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Temperature-enhanced synthesis of DMSO-Melanin

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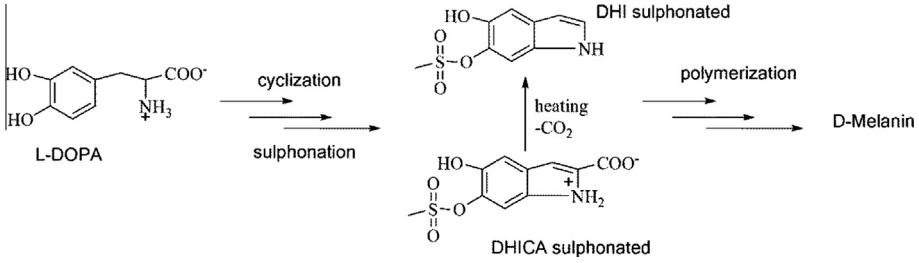
HIGHLIGHTS

- The influence of temperature on D-Melanin synthesis and its properties is studied.
- Increasing the synthesis temperature, synthesis time can be decreased down to 7 times.
- Increase in temperature causes a decarboxylation of D-Melanin monomers.
- Decarboxylation processes facilitating the polymerization of D-Melanin.

GRAPHICAL ABSTRACT



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ABSTRACT

Melanins are a class of pigmentary conjugated macromolecules found in many biological systems. Functionalization of synthetic melanin provides interesting new properties like the greater solubility of melanin synthesized in dimethyl sulfoxide, D-Melanin. In this work we have studied the influence of temperature on D-Melanin synthesis and its properties. To this end, UV–Vis, Fourier-transform infrared (FTIR) spectroscopy and Nuclear Magnetic Resonance (NMR) techniques have been employed to analyze D-Melanin synthesized within the range of 25–100 °C. Our results reveal that by increasing the synthesis temperature up to 100 °C, the synthesis time can be decreased by a factor of 7 when compared to room temperature. From FTIR and ^{13}C CP/MAS NMR analyses the increase in temperature causes a decrease in the number of carbonyl groups from carboxylic acid and from ionized carboxylic acid. The decarboxylation of D-Melanin monomers at higher temperatures shows that the use of higher synthesis temperatures influences the elimination of carbonyls present in the precursor molecules, thus facilitating the polymerization of D-Melanin.

1. Introduction

Melanins are a class of pigmentary conjugated macromolecules found in many biological systems [1]. The structure of melanin is believed to be composed of disordered cross-linked ensembles of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) in various redox states [1–4]. These ensembles form planar sheets with varying dimensions that stack in 2 or 3 sheets with interplanar distance of 3.7–4.0 Å [1,5–7]. Melanins present a broadband monotonic absorption in the UV–Vis as a consequence of the superposition of a large number of transitions associated with the different molecules that form melanin [3,5,6,8–11].

Melanins have potential bioelectronics applications [1]. In this context, there is an increasing interest in melanin thin films, as a pre-requisite to the full production of melanin-based electronic devices [12–14]. However, melanin is insoluble in common solvents, making good quality thin film deposition harder, although possible under special conditions [12,14]. In particular, free-standing melanin films synthesized electrochemically from dopa showed good photoconductivity and higher conductivity than chemically synthesized melanin [13]. Our group produced soluble melanin using organic solvents such as DMSO in the synthesis in replacement to water [15–18]. The reason for this enhanced solubility was recently described [19]. The main difference when melanin is synthesized in DMSO is the incorporation of sulfonate groups ($-SO_2CH_3$) coming from DMSO oxidation, at the phenolic hydroxyl group of DHI and DHICA. This process is illustrated in Scheme 1.

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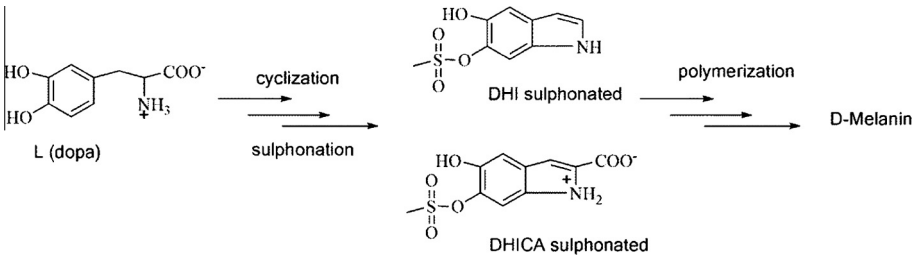


Scheme 1. Initial steps of melanin formation in DMSO.

