NANOPARTICULE DE FOSFAT DE CALCIU DOPATE CU IONI DE Cu^{2+}- POSIBIL SUBSTRAT PENTRU ELIBERAREA CONTROLATĂ A MEDICAMENTELOR

NANO CALCIUM PHOSPHATE DOPED WITH Cu^{2+} IONS – POSSIBLE CARRIER FOR DRUG DELIVERY SYSTEMS

RODICA ROGOJAN[1,2*] , ECATERINA ANDRONESCU[1], MIHAI HOTETEU[2]

[1] *University POLITEHNICA of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Science and Engineering of Oxide Materials and Nanomaterials, 1-7, Gh. Polizu street, 1-Bucharest, Romania*

[2]*National Institute of Rehabilitation, Physical-Medicine and Balneoclimatology, 11A, Ion Mihalache Bld, 1-Bucharest, Romania*

The purpose of this paper is to assess whether calcium phosphate nanoparticles may be potential carriers of different cations. Recent advances in nanotechnology show that nanoscale particles may play an important role in tissue engineering in medicine or in their use as substrates for controlled drug release. The calcium phosphate nanoparticles were obtained from $(NH_4)_2HPO_4$ 0.3 M and $Ca(NO_3)_2{\cdot}4H_2O$ 0.5 M by coprecipitation technique and the hybrids calcium phosphates - copper ions were obtained by the similar cold-wet method, in the presence of ammonium hydroxide. The synthesized samples were analyzed by X-ray diffractometry, Fourier transform infrared spectrofotometry (FT-IR), energy dispersive X-ray analysis (EDAX) and the micrographs were determined by scanning electron microscopy (SEM). Calcium phosphates are widely used as implantable bioactive agent that replaces defect bone tissues and can serve, also, as substrate for binding of cations, for provide bioactivity and biological interactions or could be used as drug deliveries.

Scopul acestui studiu este de a evalua dacă particulele de fosfat de calciu pot fi utilizate ca substrat pentru diferiți cationi. Cercetări recente în domeniul nanotehnologiei arată că dimensiunile nanometrice ale particulelor pot juca un rol important în ingineria țesuturilor, în medicină sau în utilizarea lor ca substraturi de eliberare controlată a medicamentelor. Nanopulberi de fosfat de calciu au fost obținute prin tehnica precipitării din $(NH_4)_2HPO_4$ 0.3 M şi $Ca(NO_3)_2{\cdot}4H_2O$ 0.5 M iar hibrizii fosfat de calciu – ioni de cupru au fost obținuti prin aceeaşi metodă umedă, la rece, în prezența hidroxidului de amoniu. Probele sinterizate au fost analizate prin difractometrie de raze X, spectroscopie în infraroşu cu transformata Fourier (FT-IR), energy dispersive X-ray analysis (EDAX) iar micrografiile au fost realizate prin microscopie electronică de baleiaj (SEM). Fosfații de calciu sunt utilizați pe scară largă ca agenți implantabili bioactivi care înlocuiesc țesuturi osoase defecte şi pot servi, de asemenea, ca substrat pentru legarea cationilor, pentru a oferi bioactivitate şi interacții biologice sau pot fi utilizați ca sistem de eliberare controlată a medicamentelor.

Keywords: *copper ions, calcium phosphates, hydroxiapatite, nanoparticle*

1. Introduction

The nanoparticles (particles smaller than 100 nm) are studied by many researchers due to their applications in various fields such as: medicine, electronics, optics, due to chemical and physical properties that they possess, properties that differ with as their size decreases. Nanoparticles on the basis of calcium phosphate, it can be use, due to their excellent biocompatibility properties, as the base for substitution with other atoms, which in their turn can bind a variety of organic and inorganic components (hormones, cancer drug, an antibiotic, etc.) [1-11] to be taken to the sick organ (target) or may be used, due to the similarity with biological apatite for regeneration of bone and teeth [7,8]. The calcium phosphates are known able to promote new bone formation and have been already used for repair of periodontal defects, orthopedic and maxillofacial applications [12], also may have applications in many other medical fields [13, 14]. Copper is an essential element in the body, having different roles in its functioning: the presence of the Cu^{2+} ions can have effects on the proper functioning of elastin and collagen, contribute to the synthesis of phospholipids, has anti-inflammatory and anti-infective properties, is used to treat rheumatoid arthritis, cofactor for the enzyme systems and has a angiogenic potential on blood vessels, etc.[7].

* Autor corespondent/*Corresponding author,*
E-mail: rorogojan@yahoo.com

The substitution of ions in hydroxyapatite structure may make changes in its properties such as crystallinity, morphology leading to new uses of this material doped. The purpose of this study is to prepare the compound based on calcium phosphates and copper, due to the biocompatibility of the calcium phosphate and the multiple roles that copper has in the body.

