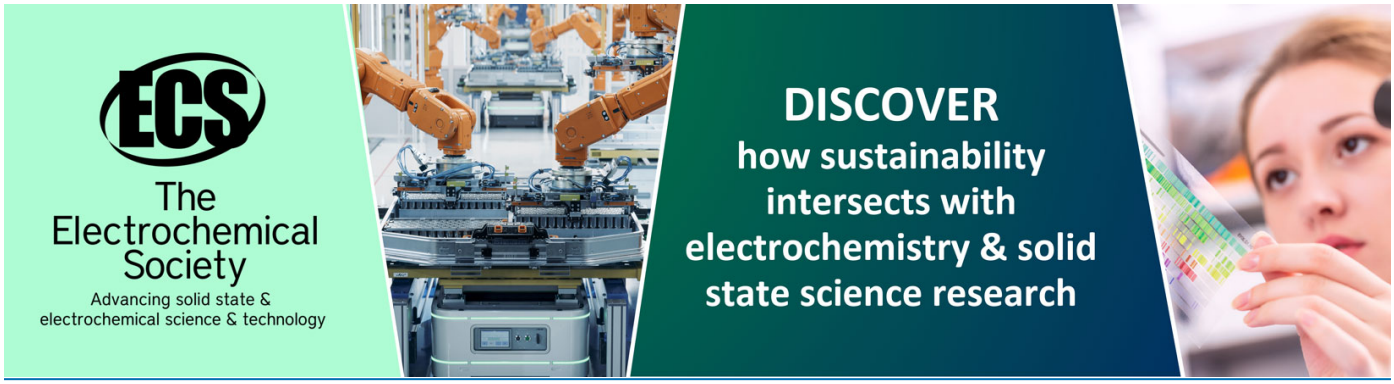
Precision study of mineralization features of enamel apatite in the development of fluorosis by means of optical spectroscopy



Precision study of mineralization features of enamel apatite in the development of fluorosis by means of optical spectroscopy

D Goloshchapov[1], V Kashkarov[1], K Nikitkov[1], I Ippolitov, Yu Ippolitov[2], J Vongsvivut[3], M Savvateev[4], A Vaxtel[4] and P Seredin[1]

[1]Department of Solid State Physics and Nanostructures, Voronezh State University, Voronezh, Russia
[2]Department of Pediatric Dentistry with Orthodontia, Voronezh State Medical University, Voronezh, Russia
[3]Australian Synchrotron (Synchrotron Light Source Australia Pty LTD), 800 Blackburn Rd Clayton, VIC 3168, Australia
[4]JSC «Nitek Instruments» - General Distributor of HORIBA Scientific
E-mail: paul@phys.vsu.ru

Abstract. We have investigated the molecular composition of the tooth enamel microregions in the normal and initial stages of the development of fluorosis by means of the IR and Raman spectroscopy. It has been shown that in the pathology of fluorosis, tooth mineralization takes place with the formation of calcium fluoroapaptite. It was found that an increased fluorine content causes a change in the characteristic profile of the bands and a redistribution of the intensity of the components of the υ_1 PO_4 and υ_3 CO_3 groups of the infrared and υ_4 PO_4 Raman spectra. The resulting IR and Raman spectra can be utilized as standards in the development of a new diagnostic approach of the early forms of the disease.

1. Introduction

The pathology of fluorosis of human teeth is known to be caused by excess fluoride in drinking water, excessive use of fluoride-containing medicines, territorial specifics, occupational factors, dietary features, metabolic disorders [1,2]. It is noted that 11 regions of Russian Federation are characterized by increased fluorine content with different concentration in water. While in some areas fluorine content is at the level that is by two or three times greater than maximum permissible concentration (MPC) [3]. At the same time the rate of dental fluorosis occurrence for the permanent teeth varies from 10% to 90 % in a dependence of excess for MPC norms for fluorine in water [3]. In terms of processes mineralization the increased fluorine content in the oral cavity causes it to be included in the inorganic part of the enamel, i.e., defective carbonate-substituted hydroxyapatite (CHAP). In the case of excess fluorine in the enamel, the formation of calcium fluorapatite (FAP) takes place whose physicochemical properties cause change in the mineralization of tooth enamel [4]. With a constant excess of fluorine concentration in the subsurface layers of enamel, calcium fluoride CaF_2 is formed [5], which can be fixed at the late stage of fluorosis in the form of cracks, chips, and a change in the color of enamel [6]. This requires the development of a diagnostic technique for the state of enamel which is sensitive to initial changes in fluorosis.

