

Spectroscopic criteria for early diagnosis of changes in the mineral and organic matrix of hard dental tissues

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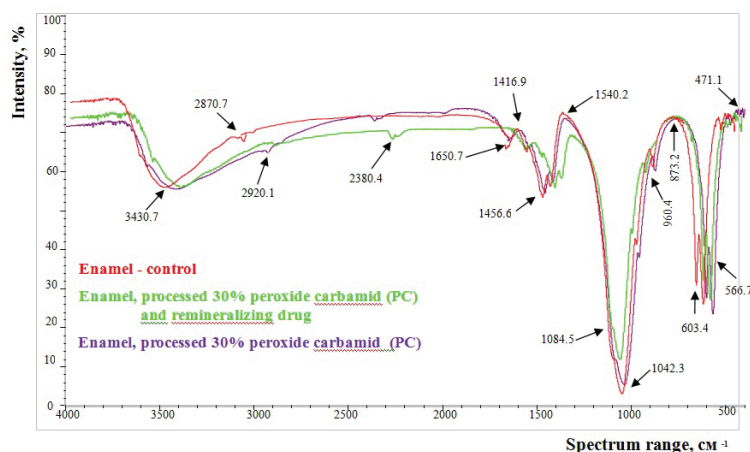
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Abstract

At the present stage of development of medical science, experimental and clinical dentistry is often used in scientific research, which allows diagnosing preclinical stages of manifestations of changes in tissues and organs of the oral cavity and biological fluids at the level of nanotechnology enabling to study the mechanisms of molecular transformation that lead to occurrence of pathological changes. One of the promising methods of research is infrared spectroscopy. The advantage of the method is that the absorption spectrum can be obtained with a small amount of the test substance (0.1–0.01 cm³) and under conditions of different aggregate states (solution, powder, liquid).

Graphical abstract



Keywords

infrared spectroscopy, hydroxyapatite, carbamide peroxide

Introduction

Infrared spectroscopy (IRS) is a part of molecular optical spectroscopy that studies the absorption and reflection spectra of electromagnetic radiation in the infrared region. This method can be used to study the state of the mineral and organic matrix of hard dental tissues and the effect of factors of exogenous and endogenous origin, as well as the state of indicators of oral fluid that reflect changes in the structure of teeth, periodontal and oral tissues, and for the purpose of planning the volume of dental intervention with further evaluation of the treatment efficacy.

The molecular structure of apatites – mineralized tissues of the tooth, can differ sharply both in the conditions of the physiological norm and in pathological conditions. The optimal composition of the hydroxyapatite corresponds to the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with a molecular ratio of Ca/P ions corresponding to 1.67 (Smith 1996; Gordetsov 2010). Occasionally there are hydroxyapatites with the index ratio 1.33 to 2.0, which is due to the presence of vacant spaces in the structure of hydroxyapatite and the ability to substitute atoms and ions in the crystal itself. The foregoing determines the variability of the crystalline structure of hydroxyapatite. The trigger mechanism for the formation of a week spot on the enamel due to caries is the demineralization process, which is characterized by changes in the structure of hydroxyapatite. Apatites in the enamel include carbonate groups and a number of chemical elements in residual (trace) amount (Na, Mg, K, Zn, Co, Sr, Pb, Al, Cu, Mn, etc.). Moreover, free crystal lattices are often stored in the structure of the crystalline lattice (vacancies) that affect the crystalline density of biological apatite and its solubility. The release of ions from apatite into the environment begins with a decrease in a certain amount of free ions that form this apatite. The first one dissolves apatites that contain carbonates – magnesium and sodium, as well as citrates and apatites with fluorides. The cationic composition of the enamel matrix varies as a result of the life of the tooth, due to the ability of hydroxyapatite crystals to isomorphic and hetero-morphological rearrangements. It should be noted that according to scientific data on the specifics of the chemical structure of the hydroxyapatite crystal, it is the anions that determine the shape, charge, crystalline polarity, which determines its structure and functions. Thus, for the early detection of pathological changes in the enamel structure, the ratio of the concentrations of anions among themselves, as well as the determination of their quantitative and qualitative characteristics by the method of IRS is significant. Cations in the IRS are not displayed or absorbed. Consequently, the change in their concentra-

tions cannot affect the study of the composition of anions on the surface of the dental enamel.

