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Study of the FTIR spectrum of whole saliva at normal

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ABSTRACT
Thanks to the integration of modern biotechnologies and information about saliva biomarkers, there are now more and more opportunities for the diagnosis of a wide range of diseases. At the same time, dental caries and its prevention remain an urgent health problem. In this article, the saliva of healthy donors was studied by FTIR spectroscopy. Areas of the spectrum were identified and confirmed, allowing to assess the state of dental health and monitor the development of caries and the effectiveness of its treatment. Ideas about the micellar structure of calcium phosphate in saliva have been obtained, which allows us to propose a mechanism for maintaining oral homeostasis in the chain: tooth enamel – saliva – the occurrence of caries – the deposition of tartar. FTIR spectroscopy, as a method for studying biological fluids and tissues, opens up new possibilities for saliva diagnostics.
Keywords*: whole (mixed) saliva; saliva diagnostics; caries; FTIR spectroscopy.*

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INTRODUCTION

Recently, saliva diagnostics has found its application in monitoring a number of diseases, including periodontal diseases and other diseases of the oral cavity [1-3]. Thanks to the integration of modern biotechnologies and information about saliva biomarkers, there are more and more opportunities for the diagnosis of diseases such as cancer[4-7], autoimmune[8,9], bacterial[10-14], cardiovascular[15-17], as well as viral and HIV infections[18,19]. These capabilities significantly expand the range of saliva-based tests, extending them from assessing the condition of the oral cavity to the entire physiological system of the body. Thus, saliva research is at the forefront of diagnostic technologies and in the near future may be recommended to clinicians as a reliable alternative to invasive technologies.

Dental caries is still the main problem of dentistry due to its wide spread in all countries of the world. It should be noted that this disease belongs to a medical and social problem, since dental caries, and mainly its complications, lead to loss or decrease in working capacity, the appearance of aesthetic defects, disorders of the gastrointestinal tract[20].

WHO experts note that the prevalence of caries among the population of economically developed countries reaches 95-98%[21,22].

The etiology, pathogenesis and treatment of caries are considered to be well studied[23-26]. However, the problem of timely detection and prevention of caries development remains relevant at the present time[27,28]. Solving this problem requires the development of effective diagnostic methods for detecting caries in the early stages in order to reduce its impact on the body.

We have previously shown that the determination of the pH of mixed saliva can become a reliable non-invasive method for assessing the acid-base balance of the body, especially in the oral cavity, and can

determine the predisposition to caries[29,30]. In this context, it is of not only scientific, but practical interest to conduct studies of mixed saliva in norm and pathology using FTIR spectroscopy methods. This formulation of the problem is also motivated by the fact that in the scientific literature available to us, the number of publications devoted to the study of FTIR spectra of oral fluid remains insufficient, and the information is sometimes contradictory, although even single articles show the effectiveness of the use of IR spectroscopy in early diagnosis and determination of the risk of caries[31-34].
The purpose of this work is to study the FTIR spectra of mixed saliva in normal.

MATERIAL AND METHODS

The work uses the FTIR spectrometer IRAffinity-1S (Shimadzu, Japan) with the prefix of a single disturbed total internal reflection MIRacle 10. To measure the spectrum of whole saliva, a sample was applied to a prism using a pipette microdosator and dried for 3–5 minutes. The IR spectrum was taken in the range of 600-4000 cm^{-1}, the number of scans was 20 with a resolution of 4 cm^{-1}.
The study involved 21 healthy volunteers (9 men, 12 women, age 21±0.9 years). Criteria for inclusion in the study group: absence of signs of active infection (including purulent processes), oral sanitation. Sampling was carried out from 12 to 14 hours to minimize circadian changes in saliva. The volunteers did not receive food for at least 1.5 – 2 hours before taking the sample. Before taking the sample directly, donors rinsed their mouths with distilled water. Saliva formed in the first 2–3 minutes after mouthwash was not used for analysis and was spat out. The collection of the following portions of saliva was carried out in plastic tubes with a volume of 3 ml.

