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The Absorption and Scattering of Light in Bovine and Human Dental Enamel

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The reflectance and transmission of thin slabs of dental enamel has been measured at all wavelengths between 220 and 700 nm by means of an integrating sphere. From the results the true scattering and absorption coefficients have been computed. The theoretical model used is an extended two-flux model, which is presented and discussed. The absorption spectrum of the dissolved organic component of enamel was also determined. An absorption peak at 270 nm is common to all the spectra. This peak in the bovine enamel spectrum is about three times as high as in the spectrum of human enamel. The peak of the dissolved material is about as high as the peak of the corresponding enamel. Hence it is concluded that the organic component, presumably aromatic amino acids, is responsible for most or all of the observed optical absorption.

Key words: Optical absorption — Enamel — Scattering.

Introduction

The measurement of optical absorption, together with other optical properties, is an effective method for the structural investigation of a material. The knowledge gained can be used for structural analysis and determination of crystal defects and impurities. Especially useful is the combination of optical absorption with luminescence measurements, performed on the same material.

Due to their turbidity, the hard dental tissues have never been investigated from the point of view of optical absorption. The scattering of light in turbid materials makes it necessary to use special measuring arrangements, to perform at least two different measurements on the one and the same sample, and to make corrections of the reflections on the front and rear surface of the sample. A theoretical model describing the scattering, absorption and reflection processes is needed to compute true absorption and scattering coefficients.

In this investigation, we have measured the total (independent of the degree of scattering) transmittance and reflectance of thin slabs of sound human and bovine dental enamel by means of an integrating sphere. Surface reflections were estimated from the refractive index measured independently.

The theoretical model used in this paper is the two-flux model (Kubelka, 1948) as described by Klein (1965) and extended by Klier (1972).

Thus, the absorption and scattering coefficients of several human and bovine dental enamel samples have been calculated. In order to investigate the cause of the absorption of the enamel, the optical absorption of the organic component of the enamel was determined independently.

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The absorption data will be used for further optical measurements such as fluorescence and phosphorescence.

Theory

Several theoretical models are available for solving the problem of scattering and absorption in plane parallel diffusing layers. It is not the purpose of this paper to discuss them, a review is given e.g. by Mudgett and Richards (1971) and Reichman (1973). The model we chose corresponds well to our experimental data and is attractive because of its relative simplicity.

The oldest and relatively the simpliest model is the two-flux model (Kubelka, 1948). In this only a forward and a backward light flux are considered and therefore a basic assumption is that the incident radiation is diffuse. In most experimental set-ups however, this conditions is not realized.

A general theory describing scattering and absorption in turbid materials is the radiative-transfer theory formulated by Chandrasekhar (1960). This theory is also applicable to collimated incident light but to solve the equations requires considerable computational effort.

Klier (1972) has shown that the two-flux equations are formally identical with a solution of the general Chandrasekhar equation for isotropically highly scattering media with relatively low absorption. He found that the absorption and scattering coefficients as obtained by the two theories are related through numerical coefficients, which depend upon the absorption-to-scattering ratio. This makes the problem much easier because one can then compute the absorption and scattering coefficients according to the simpler two-flux model, and afterwards simply correct the results. The absorption and scattering coefficients obtained are identical to the one that would have been found by application of the rigorous Chandrasekhar theory.

Also, Klier (1972) discusses the conditions under which a turbid medium can be considered as a highly scattering and isotropic scatterer. This is the case if the medium consists of densely packed randomly shaped particles whose sizes are comparable to or smaller than the wavelength of the light and if the particle separation is comparable to or smaller than the particle size.

Since dental enamel is well known to be microscopically birefringent, its particles cannot be considered isotropic scatterers. However, if one considers the crystalline ultrastructure of enamel as described e.g. by Helmcke (1964) one can see that the areas of anisotropy (~50 μm) are much smaller than the true optical path length in the material. Thus the sample can be considered as a composition of anisotropic, but randomly distributed particles. Therefore the condition of randomly shaped particles will be fulfilled. This was substantiated by our experimental observation that the refractive index as measured by means of polarised light (c.f. Materials and Methods) appeared to be independent of the rotation of the sample around the axis of the incident beam.

