

Photoacoustic studies of non-radiative relaxation of excited states in melanin

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Received February 4, 1989/Accepted in revised form September 7, 1989

Abstract. Photoacoustic measurements made at various chopping frequencies on dense aqueous melanin suspensions have revealed a significant dependence upon pH and redox state. It is shown that such behaviour, differing from the simple predictions of the Rosencwaig-Gersho theory, can be explained in terms of finite carrier diffusion and recombination times. The implications of these findings with respect to the amorphous semiconductor model proposed to describe the dynamic role of epidermal melanin are discussed. From the experimental data, values of physical parameters were calculated which allow a qualitative correlation between chemical states and electronic behaviour and, consequently, some aspects of the molecular biology of the melanosome, founded on a rigorous base.

Key words: Melanin, photoacoustics, electron relaxation

1. Introduction

Although different physiological roles have been suggested for the class of pigments collectively called melanins, depending upon their anatomical location in the organism, the most relevant one is the photoprotective function performed in the human body by epidermal melanin granules. However, despite a lot of experimental effort in this direction in the past twenty years, the understanding of the primary photoprocesses in the light excited melanin molecules is still unsatisfactory, mainly with respect to the events immediately following the electronic excitation, including both thermal relaxation and possible formation of triplet states.

The role of transient free radicals and the consequent modifications of the molecule (including interactions with oxygen) was exhaustively investigated and threw some light on this de-excitation channel (Chedekel 1982). In contrast, electronic relaxations via direct interaction with molecular vibration has received rather little interest, despite the suggestion, due to McGinness et al. (McGinness 1972; McGinness et al. 1974) that the molecular biology of the melanosome could be based on the amorphous semiconductor properties of melanin. As a consequence, electronic band structure (Crippa et al. 1978; Strzelecka 1982; Galvão and Caldas 1988) and electron-phonon interaction (McGinness and Proctor 1973) become central features in this subject.

The present work describes the applications of such ideas to a photoacoustic (PA) study of melanin under various conditions of pH and oxidation. This approach is based on the following assumptions:

- the bulk of the energy absorbed by melanin is released in radiation-less transitions as heat, so rendering PA spectroscopy particularly useful in the study these processes. The very weak fluorescence emission (Kozi-kowski et al. 1984) and the low yield of photochemical products (Felix et al. 1978) are assumed to be unimportant in the present analysis;
- the theoretical treatment of the PA effect in semiconductors has been recently extended to take into account finite carrier diffusion and recombination times (Miranda 1982). With the hypothesis that the melanosome behaves as some kind of "solid state" device at the cellular level, such parameters are essential to characterize the dynamic role of epidermal melanin;
- melanins are redox polymers containing high concentrations of o-quinone and o-hydroquinone groups and therefore show a pH dependent redox equilibrium. This effect is well known in the case of simple quinones and was discussed in several classical papers (Michaelis and Schubert 1938; Vetter 1952). We have

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studied the consequences of the possible redistribution of conjugated double bonds by investigating the effect of mild reduction at various pH values on the PA response in order to correlate variations of the extension of electronic delocalization with differences in carrier diffusion and recombination. In such a way we try to connect chemical properties with a physical description of the electronic behaviour.

2. Experimental methods

Materials

Synthetic melanin was obtained by auto oxidation of l-dopa(1-3,4-dihydroxyphenylalanine) in water at pH 9. After 48 h, the resulting black suspension was divided into three lots and the pH of each one was regulated with HCl and KOH to values of 3, 7 and 12 respectively. By incomplete evaporation at 50°C, dense samples of concentrated melanin were prepared and used immediately for the experiments. Reduced specimens were obtained by adding a slight excess of sodium dithionite.

All these manipulations were performed in air. As reported previously (McGinnes et al. 1979), a strong effect on the titration curves of melanins due to the absorption of CO₂ is observed at high pH. Measurements are in progress to evaluate the possible contribution of this reaction to our results.