Hence the optical methods of molecular identification of a substance are most promising and sensitive for recording changes generated by the development of fluorosis in the micro-areas of tooth

enamel. As a non-destructive analysis method, the Raman spectroscopy allows one to obtain precision data on the molecular composition of biological objects [5,7,8]. Hard tooth tissue of a human tooth can also be studied by means of the IR spectroscopy [8,9]. In addition, the inclusion of an IR microscope into the optical circuit using synchrotron radiation allows local changes in the mineral-organic matrix of tooth enamel from areas less than $100\mu^2$ to be investigated [10].

Note that in the literature there are no data on the initial changes in both the molecular composition of the teeth in the case of fluorosis and the one-to-one correspondence between the methods of optical spectroscopy in the pathology in question. Therefore, the aim of this study is to investigate the features of mineralization of enamel apatite in the initial stages of the development of pathology caused by an increased fluorine content in the oral cavity by means of the Raman and IR spectroscopy methods.

2. Materials and methods

2.1. Teeth preparation

We have investigated tooth samples with intact enamel and enamel with fluorosis. The study was performed on tooth samples removed from the patients aged 20-40 according to the orthodontic criteria. The studied samples of teeth were extracted for orthodontic reasons off the patients living in the South Federal district where increased fluorine content is observed in drinking water. 5 samples of teeth suspected for dental fluorosis were examined. Besides, 5 samples of the intact teeth were examined. The degree of fluorosis on the Thylstrup-Fejerskov scale (TFI) was 1-3 and corresponded to the early stage of fluorosis. In accordance with the requirements of the microspectroscopic research techniques for the geometry of the samples, we prepared plane-parallel segments of teeth similar to [7,11]. No less than 10 selected points were used for each of the samples within the surface and near-surface region for obtaining of the spectra. The spectra for each point were averaged over 10 measurements.

2.2. Raman microspectroscopy

The Raman spectra were obtained on an Xplora Plus confocal Raman microscope (HORIBA, Japan) with a spectral resolution of 1.5 cm^{-1} in the range 200–2000 cm^{-1}. The excitation was carried out by means of a laser with a wavelength of 785 nm. Precision studies of tooth microregions were carried out with a step of 100 mkm from the surface to the deep layers of enamel.

2.3. IR microspectroscopy with the use of synchrotron radiation

This part of study was conducted at the Infrared Microspectroscopy (IRM) beamline (Australian synchrotron, Victoria, Australia), using a Vertex 80v spectrometer coupled with a Hyperion 3000 FTIR microscope (Bruker Optik GmbH, Ettlingen, Germany) and a liquid nitrogen-cooled narrow-band mercury cadmium telluride (MCT) detector (Bruker Optik GmbH, Ettlingen, Germany). The synchrotron FTIR measurement of the tooth slices was performed in a reflectance mode using CsI window as an IR background reference. All the synchrotron FTIR spectra were recorded within a spectral range of 3800–700 cm^{-1} using 4-cm^{-1} spectral resolution.

3. Results

3.1. Raman microspectroscopy

The Raman spectra obtained from micro-sites of intact and fluorous teeth samples are shown in figures 1a and 2a in the range of 250–600 cm^{-1} which were collected at several enamel points in the direction from the enamel surface to the dentin (figures 1b, 2b) with a step of 100μ.

Based on the analysis of the spectral data, it was found that the most intense lines in the spectra belong to the PO_4^{3-} HAP modes where the ν_1 vibrations are localized around 962.6 cm^{-1}, ν_3 in the ranges 1006.2, 1030, 1047.4 cm^{-1} and ν_2 428.4 cm^{-1}, 448.2 cm^{-1}, and ν_4 about 579.6 cm^{-1}, 591.6 cm^{-1}. As the experimental data suggests (figure 1a), a change in the shape of the mode is characteristic of the $PO_4^{3-}\nu_4$ vibration, since there is a redistribution of the intensity of its components. The change in the intensities of the components of the $PO_4^{3-}\nu_4$ vibration is noticeable while comparing the spectra

obtained from different layers of intact enamel. In addition, a different form of the PO_4^{3-} ν_4 vibration is observed while comparing the spectra obtained from similar intact enamel and enamel with fluorosis microregions (figures 1a, 2a).

