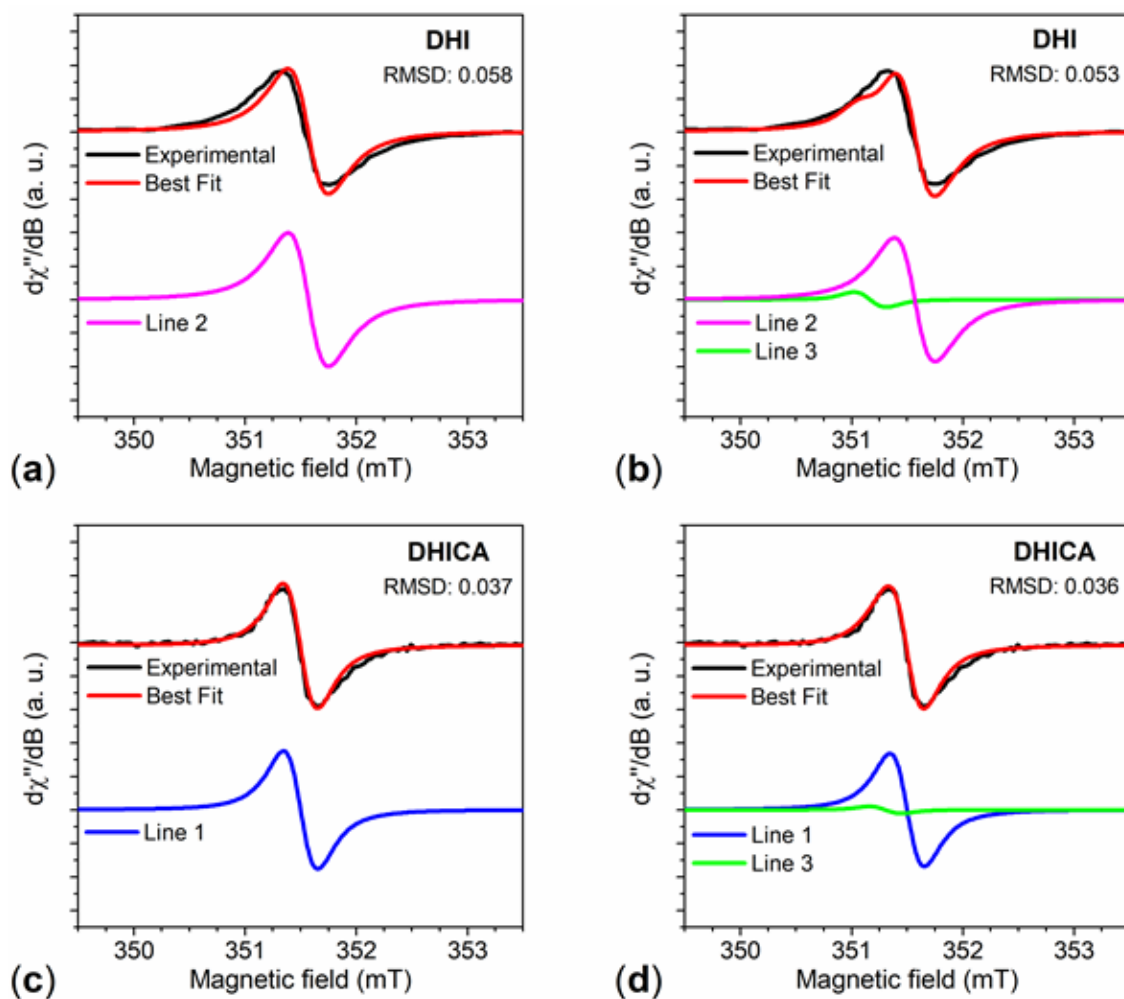


Figure C3. Melanin X-band EPR spectrum simulation from (a and b) DHI and (c and d) DHICA solid-state samples when only (a and c) one or (b and d) two paramagnetic species are considered for fitting. Experimental data reprinted with permission from Panzella *et al.* (PANZELLA *et al.*, 2013). Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

APENDIX C.2 – X-Band EPR supplementary material for Section 5.3.2 in the main-text

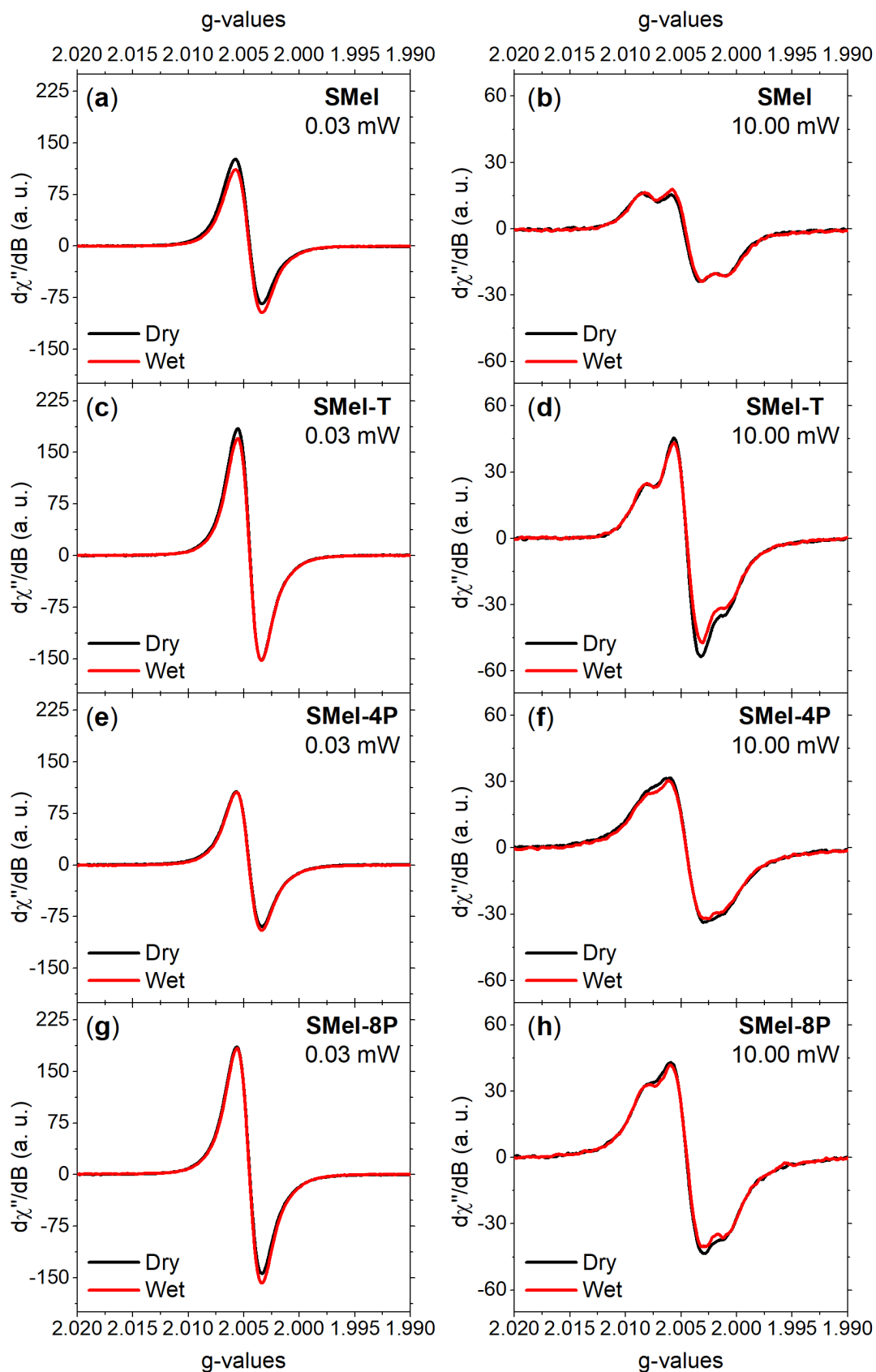


Figure C4. CW X-band EPR spectra for S-melanin under dry and wet hydration level at low (left column) and high (right column) microwave power.

Table C1. Variation in the spin concentration after the introduction of water in the system. The values present in this table were estimated from the double integration of the EPR signal present in Figure C4. However, note that such values could be a consequence of mathematical artifact coming from spectral variations in peak asymmetry and linewidth after the hydration (see Figure C5).

Melanin samples	Spin concentration changes with hydration (%)
SMel	- 20.3
SMel-T	+ 4.7
SMel-4P	+ 9.4
SMel-8P	- 4.7
NFMel-I	- 44.7
NFMel-S	- 37.7
NFMel-6P	- 32.2

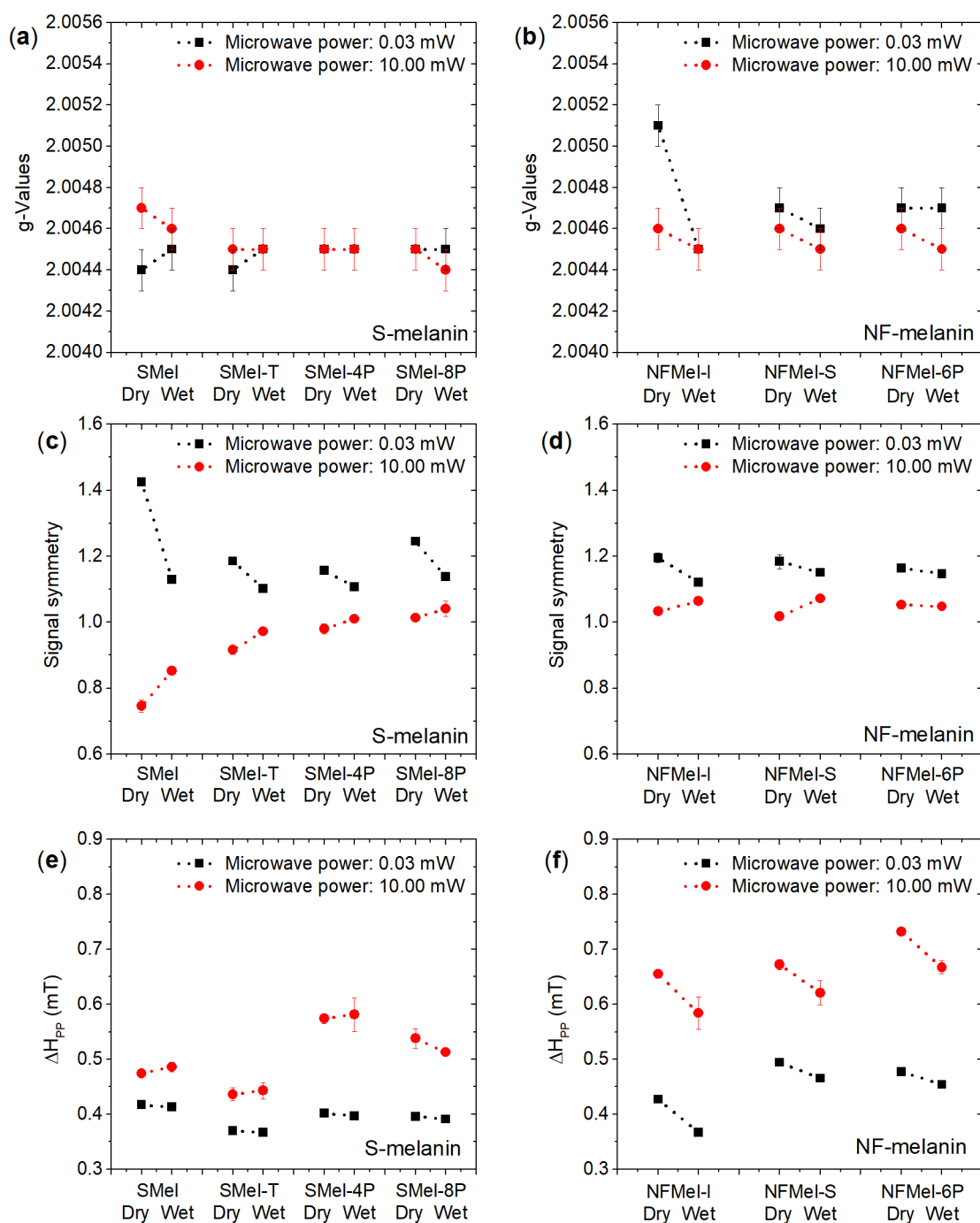


Figure C5. (a, b) g-Values calculated with the fundamental equation of EPR spectroscopy ($h\nu=g\mu B_0$). The deviations were obtained by choosing three different baselines from the positive and negative part of the spectra to get different zero position, that is, magnetic resonance fields (B_0). (c, d) EPR signal symmetry obtained from the ratio between positive and negative intensities. The closer the ratio is to 1, the more symmetrical the signal is. (e, f) The linewidth of the EPR signal. The uncertainty bars, when not shown, are smaller than the data point resolution.

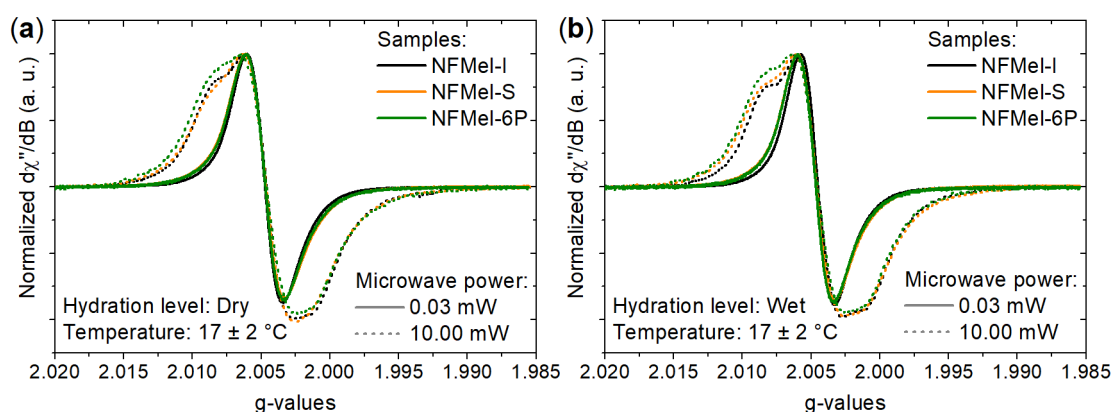


Figure C6. X-band EPR signal of NF-melanin.

