This article was downloaded by: [University of Haifa Library]
On: 13 August 2012, At: 20:33
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Thermal Annealing Effects on Synthetic Melanin

Pablo J. Gonçalves [a], Carlos A. Brunello [a] & Carlos F. O. Graeff [a]
[a] DFM-FFCLRP, USP, Av. Bandeirantes, Ribeirão Preto, 3900, Brazil

Version of record first published: 29 Oct 2010

To cite this article: Pablo J. Gonçalves, Carlos A. Brunello & Carlos F. O. Graeff (2002): Thermal Annealing Effects on Synthetic Melanin, Molecular Crystals and Liquid Crystals, 374:1, 39-44

To link to this article: http://dx.doi.org/10.1080/713738300

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Mol. Cryst. Liq. Cryst., Vol. 374, pp. 39-44
Copyright © 2002 Taylor & Francis
1058-725X/02 $12.00 + .00

Downloaded by [University of Haifa Library] at 20:33 13 August 2012

Thermal Annealing Effects on Synthetic Melanin

PABLO J. GONÇALVES, CARLOS A. BRUNELLO
and CARLOS F. O. GRAEFF*

DFM–FFCLRP–USP, Av. Bandeirantes 3900, Ribeirão Preto, Brazil

The influence of adsorbed moisture on the ESR signal of the free radical in synthetic melanin is investigated. Water is found to correspond to 10% to 20% of melanin mass in the as synthesized state. Water removal is responsible for an increase in spin density induced by extended heating at temperatures above 60 °C. This enhanced spin concentration is stable at room temperature but can be quenched by rehydratation. A systematic study on the time dependence of the spin enhancement is presented along with a macroscopic model based on rate equations.

Keywords: melanin, ESR, water, thermal annealing

I. INTRODUCTION

The black and brown colorations found so widespread in nature are generally attributed to melanins. In particular, the black melanin found in animals is thought to be an amorphous polymer based principally on the indole (5,6) quinone structural units. Aside from the photo-protective functions associated with its black color, other biological functions of melanin are only poorly understood. Similarly, neither the possible biological function nor structural origin is known for the free radical found to occur naturally in melanins. Although the featureless inhomogenously broadened electron spin resonance (ESR) signal associated with the free radical does not provide much solid information on the location or structure of the free radical site in the amorphous melanin polymer, it has been possible to observe the influence of a variety of factors on the density of free radicals, and the relaxation

* Corresponding author, e-mail: cfograeff@ffclrp.usp.br

parameters associated with these free radicals [1]. The effect of the various environmental perturbations on the ESR signal is greatly affected by the degree of hydratation of the sample. For example photoinduced ESR [2], photoconductivity [3], and electrical conductivity [4] are all reduced as the degree of hydratation of the melanin sample is reduced.

Both synthetic and natural melanins are formed in aqueous environments, although in-vitro solution studies may be useful in studying the role of adsorbed water, it appears that much can be learned from solid state hydration studies.

Downloaded by [University of Haifa Library] at 20:33 13 August 2012

II. MATERIALS AND EXPERIMENTAL DETAILS

The melanin samples were synthesized by auto-oxidation by standard techniques [1] from a starting mixture of (D)L-dopa and twice distilled de-ionized water. The water content of the wet samples was estimated from the measured weight loss after dehydration, as well as from Thermogravimetric data. Thermogravimetric data were registered on a Thermal Analyst equipment model 2100-TA in air atmosphere and at a heating rate of 10 K min-1. Electron spin resonance (ESR) spectra were obtained using a computer interfaced Varian E-4 spectrometer operating at 9.5GHz (X-band). g Values were obtained by reference to the standard diphenyl-ß-picrylhydrazyl (DPPH) signal. For the studies on the effect of heat treatments on the ESR signal, those were done inside the ESR resonance cavity to avoid sample re-positioning problems and thus increase the precision in signal changes.