It is established that the nature of chemical bonds in the enamel structure allows determining the initial changes that lead to a violation of the stoichiometry of hydroxyapatite crystals, as well as its demineralization and recrystallization (Ferraro et al. 1990; Kazarina et al. 2014). For structural analysis, IRS is applied in the frequency range between $4000\text{--}200\text{ cm}^{-1}$ (with a wavelength of $2.5\text{--}50\text{ }\mu\text{m}$). The advantage of the method is that the absorption spectrum can be obtained with a small amount of the test substance ($0.1\text{--}0.01\text{ cm}^3$) in its diverse aggregate state (Ferraro et al. 1990; Hrynovets et al. 2016; Kargapolov et al. 2009; Sidorchuk 2012).

There are two types of oscillations of the molecules: valence (oscillations of atoms occur along the axis of the valence bond) and deformation (the atoms in the oscillations deviate from the axis of the valence bond). For each chemical group, there are the corresponding oscillation frequencies (wave numbers) and absorption bands (characteristics). On the spectra, fluctuations of structural molecular and ionic units of the inorganic matrix such as phosphate, carbonate, and hydrophosphate ions are recorded, as well as numerous oscillations associated with a protein matrix. The spectrum band is described by the following indicators: intensity, width, type of polarization (Bell 1975). The intensity of the band characterizes the concentration of chemical groups that absorb the initiating light at the wavelength λ and the corresponding molecular structure of the substance.

Experimental part

Materials and methods

The main aim of the work was to investigate the enamel discoloration of vital teeth and to determine the effect of remineralizing therapy on valence grouping of the enamel surface of the experimental teeth with the help of the active ingredient of carbamide peroxide (CP), as well as to apply the IRS in order to confirm the effectiveness of the therapeutic correction results.

Results and discussion

As a result of analysis of scientific sources, short reports, patents and own research (Bell 1975; Gordetsov 2010; Hrynovets et al. 2016; Zubachyk et al. 2001), methods of dental bleaching with CP-based therapeutic compositions

have been proposed (Zubachyk et al. 2003). The therapeutic composition for clinical whitening of teeth contained the following components in weight, % concentration:

carbamide peroxide –	30
glycerol –	37
vinylin –	18
aerosol –	10
quercetin –	4
three sodium ethylene diamine tetra acetate –	1

For dental bleaching at home, the composition is:

carbamide peroxide –	15
glycerol –	46
vinylin –	22
aerosol –	12
quercetin –	4
three sodium ethylene diamine tetra acetate –	1

The components of the two prescriptions are compatible with each other, and their quantitative and qualitative characteristics correspond to the concentrations described in the Pharmacopoeial Articles, the Temporary Pharmacopoeial Articles, the specifications and the instructions for use.

Unlike most of the bleaching agents, the above-mentioned agents have a pH of 6.8–7.1, which prevents the demineralization of the surface layer of enamel. The bleach was kept for 20 days in a light-proof package.

The CP solution is unstable and is applied to the surface of the tooth and decomposes into hydrogen peroxide (up to 30% by weight of CP) and carbamide (70%). In the further decay of hydrogen peroxide, water and oxygen are formed, and urea disintegrates into ammonia and CP. These molecular compounds freely penetrate the surface structures of the enamel due to the relatively small molecular weight (34 g/mol for hydrogen peroxide and 60 g/mol for urea). One of the breakdown products of CP is free hydroxide radicals that can inactivate enzymes, alter the structure of macromolecules and disrupt cells and intracellular organelles. Therefore, when developing the composition of bleaching therapeutic compositions, they should include antioxidant agents that prevent the harmful effects of high-level hydroxide radicals, protecting the functional groups of proteins and other bioactive molecules of organs and tissues of the oral cavity. For this purpose quercetin, an active pharmaceutical ingredient with a pronounced antioxidant effect is included in the proposed bleaching compositions.

It should also be noted that carbamide $\text{CO}(\text{NH}_2)_2$ neutralizes acids (primarily milk) that produce microorganisms of the dental plaque. This process is due to the cleavage of the urea with the enzyme with the formation of carbon dioxide (CO_2) and ammonia (NH_3). Since NH_3 has an alkaline environment, it neutralizes the acid. The pres-

ence of aerosil composition, which is silicon sorbent, prolongs the action of the active component – urea peroxide and its gradual removal from the therapeutic form. Vinylin is known for its anti-inflammatory, enveloping, and regenerating effect, which also reduces the irritant effect of urea peroxide on gum in the formulation. 3-natrium EDTA was introduced into the bleaching composition as a stabilizer that prevents catalytic decomposition of urea peroxide.

