Ministry of Education and Science of Ukraine
Vinnytsia National Technical University

Instructions
for exercises in discipline
"Biochemistry"
Module "Biomaterials"
for applicants for higher education in the specialty
163 – Biomedical Engineering





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Exercise № 1
Microstructural analysis of biomaterials

Purpose and objectives:

Conduct microstructural analysis of biomaterials .

Theoretical information and guidelines

The study of the microstructure of metallic biomaterials using optical microscope nd widely used for technical control in implantology. This is due to the fact that there is a close connection between the structure of materials and their properties.

Microstructural analysis is performed on specially selected, polished, polished and etched samples. Then the samples are examined under microscopes at a certain magnification. The magnification of the microscope is set depending on the purpose of the study. To determine the type of grain, its size is set to increase by 300-400 times.

Ferrite grains under the microscope look like light areas without clear boundaries. Ferrite grains are formed in low-carbon steels (0.02-0.03% C).

Perlite can be both granular and lamellar. Granular perlite under the microscope looks like reticulate dark spots. Lamellar - the area is filled with light and dark lines that alternate with each other. In pre-eutectoid steels (0.2-0.3% C) grains of ferrite and perlite are observed, but with the approach of 0.8% C the amount of ferrite decreases and at 0.8% C there will be one perlite.

Cementite is visible under a microscope as a light grid superimposed on a dark field. That is, cementite is released along the boundaries of perlite. The appearance of the grain is depicted graphically. Find out for yourself how the grains are depicted in the pictures in the recommended literature. The size of grains in metal-graphic practice is determined by visual assessment. A microphotographic set has been created for this purpose. The conditional grain number corresponds to the number of grains per 1 mm^2 of the surface of the sections.

The first number corresponds to sixteen grains on the area of 1 mm^2 of the section. Reducing the size of grain on one number on the scale corresponds with Increase in GVA ichi number of grains per 1 mm^2.It is convenient to set the grain number using a special attachment to the eyepiece of the microscope. In the half of the field of view of the eyepiece is mounted a plate with photos of the numbers of the scale of grain sizes.

Conduct microstructural analysis of samples of metallic biomaterials (titanium , aluminum, titanium nickelide, and stainless steel) .

Find the dependence of the microstructure of the samples on the material

Make a report on the work. Record the results of the study in the table.

Table of exercise results

Material	Schematic representation of the microstructure	Magnification	Description of the microstructure	Note

Present the results of your research in the form of a presentation with references to scientific publications on the topic (at least three).

Exercise № 2
Design of biomaterials based on hydroxyapatite

Purpose and objectives:

To study the structure and technology of obtaining hydroxyapatite .

Theoretical information and guidelines

Hydroxylapatite is a type of apatite in which hydroxyl predominates among the additional anions. It is the basis of bones (about 50% of the total bone mass) and teeth (96% of enamel).

Formula - $Ca_5[OH(PO_4)_3]$. Composition in %: CaO - 44.14; P_2O_5 - 40.26; H_2O - 2.87. Impurities: SrO, Na_2O, FeO, MnO.

Syngony is hexagonal. Density 3.07. Hardness 5. Color white, waxy yellow, green. Occurs in talc-chlorite shales, but does not form deposits.

There are:

- hydroxylapatite carbonate (carbon apatite);
- manganese hydroxylapatite (cancellation of hydroxylapatite, in which divalent manganese replaces calcium; Mn: Ca = 1: 7.7);
- lead hydroxylapatite (hydroxyl pyromorphite);
- hydroxylapatite fluoride (fluorapatite hydroxyl);
- hydroxylapatite chloride (cancellation of hydroxylapatite, in which part of the hydroxyl is replaced by chlorine).

The creation of new bone and plastic materials is an important area in dentistry, traumatology and orthopedics. However, the study of the composition and properties of such materials is given insufficient attention. The use of allogeneic bioactive calcium phosphate material (hydroxyapatite, tricalcium phosphate) affecting mineral metabolism and have osteoindukt and vnym and properties has advantages over artificial. In comparison with other biomaterials, these materials are identical in composition of bone minutes tissues and mammals and induce biological reactions similar to those in the remodeling of bone. At present, a large amount of experimental and clinical material is available in the literature, which indicates the successful use of both composite biomaterials and calcium-phosphate ceramics in pure form in the treatment of patients. At the same time, insufficient attention is paid to the possibility of using natural mineral components of bone tissue, which are often formed as waste in the production of dem and neral and so- called bone matrix.