2. Experimental

In this study, the nano-particles of calcium phosphate were prepared by cold co-precipitation, and the copper was added in the form of sulfate in a percentage of 1%and 3%.

It were synthesized nanopowders from materials of purity pa: $(NH_4)_2HPO_4$ (Sigma-Aldrich) (0.3 M), $Ca(NO_3)_2{\cdot}4H_2O$ (Sigma-Aldrich) (0.5M), $CuSO_4{\cdot}5H_2O$ (Consors) and NH_4OH (Sigma-Aldrich) (0.3M). $(NH_4)_2HPO_4$ and NH_4OH was added dropwise, over reactants and they were mixed for 24 hours, according to the diagram presented in Figure 1.

After drying at 100°C the precipitates were termic treated at 500°C and 800°C, to facilitate entering copper.

The synthesized sample were analyzed by X-ray diffractometry using a SHIMATZU XRD 6000 diffractometer, with CuKα (λ=1.5405 Å) radiation, scanning speed 20/min., in 2θ = 10 - 55 grd range. Fourier transform infrared (FT-IR) spectrofotometry spectra were recorded with Bruker TENSOR 27 spectrometer, over the region of 500-4000 cm^{-1} at a scan rate of 4 cm^{-1}, for determine the possible changes in the bonds between the elements of powder obtained. The micrographs were obtained using a Quanta Inspect F scanning microscope and the elemental composition was determined by energy dispersive X-ray spectroscopy (EDAX).

3. Results and discussions

The dried and annealed powders were characterized by XRD, in order to obtain information about their crystallization tendency.

In Figure 2 it is observed that the undoped powder, thermally treated at 500°C/ 2 hours, are highlighted the specific interferences for monetite, $H(Ca(PO_4)$ and hydroxyapatite, $Ca_5(PO_4)_3(OH)$ (JCPDS file 01-080-7086). Increasing the sintering temperature at 800°C determine the mentioned phases, to turn into β-calcium phosphate, $Ca_3(PO4)_2$) (JCPDS file 04-008-8714) (Figure 3).

For samples doped with 1% and 3% of copper ion, at 500°C the single phase is hydroxyapatite, $Ca_5(PO_4)_3(OH)$ (JCPDS file 00-009-0432) as shown in Figure 4, that turns into calcium phosphate, $Ca_3(PO_4)_2$) (JCPDS file 04-006-2291) and tenorite, CuO (JCPDS file 04-007-0518) (Figure 5), at 800°C. Crystallographic parameters of the lattice of hydroxyapatite powder undoped obtained at 500°C (a (Å) 9.4500, b (Å) 9.4500, c (Å) 6.9000) and those of hydroxyapatite powder doped with copper ions (a (Å) 9.4180, b (Å) 9.4180, c (Å) 6.8840) are different, which may explain the increase in intensity of the characteristic peaks for doped hydroxyapatite. Note also that the characteristic peaks of Hap, were little changed with the addition of copper ions and the theta angle values was bigger. All of these can be attributed to the intercalation of Cu^{2+} ions in Hap lattice.

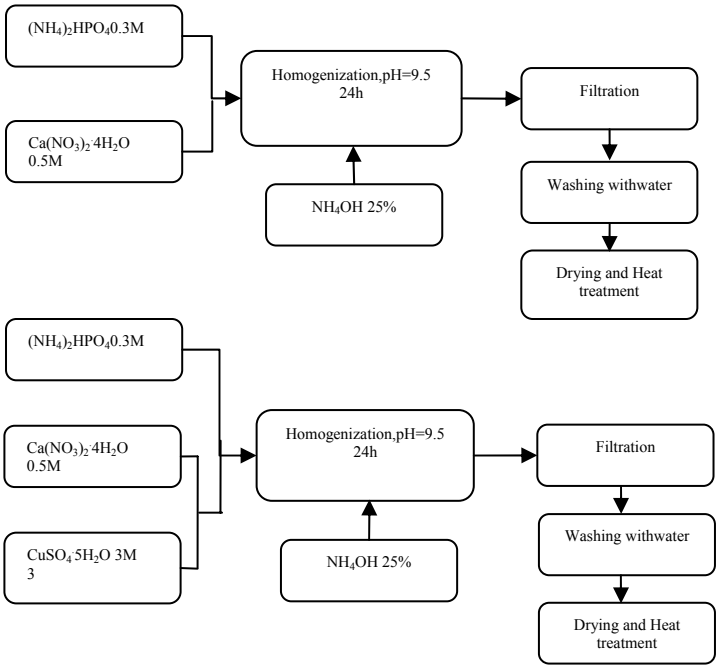


Fig. 1 - The steps for the preparation of calcium phosphate powder and calcium phosphate substituted with copper ions / *Etape în prepararea pulberilor de fosfați de calciu şi fosfați de calciu substituiți cu ioni de cupru.*

