NAP-2013

Creating Properties of Eu³⁺-doped Calcium Hydroxyapatite as Biocompatible Fluorescent Probes

E.V. Zubar, N.P. Efryushina, V.P. Dotsenko

A.V. Bogatsky Physico-Chemical Institute, National Academy of Sciences of Ukraine, 86, Lustdorfskaya Doroga, 65080 Odessa, Ukraine

The luminescent properties of Eu^{3+}-doped calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) nanopowders prepared by the chemical precipitation and sol-gel method were studied. It was shown the possibility of improving the properties of HAp as a fluorescent carrier of biological molecules and drugs by control of the defect formation.

Keywords: Calcium hydroxyapatite, Synthesis, Nanopowders, Europium, Luminescence.

1. INTRODUCTION

Fluorescence analysis is one of the most important techniques in biology and biomedicine due to its non-invasive character and high sensitivity. In recent years, lanthanide-doped nanocrystals have been developed as a new class of luminescent optical labels that have become promising alternatives to organic fluorophores and quantum dots for applications in biological assays and medical diagnostics [1]. In this context, calcium hydroxyapatite based nanophosphors might be good candidates, since this material is highly biocompatible, biodegradable, stable in biological buffer solutions [2]. It should be noted that Eu^{3+} is less sensitive than some other lanthanide ions to the decrease in luminescence intensity in the presence of water molecules or high vibration energy groups such as C-H or C-O [3]. So Eu^{3+}-doped calcium hydroxyapatite can be employed as a biological probe. HAp belongs to the hexagonal system with the $P6_3/m$ space group. In this lattice, two unequivalent calcium sites are present: Ca(I) with C_3 symmetry is surrounded by nine oxygen atoms, and Ca(II) with C_S symmetry is coordinated to six oxygen atoms and one OH^- group [4]. It is accepted that the disordering of OH^- ions in c-axis columns causes a structural complexity of HAp. Due to the different structure of coordination polyhedra of Ca(I) and Ca(II) Eu^{3+} ions at these positions exhibit different spectral properties. Despite numerous publications devoted to study of the character of isomorphic substitution of alkaline earth atoms in this structure by lanthanide ions, there is no single opinion on the matter. Also information about the ability to manage the process of distributing Eu^{3+} ions on the positions of Ca in the HAp by the formation of the charge compensation defects is strongly limited. The aim of this work is to study the influence of the preparation route and formation of different types of defects on the distribution and luminescent properties of Eu^{3+} ions in HAp.

2. EXPERIMENTAL

Samples of nominal formula $Ca_{10(1-x)}Eu_{10x}(PO_4)_6(OH)_2$ ($x = 0.01$) used in the present work were prepared by two different methods. The samples were checked by X-ray diffraction (XRD) using Cu K_α radiation. No impurity phases were detected in the XRD patterns. Morphological investigations were carried out by scanning electron microscopy (SEM) on JEOL JSM 6390LV electron microscope. The emission and excitation spectra in UV-visible region were recorded at room temperature using a Fluorolog Fl-3 (Horiba Jobin Yvon) spectrofluorometer equipped with a xenon lamp. The Fourier-transformed infrared absorption spectra were recorded using a Bruker Vertex 70 spectrometer.

3. RESULTS AND DISCUSSION

In this work, Eu^{3+}-doped $Ca_{10}(PO_4)_6(OH)_2$ with different particle sizes have been synthesized by chemical precipitation and sol-gel method. To prepare calcium phosphate nanoparticles by a precipitation process, an aqueous solution of $(NH_4)_2HPO_4$ was slowly added to an aqueous solution of calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot 4H_2O$ upon continuous stirring. The pH of the mixture was adjusted to 10 by adding NH_4OH and the reaction temperature was maintained at 60 °C. The resulting precipitate was aged for 0.5 h under stirring, and then filtered, washed with distilled water and dried at 105 °C. The preparation of HAp powders by the sol-gel method involved the use of aqueous solution of calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot 4H_2O$ and alcohol solution of trimethyl phosphate $(CH_3O)_3PO$ as the precursors. Some amount of citric acid (as a chelating agent) was added into aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$. The solutions were mixed at room temperature and kept at pH 7.5 for 48 h. The obtained gel was dried at 190 °C for 2 h, and then fired at 600 °C for 1 h. In the both cases europium nitrate was added to the calcium nitrate solutions to obtain molar ratio of Eu / (Ca + Eu) = 0.01. The luminescent properties of the samples were analyzed in the as prepared-state and after annealing at 800 °C for 2-3 h in air. The average sizes of crystallites (d) of the as-prepared polycrystalline materials were calculated from XRD-patterns by the Scherrer equation [5]. The reflections from the (300) and (002) planes were used. The obtained values

