

# Exploring the chemistry and composition of black soldier fly eumelanin, a material for a circular economy

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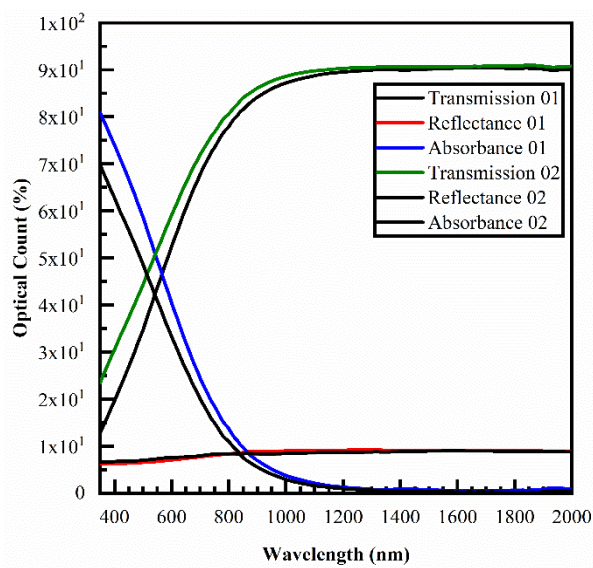
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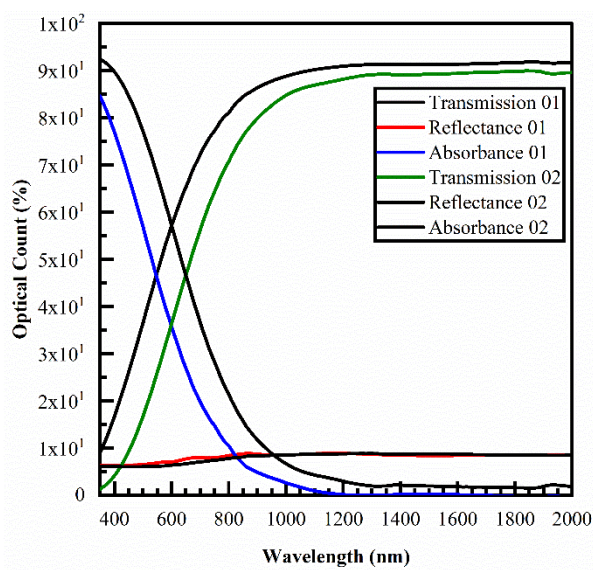
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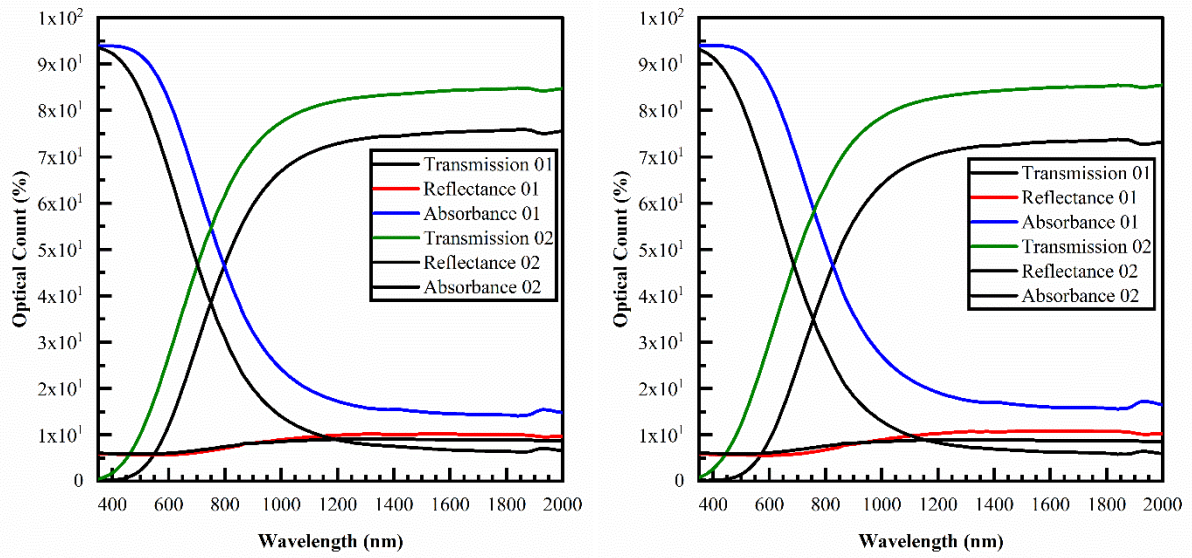
### UV-Vis Spectra of Various Solid-State Films