Due to the disordered nature of melanin and its synthetic analogues, the conditions under which the polymerization process is carried out are very important in determining the synthesis kinetics. Small changes in the synthesis conditions may modify the reaction thermodynamics affecting the final structure and, consequently, the properties of the final product. Of special interest here is that, at room temperature, it takes several days for melanin to be synthesized. Therefore, it would be of great practical importance if it were possible to decrease the synthesis time keeping melanin properties. Therefore, in this work, we report a systematic study of the synthesis of D-Melanin at different temperatures and its influence on the reaction, the material structure and optical properties.

2. Experimental

2.1. D-Melanin synthesis

D-Melanin was synthesized from a mixture of 3 g of (2*S*)-2-amino-3-(3,4-dihydroxyphenyl) propanic acid (L-dopa) with 3.7 g of benzoyl peroxide in 400 mL of DMSO [15]. The reaction mixture was kept under agitation for a few days, sealed to avoid air humidity, at room temperature (±25 °C) as well as under heating by using a silicone bath with controlled temperature at 50 °C, 80 °C and 100 °C. At the end of the synthesis (complete formation of D-Melanin), solutions were heated at 140 °C to evaporate the DMSO until 15% of the initial volume was left. Acetonitrile was added to this concentrated solution and kept at rest for 48 h for D-Melanin precipitation. A sequence of centrifugation followed by extraction of the precipitate provided a purified powder of synthetic D-Melanin. All reagents were purchased from Sigma–Aldrich and used without further purification procedures.

2.2. UV–Vis, FTIR and ^{13}C CP/MAS NMR spectroscopy characterization

In order to verify the evolution of the synthesis, an aliquot was withdrawn periodically for UV–Vis Spectroscopy analysis using a Shimadzu UV–Vis-Spectrophotometer (UVmini-1240). Aliquots removed during the synthesis of D-Melanin were diluted at a ratio of 0.07/1 in DMSO using a quartz cuvette of 1 cm path length. Absorption spectra from 290 to 700 nm were recorded at regular time intervals, until the reaction ended. The criteria for reaction end will be discussed in the results section.

D-Melanin powders synthesized at room temperature (25 °C), 50 °C, 80 °C and 100 °C were diluted in DMSO thus obtaining four stock solutions of 10 mg/ml. From these stock solutions, dilutions using the same solvent were made at six different concentrations ranged from 0.002 mg/ml to 0.010 mg/ml. Absorption coefficient was obtained following Beer-Lambert law [20]. These analyses were performed in triplicate.

The FTIR measurements were obtained on a Bruker Vertex 70 Fourier transform spectrometer in the region between 4000 and 400 cm^{-1}, at room temperature in Attenuated Total Reflectance (ATR) mode. Spectral deconvolutions of the FTIR spectra were performed in order to evaluate the relative changes in the absorption bands, the procedure used follows Paudel et al. [21]. After a selection of a region of interest, Gaussian functions were used to fit the spectra using the least square method. We used OriginPro version 8 (OriginLab Corp., MA, USA) for that purpose [21].

^{13}C CP/MAS analyses were performed on a Bruker Avance III 400 MHz spectrometer equipped with a 4 mm CP/MAS probe, operating at 100.5 MHz for ^{13}C. The ^{13}C CP/MAS spectra of the solid melanins were obtained by means of the cross-polarization technique (Cross-Polarization Magic Angle Spinning – CPMAS) with contact time of 2 ms, repetition time of 5 s and MAS rotation frequency of 5 kHz. Two Pulse Phase Modulation (tppm) proton decoupling was used.

3. Results

3.1. D-Melanin synthesis and temperature dependence

The process of D-Melanin polymerization was followed using UV–Vis spectroscopy. Fig. 1 shows the absorption spectra obtained during the synthesis of D-Melanin at a temperature of 50 °C. A similar behavior was observed for other synthesis temperature. In this synthesis the reaction was monitored for 27 days.