Experimental apparatus

PA measurements were performed with an EG & G PARC cell model 6003 equipped with the preamplifier model 6005. The light source was an Ar laser (Spectra Physics mod. 2020) used in single line mode, with the power kept at about 600 mw. The blue ($\lambda=488$ nm) and the green ($\lambda=514.5$ nm) lines were used. The beam was modulated with an EG & G PARC model 192 variable frequency chopper in the range 40–350 Hz. These limits are dependent on the geometrical characteristics of the cell and were chosen in order to avoid: *a*) saturation of the signal due to 3 D effects (at low frequency) and *b*) deviation caused by Helmholtz resonances (at high frequency). This range was selected after previous measurements on black carbon samples, used also to test the linear response of the apparatus.

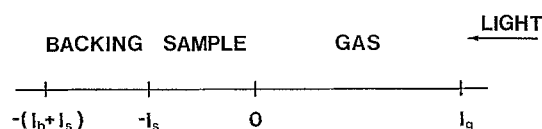


Fig. 1. Schematic geometrical configuration of a gas-microphone PA cell

The PA signal was revealed by a two phase digital lock-in amplifier (EG & G PARC model 5206). The control of the various experimental parameters and the data acquisition and elaboration were performed with a Commodore Amiga 1000 personal computer.

3. Theory

It has been established that the generation of the photo-acoustic signal may be calculated within the framework of a one dimensional heat diffusion theory (Rosencwaig and Gersho (R & G) 1976). The R & G theory assumes that the absorption of light by the valence electrons is immediately followed by non-radiative relaxations with yield 1. The fundamental parameters involved in this treatment are summarized in Table 1, together with the values used for melanin and air. Figure 1 shows a schematic of the geometrical configuration of the cell.

Assuming that the sample has an electronic energy band structure, the heat power density may be assumed to be proportional to the time derivative of the electron excess density in conduction band, $n(x, t)$:

$$Q_s(x, t) = \varepsilon h \nu \frac{\partial n(x, t)}{\partial t}. \quad (1)$$

$h \nu$ is the energy absorbed from the exciting field and $\varepsilon = \frac{\tau}{\tau + \tau_{ph}}$, where τ is the electron hole recombination time and τ_{ph} is the electron phonon collision time. Since $\tau_{ph} \ll \tau$, we have $\varepsilon \approx 1$.

With the previous assumptions we can write:

$$\frac{\partial n(x, t)}{\partial t} = \frac{\beta I_0}{h \nu} \exp \beta x \exp i \omega t \quad (2)$$

where $f = \omega/2\pi$ is the chopping frequency, ν is the frequency of the incident light and β is the absorption

Table 1. Fundamental parameters. The subscript $i=s, g$ and b denotes respectively sample, gas and backing parameters

	Melanin	Air
l = sample length (cm)	≈ 0.1	—
k_i = thermal conductivity (cal/cm s °C)	$1.5 \cdot 10^{-3}$	$5.7 \cdot 10^{-5}$
ρ_i = density (g/cm ³)	1.5	$1.29 \cdot 10^{-3}$
C_i = specific heat (cal/g °C)	0.61	0.24
$\alpha_i = k_i/\rho_i C_i$ = thermal diffusivity (cm ² /s)	$1.6 \cdot 10^{-3}$	0.19
$a_i = (\omega/2\alpha_i)^{1/2}$ = thermal diffusion coefficient (cm ⁻¹)	—	—
$\mu_i = 1/a_i$ = thermal diffusion length (cm)	—	—
β = optical absorption coefficient (cm ⁻¹)	—	—
$\mu_g = 1/\beta$ = optical penetration depth (cm)	—	—
D = carrier diffusion coefficient (cm/s)	—	—
I_0 = intensity of incident light (erg/scm ²)	—	—

coefficient of the sample. It is assumed that the Lambert-Beer law for absorption is obeyed.

