Optical properties of melanin in the skin and skin-like phantoms

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ABSTRACT

Experimental study and computer modeling were used to investigate the optical properties of melanin in the skin and skin-like phantoms. To investigate light scattering by melanosomes in skin we made skin-like phantoms on the base of gelatin with different content of melanin particles. Spectra of total transmittance and diffuse reflectance of the phantoms were obtained in the wavelength range from 400 to 800 nm. Absorption and reduced scattering coefficients of melanin were calculated. Mie theory was used to estimate the optical properties of melanin particles. Wavelength dependence of refractive indices of eumelanin particles (isolated and purified from the ink of the cuttlefish *Sepia officinalis*) and *synthetic* melanin particles was estimated.

Keywords: Melanin, optical properties, light scattering, skin, inverse problem, phantoms

1. INTRODUCTION

Melanins are the biological pigments ranging in color from the yellow and red-brown pheomelanins to the brown and black eumelanins. They are found in skin, hair, eyes, retina etc. Skin melanin attenuates incident UV and visible radiation. There are many papers describing the physical and chemical properties of melanins. [1-8] There is a point of view that photoprotection effect of melanin is connected with light absorption. However, it is well-known that melanin contains in skin as grains with size from 30 to 400 nm.[2] In addition melanin has a higher refractive index than surrounding medium and, as a consequence, the melanin grains strongly scatter light. Knowledge of optical properties of melanin grains is necessary for diagnostics of skin disease, dosimetry of laser radiation in phototherapy and photoprotective compounds development.

This paper is devoted to study of the optical properties of *natural* (isolated and purified from the ink of the cuttlefish *Sepia officinalis*) and *synthetic* melanins.

2. MATERIALS AND METHODS

In this study we used a commercially available computer-driven CARY-2415 spectrophotometer with integrating sphere to make measurements of total transmittance, and diffuse reflectance in the 340-800 nm wavelength range for various turbid media (phantoms) which model living tissues. For each phantom diffuse reflectance and total transmittance were measured. The diffuse reflectance were calibrated on the basis of reflectance from standard reflectance plate (BaF_2).

We used transparent gel, prepared from 10% aqueous solution of a food gelatin, as a ground of the phantoms. Structure of gelatin gel was described in Ref. 9. Gel was done turbid by adding of various quantities of melanin particles. In our study we used *natural* eumelanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis* (Sigma, M-2649) and *synthetic* melanin particles (Sigma, M-0418). *Synthetic* melanin was prepared by oxidation of tyrosine with hydrogen peroxide. Concentrations of melanin particles within samples were 0 mg/ml, 0.5 mg/ml, 1 mg/ml, and 2 mg/ml for *natural* melanin and 0 mg/ml, 0.1 mg/ml, 0.2 mg/ml, and 0.4 mg/ml for *synthetic* melanin. The samples were prepared as tablets with diameter of 25 mm and height of 4 mm for *synthetic* melanin and height of 3.75 mm (0 mg/ml), 3.81 mm (0.5 mg/ml), 3.78 mm (1 mg/ml), 3.59 mm (2 mg/ml) for melanin from *Sepia officinalis*. The samples were placed on thin glass plates with thickness of 0.17 mm.

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Electron microscopy was used to determine sizes of the melanin particles. These measurements were performed both for melanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis* and *synthetic* melanin particles. Melanin suspension was prepared using distilled water. A drop of suspension was placed on a grid with carbon back. During 1-2 minutes the grid was drying and after that it was observed in electron microscope JEM-7A at accelerated voltage 80 kV.

Figures 1 and 2 show typical electron-microscope photographs of *natural* and *synthetic* melanin particles. They show the size difference in melanin particles and their generally spherical shape. The particle size distributions are presented in Figs. 3 and 4. The mean value and standard deviation are 97.2 (30.2) nm and 127 (55) nm for melanin particles obtained from *Sepia officinalis* and *synthetic* melanin particles, respectively.

