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An X-Ray Electron Study of Nanodisperse Hydroxyapatite

I. V. Melikhov^a, Yu. A. Teterin^b, V. N. Rudin^a, A. Yu. Teterin^c, K. I. Maslakov^b, and A. V. Severin^a

^a Faculty of Chemistry, Moscow State University, Moscow, Russia

^b Kurchatov Institute Russian Research Center, pl. akademika Kurchatova 1, Moscow, 123182 Russia

^c Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences,
Leninskii pr. 31, Moscow, 117907 Russia

e-mail: melikhov@radio.chem.msu.ru

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Abstract—Two states of surface valence electrons localized on faces with different molecular reliefs were observed for nanodisperse hydroxyapatite. Thermal treatment of nanocrystals caused a shift $\Delta E_b = 0.5$ eV of the spectrum of valence electrons on molecularly rough faces and a shift of 0.8 eV of the spectrum from smooth faces. Similar electron spectrum shifts were observed for sorption, in particular, of sodium succinate. These results are of importance for the diagnostics of various hydroxyapatite kinds, since hydroxyapatite is a constituent mineral component of living organisms, and for the synthesis of medicines with enhanced biological activity used in treatment of various bone diseases.

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INTRODUCTION

Hydroxyapatite (HA) is the main mineral ingredient of bone tissue of animals and men. It exists in tissue in the form of nanocrystals, which interact with collagen [1]. For this reason, the problem of comprehensively describing the properties of HA nanocrystals and the state of biologically active substances adsorbed on their surface is of obvious interest. This problem is solved using atomic force and high-resolution electron microscopy, isotope exchange, and various kinds of spectroscopy [2–5]. In particular, HA and other calcium phosphate crystals are studied by X-ray electron spectroscopy [6–8].

The experimental data accumulated show that the state of atoms of the surface monoatomic layer should be studied more thoroughly [9–11]. Because of anisotropy of surface forces, the properties of this layer, which to a great extent determine the chemical activity of HA, are substantially different from those of bulk crystals. This difference is, however, difficult to reveal if we do not have samples with a fairly high fraction q_s of monolayer atoms with respect to the total number of atoms. We therefore studied the X-ray electron spectra of HA samples obtained from lamellar nanocrystals with a maximum possible q_s fraction. The X-ray electron spectra of such samples are characterized by features that can only be explained by the special features of the composition and structure of the monoatomic layer, which allowed us to describe its properties in more detail.

EXPERIMENTAL

Samples were prepared from an aqueous suspension of HA nanocrystals as recommended in [12]. A portion of the suspension was diluted with water, and part of it was transferred to a Formvar film and examined using an electron microscope [13]. The other part was deposited on a metallic plate and studied using a tunnel microscope as recommended in [14]. A portion of the suspension was deposited on a glass substrate and dried in air at 300 K and relative air humidity up to 40% until its weight ceased to change. A 7×7 mm² layer of nanocrystals 1.5 mm thick formed on the substrate (sample A). A portion of the suspension deposited on a glass substrate and dried in air was heated at 10^3 K for 10^4 s, which resulted in the formation of a ceramic film 0.5 mm thick (sample B). Sample C containing sodium succinate of kh. ch. (chemically pure) grade was prepared by wetting sample A with a solution of succinate followed by drying in air. The concentration of the solution provided the formation of a monolayer on the surface of HA particles after drying.

The X-ray electron spectra of the samples were recorded on an MK II VG Scientific electrostatic spectrometer with the use of $AlK_{\alpha 1,2}$ not monochromatized exciting X-ray radiation (1486.6 eV) in a 1.3×10^{-7} Pa vacuum at room temperature [7, 15]. The spectra were obtained without preliminarily purifying the surface of the samples with argon ions. The resolution of the spectrometer measured as the width of the $Au4f_{7/2}$ line at half-height was 1.2 eV. The electron binding energies E_b (eV) are given with respect to the C1s electron binding energy of hydrocarbons, the binding energy of electrons of the reference was taken to be 285.0 eV. Errors in E_b (eV) and spectrum line width Γ (eV) measure-