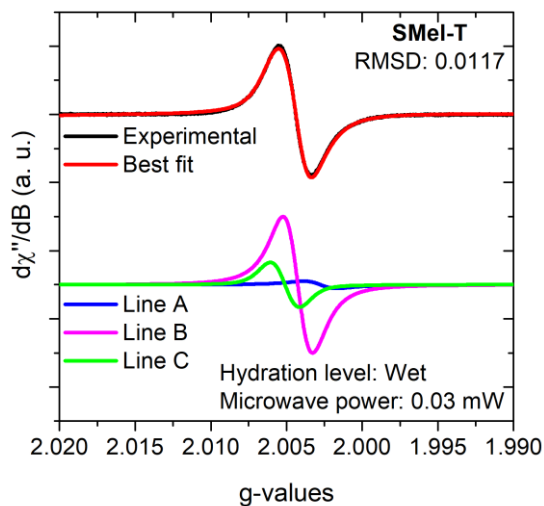


Figure C7. EPR simulation of SMel-T in wet condition (about 80 % humidity).

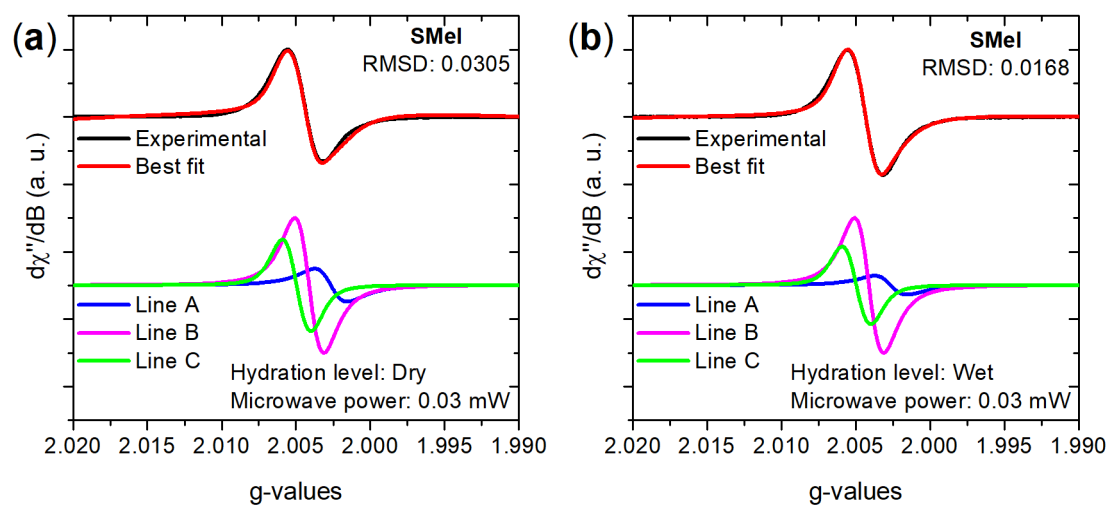


Figure C8. EPR simulation for SMel.

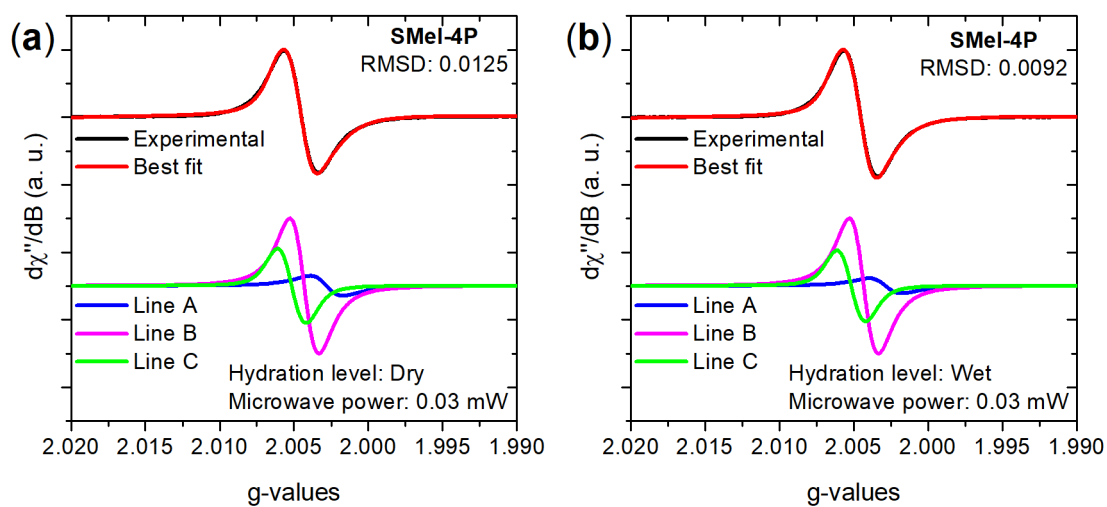


Figure C9. EPR simulation for SMel-4P.

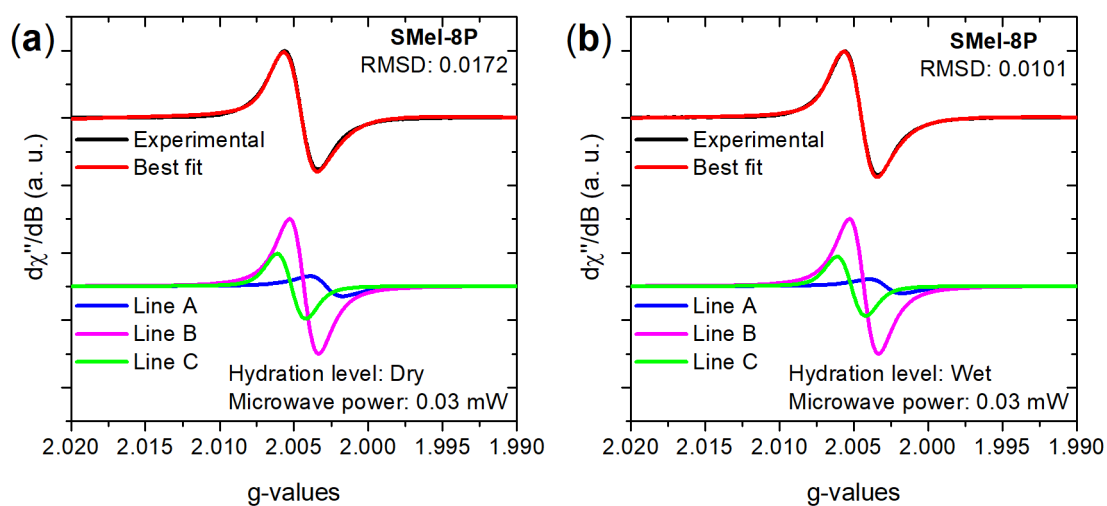


Figure C10. EPR simulation for SMel-8P.

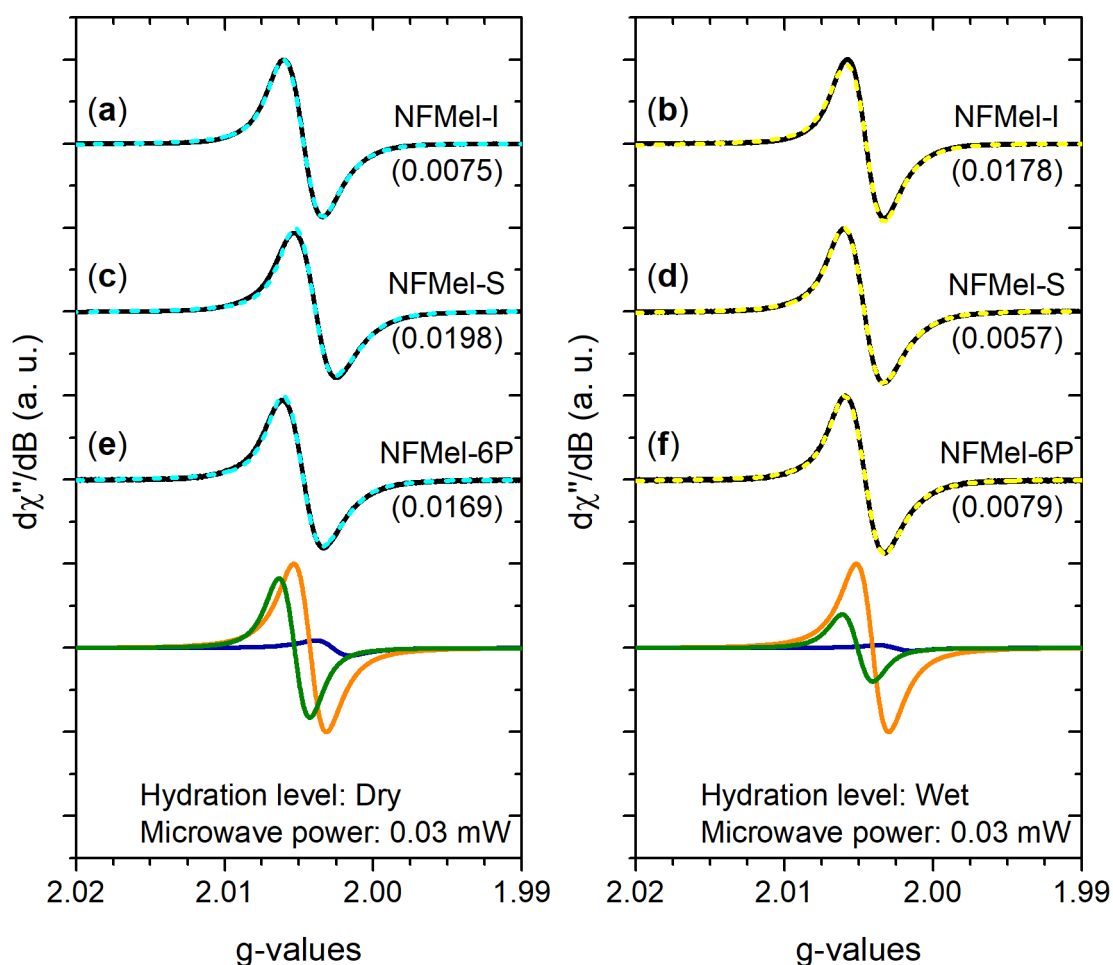


Figure C11. A rough estimation of the EPR simulation for NFMel-I (a, b), NFMel-S (c, d) and NFMel-6P (e, f) under dry (left) and wet (right) hydration level. The black lines represent the experimental spectra and cyan and yellow dashed lines the simulated one at dry and wet hydration level, respectively. Royal-orange-olive lines are the three components used in the fit. The simulated parameters related to g-value and linewidth are shown in Table C2, whereas the different intensities in Figure C12. The numbers between the brackets indicate the RMSD values obtained for each simulation.

Table C2. Spectral parameters of the simulated signals for all three NF-melanin samples at microwave power of 0.03 mW.

Lines	g-Values	ΔH_{PP} (mT)
NF-A	2.0027	0.457
NF-B	2.0043	0.468
NF-C	2.0053	0.438

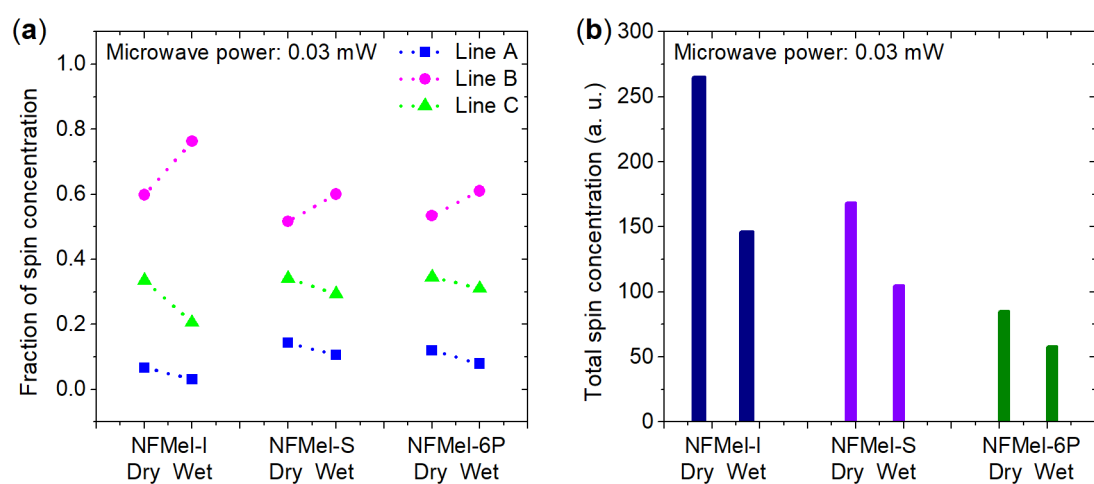


Figure C12. Estimation of the spin concentration of each component of the NF-melanin. The uncertainty bars are smaller than the data point resolution. The decrease in intensity of Line C is related to the diminished of the linewidth spectra after the addition of water (see Figure C5f and Figure C13). Such behaviour is unexpected and is not fully understood yet.