As can be seen, as D-Melanin is synthesized there is a gradual increase in absorption throughout the spectrum. The spectrum, especially in the beginning is composed of broad absorption peaks at approximately 315 nm, 445 nm and 525 nm. These peaks may be associated with π–π^* transitions of different moieties of D-Melanin products from the polymerization process. At the end of the synthesis, the absorbance is characterized by the characteristic featureless broadband spectrum, also observed in natural and water synthesized melanins [8,22]. Absorption spectra of monomers, dimmers and oligomers of natural melanin and H-Melanin can be found in the literature, and the absorption spectrum of melanin

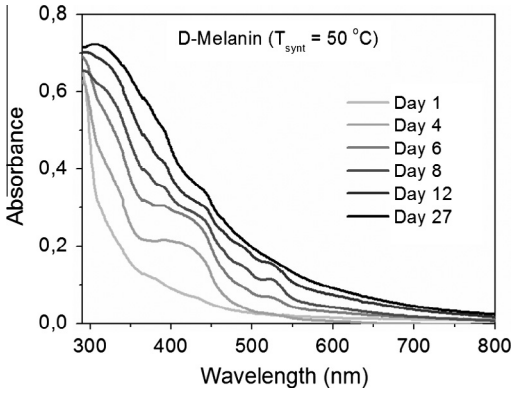


Fig. 1. UV–Vis spectra taken at different times during the synthesis of D-Melanin at 50 °C (T_{synt} = 50 °C). The absorbance increases as reaction proceeds.

is a superposition of a broad distribution of absorptions from different oligomeric units [6,8–10,23]. Furthermore, Stark et al. showed that the stacking of H-Melanin single sheets increases the absorption in the red-end part of the spectrum and destroys absorption shoulders, promoting the smooth absorption spectrum normally observed [10]. Considering that the polymerization reaction of D-Melanin is similar to that of H-Melanin, we assume they have similar origins.

Fig. 2 shows the evolution of D-Melanin absorbance at 445 nm as a function of time, for different synthesis temperatures. As can be seen during synthesis the absorbance increases linearly with time until it reaches a plateau with constant absorbance. From this plateau, it was considered that the polymerization reaction was complete when the difference in absorption values between two or more consecutive measurements were lower than 5%. This behavior is similar to that of DHI in the presence of poly(vinylalcohol),[3] as well as of melanin formed from mushroom tyrosinase [24].

The stabilization of the absorbance curve, i.e. the end of reaction, depends on the reaction temperature. Therefore the higher the temperature, the earlier the stabilization is achieved. Our results indicate that by increasing the synthesis temperature up to 100 °C, the synthesis time can be decreased by a factor of 7 compared to room temperature (RT).

One can observe that Fig. 2 is related to the formation rate of the chromophore structures that absorb at 445 nm. However, due to the large structural variety of D-Melanin, one may think that it is possible that the structures responsible for absorption at other wavelengths are formed at different rates. Nevertheless, the difference between these rates is probably not significant, considering that the increase in absorption occurs uniformly independent on absorption wavelength, as seen in Fig. 1.

Fig. 3 shows the absorption coefficients of D-Melanin as a function of wavelength. It is observed that with the increase in wavelength, the absorption coefficient of melanin decreases, being consistent with the results obtained for natural melanin [25–27]. The absolute values of the absorption coefficients of the D-Melanin are an order of magnitude greater than those reported by Sarna and Swartz for natural melanin [27]. Note that, for shorter wavelengths, the absorption coefficient is higher for D-Melanin synthesized at 25 °C compared to the material synthesized at higher temperatures.

Fig. 4(a) shows the FTIR spectra for D-Melanin synthesized at 25 °C and 100 °C. These spectra were taken after the synthesis was considered complete. Similar features are observed in intermediate synthesis temperatures. It is possible to observe that the products obtained exhibit similar functional groups, confirming that D-Melanin has a similar structure independent of the synthesis

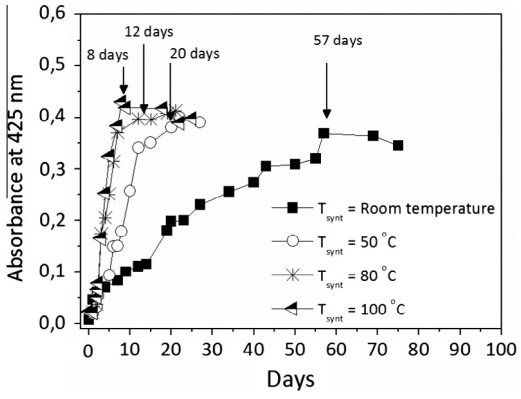


Fig. 2. Absorbance at 445 nm of D-Melanin solutions as a function of days for synthesis done at different temperatures. The arrows indicate the point at which the absorbance becomes constant, more details in the text.

