

## ANALYSIS OF THE CHEMICAL COMPOSITION OF A BIOCERAMIC BASED ON HYDROXYAPATITE AND TRICALCIUM PHOSPHATE

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UDC 543.621:543.05

In modern medicine, implants, pellets, and coatings are commonly prepared by using a biologically active ceramic based on hydroxyapatite (HAP) and tricalcium phosphate (TCP). These are substances that actively participate in the biochemical processes of living organisms. Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is identical in its composition and crystalline structure to the mineral substance of natural bone. Thus, a ceramic made from HAP is strongly integrated with living bone matter (which grows into pores in the material) and is partially converted by the organism into new bone matter. The compound TCP ( $\text{Ca}_3(\text{PO}_4)_2$ ) actively stimulates the growth of bone matter and is gradually replaced in the organism by natural bone.

The rate of these processes, as other details of the behavior of HAP and TCP in an organism, depends appreciably on the chemical composition and defectiveness of the crystalline structure of the material. At the same time, the synthesis of HAP and TCP – as the synthesis of other monophase orthophosphates – is very complex because they are easily hydrolyzed in aqueous solutions and form phases or surface layers whose composition depends on temperature, the rate of introduction of the reactants, and other factors. In addition, due to the intensive interaction of HAP and TCP with organisms, stringent standards must be observed with respect to the composition of the impurities in these substance. This applies in particular to the content of heavy metals. For example, according to the requirements of U.S. standards ASTM F 1185–88 and ASTM F 1088–87, materials based on HAP and TCP must contain at least 95% of the main substance, while the concentrations of As, Cd, Hg, and Pb in them cannot exceed 3, 5, 5, and 30 ppm, respectively. However, the methods described in those standards for analyzing the main components are based on the appearance of new lines in the x-ray diffraction pattern. This makes it impossible to account for changes in the composition and properties of these substances in the homogeneous region, which for HAP is equal to 1.41-1.75 Ca:P [1].

In connection with the development of methods of synthesizing HAP and TCP and a technology for obtaining ceramics from them, we have devised optimum methods to analyze the composition of the main components and the impurities in these substances.

**Chemical Analysis.** To perform the chemical analysis, a weighed quantity of powdered calcium orthophosphate was dissolved in dilute (1:1) nitric acid. Calcium content was determined from an aliquot part of the solution by reverse complexometric titration, i.e., by titration of the excess of complexone III with the use of a solution of nickel(II) chloride in ammonia. Murexide was used as the indicator. Phosphorus content was determined by the photometric method on the basis of the formation of yellow phosphomolybdic acid and its subsequent reduction to a blue complex compound in a hydrochloric acid solution of thiocarbamide in the presence of copper(II) sulfate. The error of the analysis is 0.5% Ca and 0.1% P.

**Emission Spectrum Analysis of Microimpurities.** The stringest requirements that have been established on the content of heavy metals dictate the choice of analytical method.

Emission spectrum analysis (ESA) makes it possible to determine Cd, As, Hg, and Pb with the necessary sensitivity and speed. In the analysis we performed, the spectrum was excited in a carbon dc arc. The sample was vaporized from a cavity in the anode. The above-mentioned metals are highly volatile elements. Thus, their vaporization rate must be reduced somewhat in order to increase the sensitivity of the method. To do this, we used electrodes 11-mm deep. The choice of

electrode configuration was also dictated by the mass of the sample, which was 150 mg. The weighed quantity of powder was compacted in a compression mold with a rubber insert under a force of 700 kg. Use of the insert made it possible easily press out the resulting cylindrical specimens, which had a diameter of 2 mm and a length of 10 mm. The operation could not be performed using a steel mold, due to the adhesion of the HAP powder to the mold. The analysis was performed with an arc current of 15 A. The time of exposure was chosen with allowance for the concentrations of the samples and the sensitivity of the photographic plates. The analysis was performed using the atomic lines Gd I (228.80 nm), As I (234.98 nm), Hg I (253.65 nm), and Pb I (283.31 nm). The base of the sample (Ca and P) is a good spectroscopic buffer which creates optimum conditions in the plasma to excite the indicated lines. The intensity of the spectral lines integrated over time was used as the analytical signal. The analysis was performed by the method of additions, which involved adding known amounts of the elements being determined to the initial HAP powders. The additions were made in the form of aqueous solutions of nitrates and chlorides. The mixture was then evaporated and the dry residue was homogenized. The initial standard solutions of Pb, Cd, Hg, and As, with a concentration of 1 mg/cm<sup>3</sup>, were prepared by dissolving precisely weighed quantities of metals with a purity no lower than 99.9% in a minimal amount of nitric acid in accordance with the requirements of the standard (GOST 28473–90). The sensitivity of the proposed method is 0.4, 1, 5, and 0.2 ppm for Cd, As, Hg, and Pb, respectively, which is adequate for the certification of materials of the given designation.