varied from 34 to 70 nm, in agreement with the results reported previously for HAp nanopowders prepared either by chemical precipitation method [6, 7] or by the sol-gel process [8]. A typical SEM image of HAp prepared by the sol-gel method is presented in Fig. 1. It is seen that the material is composed of crystallites with an irregular form, and which exhibit a high degree of aggregation.
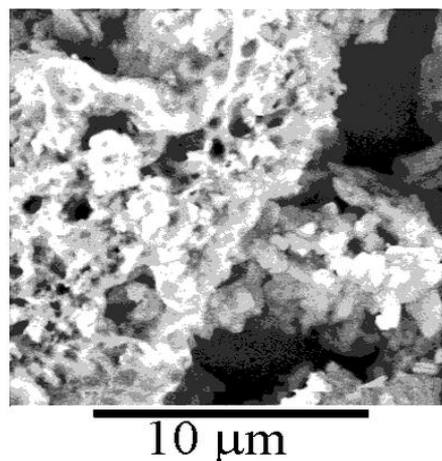

Fig. 1 – SEM photograph of $Ca_{10(1-x)}Eu_{10x}(PO_4)_6(OH)_2$ ($x = 0.01$) synthesized by the sol-gel method

Fig. 2 shows FTIR absorption spectra of HAp prepared by precipitation from aqueous solution at 60 °C, precipitation followed by calcination at 1000 °C and the sol-gel method. Regardless of the synthesis method the spectra contain the stretching and bending vibration bands of OH^- groups at 3572 and 631 cm^{-1}, asymmeric (v_3) and symmetric (v_1) stretching vibration bands of the isolated PO_4^{3-} groups at 1090, 1039 and 960 cm^{-1}. The bending vibrations (v_4, v_2) of the PO_4^{3-} groups cause the bands at 601, 568 cm^{-1} and 472 and 435 cm^{-1}, respectively. In addition to these features, the FTIR spectrum of HAp prepared by the sol-gel method contains bands at 1457, 1416 and 873 cm^{-1}, which must be attributed to the stretching and bending vibrations of the CO_3^{2-} anions occupying both the OH^- and PO_4^{3-} positions [7-9]. The presence of these bands indicates that the sol-gel method causes the formation of carbonate substituted calcium hydroxyapatite of mixed AB-type. Since these bands are absent in the FTIR absorption spectrum of HAp prepared by the chemical precipitation method, one can expect that the incorporation of the CO_3^{2-} groups into the crystal lattice is a consequence of the use of citric acid as a reagent.

Fig. 3 presents the luminescence spectra of Eu^{3+} ions in $Ca_{10}(PO_4)_6(OH)_2$ prepared by precipitation from aqueous solution at 60 °C, precipitation followed by calcination at 1000 °C and the sol-gel method at 600 °C. A comparison of the luminescence spectra shown in Fig. 3 indicates that the distribution of Eu^{3+} ions at Ca positions in the structure of $Ca_{10}(PO_4)_6(OH)_2$ depends strongly on the synthesis conditions. It is seen that the luminescence spectrum Eu^{3+} ions in HAp, obtained by precipitation from aqueous solution at 60 °C, consists of three main groups of bands in the 580-600, 608-630, 685-715 nm regions. It is clear that this spectrum is due to $^5D_0 \rightarrow {}^7F_J$ ($j = 1, 2, 4$) transitions in Eu^{3+} ions. It

is well known that the spectrum of luminescence of Eu^{3+} in Ca(II) positions in the apatite structure is characterized by an extremely intense band at 574 nm associated with $^5D_0 \rightarrow {}^7F_0$ transition [10]. According to Chen and Liu [10], high energy and a relatively high intensity of $^5D_0 \rightarrow {}^7F_0$ transition of Eu^{3+} ions in inorganic crystals are due to the low point symmetry of the lattice sites occupied by Eu^{3+}. It is assumed that in the case of $Ca_{10}(PO_4)_6(OH)_2$ and BaFCl this effect is caused by compensation of the excess positive charge of Eu^{3+} ions in Ca^{2+}/Ba^{2+} positions due to substitution $O^{2-} \rightarrow OH^-$ or $O^{2-} \rightarrow F^-$ [10].