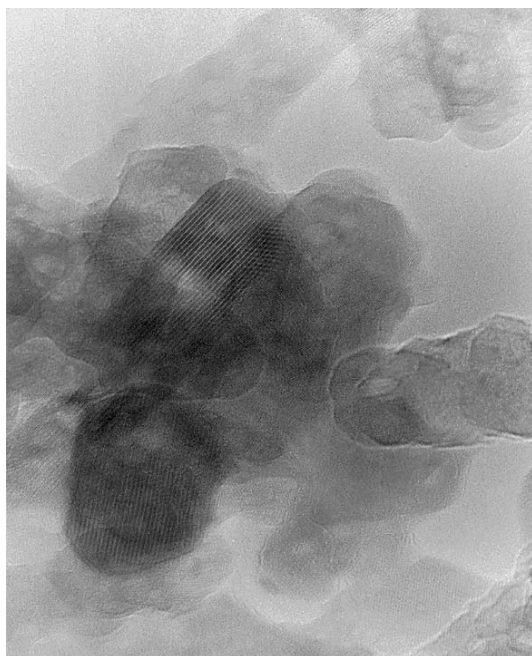


Fig. 1. Single HA crystals in the initial suspension (3×10^6). The image was obtained at Lausanne University as described in [13], a CM-300 Philips microscope.

ments were of 0.1 eV, and relative intensities were measured to within $\sim 10\%$. To exclude the influence of sample charging on measurement results, the width of each line at half-height was related to the corresponding value for the line of C1s electrons ($\Gamma(\text{C1s}) = 1.3$ eV). This allows the values obtained for various samples to be compared. The samples experienced strong charging (to 10 eV with respect to electron binding energies), and the charging was unstable in time. For this reason, the spectra were calibrated against the carbon line for each separate line over the energy range 0–1000 eV. In other words, the C1s line was measured before recording the line under study and afterward. This decreased the error in binding energies to 0.1 eV.

For all the samples, quantitative elemental and ionic analyses were performed on the basis of proportional relation between the intensity of spectral lines and the concentration of atoms in the sample. Analyses were performed using the equation $n_i/n_j = (S_i/S_j)(k_j/k_i)$, where n_i/n_j is the relative concentration of the atoms under study, S_i/S_j is the relative intensity of the lines of inner shell electrons of these atoms, and k_j/k_i is the experimental relative sensitivity coefficient. We used the coefficients (with respect to carbon) 1.00 (C1s), 2.64 (O1s), 6.32 (Ca2p), 1.56 (P2p), and 1.16 (P2s) [16]. The spectrum lines were also identified using the spectra of reference Ca, CaO, and CaCO_3 samples [17, 18]. Reference samples contained fairly large-sized crystals with a small fraction of atoms in surface monoatomic layers. The Ca sample was a metallic plate, and Ca oxide formed on its surface. The $\text{Ca}(\text{CO}_3)_2$ carbonate sample



Fig. 2. Atomic force microscopic image of aggregates of HA nanocrystals (3.6×10^5). SMENA NT-MDT microscope, Russia.

was in the form of a powder. In all cases, we obtained survey spectra and detailed valence zone spectra over the range from zero to 50 eV. In addition, we specially studied the most intense lines of inner-shell calcium and phosphorus electrons and the O1s and C1s electrons of oxygen and carbon. The spectra were processed taking into account that the survey spectrum should contain $\text{AlK}_{\alpha 3,4}$ satellites. The contributions of these satellites were calculated using a special program.

RESULTS

Immediately after its preparation, the aqueous suspension from which the samples were obtained consisted of lamellar nanocrystals having the composition $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ and the structure of hydroxyapatite (Fig. 1). Lamina bases were (100) faces, and side surfaces of laminae had no well-defined faceting. According to the high-resolution electron and atomic force microscopy data, the fractions of Ca atoms in a monolayer on base faces and side surfaces of laminae were about $q_{s1} \sim 0.32$ and $q_{s2} \sim 0.16$ of the total number of suspension atoms. In the volume of nanocrystals, the q_v value is approximately 0.52.

The major part of nanocrystals in the suspension were combined into aggregates, whose number and degree of ordering increased with time (Fig. 2). The transfer onto the glass substrate and drying in air accelerated aggregation. When a layer of aggregates was heated, we observed coalescence of nanocrystals in their volume and faceting of aggregates, which transformed into crystalline particles. As was shown in [12], this caused a decrease in the specific surface area of hydroxyapatite. As a result, samples A, B, and C had specific surface areas F listed in Table 1 (the specific surface areas were determined by the adsorption of nitrogen). Table 1 also contains the fractions q_{si} of Ca

atoms in monoatomic layers on (100) faces ($i = 1$) and side surfaces ($i = 2$) calculated on the assumption that

$$q_{si} = \hat{q}_{si} F / \hat{F},$$

where $\hat{F}$ is the specific surface area of the initial suspension particles found by atomic force and high-resolution electron microscopy.