At the initial stage, the vestibular surface of the enamel of intact teeth treated by the method of home (14 teeth) and clinical (12 teeth) bleaching in patients aged 17–35 years old was removed by orthodontic indexes. The oral surface of the same experimental teeth served as control.

After the last bleaching procedure, the teeth to be prepared were purified by the end of the brush and washed with distilled water. Enamel was ground with a diamond bur at a rate of 2000 rpm. Overheating of solid tissues was prevented by periodic irrigation of tissues with distilled water and by drying them in a stream of air, as well as by intermittent loading of a rotating tool on a tooth. The sample of enamel was thoroughly triturated in an agate mortar and sieved through a sieve with a diameter of holes of 0.04 mm. To remove moisture and to prevent the chemical activity of potassium bromide (KBr) against hydroxyapatite, the powder was dried at 100–105 °C for 3 hours in a drying cabinet. A hard powder of enamel (3 mg) was mixed in an agate mortar with 300 mg of potassium bromide, the particle size of which was about 30 ... 40 microns. The obtained samples were the same mass, which made it possible to quantify the relative optical density of absorption bands of the IRS, in particular, to determine the content of the test substance in the sample. The samples were prepared by compression of KBr tablets under pressure of 1200–1500 kg/cm² under vacuum conditions. Infrared absorption spectra of enamel were obtained in the range of 4000–400 cm⁻¹ on the Avatar 320 FT-IR IRS meter (“Nicolet”, Japan) with Fourier transform, which allows more accurate determination of the nature of experimental objects (Bell 1975; Stuart 2004).

After obtaining the results, the parameters of each individual absorption band were analyzed and the concentration of the substance in the samples was determined. The analysis was based on the dependence of the intensity of absorption bands on the concentration of the test substance according to the Beer-Lambert-Bouguer formula (Bell 1975):

$$D = \lg \frac{g}{g_0}, \quad (1)$$

at that D – optical density; \lg – logarithm; g – the intensity of the incident beam of light; g_0 – the intensity of the passage of radiation.

In order to process and analyze the results obtained, methods of variation statistics were used: the estimation of the difference between the frequency of occurrence of the characteristic in separate series of observations, the comparison of serial values and the mean square deviation. To

assess the significance of the difference in the indices of the two populations obtained during the study, the degree of difference between their mean scores was determined using the Student's t-test. The difference in statistical variables was considered significant at $p < 0.05$. "Statistica for Windows" advanced analytics software, "Excel Statistica 7.0" spreadsheets ("Microsoft", USA) were used.

An analysis of the results of infrared spectra of intact enamel revealed the types of oscillations of valence grouping of organic and mineral components characteristic of enamel. In the range of $4000 \dots 400 \text{ cm}^{-1}$ (Fig. 1), characteristic absorption bands are distinguished in the enamel of intact teeth with the maximum at:

- 565 cm^{-1} and 603 cm^{-1} – asymmetric deformation vibrations ν_4 (PO_4^{3-})
- 872 cm^{-1} – non-expressed vibrations ν_2 (CO_3^{2-})
- 960 cm^{-1} – complete metric valence fluctuations ν_1 (PO_4^{3-})
- 1040 cm^{-1} and 1085 cm^{-1} – asymmetric valence fluctuations ν_3 (PO_4^{3-})
- 1417 cm^{-1} , 1457 cm^{-1} – asymmetric flat valence vibrations ν_3 of CO_3^{2-} ion
- 1540 cm^{-1} – Amide II valence oscillations (NH)
- 1650 cm^{-1} – Amide I and valence oscillations (C-O)
- 2849 cm^{-1} and 2920 cm^{-1} – valence fluctuations (C-H), (NH)
- 3430 cm^{-1} – oscillations of OH-group of molecules of tightly bound water

In assessing the IRS of the enamel of the tooth, the band $1100\text{--}900 \text{ cm}^{-1}$ is the most intense, which characterizes the valence fluctuations of the phosphate anion PO_4^{3-} . In the complex contour of this band, three maxima are distinguished: 1040 cm^{-1} – the highest, 1085 cm^{-1} – medium, 960 cm^{-1} – the weakest intensity. Along with the absorption bands inherent in the anion PO_4^{3-} , there are $1090\text{--}1030 \text{ cm}^{-1}$ bands characteristic for the two-substituted orthophosphate HPO_4^{2-} , which are weak intensity due to the low content of soluble phosphate forms. In addition, the composition of hydroxyapatite crystals compounds includes Me^*CO_3 ($900\text{--}850 \text{ cm}^{-1}$). Comparison of the contents of Me^*CO_3 with phosphates is insignificant. In the region of the oscillations of the connections of the anion CO_3^{2-} , absorption bands are determined due to valence vibrations ν_1 ($1430\text{--}1405 \text{ cm}^{-1}$, $1460\text{--}1450 \text{ cm}^{-1}$), as well as valence vibrations ν_2 ($880\text{--}870 \text{ cm}^{-1}$). The analysis of the strips of compounds CO_3^{2-} determines the nature of the mineralization of enamel, that is, the low content of carbonates indicates high mineralization of enamel.