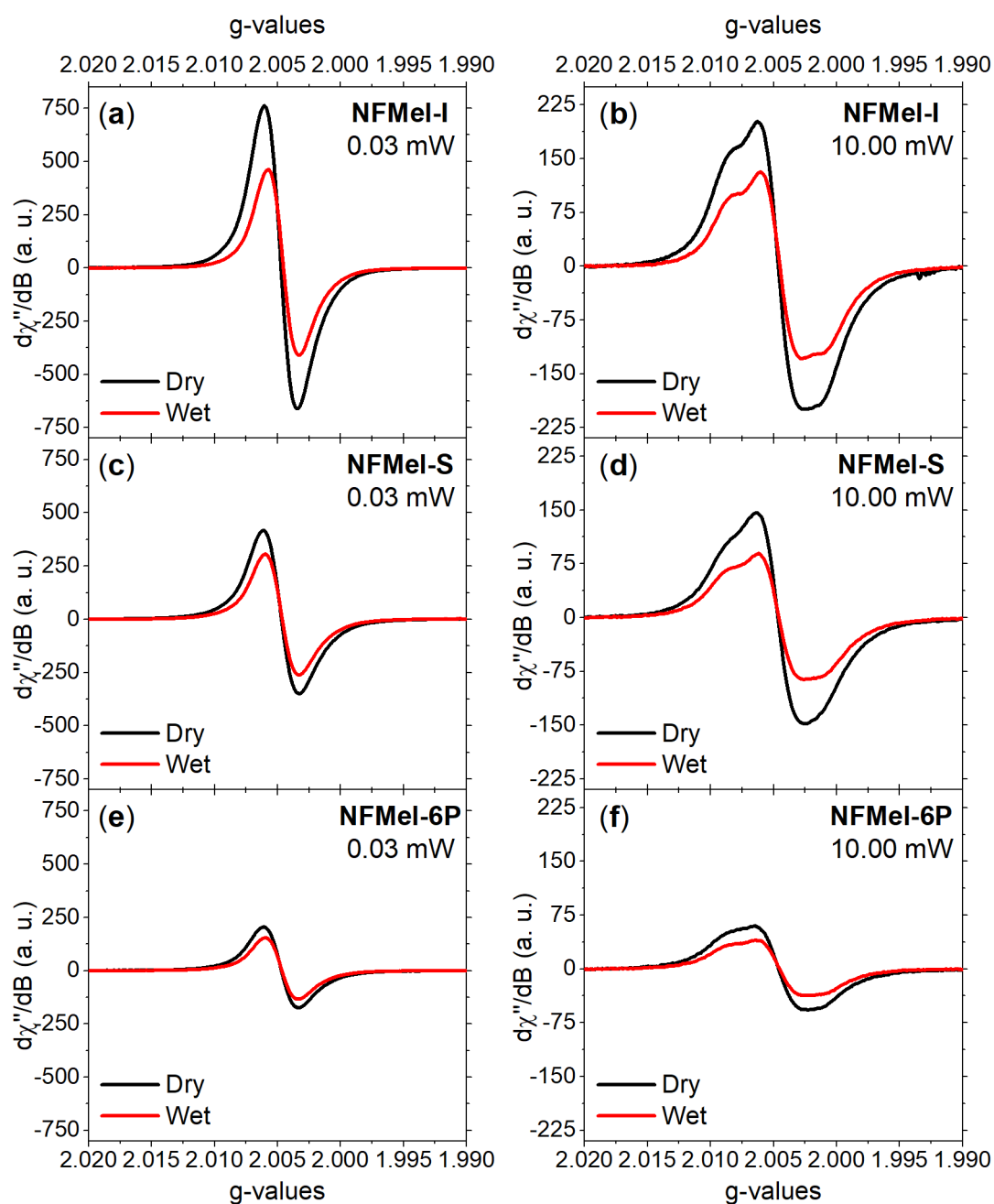


Figure C13. CW X-band EPR spectra for NF-melanin under dry and wet hydration level at low (left column) and high (right column) microwave power.

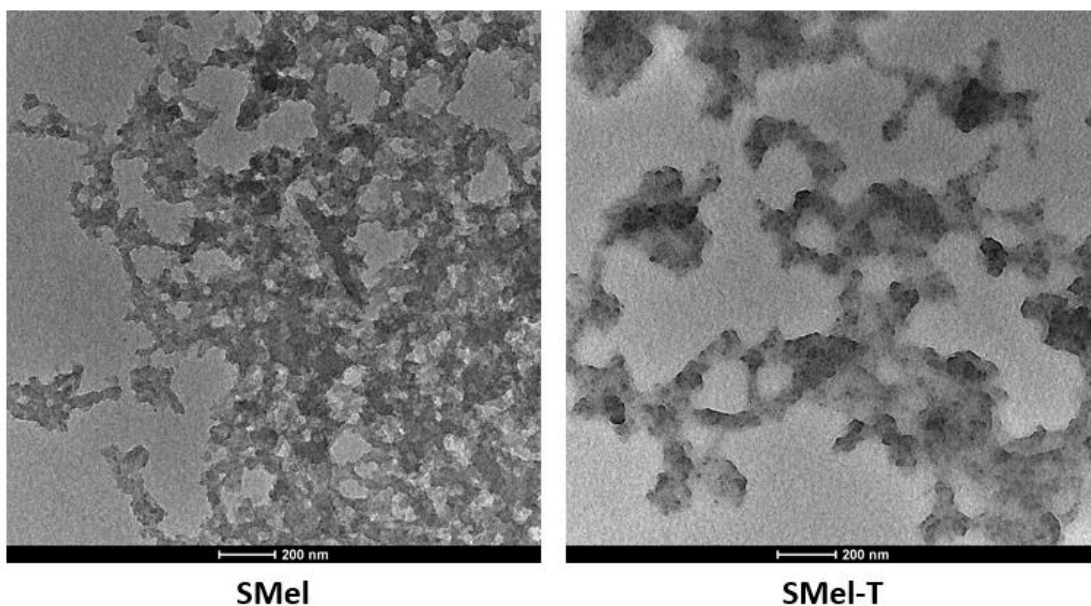


Figure C14. TEM images of SMel and SMel-T. The images were obtained with an FEI Tecnai G2 F20 X-Twin 200 kV microscope (LIEC, Federal University of São Carlos, Brazil).

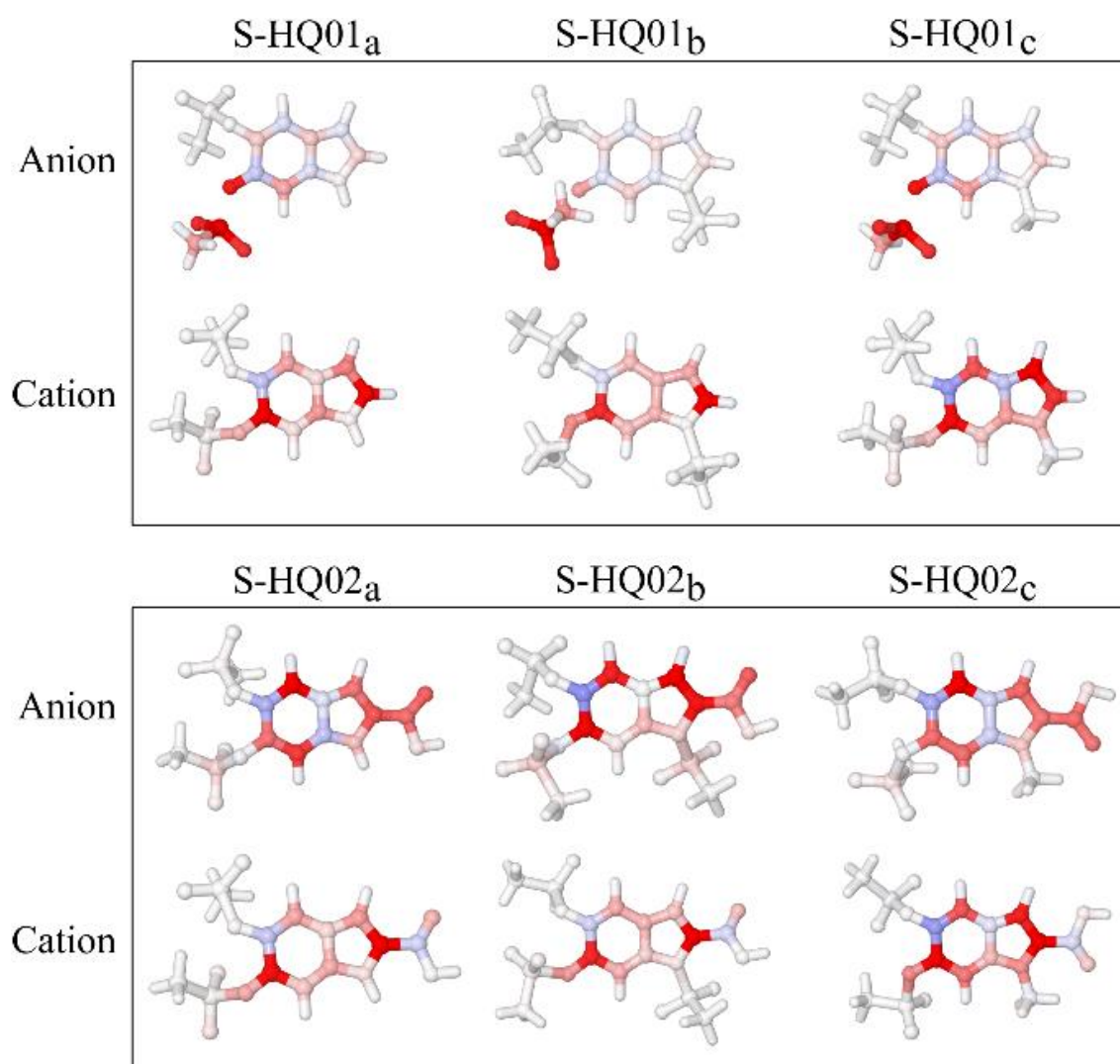


Figure C15. Representation of the spin population over the structure of S-HQ units.

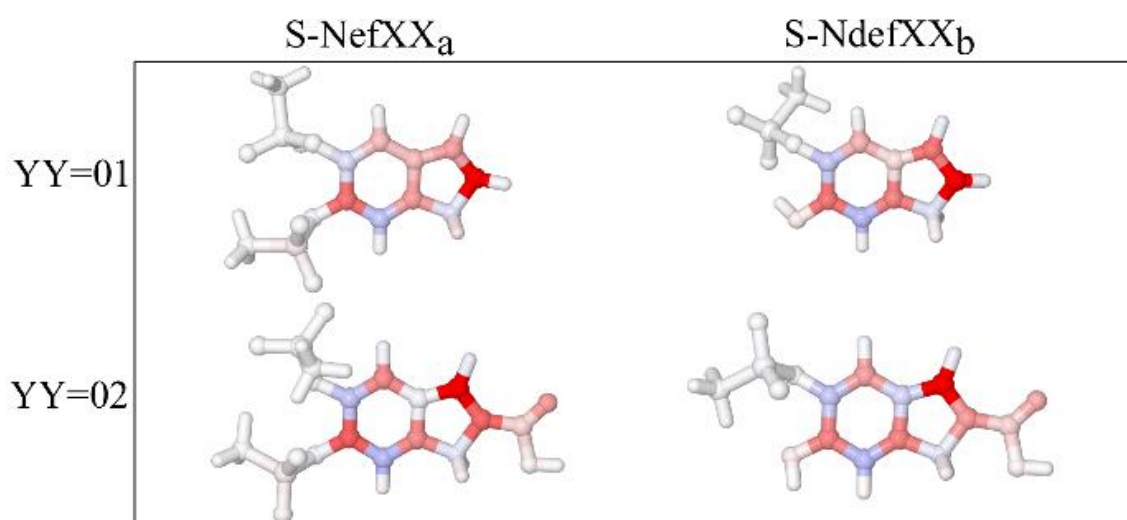


Figure C16. Representation of the spin population over the structure of S-Ndef units.