The X-ray electron spectrum of each sample contained 20 lines over the binding energy range 0–10³ eV (Figs. 3, 4). Table 2 contains the binding energies E_b of valence and inner shell electrons, widths Γ at half-height at the reference width $\Gamma = 1.3$ eV of the C1s line, and relative intensities $I = S/S_0$ of some lines calculated as the ratio between the area S of a line and the area S_0 of the line of Ca2p electrons used as a reference. The characteristics of the spectra of reference samples, which coincide with the literature data [19], are listed in Table 3.

For part of lines, the energy E_b distribution of photoelectrons was canonical. For the other lines, the distribution could be described as the superposition of canonical distributions (Fig. 5), and, most often, the main and “satellite” components could be identified.

Table 1. Characteristics of the samples subjected to spectral studies

Sample	$F, \text{m}^2/\text{g}$	q_{s1}	q_{s2}	q_v
H	400	0.32	0.16	0.52
A	170 ± 10	0.25	0.07	0.68
B	65 ± 5	0.04	0.02	0.94
C	120 ± 10	<0.01	<0.01	>0.98

Note: H are nanocrystals in suspension.

DISCUSSION

The state of surface layer atoms of HA crystals (as of all solids) was different from the state of atoms in the bulk. This difference was observed in the X-ray electron spectra of the samples. We were able to record lines I–V with energies $E_b < 12$ eV, which, as was shown in [15, 19], characterized valence electrons. Of these lines, those with $E_b = 6.8 \pm 0.1$, 9.5 ± 0.1 , and 11.9 ± 0.1 eV were present in samples A, B, and C. The line $E_b = 5.9 \pm 0.1$ eV was only characteristic of sample A, and the line $E_b = 4.7 \pm 0.1$ eV shifted by $\Delta E_b = 0.5 \pm 0.2$ eV in samples B and C compared with A. These

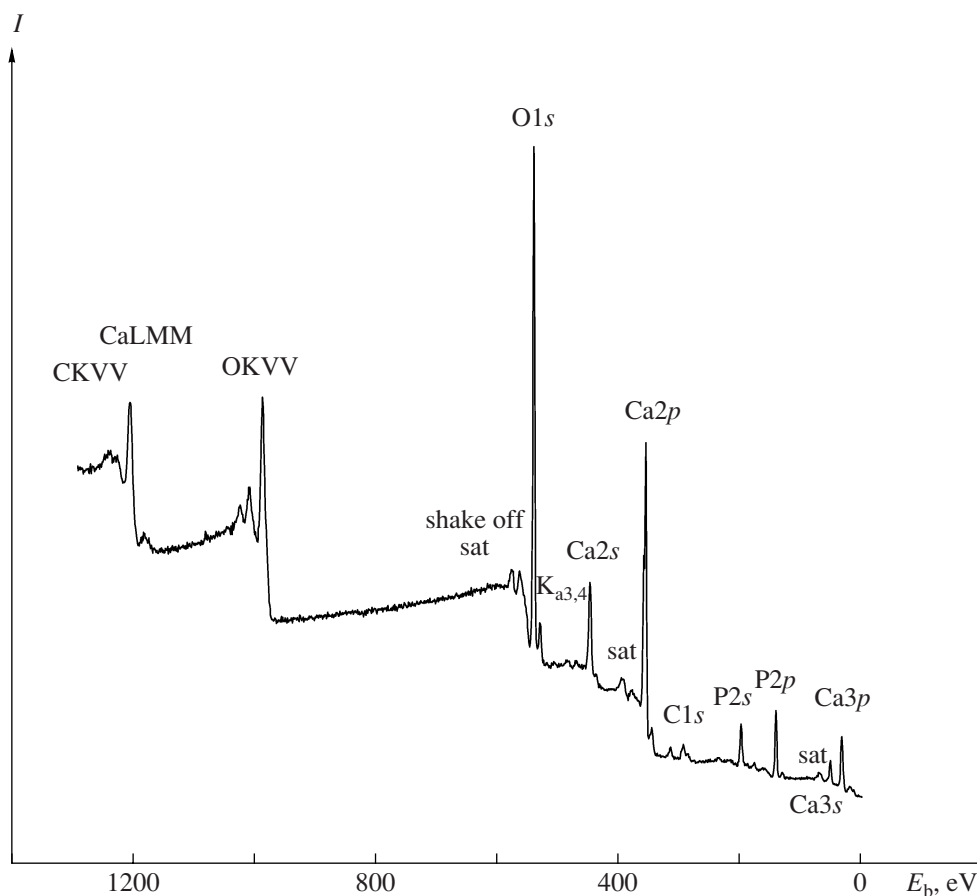


Fig. 3. Survey X-ray electron spectrum, sample B.

