12.1 Summary

Micro-Raman spectroscopy is a technique which enables us to obtain infrared and far infrared vibrational (Raman) spectra of molecules or compounds in materials using visible (or near) visible light. Localised molecules or components concentrated in a small volume of a few cubic micrometers can be identified and quantified.

Main emphasis in this thesis is on biological materials and tissues consisting of mineral as well as of organic components. The essential mineral phases in the biological materials investigated are calcium phosphates of the calcium hydroxyapatite-"like" type. Therefore also attention was given to pure synthetic single crystals of calcium hydroxyapatite.

The main obstacle in performing "normal" Raman spectroscopy using visible laser light as an excitation source, is fluorescence emitted by organic components present in all biological materials. For this reason, enamel which contains a relatively small organic content, had been the only material studied by the Raman technique among the dental hard tissues (enamel, dentine and cementum) and mineralised dental plaque (calculus), at the time this project was initiated.

A series of micro-Raman spectroscopic investigations have been undertaken with the basic aim of obtaining Raman spectra of the materials mentioned above and developing analytical methods based on the micro-Raman technique, for various aspects in dental research.

In this investigation the principal interests were focussed on:
- the mineral hard tissues enamel and dentine,
- the effects of fluoridation of these tissues,
- the properties of hydroxyapatite single crystals,
- calculus: mineralised plaque.

Since fluoride(s) plays a dominating role in the prevention of enamel- and root-caries, attention to the effects of fluoridation of the hard tissues is relevant. In nearly all practical applications, the fluoride concentration used is high enough to form at least in principle calcium fluoride or calcium fluoride-"like" material on and or in the surface of teeth. Because calcium fluoride is formed in all applications in which fluoride is used, it is important to detect in small amounts of CaF₂ in and on teeth.

In Chapters 1 and 3, a literature survey on tissues and apatites is mentioned; only Raman spectroscopy using visible light as an excitation source, is fluorescence emitted by organic components present in all biological materials. For this reason, enamel which contains a relatively small organic content, had been the only material studied by the Raman technique among the dental hard tissues (enamel, dentine and cementum) and mineralised dental plaque (calculus), at the time this project was initiated.

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In Chapters 5 and 6, a literature survey on tissues and apatites is mentioned; only Raman spectroscopy using visible light as an excitation source, is fluorescence emitted by organic components present in all biological materials. For this reason, enamel which contains a relatively small organic content, had been the only material studied by the Raman technique among the dental hard tissues (enamel, dentine and cementum) and mineralised dental plaque (calculus), at the time this project was initiated.

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in all applications in very small amounts and because it is very difficult to
detect in small amounts, many papers in the literature "suggest" the presence
of CaF$_2$ in and on teeth without real evidence.

In Chapters 1 and 2 of this thesis general information and background
information is presented on the subjects to be considered later. In Chapter
3, a literature survey on Raman spectroscopy measurements of the dental hard
tissues and apatites is given. It shows that very few applications of the Raman
technique were published previously on the focusing points of this thesis
mentioned; only Raman spectra of enamel had been published showing the
presence of mineral and organic materials. In Chapter 4, the micro-Raman
instrument used, the procedures employed as well as technical and analytical
details are presented.

In Chapters 5 and 6, special attention has been given to CaF$_2$ and CaF$_2$-
formation after fluoridation by means of acidic APF on enamel. In the former
chapter, the CaF$_2$ determination by micro-Raman in the presence of large
amounts of mineral hydroxyapatite was studied. The intensity ratio of the CaF$_2$
Raman band (322 cm$^{-1}$) and the phosphate $v_2$ Raman bands (432/447 cm$^{-1}$)
was found to be linearly related to the CaF$_2$ concentration. The lower limit of
CaF$_2$ quantification was found to be about 3 %.

In the latter chapter, the formation of "CaF$_2$-like" material was
investigated on wedge-shaped enamel samples after an APF application. A
major result is that the bandwidth of the CaF$_2$ band is approximately a factor 2
broader than that of pure CaF$_2$, implying that the lattice dynamics of CaF$_2$
formed by APF fluoridation is different from pure CaF$_2$ and that the material
formed is "CaF$_2$-like" or disordered CaF$_2$. The results show furthermore that
about 1/3 of the total amount of the CaF$_2$-like material formed is expected to
be concentrated within a narrow depth < 2 µm and that the majority of the
CaF$_2$ was distributed over the depth up to 26 µm; this result is consistent with
SEM observations of fractured fluoridated samples.