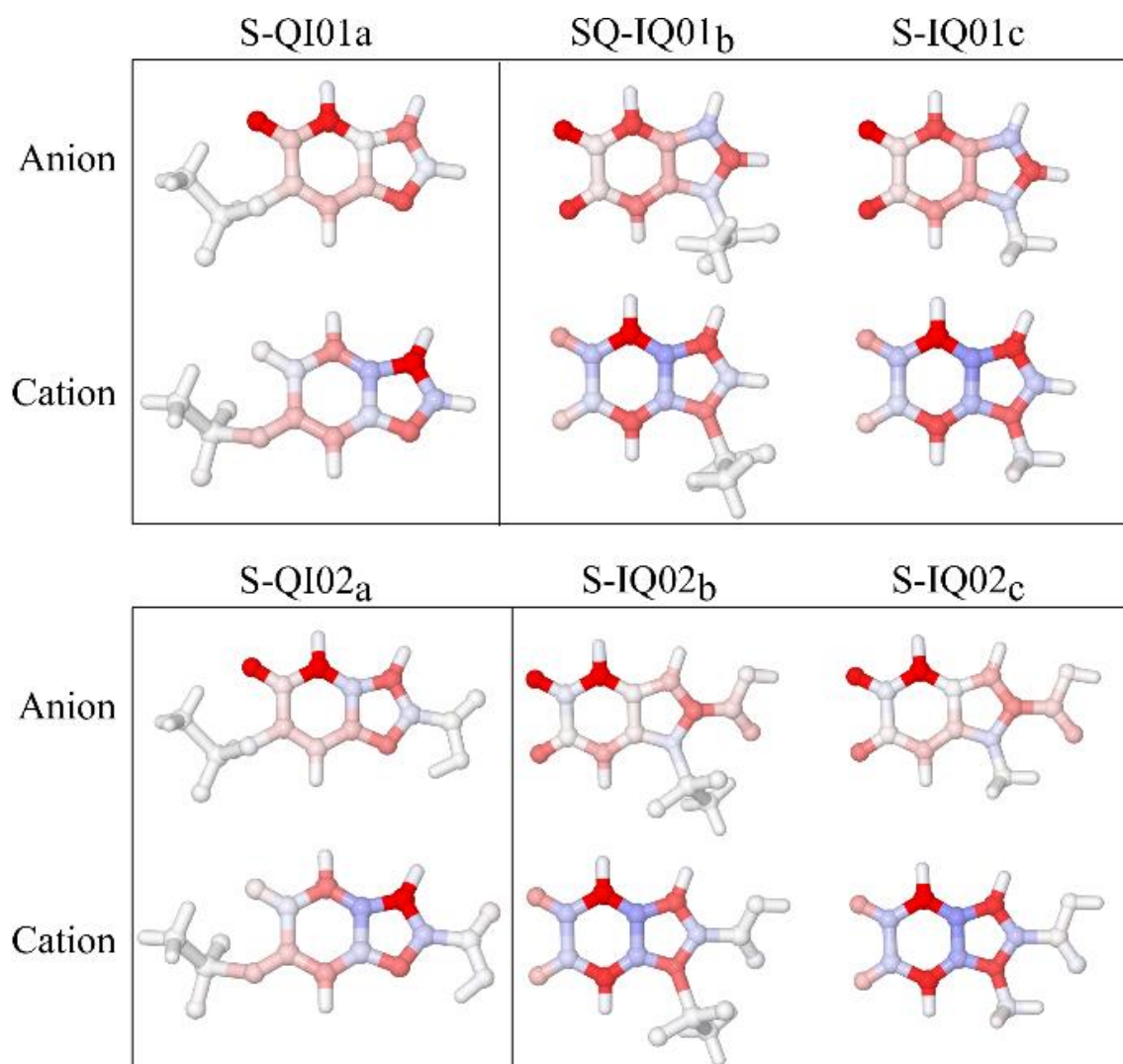


Figure C17. Representation of the spin population over the structure of S-QI and S-IQ units.

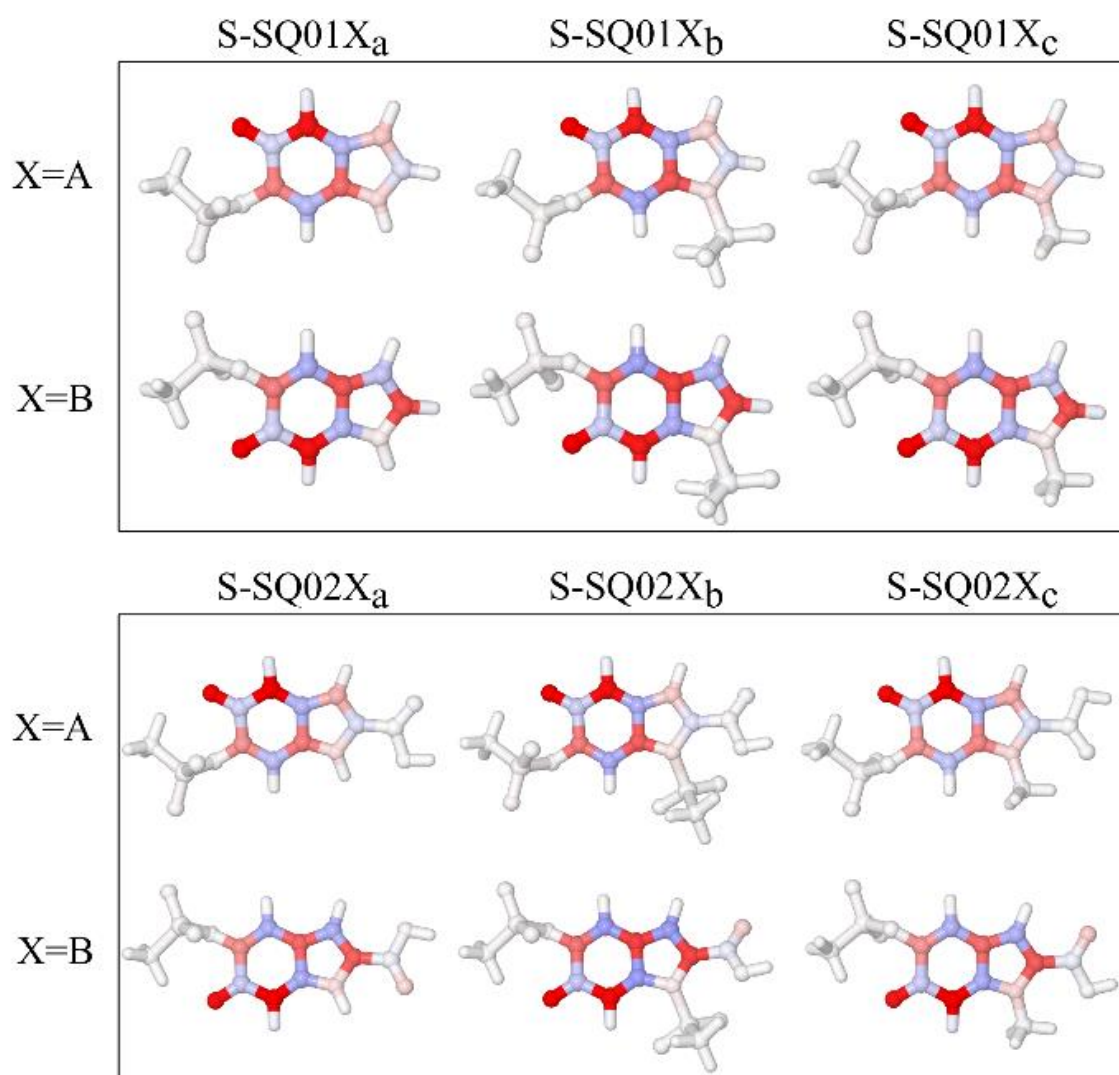


Figure C18. Representation of the spin population over the structure of S-SQ units.

APENDIX C.3 – HFEPR supplementary material for Section 5.3.3 in the main-text

Table C3. Experimentally Determined g-Values for NFMel-S with different resonant lines.

Resonant Line	g_{xx}	g_{yy}	g_{zz}	g_{iso}
A-I	2.0038	2.0033	2.0023	2.0031
B-I	2.0043	2.0030	2.0023	2.0032
B-II	2.0037	2.0032	2.0022	2.0030
C-I	2.0050	2.0031	2.0023	2.0035
C-II	2.0038	2.0033	2.0023	2.0031
C-III	2.0038	2.0033	2.0022	2.0030

Table C4. Estimated spin concentration for one and two RL obtained from fitting.

RL	Microwave power (mW)	NFMel-S	NFMel-6P	SMel
One	0.1	1	1	1
	10	1	1	1
Two	0.1	0.37 / 0.63 [§]	0.09 / 0.91 [§]	0.36 / 0.64 [§]
	10	0.59 / 0.41 [§]	0.16 / 0.84 [§]	0.65 / 0.35 [§]

[§] x / y refers to specie A and specie B, respectively.

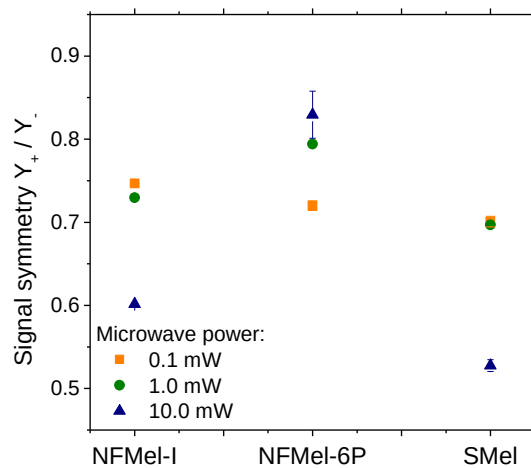


Figure C19. HFEPR signal symmetry of NFMel-S, NFMel-6P, SMel. The closer ΔY_{pp} is to 1, more symmetrical the signal is.

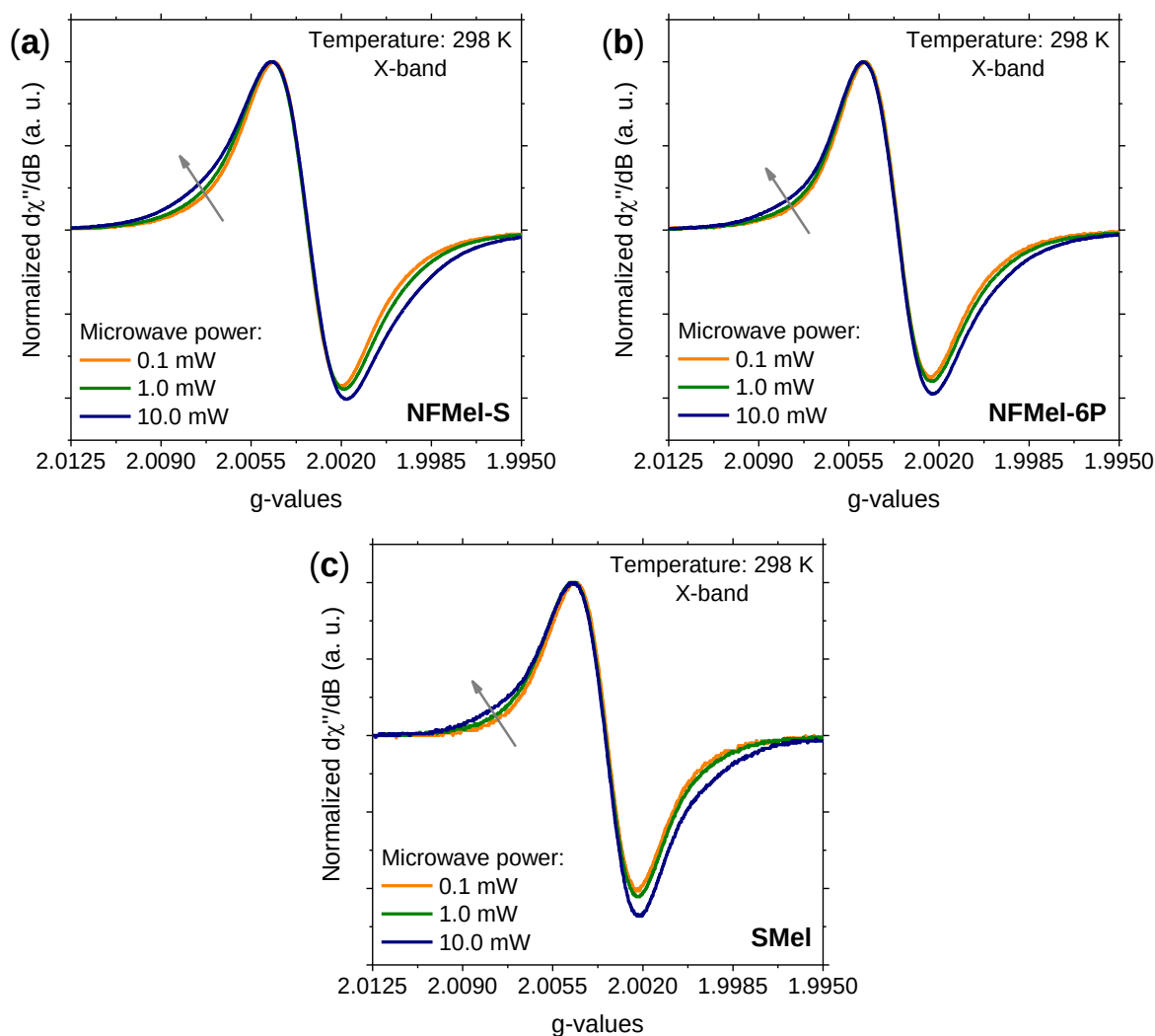


Figure C20. X-band EPR absorption signal of (a) NFMel-S, (b) NFMel-6P and (c) SMel at different microwave powers. Gray arrow indicates the shoulder-like feature increases. The measurements of Figure C20 were performed in solid-state using an X-band spectrometer MiniScope MS300 (Magnetech) coupled with an Agilent Frequency Counter 53181A RF. For g calibration, it was used 2,2-diphenyl-1-picrylhydrazyl (DPPH, $g = 2.0036$).