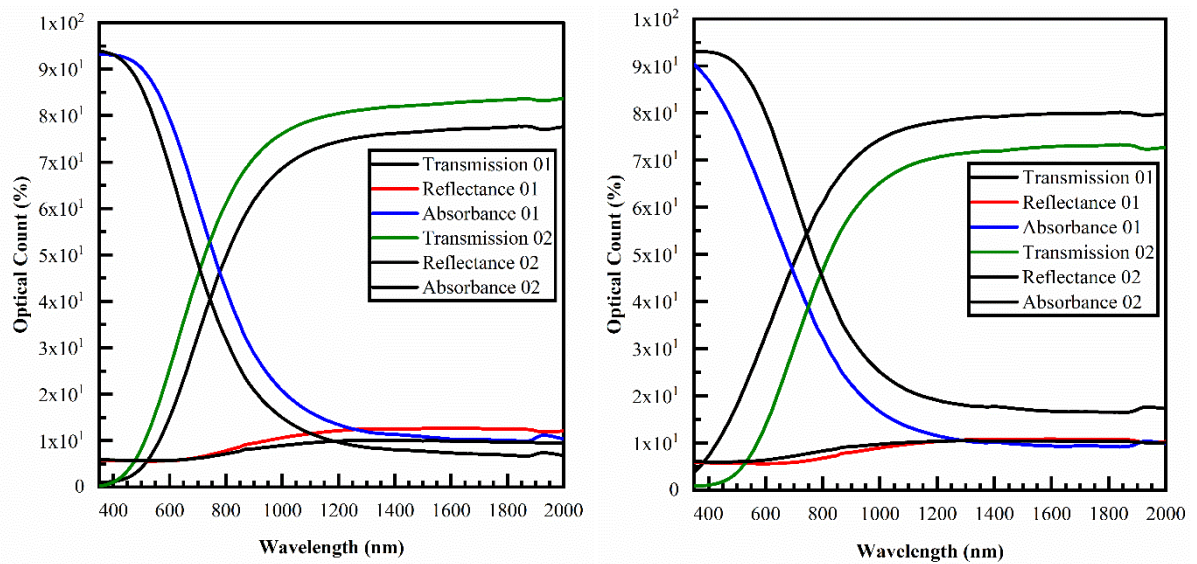
**Figure S1:** The optical data obtained on two films of BSF eumelanin. BSF eumelanin was filtered, and substrate ozone treated.



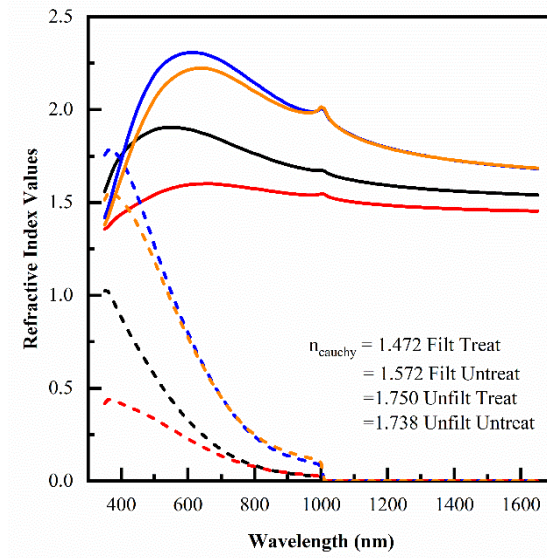
**Figure S2:** The optical data obtained on two films of BSF eumelanin. BSF eumelanin was filtered, and substrate was left untreated.



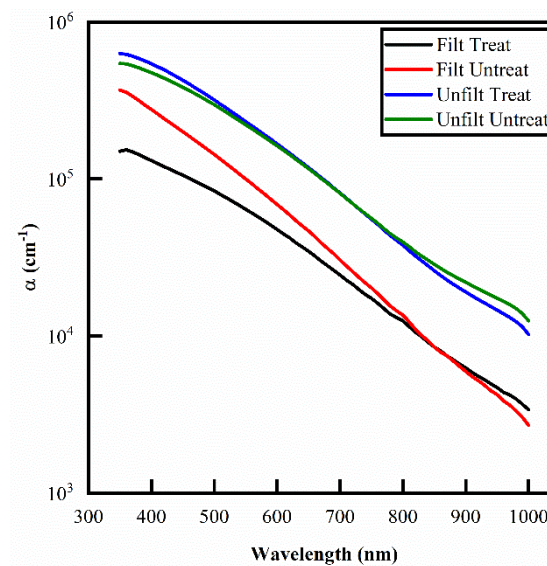
**Figure S3:** The optical data obtained on four films of BSF eumelanin but depicted across two sub figures for clarity. BSF eumelanin was unfiltered, and substrate was treated with ozone.



**Figure S4:** The optical data obtained on four films of BSF eumelanin but depicted across two sub figures for clarity. BSF eumelanin was unfiltered, and substrate was left untreated.