The valence fluctuations of the amide groups of proteins are in the range of $2920\text{--}2860 \text{ cm}^{-1}$, $2390\text{--}2370 \text{ cm}^{-1}$, and $1700\text{--}1500 \text{ cm}^{-1}$. The valence fluctuations of NH groups are the most important among them, which are designated as Amide II and the carbonyl strip Amid I. The valence vibrations in the region of $2920\text{--}2860 \text{ cm}^{-1}$ belong to weakly evident valence fluctuations of CH- and NH-groups. Valid oscillations of Amide II, with a maximum at 1540 cm^{-1} , are due to plane fluctuations accompanied by the rotation of

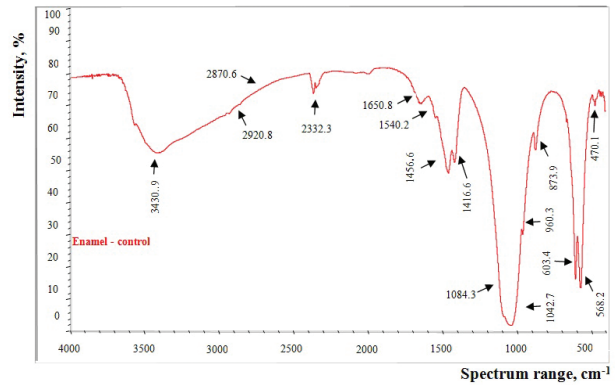


Figure 1. The infrared spectrum of intact enamel.

the N-H bond. Therefore, the band Amide I, with a maximum at 1660 cm^{-1} (carbonyl), is characteristic of oscillations, in which the length of the C-O bond changes. The position of these bands is a function of conformation of the protein, in addition, they are connected with deformation oscillations of the bond $\text{O} = \text{C-N-H}$. The absorption band of Amide II band exceeds the absorption area of Amide I, probably due to the absence of collagen proteins in the enamel. The protein matrix of the enamel is represented by the three-dimensional protein-calcium-protein structure, the basis of which is glycosaminoglycans and Ca^{2+} ions. The mature, formed enamel almost does not contain proline and oxyproline (Ciurczak et al. 2002; Mathur et al. 2003; Kazarina et al. 2016).

The absorption bands of groups that have hydrogen atoms are located at the short-wave end of the fundamental frequency of oscillations of $4000\text{--}2000 \text{ cm}^{-1}$ molecules. The greatest frequencies are characterized by the band of valence fluctuations of free hydroxide groups, which are in the region of $3650\text{--}3590 \text{ cm}^{-1}$. The absorption band with a maximum at 3340 cm^{-1} relates to oscillations of strongly coupled water molecules (OH^-) (Ciurczak 2002; Workman et al. 2016). The formation of associations, due to the hydrogen bond $\text{O-H} \dots \text{O}$, is observed in the IR spectrum, due to the decrease in the frequencies of the valence oscillation of O-H. The localization of the bands of the associated hydroxide groups depends on the strength of the hydrogen bonds. A less intense absorption band in the region of 3730 cm^{-1} characterizes the valence fluctuations of the OH group of coordinated water molecules and reflects the interaction between molecules due to hydrogen bonds of OH.

Thus, the mineral enamel matrix is mainly composed of insoluble forms of trisubstituted calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$, which is part of the hydroxyapatite. In addition, valence oscillations of orthophosphates, characterized by different intensive bands, have been detected; For example, strips of weak intensity in the range of $1090\text{--}1030 \text{ cm}^{-1}$ characterize soluble forms of phosphates (HPO_4^{2-}). At the same time, the presence of carbonates has been noted with orthophosphates in crystalline hydroxyapatite (Ciurczak 2002; Workman et al. 2016). The basis of the organic enamel matrix is collagen-like protein and aminoglycan glycosides.