We apply here the two-flux model as improved by Klein (1965), who incorporated the reflections on the external and internal surfaces of the slab, and subsequently apply the corrections after Klier.

The basic equations are:

$$\frac{dI}{dx} = -(a+s)\ I + sJ \tag{1}$$

$$\frac{dJ}{dx} = (a+s)\ J - sI \tag{2}$$

with the conditions at the reflecting front and rear boundaries:

$$I_0 = (1-\varrho_0)\ I_i + \varrho_i J_0 \tag{3}$$

$$J_D = \varrho_i I_D \tag{4}$$

Where:

x = distance from the front surface

a = absorption coefficient for diffusely incident light

s = scattering coefficient for diffusely incident light

ϱ_0 = reflectance at an interface where the refractive index is increasing

ϱ_i = reflectance at an an interface where the refractive index is decreasing

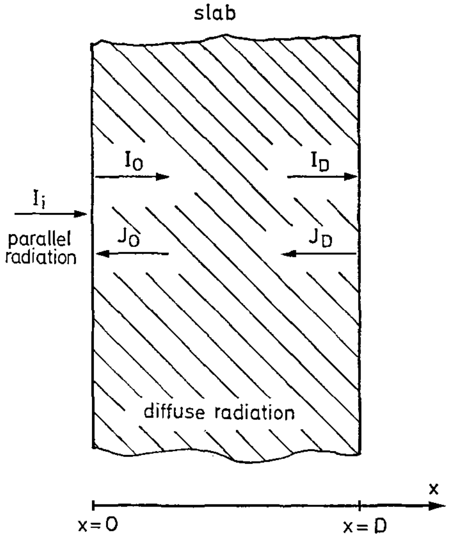

Fig. 1. A scheme of the two-flux model, in which the treatment of diffuse radiation is simplified by the use of only two one-dimensional fluxes, $I(x)$ and $J(x)$. The fluxes, not the directions of the light, are indicated

I = energy flux in the direction of the positive x-axis
J = energy flux in the direction of the negative x-axis
I_i = incident flux at $x = 0$
I_0 = forward flux inside slab at interface $x = 0$
I_D = forward flux inside slab at interface $x = D$
J_0 = backward flux inside slab at interface $x = 0$
J_D = backward flux inside slab at interface $x = D$
as schematically shown in Fig. 1

The solution is given by Klein (1965).

$$\tau = \frac{2\beta(1-\varrho_0)(1-\varrho_i)}{[\beta^2(1+\varrho_i)^2+(1-\varrho_i)^2]\sinh \sigma D + 2\beta(1-\varrho_i^2) \cosh \sigma D} \tag{5}$$

$$\varrho = \frac{[(1-\varrho_i)^2-\beta^2(1-\varrho_i-2\varrho_0)(1+\varrho_i)]\sinh \sigma D + 2\beta(\varrho_0+\varrho_i)(1-\varrho_i)\cosh \sigma D}{[\beta^2(1+\varrho_i)^2+(1-\varrho_i)^2]\sinh \sigma D + 2\beta(1-{}_i^2)\cosh \sigma D} \tag{6}$$

where:
τ = transmittance of the slab
ϱ = reflectance of the slab

$$\beta = \sqrt{a/(a+2s)} \tag{7}$$

$$\sigma = \sqrt{a(a+2s)} \tag{8}$$

D = thickness of the slab.

Firstly we focus our attention on ϱ_0 and ϱ_i. The reflection coefficient ϱ_0 concerns the light incident on the front surface from outside. For polished surfaces ϱ_0 can be calculated simply from the Fresnel formula, ϱ_i at the rear surface has to be determined experimentally (Spitzer and Ten Bosch, 1973). However, in this study we employed diffusely reflecting (etched) surfaces since these are easier to prepare than optically polished ones, and because then the assumption of isotropy inside the slab is better fulfilled. The ϱ_0 can then be computed according to Walsh (1926). This relation for ϱ_0 is also tabulated (Orchard, 1970) and available as a curve (Egan *et al.*, 1973) which follows:

$$\varrho_0 \cong -0.4399 + 0.7099\, n - 0.3319\, n^2 + 0.0636\, n^3$$

where n is the refractive index.