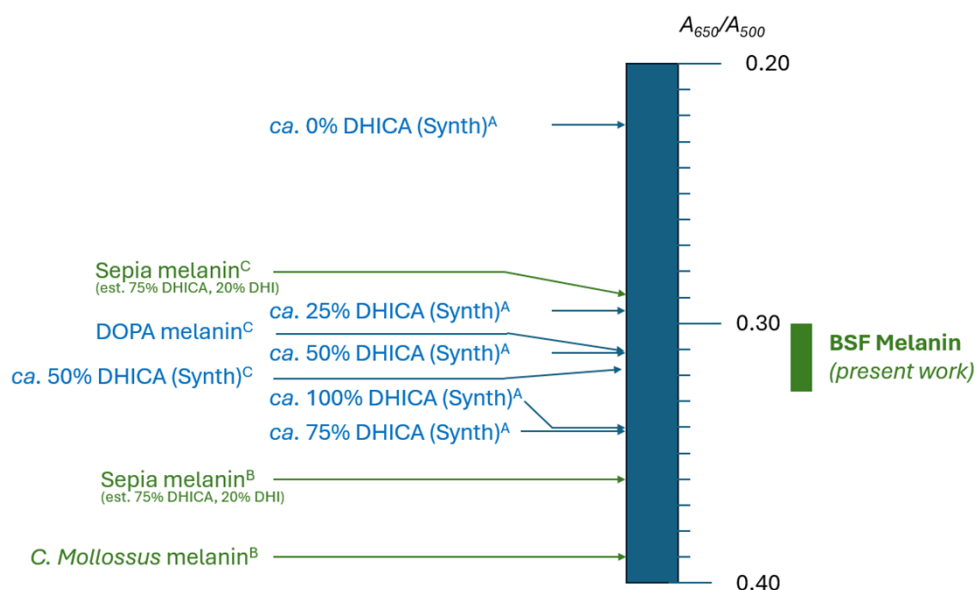


**Figure S5:** The overall refractive indices  $n$  (solid lines) and  $k$  (dashed lines) for films that were (blue) unfiltered BSF eumelanin on ozone treated glass, (orange) unfiltered BSF eumelanin on untreated glass, (black) filtered eumelanin on untreated glass and (red) filtered eumelanin on ozone treated glass. The Cauchy refractive index ( $n(\lambda \rightarrow \infty)$ ) is indicated in the legend.



**Figure S6:** The absorption coefficients for the various sample types (see legend).

## UV-Vis $A_{650}/A_{500}$ ratio analysis of BSF eumelanin Solutions



**Figure S7:**  $A_{650}/A_{500}$  ratios reported in the literature for synthetic (blue) and natural (green) melanins. Values are taken from: Itou *et al.* (A) where the values of %DHI 0%, 25.1%, 49.9%, 74.7%, 99.9% correspond to ratio values of 0.22, 0.30, 0.31, 0.34, 0.34;<sup>1</sup> Xin *et al.*<sup>2</sup> (B) where *Sepia* eumelanin from sigma was referenced as yielding a ratio of 0.36 for 75%/20% DHICA/DHI<sup>3</sup> and where they reported a eumelanin for *C. mollossus* ratio of 0.39; Ito *et al.* (C) where *Sepia* eumelanin yielded a ratio of 0.29 and a synthetic eumelanin of 50%DHI/50%DHICA yielded a ratio of 0.318.<sup>4</sup>

The literature contains a range of reports of how  $A_{650}/A_{500}$  ratios relate to %DHI, recorded in varying conditions. For synthetic (DHI/DHICA) eumelanin of varying %DHI, a range of  $A_{650}/A_{500}$  ratios from ~0.22 (no DHI content) to 0.34 (100% DHI) was reported.<sup>1</sup> This dependence is not linear but monotonically increases as DHI content increases, with  $A_{650}/A_{500}$  for 80% DHI as essentially indistinguishable from 100% DHI.<sup>1</sup> Another report observed an  $A_{650}/A_{500}$  ratio for *Catharsius molossus* L. (dung beetles) of 0.39,<sup>2</sup> and for *Sepia officinalis* (cuttlefish)  $A_{650}/A_{500}$  ratio of 0.36 (noting that *Sepia* is known to have a DHICA/DHI ratio of up to 75%/20%).<sup>3</sup> A previous report observed an  $A_{650}/A_{500}$  ratio of 0.291 for ‘*Sepia* eumelanin’, and 0.318 for synthetic (1:1 DHICA/DHI eumelanin).<sup>4</sup>

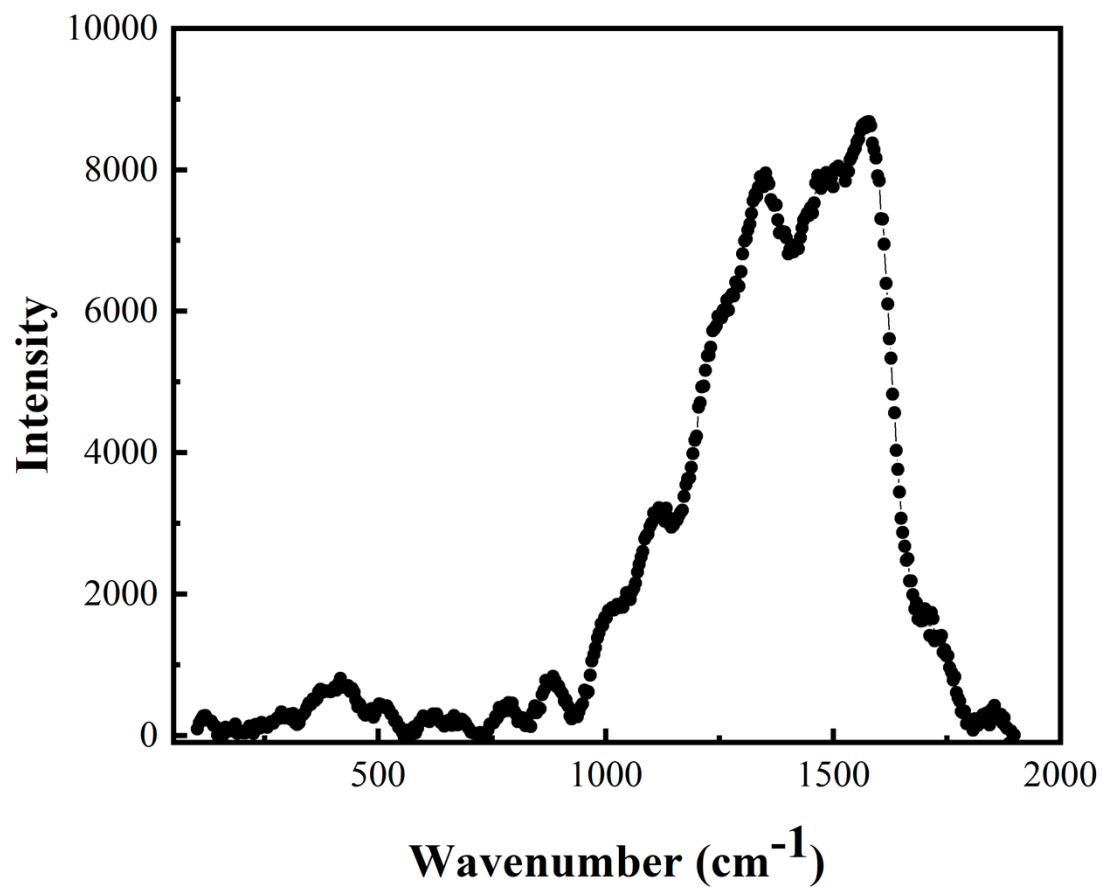
Applying this analysis to our UV-Vis spectra of solutions/suspensions of BSF eumelanin, we obtain an average result for the  $A_{650}/A_{500}$  ratio of 0.3 on unfiltered samples and 0.33 on filtered samples. Considering the literature results above together, it is not appropriate to draw quantitative conclusions of DHI/DHICA ratio from our  $A_{650}/A_{500}$  data for BSF eumelanin. However, we note that values for BSF eumelanin values fall into the range in which mixed DHI/DHICA have been reported, and therefore that our observations do not appear inconsistent with the presence of DHICA. Indeed, ca. 20% DHICA, as estimated by AHPO, would not be readily differentiated from 0% DHICA by comparing  $A_{650}/A_{500}$  ratios, following the literature.<sup>1</sup>