Let us finally consider the model proposed by Miranda (1982), in order to describe a semiconductor specimen: once the electrons are excited in the conduction band, they undergo the following de-excitation processes:

- radiative recombination with the holes left in the valence band;
- non-radiative recombination through interaction with vibrational modes.

Superimposed on both processes is the diffusion through the sample.

The excess electron Eq. may thus be written:

$$\frac{\partial n}{\partial t} = -\frac{n}{\tau} + D \frac{\partial^2 n}{\partial x^2} + \frac{\beta I_0}{h\nu} \exp \beta x \exp i \omega t \quad (3)$$

The solution of this equation with the appropriate boundary conditions leads to the expression for $Q_s(x, t)$. The heat diffusion equation can then be solved with the usual techniques giving the expression for the temperature in the sample, backing and gas.

The gas piston model of R & G gives the fluctuation of the pressure amplitude in the gas:

$$\delta P(t) = Q \exp i \left(\omega t - \frac{\pi}{4} \right) \quad (4)$$

with

$$Q = \frac{\gamma P_0 \theta}{\sqrt{2} l_g T_0 a_g} \quad (5)$$

where P_0 and T_0 are ambient pressure and temperature, l_g is the gas length and θ is the temperature amplitude in the gas at $x=0$.

For an optically opaque ($\mu_\beta \ll l$) and thermally thick ($\mu_s \ll l$) semiconductor, the expression for θ becomes:

$$\theta \approx -\frac{1}{1+g} \left\{ (r-1) B + \frac{-i \omega \beta^2 I_0}{k_s (\beta^2 - \alpha^2) D \alpha (\alpha^2 - \sigma_s^2)} \cdot \left[\lambda - \frac{\exp l \alpha + \exp (-l \alpha)}{\exp l \alpha - \exp (-l \alpha)} \right] \right\}$$

with:

$$B = \frac{i \omega \beta I_0}{k_s (\beta^2 - \sigma_s^2) D (\beta^2 - \alpha^2)}$$

$$\alpha^2 = \frac{(1+i \omega t)}{D \tau}$$

$$\sigma_s = (1+i) a_s$$

$$g = \frac{k_g \sigma_g}{k_s \sigma_s}$$

$$r = \frac{\beta}{\sigma_s}$$

We report also the expression for θ in the approximation studied by Miranda ($\mu_\beta \ll l$, $\mu_s \ll l$, $\mu_s > \mu_\beta$):

$$\theta \approx \frac{i \omega I_0}{k_s D \alpha (\alpha^2 - \sigma_s^2)} \left[\lambda - \frac{\exp l \alpha + \exp (-l \alpha)}{\exp l \alpha - \exp (-l \alpha)} \right] \quad (7)$$

Equation (6) is more general than the one obtained by Miranda since it makes no assumptions about the relative magnitude of μ_β and μ_s . However, in the case of strongly absorbing samples, for which at every chopping frequency $\mu_\beta \ll \mu_s$ (6) and (7) yield indistinguishable results. In this case (7) is preferable since it is more simple. The expressions (4), (5) and (6) (or (7) in the approximation of Miranda) give the amplitude of the PA signal. By varying the chopping frequency and recording the PA signal amplitude, we obtain data to be compared with these theoretical expressions. Fitting of these data allows us to obtain information on the electron diffusion and absorption parameters for melanin. We have used the chi-square minimization program MINUIT from CERN-LIB to perform such a calculation.

4. Results

It has been reported (Gallas et al. 1987) that the dependence of the PA signal on frequency for synthetic melanin powder, calculated from a log-log plot, follows a straight line with slope-1 in agreement with the R & G theory. Control measurements performed in our laboratory on natural (from equine eyes and melanoma) and synthetic melanin (both from l-dopa and dopamine) in the form of powder or pastille, confirm these results only to some extent. A rather broad range of slopes was found, ranging from -0.9 for acid extracted melanoma melanin to -1.1 for the synthetic preparation used for the experiments reported in the present paper.