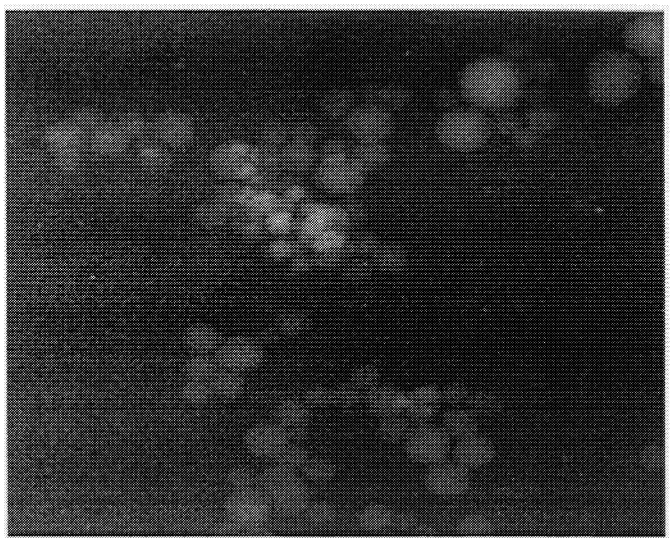

Figure 1. Typical electron microscope photograph of *natural* melanin particles. (magnification x22000)

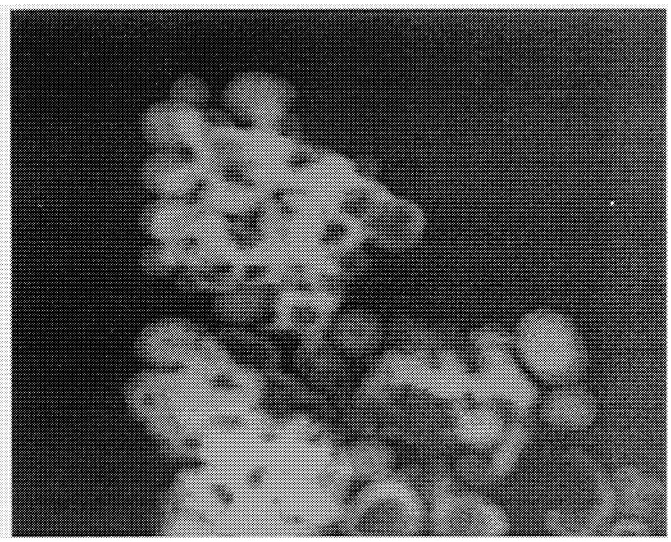

Figure 2. Typical electron microscope photograph of *synthetic* melanin particles. (magnification x19000)

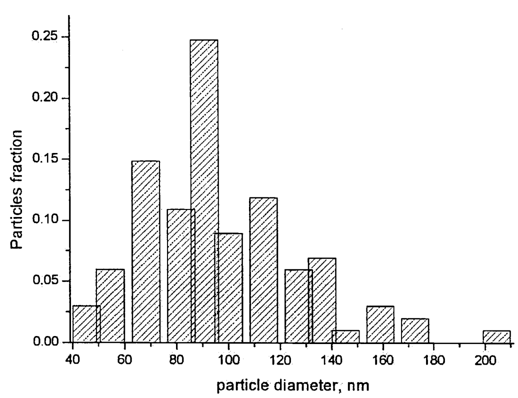

Figure 3. *Natural* melanin particles size distribution determined by electron microscopy.

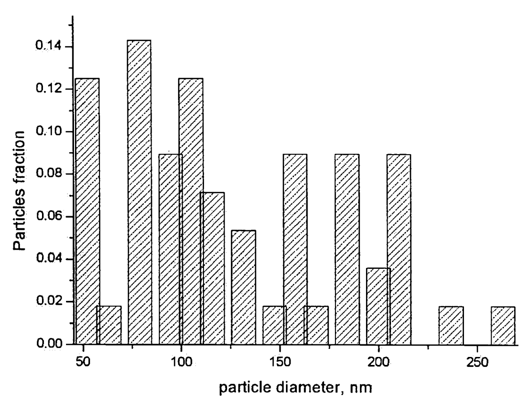

Figure 4. *Synthetic* melanin particles size distribution determined by electron microscopy.