### *UV-Vis $A_{650}/A_{500}$ ratio analysis of Various Solid-State Films*

For various solid-state film samples, the  $A_{650}/A_{500}$  ratios obtained are shown in Table S1. What is notable is that the ratios obtained are significantly different to the suspension results obtained from the data in Figure 2. Furthermore, there are two clear categories for the solid films: data with and without treated glass slides. Clearly substrate preparation is a key determinant of the results, with glass that was treated with UV-Ozone yielding results closer to the solution/suspension data. Differences between filtered and unfiltered eumelanin was minimal, like the solution/suspension data. Still, even with cleaned substrates, the difference to Figure 2 data is substantial with almost twice the ratio values. As such, the solid-state data currently is not determinative and may indeed indicate that the solid state is not a suitable approach for ratio analysis as it is too far out of scope with what has been observed in solution based studies.

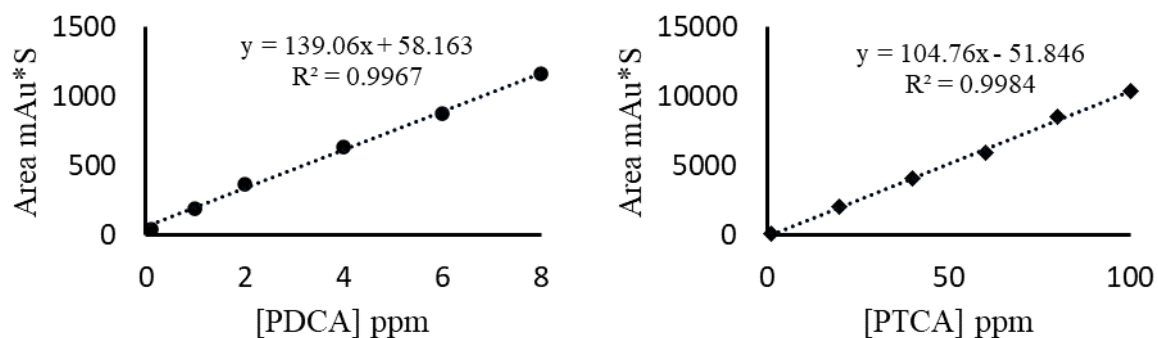
<b>Sample Type</b>	<b>Average <math>A(650nm)/A(500nm)</math></b>	<b>Uncertainty <math>A(650nm)/A(500nm)</math></b>
Filtered on treated glass	0.544	0.003
Unfiltered on treated glass	0.54	0.13
Filtered on untreated glass	0.75	0.18
Unfiltered on untreated glass	0.74	0.11

**Table S1:** Absorbance ratio analysis performed on thin films of BSF-eumelanin. Averages and uncertainty (range) given for various sample preparations.



**Figure S8:** Full Raman spectrum of BSF-EuMel. No visible peaks are attributed to the pheomelanin.

HPLC



**Figure S9:** Standard curve of eumelanin markers PDCA and PTCA. 30  $\mu$ L of standard solutions containing 0.1 to 100  $\mu$ g  $mL^{-1}$  of markers, for PTCA (1, 20, 40, 60, 80, 100  $\mu$ g  $mL^{-1}$ ) and for PDCA (0.1 to 8  $\mu$ g  $mL^{-1}$ ), were injected.