It can be shown (Egan *et al.*, 1973) that

$$\varrho_i = 1 - \frac{1-\varrho_0}{n^2}\,.$$

Thus, when n is known, ϱ_0 and ϱ_i can be calculated.

The coefficients β and σ may then be computed through (5) and (6) from the experimental values of τ and ϱ. We used a simple algorithm for the finding of zeros of a transcendental equation as described by Kristiansen (1963). a and s are then calculated from Eqs. (7) and (8).

Klier (1972) related these coefficients to the true energy absorption and scattering coefficients a_c and s_c for a parallel beam by the relations

$$a_c = \eta \cdot a \qquad (9)$$

$$s_c = \chi \cdot s \qquad (10)$$

Thus a_c and s_c equal the coefficients that would have been found by application of the rigorous theory presented by Chandrasekhar (1960). Experimentally, a_c is the absorption coefficient that would be found when no scattering would take place. η and χ are numerical correction factors for the absorption and scattering respectively.

They are calculated by Klier and tabulated (Klier, 1972) as functions of the ratio a/s (Noted K/S by Klier, 1972). In our case these factors vary within the boundaries

$$0.50 < \eta < 0.53,$$

$$1.33 < \chi < 1.44.$$

Independently, Mudgett and Richards (1971) developed a many-flux treatment of the problem. A comparison of their results with the two-flux model also led to $\eta \cong 0.5$. Again, the agreement with this value is best in conditions of absorption and scattering that are fulfilled in our case. The same conclusion, $\eta \equiv a_c/a \cong 0.5$, was reached by Brinkworth (1971) and Gate (1971) using a photon diffusion model for light absorption in diffusing materials.

Material and Methods

Both human and bovine samples were prepared from sound enamel of mature freshly extracted incisors. The bovine incisors were from 2 to 4-year-old animals. An enamel layer was cut off the tooth and ground from the dentine-enamel junction side until no dentine was detectable by visual inspection and under UV-light. The layer was then ground from the other side until a slab of desired thickness was obtained (approximately 0.2 mm for bovine enamel and 0.3 mm for human enamel). The area of the samples was about 50 mm^2 for bovine enamel and about 40 mm^2 for human enamel. One side of the sample was optically polished for specular reflectance measurements. In order to determine the reflections on the front and rear surfaces the refractive index was measured as a function of the wavelength. This was done by measuring the specular reflection on the perfectly polished enamel slabs with a home-made accessory to the Perkin-Elmer 124 spectrophotometer. The refractive indices were then computed from the Fresnel's equation

$$\varrho_s = \frac{1}{2}\left\{\left(\frac{\sqrt{n^2-\sin^2\Theta}-\cos\Theta}{\sqrt{n^2-\sin^2\Theta}+\cos\Theta}\right)^2 + \left(\frac{n^2\cos\Theta-\sqrt{n^2-\sin^2\Theta}}{n^2\cos\Theta+\sqrt{n^2-\sin^2\Theta}}\right)^2\right\} \qquad (11)$$

where ϱ_s *is the specular reflection coefficient and* Θ *the angle of incidence.*

These results have been substantiated by Bril (personal communications) who determined the refractive index of the enamel by means of Brewster angle measurements and by Castelijns (personal communications) by means of ellipsometric measurements.

After these measurements were performed, both surfaces of the sample were etched in 50% phosphoric acid for 30 s for the volume transmittance and reflectance measurements. The samples were always stored in water. After superficial drying of the sample surfaces, the total reflectance and transmittance were measured with an integrating sphere Perkin-Elmer 124-0049. Five samples of both bovine and human enamel were used for measurements. No influence of the water content of the sample on the optical measurements was found between 5 min and 48 h after taking the sample out of the water. This is in agreement with measurements of the water content of the enamel as performed by Arends (personal communications).

As known, dry enamel consists of about 99% of crystallites of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and less than 1% of organic material (mostly protein).