Such data may be contrasted with those which we now report for dense aqueous suspensions of synthetic l-dopa melanin at various pH values. Figure 2 reports the results of a first experiment. It clearly shows a different slope for the data obtained with samples at pH 3 and pH 7 (both with a slope of -1.25) with respect to the data at pH 12 (with a slope of -0.95). The apparent displacement in the pH 3 line at $\log f = 2.34$ is due to a scale change on the lock-in amplifier and happens in some other plots both at low and high frequencies. No significant difference was noticed between experiments performed under blue and green light. Therefore we have reported here only the data obtained with $\lambda = 514.5$ nm. Figure 3 shows the results of the series of measurements on samples reduced with sodium dithionite. A quite different behaviour is evident in these cases: the experimental points cannot be

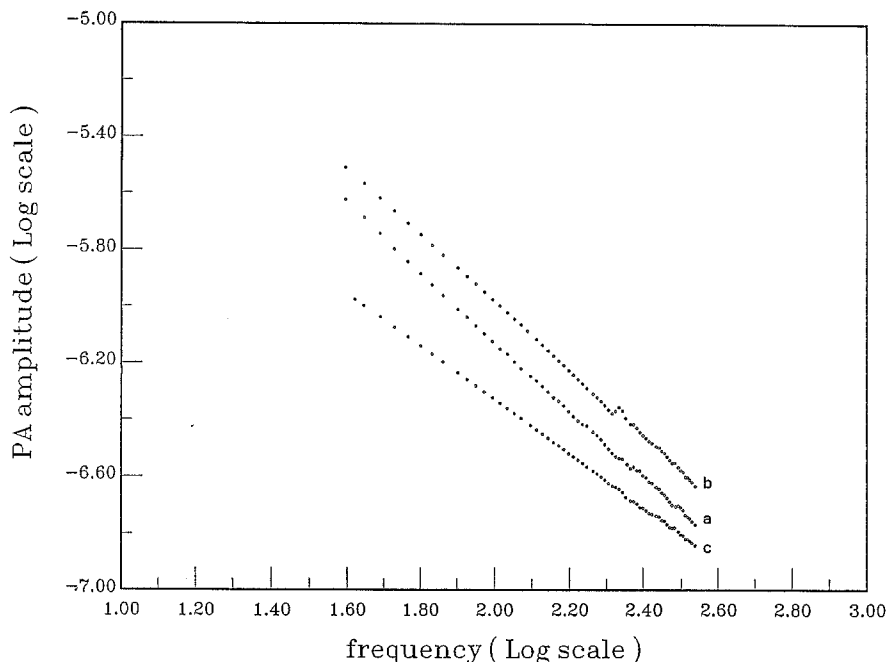


Fig. 2. PA intensity vs. chopping frequency of synthetic melanin suspension at various pH. *a*) pH 3 (slope = -1.25); *b*) pH 7 (slope = -1.25); *c*) pH 12 (slope = -0.95)