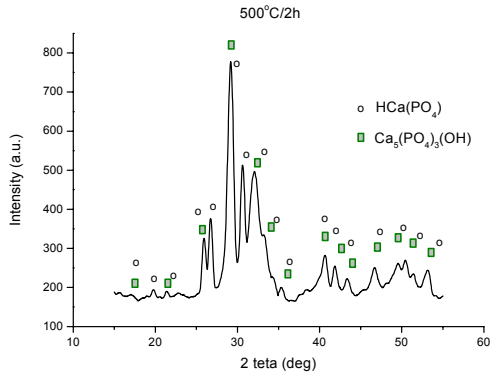


Fig. 2- Diffraction pattern for the calcium phosphate powders, annealed at 500°C/2h/ *Difractograma pulberilor de fosfat de calciu, tratate termic la 500°C/2h.*

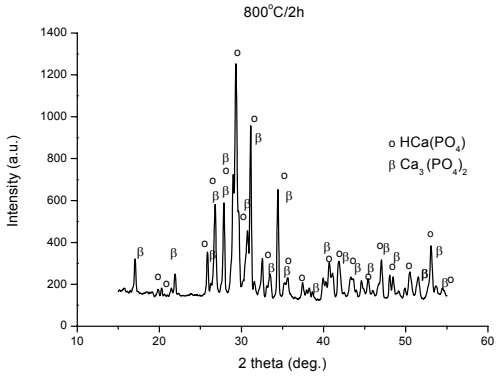


Fig. 3 - Diffraction pattern for the calcium phosphate powders, annealed at 800°C/2h/ *Difractograma pulberilor de fosfat de calciu, tratate termic la 800°C/2h.*

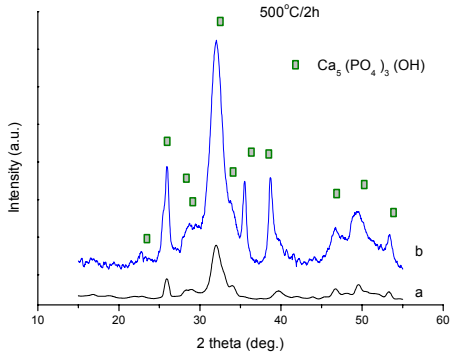


Fig. 4 - Diffraction pattern for the calcium phosphate powdersdoped with 1% (a) and 3%(b) copper ions annealed at 500°C/2h / *Difractograma pulberilor de fosfat de calciu dopate cu 1% (a) şi 3%(b) cupru, tratate termic la 500°C/2h.*

In Figures 4 and 5 are shown the diffraction peaks of the hydroxyapatite powder doped with copper at 1% and 3%, thermal treated at temperatures of 500°C or 800°C for 2 hours. It can be seen that for the sample containing 3% copper, at both temperatures, the peaks grow in intensity.

Figure 6 a, b shows the FT-IR absorbance spectra of the undoped and copper doped samples obtained by thermal treatment at the 500°C and

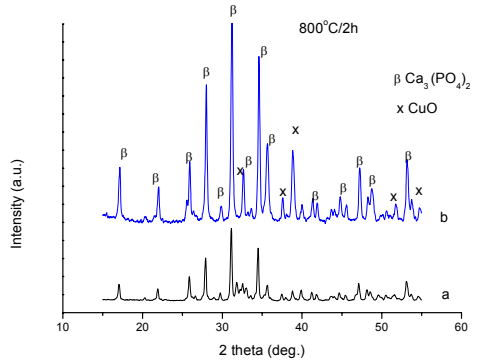


Fig. 5 - Diffraction pattern for the calcium phosphate powders doped with 1% (a) and 3% (b) copper ions annealed at 800°C/2h / *Difractograma pulberilor de fosfat de calciu dopate cu 1% (a) şi 3% (b) cupru, tratate termic la 800°C/2h.*

800°C. The –OH bending frequency is observed in the range 600 cm^{-1}- 700 cm^{-1} vibrational mode for hydroxyapatite. The spectra in the 900 cm^{-1}- 1250 cm^{-1} range are due to the vibration mode of the group PO_4^{3-} in both compounds.