Attempts were made to separate the organic component of the bovine enamel from the inorganic one in the following manner. Enamel slabs were ground and the powder was suspended into 0.01 M HCl. During the dissolution process the pH was kept constant by adding acid. The resulting suspension was then dialysed (Viskin 18/32) against water. Both the content of the bag and the surrounding solution were decreased in volume until 10 ml by evaporation of water at 50°. Subsequently, to the contents of the bag urea was added to 6 M, and kept at 80° for 48 h. After this process some undissolved material was still present. The absorption spectra of the supernatant and of the dialysis liquid were determined.

Results

We found that the refractive index of different slabs of enamel did not differ much. No substantial difference between the refractive indices of human and bovine samples was found. No substantial anisotropy was found. Fig. 2 shows the general relation for the refractive index as a function of the wavelength.

These values are in good agreement with the values of the refractive index of hydroxyapatite (the main component of the dental enamel) as found by Posner and Perloff (1957).

Fig. 3 shows, as an example, the total reflectance and extinction, log $(1/\tau)$, for one sample.

The absorption and scattering coefficients were computed from these measurements by procedures explained in the theoretical section. For two different samples the curves are shown in Figs. 4 and 5. For convenience of the reader familiar with spectrophotometry, the decadic absorption and scattering coefficients are presented.

The main absorption peak is found at 270 ± 5 nm for the human enamel and 260 ± 5 nm for bovine enamel. A peak at about 235 nm was noticed in several cases but due to the rapid increase of absorption in this region this peak is not found back in the computed results.

An important difference was found between the absorption coefficients of bovine and human enamel, the former being about three times larger in the region between 220 and 300 nm. No such difference was found for the computed scattering coefficients of different samples as shown in Fig. 5.

Both solutions resulting from the dissolution of bovine enamel powder showed an absorption peak at 260–280 nm. Control experiments without enamel demonstrated that at the pH used, components extracted from the bag itself caused optical absorption also. However, neutralisation before dialysis caused precipitation of material. The spectra of the supernatant of the urea solution of the bag content were corrected for the absorption of the bag material by using the absorption spectrum of the liquid used for dialysis. The corrected spectrum was used to calculate the decadic absorption coefficient of enamel as caused by the dissolved material. We estimate the error in these values to be of the order of 10%.

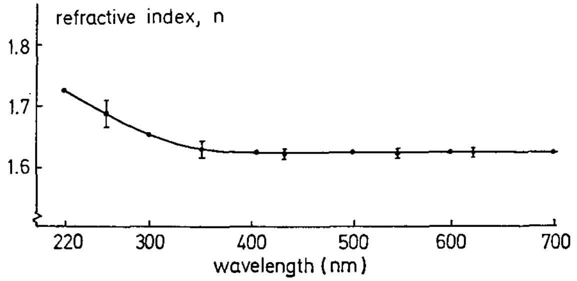

Fig. 2. The refractive index n of the enamel as a function of the wavelength. The errors, covering the differences between the samples, are indicated in several points where specular reflection as well as Brewster angle were measured. Between these points the shape of the curve is derived from the reflectance measurements