Mie theory provides an exact solution for finding the scattering coefficient and the anisotropy factor of perfect spheres of arbitrary size.[10,11] To calculate optical properties of spherical particles it is necessary to know diameter of the particle and refractive indices of sphere (particle) and surrounding medium. The mean diameter of particles we obtained using electron microscopy data. We also assume that surrounding medium has refractive index equal to refractive index of water. Wavelength dependence of refractive index of water is defined as:[12]

$$n_{H_2O}(\lambda) = 1.31848 + \frac{6.662}{\lambda[nm] - 129.2} \quad (1)$$

Refractive index of *synthetic* melanin particles is 1.65 at wavelength 550 nm.[2] Refractive index of *natural* melanin particles is 1.7 at wavelength 589 nm.[4]

We used the inverse adding-doubling method developed by *Prahl et al.*[13] to calculate the absorption and reduced scattering coefficients of turbid media (phantoms) from the measured values of the total transmittance and diffuse reflectance. To obtain optical properties of the investigated phantoms we used a computer program of *S.A. Prahl* (Oregon Medical Laser Center, USA; www.omlc.ogi.edu).

The reduced scattering coefficient can be calculated using the following formula:[14]

$$\mu_s' = N_0 \pi \frac{d^2}{4} Q_{sca}\left(n_{ex}, n_{in}, d, \lambda\right) \cdot \left(1 - g\right), \tag{2}$$

where d is diameter of particles, N_0 is the number of particles in the unit volume, g is the average cosine of the scattering angle (anisotropy factor), λ is the wavelength, n_{ex} is the refractive index of the surrounding medium, n_{in} is the refractive index of the scatterers and $Q_{sca}(n_{ex}, n_{in}, d, \lambda)$ is the efficiency factor for scattering. N_0 can be calculated as:

$$N_0 = \frac{\eta}{\frac{\pi d^3}{6}} \tag{3}$$

where η is the volume fraction of the scatterers.

Knowing the diameter of particle and the values of refractive indices of scatterer and surrounding medium we can calculate the value of efficiency factor for selected wavelength. Equation (2) allows us to use the reduced efficiency factor for scattering instead of reduced scattering coefficient.

As the mean diameter of scatterers (melanin particles) is known and we assume that refractive indices of surrounding medium is equal to refractive indices of water, then we can reconstruct refractive index of scatterers for the investigated spectral range. For that we have developed the computer program. It uses algorithm described in Ref. 10 for solving of direct problem. To minimize target function our program uses *complex* method described in Ref. 15. This program allows to reconstruct the value of refractive index of spherical scatterer at selected wavelength if diameter of scatterer and refractive index of surrounding medium are known. The example of program test is shown in Table 1.

We calculated the optical properties for the scatterer (column 4-6) on the base of algorithm descibed in Ref. 10 using parameters in column 1-3 and setting the scatterer diameter equal to 0.1 μm. Then we solved the inverse problem knowing the scatterer diameter and data from column 1, 2, 6, and reconstructed the optical properties of the scatterer (column 8-10). It is well seen they are in a good agreement with data used for direct problem solving (column 3-6).

Table 1.

