

## Research of mineral composition of human salivary calculi

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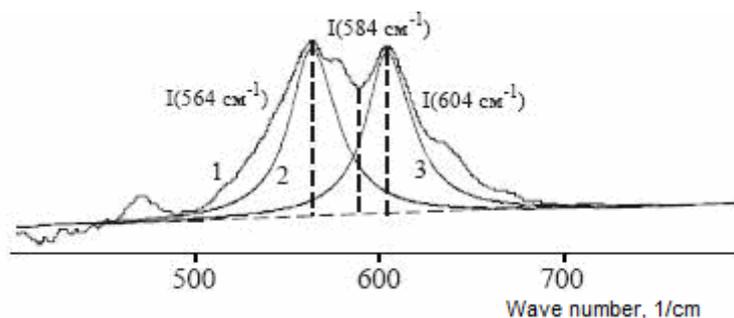
Salivary calculi - pathogenic organomineral concretions of maxillofacial areas, which are formed from the saliva more often in submandibular gland and its ducts. They are folded, usually because of rhythmically deposited layers of organic and inorganic components around a central core. Formation of salivary stones may be related to a violation of the outflow of saliva or exchange any of its elements (mostly calcium metabolism), with inflammatory processes in the salivary glands with a significant participation of microorganisms, poor nutrition, with the state of the living environment of patients, etc. [Denisov, 2003; Corago, 1992; Palchick et al, 2004]. *Objective:* to study the mineral composition of salivary calculi residents of the Omsk region.

**Materials and methods.** Study of phase composition of salivary calculi (14 samples) was carried out by using XRD, IR spectroscopy and thermal analysis. Diffraction patterns were obtained by "the powder method" on a stationary X-ray DRON-3. During of the phase analysis used monochromatic copper ( $\lambda = 1.54178 \text{ \AA}$ ), cobalt ( $\lambda = 1.79021 \text{ \AA}$ ) and molybdenum radiation ( $\lambda = 0.71069 \text{ \AA}$ ). Operating mode:  $U = 35 \text{ kV}$ ,  $I = 15 \text{ mA}$  for cobalt and copper radiation;  $U = 38 \text{ kV}$ ,  $I = 10 \text{ mA}$  for Mo radiation, the rotation speed detector  $1^\circ/\text{min}$ , the velocity of the chart of  $720 \text{ mm/h}$ , operating scale grapher  $1 \cdot 10^3 \text{ pulses/sec}$ , alignment gap:  $1 \text{ mm}$ ,  $0.25 \text{ mm}$ . Angles  $2\Theta$  Bragg reflections and their relative intensity were determined from the diffraction patterns. Qualitative analysis of phase composition of the sample was conducted by comparing the experimental values of interplanar distances and relative intensities of the diffraction peaks corresponding to a set of tabular values for each of the proposed phases. Sensitivity of the XRD data of the measurements was 3%.

The method of infrared spectroscopy was used in order to obtain more information on the composition of salivary calculi (spectrophotometer "FT-801"). Samples were prepared by pressing pellets with KBr:  $0.5 \text{ mg}$  of salivary calculus, pounded into powder, mixed with the  $50 \text{ mg}$  of KBr, and then the mixture was transferred into a mold and pressed into disk-shaped tablet with a diameter of  $3 \text{ mm}$  at room temperature. Record the spectrum of the samples was carried out in  $4000$  to  $500 \text{ cm}^{-1}$  with the total number of scans is 32. The program «ZaIR 3.5» was used for receiving, processing and retrieval of infrared spectra in the databases, which contain more than 130000 spectra. Sensitivity of infrared spectroscopy for measurements was 5%.

Thermal studies were carried out on derivatograph firm Perkin Elmer SII Diamond - TG-DTA software PYRIS 7.0; investigated temperature range was  $25$ - $1000 \text{ }^\circ\text{C}$ ; heating rate -  $5$ - $20 \text{ }^\circ\text{C/min}$ , were used samples weighing  $25$ - $30 \text{ mg}$ , the sensitivity of weight measurement was  $0.2 \text{ mg}$  (the error in determining the weight loss  $\sim 0.1\%$ ), sensitivity of the measurement thermal effects (peak position at DTA)  $0.06 \text{ mV}$ . Obtained by thermo gravimetric (TG), differential thermal (DTG) curves were determined massive changes upon heating, the differential-gravimetric curve (DTA) was used for the characteristics of the observed thermal effects. The results are presented in digital form and processed statistically using the software Statistic Soft 2006.