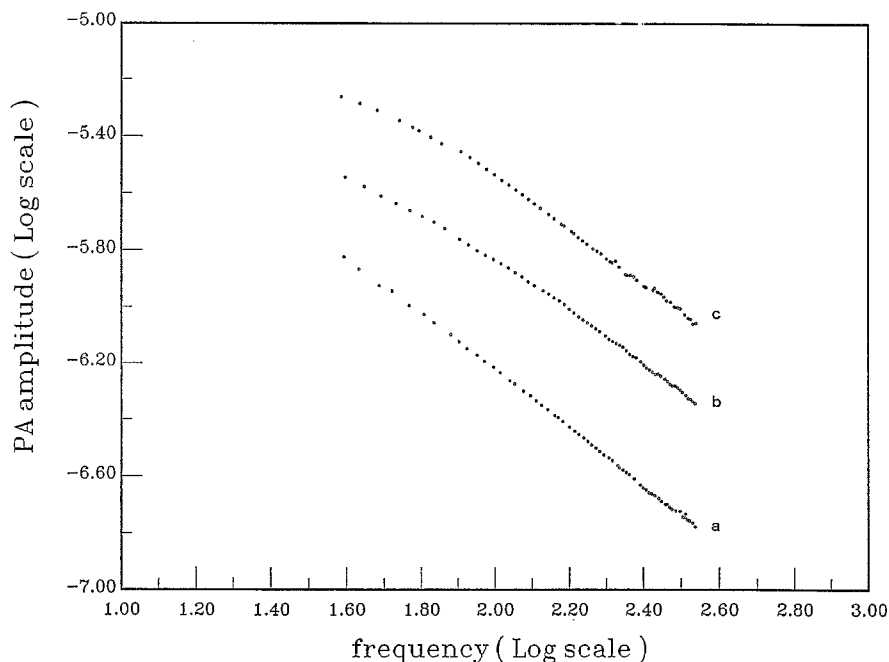


Fig. 3. PA intensity vs. chopping frequency of synthetic melanin suspensions at various pH after reduction with sodium di-thionite. *a*) pH 3; *b*) pH 7 and *c*) pH 12

fitted by straight lines because for frequencies lower than about 100 Hz a saturation effect appears. In any case, if one considers the range 100–350 Hz as linear, the slope of the interpolating lines is about 0.66, so raising the problem of the applicability of the simple R & G treatment to these data.

5. Discussion

On the basis of our experimental data we now evaluate the role of pH and oxidation state on the PA response

of melanin, including some implications related to its physical and chemical properties.

Despite the significant differences between well characterized synthetic polymeric quinones and the irregular and scarcely known melanin molecules, we can base a part of our interpretation on the data reported in the Lindsey review (1974) on polyquinones. In particular we must consider the factors affecting the mid-point potentials in potentiometric titration curves, that should play a role in electron transfer reactions in melanins. Among others, particularly relevant are: pH, presence of semi-quinones and quinidrones,

