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A.N. Sagidugumar*[1], A. Turlybekuly[2], D. Dogadkin[1], A.D. Pogrebnjak[4], N. Kantay[3], A. Sadibekov[1]

[1]*D. Serikbayev East Kazakhstan Technical University, Oskemen, Kazakhstan*
[2]*LLP "Aman Technologies", Nur-Sultan, Kazakhstan*
[3]*S. Amanzholov East Kazakhstan University, Oskemen, Kazakhstan*
[4]*Sumy State University, Sumy, Ukraine*
*(*E-mail: sagidugumar@gmail.com)*

Phase transformations during the doping of zinc chloride and silver nitrate into calcium phosphates

This article shows the results of a study on a biocomposite material based on calcium phosphate doped with ZnCl and $AgNO_3$. Calcium phosphates are mainly used in dentistry and orthopedics due to their excellent biocompatibility, osteoconductive properties and similarity to the inorganic components of human bone. The main objective of the study is to investigate the morphology, elemental and phase composition, and physicochemical properties of the obtained material. The test material is obtained in the form of a suspension and subjected to ultrasonic treatment. The microstructure and phase composition of the obtained biocomposites are studied by SEM, XRD, FTIR methods. It is possible to obtain dicalcium phosphate dihydrate (DCPD) by the presented methods, and the results demonstrate a partial replacement of calcium atoms by zinc atoms. X-ray phase analysis shows that $Ca(HPO_4)(H_2O)_2$ phases as well as $CaZn_2(PO_4)_2(H_2O)_2$ and AgCl phases were formed during the reaction. Fourier transform infrared spectroscopy revealed that the obtained samples contain the groups HPO_4^2 and PO_4^3, with the group PO_4^2 replacing the group CO_3^2. The biocomposite materials could be of great interest in the biomedical field, including the development of coatings that prevent or delay the development of bacterial biofilm.

Keywords: biocomposite material, calcium phosphates, morphology, Ag-doped, hydroxyapatite, bioactivity, biocompatibility, osteoconductive.

Introduction

Currently, biomaterials are an area of great interest in medicine, especially new biomaterials with osteoinductive properties and a bactericidal effect [1]. The fact is that during surgery, there is a risk of bacterial infection. In the event of septic inflammation of the tissues that come into contact with the implant, bacteria can adhere to the surface of the implant and form a biofilm [2]. This leads to the development of "implant-associated infections", which are one of the main complications of orthopedic surgery. Such infections must be treated with systemic antibiotics, which are not always effective because there is another problem - antibiotic resistance of microorganisms [3]. One of the ways to solve these problems is to create materials that have antibacterial properties in addition to biocompatibility.

Silver nanoparticles are effective in treating wounds and ulcers. Currently, antimicrobials containing silver nanoparticles are widely used [4]. It has been shown that $AgNO_3$ demonstrates significant antibacterial activity against various types of bacteria, both Gram-positive and Gram-negative: Salmonella, Staphylococcus, and Pseudomonas, etc. [5]. In the works of the authors Qiuju Zhou et al. it has been reported that $AgNO_3$-doped nanocomposite microparticles showed good cytocompatibility and effective antibacterial activity against Gram-negative E. coli and Gram-positive S. aureus [6]. Zinc ions also show an antibacterial effect. In addition, trace elements of zinc are present in human bone tissue and stimulate the process of osteoinduction and play an important role in the human immune system [7].

The use of biocomposite materials with antimicrobial activity based on calcium phosphates in medicine is promising because human bone tissue is a natural nanocomposite material [8, 9], in which hydroxyapatite nanoparticles (HA) are embedded in collagen fibrils and contain microelements, such as Si, Fe, Zn, Cu, I, Ag, etc., which affect bone tissue, its formation and properties. As an inorganic bone component, HA nanoparticles have excellent biocompatibility, osteoconductivity, and are widely used as a basis for bone tissue engineering, as well as a material for coating implants [10,11].

Calcium phosphates was mainly used in dentistry and orthopedics as dental fillers, coatings for titanium dental implants, as bone substitutes, and for bone reconstruction and regeneration, due to their excellent biocompatibility, osteoconductive properties, and similarity to the inorganic constituents of human bone [12–

16]. This work is devoted to the synthesis and comprehensive study of a biocomposite material based on calcium phosphate doped with ZnCl and $AgNO_3$.