One of the important properties of biomaterials that determine their biocompatibility and ability to affect osteogenic and bone cell potential is the ratio of calcium and phosphorus. According to the available literature, the stoichiometric ratio of these elements in hydroxyapatite is 1.37, weight - up to 2.15. In amorphous phosphate, these values are 1.5 and 1.94, respectively.

Depending on the method of obtaining synthetic and natural calcium-phosphate materials, this ratio may vary. The available literature presents the results of research on obtaining and studying the properties of hydroxyapatite of various origins. In view of the contradictory data of

different authors explore important ratio of calcium and phosphorus as the main criterion bioavailability and biocompatibility implantation s calcium phosphate materials.

Interest in hydroxyapatite is due to its great importance for biological research and practical medicine due to the fact that it is the main inorganic component of bone tissue of a living organism and has a high biocompatibility. Hydroxyapatite is used as a material during implants and coatings in dentistry and orthopedics. Both natural (biological) hydroxyapatite and artificially synthesized are used for these purposes. This produces powders with a wide range of particle dispersion from 2 to 100 μm. The interaction of HA with a living object depends on its chemical purity, size and morphology of crystals. Therefore, obtaining a chemically pure synthetic analogue of the mineral component of bone is an urgent and popular task.

When creating biocompatible materials of a given composition and morphology, the problem of developing methods for their synthesis is acute.

The main methods of HA synthesis are soluble and solid-phase. Soluble methods include precipitation, hydrothermal synthesis, hydrolysis of calcium phosphates. These methods are the most diverse and, using different techniques, allow to obtain chemically pure powders with adjustable crystal shape, degree of crystallinity, a given ratio of Ca / P (for biological HA determined the ratio of Ca / P = 1.67).

Good results in terms of crystallinity and chemical purity were obtained using methods of precipitation from aqueous solutions using different sources of calcium and phosphorus.

The aim of this work was to determine the most effective methods for obtaining chemically pure HA powders with a high degree of crystallinity and stoichiometric composition.

Consider three methods of synthesis of hydroxyapatite powder for medical purposes.

The first method of synthesis is a modification of the earlier one. This method is carried out by simple mixing of calcium and phosphate-containing suspensions in a stoichiometric ratio, leading to the formation of hydroxyapatite. A solution of $Ca(OH)_2$ and H_3PO_4 was used as starting components (precursors). The chemical reactions of this method of synthesis are given below

$$CaCO3_{(s)} \rightarrow CaO_{(s)} + CO2_{(g)} \quad (1)$$

$$CaO_{(s)} + H2O_{(l)} \rightarrow Ca(OH)2_{(s)} \quad (2)$$

$$10Ca(OH)2_{(l)} + 6H3PO4_{(l)} \rightarrow Ca10(PO4)6(OH)2_{(l)} + 18H2O_{(l)} \quad (3)$$

The first step was to obtain CaO from $CaCO_3$ followed by the use of hydrated calcium oxide as a water- dissolved source of calcium. In the process of preparation of $Ca(OH)_2$ first 100 grams of $CaCO_3$ calcined at 1010 ° C for 2.5 h, followed by obtaining CaO. Then $Ca(OH)_2$ was obtained by mixing a suspension of CaO with distilled water. The resulting suspension was dried at 60 ° C for 2 hours to obtain $Ca(OH)_2$.

To prepare HA powders separately mixed 60 gr $Ca(OH)_2$ and 30 ml H_3PO_4 with 1000 mL of where and on and zovan first water. Preparation of suspensions $Ca(OH)_2$ produced by

mixing these ingredients for 2 hours, adding drops La for a drop in the stock solution ortofosfo was but her acid. The resulting mixture was kept for 30 days. The final suspension containing about with Ada , filtered and dried in vacuum at 80 ° C for 2 hours. After that, the solid powder products were fried at a temperature of 1000 ° C for 3 hours .