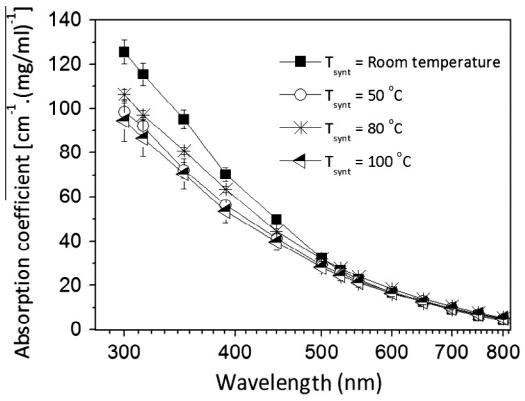


Fig. 3. Absorption coefficients as a function of wavelength for D-Melanin synthesized at different temperatures.

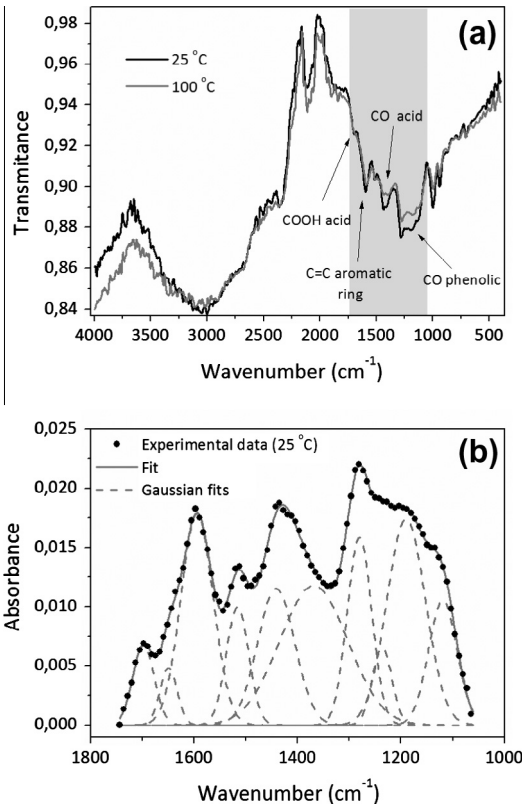


Fig. 4. (a) FTIR spectra for D-Melanin synthesized at different temperatures. The highlighted area is converted into absorbance in (b). (b) Absorbance spectrum for the synthesis at 25 °C, showing the least square fitted curves.

temperature. The bands between 1500 cm^{-1} and 500 cm^{-1} are related to $-SO_2CH_3$ groups present in the structure. The other bands are typical of natural and water synthetic melanin, as described in the literature [28].

The highlighted area in Fig. 4(a) corresponds to the stretching of: carbonyl group from carboxylic acid (1685 cm^{-1}), C=C from the aromatic ring (1596 cm^{-1}), C—O from ionized carboxylic acid (1427 cm^{-1}) and C—OH from phenolic or carboxyl OH (1180–1280 cm^{-1}) bands. It is possible to observe that he stretching bands related to carboxylic acid (C—O and —COOH) and phenolic groups (C—O/carboxyl OH) have a greater intensity for the D-Melanin obtained at RT.

Fig. 4(b) shows the results of spectral deconvolutions of the FTIR spectra of D-Melanin, in the carbonyl and C=C region, synthesized

at RT. A similar analysis was done for D-Melanin synthesized at 100 °C, using the same number and positions of the Gaussians absorption peaks in the fitting procedure.

In Table 1 the intensities of the individual vibration bands are presented for D-Melanin synthesized at 25 °C and 100 °C. The band corresponding to the stretching vibration of C=C (1600 cm^{-1}) of the phenyl structures was used to normalize the spectra.

Fig. 5(a) and (b) shows ^{13}C CP/MAS-NMR measurements for D-Melanins obtained at RT and 80 °C respectively. All signals were normalized to the integral area of the sulfonated carbon at 39.5 ppm. These spectra show broad resonances due to the polymer heterogeneity [29]. Due to the large number of resonances peaks that overlap, it is not possible to identify individual signals of melanin carbons. However previous works found in the literature have determined the protonated carbons, 95–114 ppm, and non-protonated regions, 110–147 ppm, using conventional CP/MAS combined with short-contact time CP/MAS and dipolar dephasing techniques [29–32]. Therefore the spectra shown in Fig. 5(a) and (b) can be divided in three main regions: (1) aliphatic groups (0–90 ppm) from unreacted L-dopa and carbon signals at 39.5–42.22 ppm) related with first and second sulfonation, respectively, of phenolic hydroxyls present in the synthesis process, (2) aromatic carbons (95–155 ppm), including pyrrole and indole groups (CH_x, C—O, C_2NH) and (3) carbonyl groups (160–200 ppm) from carboxylic acids and carboxyl of indolequinones (COO^- and C=O) [33,34].