From the data on dental calculus presented in chapters 7 and 8, the
following main conclusions can be drawn:

- Dental calculus gave micro-Raman spectra characterised as
  phosphate bands due to $v_1$, $v_2$ and $v_4$ modes consistent with
  impure hydroxyapatite containing CO$_3^{2-}$ and HPO$_4^{3-}$ ions.
In comparison with pure synthetic HAP, the intensity of the OH-stretch band relative to that of the $\text{PO}_4^{3-} \nu_1$ band was 70% weaker and the bandwidth of the $\text{PO}_4^{3-} \nu_1$ band was 200% broader, reflecting various crystal imperfections in the calculus mineral.

The intensity of the $\text{CO}_3^{2-}$ Raman band (of calculus mineral) relative to that of the phosphate band was equivalent to that of synthetic carbonated HAP with $\text{CO}_3^{2-}$ concentrations in the range 2 - 8 wt%.

The micro-Raman mineral-spectra of calculus removed from patients after 1, 2, 3 and 6 months of calculus formation were nearly identical. From this observation, we can conclude that the mineral phase in calculus is mainly an impure carbonate-containing hydroxyapatite after using fluoridated dentifrices during 1 month.

- Micro-Raman measurements on fractured calculus indicated that the mineral phase was nearly identical near the saliva and near the dentine interface.

In Chapter 9, the first micro-Raman investigation on human dentine mineral is presented. The spectral features observed indicate that the mineral is an impure hydroxyapatite containing $\text{CO}_3$ and $\text{PO}_4$. Main results of this chapter showed that

- There was no measurable difference between the mineral in coronal and root dentine.
- There are clearly observable differences between enamel and dentine mineral such as the bandwidth of the $\text{PO}_4^{3-} \nu_1$ band and the (relative) intensities of the OH-, $\text{CO}_3^{2-}$ and $\text{HPO}_4^{2-}$ bands.
- After treatment of the dentine mineral by NaOCl, NaF or an APF-gel, changes in $\text{CO}_3$, $\text{HPO}_4$ or protein bands are clearly detectable by the micro-Raman technique.

Human dental enamel mainly consists of hydroxyapatite up to 98% in the form of micro-crystals so-called crystallites. The enamel crystallites are single-crystals of 25-1000 nm in length and 30-40 nm in diameter. To obtain more information on measurements, single chapter 10. The Ramans point group C₆ of HAp including the $\text{PO}_4^{3-}$ axes and spectral range 180 - 3000 cm⁻¹ the rotation about the a-axis with respect to the rotations about other axes. The useful bands for this, which contains E₁ (581 cm⁻¹).

Crystallites near a strong orientational direction crystals, indicating the area of 1 μm diameter.

In conclusion, this research will make in situ possible especially if fluorescence effects are considered.
more information on orientational effects of the crystallites on Raman measurements, single crystals of synthetic hydroxyapatite were studied in chapter 10. The Raman active symmetry species (A, E₁ and E₂) derived from the point group C₆ of HAP have been assigned to all the observed Raman bands including the PO₄³⁻ and OH⁻ internal modes and external modes over the spectral range 180 - 3600 cm⁻¹. The invariance of the Raman band intensity to the rotation about the c-axis can be used to determine the orientation of the c-axis with respect to the known excitation/observation coordinates since other rotations about other crystal axis can cause changes in the spectral profile. The useful bands for this purpose are those of the PO₄³⁻ v₄ vibrational mode which contains E₂ (581 cm⁻¹) and A (593 cm⁻¹) symmetry species.

Crystallites near the intact outer surface of human enamel showed strong orientational dependencies similar to those of hydroxyapatite single crystals, indicating that the crystallites are highly oriented at least within the area of 1 μm diameter.

In conclusion, the micro-Raman technique is a valuable tool in caries research and in the field of caries prevention. Considerable progress is still possible especially if more instrumental sophistication is applied and the fluorescence effects are better controlled. In the future also the use of fiber optics will make in situ and in vivo spectroscopic studies possible.