RESULTS AND DISCUSSION

Infrared spectroscopy is a branch of molecular optical spectroscopy that studies the absorption and reflection spectra of electromagnetic radiation in the IR region. This region of the spectrum is used to identify substances, because it allows, by analogy with a "fingerprint", to identify a given substance by the presence in it of certain functional groups of organic compounds having characteristic vibrational frequencies (modes). Vibrations are mainly caused by certain groups of atoms, and despite the change in the bond length and angles between them with each oscillation, the contribution of the rest of the molecule is small. Thus, any organic substance or mixture of substances has separate unique IR spectra that can be interpreted both qualitatively and quantitatively. This provision applies to the IR spectrum of saliva and other biological fluids and tissues. Figure 1 shows the IR spectrum of saliva of healthy people.
Three main groups of macromolecules can be distinguished in the IR spectrum of saliva: lipids (3000-2800 cm^{-1}), proteins (1700-1600 and 1560-1500 cm^{-1}) and nucleic acids (1250-1000 cm^{-1}). The widest absorption band at 3273 cm^{-1} corresponds to amide A. The narrow absorption band of medium intensity at 2058 cm^{-1} refers to thiocyanate anions (SCN^-). This absorption band is characteristic of saliva and manifests itself in an area that usually does not contain any absorption peak for other biological samples[31,37]. This statement is confirmed by other studies[38,39]. For example, a comparison with the IR spectra of blood plasma, semen and vaginal secretions confirms that the absorption bands of thiocyanate anions is observed only in the IR spectra of saliva, although as a result of natural fluctuations in the concentration of this anion, the intensity may vary.
Two narrow absorption bands at 1651 and 1541 cm^{-1} are classified by us as amide I and II.
The band at 1074 cm^{-1} in the spectra of saliva represents fragments of sugars. Other, less intense absorption bands correspond to methylene groups of amino acid side chains in proteins and lipids (1456 cm^{-1}), amino acid side chains (1404 cm^{-1}), amide III/phospholipids (1338-1317 cm^{-1}) and fragments of sugars, glycosylated proteins and phosphate groups in nucleic acids (1080-950 cm^{-1}). The above is more fully presented in Table 1.
In[31], the IR spectra of α-amylase and saliva were compared, which shows the presence of overlapping maxima in the sugar absorption region. Consequently, the oscillation modes in the range 1080-950 cm^{-1} can be attributed to glycosylated α-amylase, as well as to other sugar residues present in saliva.
In addition to these vibrational modes, IR spectra can display absorption bands characteristic of the presence of caries[39]. These include vibrational modes in the range of 2150-1950 cm^{-1} of the IR spectrum, which should be attributed to the coupling of -N=C=S thiocyanate anions contained in mixed saliva. We found for this absorption band correspond to 2058 cm^{-1}. Compared with the norm in patients with multiple caries, the intensity of this absorption band is significantly higher. It should be taken into account that NCS^-ions are local antibacterial agents for anaerobic microorganisms[39], which means that the presence of thiocyanates in saliva may also indicate the state of the local immune status of the oral cavity[40].
We also noted that the intensity of this absorption band in people who abuse tobacco is also higher. This fact can be used for forensic - medical research and criminology

The mineralizing function of saliva is due to the presence of Ca^{2+} cations and HPO_4^{2-}hydrophosphate anions in the salivary fluid, which are in saturation. The mechanism of mineralization of dental tissue by saliva is explained by the fact that calcium phosphate is in a micellar state. The scheme of the structure of the micelle $Ca_3(PO_4)_2$ is shown in Figure 2.

The core of the micelle consists of $Ca_3(PO_4)_2$, potential – determining ions – HPO_4^{2-}, counterions – Ca^{2+}, they are also part of the diffuse layer[41,42].

At the same time, it is absolutely possible that the composition of the colloidal phase of calcium phosphate will be stabilized by calcium hydrophosphate. The micelle is protected from aggregation by glycolized proteins, mainly mucin, which is adsorbed on colloidal particles, thus showing a protective effect.

It is noted in[42] that a significant increase in the concentration of sodium and potassium ions in saliva leads to a decrease in the protective properties of biofilms, which is apparently due to the protective hydrate shells of glycoprotein macromolecules and their denaturation.

The stability of saliva micelles also depends on the pH of the medium. So, in an acidic environment, the charge of the micelle can decrease by half, for the reason that the HPO_4^{2-} ions bind H^+ ions and turn into dihydrophosphate-$H_2PO_4^-$ - ions. This reduces the stability of the micelle, and the dihydrophosphate ions of such a micelle cannot participate in the process of remineralization of tooth enamel. It was found that at pH < 6.4, the process of demineralization of tooth enamel increases, while alkalinization, on the contrary, leads to an increase in the concentration of phosphate ions, which combine with Ca^{2+} ions and a poorly soluble compound $Ca_3(PO_4)_2$ is formed. However, even at pH > 7.5-7.8, it is possible to increase tartar formation on the teeth, which also adversely affects the health of the oral cavity. Thus, the optimal saliva pH value for the mineralization-remineralization process of tooth tissues is a pH value of ~7.2 -7.3. This conclusion is indirectly confirmed by the results of our studies on measuring saliva pH[30].