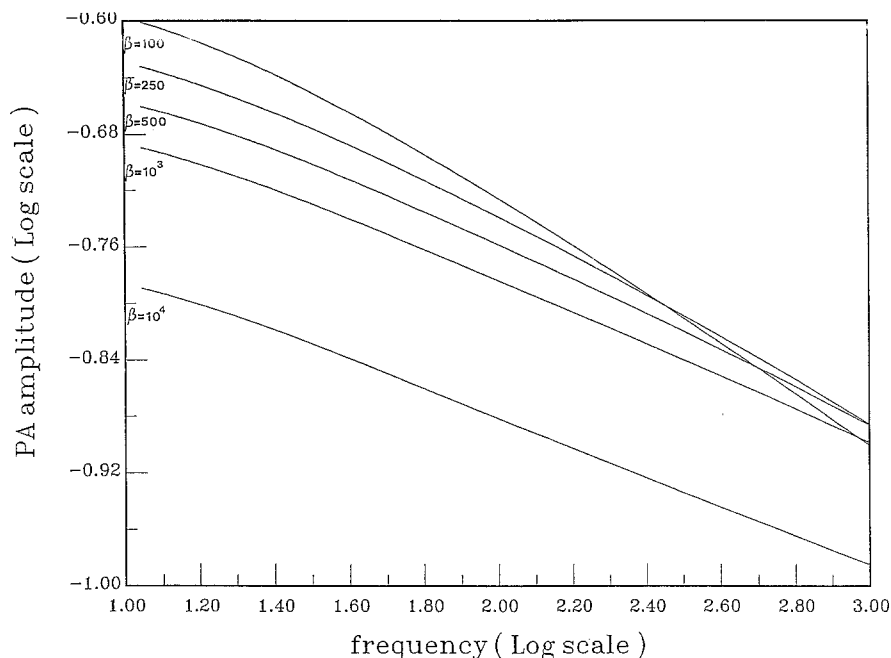


Fig. 4. Calculated PA intensity curves vs. frequency for various values of β (shown in the figure) for an optically opaque ($\mu_\beta \ll l$) and thermally thick ($\mu_s \ll l$) sample. The values $D = 4.9 \cdot 10^{-2} \text{ cm}^2 \text{ s}^{-1}$ and $\tau = 9.7 \cdot 10^{-3} \text{ s}^{-1}$ have been used in the calculation

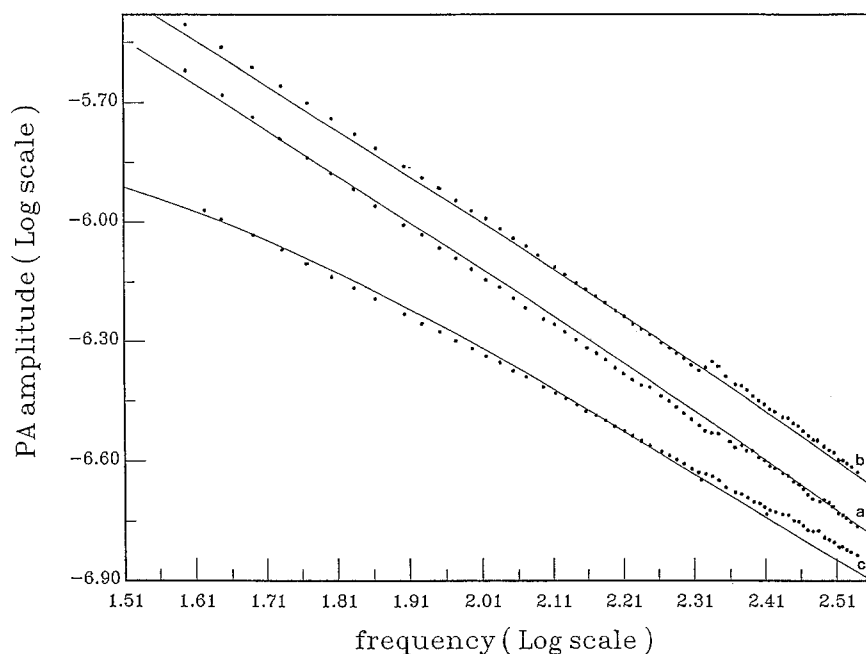


Fig. 5. Fitting of the experimental points of Fig. 2 using (6). *a*) pH 3 ($\chi^2 = 10^{-4}$); *b*) pH 7 ($\chi^2 = 10^{-4}$) and *c*) pH 12 ($\chi^2 = 10^{-3}$). The values of the parameters used in the calculations are listed in Tables 1 and 2

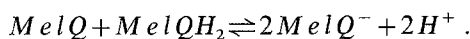
electrostatic field effects typical of polyelectrolytes and solubility variations due to reversible aggregation processes. Such a complexity is reflected in hydrogen ion titrations and involves the modulation effect on the pK_a of the surface functional groups, dependent on the electronic state (McGinness et al. 1979). For a quantitative discussion of the electronic behaviour of melanins we assume the presence of a dynamic distribution of extended regions of conjugate double bonds and of localized net charges on ionizable groups. Both the carrier diffusion coefficient, D , and the recombination time, τ , that can be evaluated by PA measure-

ments are supposed to be dependent upon this chemical situation.

Let us discuss firstly the limits of applicability of our theoretical model. For an optically opaque and thermally thick sample, the frequency dependence of the PA signal is shown in Fig. 4 for various values of β , corresponding to different physical states (namely $\mu_\beta > \mu_s$, $\mu_\beta < \mu_s$ and intermediate cases where $\mu_\beta < \mu_s$ at low frequencies and $\mu_\beta > \mu_s$ at high frequencies). These plots present an interesting feature. The high frequency limiting slope of the curves increases as β decreases until a value about -1.5 , corresponding to a $\beta \leq 10^2$.

