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METHODOLOGY OF BIOCHEMICAL STABILITY DETERMINATION OF COMPOSITIONAL SYSTEMS BASED ON HYDROXYAPATITE

В.М. Тіхенко, Ю.О. Федоренко, І.В. Прокопович. **Методика визначення біохімічної стабільності композиційних систем на основі гідроксиапатиту.** Зростання кількості людей з патологіями кісткової тканини обумовлює актуальність питання пошуку нових реконструктивних матеріалів. Водночас, при їх застосуванні можуть виникати різноманітні побічні ефекти, що є наслідком реакції відторгнення. Найбільшого поширення в остеопластиці отримали матеріали на основі гідроксиапатиту, оскільки цей матеріал характеризується своєю нетоксичністю, біосумісністю і корозійною стійкістю. Гідроксиапатит також за своїм хімічним складом є аналогом мінеральної складової кісткової тканини, що сприяє її регенерації. ***Мета:*** Метою роботи є розробка комплексної методики дослідження поведінки базальтової луски і композиційної системи «гідроксиапатит – базальтова луска» в середовищах, які імітують рідини живого організму. ***Матеріали і методи:*** Для оцінки біохімічної стабільності досліджуваних матеріалів було застосовано комплексний підхід, який включає спеціальну підготовку проб біологічних середовищ і проведення комплексного хімічного, фотоелектрокалориметричного й рентгеноструктурного досліджень. При проведенні фотоелектрокалориметрії для визначення кількості речовини було складено калібрувальні криві. Під час дослідження біохімічної стабільності композиційних систем «гідроксиапатит – базальтова луска» у фізіологічних середовищах для визначення кількості загального заліза у фільтратах було використано дві методики підготовки проб. ***Результати:*** На основі проведеного багатокомпонентного хімічного аналізу було розроблено методику, яка дозволяє зробити висновок про фізико-хімічну стабільність у взаємодії досліджуваних порошків базальтової луски і композиційних систем «гідроксиапатит – базальтова луска» з плазмою крові і розчинами Рінгера й Рінгера-Локка, які імітують рідини живого організму.

Ключові слова: біоматеріали, базальтова луска, гідроксиапатит, метрологічне забезпечення.

V.M. Tihenko, Yu.O. Fedorenko, I.V. Prokopovich. **Methodology of biochemical stability determination of compositional systems based on hydroxyapatite.** The growing quantity of people with bone pathology causes the urgency of finding the new reconstruction materials. However, their application may experience different side effects that result the rejection reactions. Materials based on hydroxyapatite are the most widely used in osteoplasty as this material is characterized by its non-toxicity, biocompatibility and corrosion resistance. Hydroxyapatite in its chemical composition is analogous to the mineral constituent of bone, contributing to its regeneration. ***Aim:*** The aim is to develop a comprehensive methodology to study the behavior of basalt scales and compositional system "hydroxyapatite – basalt scales" in environments that simulate fluids of living body. ***Materials and Methods:*** A comprehensive approach that includes specific preparing of samples and biological environments and complex chemical, photoelectric colorimetric and X-ray studies were applied to evaluate the biochemical stability of the studied material. Calibration curves were prepared during photoelectric colorimetry to determine the amount of substance. Two methods of sample preparation were used to determine the amount of total iron in the filtrate during the study of biochemical stability of the composition systems "hydroxyapatite – basalt scales" in physiological environments. ***Results:*** Based on the multi-chemical analysis it can be concluded about the physical and chemical stability in interaction of studied basalt scales powders and composite systems "hydroxyapatite – basalt scales" with the blood plasma and Ringer's solution and Ringer-Locke solution that imitate the fluid of a living body.

Keywords: biomaterials, Basalt scales, hydroxyapatite, metrological support.

Introduction. Improving methods of research of new medical materials remains to be a topical issue today. Biomaterials used for manufacturing endoprosthesis must meet certain requirements.