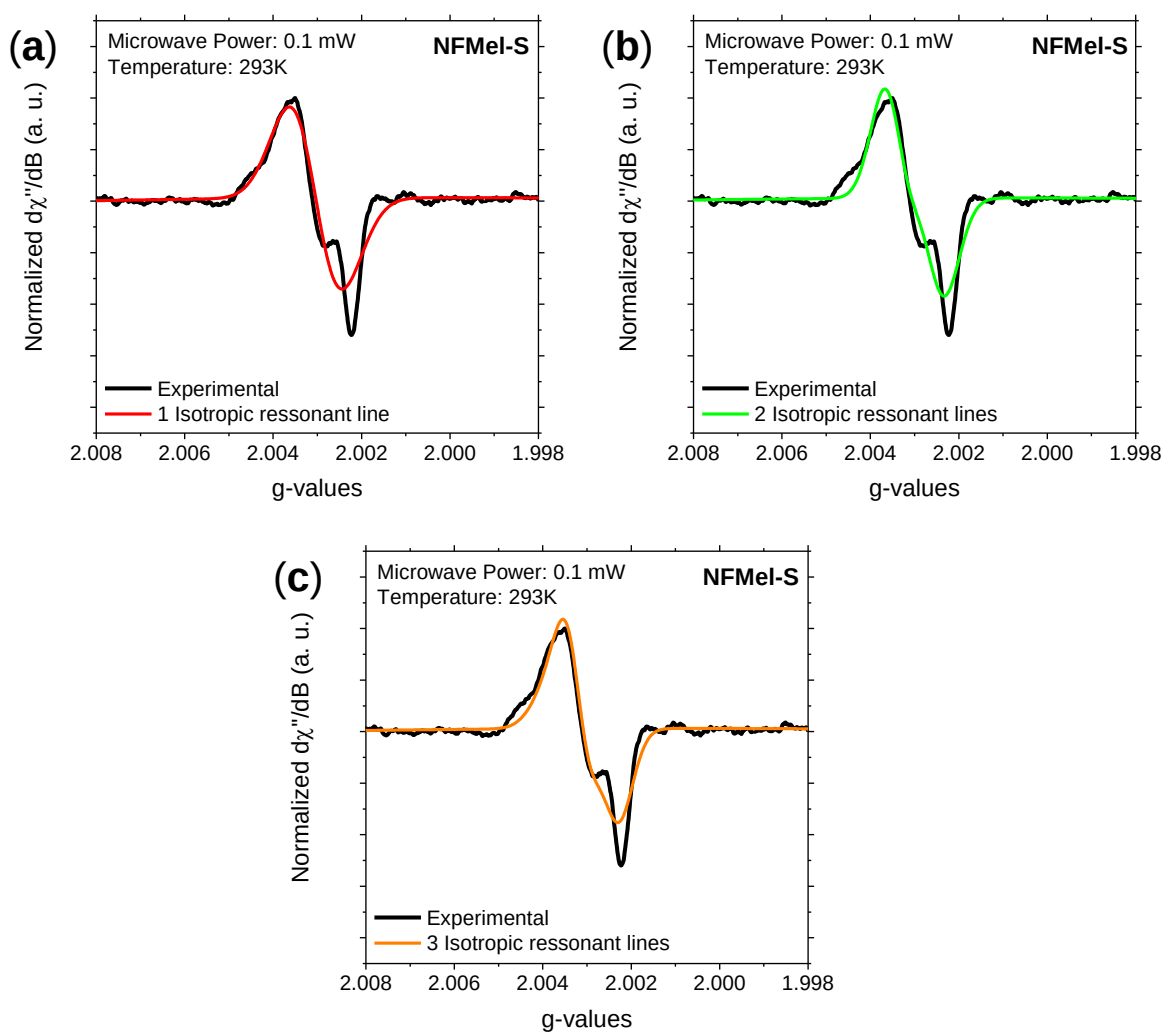


Figure C21. HFEPR absorption signal simulation of NFMel-S with different isotropic resonant lines at low microwave power (0.1 mW).

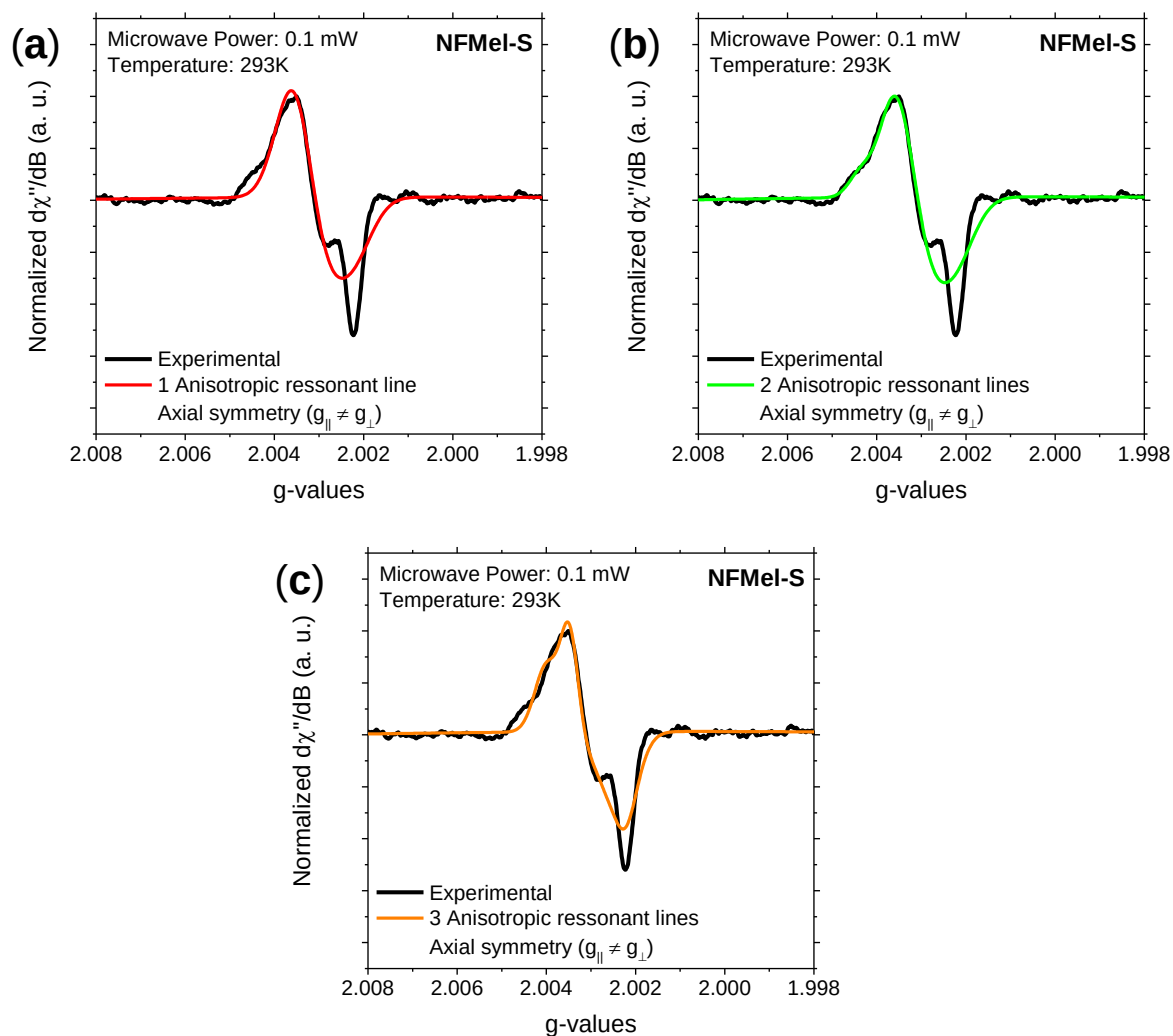


Figure C22. HFEPR absorption signal simulation of NFMel-S with different anisotropic resonant lines and axial symmetry at low microwave power (0.1 mW).

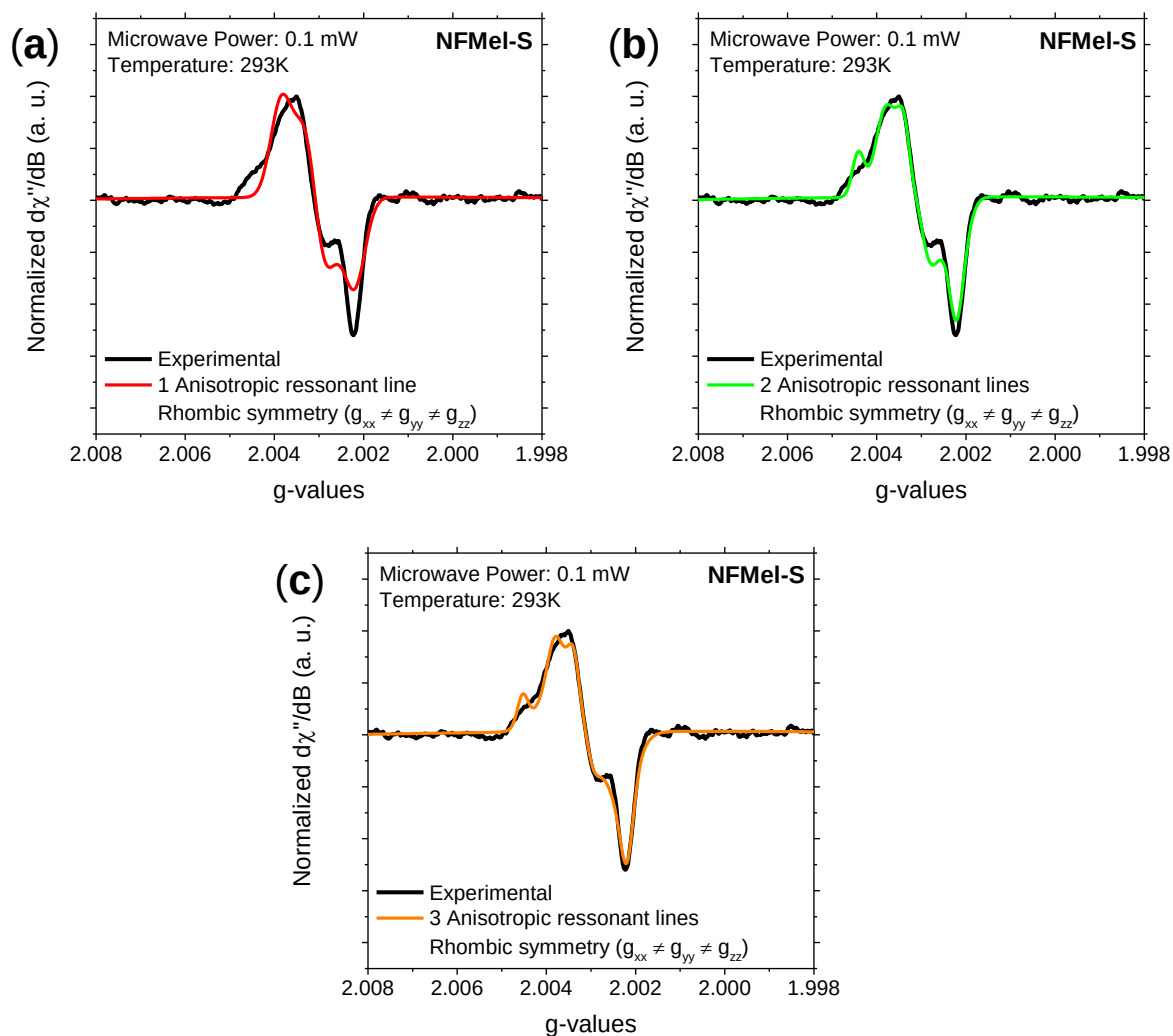


Figure C23. HFEPR absorption signal simulation of NFMel-S with different anisotropic resonant lines and rhombic symmetry (without g strain) at low microwave power (0.1 mW).

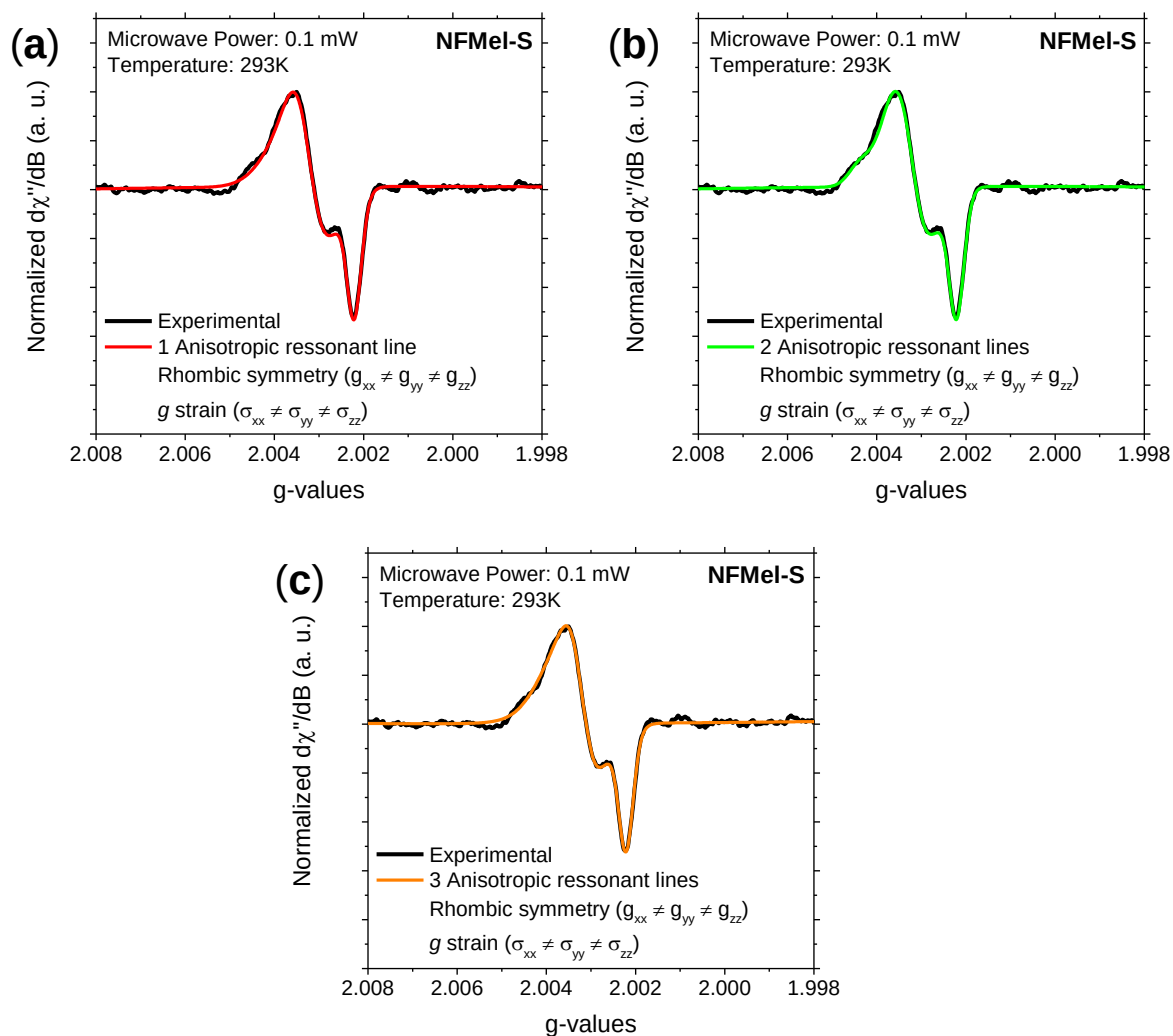


Figure C24. HFEPR absorption signal simulation of NFMel-S with different anisotropic resonant lines, rhombic symmetry and g strain at low microwave power (0.1 mW).