A detailed study of the structural features was performed semi-quantitative analysis of the spectra using the software package PeakFit\_v 4.11 [Shi et al, 2005]. The degree of crystallinity of the sample was estimated by the parameter of the infrared splitting of the antisymmetric bending vibration  $\nu_4$  of O-P-O (spectral range  $500$ - $650 \text{ cm}^{-1}$ ), defined as the ratio of the intensities of the two peaks to the intensity of "depression" among them:  $\text{IRSF} = (I(564 \text{ cm}^{-1}) + I(604 \text{ cm}^{-1})) / I(584 \text{ cm}^{-1})$ . By reducing the crystallinity bands with maxima  $564$  and  $604 \text{ cm}^{-1}$  broadened, and resolution between them becomes smaller. This reduces the value of the splitting of the infrared (K) (Fig. 1).



**Fig. 1.** Decomposition of the infrared spectrum in the 500-650  $\text{cm}^{-1}$  vibration of O-P-O bonds of phosphate groups at two elementary absorption bands

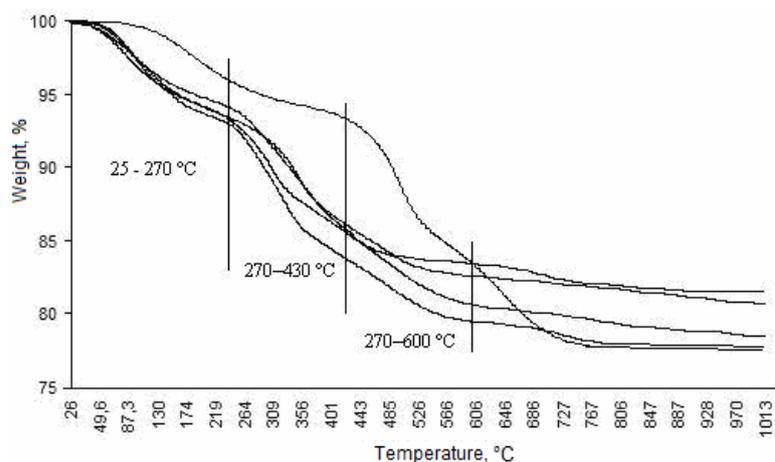
**Results and discussion.** Established that the mineral component of salivary calculi inhabitants of Omsk presented hydroxyapatite  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ , in one sample with hydroxyapatite present whitlockite  $[\beta\text{-Ca}_3(\text{PO}_4)_2]$ , in another - brushite  $[\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}]$ . According to preliminary estimates from saliva thermodynamically likely to form brushite, octacalcium phosphate  $[\text{Ca}_8\text{H}(\text{PO}_4)_3 \cdot 2.5 \text{H}_2\text{O}]$  and hydroxyapatite. Comparing the indices of supersaturation calculated for calcium phosphates of different stoichiometric composition, it should be noted that within the chosen thermodynamic model in saliva (pH 5.2 – 8.0) has the greatest degree of supersaturation of hydroxyapatite. It is known that brushite is crystallized at lower pH values than apatite, and according to our thermodynamic calculations, is a metastable phase. Presence of brushite in some samples indicates marked variation of pH of saliva in the growth of pathogenic entities [Belskaya and Golovanova, 2008].