Experimental

The suspension based on calcium phosphate was obtained by dissolving $CaCl_2$ $2H_2O$: Na_2HPO_4 $12H_2O$ in distilled water in the ratio of 1:2. The solution was mixed in a ball mill for 60 min. ZnCl, $AgNO_3$ were added to the resulting suspension in different ratios (Table 1). The resulting solution was subjected to ultrasonic treatment for about 40 minutes. The solution was mixed again in a ball mill for 40 minutes. Then the solid fractions were separated by centrifugation and dried at room temperature. The resulting composite material was analyzed using analytical equipment.

XRD analysis. The crystallographic structure investigation was performed by the PANalytical X'PertPro diffractometer. The detector diffraction aperture is 100 µm, anode material - Cu/K-Alpha 1,54060Å.

FTIR analysis. The functional groups study was carried out by PerkinElmer Spectrum BX Fourier transform infrared spectrometer. Spectra were recorded over the range 4000–400 cm-1 at 1 cm-1 resolution.

SEM analysis. Surface morphology was performed on the JSM-6390LV microscope with the INCA Energy Penta FET X3 energy dispersive microanalysis system at 20 kV acceleration voltage. The chemical elements distribution analysis was performed in the selected area.

T a b l e 1

Concentrations of ZnCl, $AgNO_3$

Samples	$AgNO_3$	ZnCl
1	1,5%	3,5%
2	2,5%	2,5%
3	3,5%	1,5%

Results and Discussion

According to the results of X-ray phase analysis (Fig. 1), all samples were found to contain dicalcium phosphate dihydrate (DCPD) (Brushite) $Ca(HPO_4)$ $(H_2O)_2$ phases, as well as $CaZn_2$ $(PO_4)_2(H_2O)_2$ and AgCl phases. ICSD databases were used in the analysis of the obtained data. The main phase is $Ca(HPO_4)(H_2O)_2$, which crystallizes in a monoclinic unit cell, and the results show that there is a partial replacement of calcium atoms by zinc atoms, and AgCl synthesis also occurred in the reaction. From a comparison of the diffraction patterns (Fig.1) of the obtained samples, a change in the shape of the diffraction lines with an increase in the zinc concentration is visible. This is probably due to deformation in the crystal lattice and a decrease in the size of nanocrystals of zinc-substituted samples.

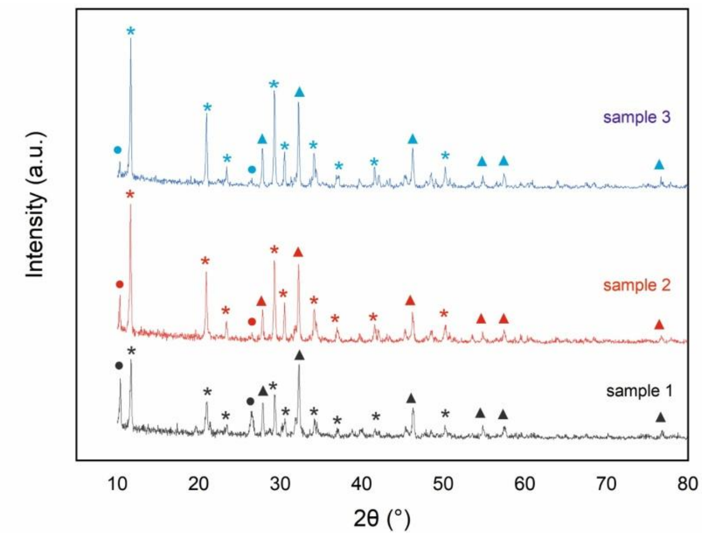

Figure 1. X-ray diffraction patterns of the obtained samples.
***** $Ca(HPO_4)(H_2O)_2$ (ICSD code16738), ● $CaZn_2(PO_4)_2(H_2O)_2$ (ICSD code 040146), ▲ AgCl (ICSD code 64734).