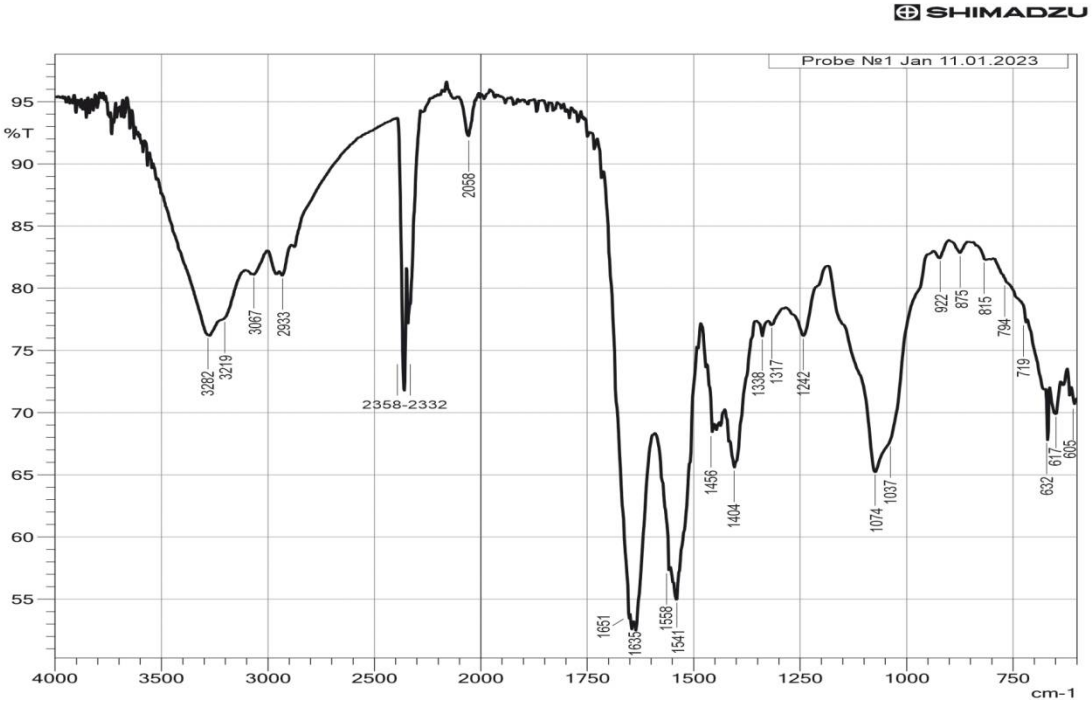

Figure 1. IR spectrum of saliva of healthy donors.

$$\{[mCa_3(PO_4)_3]\bullet nHPO_4^{2-}\bullet(n\text{-}x)\ Ca^{+2}\}^{2x-}\bullet xCa^{2+}$$

Core | Potential determining ions | Counterions | Diffuse later

Figure 2. Scheme of the calcium phosphate micelle.

Table 1. The main absorption bands (cm^{-1}) in the IR spectra of saliva

The present work	Types of vibration	Assignment	Reference
3282	νNH	amide A	3270-3285 [34]
3067	R-NH_2 (ν_{as}NH, ν_sNH) R-NH (νNH)	Primary and secondary amines	3067 [33]
2933	νCH_2, νCH_3	methylene groups of lipids of the oral mucosa	2933, 2875 [32]
2358-2332	ν_{as} O=C=O	Atmospheric carbon dioxide (used for calibration)	2349 [43]
2058	νSCN^-	thiocyanates	2058 [31,38,39]
1651	νCO	amide I	1645 [34, 35]
1541	δNH, νCN	amide II	1544 [31] 1548 [34,35]
1456-1404	δCH_2, δCH_3	methylene groups of side chains of amino acids, lipids and proteins	1450, 1395-1410 [34, 35]
1338-1317	δNH, νCN	amide III	1330 -1300 [31, 34, 35]
1242	ν_{as}PO	phospholipids	1244 [36]
1074	νCN, $\nu_s PO_2^-$	DNA, RNA	1078 [31]
1037	νCC, νCO, νCH_2OH	sugars, glycosylatedproteins	950-1080 [37]
922 - 794	ν –C-O-C-	aliphatic compounds, vibrations of the carbon skeleton.	920-800 [43]
794-719	ν$PO(P_2O_7)$	Oligo- and polysaccharides, phosphatases, phospholipids	735-775 [31]
632-617	δOCN	amide IV	625-765 [31]
632-813	δNH	amide V	640-800 [31]
605	δCO	amide VI	535-605 [31]

Note: ν- stretching (ν_s — symmetric; ν_{as} — asymmetric), δ — bending vibrations.

CONCLUSIONS

During the experiment on the study of the FTIR spectrum of saliva, sections of the spectrum were identified and confirmed, allowing assessing the state of dental health and monitoring the development of caries and the effectiveness of its treatment. Ideas about the micellar structure of calcium phosphate in saliva allow us to propose a mechanism for maintaining oral homeostasis in the chain: tooth enamel – saliva – the occurrence of caries – the deposition of tartar, which provides an opportunity to implement preventive measures aimed at maintaining and preserving the functions of saliva and oral health in general.

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CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest

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