In contrast, for high values of β , the limiting slope becomes -1 and the shape of the curves doesn't change when β is higher than 10^3 to 10^4 (saturating values).

Figure 5 shows the results of the parametric fitting of the experimental data at different pH values by the use of (6). To fit the points with higher slope (pH 3 and pH 7), a lower value of β was adopted compared to the data obtained at pH 12. In this case, for the entire frequency range, $\mu_\beta \ll \mu_s$ (see Tables 1 and 2). At present it seems rather difficult to justify such large variations of the optical absorption coefficient. It is well known that the melanin free radical population depends on pH through the equilibrium (Sealy et al. 1980)



We can suggest that the consequent redistribution of π -electrons increases the concentration of optical absorbers, but the lack of detailed chemical data does not allow any quantitative correlation.

Table 2. Parameters from fitting

pH	β (cm ⁻¹)	D (cm ² s ⁻¹)	τ (s ⁻¹)	μ (cm ² V ⁻¹ s ⁻¹)
Oxidized forms				
3	500	$0.49 \cdot 10^{-1}$	$0.18 \cdot 10^{-1}$	17
7	500	$0.5 \cdot 10^{-1}$	$0.29 \cdot 10^{-1}$	17
12	$\geq 10^4$	$0.95 \cdot 10^{-2}$	$0.3 \cdot 10^{-2}$	3.3
Reduced forms				
3	$\geq 10^4$	$0.49 \cdot 10^{-1}$	$9.7 \cdot 10^{-3}$	17
7	$\geq 10^4$	$0.95 \cdot 10^{-2}$	$1.5 \cdot 10^{-3}$	3.3
12	$\geq 10^4$	$0.99 \cdot 10^{-2}$	$2.3 \cdot 10^{-3}$	3.4

In melanins, the only chemical groups sensitive to high pH are the phenolic hydroxyls that have a pK_a around 10.5. Their ionization greatly accelerates the oxygen consumption rate (Sarna et al. 1980). Simultaneous electrochemical-ESR studies under alkaline conditions (Lukiewicz et al. 1980) demonstrated the role of high pH in shifting the redox equilibrium of melanin oxidizable groups towards increased amounts of forms in the one electron state of reduction. Moreover, the ionization state can influence the hydrated structure of the macromolecule and consequently the redistribution of hydrogen bonds.

The intervention of such structural, configurational and environmental factors does influence both thermal and optical properties. Gallas et al. (1987) were able to reveal, in acid precipitated dry melanin, a transition between a thermally thick solid where $\mu_s > \mu_\beta$ and a thermally thin solid where $\mu_s < \mu_\beta$, with values of thermal diffusivity two orders of magnitude lower than the value adopted by Wissler (1976) for eye choroid. They attribute this behaviour to acid aggregation of the polymer due to the change of the ionization state and, consequently, of the binding of water.

Table 2 reports the calculated values of the parameters β (absorption coefficient), D (carrier diffusion coefficient) and τ (electron hole recombination time). The biophysical relevance of such parameters must be emphasized as one of the central points in the proposed mechanism of the non-radiative de-excitation process in pigment cells. The values of τ are long enough to allow the decay of the excited carriers through phonon collisions ($\tau_{ph} \approx 10^{-12}$ s). This de-excitation channel can thus be favoured as was suggested by McGinness and Proctor (1973). The carrier diffusion coefficient