Comparison of cell parameters of salivary calculi ( $a = 9.449$  (5),  $c = 6.883$  (3) Å) with a stoichiometric hydroxyapatite ( $a = 9.418$ , with  $a = 6.884$  Å) shows that the parameter and the hydroxyapatite of salivary calculi at 0.33% more than at stoichiometric, and the value of virtually the same. These lattice parameters are characteristic of non stoichiometric calcium-deficient hydroxyapatite, including carbonate. Parameter and investigated hydroxyapatite close to the upper limit of this parameter variation in apatite of tooth enamel. Earlier [Yelnikov *et al*, 2005] a formula for hydroxyapatite of salivary calculi:  $[\text{Ca}_{8.09-8.19}\text{Mg}_{0.13}\text{Na}_{0.21}\text{K}_{0.02}\square_{1.55-1.45}](\text{PO}_4)_{4.22-4.39}(\text{HPO}_4)_{0.97-0.87}(\text{CO}_3)_{0.75-0.76}(\text{SO}_4)_{0.06-0.00}[\text{OH}_{0.12-0.21}\text{F}_{0.10}\text{Cl}_{0.02}(\text{CO}_3)_{0.11}(\square, \text{H}_2\text{O})_{(1.66-1.56)}]$ . According to this formula, the vacancy in the positions of calcium in the structure of the investigated apatite is 15%, significantly higher than in the apatite structure of enamel and dentin (10 and 6% respectively [Yelnikov *et al*, 2005]).

The IR spectroscopy confirmed the phosphate composition of the inorganic component of salivary calculi. In the spectra of all samples the absorption bands corresponding to vibrations of P-O bonds hydroxyapatite, which coincides with the XRD data. Not detected absorption bands characteristic for OH groups, however, all examined samples contain carbonate ions. The presence of bands of vibration C-O bond ( $1420, 1460 \text{ cm}^{-1}$ ) indicates substitution of  $\text{RO}_4^{3-}$ -tetrahedral carbonate ions (the so-called substitution of B-type). The band  $1550 \text{ cm}^{-1}$  indicates the substitution of OH - groups of carbonate ions in the channels of the structure of hydroxyapatite, which corresponds to the replacement of A-type. Ratio of carbonate ions, corresponding to the two types of replacements for all the samples is 2:1. In addition, on most of the IR spectra present a broad band of stretching vibrations of water molecules at  $3440 \text{ cm}^{-1}$ , indicating the presence of water molecules in structural channels of hydroxyapatite and a band of deformation vibrations of water at  $1650 \text{ cm}^{-1}$ .

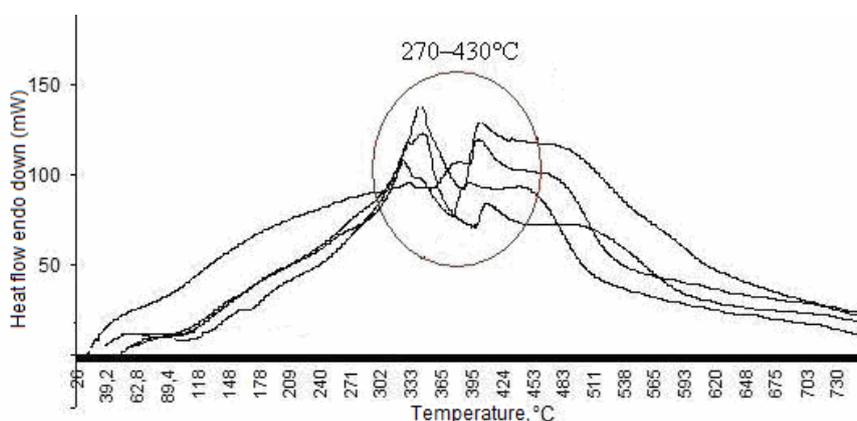
The calculated values of crystallinity of the samples (IRSF = 4.85) show that salivary calculi is typical of apatite formation of less ordered structures characterized by less than the value of splitting the infrared peak of the antisymmetric bending vibration of the O-P-O than in the control sample (stoichiometric hydroxyapatite, IRSF = 5.35).

The results of the thermal studies on all derivatogram observed mass loss in the four main temperature ranges (Fig. 2): 25-270 °C (corresponds to the loss of adsorbed water); 270-430 °C (evaporation of structural water and the removal of low molecular weight organic substances - amino acids, proteins of low molecular weight, glucose, etc.); 430-600 ° (conversion of macromolecular organic compounds, including glycoprotein's such as mucin) and 700-900 °C (corresponds to the transition stoichiometric carbonate hydroxyapatite, forming a crystalline substance of salivary calculi in stoichiometric due to the removal of volatile components, mainly carbon dioxide).



**Fig. 2.** Weight losses of samples of salivary calculi

It was established that the maximum mass loss observed in the decomposition of the organic phase of calculi in the temperature range 270-600 °C, with most of the energy effect is observed for the loss of low molecular weight organic matter (Fig. 3).