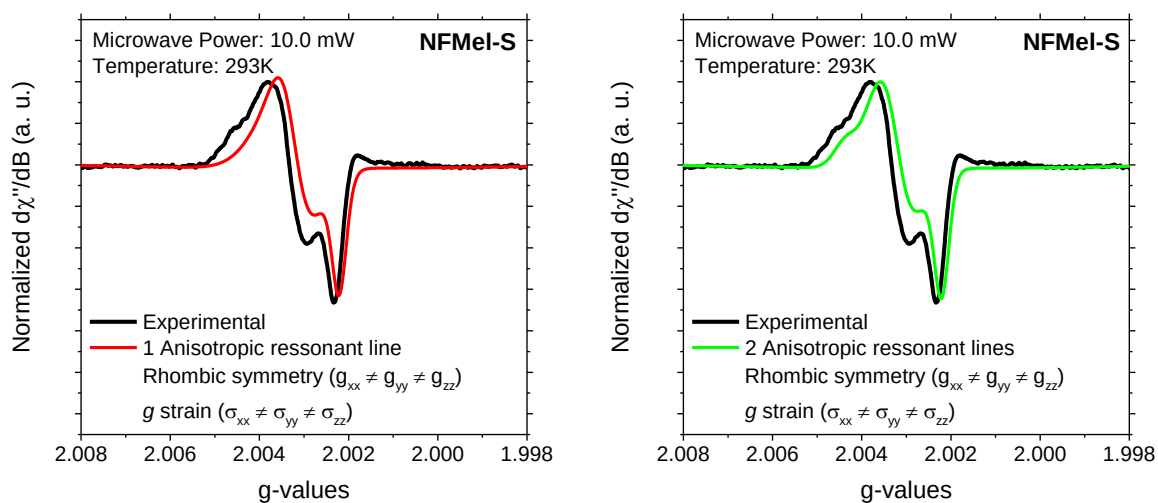


Figure C25. HFEPR absorption signal simulation of NFMel-S with different anisotropic resonant lines, rhombic symmetry and g strain at high microwave power (10.0 mW).

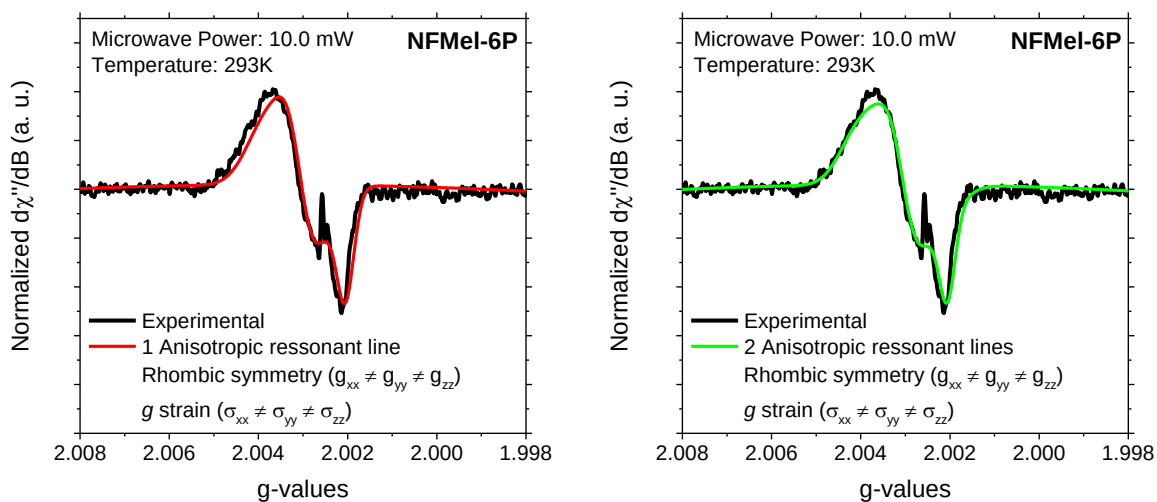


Figure C26. HFEPR absorption signal simulation of NFMel-6P with different anisotropic resonant lines, rhombic symmetry and g strain at high microwave power (10.0 mW).

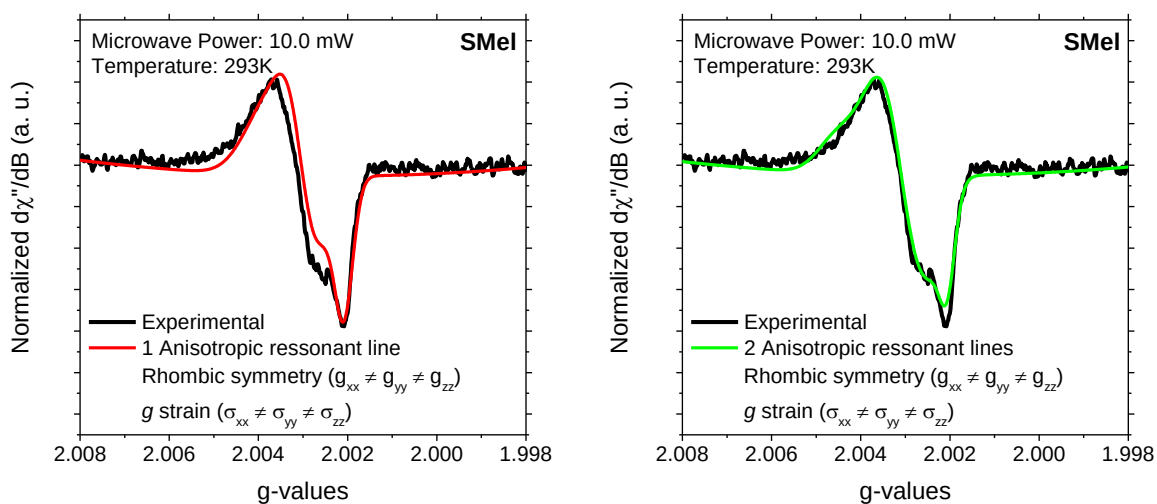


Figure C27. HFEPR absorption signal simulation of SMel with different anisotropic resonant lines, rhombic symmetry and g strain at high microwave power (10.0 mW).

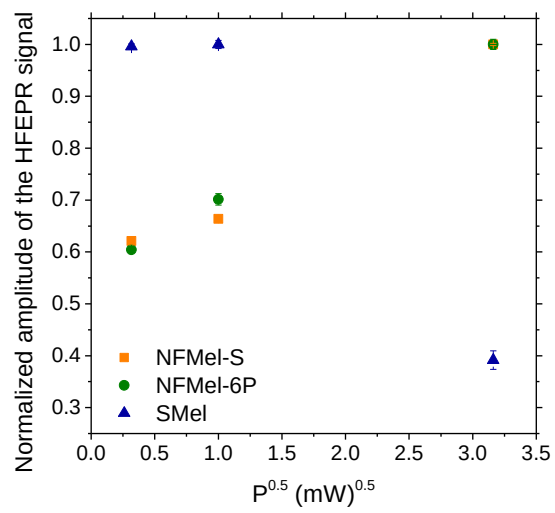


Figure C28. Normalized amplitude as function of the square root of the power intensity for NFMel-S, NFMel-6P and SMel.

APENDIX C.4 – Phase correction procedure for HFEP spectra

The phase correction procedure was based on Earle, 1993:

“The phase correction was accomplished by taking a linear combination of the measured signal and a calculated quadrature signal (generated from the Hilbert transform of the measured signal) and adjusting the admixture of both components across the spectrum until the integrated combination spectrum displayed a flat, zero base line.” (K. A. Earle, et al. J. Phys. Chem, 1993,13289-13297, page 13290.)

Initially, we create a hypothetical and arbitrary absorption spectrum from $A = \exp \left[-\frac{(1-x)^2}{0.005^2} \right]$, Figure C4-1a. The Hilbert transform of the absorption A spectrum was used to obtain the dispersion signal D . As mentioned in section 2.2.5, we usually measure the first derivative of the absorption. Hence, Figure C4-1b presents the first derivative of the absorption (A') and the first derivative of the dispersion (D').

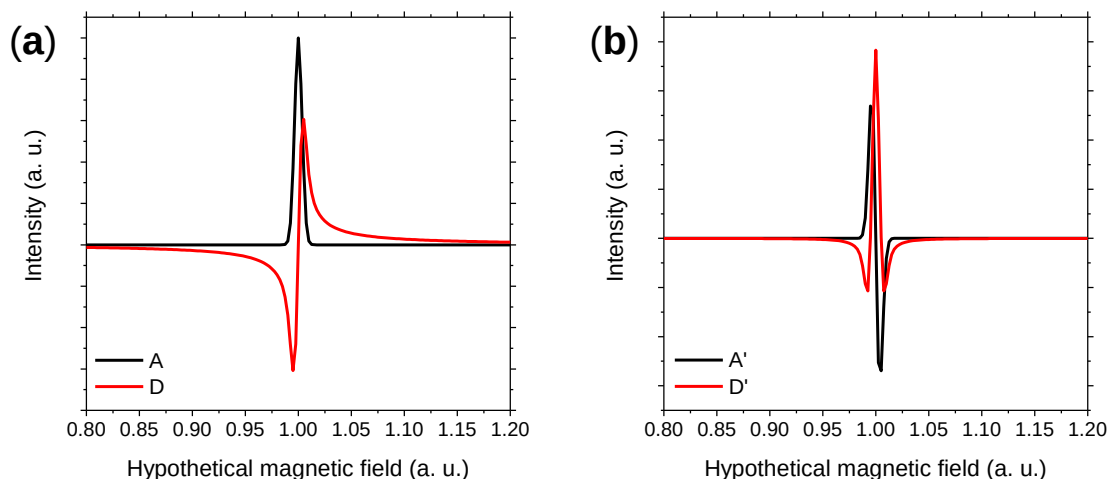


Figure C4-1. (a) Hypothetical absorption A and dispersion D spectra. (b) The first derivative of the signals in (a).

Figure C4-1 shows the *pure* spectra. In our experimental case, there is the possibility that the spectra we got from the HFEP spectrometer are a mixture of both absorption and dispersion. Therefore, we create a hypothetical experimental spectrum S by taking a linear of A' and D' (C4-1). Additionally, the Hilbert transform of S (named H) is given by (C4-2).

$$S = A' + \alpha D' \quad (\text{C4-1})$$

$$H = D' + \alpha A' \quad (\text{C4-2})$$

Figure (C4-2) shows the resulted spectra for S and H considering $\alpha = 0.5$.

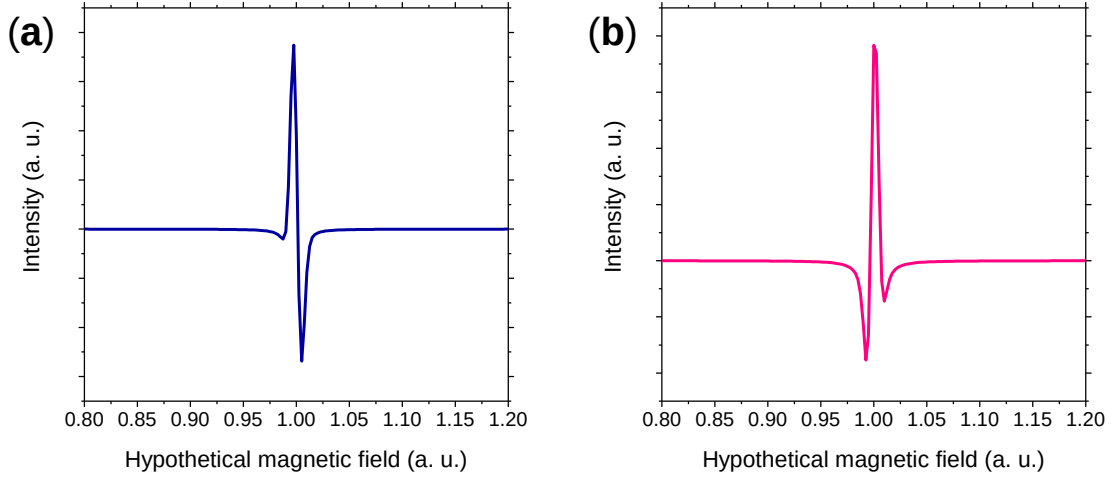


Figure C4-2. (a) Hypothetical experimental spectra S, with $\alpha = 0.5$ with its (b) Hilbert transform H.