The second method of synthesis methods is th , which is used as a precursor powder $CaCO_3$ and $(NH_4)_2HPO_4$,. They have relatively low solubility values compared to those used in the first method.

The chemical reaction for this method of synthesis is as follows:

$$10CaCO3_{(s)} + 6(NH4)2HPO4_{(s)} \rightarrow Ca10(PO4)6(OH)2_{(s)} + H2O_{(l)} + CO2_{(g)} \qquad (4)$$

First, in the same amount where and on and zovano her water separately prepared solution consisting of 0.5 mol of $CaCO_3$ and 0.3 mol $(NH_4)_2HPO_4$ with an excessive amount of a substance that dissolves (5%) by weight. Excessive amounts of $(NH_4)_2HPO_4$ were added to accelerate the dissolution of sparingly soluble $CaCO_3$ powders. A solution of $(NH_4)_2HPO_4$ was poured into a suspension of $CaCO_3$, and heated with vigorous stirring to 80 ° C.

During cooking, pH values were recorded.

In the process of exposure, at regular intervals, samples were taken to study the precipitated phases.

After holding for 2 days, an aqueous suspension was filtered to recover the solid sediment and excess phosphate was removed by washing in where and on and zovan first water. Then the solid product was dried at 80 ° C for 2 hours. Then calcination was performed at 1000 ° C for 3 hours

The third method of synthesis was performed according to an advanced technique . In this case, $Ca(NO_3)4H_2O$ and $(NH_4)_2HPO_4$ were used as precursors . In this case, the solid precursor calcium had a relatively plumb th solubility th . The chemical reaction for this method of synthesis is as follows:

$$10Ca(NO3)2{\cdot}4H2O_{(s)} + 6(NH4)2HPO4_{(s)} + 8(NH4OH)_{(l)} \rightarrow$$
$$Ca10(PO4)6(OH)2_{(s)} + 20(NH4NO3)_{(g)} + 40(H2O)_{(g)} \qquad (5)$$

First, 0.5 mol of $Ca(NO_3)_2 \cdot 4H_2O$ and 0.3 mol $(NH_4)_2HPO_4$ were separately mixed with 50 ml of deionized water and stirred vigorously. The pH value of the pH solutions of Ca and P was adjusted to pH = 11-12 by adding NH_4OH.

After adding all the components, the final solution was stirred for 2 hours after adjusting the pH = 11-12. Then the solution was heated to 80 ° C and stirred for 1 h at this temperature. After heating, the pH value was 6.5. After 1 h, the heated mass was cooled to room temperature by natural cooling in air and stirred for about 20 hours. The solution was filtered and washed

continuously with water to remove any excess NH_4OH. The filtered precipitate was dried at 80 ºC for 8 hours and calcined at 1000 ° C for 3 hours.

According to the results of X-ray diffraction analysis, it was found that the most effective way to obtain chemically pure hydroxyapatite powders with a high degree of crystallinity and compliance with the stoichiometric composition is the synthesis of hydroxyapatite powders, carried out using precursors $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$. The resulting products of this deposition method do not contain any other phase, in addition to hydroxyapatite, and have a high degree of crystallinity and stoichiometric composition.

Conduct each method for obtaining hydroxyapatite.

Exercise № 3
Study of shape-memory in structural biomaterials

Purpose and objectives:

To study the phenomenon of shape memory and its application to biomaterials .

Theoretical information and guidelines

The shape memory effect is a phenomenon of return to the original shape when heated, which is observed in some materials after previous deformation.

One of the basic people's perception of the phenomena of the external world - is the stability and reliability of metal products and designs remain stable functional form for a long time, unless of course they are not subjected to supercritical of impact am .

However, there are a number of materials, metal alloys, which when heated after pre-deformation show the phenomenon of returning to its original shape.

To understand the effect of shape memory, just conduct an experiment (Fig. 1).