The surface morphology of the samples was studied (Fig. 2) using a scanning electron microscope (SEM) with INCA analysis. Analysis results illustrate that the samples have similar surface morphology. This indicates that for the samples, the introduction of zinc atoms into the crystal lattice of dicalcium phosphate dihydrate does not change the materials morphology. Images show that these samples have calcium phosphate agglomerates and calcite grains. According to the INCA analysis data (Fig. 2d), we can conclude that white inclusions belong to the AgCl crystals. Our supposition is also proved by XRD analysis data, where the peaks of AgCl (ICSD code 64734) are clearly shown. However, it is difficult to identify certainly because INCA and XRD integrate signals from the material.

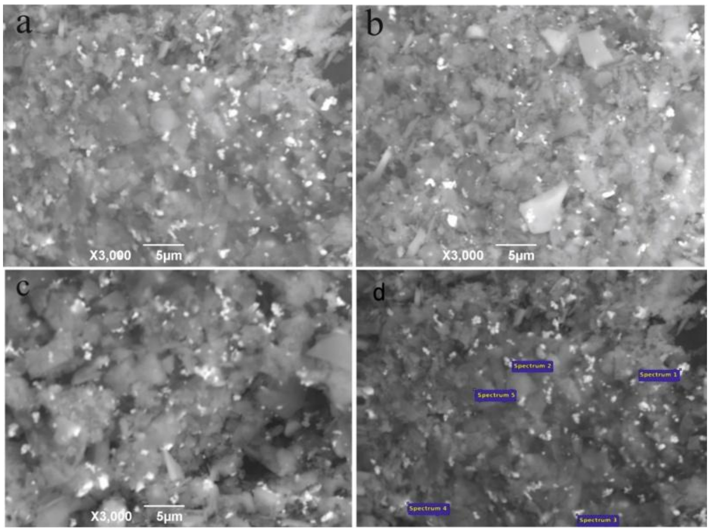

Figure 2. SEM image of samples (a – sample 1; b – sample 2; c – sample 3, d – EDX analysis of the sample 1)

Table 2

Elemental composition of the obtained samples, all results in weight %

Samples	O	Na	**Ag**	**Zn**	Cl	**Ca**	**P**	**Ca/P**
Sample 1	58.7±1.38	0	**9.3±4.5**	**8.39±3.4**	2.6±0.2	**11.18±2.5**	**9.02±1.6**	**1.23**
Sample 2	55±1.6	1.25	**5.58±2.8**	**5.94±3**	2.3±0.13	**16±1.07**	**13.8±0.68**	**1.16**
Sample 3	60±2.1	1.28±0.11	**8.44±1.6**	**1.35±0.22**	2.2±0.09	**14.076±0.46**	**11.65±0.46**	**1.2**

From the results of INCA analysis (Table 2), the average Ca/P ratio in Sample 1, Sample 2, and Sample 3 is 1.23, 1.16, and 1.2, respectively. It can be seen that with an increase in Zn concentration, Ca concentration decreases, which could indicate a partial replacement of calcium atoms by zinc atoms, which is confirmed by X-ray diffraction analysis.