Features of valence groupings of enamel bleached teeth

According to the authors (Zubachyk et al. 2001; Zubachyk et al. 2003), the mechanism of action of bubble preparations on the basis of the CP represents the oxidation of pigments, which occurs as a result of the oxidation reaction and leads to the breakup of the double carbon bonds in the organic structure of the pigment molecules. Strengthening of the teeth whitening process is due to the influence of urea, which increases the level of physical-chemical exchange of enamel. There is a possibility of changes in the enamel matrix, as bleaching agents cannot have a clear directed effect on organic molecules of pigments and do not affect the valence grouping of the organic component of the enamel.

IRS of bleached teeth allowed determining the state of valence compounds of enamel under the influence of different concentrations of bleaching preparation.

As evidenced by the results of the enamel study, after the application of the drug composition with 30% or 15% of CP (Figs 2, 3), the IRS of the samples does not reveal changes in the number of absorption bands, their configuration, and localization in comparison with the control samples. However, the study of the parameters of characteristic bands enabled to determine the following differences: the change in intensity was determined in the absorption bands with maxima at 3430 cm^{-1} , which characterizes the hydrogen bonds ν (O-H); 2920 cm^{-1} , 2870 cm^{-1} , 2380 cm^{-1} – valence fluctuations of the CH- and NH-groups of the organic component of the enamel. The preserved comparison of the optical density of the Amide II to Amide I bands remains.

Analysis of the mineral component of the enamel allowed determining that the parameters of the characteristic band with a maximum at 1040 cm^{-1} remain the largest. The decrease in the intensity of the valence fluctuations of the PO_4^{3-} phosphate anion in the spectral region of $1090\text{--}1040\text{ cm}^{-1}$ was noted. No condensed phosphate groups were found that would be characterized by two types of bridge-links P-O-P with fluctuations of 750 cm^{-1} and 710 cm^{-1} . In our opinion, their absence is due to the greater sensitivity of the bridge links of P-O-P to the influence of oral fluid, in contrast to the stable orthophosphate groups contained in the hydroxyapatite crystal.

In the oscillation region of the connections of the SO_3^{2-} anion, the absorption bands were determined due to asymmetric flat valence vibrations ν_3 with maxima at 1417 cm^{-1} and 1457 cm^{-1} , as well as unexpressed valence fluctuations ν_2 ($880\text{--}875\text{ cm}^{-1}$). The parameters of these absorption bands are similar in the control group of samples.

Our obtained results indicate that the absorption band intensity of the hydroxide groups of the short-wave spectrum is reduced, as well as the bandwidths of the valence oscillations of the groups (CH- and NH-) with maxima at 2920 cm^{-1} , 2870 cm^{-1} , 2380 cm^{-1} .

To determine the quantitative changes in the organic and mineral components of the enamel processed by

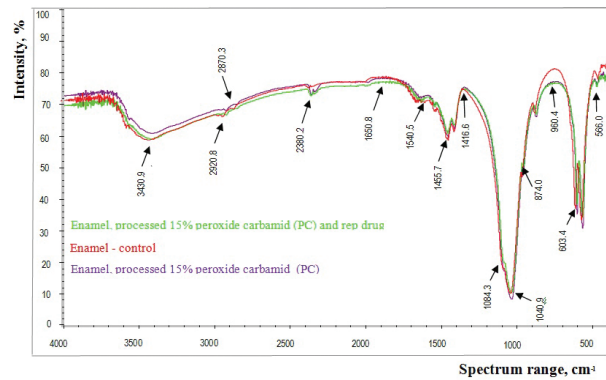


Figure 2. The infrared spectrum of enamel after treatment with bleaching preparations with different CP concentrations and performed remineralizing therapy.

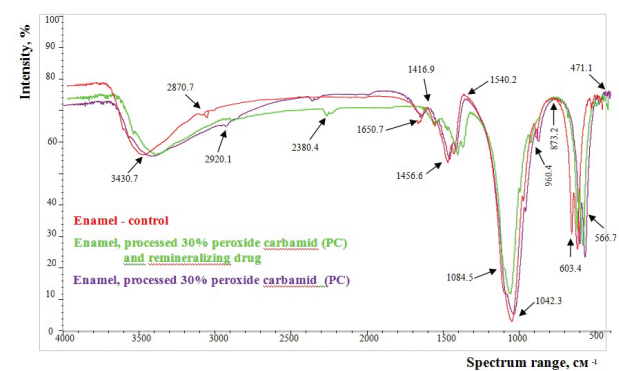


Figure 3. The infrared spectrum of enamel after the action of bleaching preparations with different concentrations.