III RESULTS AND DISCUSSION

Initially we had considered that water removal by a combination of 'pumping' and 'heating' processes would be sufficient for a qualitative study of the water effect in melanin; however, we noted that pumping alone, no matter how good the vacuum, had little effect on the ESR signal, while heating in high vacuum (10^{-5} mbar), or in a rough vacuum (lower than 10^{-2} mbar), or even in the absence of vacuum, produced a significant increase of the ESR signal. The fact that a high temperature is required for removal of the water interacting with the free radical indicates that this water is bound to the solid. The melanin free radical is relatively stable and is not destroyed by heating to temperatures above 300 °C. The polymer, however, will eventually break down and lose mass at elevated temperatures. In order to determine the high

temperature limit, we examined DOPA-melanin in a thermogravimetric (TGA) and differential thermal analysis (DTA) system. The results indicated that melanin is a relatively stable organic compound losing half of its mass just at about 800 K, and that about 20% of its mass is water [a].

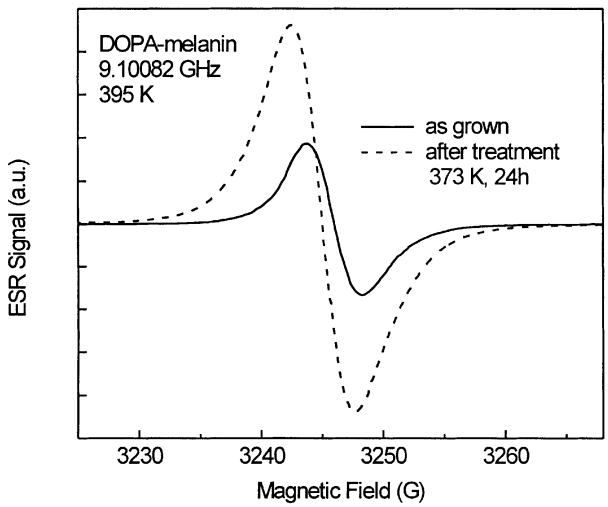

FIGURE 1. ESR spectra of as grown (solid line) and thermally treated (dashed line) DOPA-melanin samples at room temperature. The heat treatment consisted of letting the sample for 24 h at 373 K. Notice the increase in amplitude and shift in g-factor after the thermal treatment.

In Figure 1 the effect of heat treatment on the ESR signal is presented. As can be seen the spin density has increased by a factor of 3.5 after the heat treatment. The g-factor does also change after the heat treatment from (2.0032 ± 0.0001) to (2.0038 ± 0.0001), as well as the linewidth, which grows from (4.5 ± 0.1) G to (5.3 ± 0.1) G. The mass (weight) was also monitored during the process, decreasing by a factor of 22% after the treatment, in good agreement with the TGA data.

In order to further investigate the effects of water on the ESR signal, experiments were made on the rate of creation of ESR signal induced by the removal of water, when the sample is left above room temperature. The annealing temperatures used in this study were between 60°C and 100°C, however the spin density change was monitored at room temperature. Thus cycles of heating and cooling down to room temperature of typically 30 min. were used. Notice that

Downloaded by [University of Haifa Library] at 20:33 13 August 2012

for each series of measurement at a given treatment temperature a new sample was used.

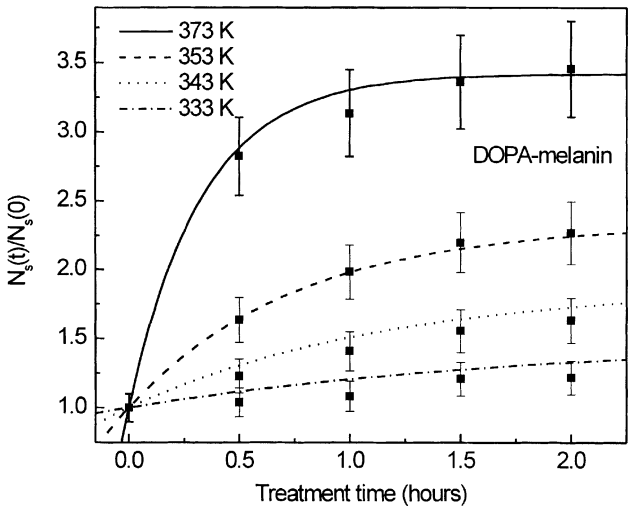

FIGURE 2. Normalized spin density of DOPA-melanin as a function of treatment time for different treatment temperature. All measurements were done at room temperature. The lines are fits using Equation 2.

The effect of these thermal treatments is shown in Figure 2, where the normalized spin concentration is shown as a function of treatment time, for different annealing temperatures. Dividing the spin concentration by the initial value did the normalization. The lines are the result of fitting procedures that will be explained in the following. As can be seen the spin density does vary with the temperature and treatment time. Important to notice is that for each temperature a different equilibrium N_s ($\tau \rightarrow \infty$) is found.