Input parameters			Calculated parameters			Reconstructed parameters			
wavelength, nm	refractive index of the surrounding medium	refractive index of the scatterer	efficiency factor	anisotropy factor	reduced efficiency factor	reduced efficiency factor	efficiency factor	anisotropy factor	refractive index of the scatterer
1	2	3	4	5	6	7	8	9	10
350	1.34865	1.70469	0.11176	0.26991	0.0816	0.0816	0.11176	0.26991	1.70469
400	1.34308	1.67032	0.06094	0.19983	0.04877	0.04877	0.06094	0.19983	1.67032
500	1.33645	1.60653	0.01879	0.1231	0.01648	0.01648	0.01879	0.1231	1.60657
600	1.33263	1.54881	0.00612	0.0835	0.00561	0.00561	0.00612	0.0835	1.54875
700	1.33015	1.49659	0.00203	0.06024	0.00191	0.00191	0.00203	0.06024	1.49662
800	1.32841	1.44933	6.46E-4	0.04541	6.1666E-4	6.1667E-4	6.46E-4	0.0454	1.44935

3. RESULTS AND DISCUSSION

In Figs. 5, 6 it is presented the diffuse reflectance and total transmittance spectra of experimental samples (gelatin phantoms with various content of *natural* melanin). In these figures it is seen that with increasing of melanin concentration in samples the diffuse reflectance is risen and total transmittance is decreased. Consequently, melanin has not only the absorption properties, but the scattering ones. In the case of *synthetic* melanin the situation is quite differed. Figs. 7, 8 present the diffuse reflectance and total transmittance spectra of the experimental samples (gelatin phantoms with various content of *synthetic* melanin). Fig. 7 shows that with increasing of melanin content in the samples the reflectance is decreased.

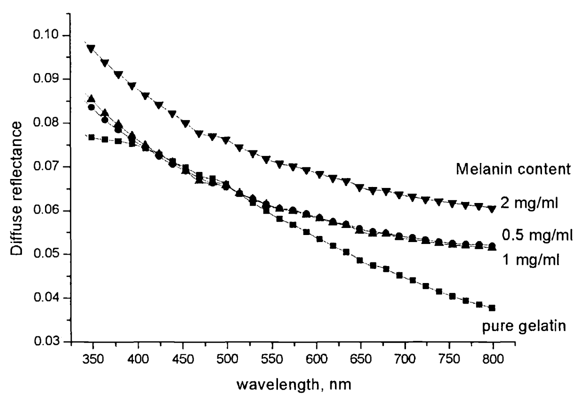

Figure 5. Diffuse reflectance spectra of the experimental phantoms with various content of melanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis*

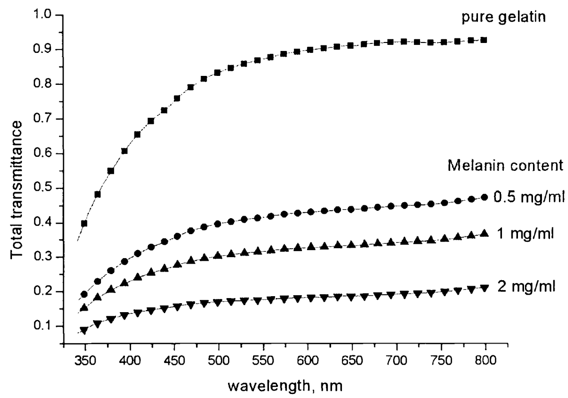

Figure 6. Total transmittance spectra of the experimental phantoms with various content of melanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis*

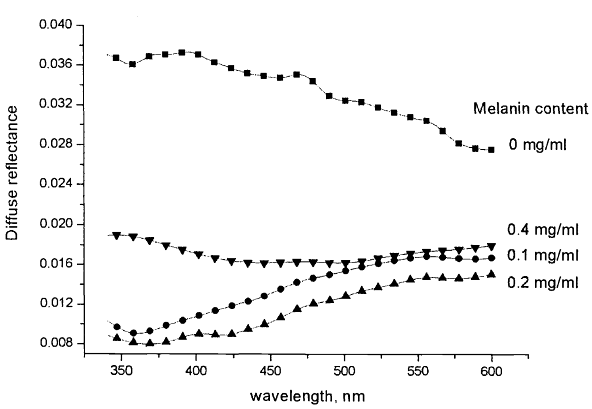

Figure 7. Diffuse reflectance spectra of the experimental phantoms with various content of *synthetic* melanin particles