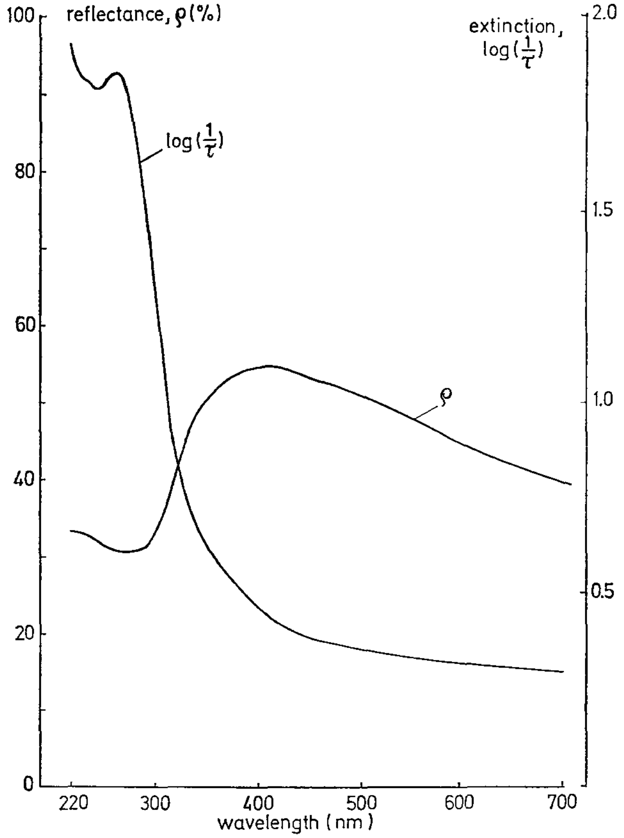

Fig. 3. The extinction and reflectance of a sample of bovine enamel, thickness 0.17 mm, as measured with the integrating sphere

Discussion

There is a strong presumption that the absorption of the organic components is either fully of partly responsible for the absorption of the enamel in the region under 300 nm.

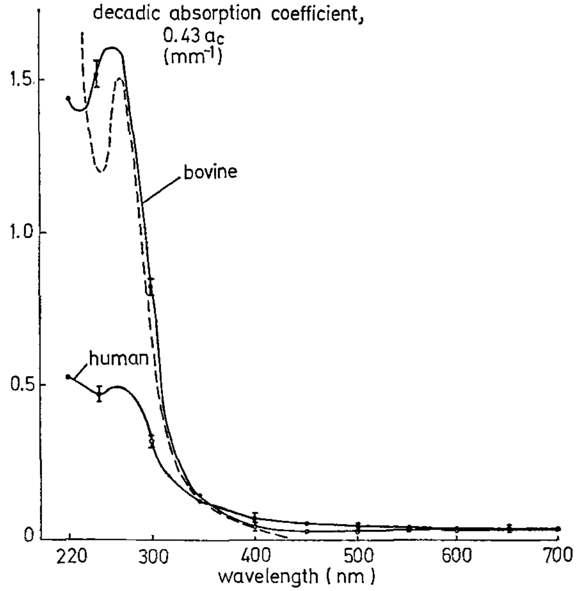

Fig. 4. The decadic absorption coefficients of one sample of human and one sample of bovine enamel as computed from the total transmittance and reflectance data. For the bovine sample these are taken from Fig. 3. The calculations were performed at 10 nm intervals, for clarity, points are shown at 50 nm intervals. Estimated errors are indicated in several points. The height of the absorption peaks of other bovine enamel samples differ less than 5% from the values shown in the figure. This is true also for the absorption of the human enamel samples. The dashed line shows the absorption coefficient caused by some, or all, of the organic material as determined from a urea solution of enamel dissolved in HCl at pH 2

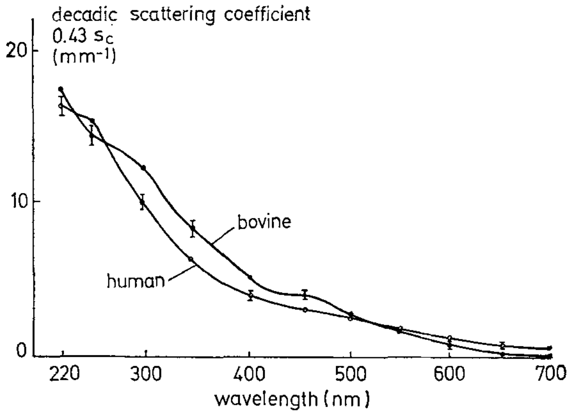

Fig. 5. The scattering coefficients of the same human and bovine samples as used in Fig. 4. The values of scattering coefficients of the other samples can differ 40% from these values

The absorption of proteins in the region between 240 and 300 nm is mainly caused by the absorption of the aromatic amino acids tryptophan, tyrosine and phenylalanine. Tryptophan and tyrosine have absorption peaks at about 275 nm, the former being about four times larger (Barenboim *et al.*, 1969).