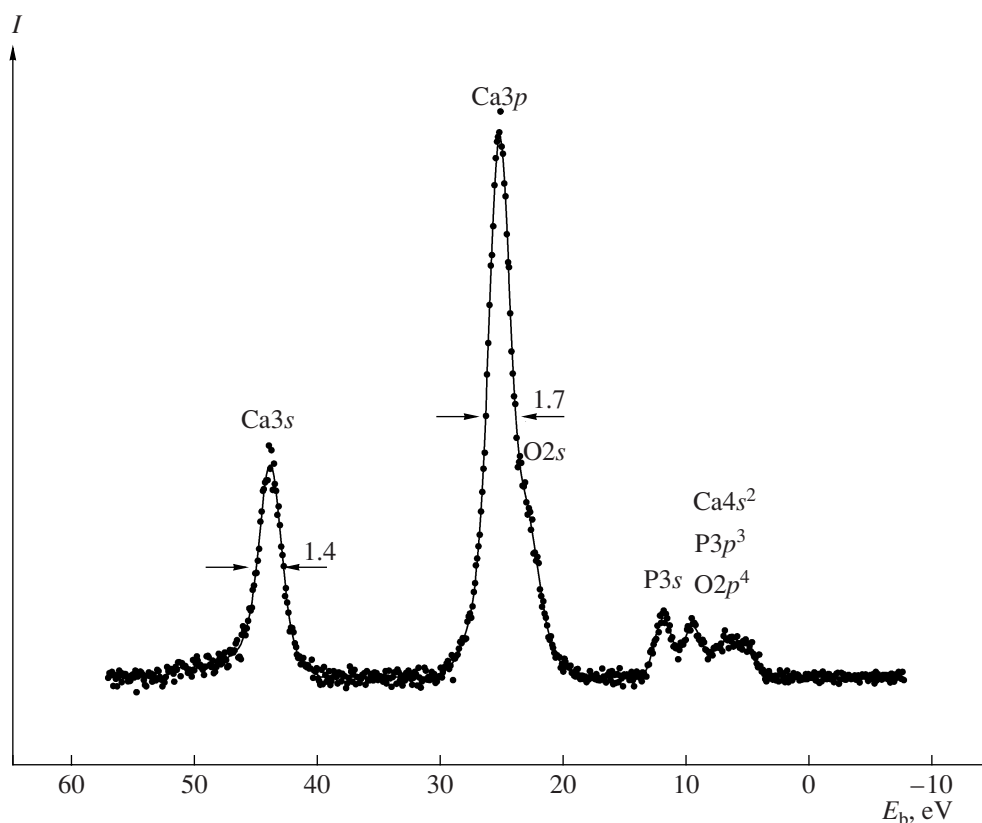


Fig. 4. Spectrum of valence electrons, sample B.

results show that line I characterizes electrons on nanocrystal side faces, line II, electrons of the surface monolayer on the (100) face, and lines III–V, electrons in the volume of nanocrystals.

Indeed, the shift of line I is evidence that this line is related to rearranging crystal regions, and, according to the electron microscopy data, these are crystal side surfaces, where relief ordering occurs. Line II is only

Table 2. Electron binding energies E_b , X-ray electron spectrum line widths at half-height Γ , and line intensities I_i for samples A, B, and C