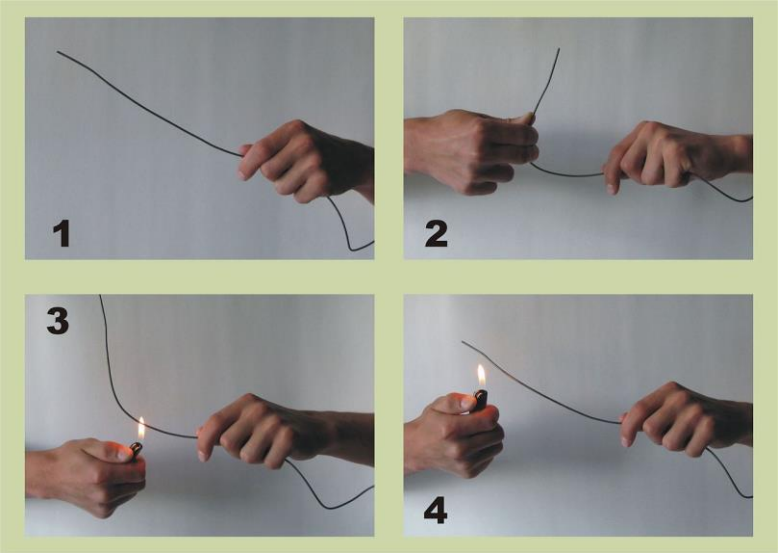


Fig. 1. Demonstration of the shape memory effect

What's going on?

1. There is a metal wire.
2. This wire is bent.
3. We begin to heat the wire.
4. When heated, the wire straightens, restoring its original shape.

Explanation of the phenomenon.

In the initial state in the material there is a certain structure.

During deformation (for example, bending), the outer layers of the material are pulled out, and the inner ones are compressed (the middle ones remain unchanged). These elongated structures are so-called martensitic plates, which is not uncommon for metal alloys. It is unusual that in materials with shape memory martensite is thermoelastic .

When heated, begins to appear so ermopruzhnist martensitic plates, ie they have a inner tension as well seek after is to return the structure to its original state, that is compressed elongated plate and flattened stretch.

As the outer elongated plates are compressed and the inner flattened ones are stretched, the material as a whole conducts self-deformation in the opposite direction and restores its original structure, and with it the shape.

The shape memory effect is characterized by two features:

1) brand of alloy with a strictly sustained chemical composition;

2) temperatures of martensitic transformations.

In the process of manifestation of the memory effect of the form involved martensitic transformations of two types - direct and reverse. Accordingly, each of them is manifested in its temperature range: direct martensitic transformation during cooling and inverse martensitic transformation during heating.

The temperatures of martensitic transformations are a function of both the brand of the alloy (alloy system) and its chemical composition. Small changes in the chemical composition of the alloy (intentional or accidental) lead to a shift in these temperatures.

Hence the need for strict exposure to the chemical composition of the alloy for unambiguous functional manifestation of the shape memory effect, which translates metallurgical production into the field of high technology.

The shape memory effect manifests itself over several million cycles; it can be enhanced by pre-heat treatment.

Reversible effects of shape memory are possible when the material at one temperature "remembers" one shape, and at another temperature - acquires another shape .

The higher the temperature of the inverse martensitic transformation, the less pronounced the shape memory effect. For example, a weak shape memory effect is observed in alloys of the Fe-Ni system (5-20% Ni), in which the inverse martensitic transformation temperature is 200-400˚C.

In a number of functional properties of the memory of the form, an important theoretical and practical significance belongs to the phenomenon of the so-called deformation of the oriented transformation. The meaning of this consequential phenomenon is as follows. If tensions cooled by Jenny body dump in the temperature ductility implementation of direct martensite transformation not stop lowering the temperature, not always continued cooling will not cause macroscopic deformation. On the contrary, most often the deformation continues to accumulate, as if the material was almost not unloaded. In other cases, there is intense is the return cooling. These

properties, especially in of which is called deformation based transformation, the second - an abnormal return deformation associated with pidrostannyam formed under load martensite crystals - in the case of deformation of crystals oriented transformation of positive orientation and, if abnormal return - negative orientation. The above phenomena can be initiated, particularly by the focused microloads.