**x-Ray Fluorescence Analysis of Ca and P.** In accordance with the requirements of the standard ASTM F 1185–88, powdered HAP should contain no more than 5% TCP. In connection with this, the error of the determination of the ratio Ca:P should be no greater than 0.5%. These requirements were met by using x-ray fluorescence analysis (XFA). The analysis was performed on a VRA-30 unit made by "Carl Zeiss" company. x-Ray fluorescence analysis makes it possible to easily perform nondestructive tests on finished dental implants, which makes it indispensable to checking the quality of finished products. One distinctive feature of the method is that information on composition (Ca and P) is emitted by a surface layer of the specimen having a thickness on the order of 10 μm. This feature makes it possible to perform layer-by-layer analyses of the ratio Ca:P, and it has been used to study the biodegradation of implants in *in vivo* experiments.

Our main goal was to obtain emitters that were stable in terms of density and to use a method of calculation that reduced the effect of instabilities. As is known, in x-ray fluorescence the intensity of the lines depends heavily on the structure of the emitters – their density and the dimensions of the particles of the powder. The powders are usually dissolved in different fluxes, such as lithium tetraborate. However, this method did not ensure the accuracy required in our investigation, since there might have been fluctuations in the homogeneity of the fluxes that would have been difficult to monitor. Thus, to obtain reliable results, we used a modification of the internal standard method in which the intensities were normalized with respect to the intensity of the main component (Ca). The calculation was performed in accordance with the formula  $I_i/I_m = kC_i/C_m$ , where  $I_i$  and  $C_i$  are the intensity and concentration of the *i*-th element;  $I_m$  and  $C_m$  are the intensity and concentration of the comparison element.

The problem posed in the investigation could be solved by obtaining information on the ratio of the concentrations. However, if the sum of the concentrations  $C$  is known, then the system of equations can be supplemented by an expression of the form  $\sum C_i = C$ . It then becomes possible to calculate absolute values of concentration. This approach makes it possible to achieve the required accuracy. The main error that limits the accuracy of the analysis is still the instrument error. We were able to reduce this error to 0.3% by efficiently choosing the dimensions of the emitters and the parameters of the instrument and by stabilizing the temperature regime of the gas used in the experiment.

The results that were obtained were checked by the method of x-ray diffraction (even though detecting the TCP phase at the given concentration level by this method is in itself a complex problem) and by the traditional methods of classical analytical chemistry. Standard specimens were prepared from synthesized batches of powders that were chemically analyzed. The powders were examined for the presence of other phases by the methods of x-ray phase analysis and x-ray fluorescence. Certified calcium pyrophosphate was used in the latter analysis. The composition of this compound is stably reproduced, which allows it to be used as a standard. The differences from the composition of hydroxyapatite made it necessary to introduce corrections for the mutual effects of the elements. These effects were accounted for by the method of fundamental parameters [2].

One feature of HAP is that it changes color when exposed to an x-ray beam (becoming yellowish brown). This distinguishes it from TCP. The color change is related to the degradation of the surface layer and a change in the ratio Ca:P, and it must be taken into account by periodically checking the composition of the tablets of the standard specimen.

The methods of analysis developed here, based on the use of modern instruments, make it possible to determine the ratio of the concentrations of calcium and phosphorus to within 1% and to determine the concentration of toxic metals with a sensitivity of  $1 \cdot 10^{-5}$ - $1 \cdot 10^{-6}$ %. These figures appreciably surpass the requirements established by the standards ASTM F 1185-88 and ASTM F 1088-87 in regard to the composition of HAP and TCP.

Properties of Materials Synthesized on the Basis of TCP and HAP

Content, % (mass):	
HAP	>95 / >95*
TCP	>95 / >95
Maximum allowable mass concentration of heavy metals, ppm:	
in the total	50/4
including:	
As	3/2
Cd	5/0.7
Hg	5/<1
Pb	30/0.5

\*The numerator indicates the requirements of ASTM F 1185-88 and ASTM F 1088-87. The denominator shows the characteristics of the powder synthesized at the Materials Science Institute

## REFERENCES

1. W. Jolly (ed.), Synthesis of Inorganic Compounds [Russian translation], Mir, Moscow (1967).
2. H. Erhardt (ed.), x-Ray Fluorescence Analysis [Russian translation], Metallurgiya, Moscow (1985).