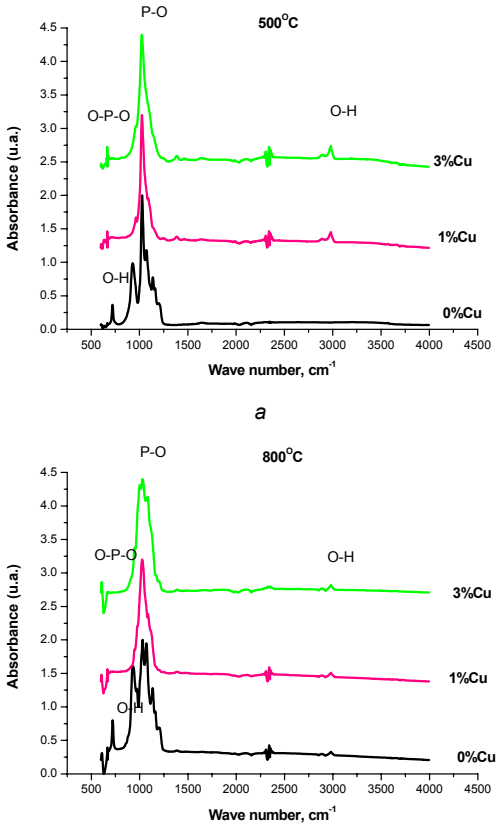


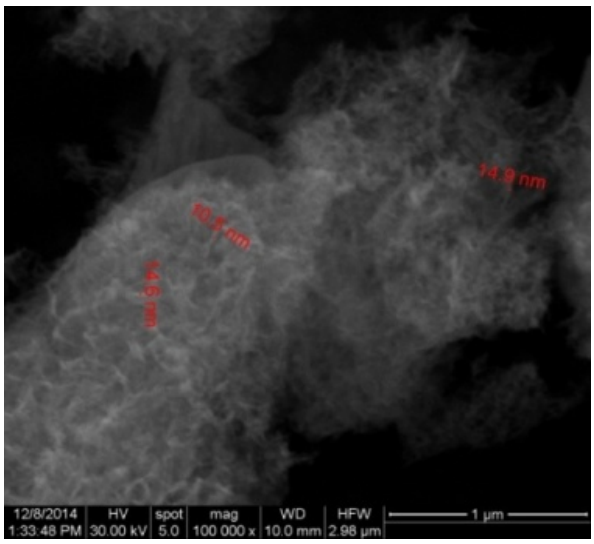
Fig. 6 - FT-IR spectra obtained on samples of calcium phosphate and copper-doped calcium phosphate (1% and 3% Cu^2) obtained by precipitation, annealed at 500°C (a) and 800 °C (b) /2h / *Spectrele FT-IR ale probelor de fosfați de calciu și fosfați de calciu dopate cu cupru (1% and 3%Cu^{2+}) obținute prin coprecipitare, tratate termic la 500 °C (a) şi 800°C (b) /2h.*

Note that the addition of Cu and increased concentration makes the characteristic band of the group P-O become sharper.

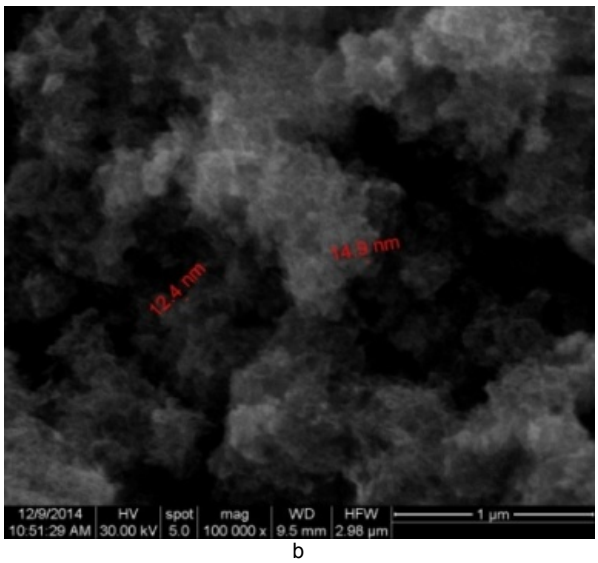
The presence of these copper ions lead to the disappearance of the peaks of the bands of hydroxyapatite in the range 800 cm^{-1}- 1300 cm^{-1}. With the increasing concentration of copper is an increase in the intensity of the OH band from about 3000 cm^{-1}. In addition, it decreases drastically with increasing temperature heat treatment. It was noted an increase in intensity of the band - OH, from around 700 cm $^{-1}$ and P-O band from around 800 cm^{-1} for the powder undoped, with increasing temperature from 500 C to 800 C, due to higher crystallinity thereof.It was no It can be also evidence interleaving Hap network. These experimental data are in agreement with data obtained by XRD results and by other researchers [6, 9 - 11].

SEM images (Figures 7, 8) of the powders obtained shows that they are clustered composed of very fine particles with nanometric dimensions up to 100 nm. It is observed that at the increase the heat treatment temperature, from 500°C to 800°C, these powders change shape, become rounded and their size increases. In the presence of copper, the nanoparticles dimensions are smaller than the undoped calcium phosphate powder at temperature of 800°C. This particulate morphology show that calcium phosphate nanoparticles were influenced by the presence of copper, especially the concentration of 3%.