As we intend to do a phase correction to obtain a pure absorption (or its first derivative) of the experimental spectra, we need to discover α . Following the proposed methodology of Earle (1993), we need to take a linear combination of the S and H. In this case, we got:

$$\begin{aligned} C &= S + \beta H \\ &\quad \downarrow \\ C &= (1 - \alpha \beta) A' + (\alpha + \beta) D' \end{aligned} \quad (\text{C4-3})$$

If,

$$\alpha \beta = 1 \Leftrightarrow \beta = \frac{1}{\alpha} \quad (\text{C4-4})$$

then, C is proportional to only D' . This means that for an arbitrary value of β , the double integration of C results in a total area other than zero due to the term A' . However, for $\beta = \alpha^{-1}$ the term A' disappears and the double integration results in zero total area because D' has an equal zero area. The double integration of the linear combination of C is present in Figure C4-3. As can be seen, only $\beta = 2$ results in a flat and zero baseline, as expected by $\alpha = 0.5$.

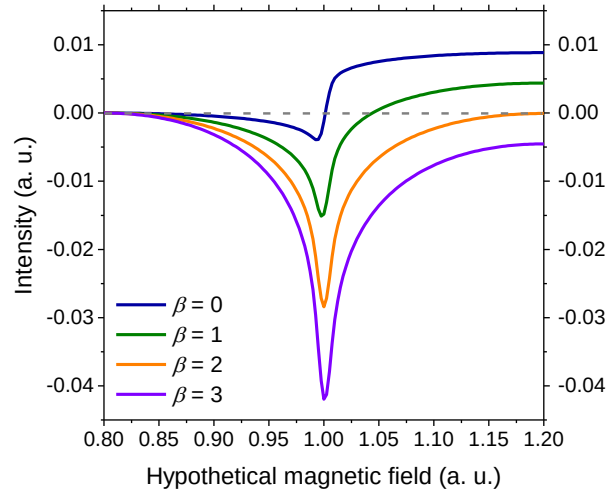


Figure C4-3. Double integration of C for different values of β .

As a real example, we applied the above procedure in an experimental NFMel-S HFEPR dispersion spectrum (S_{NFMel}) and its calculated Hilbert transform (H_{NFMel}), see Figure C4-4(a). We then take a linear combination of S_{NFMel} and H_{NFMel} following C4-3. The double integration of the linear combination of C_{NFMel} with different values of β is shown in Figure C4-4(b).

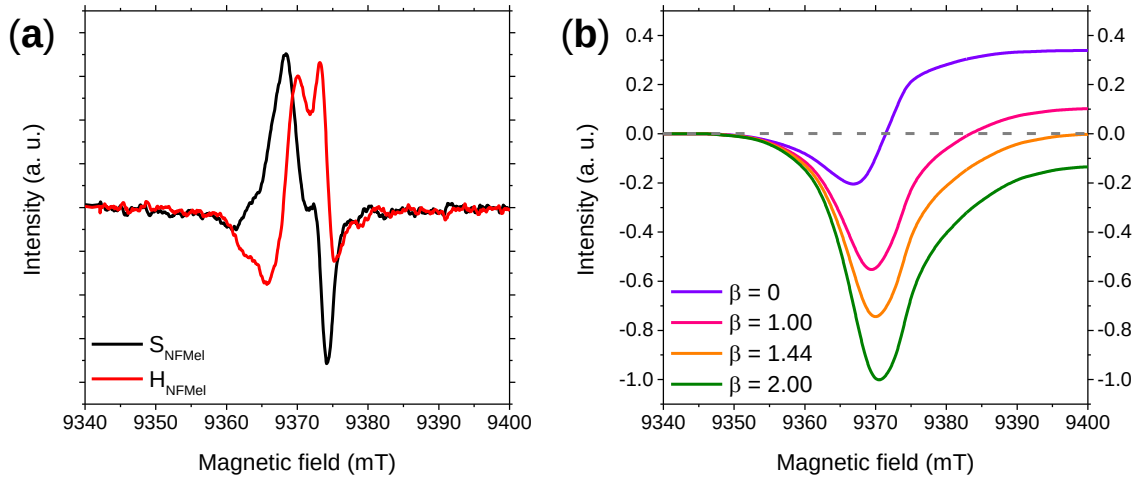


Figure C4-4. (a) Dispersion HFEPR experimental spectrum (S_{NFMel}) and its Hilbert transform (H_{NFMel}). (b) Double integration of C for different values of β .

Based on Figure C4-4, it is possible to observe that a flat and zero baseline is obtained with $\beta = 1.44$. This implies that, considering relation C4-4, $\alpha = 0.69$. Thus, with relation C4-1, we can get the phase-corrected HFEPR experimental spectrum.

APPENDIX D

APENDIX D.1 – DC and AC supplementary material for Section 6.2.2 in the main-text

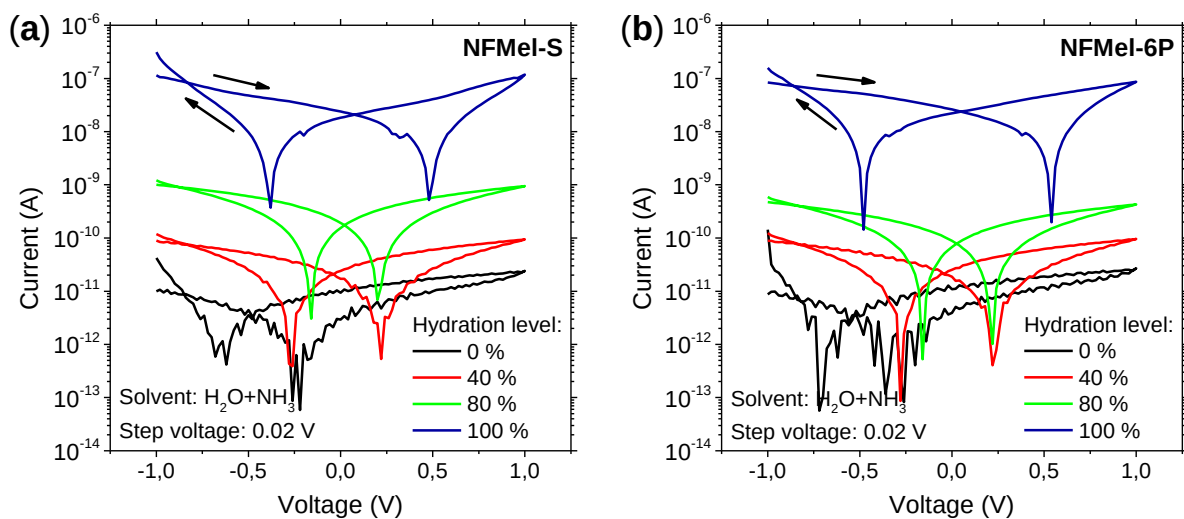


Figure D1. Current as a function of voltage for (a) NFMel-S and (b) NFMel-6P in $\text{H}_2\text{O}-\text{NH}_3$ at different hydration levels.

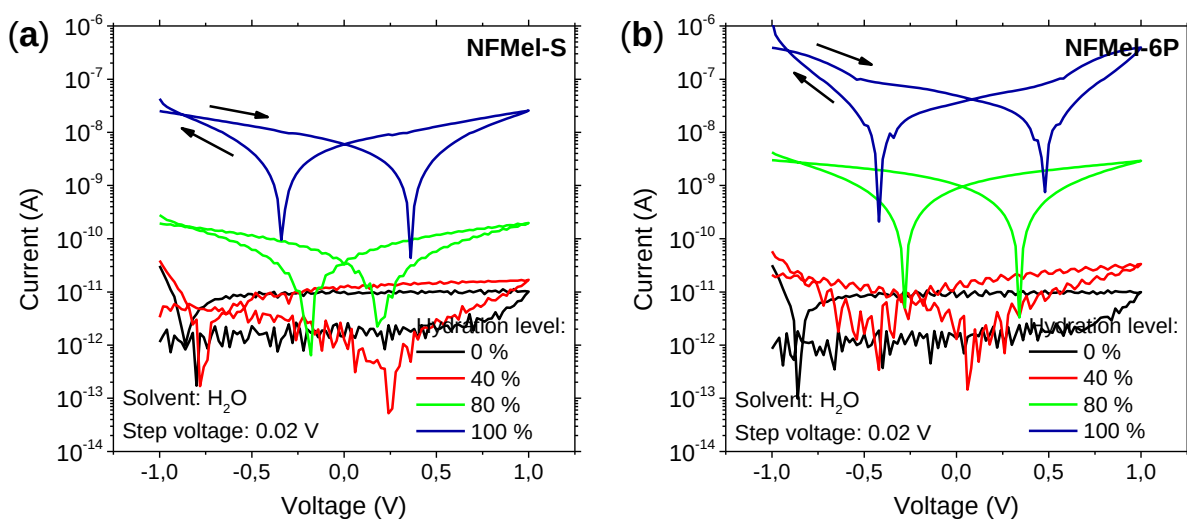


Figure D2. Current as a function of voltage for (a) NFMel-S and (b) NFMel-6P in H_2O at different hydration levels.

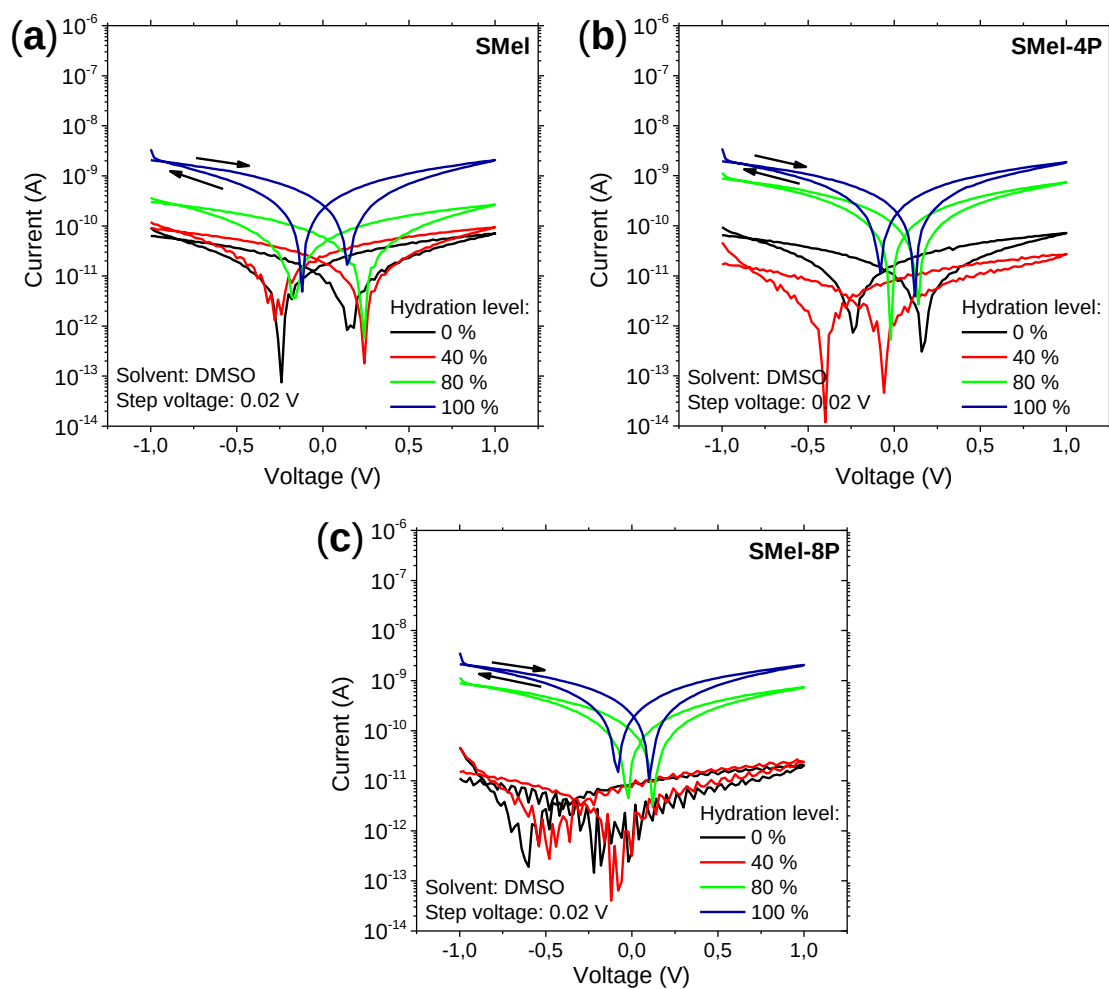


Figure D3. Current as a function of voltage for (a) SMel, (b) SMel-4P and (c) SMel-8P in DMSO at different hydration levels.

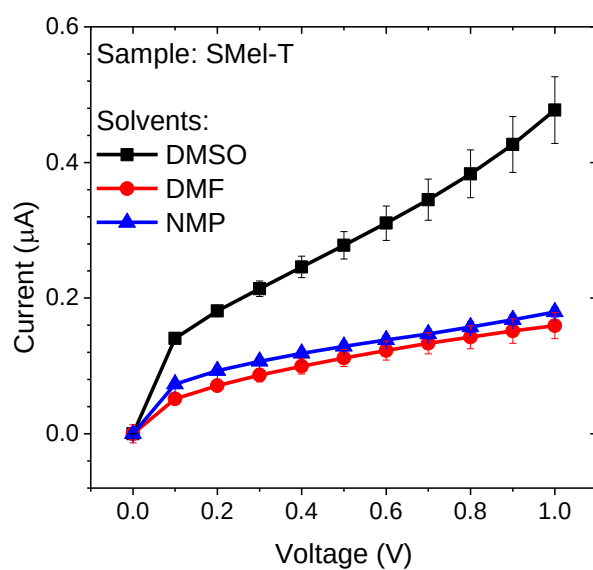


Figure D4. Current vs. voltage curve for SMel-T in different solvents at room atmosphere.