In the luminescence spectrum of Eu^{3+} ions in the HAp precipitated from aqueous solution, the band corresponding to $^5D_0 \rightarrow {}^7F_0$ transition is absent (Fig. 3(1)). This means that the Eu^{3+} ions predominantly occupy Ca(I) positions. Fig. 3 shows that the high temperature calcining of the sample not only leads to a significant increase in the luminescence intensity, but is accompanied by changes in the spectral composition of the emission. In particular, the fine structure appears in the spectrum. Moreover, very intense bands at 574 and 627 nm are present.
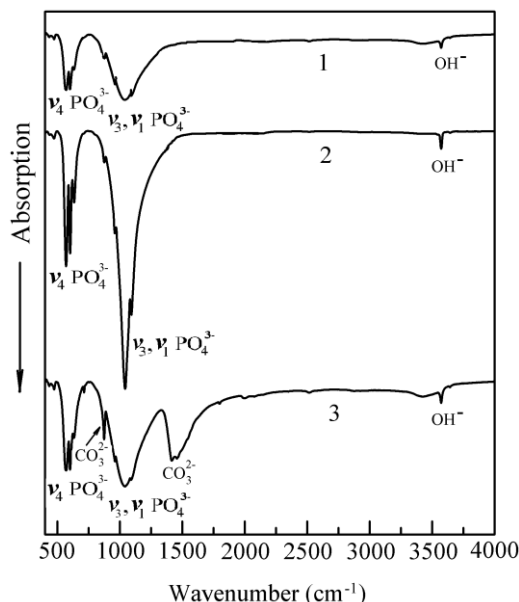

Fig 2 – FTIR absorption spectra of HAp prepared by precipitation from aqueous solution at 60 °C (1), precipitation followed by calcination at 1000 °C (2) and the sol-gel method (3)

From a comparison of the Eu^{3+} luminescence spectra (Fig. 3) for the sample HAp precipitated from an aqueous solution and the product of its high-temperature firing one can conclude that in the second case the ions Eu^{3+} are in both Ca(I) and Ca(II) positions. Actually, apart from a number of the $^5D_0 \rightarrow {}^7F_2$ emission bands of Eu^{3+} in Ca(I) position, a band with a maximum at 574 nm due to the transition $^5D_0 \rightarrow {}^7F_0$ of Eu^{3+} in the Ca(II) positions is present in the spectrum of the product of high temperature firing. Note also that the bands corresponding to the $^5D_0 \rightarrow {}^7F_0$ and $^5D_0 \rightarrow {}^7F_2$ transitions are of comparable intensity.

The fact of increasing the integrated intensity of luminescence of Eu^{3+} is attributed to the increase in the solubility of europium oxide in the HAp and an
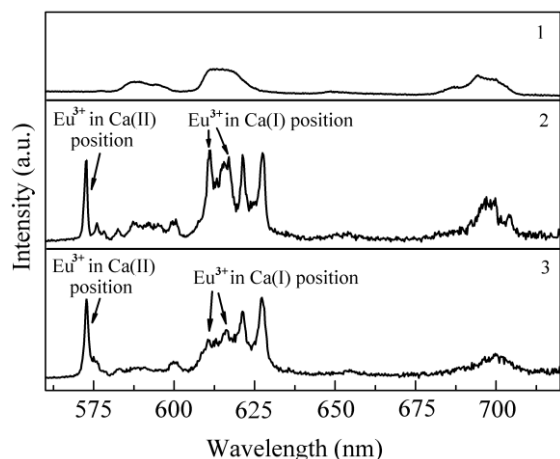
Fig. 3 – The emission spectra of $Ca_{10(1-x)}Eu_{10x}(PO_4)_6(OH)_2$ ($x = 0.01$) prepared by precipitation from aqueous solution at 60 °C (1), precipitation followed by calcination at 1000 °C (2) and the sol-gel method (3). The spectra were recorded at 293 K upon excitation at 396 nm