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These materials are covered by the Technical Regulations on medical devices. Circulation and operation of the products can be permitted only when they are fully compliant with the Technical Regulations on medical devices under the following conditions: adequate supply, implantation and installation; proper maintenance and use to their destination; existence of the national mark of conformity.

With the growing quantity of people with bone pathology the attention is paid to reconstruction materials. Various metals, alloys, polymers and ceramics are used to restore bone defects. However, when applied there is a number of adverse side effects, which are the result of resentment. Materials based on hydroxyapatite (HA) are most widely used in osteoplasty, as it is nontoxic, biocompatible and corrosion-resistant. Hydroxyapatite in its chemical composition is analogous to the mineral component of bone, contributing to its regeneration. Calcium orthophosphates are of limited use as the main materials for orthopedics and dentistry due to the poor mechanical properties (they are very fragile and hard). So the research, development and production of Bio-ceramic materials with improved mechanical properties are a significant segment of modern high technologies [1]. Scientists have developed various methods of strengthening HA (although a number of shortcomings remain) [2...4].

At the I.M. Frantsevich Institute for Problems of Materials Sciences of National Academy of Sciences of Ukraine the series of studies that allow choosing desired mass fraction of basalt scales (BS) as dispersion-strengthening supplements and temperature regime of treatment required to achieve optimum mechanical properties of the composite system were conducted [5]. Efficacy and safety of using such system requires significant research of its physical and chemical stability in the human body. Today only safety of HA in medicine is found, while BS is used to improve the mechanical properties of materials used in various fields as bearing elements. However, any studies of BS safety for living organism environments were not conducted.

In work [6] the physical and chemical stability of basalt scales in biological environments was studied. But today the urgent question is to study the behavior of composite systems based on hydroxyapatite in physiological solutions. Metrological support of research is also important to obtain the reliable data.

The aim is to develop a comprehensive methodology to study the behavior of basalt scales and composition system "hydroxyapatite – basalt scales" in environments that simulate fluids of living body.

Basic physical and chemical methods, which were modified due to the specific of considered compositional system and the environment, were taken as a basis.

Materials and Methods. The depth and character of assessment of physical and chemical and physical and mechanical properties of biocompatible materials depend on their field of application, since it is impossible and unnecessary to use all known methods to characterize the material. Attention is drawn to the fact that the use of tests should be closely linked to the conditions of operation endoprosthesis (statics, dynamics, availability of cyclic loads and long-term contact with blood). To take into account all the processes it is necessary to apply the system tests and measurements [7].

To assess the biochemical stability of BS and the composition system HA + BS we apply a comprehensive approach that includes specific training samples and biological environments of complex chemical, photoelectric colorimetric and X-ray studies.

The main task of the chemical analysis is to extract information on the matter with various measuring tools. Thus, the method of analysis is a complex, multi-measurement procedure. Just during the measurement process (with further processing of results) the internal unity of various methods of analysis manifests and patterns of chemical quantities measurement are fundamental to all sections of Analytical Chemistry. It is important to remember that the metrological support of chemical analysis guarantee the accuracy of all necessary measurements [8].

From the metrological point of view the quantitative chemical analysis is a complex process which uses auxiliary equipment and advanced techniques. In this case it is a photoelectric colorimetry (PEC). Due to multicomponent composition of biologically active solutions (blood plasma, Ringer's solution and Ringer-Locke solution), the known methods of research of their chemical composition were modified in this work.

At conducting of PEC for determination of amount of substance it is necessary to make a standard curve, which shows the dependence of optical density of the solution on the amount of

substance. To build a curve a series of stained solutions is prepared with known amount of substance (a different amount of standard solution is used). Tinted standard solutions should be prepared under conditions identical to those in which the study is conducted, and in full compliance with the methodology of work. Optical density measuring is performed and a standard curve is built by plotting the known concentration on the horizontal axis and the corresponding values of optical density on the vertical axis.