The spectra shown in Fig. 5(a) and (b) are similar in shape, however the area of the carbons signals have differences depending on synthesis temperature, indicating structural changes in polymeric material. It is noteworthy that NMR spectra of D-Melanins are similar to those obtained for melanin synthesized in water, with the exception of the signals related to sulfonation of phenolic hydroxyls, in the regions of 39.48 and 42.22 ppm. One important indicator of structural changes induced by the synthesis temperature is associated with the signals at 90–150 ppm. As can be seen in D-Melanin prepared at 80 °C protonated carbons are absent (90–105 ppm). Furthermore, with the increase in the synthesis temperature there is an increase of the second phenolic hydroxyl sulfonation peak in 42.22 ppm [33,34].

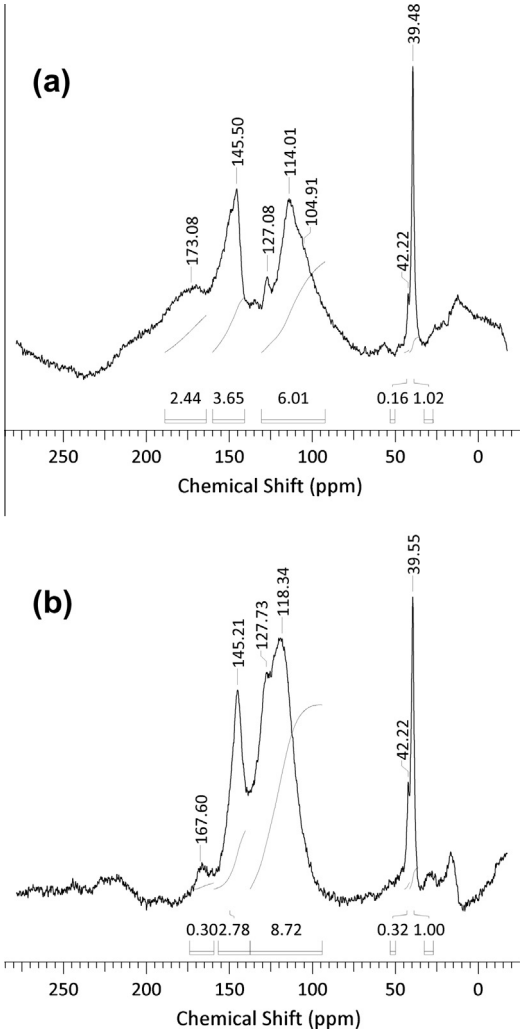


Fig. 5. ^{13}C CP/MAS NMR spectra of D-Melanins synthesized at room temperature (a) and 80 °C (b).

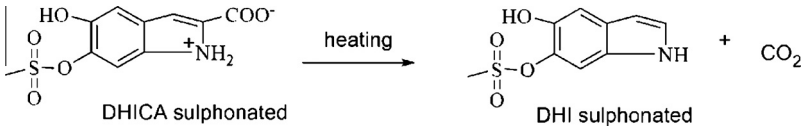
4. Discussion

In order to understand the effects of temperature on the synthesis and structure of D-Melanin we shall start with a discussion on the present knowledge of melanin synthesized in water or H-Melanin. The initial mechanism of H-Melanin synthesis, follows two paths briefly described here: L-dopa, is oxidized to form dopaquinone and then dopachrome, forming 5,6-dihydroxyindole,2-carboxylic acid (DHICA) or suffers a decarboxylative rearrangement to 5,6-dihydroxyindole (DHI) (route I); or L-dopa is oxidized to dopaquinone, cyclizes turning to cyclodopa and then dopachrome, forming DHI and DHICA (route II). H-Melanin is then formed from the oxidative polymerization of DHI and DHICA [1,28,35]. Young et al. determined the first-order rate constants for routes I and II at different temperatures [28]. It was found that in both cases, the kinetic constants increase with rising temperature and that the kinetic constants of route II is higher than those of route I, ~100 s^{-1} and 0.24 s^{-1} respectively, at 30 °C, indicating that the reaction occurs preferentially through route II. However, both routes are observed during synthesis and it is believed that route I is the limiting step in the overall polymerization velocity. In the present case, we are assuming that D-Melanin synthesis is similar to H-Melanin, especially in the initial stages, thus we believe that the effect of synthesizing D-Melanin at higher temperatures, is associated with an increase in the reaction velocity of the slowest step in the initial stages of the polymerization [28].