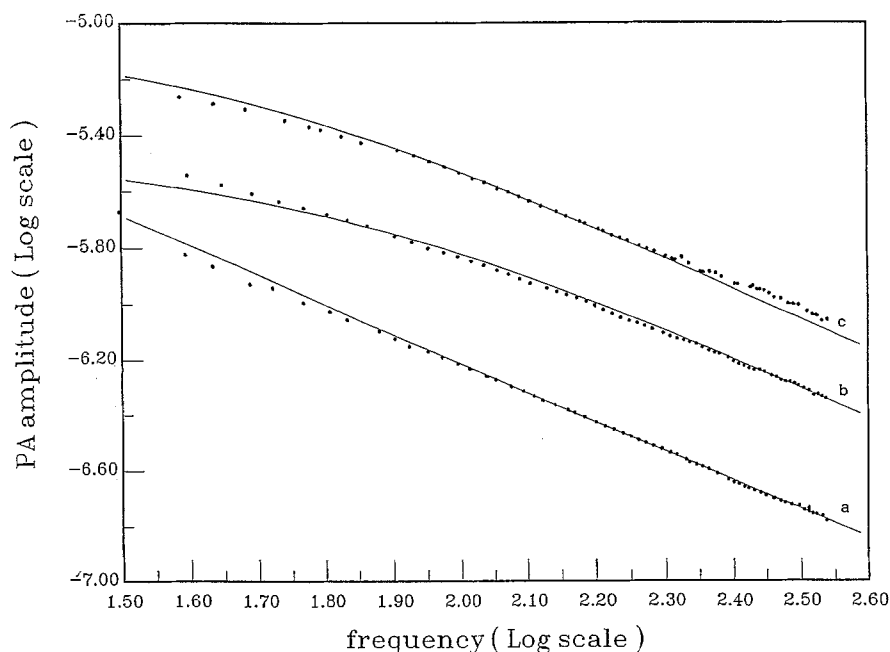
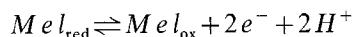


Fig. 6. Fitting of experimental data of Fig. 3 using (7). a) pH 3 ($\chi^2 = 10^{-6}$); b) pH 7 ($\chi^2 = 10^{-4}$) and c) pH 12 ($\chi^2 = 10^{-4}$). The values of the parameters used in the calculations are listed in Tables 1 and 2

allows one to estimate the carrier mobility μ through the Einstein relation, $D = k_B T \mu / e$, giving the values reported in the table for the various samples. These values fulfill the criterion for the applicability of the band theory to organic molecular solids (Bright et al. 1974) and can be compared with the data reported by Trukhan et al. (1970) that estimated a value for the mobility of $15 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for hole conductivity in melanin granules extracted from the pigment epithelium of frog eyes.

Table 2 reports also the values of the various parameters calculated by fitting of the data shown in Fig. 6 for reduced samples at various pH values by means of (7). Although the redox equilibrium



is considered generally valid, the exact proportions of quinols and quinones within the melanin molecule are not known with certainty, even if a general agreement exists in considering the stable state of isolated melanin as practically fully oxidized (Horak and Gillette 1971). However, the true state of oxidation within the cell is unknown and can depend upon different physico-chemical factors, including UV and visible absorption. It is therefore very interesting that significant variations in electronic and optical properties of melanins are dependent on the chemical state of their environment. The biophysical implications of such an effect are of concern for many cellular processes.

For example:

- the UVA dependent immediate pigment darkening of the human skin in the presence of oxygen, a defense mechanism probably based on a passive photochemical reaction (Hönigsmann et al. 1986), as suggested by the higher values of β in the case of oxidized samples;
- the pH dependence of the thermal diffusivity in pigmented tissues such as retinal pigment epithelium in the case of retinal light damage (Gallas et al. 1987);
- the proposed mechanism of energy storage in neuro-melanin, that can be influenced by ionic and electronic gradients produced by pigmented neurons (McGinness 1985).

Acknowledgements. The authors wish to acknowledge Dr. G. Melegari and Dr. G. Rivera for their helpful collaboration. This work was partially supported by Ministero della Pubblica Istruzione, Italy.

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