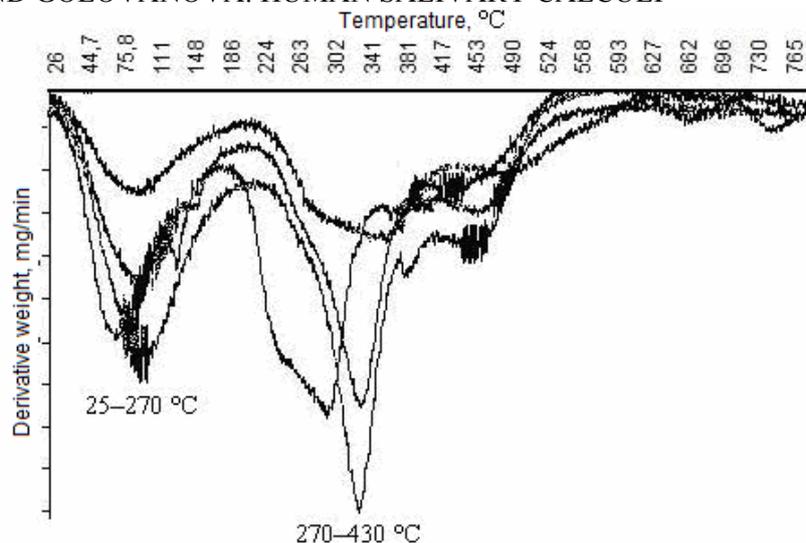
**Fig. 3.** Energy effect of the thermal decomposition of samples of salivary calculi

Mathematical treatment of the consideration of the thermal characteristics showed that the mass loss during thermal decomposition of salivary stones is  $42.15 \pm 4.97$  wt.% (Table 1) mainly due to the decomposition of organic components, namely, low molecular weight substances, the loss of volatile components and adsorption of water.

**Table 1.** Thermal characteristics of samples of human salivary calculi

№ sample	Total mass loss	The temperature range		
		25 – 270°C	270 – 600°C	600 – 900°C
3	40.74	6.8	32.51	1.43
8	42.96	7.4	33.59	1.97
9	36.27	6.5	28.71	1.06
10	44.21	6.4	35.43	2.38
14	40.49	7.5	30.65	2.34
average	$42.15 \pm 4.97$	6.9	32.18	1.84

When heated in the temperature range of 50–580 °C the samples of salivary calculi are two-stage dehydration (Fig. 4). About 600–700 °C due to chemical interaction of components, are a partial decarbonization and the crystallization of products.



**Fig. 4.** Differential curves of mass loss of samples of salivary calculi

According to [Votyakov *et al*, 2009], the selection of gaseous water, carbon and nitrogen oxides occurs synchronously with the burning of organic matrix (230–600 °C); with the mass spectra of CO<sub>2</sub> and NO, in general, repeated profiles of the different curve of mass loss (two-stage combustion of organic matter) in while the peak water output corresponding to the decomposition of organic matter in the second phase is not observed (at temperatures above 350 °C separation of gaseous H<sub>2</sub>O decreases monotonically with no defined maximum), this indirectly confirms the presence of less ordered, low molecular weight fractions of organic matter, weak hydrogen bonds which are broken at lower temperatures, and hydrogen to form water is allocated. Authors [Nedoseko *et al*, 2004] attempted to link the thermal desorption of water in synthetic hydroxyapatite with its crystal-state: the first thermo desorption peak at 100–300 °C was correlated with the strongly chemisorbed water on the porous surface of hydroxyapatite, and the second in the 350°C - with a crystalline hydrate with water, a division of hydroxyapatite the reaction:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \cdot n\text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + n\text{H}_2\text{O}$ . Weak maximum at about 700 °C, apparently due to the release of CO<sub>2</sub> from the structure of non-stoichiometric low-crystalline carbonate hydroxyapatite by the reaction:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{CO}_3)(\text{OH})_2 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{CO}_2\uparrow$ .

Thus, it is shown that the mineral component of salivary stones represented carbonate containing hydroxyapatite, which was confirmed by X-ray diffraction and thermal analysis and infrared spectroscopy.

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