Another phenomenon closely related to the shape memory effect is superelasticity - the property of a material subjected to a load to stresses significantly exceeding the yield strength to completely restore the original shape after removal of the load. The elasticity is observed in the temperature range between the beginning of the direct martensitic transformation and the end of the reverse.

Materials with shape memory effect.

Nickelide of titanium.

The leader among the materials with shape memory in terms of application and study is n and kel and d titanium (n and tinol) - intermetallics of equiatomic composition with 55% Ni (by weight). Melting point - 1240-1310 ˚C, density - 6.45 g / cm ³. The initial structure of nickelide in titanium is a stable volume-centered cubic lattice of the CsCl type during deformation undergoes thermoelastic martensitic transformation with the formation of a phase of low symmetry.

The element made of nickel in titanium can perform the functions of both the sensor and the actuator. Titanium nickelide has the following properties:

- very high corrosion resistance;
- high in strength;
- good characteristics of the form of memorization; high form recovery factor and high restorative force; deformation up to 8% can be completely restored; the recovery load can reach 800 MPa;
- good biocompatibility;
- high dempfer in onshore capacity.

The disadvantages of the material include poor manufacturability and high price:

- due to the presence of titanium, the alloy easily attaches nitrogen and oxygen, to prevent oxidation during production it is necessary to use vacuum;
- the reverse side of high strength is the complexity of processing in the manufacture of parts, especially cutting;
- at the end of the XX century, titanium nickelide was slightly cheaper than silver.

At the current level of industrial production, nickel - titanium products (along with Cu-Zn-Al alloys) have found wide practical application and market sales.

Other alloys.

At the beginning of the XX and age shape memory effect was discovered more than 20 alloys. In addition to titanium nickelide, the shape memory effect is detected in the following systems:

Au-Cd - developed in 1951 at the University of Illinois (USA); one of the pioneers of form memory materials;

Zn--Cu Al - along with titanium nickelide has a practical application; temperatures of martensitic transformations in the range from -170 to 100 ˚C; compared to titanium nickelide is not prone to rapid oxidation in air, is easily processed and five times cheaper, but worse in mechanical properties (due to grain enlargement during heat treatment), anti-corrosion and technological properties (problems of grain stabilization in powder metallurgy), characteristics form memorization;

Cu-Al-Ni - developed at Osaka University (Japan); martensitic transformation temperatures in the range from 100 to 200 ˚C;

Fe-Mn-Si - alloys of this system are the cheapest;

Fe-Ni;

Cu-Al;

Cu-Mn;

Co-Ni;

Ni-Al.

Some researchers consider, that the shape memory effect is possible in principle to any materials that undergo a martensitic transformation, including pure metals such as titanium, zirconium and cobalt.

The use of materials with the effect of shape memory in medicine (option) .

1. Production of stents that are widely used in X-ray - Endovascular second surgery.
2. Gloves used in the rehabilitation process and designed to reactivate groups of active muscles with functional insufficiency. Can be used in the wrists, elbows, shoulders, ankles and knees.
3. Contraceptive spirals that become functional after the introduction of the second form and under the influence of body temperature.
4. Filters for introduction into vessels of circulatory system. Just introduced as the first wire for the catheter, and then they take the form of a filter with a fixed location.
5. Clamps for pinching weak veins.
6. Artificial muscles that are driven by electric current.
7. Fastening pins designed to fix dentures on the bones.
8. Artificial extension device for so-called growth prostheses in children.
9. Replacement of the cartilage of the femoral head. The replacement material becomes self-tightening under the action of a spherical shape (femoral head).
10. Rods for correction of the spine in scoliosis.

11. Temporary clamps fix the elements during implantation of an artificial lens.
12. Spectacle frame. At the bottom, where the glass is attached with wire. Plastic lenses do not slip out when cooled. The frame does not stretch when wiping the lenses with prolonged use. The effect of elasticity is used. .
13. Orthopedic implants.
14. Orthodontic arch to correct the dentition.
15. Dental implants (self-fixation of diverging elements in the bone).

Assignment

According to the option (see the list of applications in medicine) prepare a material report with a presentation and reference to scientific sources. Students are divided into the pairs by a lecturer randomly and exchange their topics. As a result, each student makes a short report on the topic and materials of his partner from the pair.

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