30% and 15% preparations of dental CP, we carried out the calculation of the optical densities of absorption bands of enamel. The analysis of the mineral enamel matrix has established the stability of the valence grouping parameters in enamel samples processed by different CP concentrations, as well as the lack of changes characterizing the destruction of the organic enamel matrix of the tooth in the Amide I and Amide II regions.

According to the results of the study, after the action of 30% CP, statistically significant changes ($p < 0.01$) of optical density occurred in the region of the band with a frequency of 3430 cm^{-1} (water, hydroxide groups), 2920 cm^{-1} (CH), 2380 cm^{-1} (HPO_4^{2-} , CH, NH), as well as a tendency ($p > 0.1$), in relation to changes in the frequencies of Amide I (1650 cm^{-1}) and Amide II (1540 cm^{-1}).

In the group of enamel samples, a 30% carbamide peroxide preparation determined a statistically significant decrease of 2.17% of the optical density of the band with a frequency of 3430 cm^{-1} ($p < 0.01$). Such changes can be assumed to occur due to the ability of urea to form strong hydrogen bonds and to “drag” free water to “itself”, that is, towards the surface of the enamel. The water balance of the enamel is restored due to the ability of the centripetal motion of the enamel liquor in the state of the pulp. Taking the above mentioned into consideration, subject to the

Table 1. Indices of the optical density of absorption bands of enamel ($M \pm m$), exposed to bleaching agents with different concentrations of urea peroxide.

Bandwidth absorption (cm^{-1})	Optical density of absorption bands of enamel (conditional units)		
	Control (n = 17)	The enamel exposed to the medications	
		30% carbamide peroxide (CP) (n = 12)	15% carbamide peroxide (CP) (n = 14)
3430	0.138 ± 0.004	0.135 ± 0.021**	0.136 ± 0.018**
2920	0.062 ± 0.040	0.058 ± 0.06**	0.061 ± 0.002*
2870	0.041 ± 0.003	0.039 ± 0.003**	0.04 ± 0.002*
2380	0.006 ± 0.007	0.0078 ± 0.002**	0.066 ± 0.060**
1650	0.047 ± 0.006	0.046 ± 0.048***	0.046 ± 0.021***
1540	0.064 ± 0.012	0.063 ± 0.087***	0.063 ± 0.046***
1456	0.138 ± 0.002	0.138 ± 0.013***	0.138 ± 0.025***
1417	0.128 ± 0.002	0.128 ± 0.023***	0.128 ± 0.044***
1084	0.846 ± 0.012	0.845 ± 0.011***	0.845 ± 0.016***
1040	1.326 ± 0.015	1.323 ± 0.093**	1.325 ± 0.017**
960	0.255 ± 0.003	0.255 ± 0.043***	0.255 ± 0.062***
874	0.075 ± 0.003	0.077 ± 0.012*	0.077 ± 0.014*
603	0.432 ± 0.033	0.427 ± 0.035**	0.426 ± 0.073**
566	0.437 ± 0.014	0.436 ± 0.023*	0.436 ± 0.013*

* – indicator of the likelihood of difference, compared with control at $p < 0.05$; ** – $p < 0.01$; *** – $p > 0.1$

bleaching of the vital teeth, it can be assumed that changes in the number of hydroxide groups of free and bound water are temporary and reversible.

When comparing the prolonged effect of a bleaching agent of lower concentration (15% CP), the character of the changes in the band of hydroxide groups with a frequency of 3430 cm^{-1} indicates that the tendency to enamel deamination is preserved ($p < 0.05$). Compared with the control, the optical density of this band decreased by 1.44%. The figures obtained are summarized in Table 1.

The results indicate that teeth whitening with 15% CP at home, compared to the clinical application of 30% CP, causes a smaller (0.73%) change in the hydrated membranes of enamel prisms. Increased sensitivity of the teeth should be expected to appear during clinical examination in patients due to the processes of dehydration and changes in osmotic pressure of the enamel liquor. Therefore, in the event of hyper-transitions, it is advisable to increase the time interval between sessions of teeth whitening to restore the water balance of the enamel naturally.

Valence oscillations of the amide groups of proteins are localized in the range of $2920\text{--}2860 \text{ cm}^{-1}$, $2390\text{--}2370 \text{ cm}^{-1}$, $1700\text{--}1500 \text{ cm}^{-1}$. In the analysis of the oscillation frequency of 2920 cm^{-1} and 2870 cm^{-1} , it should be noted that these absorption bands, in addition to the fluctuations of C-H groups, characterize the variations of the hydroxide groups bonded by hydrogen bonds, as well as the R-NH^+ , aldehyde, and carboxyl groups. Carboxylic acids and aldehydes should be excluded from consideration since they do not have an absorption characteristic on the spectrum with a frequency of 1700 cm^{-1} .