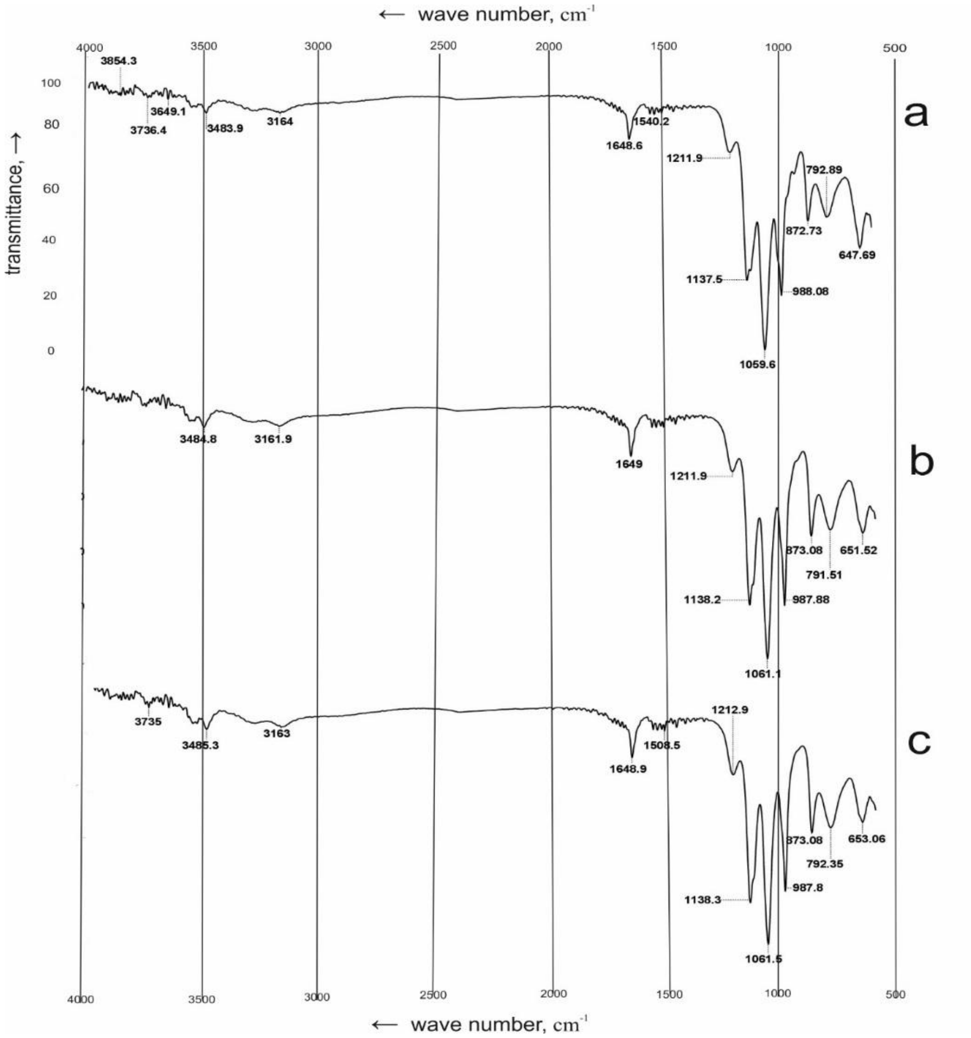

Figure 3. FTIR spectra of the synthesized samples (a – sample 1; b – sample 2; c – sample 3)

In the IR spectra (Fig. 3) of the investigated samples, high-intensity groups of modes in the range of 987.8 and 1061.5 cm^{-1} were observed, which belong to the PO_4^{3}- complex. The vibrational modes at 1038.3 cm^{-1} should be attributed to the asymmetric stretching mode (ν3) of the P–O bond and bending vibrations of the PO_4^{3} groups. In addition, vibrational modes in the range of 792, 873 and 1212 cm^{-1} can be observed in all samples belonging to the HPO_4^{2-} group. The vibrational modes in the range of 653 and 3485 cm^{-1} can be attributed to the group OH. Low-intensity vibrational modes at 1508 and 1648 cm-1 show that the PO_4^{2-} group is replaced by the CO_3^{2-} group, which is due to the absorption of CO_2 from the atmosphere during synthesis [17]. In spite of doping with Ag and Zn ions, the relative intensity of CO_2 modes on all samples remained almost constant. When comparing the spectra, the samples doped with Ag and Zn ions show a gradual slight broadening and shift of the absorption bands due to an increase in foreign ions.

Conclusions

The results of X-ray diffraction contributed to the following conclusions: It is possible to obtain dicalcium phosphate dihydrate (DCPD) by the method presented in this work; There is a partial replacement of calcium atoms by zinc atoms; All samples of dicalcium phosphate dihydrate (DCPD) (brushite) contain $Ca(HPO_4)$ $(H_2O)_2$ phases as well as $CaZn_2$ $(PO_4)_2(H_2O)_2$ and AgCl phases.

The SEM study outlined that the specimens have similar morphology. Calcium phosphate agglomerates are observed on the surface, as well as crystalline AgCl inclusions 1-2 μm in size. The INCA analysis demonstrated that with an increase in Zn concentration, Ca concentration decreases, indirectly confirming the partial replacement of calcium atoms by zinc atoms.

According to IR spectroscopy, the obtained samples contain the HPO_4^{2} and PO_4^{3} groups, with the PO_4^{2} group replaced by the CO_3^{2} group. The results of a comprehensive study provide information for a better understanding of the processes that occur during the synthesis of biocomposite materials.

