Optical properties of dental hard tissues
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Chapter 3  **Optical properties of dentin**


3.1 ABSTRACT
Absorption, scattering and fluorescence together determine the color of dentin. These properties can probably be used as the basis of quantitative diagnostic methods for caries. It is useful therefore to quantitate these properties when possible. The absorption and scattering coefficient of bovine and human dentin were determined from 170 µm thick wet sections. Bovine incisors and human premolars were used. Several sections, parallel to the labial plane, were cut from the same tooth. Diffuse reflection and transmission of these sections was measured at visible wavelengths with a spectrophotometer equipped with an integrating sphere. Kubelka-Munk theory was used. Transversal microradiography was used to determine the mineral content of a section. The absorption coefficient for fluxes was found to be ~ 0.4 mm\(^{-1}\), rather independent of wavelength, the scattering coefficient for fluxes was found as ~ 3 - 20 mm\(^{-1}\), slowly decreasing with wavelength and hardly related to mineral content. The literature on fluorescence properties is reviewed and discussed. Four emission bands have been observed, at 350, 400, 450 and 520 nm. The chromophores of the first two bands have been identified, the others have not.

3.2 INTRODUCTION
In this paper we will consider some of the macroscopical optical properties of coronal dentin: absorption, scattering and fluorescence. These properties are of twofold relevance. First, they together determine the visual appearance, i.e. the color, of dentin which plays a large role in the color of the complete tooth crown. This aspect is of importance for visual dental diagnosis of dental problems and also for the matching of a prosthetic tooth crown to an adjacent natural crown. Quantitative data will be needed to improve these processes, which up to now are qualitative. Secondly, in enamel these properties have been successfully used to quantitate enamel caries. Development of a similar method for dentinal caries would be of relevance for caries research.

3.3 MATERIALS AND METHODS
3.3.1 Samples
Dentin samples were obtained from bovine incisors and human premolars by cutting slices with an inner hole diamond saw (Borsboom *et al.*, 1987). Slices were cut parallel to the buccal surface of the tooth, from the buccal dentin-enamel junction inward. Slices were roughened on 1200 mesh abrasive paper to increase diffusivity of the light. As many slices as possible were cut from a single tooth, the separation (saw blade thickness) being ~ 220 µm. Slice thickness was of the order of 170 µm. Samples were kept wet until their insertion in the spectrophotometer. Effects of drying during spectrophotometric measurement (~ 15 min) could not be observed.
3.3.2 Absorption and scattering coefficients
For determination of these coefficients we measured the diffuse reflection and diffuse transmission of dentin slices with a dual-beam integrating sphere-attachment (type 124-49) to a Perkin-Elmer type 124 UV-Vis spectrophotometer. Since the samples were smaller than the regular port holes of the sphere, both sample and reference hole were decreased in size with black diaphragms with a 2 x 5 mm opening. In reflection measurements a BaSO$_4$-pill prepared according to standard procedures (DIN 5033, part 9) was used in the reference port hole of the sphere. In transmission measurements, the reference port hole was open. The Kubelka-Munk theory was used as was done previously for enamel (Spitzer and ten Bosch, 1975). A refractive index value, necessary to estimate surface reflections, was estimated at $n = 1.45$ based on an estimate of mineral content of 40 volume% and an apatite refractive index of hydroxyapatite of 1.64 (Perloff and Posner, 1956).

3.3.3 Luminescence Spectra
Luminescence data were all taken from the literature.

3.3.4 Mineral Content Determination
Mineral content was determined by traditional Transversal Micro Radiography with a semi-automated densitometer recently described (de Josselin de Jong et al., 1987). A single scan was made across the area that was measured optically.

3.4 RESULTS
3.4.1 Absorption and scattering
In between 8 and 11 slices per tooth of 2 bovine incisors and 2 human premolars were measured. Figure 3.1 shows examples of the spectral dependence of absorption and scattering. This spectral dependence is similar for all samples. Figure 3.2 shows examples of averages of values at wavelengths of 535, 550 and 565 nm as a function of position in the tooth. From the other teeth similar curve shapes were obtained. The height of these curves varies from tooth to tooth, values at the tooth center are given in Table 3.1. Another impression of the distribution of scattering coefficients can be obtained from Figure 3.3, which depicts the scattering coefficients of all slices versus the mineral content of these slices.

| Table 3.1 Absorption and scattering coefficients in mm$^{-1}$ for the center part of the coronal dentin (with estimated s.e.). |
|---|---|---|
| bovine samples | A | S |
| 1 | 0.45 (0.1) | 14 (2 ) |
| 2 | 0.25 (0.1) | 26 (4 ) |
| human samples | | |
| 3 | 0.4 (0.1) | 3 (0.5) |
| 4 | 0.8 (0.1) | 8 (1 ) |
Figure 3.1 Examples of spectral dependence of absorption (Ο) and scattering (□).

Figure 3.2 Dependence of absorption (Ο) and scattering coefficients (□) at 500 nm on the position of the slice in the tooth. Other teeth showed similar dependences.
Table 3.2 Fluorescence peak positions.