increase of its crystallinity due to the high temperature firing. It is believed that in the precipitated from aqueous solution Eu^{3+}-doped HAp only restricted amount of the europium ions introduced into the crystal lattice. As mentioned above, the Eu^{3+} ions in the sample HAp precipitated from aqueous solution at 60 °C, preferably occupy Ca(I) positions. High-temperature annealing is accompanied by an increase in the solubility of Eu$_2$O$_3$ and the changing character of the distribution of Eu^{3+} ions in the lattice. Karbowiak and Hubert [11] have studied the luminescent properties Eu^{3+} ions in fluorapatite calcium Ca$_{10}$(PO$_4$)$_6$F$_2$ and found out that in the apatite obtained by solid state reaction, the ions Eu^{3+} predominantly occupy Ca(II) positions, whereas in apatite prepared by precipitation from aqueous solution - position Ca(I). In the authors opinion, subsequent annealing at 900 ºC of the sample precipitated from aqueous solution, resulted in the Eu^{3+} ion migration from the position Ca(I) to position Ca(II) [11]. According to Ref. [11], synthesis condition influence strongly on site preference of Eu^{3+} in HAp. Our data for calcium hydroxyapatite are in agreement with the results presented by the authors [11] for the Ca$_{10}$(PO$_4$)$_6$F$_2$, but our interpretation of the experimental results is different. The changing character of the distribution of Eu^{3+} ions at the positions of Ca due to high temperature firing we relate not so much to the migration of ions Eu^{3+}, but mainly with the introduction of an additional amount of lanthanide ions to the crystal lattice of HAp, i.e. with the increase in concentration of lanthanide ions.

Fig. 3(3) shows that the luminescence spectrum of Eu^{3+} ions in Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ prepared by the sol-gel method is generally similar to that of the product of high temperature calcination of the sample precipitated from aqueous solution. However, it can be noted slightly lower relative intensity of the bands at 612 and 616 nm which are most characteristic bands of Eu^{3+} ions in the Ca(I) position. The probable reason for this is the carbonate substitution in the structure of Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ prepared by sol-gel method. As noted above, the sol-gel method results to the formation of carbonate substituted calcium hydroxyapatite of mixed

AB-type, i.e. CO$_3$$^{2-}$ substitute OH$^-$ group (A-type) as well as PO$_4$$^{3-}$ group (B-type). Substitution of B-type (CO$_3$$^{2-}$ → PO$_4$$^{3-}$) leads to appearance of the excess positive charge which can be compensated by calcium vacancies. Also, calcium vacancies formation is the most likely mechanism for compensation of excess positive charge of Eu^{3+} ions in the Ca(I) position. However, the ability of the HAp structure to generate the calcium vacancies is limited and therefore the number of ions Eu^{3+} holding Ca(I) position in B-type carbonate substituted HAp is less than in pure HAp. On the other hand, the CO$_3$$^{2-}$ → OH$^-$ substitution can be considered as an independent mechanism to compensate the excess positive charge of Eu^{3+} in Ca(II) positions. Analyzing the spectrum in Fig. 3(3), we conclude that the compensation of the excess positive charge due to CO$_3$$^{2-}$ groups in OH$^-$ positions is not locally. Otherwise, the formation of $(Eu_{Ca(II)} \cdot CO_3 \, _{OH})^x$ associates would lead to the appearance of additional bands in the spectra. Thus, the interaction of defects in carbonate substituted calcium hydroxyapatite of mixed AB-type obtained by the sol-gel method promote occupancy of Ca(II) positions by Eu^{3+} ions.