The analysis of the results of the infrared spectrum of the enamel processed by 30% of the CP product shows a probable reduction in the optical density of absorption bands at maxima of 2920 cm^{-1} and 2870 cm^{-1} by 6.8% and 5.1%, according to the control group of samples ($p < 0.01$). Similar changes were confirmed after tooth brushing with 15% CP: 1.72% and 2.56% ($p < 0.05$). The obtained results

can be assumed to indicate partial destruction of carbon bonds in the range of $2920\text{--}2860 \text{ cm}^{-1}$, which is likely to change the dental color in the direction towards lighter colors, due to the transition of absorption of the light beam to the other part of the spectrum.

In addition, attention should be paid to the changes in the region of the spectrum of $2390\text{--}2370 \text{ cm}^{-1}$, where the intensity band was reached at a maximum of 2380 cm^{-1} (HPO_4^{2-} , C-H- and N-H-grouping). The optical density of this band after the application of 30% of the CP product increased significantly by 30.0%, and after application of 15% of the preparation of CP – by 9.95% ($p < 0.01$) (Table 1). An increase in the optical density of this absorption band can be explained by the fact that carbamide has the ability to bind metal ions. In this case, the binding of the Ca^{2+} ion increases the concentration of PO_4^{3-} and HPO_4^{2-} . As the results show, the carbamide content is correspondingly higher in the bleaching agent of a higher concentration. The chemical processes that led to changes in the absorption band of the HPO_4^{2-} ion and the hydrogen bonds of the CH- and NH- groups are more pronounced. After the application of 30% and 15% of CP preparations, no significant changes were observed in the optical density of the Amide II and Amide bands ($p > 0.1$). The preservation of their ratio, with the predominance of the optical density of Amide II to Amide I, is specific, indicating the absence of destruction of the organic enamel matrix of the tooth.

To determine the state of the mineral component of bleached teeth, the analysis of the optical densities of absorption bands of orthophosphate-specific bands with maxima of 1085 cm^{-1} , 1040 cm^{-1} , 960 cm^{-1} , 566 cm^{-1} and carbonates with maxima at 1456 cm^{-1} and 1417 cm^{-1} , respectively was conducted. The frequencies in the range of $1000\text{--}1100 \text{ cm}^{-1}$ are due to the fluctuation of the H_2PO_4^+ , HPO_4^{2-} and HPO_4^{3-} ions, and at the frequency of 960 cm^{-1} , the absorption of the PO_4^{3-} group supplements the absorption of δ (C-H). Accordingly, it is problematic to deter-

Table 2. Indices of the optical density of absorption bands of enamel ($M \pm m$), after treatment with bleaching agents with different CP concentrations and remineralizing therapy.

Frequency of absorption bands, (cm^{-1})	Optical density of absorption bands of enamel (conditional units)		
	Control (n = 17)	The enamel exposed to the medications	
		30% carbamide peroxide (CP), «Belagel Ca/P» (n = 12)	15% carbamide peroxide (CP), «Belagel Ca/P» (n = 14)
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2920	0.062 ± 0.040	0.061 ± 0.005*	0.06 ± 0.004**
2870	0.041 ± 0.003	0.04 ± 0.024*	0.04 ± 0.009*
2380	0.006 ± 0.007	0.0076 ± 0.003**	0.0075 ± 0.007**
1650	0.047 ± 0.006	0.046 ± 0.072***	0.046 ± 0.061***
1540	0.064 ± 0.012	0.063 ± 0.058***	0.063 ± 0.092***
1456	0.138 ± 0.002	0.139 ± 0.043***	0.138 ± 0.074***
1417	0.128 ± 0.002	0.127 ± 0.013***	0.127 ± 0.042***
1084	0.846 ± 0.012	0.845 ± 0.01***	0.845 ± 0.013***
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603	0.432 ± 0.033	0.429 ± 0.034**	0.427 ± 0.038**
566	0.437 ± 0.014	0.438 ± 0.037***	0.437 ± 0.052***

* - indicator of the likelihood of difference, compared with control at $p < 0.05$; ** - $p < 0.01$; *** - $p > 0.1$

mine the changes in the valence oscillations inherent in the PO_4^{3-} anion, or C-N groups.