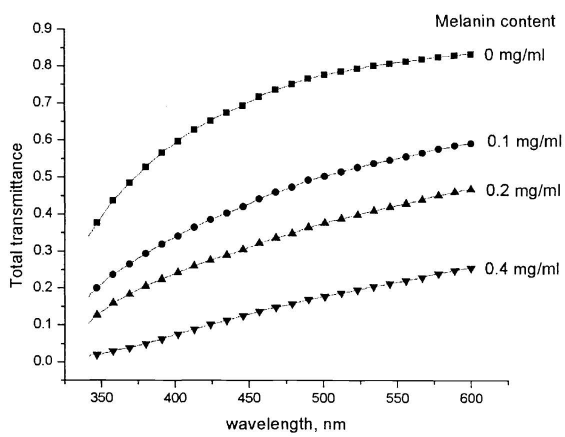

Figure 8. Total transmittance spectra of the experimental phantoms with various content of *synthetic* melanin particles

To study the optical properties of the *natural* and *synthetic* melanins we have calculated the absorption and reduced scattering coefficients of the investigated samples using inverse adding-doubling method. Results of our calculations are presented in Figs. 9, 10 (*natural* melanin) and Figs. 11, 12 (*synthetic* melanin). Figs 9, 11 show that with increasing melanin content in the investigated sample the absorption coefficient of the sample is also increased. From Figs 10, 12 it is follows that with increasing melanin concentration the reduced scattering coefficient is increased.

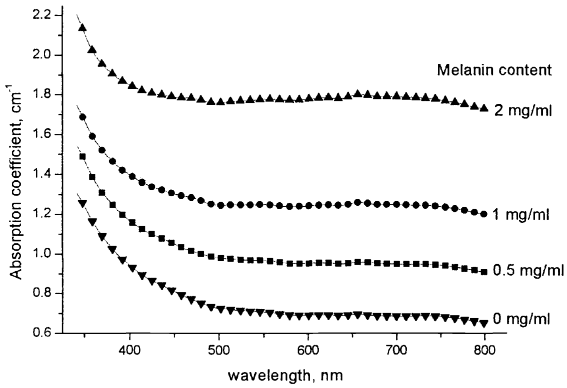

Figure 9. Spectra of absorption coefficient of the experimental phantoms with various content of the melanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis*

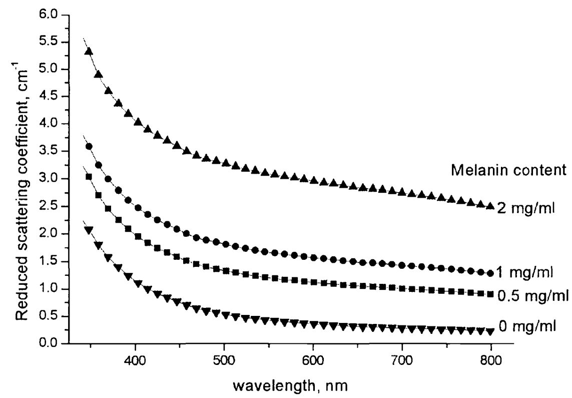

Figure 10. Spectra of reduced scattering coefficient of the experimental phantoms with various content of the melanin particles isolated and purified from the ink of the cuttlefish *Sepia officinalis*