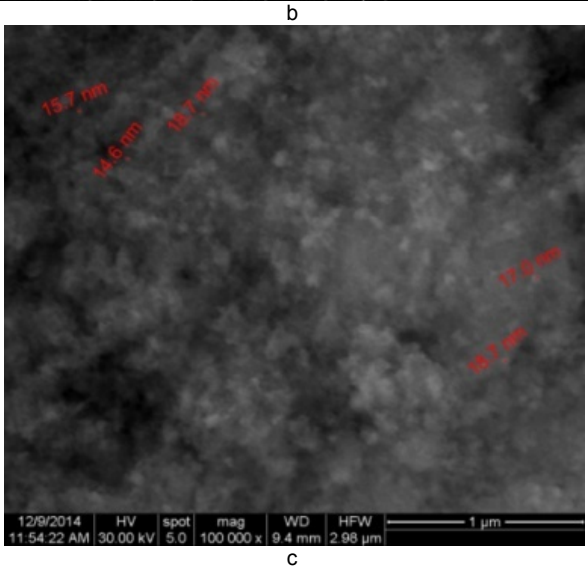
EDAX spectra (Figures 9, 10 and 11) reveal the presence of components of the compound formed after heat treatments at temperatures of 500°C and 800°C; indicate the presence calcium, phosphorus and oxygen, the chemical elements that make up calcium phosphates and copper presence and the relationships between these elements (11).



a

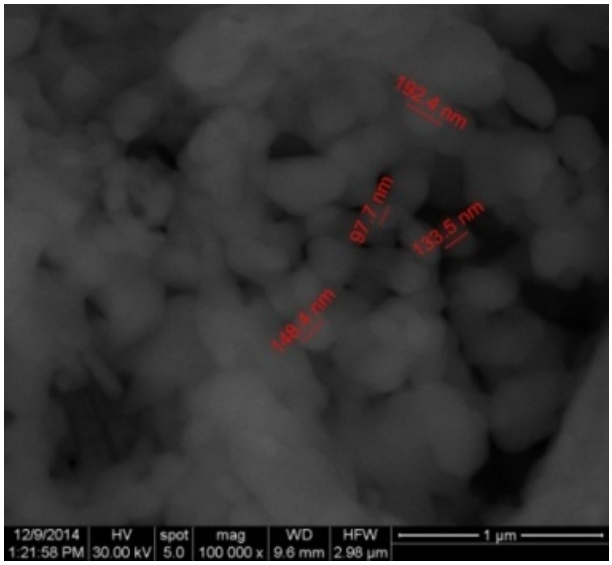


b

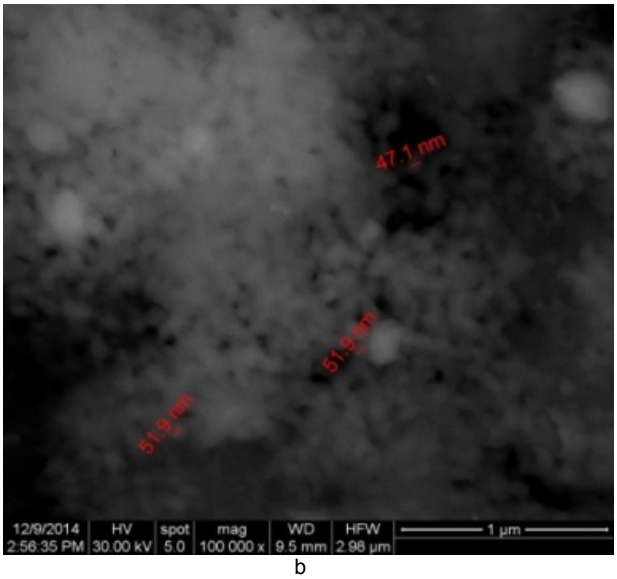


c

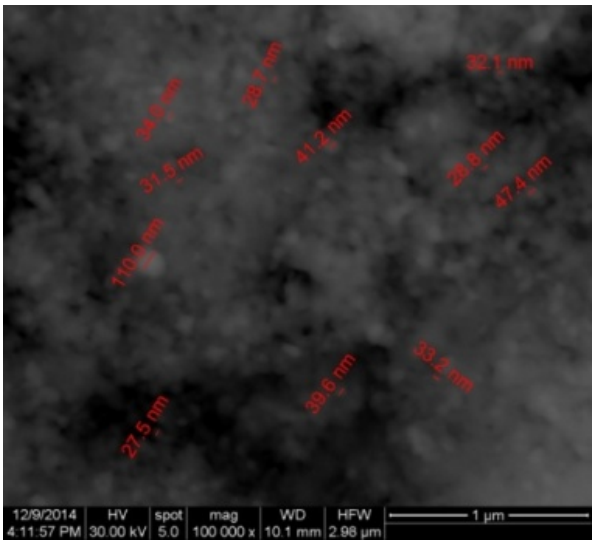
Fig. 7 - SEM images obtained on calcium phosphates(a) and calcium phosphate doped (1,3%Cu^{2+} (b,c)), annealed at 500 ^{o}C /2h; (a, b, c – x 100 000)/ *Imagini SEM pentru pulberile de fosfați de calciu (a) şi fosfatul de calciu dopat cu (1,3% Cu^{2+} (b,c)), tratate termic la 500 ^{o}C /2h; (a, b, c – x 100 000).*