Line	E_b , eV			Γ , eV			I_i		
	A	B	C	A	B	C	A	B	C
I	4.7	5.1	5.2	0.6	1.6	0.8	2.6×10^{-3}	3.1×10^{-3}	4.6×10^{-3}
II	5.9	–	–	1.3	–	–	4.2×10^{-3}	–	–
III	6.9	6.7	6.7	1.4	1.7	1.6	7.4×10^{-3}	9.8×10^{-3}	8.9×10^{-3}
IV	9.4	9.6	9.5	0.8	0.6	0.7	9.7×10^{-3}	8.8×10^{-3}	9.0×10^{-3}
V	11.8	11.9	11.9	1.1	0.4	0.6	12.9×10^{-3}	9.6×10^{-3}	8.7×10^{-3}
Ca2p	347.1 350.7	347.1 350.6	346.9 350.4	1.2	1.2	1.3	1.00	1.00	1.00
Ca3s	44.0	44.0	44.0	1.6	1.4	1.4	0.07	0.06	0.06
Ca3p	25.3	25.3	25.3	1.6	1.7	1.7	0.17	0.16	0.17
P2s	190.8	190.7	190.5	2.2	2.1	2.1	0.15	0.145	0.15
P2p	133.5	133.3	133.4	1.4	1.4	1.4	0.17	0.15	0.17
O1s	530.9 533.1	530.9 533.1	530.7 532.9	1.3	1.3	1.3	1.28 0.13	1.23 0.08	1.32 0.11

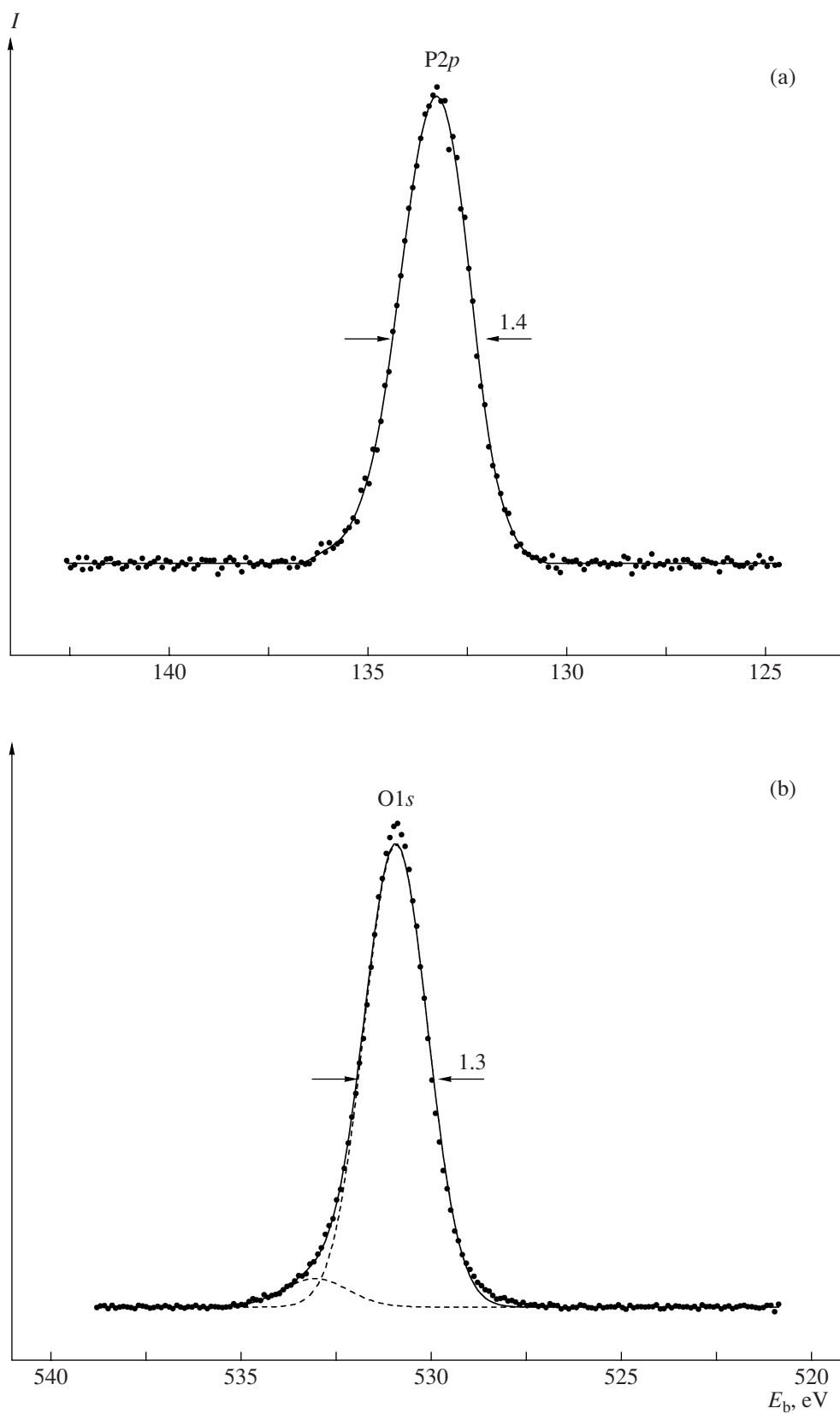


Fig. 5. (a) $P2p$ and (b) $O1s$ electron lines, sample B.