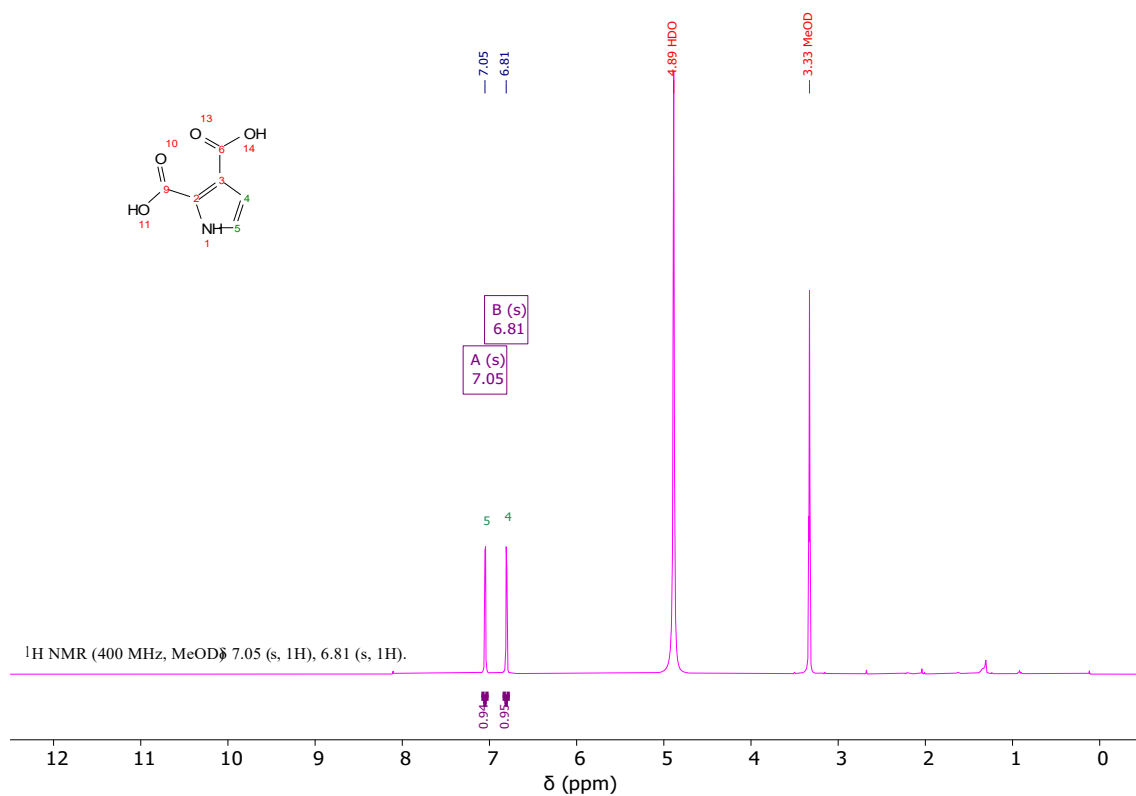
Markers	Regression Equation	Correlation ( $R^2$ )	Linearity Range ( $\mu$ g $mL^{-1}$ )
PDCA	$y=139.06x+58.163$	0.9967	0.1-8
PTCA	$y=104.76x-51.846$	0.9984	1 -100

**Table S2:** Regression equation of eumelanin markers: pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA).

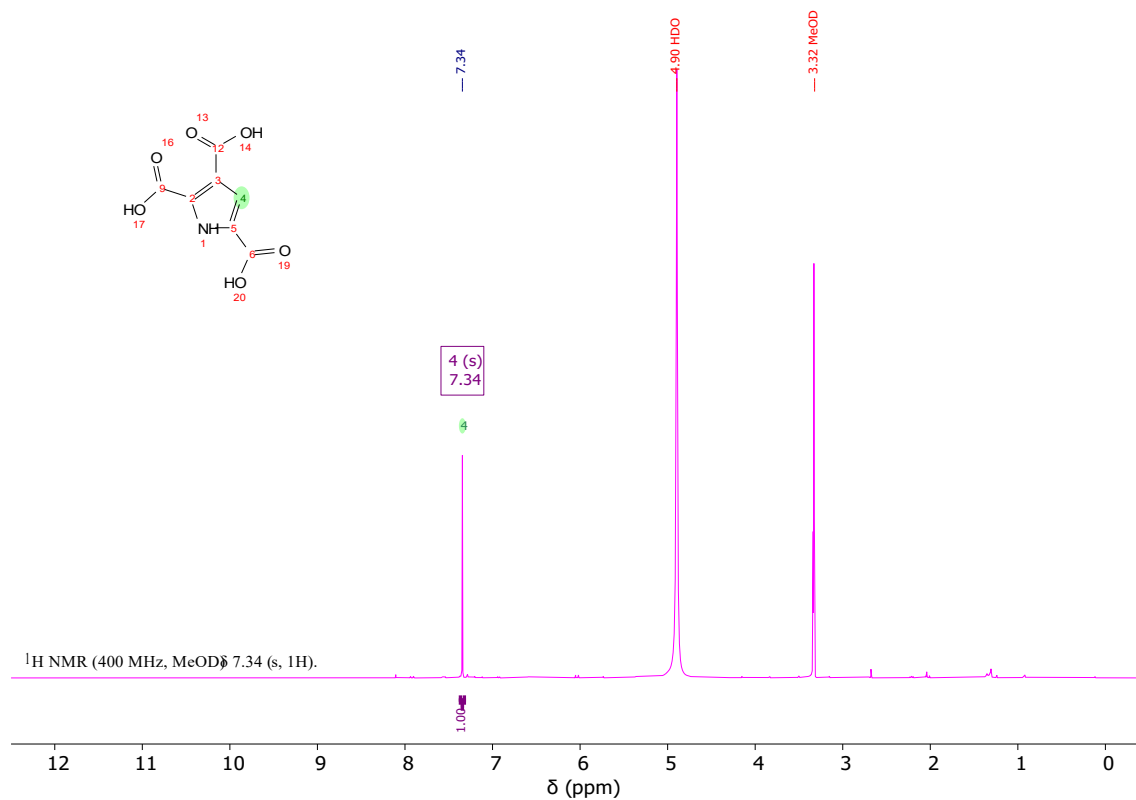
Sample	PDCA	PTCA	PDCA/PTCA Ratio
Native	$197.269 \pm 72.98$	$887.942 \pm 66.51$	0.22
HCl-Treated	$55.728 \pm 12.65$	$475.051 \pm 11.19$	0.12
Filtered	$309.586 \pm 23.30$	$791.714 \pm 20.26$	0.39