The excitation spectra for the emissions of Eu^{3+} in Ca(I) and Ca(II) positions are markedly different. The spectrum for Eu^{3+} ions in Ca(I) positions with $N = 9$ ($\lambda_{ex} = 612$ nm) is typical for Eu^{3+} in inorganic compounds (Fig. 4a). It is dominated by an intense band with a maximum at 243 nm, which is caused by charge transfer from the 2p orbitals of O^{2-} to empty Eu^{3+} 4f^6 orbitals. As can be seen from Fig. 4, the excitation spectrum for the Eu^{3+} ions in Ca(II) position with $N = 7$ ($\lambda_{ex} = 574$ nm) has a fundamentally different character. It is characterized by a relatively intense charge transfer band (CTB) 2p O → 4f^6 Eu^{3+}, the maximum of which ($\lambda_{max} = 312$ nm) is substantially shifted to longer wavelengths compared with other alkaline-earth oxide compounds. Also, there is an intense band at 459 nm, corresponding to $^7F_0 \to {}^5D_2$ transition in Eu^{3+} ions (Fig. 4b). One can expect that the long-wavelength position of the CTB ($\lambda_{max} = 312$ nm) of Eu^{3+} ions in Ca(II) position is due to a high degree of covalency of the Eu-ligand bond caused by formation of the neutral $(Eu_{Ca(II)} \cdot O_{OH})^x$ associates.
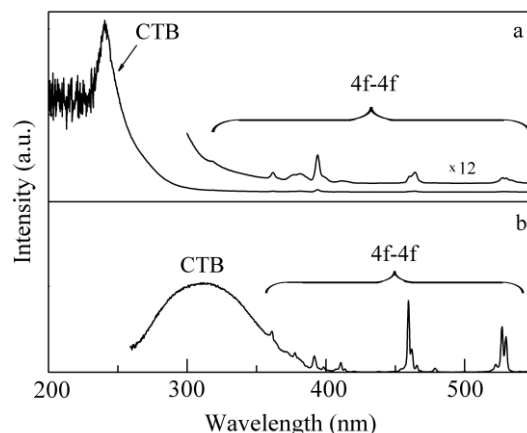

Fig. 4 – Exitation spectra for the Eu^{3+} emissions in product of high temperature calcination of the sample HAp precipitated from aqueous solution: a − $\lambda_{em} = 612$ nm, b − $\lambda_{em} = 574$ nm

Due to the availability of effective excitation sources in the 300-500 nm region HAp in which Eu^{3+} ions are preferably in Ca(II) positions should be used as a fluorescent carrier of biological molecules and drugs. As follows from the spectra shown in Fig. 3 stabilizing factor Eu^{3+} ions mainly in the Ca(II) position is the introduction of CO_3^{2-} groups in the structure of the HAp.

4. CONCLUSIONS

Eu^{3+}-doped $Ca_{10}(PO_4)_6(OH)_2$ with different particle sizes have been synthesized by the chemical precipitation and sol-gel method. The site preference of Eu^{3+} ions for the two calcium sites Ca(I), Ca(II) in the apatite structure was studied by means of luminescent spectroscopy. The effect of synthesis conditions and Eu^{3+}-concentration on the distribution of lanthanide ions on Ca-positions in the structure of $Ca_{10}(PO_4)_6(OH)_2$ was studied. It was found that at relatively low concentrations ($\ll 1$ at. %) Ln^{3+} ions occupy mainly the Ca(I) positions. With increasing concentration of lanthanide ions their fraction in the Ca(II) position increases significantly.

It was demonstrated the prospects for improving the composition and properties of the Eu^{3+}-doped HAp as a fluorescent carrier of biomolecules and drugs by control the defects formation. In particular, it is shown that the carbonate substitution of mixed AB-type influences on the distribution of Eu^{3+} ions at calcium positions and promotes to the stabilization of Eu^{3+} ions in Ca (II) positions.

REFERENCES

1. F. Wang, X. Liu, *Chem. Soc. Rev.* **38**, 976 (2009).
2. J.Y. Chane-Ching, A. Lebugle, I. Rousselot, A. Pourpoint, F. Pelle, *J. Mater. Chem.* **17**, 2904 (2007).
3. A. Doat, F. Pelle, N. Gardant, A. Lebugle, *J. Solid State Chem.* **177**, 1179 (2004).
4. J.M. Hughes, M. Cameron, A. Mariano, *Am. Mineral.* **76**, 1165 (1991).
5. H.P. Klug, L.E. Alexander, *X-ray diffraction procedures for polycrystalline and amorphous materials* (New York: Willey-Interscience: 1974).
6. S.P. Mondejar, A. Kovtun, M. Epple, *J. Mater. Chem.* **17**, 4153 (2007).
7. L.M. Rodriguez-Lorenzo, M. Vallet-Regi, *Chem. Mater.* **12**, 2460 (2000).
8. C.L. Chu, P.H. Lin, S. Dong, D.Y. Guo, *J. Mater. Sci. Lett.* **21**, 1793 (2002).
9. S. Angelov, R. Stoyanova, R. Dafinova, K. Kabasanov, *J. Phys. Chem. Solids* **47**, 409 (1986).
10. X.Y. Chen, G.K. Liu, *J. Solid State Chem.* **178**, 419 (2005).
11. M. Karbowiak, S. Hubert, *J. Alloy. Compd.* **302**, 87 (2000).