To explain such behavior we will develop a macroscopic model based on simple assumptions about the spin system, in the following we will discuss in more detail this model. We will assume that the spins responsible for the ESR signal can reversibly change from a paramagnetic to a non-paramagnetic state, and that they are not degenerate, or in other words one state has a lower energy than the other. It is assumed that the number of spins states is fixed, $N = N_p + N_n$, where N_p is the number of paramagnetic states, or number of spins that give rise to an ESR signal, and N_n is the number of non-paramagnetic states, ESR inactive. The transition from one state to the other can in this case

Downloaded by [University of Haifa Library] at 20:33 13 August 2012

be described by simple rate equation. For example, the rate of change of the paramagnetic state can be written as:

$$\frac{dN_p(t,T)}{dt} = \frac{N_n(t,T)}{\tau^*(T)} - \frac{N_p(t,T)}{\tau(T)}, \qquad (1)$$

where τ is the characteristic lifetime of the paramagnetic state and τ^* is the characteristic lifetime of the non-paramagnetic state. The second important assumption is that there are energy barriers to make a transition from one state to the other, in that case the characteristic times can be written as: $\tau = \tau_o \exp(E_a/kT)$ and $\tau^* = \tau_o^* \exp(E_a^*/kT)$.

As already mentioned different samples were used which not necessarily had the same initial number of spins, thus normalization was required. The solution of Equation 1, normalized is:

$$N'_p(t,T) = \frac{N'\tau'(T)}{\tau^*(T)} + \left(1 - \frac{N'\tau'(T)}{\tau^*(T)}\right)\exp\left(\frac{-t}{\tau'(T)}\right), \qquad (2)$$

where $N'_p(t,T) = N_p(t,T)/N_p(0, 300\ K)$, $N' = N/N_p(0, 300\ K)$ and $\tau' = \frac{\tau.\tau^*}{\tau^* + \tau}$.

Equation 2 was used in order to fit the results presented in figure 2, using a nonlinear least-mean square procedure based on Levenberg-Marquardt algorithm. It was found that the best parameters in Equation 2 are: $N' = 21 \pm 2$, $\tau_0 = (1 \pm 2)\ 10^{-3}$ s, $E_a = (0.46 \pm 0.05)$ eV, $\tau_0^* = (4 \pm 9)\ 10^{-6}$ s and $E_a^* = (0.69 \pm 0.06)$ eV.

The microscopic origin of the changes in spin density are discussed elsewhere [a]. Although most of our studies started with wet samples and concentrated on the changes found when water was removed, we noted that samples stored in a water vapor atmosphere would recover the original wet samples properties. The time required for this recovery depends on the concentration of water vapor, but in any event the rehydratation process is quite slow.

IV CONCLUSIONS

We have investigated the influence of absorbed moisture of the free radical properties of melanin and find additional free radicals can be induced by reduction of the fraction of strongly bound water. The removal of strongly absorbed water required incubation at high

Downloaded by [University of Haifa Library] at 20:33 13 August 2012

temperatures (≈ 70 °C). For the DOPA melanin prepared in water, water is found to correspond to 10% to 20% of melanin mass in the as synthesized state.

A systematic study on the time dependence of the spin enhancement with treatment temperature was performed. To describe the variations in the spin density in a quantitative way a simple rate equation is proposed. Rehydratation of samples, which have been dehydrated by high temperature treatment, results in quenching of the incremental free radical concentration. The time required for recovery back to the initial wet state is quite long (days, weeks)

ACKNOWLEDGMENT
The authors' thanks FAPESP, CNPq and CAPES for financial support.

References

[1] R.C. Sealy, Methods Enzymol. **105**, 479 (1984).
[2] E.M. Trukhan, V.N. Deryabkin, and M.A. Oskovskii, Biofizica **18**, 392 (1973).
[3] A.M. Potts and P.C. Au, Agressologie **9**, 225 (1968).
[4] M.M. Jastrzebeska, H. Isotalo, J. Paloheimo and H. Stubb, J. Biomat. Sci. Polymer Edn. **7**, 577 (1995).
[5] P.J. Gonçalves, C.A. Brunello, C.F.O. Graeff and M. Eisner, unpublished.

Downloaded by [University of Haifa Library] at 20:33 13 August 2012