**Table S3:** Content of markers in eumelanin samples ( $ng\ mg^{-1}$ ). [values are means  $\pm$  SD]

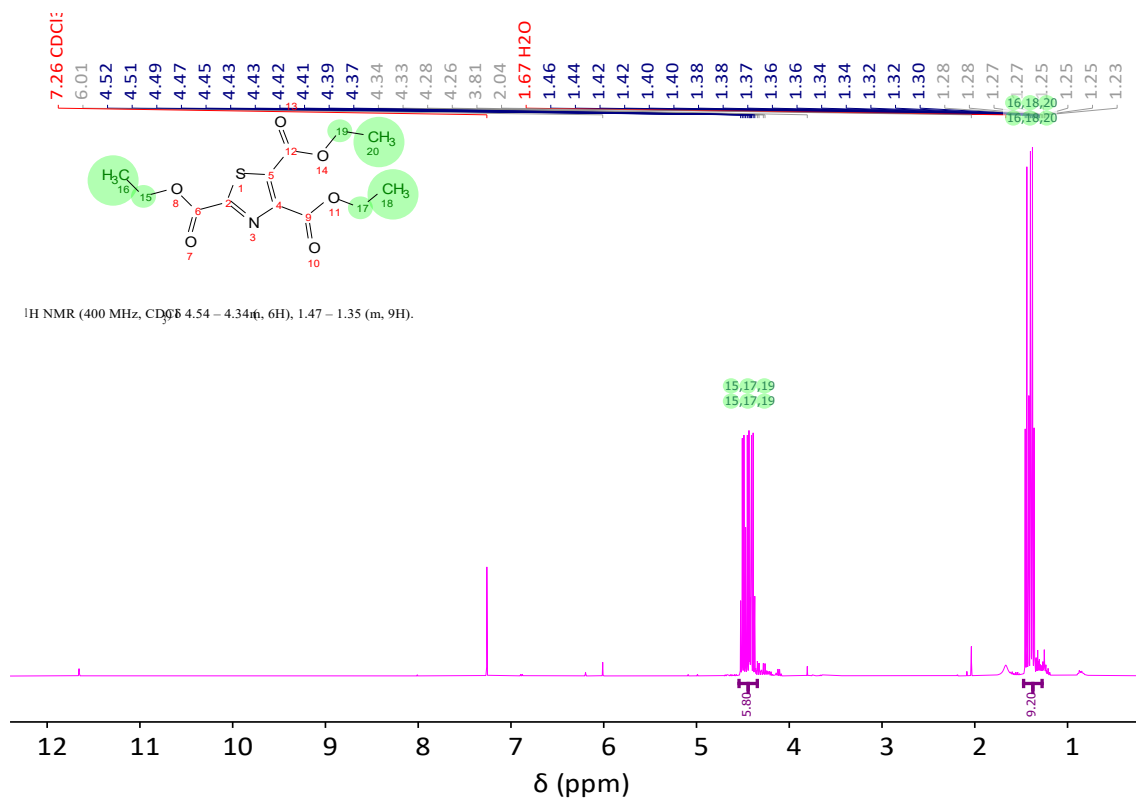




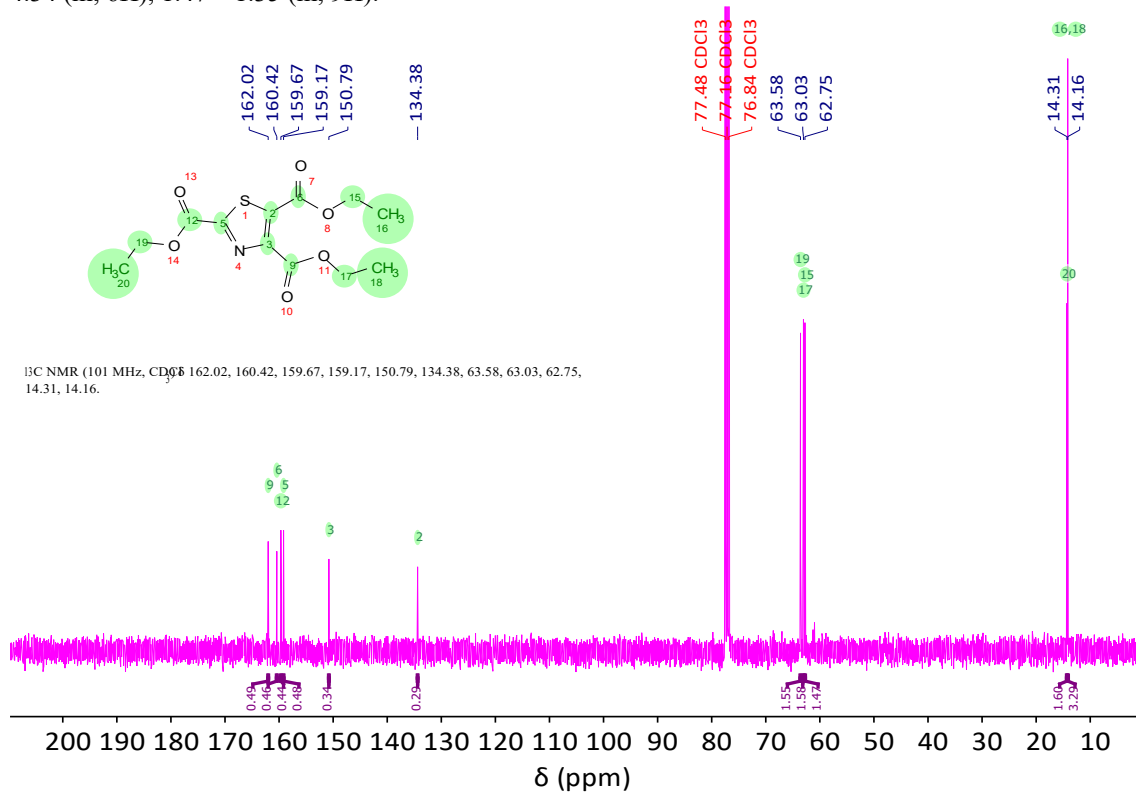
**Figure S10:** <sup>1</sup>H NMR spectrum of Pyrrole-2,3-dicarboxylic acid (PDCA).