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А.Н. Сағидұғұмар, А. Тұрлыбекұлы, Д. Догадкин, А.Д. Погребняк,
Н. Қантай, А. Садибеков

Мырыш хлориді мен күміс нитратын кальций фосфаттарына қосу кезіндегі фазалық өзгерістер

Мақалада ZnCl және $AgNO_3$ қосылған кальций фосфаты негізіндегі биокомпозиттік материалының зерттеу нәтижелері көрсетілген. Биоүйлесімділігі мен остеокондуктивті қасиеттері және адам сүйектерінің бейорганикалық компоненттеріне ұқсастығынан кальций фосфат негізіндегі биокомпозитты материалдар, стоматология мен ортопедияда кеңінен қолданылады. Алынған биокомпозиттің микроқұрылымы мен фазалық құрамы СЭМ, РФА, ИҚ-Фурье әдістерімен зерттелді. Дайындалған сынақ материалы суспензия ретінде алынды және ультрадыбыстық өңдеуден өтті. Зерттеудің негізгі мақсаты алынған материалдың морфологиясын, элементтік және фазалық құрамын, сондай-ақ физика-химиялық қасиеттерін зерттеу. Алынған нәтижелерге сәйкес, ұсынылған әдіс бойынша дигидрат дикальцийфосфатын (ДКФД) алуға болатындығы, сонымен қатар нәтижелер кальций атомдарының мырыш атомдарымен ішінара алмастырылатындығы көрсетілген. Рентгенфазалық талдау нәтижелері бойынша реакция барысында $Ca(HPO_4)$ $(H_2O)_2$ фазалары, сондай-ақ $CaZn_2$ $(PO_4)2(H_2O)_2$ және AgCl фазалары пайда болғаны анықталды. ИҚ спектроскопия әдісімен алынған үлгілерде HPO_4^2 және PO_4^3 топтары бар екендігі анықталған, сонымен қатар PO42 тобы CO_3^2 тобымен алмасқанын байқауға болады. Биокомпозиттік материалдар биомедицина саласында үлкен қызығушылық тудыруы мүмкін, соның ішінде бактериялық биопленканың дамуын болдырмайтын немесе баяулататын жабындарды әзірлеу.

Кілт сөздер: биокомпозитті материал, кальций фосфаты, морфология, гидроксиапатит, легирленген Ag, биологиялық белсенділік, биосәйкестік, остеокондуктивтілік.

А.Н. Сағидұғұмар, А. Тұрлыбекұлы, Д. Догадкин, А.Д. Погребняк,
Н. Қантай, А. Садибеков

Фазовые превращения при доппировании хлорида цинка и нитрата серебра в фосфаты кальция

В статье показаны результаты исследования биокомпозитного материала на основе фосфата кальция, доппированного ZnCl и $AgNO_3$. Фосфаты кальция находят широкое применение, особенно в стоматологии и ортопедии, из-за их превосходной биосовместимости, остеокондуктивных свойств и сходства с неорганическим компонентом костей человека. Микроструктуру и фазовый состав полученных биокомпозитов исследовали методами СЭМ, РФА, ИК-Фурье. Исследуемый материал был получен в виде суспензии и подвергнут ультразвуковой обработке. Основной целью исследования было изучение морфологии, элементного и фазового состава, а также физико-химических свойств полученного материала. Согласно результатам, возможно получение дикальцийфосфата дигидрата (ДКФД) по представленной методике, кроме того, результаты показывают, что происходит частичное замещение атомов кальция атомами цинка. По результатам рентгенофазового анализа было установлено что, в ходе реакции образовались фазы Ca (HPO_4) $(H_2O)_2$, а также $CaZn_2$ $(PO_4)_2(H_2O)_2$ и AgCl. Методом ИК-спектроскопии доказано, что полученные образцы содержат группы HPO_4^2 и PO_4^3, кроме того, происходит замещение группы PO_4^{2-} группой CO_3^2. Биокомпозитные материалы могут представлять большой интерес в области биомедицины, включая разработку покрытий, предотвращающих или замедляющих развитие бактериальной биопленки.

Ключевые слова: биокомпозитный материал, фосфат кальция, морфология, гидроксиапатит, легированный Ag, биологическая активность, биосовместимость, остеокондуктивность.