Table 3. Characteristics of reference sample spectra

Line	Ca		CaO		CaCO ₃	
	E_b , eV	Γ , eV	E_b , eV	Γ , eV	E_b , eV	Γ , eV
I	−0.6	1.2	4.3	2.1	5.3	2.2
II	–	–	20.1	2.6	–	–
Ca3p	23.4	–	24.3	2.0	25.2	2.0
Ca3s	42.5	1.8	42.9	1.9	44.1	1.8
Ca2p	344.7	1.7	346.0	1.7	347.3	1.7
	348.4	1.7	349.7	1.7	351.0	1.7
Ca2s	436.6	–	437.9	3.3	439.1	3.0
O1s	–	–	528.9	1.4	531.6	1.6
C1s	–	–	–	–	285.0	1.3
					289.7	1.3

observed for sample A with a high q_{s1} fraction. It does not appear in the spectrum of sample B because of the low q_{s1} value and in the spectrum of sample C because its surface is filled with succinate. Lastly, lines III–V are identical for samples with different specific surface areas F , which shows that they are not related to crystal surface layers and characterize crystal volume.

At $E_b = 12$ –600 eV, the spectra of samples A, B, and C are almost identical, not counting differences in the Γ and I_i values, probably, caused by the variability of the emission of electrons and the difficulties of recording it. We see from Table 2 that orbitals with energies $E_b > 12$ eV are not sensitive to changes in the shape of nanocrystals (Figs. 1, 2). The similarity of the spectra of samples A and B with substantially different q_{s1} values shows that the nearest environment of Ca and P atoms in the surface monolayer is identical to that in the bulk. Otherwise, the spectra would be different, as for crystals of Ca compounds (Table 3). Crystals are likely covered by a “carpet” of O atoms, which provide the same oxygen environment of surface Ca atoms as in the bulk of crystals. In this carpet, valence electrons with $E_b = 5.9$ eV are localized on base faces, and valence electrons with $E_b = 4.7$ eV, on side faces.

The spectra of samples A, B, and C reveal sodium succinate effects on the properties of the surface of nanocrystals. The shift of line I in sample C compared with A shows that succinate molecules cause the rearrangement of structures characterized by $E_b = 4.7$ eV, similarly to what occurs in sample B as a result of calcining. Calcining, however, does not cause an increase in the intensity of line I, whereas succinate increases it almost twofold, probably, because of an increase in the number of surface structures with $E_b = 5.2$ eV. The disappearance of line II in the passage from sample A to C shows that, when succinate is adsorbed, the surface layer of Ca, P, and O atoms on (100) faces loses its special features and becomes indistinguishable from layers in the volume of crystals.

The spectra shown in Figs. 3–5 and Tables 2, 3 are close to those given in [6–8, 19] over the binding energy range $E_b = 12$ –1000 eV. In this work, they are refined and augmented by the data on line widths and intensities. It was proved that the spectrum given in Table 2 over the specified binding energy range is independent of the method of sample preparation, and can be considered characteristic of hydroxyapatite. Conversely, over the binding energy range $E_b = 0$ –12 eV, information about which was obtained in this work for the first time, the spectrum of hydroxyapatite depends on the method for sample preparation.

CONCLUSIONS

To summarize, because of anisotropy of surface forces, the electronic state of the atoms of the surface monolayer of HA differs from the state of atoms in the volume of crystals, and the E_b energy of monolayer valence electrons is lower by 2–6 eV than that in the volume. The state of electrons populating inner-shell orbitals of the Ca and P atoms is insignificantly different from that in the bulk for Ca and is almost identical in HA and CaCO₃ crystals. This is likely related to similar oxygen environments of calcium atoms in carbonate and phosphates. When the surface of nanocrystals is covered by a monolayer of Na succinate molecules, the E_b energies of valence electrons on the surface increase by about 0.5 eV, and their state approaches that in the volume of crystals. The results obtained lead us to conclude that X-ray and photoelectron spectroscopy methods can be used to study the interaction of biologically active substances with hydroxyapatite at the level of obtaining detailed information about electronic states.

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