<table>
<thead>
<tr>
<th>Optimum excitation</th>
<th>Maximum emission</th>
<th>Chromophore</th>
</tr>
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<tbody>
<tr>
<td>&lt; 300 nm</td>
<td>~ 350 nm</td>
<td>traces of tryptophan</td>
</tr>
<tr>
<td>325 nm</td>
<td>~ 400 nm</td>
<td>hydroxypyridinium</td>
</tr>
<tr>
<td>380 nm</td>
<td>450 nm</td>
<td>unknown</td>
</tr>
<tr>
<td>410 nm</td>
<td>520 nm</td>
<td>unknown</td>
</tr>
</tbody>
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3.4.2 Luminescence
Numerous publications deal with luminescence of dentin, most consider fluorescence at room temperature only. Such fluorescence occurs at 4 bands. A summary is given in Table 3.2.

Excitation around and below 300 nm leads to emission around 350 nm (Hoerman and Mancewicz, 1964; Perry et al., 1969), which has been ascribed tentatively to traces of tryptophan (0.5 residue/1000). The next emission band shows a maximum around 400 nm. It has been investigated by several research groups (Perry et al., 1969; Armstrong and Horsley, 1966). The origin of this emission is a collagen crosslink: hydroxypyridinium, of which there exist two forms (Fujimoto et al., 1977; Walters and Eyre, 1983). The fluorescence of this chromophore is excited optimally at 325 nm in neutral or alkaline conditions and at 300 nm in acid conditions (Fujimoto et al., 1977).

Fluorescence emission with a peak around 450 nm has been reported by Scharf (1971) and by Alfano and Yao (1981). Optimum excitation seems to be around 380 nm. Finally, a broad peak, often appearing as a shoulder on the 450 nm peak, is present at 520 nm but slowly decreases at higher wavelengths. This emission is excited optimally at 410 nm (ten Bosch et al., 1986). It is also caused by the organic component of the tissue but the chromophore is unknown. The color coordinates of these emissions, when excited by 365 nm radiation, have been given by Scharf (1971).

Other types of luminescence have been studied scarcely. Hefferren et al. (1971) report phosphorescence at liquid nitrogen temperature with an emission around 445 nm. Kolberg et al. (1974) report X-ray induced thermoluminescence peaking at 480 nm, which is ascribed to the dentin mineral.

3.5 DISCUSSION
3.5.1 Absorption and scattering
The scattering results show first of all that the scattering coefficient is not related to mineral content (Figure 3.3). This implies that the mineral crystals are not the predominant cause of scattering of light in dentin. Accordingly, larger structures such as the dentinal tubules, must be the cause.

This is confirmed by the results which show the wavelength dependence of the scattering (Figure 3.1), which is somewhat but not much higher at shorter wavelengths than at longer wavelengths. This rather small wavelength dependence points at large scatterers. Also, near the enamel-dentinal junction the scattering is low (low tubule density and small tubuler size)
compared to in the dentin directly adjacent to or above the pulp (high tubule density and large tubule size) (Figure 3.2).

Figure 3.3 Scattering and absorption coefficients of all samples at 500 nm versus mineral content of these samples in volume percent mineral. (□ ○ ): human premolar samples, (■ ● ): bovine incisor samples.

The absorption results also are not strongly wavelength dependent. At 400 nm the beginning of a rise of the coefficient in the ultraviolet can be seen, possibly due to collagen crosslinks (viz. fluorescence). In the visible the absorption spectrum is rather flat. Although this is consistent with the greyish-yellowish color, it does not give any clue to the cause of the absorption. There is no systematic change of absorption with position in the tooth.

The scattering coefficients in human premolars found are of the order of 3 mm$^{-1}$ near the EDJ, of the order of 8 mm$^{-1}$ near the pulp. Values for bovine incisors are higher by a factor 2 - 3. These values can be compared with those of sound enamel: ~ 3 mm$^{-1}$ for scattering, ~ 0.1 mm$^{-1}$ for absorption (Spitzer and ten Bosch, 1975). Since the penetration depth of light in a material is roughly the inverse of the sum of both coefficients it can be understood quantitatively that the dentin only, and even the inner part of the dentin predominantly, determines the opacity of a non-carious tooth. Specifically, scattering is the dominating process, as it is in dental enamel (Spitzer and ten Bosch, 1975).

3.5.2 Fluorescence

The fluorescence bands of dentin can be compared with those of dental enamel. The 400 nm emission in enamel was rather carefully investigated by Booy and ten Bosch (1982) and ascribed to a compound very similar to dityrosine. This conclusion was based on a
comparison of emission and excitation spectra and their pH-dependence and on Biogel P2- and thin-layer-chromatography. Although the positions of the emissions and excitation maxima of the corresponding band in dentin are very similar, the emission has been ascribed to an entirely different chromophore on the basis of many different techniques. The fluorescence emissions in the visible have also been observed in enamel (ten Bosch et al., 1986, Sundström et al. 1985). They show the same spectral characteristics. Biochemical analysis of either chromophore has, as far as we know, not yet been performed. In view of the demonstrated possibility to use these emissions for caries diagnosis (Bjelkhagen, 1982), such research seems useful.

3.6 CONCLUSION
In conclusion, the visual appearance of dentin can semi-quantitatively be ascribed to the rather flat wavelength dependence of absorption and scattering, the latter specifically being due to the high density of tubules in the inner dentin layers. The fluorescence bands of dentin and enamel are very similar, but the 400 nm emissions band of both tissues are nevertheless due to different chromophores. The cause of emissions at higher wavelengths is unknown.

3.7 REFERENCES


Scharf F (1971) Über die natürliche Lumineszenz der Zahnharthgewebe "Schmelz und Dentin". *Stoma* 1:10-25