After application of 30% or 15% of the CP preparation, no significant decrease in the optical density in the orthophosphate region with absorption band maxima at 1085 cm^{-1} , 1040 cm^{-1} , 960 cm^{-1} ($p > 0.1$) (Tab. 1) was observed, indicating the absence of destructive processes in the crystals of hydroxyapatite enamel of the tooth. It should be noted that a statistically significant reduction of orthophosphates was determined in the area of the spectrum $610\text{--}550 \text{ cm}^{-1}$ after the use of bleaching agents: in the case of 30% CP – by 1.67% ($p > 0.1$), and in the treatment of teeth with 15% CP – by 1.39% ($p > 0.1$). We can assume that such results characterize the spontaneous washing of a small number of soluble phosphate forms from uncertain factors.

In the frequency region of 1456 cm^{-1} and 1417 cm^{-1} , corresponding to the variation ν_1 of the CO_3^{2-} ion, and also δ C-H, no probable changes in optical absorption density were detected ($p > 0.1$). In the range of $900\text{--}850 \text{ cm}^{-1}$ spectrum, after the treatment of the teeth with 15% and 30% CP, an insignificant increase of 2.7% of the optical density of CO_3^{2-} was determined in comparison with the control group of samples ($p > 0.1$). Thus, the results of the study indicate that there are no significant changes in the organic or mineral component of the enamel of the tooth, exposed to the action of various concentrations of CP. However, due to the sensitivity of the IRS method to the slightest changes in the nature of valence groups, a tendency has been observed regarding the reduction of soluble forms of phosphates, hydroxide groups, and the nature of ether-phosphorous bonding of enamel. In view of the results obtained, it is advisable to study the effect of remineralizing therapy on the nature of valence groupings of enamel-bleached teeth in order to prevent possible changes in the crystal lattice and organic enamel matrix.

The character of valence groups of bleached enamel after remineralization therapy is as follows. It should be noted

that remineralization therapy has taken place since the fifth session of the use of bleaching substances. To do this, at the end of the teeth whitening session, the “Belagel Ca/P” gel preparation was applied on the cleaned and dried surface of the enamel, which was saturated with Ca and P ions for a prolonged time. The IRS results of the enamel (Figs 2, 3) allowed determining the nature of the changes in valence groupings of bleached enamel after the use of Belagel Ca/P.

The study of the spectrograms of the experimental samples did not reveal changes in the number of absorption bands and their localization in comparison with the control group, which indicates the absence of additional inclusions in the chemical structure of the enamel (Table 2).

Further analysis of the absorption strips of bleached enamel samples and the Belagel Ca/P was performed at $2920\text{--}2860 \text{ cm}^{-1}$ ($p < 0.01$). The obtained results indicate that the use of remineralizing agent does not affect the process of destruction of the carbon bonds of CH- and NH-groups and the removal of the restructured organic component of the enamel (probably this process relates to the chromoform of the pigments) when bleaching the teeth. A probable reduction of the absorption band with a maximum at 603 cm^{-1} was determined, and an increase in the optical density index with a maximum at 874 cm^{-1} and 1040 cm^{-1} , indicating a process of saturation of enamel with HPO_4^{2-} ions ($p < 0.05$).

The use of remineralizing therapy leads to a possible increase in the optical density of the band with a maximum at 3430 cm^{-1} ($p < 0.01$) in the case of teeth whitening in clinical conditions by 5.79%, and at home – by 3.74% – according to indicators of bleached teeth.

Taking into account the obtained results, it can be assumed that the remineralization capacity of the oral fluid and the pulp, the state of hydration membranes, osmotic pressure of the enamel liquor is restored in the enamel due to the compensatory processes caused by the action of the Belagel Ca/P. Because of these activities, the processes of

ion exchange are improved. Therefore, the use of remineralizing therapy in a clinical study of bleaching agents can lead to the disappearance of an unpleasant sensation during tooth whitening due to changes in the pulp.

Conclusions

Thus, the obtained results of the study indicate that the process of discoloration of teeth is accompanied

by changes in carbon bonds of CH- and NH-groups, a decrease in the number of OH-groups and dissolved forms of phosphates. When applying medicinal compositions, in accordance with the elaborated methods of bleaching the vital teeth, no significant changes in the enamel are revealed, which would indicate the destruction of the hydroxyapatite crystals or its organic matrix. To seal the crystal lattice of enamel, the use of remineralizing therapy with drugs containing Ca and R ions is recommended.

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