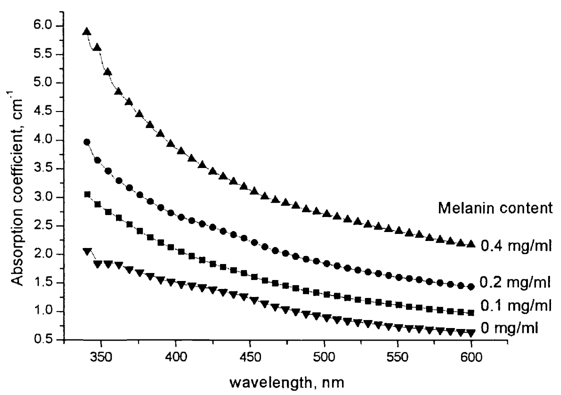

Figure 11. Spectra of absorption coefficient of the experimental phantoms with various content of the *synthetic* melanin particles

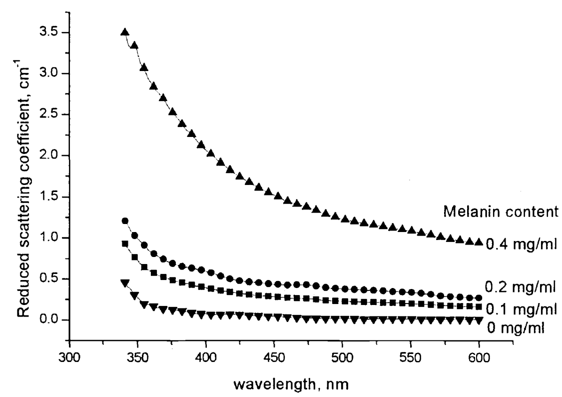

Figure 12. Spectra of reduced scattering coefficient of the experimental phantoms with various content of the *synthetic* melanin particles

Using data presented in Figs. 10, 12 we have calculated the absorption coefficient (Fig. 13) and reduced scattering coefficient (Fig. 14) of *natural* and *synthetic* melanins at concentration of 1 mg/ml. From Fig. 13 it can be seen that *synthetic* melanin at the same concentration has the absorption coefficient much higher than that of *natural* melanin. Furthermore absorption coefficients of *natural* and *synthetic* melanins have different spectral behavior. Absorption coefficient of *synthetic* melanin decreases with increasing of wavelength. *Natural* melanin has weakly expressed spectral dependence and weak absorption band in red region of wavelengths. Fig. 14 presents similar situation. *Synthetic* melanin has more strongly expressed scattering properties in comparison of *natural* melanin. Spectral behavior of *natural* and *synthetic* melanins is also different. For *synthetic* melanin the strongly expressed spectral dependence with increasing of wavelength is observed. For *natural* melanin this dependence is more weak. However, with increasing wavelength the reduced scattering coefficient decreases.

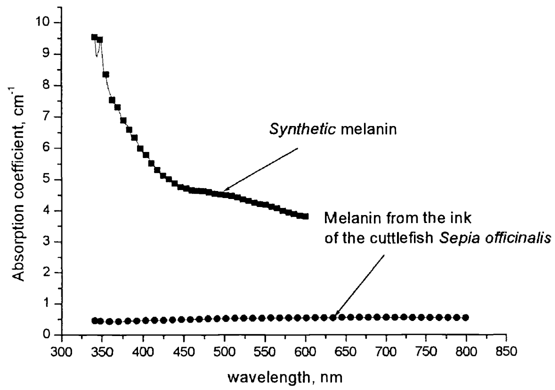

Figure 13. Spectra of absorption coefficient of the *synthetic* melanin particles and melanin particles obtained from *Sepia officinalis* at concentration of 1 mg/ml

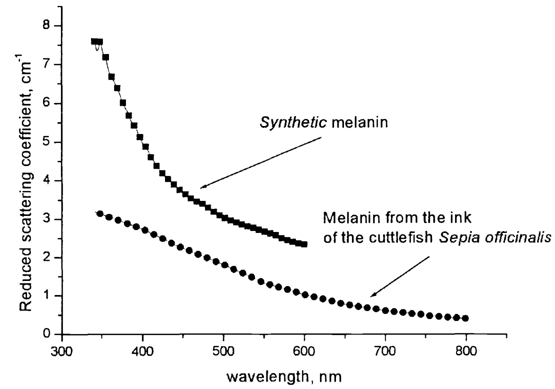

Figure 14. Spectra of reduced scattering coefficient of the *synthetic* melanin particles and melanin particles obtained from *Sepia officinalis* at concentration of 1 mg/ml

We have reconstructed spectra of refractive indices of *natural* and *synthetic* melanins in the studied spectral range (Fig. 15). It was found that *natural* and *synthetic* melanins have anomalous spectral behavior of refractive indices. Consequently, there is absorption in the red spectral region.