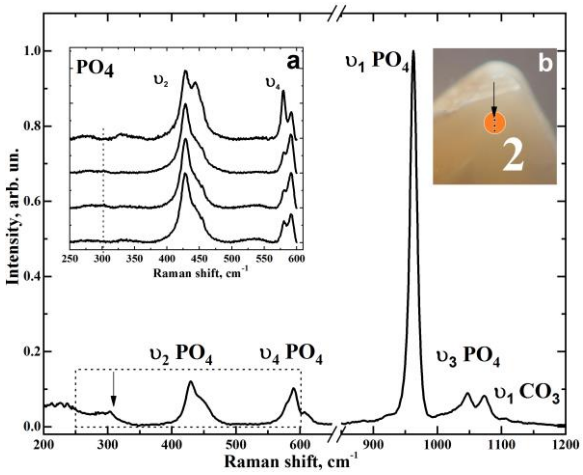


Figure 1. The Raman spectra of enamel of intact tooth.

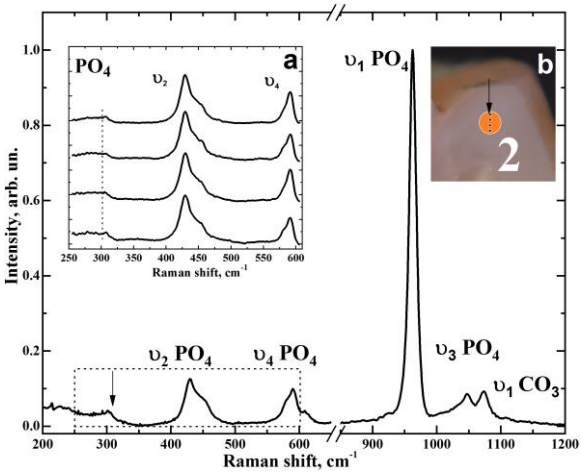


Figure 2. The Raman spectra of tooth enamel with fluorosis s.

Note that in all the Raman spectra (figures 1a, 2a) there are maxima associated with the inclusion of carbonation CO_3^{2-} into the apatite lattice. The peak located at about 1073 cm^{-1} is the CO_3^{2-} vibration which replaced the PO_4^{3-} group in the CHAP lattice (B type of substitution). A low-intensity vibration localized in the area of 1106 cm^{-1} is associated with the inclusion of the carbonation CO^{2-}_3 into the OH^- group lattice in the position of the OH^- group (A-type substitution). A low-intensity broad band is also observed in the spectra in the region of 280–311 cm^{-1}, which relates to the Ca_{II} – OH vibrations of the hydroxyapatite and Ca_{II} -F from the fluoroapatite (FAP) [12].

3.2. Results of the IR-microspectroscopy of the reflection

Based on the analysis of the data in Figure 3.4, it was found that in the IR reflection spectra of all the samples there are vibrations associated with the enamel mineral matrix.

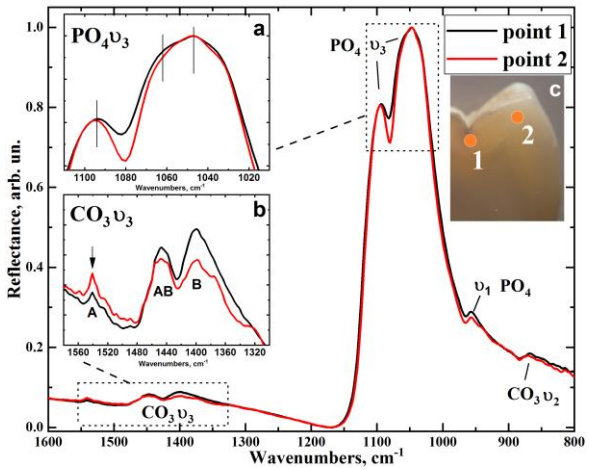


Figure 3. The IR-spectra of enamel of intact tooth.

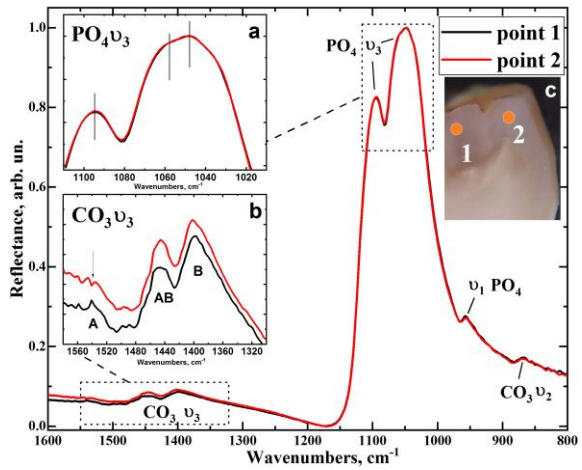


Figure 4. The IR-spectra of tooth enamel with fluorosis.