This means that the spectrophotometric measurements of the enamel could be an effective method of investigation of concentration and spatial distribution of these amino acids in the enamel, as well as the changes in concentration and distribution due to any diffusion or decomposition process. For instance, under the assumption that all the absorption at 275 nm is caused by tryptophan, one can show that the concentration of the tryptophan residues is $3\times10^{-6}\pm3\times10^{-7}$ moles/g bovine enamel. The amino acid composition of protein from mature enamel has been investigated by several authors and summarized by Smillie (1973). The number of the tryptophan residues has not been determined.

The strong absorption in this wavelength region was also concluded from tentative luminesence measurements, where it was found that the excitation spectrum has a peak at about 280 nm causing emission with a peak at about 350 nm which is the region of emission of tryptophan (Barenboim *et al.*, 1969).

The difference between the absorption of human and bovine enamel should indicate that a substantial difference in the composition of those two calcified tissues exists in the investigated part of the enamel layer. Although hypochromicity may play a role, it seems unlikely that these differences in absorption are due only to hypochromicity effects of the protein chromophores.

The smaller differences in the absorption of different samples of one and the same type of enamel can also be expected due the natural differences in composition and structure of each natural product of one and the same kind.

It can be seen from a comparison of the absorption spectra of bovine enamel and the dissolved organic component therein (Fig. 4), that the absorption coefficients are in a good agreement. Since it may be possible that either not all organic material is dissolved in urea, or that small absorbing molecules disappeared out of the dialysis bag, we conclude that no absorption of the inorganic component can be inferred from our results.

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References

Barenboim, G. M., Domanskii, A. N., Turoverov, K. K.: Luminescence of biopolymers and cells, p. 18–75. New York-London: Plenum Press 1969

Brinkworth, B. J.: On the theory of reflection by scattering and absorbing media. J. Phys. D.: Appl. Phys. **4**, 1105–1106 (1971)

Chandrasekhar, S.: Radiative transfer, p. 9. New York: Dover 1960

Egan, W. G., Hilgeman, T., Reichman, J.: Determination of absorption and scattering for nonhomogeneous media: II. Experiment. Appl. Opt. **12**, 1816–1821 (1973)

Gate, L. F.: The determination of light absorption in diffusing materials by a photon diffusion model. J. Phys. D.: Appl. Phys. **4**, 1049–1056 (1971)

Helmcke, J. G.: Ultrastructure of enamel. In: Structural and chemical organisation of teeth (Miles, A. E., ed.), vol. II, p. 135–163. New York and London: Academic Press 1967

Klein, J. D.: Radiation heat transfer through scattering and absorbing nonisothermal layers. Techn. Rept. NASA SP-55, 73–81 (1965)

Klier, K.: Absorption and scattering in plane parallel turbid media. J. opt. Soc. Amer. **62**, 882–885 (1972)

Kristiansen, G. K.: Zero of arbitrary function. BIT **3**, 205–207 (1963)

Kubelka, P.: New contribution to the optics of intensely light-scattering materials. Part I. J. opt. Soc. Amer. **38**, 457–488 (1948)

Mudgett, P. S., Richards, L. W.: Multiple scattering calculations for technology. Appl.Opt. **10**, 1485–1501 (1971)

Orchard, S. E.: Reflection and transmission of light by diffusing suspensions. J. opt. Soc. Amer. **59**, 1584–1597 (1970)

Posner, A. S., Perloff, A.: Apatites deficient in divalent cations. J. Res. Nat. Bur. Stand. **58**, 268–279 (1957)

Reichman, J.: Determination of absorption and scattering coefficients for nonhomogeneous media. I. Theory. Appl. Opt. **12**, 1811–1815 (1973)

Smillie, A. C.: The chemistry of the organic phase of teeth. In: Biological mineralisation (Zipkin, I., ed.), p. 139–163. New York: John Wiley & Sons 1973

Spitzer, D., Ten Bosch, J. J.: A simple method for determination of the reflection coefficient at the internal surfaces of turbid slabs. Opt. Comm. **9**, 311–313 (1973)

Walsh, J. W. T.: Dept. Sci. Int. Res. (Gr. Brit.) 10, Res. Comm., Techn. Paper No **2**, 10 (1926)