We have reconstructed the values of anisotropy factor and scattering coefficient (Figs. 16, 17) for *natural* and *synthetic* melanins at concentration of 1mg/ml using Mie theory. The corresponded values of diameter and refractive indices of melanin particles were calculated. Figs. 16, 17 show that *synthetic* melanin has more expressed scattering properties than *natural* melanin. It is because of *synthetic* melanin has larger scattering particles.

Comparison of our results with data presented by Steven Jacques from Oregon Medical Laser Center[7] shows a good fit between them (Fig. 18).

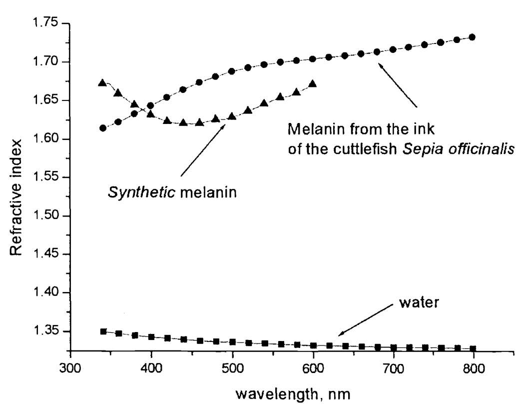

Figure 15. Wavelength dependence of refractive indices of the *synthetic* melanin particles and melanin particles obtained from *Sepia officinalis*.

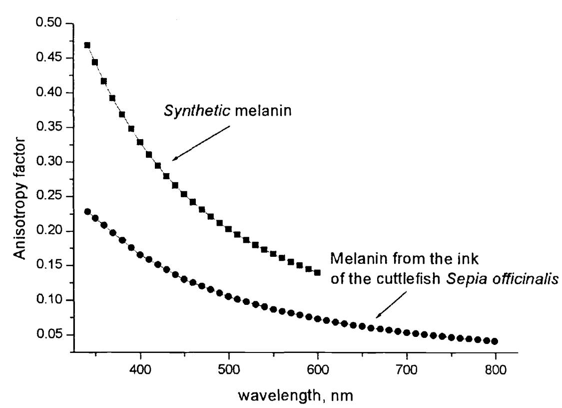

Figure 16. Wavelength dependence of the anisotropy factor of the *synthetic* melanin particles and melanin particles obtained from *Sepia officinalis*.

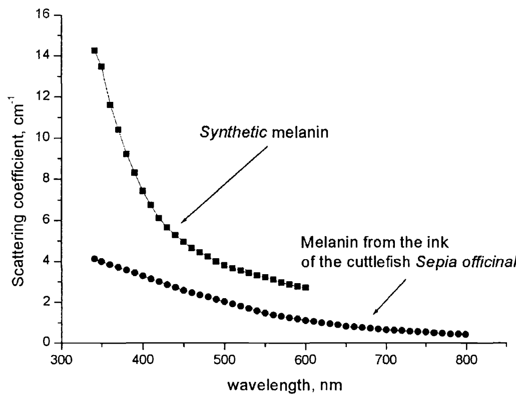

Figure 17. Scattering coefficient spectra of the *synthetic* melanin particles and melanin particles obtained from *Sepia officinalis* at concentration of 1 mg/ml