a

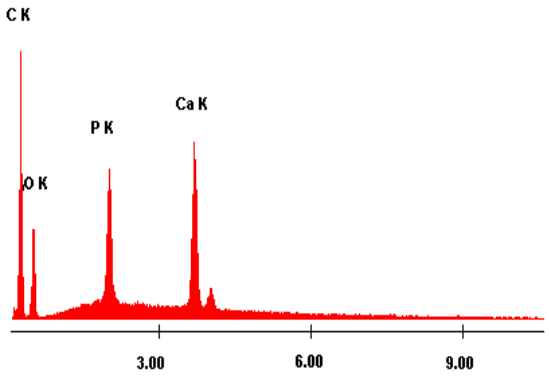


b

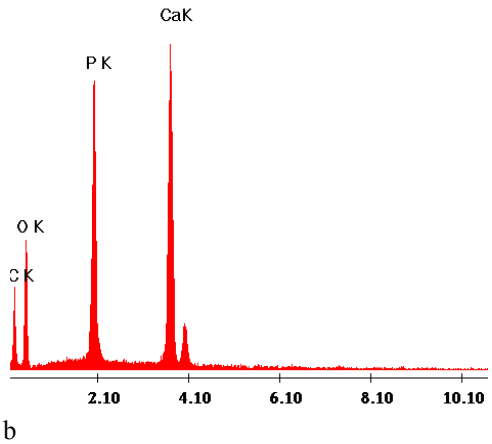


c

Fig. 8 - SEM images obtained on calcium phosphate(a) and calcium phosphate doped (1% and 3%Cu^{2+} (b,c)), annealed at 800 °C /2h; (a, b, c – x 100 000)/ *Imagini SEM pentru pulberile de fosfat de calciu (a) şi fosfatul de calciu dopat cu (1% şi 3%Cu^{2+} (b,c)), tratate termic la 800°C /2h; (a, b, c – x 100 000).*



a



b

Fig. 9 - EDAX spectra of calcium phosphate obtained by precipitation, annealed at 500 °C/2h (a) and 800 °C (b) /2h / *Spectrele EDAX ale fosfatului de calciou obținut prin coprecipitare la 500°C/2h (a) şi 800°C (b) /2h.*

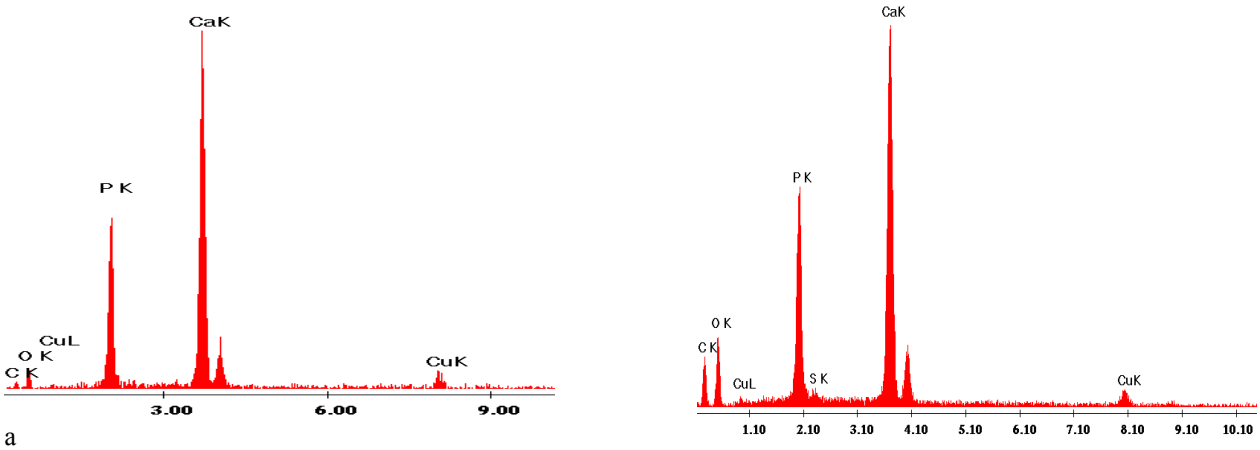


Fig. 10 - EDAX spectra obtained on samples of copper-doped calcium phosphate (1%Cu^{2}) obtained by precipitation, annealed at 500 °C/2h (a) and 800 °C (b) /2h / *Spectrele EDAX ale fosfatului de calciu dopat cu ioni de cupru 1% obținut prin coprecipitare la 500°C/2h (a) şi 800°C (b) /2h.*