As discussed earlier, D-Melanin formation occurs through a complex reaction in which heterogeneous oligomers are obtained and oligomers of different sizes will affect the absorption coefficient at a given wavelength [24,25,36]. Moreover, the experimental conditions like temperature or oxygen content in the mixture can

Table 1
Intensity (I) of the fitted vibrational bands found in the FTIR spectra of D-Melanin synthesized at different temperatures. The intensities were normalized using the absorption band of the C=C aromatic ring.

Stretching mode	Peak position (cm^{-1})	Normalized intensity T_{synt} = 25 °C	Normalized intensity T_{synt} = 100 °C	$\frac{I(25\ °C)-I(100\ °C)}{I(25\ °C)}$ (in%)
C—O ionized carboxylic acid	1140/1370	1.866	1.294	30.7
C—OH phenolic	1180/1280	1.607	1.609	−0.1
Carbonyl group from carboxylic acid	1685	0.219	0.109	50.4



Scheme 2. Decarboxylation of DHICA sulfonated due to heating.

result in large differences in absorption coefficients as found for example between natural and synthetic melanin reported by different research groups [20,24,26,27]. This same argument explains the differences between H-Melanin and D-Melanin absorption coefficients. On the other hand, the results presented in Fig. 3 shows that the absorption coefficients above 500 nm are weakly dependent on synthesis temperature, indicating that the higher molecular weight oligomers are weakly dependent on synthesis temperature.

Peles et al. studied the absorption coefficient of bovine retinal pigment epithelial melanosome in order to understand how the absorption of melanin varies with molecular composition [36]. They found that the absorption coefficient is a function of the compositional ratio of the two monomeric units and the higher the absorption coefficient the greater the proportion of DHICA present [36]. This may explain the behavior for the absorption coefficient in Fig. 3, for wavelengths below 500 nm, if one assumes that as the synthesis temperature is increased, decarboxylation of sulfonated DHICA occurs, as expected from previous results in the literature [37] see Scheme 2.

Thus, for D-Melanins synthesized at 25 °C, the higher absorption coefficient can be associated to a higher concentration of DHICA [36]. FTIR and NMR findings support this hypothesis. Note that from FTIR the increase in temperature causes a decrease of the relative stretching of the carbonyl group from carboxylic acid of 50.4%, and from ionized carboxylic acid C—O bands of 30.7%. This is a clear indication of decarboxylation of D-Melanin monomers at higher temperatures. Concurrently, from NMR a large decrease, by a factor 8, in the C=O and COO^- absorption peaks is observed and the reduction of the signal C=O indole occurs concomitantly with the increase of sulfonation, by a factor of 2 (0:16 to 0:32). Therefore one possibility is that these decreases occur due to decarboxylation of DHICA. A great reduction of carbonyl groups is observed when the synthesis temperature is increase, confirming the absorption coefficient data, indicating a lower proportion of DHICA constituting the D-Melanin made at higher temperatures [29,31].

Our results indicate that the increase in temperature accelerates the synthesis for obtaining D-Melanin through a decrease in carboxyl groups in the final polymer, as well as in the synthesis of intermediate products. This hypothesis is supported by other results found in the literature. It is known that DHI is more reactive when compared to DHICA [8,38]. This higher reactivity is related in part to the favorable dimerization of DHI via the two-position of the indole ring, and thus polymerization via radical reactions [3,38]. Thus, as the reacting solution is heated, sulfonated-DHICA suffers decarboxylation forming sulfonated-DHI, which polymerizes more rapidly.

Finally, this study suggests that D-Melanin synthesized at 100 °C may be more suitable for use in organic electronics, since the synthesis is accelerated and the reaction is better controlled. More studies on the characterization of D-Melanins are being conducted in order to better elucidate the structure and electronic differences of D-Melanin prepared at different temperatures.

5. Conclusions

In this study we evaluated the influence of temperature in D-Melanin synthesis through the oxidative polymerization of L-dopa. We observed that the rise in the synthesis temperature up to 100 °C leads to an increase in the reaction velocity, decreasing the total time required for synthesis by a factor of 7 when compared to room temperature.

We propose that the mechanism for the increase in reaction velocity is due to the decarboxylation of DHICA during the synthetic process at higher temperatures, thus facilitating the polymerization of D-Melanin.

Acknowledgments

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