The maxima in the spectra located near 1094, 1060, 1048, 1040 cm^{-1} are the ν_3 vibrations of the PO_4^{3-} radical and a peak at about 956.4 cm^{-1} can be attributed to PO_4^{3-} ν_1. In addition, the reflection spectra contain bands associated with the CO_3^{2-}carbonation which replaces phosphate or hydroxyl radicals (A and B-type substitutions, respectively). The active modes in the spectra are the CO_3^{2-} ν_3 in the area of about 1540.7 (A-type), 1446.6 (AB-type), 1401.3 (B-type) cm^{-1} as well as the CO_3^{2-} ν_1 879 ((A-type - shoulder), 870.6 (B-type) cm^{-1}. Figures 3a and 4a show separately the areas of the main peak ν_3 of the PO_4^{3-} radical in the area of 1000-1100 cm^{-1} and the vibrational area in Figures 3b and 4b 1600–1300 cm^{-1} correlated with the CO_3^{2-}carbonation in the A- and B-type positions. As can be seen in figure 3, in the case of intact teeth, the IR reflection spectra from enamel areas located at different depths are characterized by a redistribution of high-intensity components band ν_3 of the PO_4^{3-} radical, which is in agreement with the previously obtained data [8]. Similarly, a redistribution of intensity is observed for the components of the carbonate anion band CO_3^{2-} ν_3 corresponding to the A and B substitution types located both in the range 1550–1350 cm^{-1}, so and in the area of 890–860 cm^{-1}. This corresponds to different contents of the carbonate ion CO_3^{2-} in the enamel apatite structure. The analysis of the IR spectra obtained from tooth enamel microregions with dental fluorosis shows that the profile of the spectral band of the PO_4^{3-}radical in enamel zones on its varying depth is almost identical (figure 4).

4. Discussion

As shown in [12], through the course of the study of severe cases of fluorosis, the inclusion of a large proportion of fluorine in the OH^- position resulted in a shift of the Raman scattering band ν_1 PO_4^{3-} to the high frequency area. Our experimental data show that in the spectra of the samples of enamel with fluorosis (figure 2) there is no shift of the main band relative to samples of intact enamel, which indirectly confirms the fact that a low amount of fluorine is found in the apatite. Simultaneously, the redistribution of the intensity of the components of the PO_4^{3-} ν_4 mode in the area of 579.6 cm^{-1}, 591.6 cm^{-1} observed during scanning of the microregion (figure 2a) may indirectly indicate the presence of fluorine in the structure of the CHAP enamel in accordance with the data in [13]. However, in accordance with the data in [14], the redistribution can also be generated by different orientations of the apatite crystals in the enamel prisms. In the case of the enamel with fluorosis, such a redistribution is not observed within the same microregion (figure 2 a,b). This might mean that the contribution of fluorine inclusion into the apatite structure is key to the intensity transformation. This is also confirmed by an increase in the intensity of the vibrations of a wide low-intensity band of 280-311 cm^{-1}, which relates to the Ca_{II} – OH vibrations of the hydroxyapatite and Ca_{II}-F from the fluoroapatite (FAP) [12], but becomes more prominent in the case of fluorosis with a maximum shift to 311 cm^{-1} (figure 2a). Simultaneously, based on the analysis of the experimental spectral data (figures 1,2), in the samples of the intact and enamel with fluorosis there are A and B substitution types with the presence of the corresponding modes in the area of 1106 and 1073 cm^{-1}, respectively. Moreover, in the case of the dental fluorosis the intensity of the CO^{2-}_3 ν_1 A-type modes is several times lower both in the surface and in the deep layers of the enamel (figure 2), which is most likely due to the inclusion of fluorine atoms into the apatite structure.

The investigation of the samples of enamel with fluorosis by means of the IR spectroscopy in the reflection using synchrotron radiation in various layers of enamel shows a high stability of the ratio of mode intensities of the PO_4^{3-} ν_3 1094, 1060, 1048, 1040 cm^{-1}, PO_4^{3-} ν_1 956.4 cm^{-1}, which is in agreement with the Raman data spectroscopy on the stability of the apatite crystal lattice in the case of fluorosis. Moreover, in the IR spectra, similarly to what was observed by means of the Raman spectroscopy, there is a redistribution of the CO_3 ν_3 intensity modes localized around 1540.7, 1446.6, 1401.3 cm^{-1} and correlated with the A and B type of substitution.