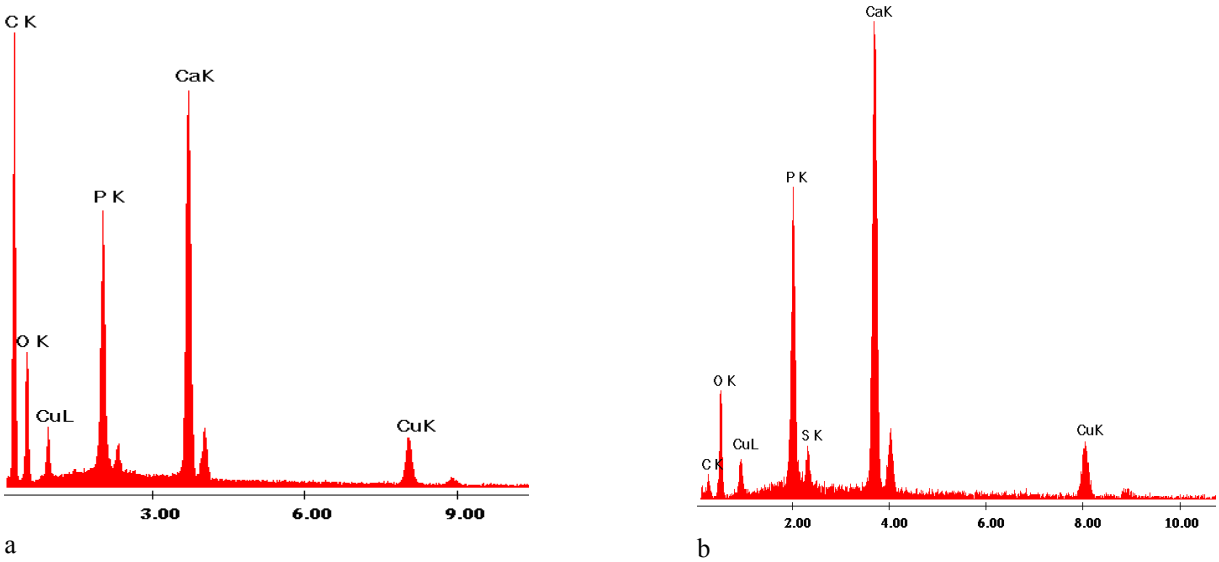


Fig. 11 - EDAX spectra obtained on samples of copper-doped calcium phosphate (3%Cu^{2}) obtained by precipitation, annealed at 500 °C/2h (a) and 800 °C (b) /2h / *Spectrele EDAX ale fosfatului de calciu dopat cu ioni de cupru 3% obținut prin coprecipitare la 500°C/2h (a) şi 800°C (b) /2h.*

4. Conclusions

In this study, we have prepared the materials based on calcium phosphate and copper ions, with potential uses in medicine. X-ray analysis indicates that the addition of 1% and 3% Cu^{2+} does not change the structure of calcium phosphate until at temperature of 800°C, when a mixture of calcium phosphate and tenorite was obtained. X-ray and FT- IR analyzes showed that copper was integrated in network hydroxyapatite. EDAX spectra indicate the presence of copper in powders obtained. SEM images show that the powders, thermal treated at 500°C and 800°C shows nanometric sized. Calcium phosphate nanoparticles may be potential carriers of copper ions and bridge to various substances as hormons, antibiotics or anticancer drugs.

Acknowledgements

The work has been funded by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/134398.

REFERENCES

1. M. Sunder, N. Babu Ramesh, V. Sunita Prem, K. Kumar Ram and T.S.Sampath, Biphasic calcium phosphates for antibiotic release, Trends in Biomaterials and Artificial Organs, 2005, **18** (2), 213.
2. M. Dirk, E. Duygu, F. Kirifl, D. Schneider Oliver, I. Thomas, R. Boccaccini Aldo, Spherical calcium phosphate nanoparticle fillers allow polymer processing of bone fixation devices with high bioactivity, Polymer Eng and Science May 2010, **50**, 952.
3. Sun Limin, C. Chow Laurence, A. Frukhtbeyn Stanislav, E. Bonevich John, Preparation and properties of nanoparticles of calcium phosphates with various Ca/P ratios, Journal of Research of the National Institute of Standards and Technology, 2010, **115**(4), 243.