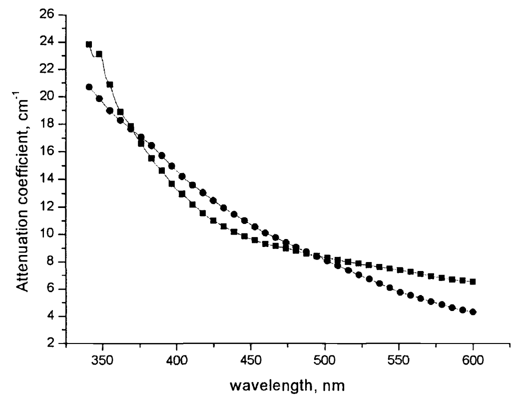

Figure 18. Comparison of calculated data (squares) and data presented by S. Jacques[7] (circles) for *synthetic* melanin particles at concentration of 1 mg/ml.

It was presented in Ref. 2 that melanin is concentrated in the epidermis of the skin as grains with various diameters. In the upper layers of epidermis the diameter of melanin grains is about 30 nm. In more deep layers grains are about 159 nm. At the boundary between epidermis and dermis there are melanosomes with diameter 400 nm. Using calculated values of refractive indices and Eqs. 2, 3 we have obtained the values of scattering coefficients (Fig. 19) and anisotropy factor (Fig. 20) for *natural* melanin grains with various diameter at concentration of 1 mg/ml. At that we assumed the melanin specific weight is 1.5.[2] The comparison of data presented (Fig. 19) and by S. Jacques results in conclusion that S. Jacques used melanin particles with diameter about 130 nm.

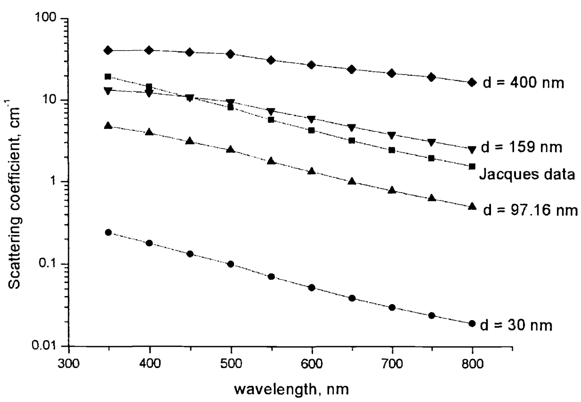

Figure 19. Comparison of calculated data (for particles with various diameters) and data presented by S. Jacques[7] for *natural* melanin particles at concentration of 1 mg/ml. d is diameter of particles.

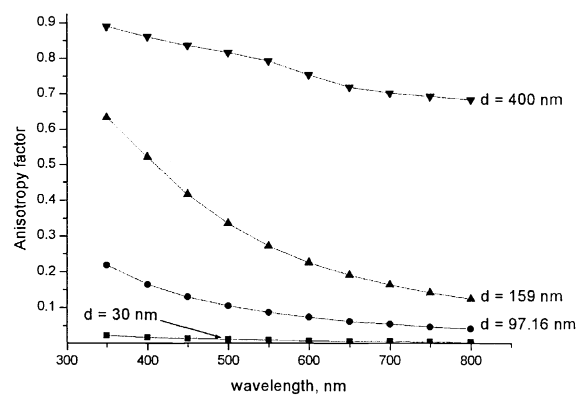

Figure 20. Calculated anisotropy factor for melanin particles with various diameters. d is diameter of particles.

4. CONCLUSION

In this study we have measured and calculated the optical properties of *natural* and *synthetic* melanin. We have separated the scattering and absorption properties of *natural* and *synthetic* melanins and have shown that melanin attenuates light due to scattering properties is general. Using wavelength dependence of refractive index of *natural* melanin we have calculated the anisotropy factor and the scattering coefficient spectra for various melanin aggregates in the human skin at melanin particles concentration of 1 mg/ml. It is necessary to note that our results have preliminary character and demand subsequent investigation.

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