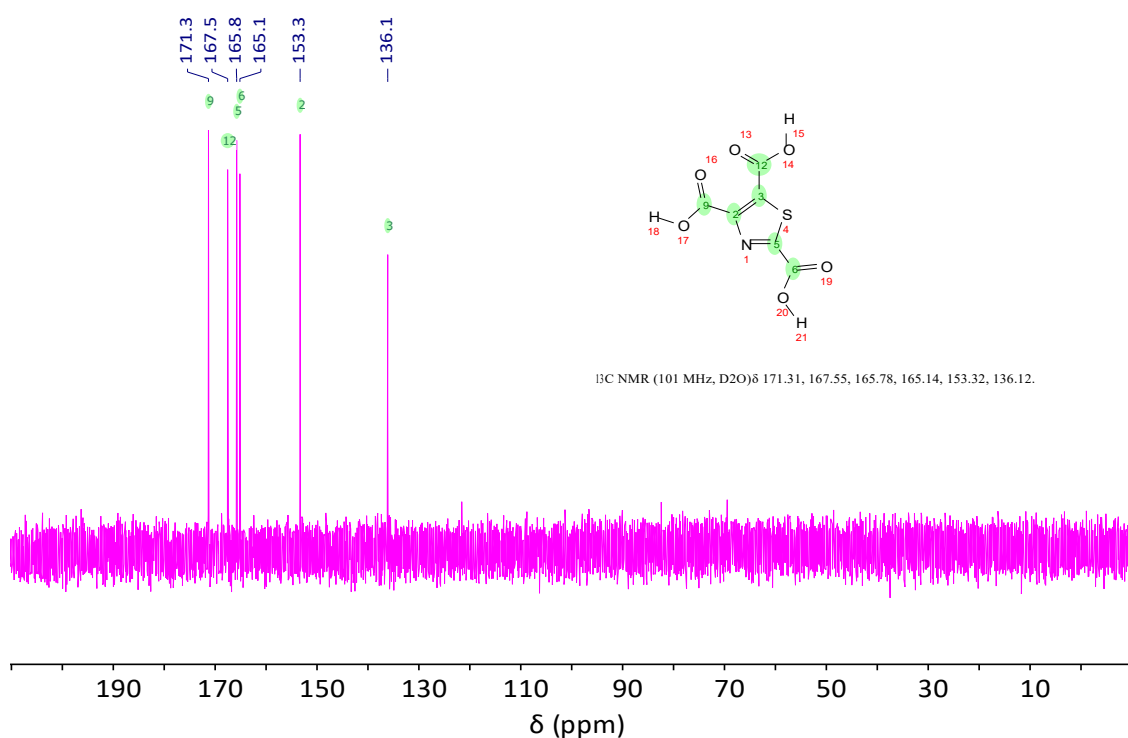
**Figure S11:** <sup>1</sup>H NMR spectrum of pyrrole-2,3,5-tricarboxylic acid (PTCA).