Concentration of the substance in the test solution is determined by the calibration curve. To provide this the solution is poured into the same cuvette for which a curve is constructed and the same filter is turn on, and its optical density is determined. Then the concentrations of substance are found on a curve, which is determined according to the optical density.

Another important component of the chemical analysis is the correct sample preparation.

The study used multicomponent solutions that are approximated to the tissue fluid by its saline composition:

1. Saline 9 g/l NaCl, pH 6.7. Sodium chloride is found in blood and other physiological fluids (blood concentration is about 0.5 %), which provides a stable osmotic concentration of blood.

2. Ringer (g/l: NaCl – 8.6; KCl – 0.3; $CaCl_2$ – 0.33) is the source of water and electrolytes in blood and tissue fluid. Sodium is a primary cation of extracellular fluid primarily involved in the control of water distribution, water balance and osmotic pressure of body fluids. Sodium is also associated with chlorine and bicarbonate in the regulation of acid-base balance of body fluids. Potassium is a main cation of intracellular fluid involved in the utilization of carbohydrates and protein synthesis and is needed for regulation of nerve conduction and muscle contraction (especially the heart muscle). Chlorine as the main extracellular anion is closely related to the metabolism of sodium, and changes in acid-base balance of the body reflect changes in the concentration of chlorine. Calcium – important cation, which provides the formation of bones and teeth (in the form of calcium phosphate and calcium carbonate). In the ionized form the calcium is needed for functional mechanism of blood clotting, proper functioning of heart and regulation of neuromuscular excitability.

3. Ringer-Locke (g/l: sodium chloride – 9.0, sodium bicarbonate – 0.2; calcium chloride – 0.2; potassium chloride – 0.2; glucose – 1) refers to plasma substituting saline fluids. It contains a balanced (isotonic) mixture of basic cations (Na^+, K^+, Ca^+, Sl^-, NSO_3^-) of blood in its corresponding concentrations and is more physiological in compare with 0.9 % isotonic sodium chloride solution.

4. Human blood plasma, which was obtained by separating suspended cellular elements from donated human blood by centrifugation. Blood plasma is a transparent and viscous liquid, consisting of 90...91 % water and 9...10 % solids, which part of proteins is 6.6...8.2 %, 0.9 % is inorganic salts and the rest – nonprotein organic compounds. Proteins are made up of factions: 4...4.5 % albumin, 2.8...3.1 % globulin and 0.1...0.4 % fibrinogen. In blood plasma the iron is in persistent state of 50...180 μg per 100 ml.

During the study of the biochemical stability of the composition system HA + BS in physiological environments two methods of sample preparation were used to determine the amount of total iron in the filtrate.

According to the first technique, the filtrate was repeatedly diluted with distilled water (30...60 times), and then the procedure described in [9] was used, which is based on the formation of complex compounds of iron sulfosalicylic acid. To measure optical density, the blue filter (440 nm) and cuvette with a layer thickness of 10 mm were taken. Multiple diluted plasma with reagents was used as the standard solution.

The second method involves a preliminary deposition of iron in the form $Fe(OH)_3$, its allocation to the filtrate, followed by hot solution (1:1) dissolving according to the method [10]. The advantage of this method is that coagulated plasma proteins remain on the filter; working solutions for PEC are more transparent than in the first case.