4. S. Jabr Al-Sanabani, A. Ahmed Madfa and A. Fadhel Al-Sanabani, Application of Calcium Phosphate Materials in Dentistry, Review Article, International Journal of Biomaterials, 2013, **2013**, Article ID 876132.
5. S.M. Sagar, MD, D. Yance, MH and R.K. Wong, MD, Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer—Part 1, Curr Oncol. Feb. 2006 , **13**(1), 14.
6. X. Dong-Qin, W. Dong-Wei, R. Jun-Chen, D. Ke, Y. Ning, Lu Xiong, Z. Xiao-Tong and W. Jie, Synthesis and Characterization of Copper-substituted Hydroxyapatite Micrspheres, Journal of Inorganic Materials , 2014, **29** (7), 769.
7. X. Cheng, Hong L., L. Wei, C. Fa-Ming, Y. Min Zhao, The use of calcium phosphate-based biomaterials in implant dentistry, JMS, Materials in Medicine, 12/2011, **23**(3), 853.
8. M. V. Regí, and J. M. González-Calbet, Calcium phosphates as substitution of bone tissues, Progress in Solid State Chemistry, 2004, **32** (1–2), 1.
9. V. Stanic, S. Dimitrijevi, J. Antic Stankovic, M. Mitric, B. Jokic, I. B. Plecas and S. Racevic, Synthesis, characterization and antimicrobial activity of copper and zinc doped hydroxyapatite nanopowders. Appl Surf Sci, Applied Surface Science, 2010, **256**, 6083.
10. E. P. Domashevskaya, A. A. Al-Zubadi, D. L. Goloshchapov, N. A. Rumyantseva, and P. V. Seredin, Structure and composition of metal-substituted calcium-deficient hydroxyapatite, 2014, **9**(6), 1128.
11. F. E. Imrie, J. M.S. Skakle and I. R. Gibson, Preparation of Copper-Doped Hydroxyapatite with Varying x in the Composition $Ca_{10}(PO_4)_6Cu_xO_yH_z$, Bioceram. Dev. Appl., 2013.
12. C. Tardei, M. Spataru, F. M. Albu, Ş. Stoleriu and A. Ioncea, Prepararea şi caracterizarea microsferelor ceramice poroase pe bază de fosfat tricalcic , Romanian Journal of materials, 2013, 43 (1), 41.
13. E. Dinu, M. Bîrsan, C. Ghiţulică, G. Voicu, and E. Andronescu, Synthesis and characterization of hydroxyapatite obtained by sol–gel method, Romanian Journal of Materials, 2013, **43**(1), 55.
14. Hydroxyapatite (HAp) for Biomedical Application, edited by Michael Mucalo, Woodhead Publishing.

****

MANIFESTĂRI ŞTIINŢIFICE / SCIENTIFIC EVENTS

Al XII-lea SIMPOZION NAŢIONAL - PULBERI CERAMICE, Bucureşti, 20.11.2015

organizat de

SOCIETATEA ROMÂNĂ DE CERAMICĂ

INSTITUTUL DE CHIMIE FIZICĂ AL ACADEMIEI ROMÂNE

UNIVERSITATEA POLITEHNICA BUCUREŞTI

Acest simpozion, ajuns la a XII-a ediţie, constituie un mod direct şi eficient de informare a specialiştilor asupra principalelor noutăţi în acest domeniu.

Au fost invitaţi să participe toţi specialiştii care au preocupări în domeniul obţinerii sau utilizării pulberilor pentru producţia de materiale ceramice.

În acest an, s-a susţinut o lucrare invitată şi alte 15 lucrări ale unor cadre didactice din cadrul Universităţii POLITEHNICA Bucureşti, lucrări prezentate de câţiva studenţi şi masteranzi ai Facultăţii de Chimie Aplicată şi Ştiinţa Materialelor, precum şi de la Facultatea de Ştiinţa şi Ingineria Materialelor, lucrări ale multor cercetători din diverse instituţii de profil: Institutul de Chimie Fizică ”Ilie Murgulescu” al Academiei Române, Institul de Cercetări Metalurgice (ICEM), Institutul Naţional de Cercetare Dezoltare pentru Fizica Materialelor (INCDFM), Institutul Naţional de Cecetare Dezvoltare pentru Inginerie Electrică (INCDIE ICPE-CA), Institutul Naţional de Fizica Laserilor, Plasmei şi Radiaţiei (INFLPR), Institutul Naţional pentru Metale Neferoase şi Rare (IMNR), Centrul de Cercetare Ştiinţifică pentru Apărare şi Ecologie, SC Ceprocim SA.

This symposium, now at its twelfth edition, is a direct and effective way to inform the specialists on key news in this field.

All the specialists with concerns in the field of obtain or use powders for the production of ceramics were invited to attend.

This year it was held a paper invited and 15 other works of academics from the University POLITEHNICA of Bucharest, papers submitted by some students from the Faculty of Applied Chemistry and Materials Science and the Faculty of Materials Science and Engineering, works of many researchers from various specialized institutions: Institute of Physical Chemistry "Ilie Murgulescu" of the Romanian Academy, Institute for Metallurgical Research (ICEM), the National Institute for Research and Development of Materials Physics (INCDFM), the National Institute for Research and Development in Electrical Engineering (ICPE-CA), the National Institute for Laser, Plasma and Radiation (INFLPR), the National Institute for Nonferrous and Rare Matels (IMNR), the Center for Defence and Ecology Scientific Research, SC CEPROCIM SA.

Material realizat de Ş.l. dr. Ing. Alina Melinescu
Co-organizator Pulberi Ceramice 2015