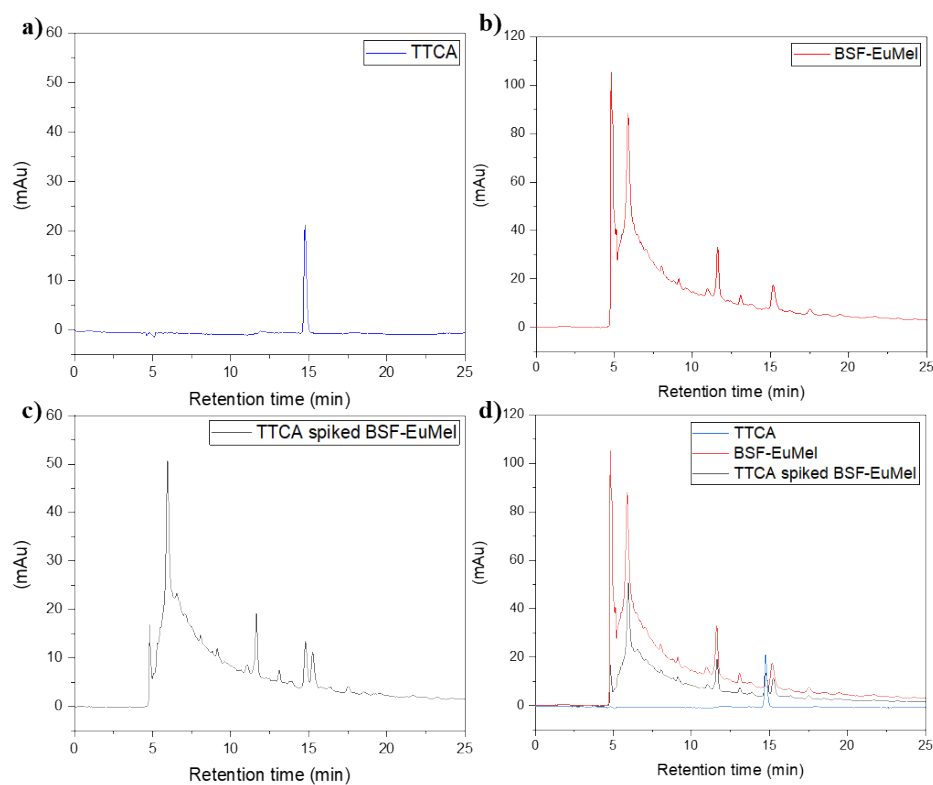
**Figure S12:** <sup>1</sup>H NMR of TTCA precursor: triethyl thiazole-2,4,5-tricarboxylate. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.54 – 4.34 (m, 6H), 1.47 – 1.35 (m, 9H).



**Figure S13:** <sup>13</sup>C NMR of TTCA precursor: triethyl thiazole-2,4,5-tricarboxylate. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 162.02, 160.42, 159.67, 159.17, 150.79, 134.38, 63.58, 63.03, 62.75, 14.31, 14.16. Cal. mass: 301.062 found: 302.0 [M+H<sup>+</sup>].



**Figure S14:**  $^{13}\text{C}$  NMR of TTCA: thiazole-2,4,5-tricarboxylic acid.  $^{13}\text{C}$  NMR (101 MHz, D<sub>2</sub>O)  $\delta$  171.31, 167.55, 165.78, 165.14, 153.32, 136.12.



**S15: HPLC analysis showing no/negligible TTCA in the BSF-EuMel sample.** a) TTCA eumelanin marker. b) AHPO-digested solution of BSF-EuMel. c) AHPO-digested solution of BSF-EuMel spiked with TTCA marker. d) Superimposition of three chromatograms: no peak is observed in the AHPO-digested solution of BSF-EuMel at the retention time of TTCA.

NMR

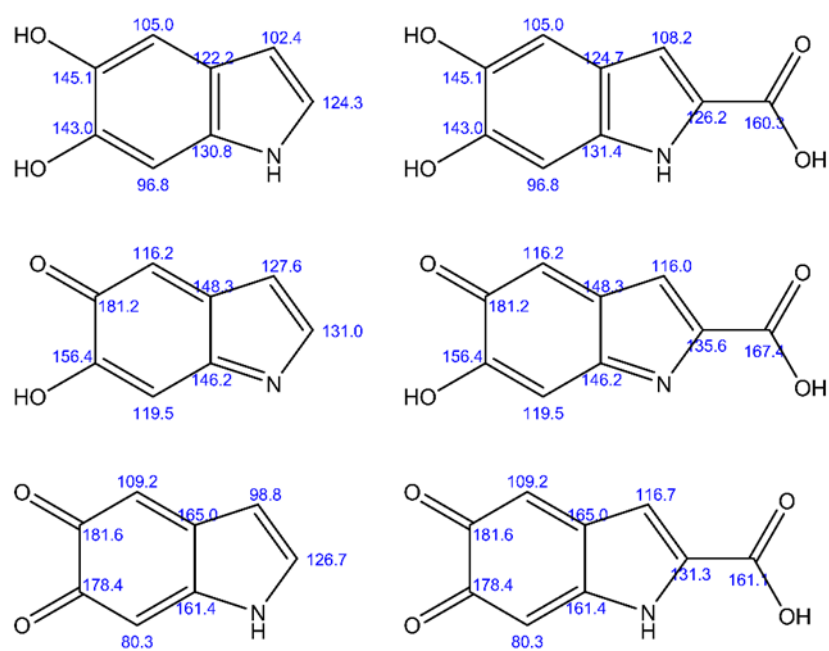


Figure S16: NMR Peak assignment for different monomer redox states of eumelanin.

## Elemental Analysis

The elemental analysis on the BSF eumelanin is shown in Table S4. Sulphur was measured for, but not detected, which indicates that no significant pheomelanin is present. To demonstrate this assertion further, it should be noted that natural pheomelanin sulphur content has been quantified to be between 6 – 16% w/w.<sup>5</sup> If one assumes a lower limit of sulphur content to be 5%, and given that the elemental analysis has a detection limit of 0.01% w/w, then if one was able to detect sulphur at this lower limit, it would correspond with a material containing 0.2% w/w pheomelanin. As such, if pheomelanin is present in BSF eumelanin, it should be below 0.2% w/w and thus should be considered irrelevant to the rest of the analysis.

Sample ID	C%	H%	N%
QR24011	50.85	5.8	9.04
QR24007	49.3	5.52	9.08

**Table S4:** Content of markers in BSF eumelanin samples (ng mg<sup>-1</sup>). [values are means ± SD]

## References

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