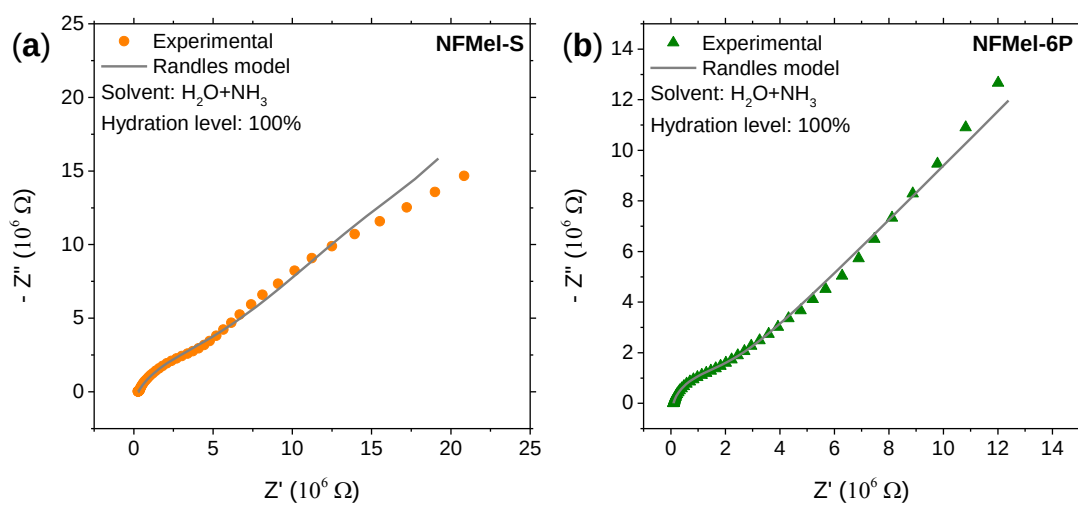


Figure D5. Nyquist plot of the EIS measurement of NF-Melanin using H_2O+NH_3 as a solvent at 100% hydration level.

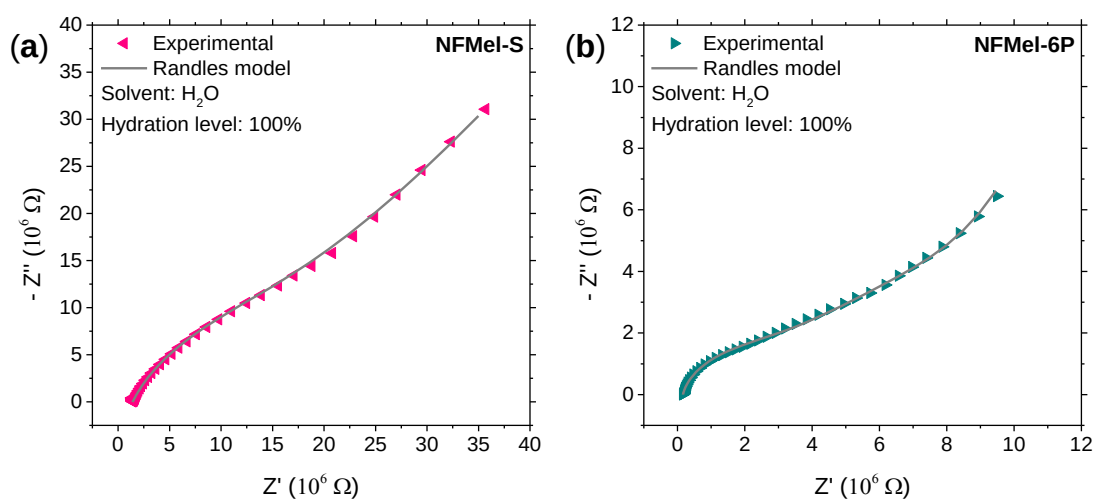


Figure D6. Nyquist plot of the EIS measurement of NF-Melanin using H_2O as a solvent at 100% hydration level.

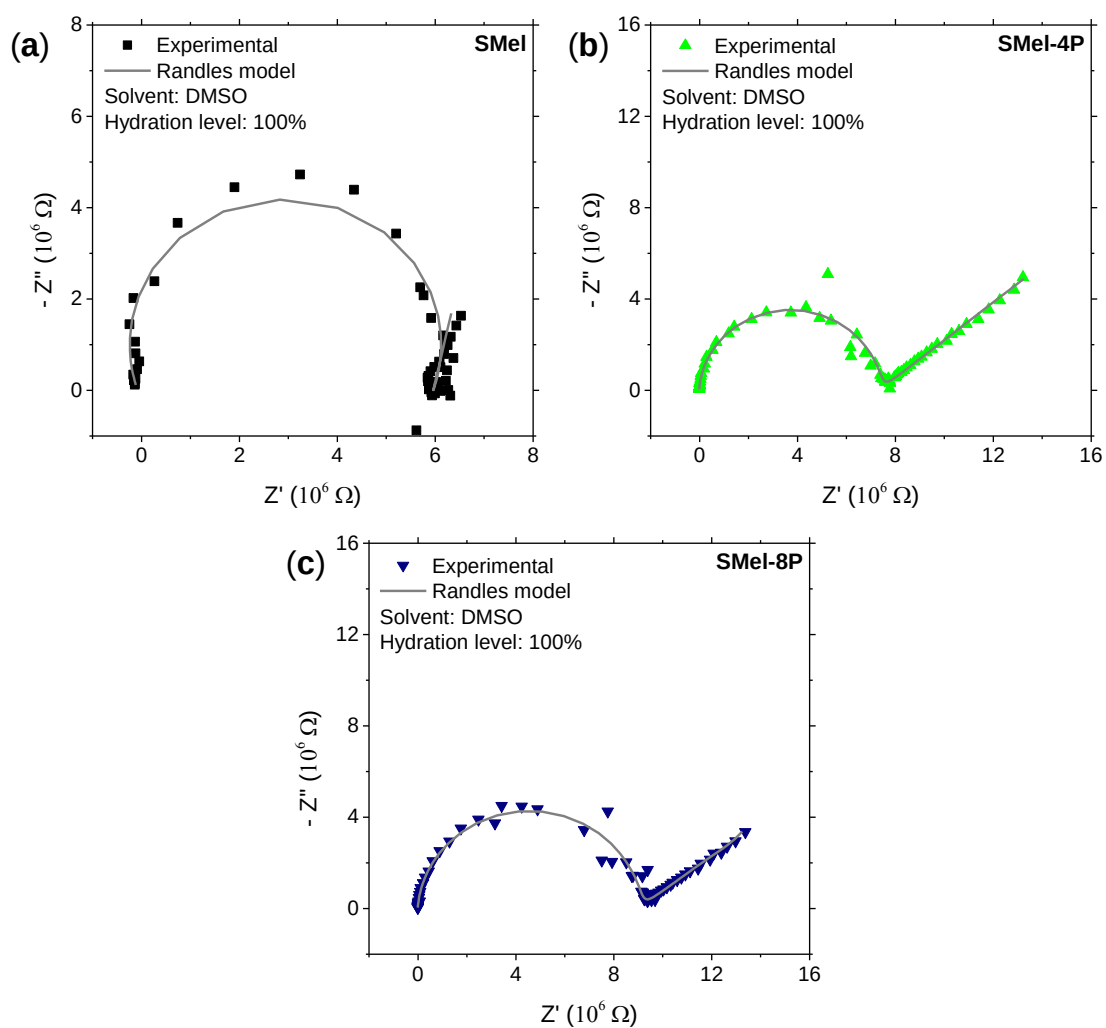


Figure D7. Nyquist plot of the EIS measurement of S-Melanin using DMSO as a solvent at 100% hydration level.

Table D1. Fitting parameters used to fit the data according to the equivalent circuit (Figure D8) for all samples at 100% hydration level.

Sample	R1	R2	W-R	W-T	W-P	CPE-T	CPE-P
<i>H₂O+NH₃</i>							
NFMel-I	8.99E+05	1.73E+07	9.03E+07	8.65E+01	6.25E-01	6.81E-08	7.38E-01
NFMel-S	2.87E+05	5.84E+06	6.57E+07	1.06E+02	4.92E-01	4.91E-08	7.06E-01
NFMel-6P	1.02E+05	2.01E+06	1.39E+05	1.71E-03	2.62E-01	3.75E-08	8.68E-01
<i>H₂O</i>							
NFMel-S	1.41E+06	2.39E+07	9.06E+05	1.69E-02	2.82E-01	9.45E-08	7.12E-01
NFMel-6P	1.62E+05	2.11E+06	2.02E+07	7.79E+01	4.00E-01	4.25E-08	8.64E-01
<i>DMSO</i>							
SMel	7.80E+06	6.04E+08	2.10E+03	3.22E-05	4.32E-01	6.55E-16	1.00E+00
SMel-T	1.95E+06	3.58E+08	3.74E+08	2.44E+01	4.15E-01	4.19E-13	9.69E-01
SMel-4P	1.61E+06	7.36E+08	4.55E+10	1.69E+05	4.41E-01	4.68E-13	9.68E-01
SMel-8	1.07E+06	9.05E+08	1.35E+09	1.24E+02	4.24E-01	6.57E-13	9.60E-01

APENDIX D.2 – QENS supplementary material for Section 6.3.2 in the main-text

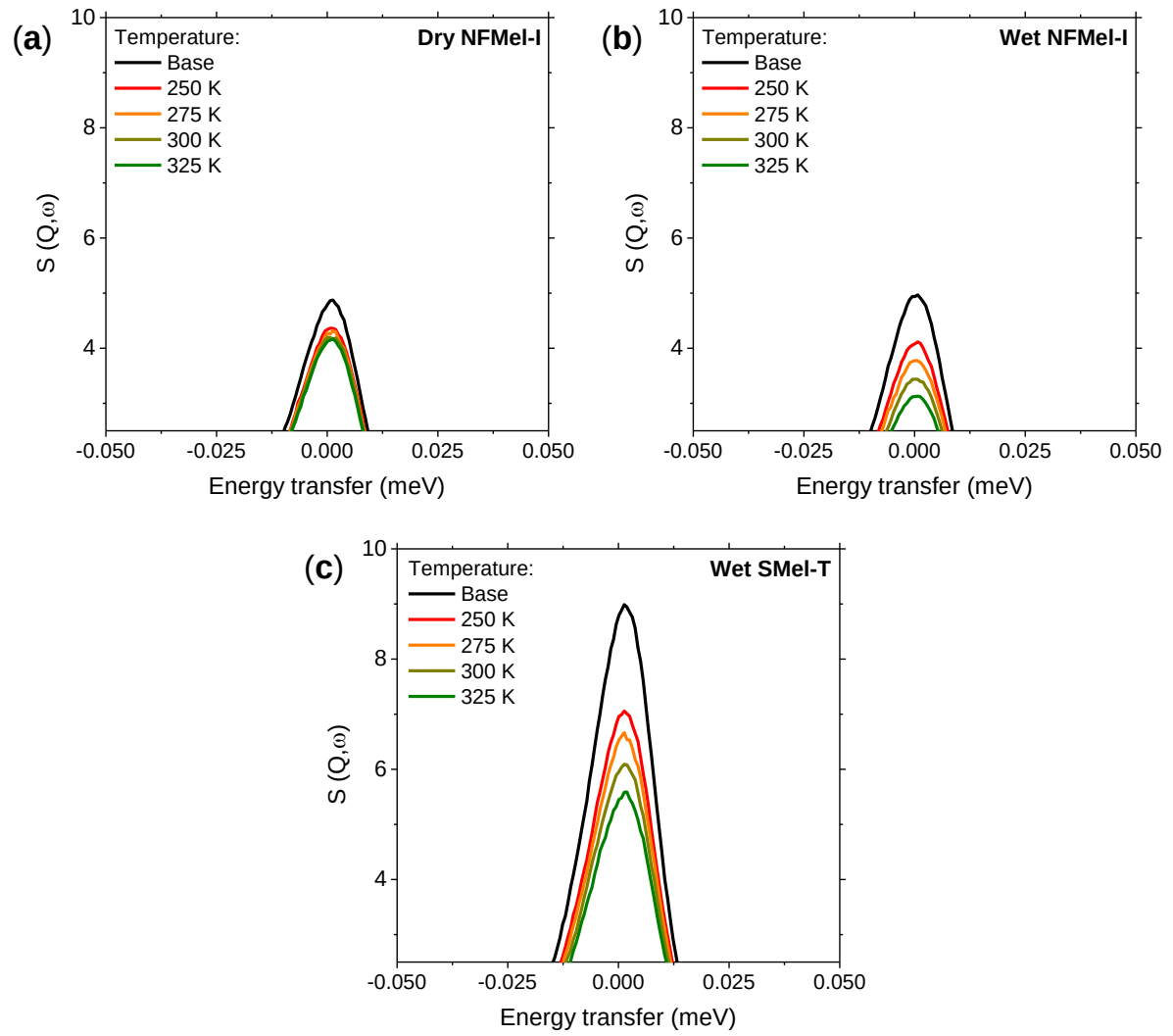


Figure D9. QENS spectra for (a) dry NFMel-I, (b) wet NFMel-I and (c) wet SMel-T at different temperatures.