The use of the Raman and IR spectroscopy data on the mineralization processes of enamel apatite will be instrumental in distinguishing between healthy and damaged hard tissues of the tooth in the initial stages of fluorosis.

5. Conclusion

According to the data of optical spectroscopy, in the case of the initial stages of the fluorosis disease, fluorine atoms are introduced into the enamel apatite structure causing the formation of the fluorine-substituted apatite. It was shown that the microregions of the enamel with fluorosis contain defective hydroxyapatite where the positions of the hydroxyl groups are replaced by fluorine atoms with the CO^{2-}_3 carbonate anion being replaced from the positions of A-type defects in the apatite lattice. Simultaneously, the analysis of the spectral data shows that the inclusion of fluorine atoms in the initial stages of the disease does not exclude the carbonate anion from the position of B-type defects in the enamel apatite. Mineralization of enamel in the presence of a high amount of fluorine also leads to the stabilization of the apatite structure based on the stable ratio of the PO_4/CO_3intensity modes (580-615cm^{-1} and 1045-1080cm^{-1}) in different layers of the enamel.

The above features can be employed in the development of a new method for diagnosing fluorosis.

Acknowledgments

This work was supported by the grant of Russian Science Foundation, grant number 16-15-00003. The part of this research was undertaken with The Infrared Microspectroscopy (IRM) beamline at the Australian Synchrotron.

References

[1] Carey C M 2014 Focus on Fluorides: Update on the Use of Fluoride for the Prevention of Dental Caries *J. Evid. Based Dent. Pract.* **14** Supplement 95–102
[2] O'Mullane D M 2016 Fluoride and Oral Health *Community Dent. Health* 69–99
[3] Makeeva I M, Volkov A G and Musiev A A 2017 Endemic fluorosis of the teeth - causes, prevention and treatment *Russ. J. Dent.* **21** 340–4
[4] Campillo M, Lacharmoise P D, Reparaz J S, Goñi A R and Valiente M 2010 On the assessment of hydroxyapatite fluoridation by means of Raman scattering *J. Chem. Phys.* **132** 244501
[5] Gerth H U V, Dammaschke T, Schäfer E and Züchner H 2007 A three layer structure model of fluoridated enamel containing CaF2, Ca(OH)2 and FAp *Dent. Mater.* **23** 1521–8
[6] Ramya R, Thanveer K and Ajithkrishnan C 2014 Development and psychometric testing of a visual analog scale for dental fluorosis *J. Indian Assoc. Public Health Dent.* **12** 4
[7] Seredin P V, Goloshchapov D L, Prutskij T and Ippolitov Yu A 2018 A Simultaneous Analysis of Microregions of Carious Dentin by the Methods of Laser-Induced Fluorescence and Raman Spectromicroscopy *Opt. Spectrosc.* **125** 803–9
[8] Jegova G, Titorenkova R, Rashkova M and Mihailova B 2013 Raman and IR reflection micro-spectroscopic study of Er:YAG laser treated permanent and deciduous human teeth *J. Raman Spectrosc.* **44** 1483–90
[9] Fleet M E 2017 Infrared spectra of carbonate apatites: Evidence for a connection between bone mineral and body fluids *Am. Mineral.* **102** 149–57
[10] Seredin P, Goloshchapov D, Ippolitov Y and Pimm Vongsvivut 2018 Pathology-specific molecular profiles of saliva in patients with multiple dental caries—potential application for predictive, preventive and personalised medical services *EPMA J.* **9** 195–203
[11] Seredin P, Goloshchapov D, Prutskij T and Ippolitov Y 2015 Phase Transformations in a Human Tooth Tissue at the Initial Stage of Caries *PLoS ONE* **10** e0124008
[12] Penel G, Leroy G, Rey C, Sombret B, Huvenne J P and Bres E 1997 Infrared and Raman microspectrometry study of fluor-fluor-hydroxy and hydroxy-apatite powders *J. Mater. Sci. Mater. Med.* **8** 271–6
[13] Penel G, Delfosse C, Rey C, Hardouin P, Jeanfils J, Delecourt C, Lemaître J and Leroy G 2003 Raman microspectrometry studies of calcified tissues and related biomaterials *Dent. Med. Probl.* **40** 37–43
[14] Tsuda H and Arends J 1997 Raman spectroscopy in dental research: a short review of recent studies *Adv. Dent. Res.* **11** 539–47