The titration method is also based on a complex interaction of sulfosalicylic iron and ammonia complex [11]. In this case, the aliquots of the filter (5 ml) was diluted with distilled water for 50 times, and then to transfer iron in oxide form the 10 ml hydrochloric acid were added (1:1) and one

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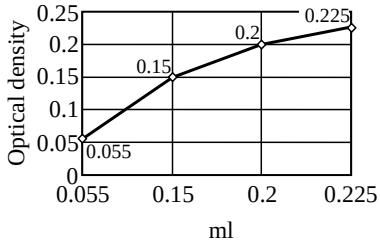

Fig. 1. Calibration curve for calcium, light filter № 7, cuvette – 10 ml

milliliter of concentrated nitric acid solution followed by boiling for a few minutes. 10 ml of 10 % sulfosalicylic acid was added. In the cooled solution and with drop method ammonia solution (1:1) was neutralized until the color was brown. Then 10 ml of 1 % hydrochloric acid were added. The contents of the flask were heated to 50...60 °C and titrated with Trilon B to transfer color from brown to green. This complex technique using a special sample preparation of biological media filtrate is used to study biochemical stability on iron, silicon and calcium of various BS modifications and composition of HA + BS.

Since calcium is a member of saline and plasma the calibration curve is constructed (see Fig. 1), further calculations will be performed due to the curve.

To construct the calibration graph Ringer's solution containing 0.048 g of $CaCl_2$ per 100 cm^3 of liquid was taken. It was established that the total weight of calcium chloride in solution is 110.984 g. To ensure the accuracy of further calculations, the mass fraction of calcium was calculated in two ways (in volume 100 cm^3 and 200 cm^3).

Approximately equal values were received in both cases, so we can say that calcium titer T_{Ca}=0.00088 g/ml.

The quantitative assessment of calcium in Ringer's solution and plasma was conducted based on the calibration graph.

Since the determination of silicon, iron and calcium was held in dynamics – at the fifth and tenth day of the experiment – the overall volume of solution decreased as shown in the calculations.

The following composition were used as comparative solution: 2 cm^3 of Arsenazo I, 2 drops of 10 % solution of NaOH, 0.1 % solution of NaOH, which were added till the 100 cm^3 value. The mixture was thoroughly mixed and after 30 minutes the necessary measuring were conducted to determine optical density (α). The optical density of the solution was determined at the appropriate wavelength and with the same light filter, using the same cuvette.

Concentration of the substance to be determined was calculated by comparing the optical density.

The iron content in the filtrate was determined by the method [11] based on the formation of complexes with sulfosalicylic acid.

In the first phase of chemical analysis of the BS conduct research in saline a blue filter λ_{eff}=440 nm with cuvette layer thickness of 10 mm were used.

In the second phase of quantitative chemical analysis, i.e. the study of biochemical stability of composition systems HA+BS – for determination of iron were used: light filter № 4, aliquot 1/45, 10 mm cuvette and value titer T_{Fe}=0.00001155 g/ml. The quantity of iron was measured in dynamics – after 24, 72, 120 and 240 hours.

Also, the calculations in mg/100 ml taking into account the correction factor, as the volume in the flask decreased after each measurement, and the concentration of elements increased.

Determining the amount of calcium in physiological solutions for a variety of samples and construction of the calibration curve was performed by the following procedure. Such components were added to test solution: 2 cm^3 of 0.1 % Arsenazo I (aqueous) solution or 4 cm^3 of 0.05 % Arsenazo I aqueous solution, then 2 drops of 10 % solution of NaOH, and to fill up to the 100 cm^3 value the 0.1 % solution of NaOH were added. Then, the mixture was gently stirred and after 30 minutes the optical density was measured.

To determine the optical density of the solution, the yellow light filter № 4 was used, which transmits the wave length in the range of 560...570 nm.

Calculations of the amount of calcium in the filtrate as the amount of iron and silicon were made using built calibration charts.

Conclusion. Based on a modified multi-chemical analysis we can conclude about the physical and chemical stability in interaction of basalt scales powders and composite systems "hydroxyapatite – basalt scales" with the blood plasma and Ringer's solution and Ringer-Locke solution, that imitate the fluid of a living body. Modification of the analysis was carried out by special sample preparation, due

to the composition of solutions that imitate body fluids and the material. In consequence of metrological support of this process you can talk about the feasibility of biomaterial using for bone reconstruction in osteoplastic surgery, dentistry and orthopedics.

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