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MEETING POINT OF THE SCIENCE AND PRACTICE IN THE FIELDS OF  
CORROSION, MATERIALS AND ENVIRONMENTAL PROTECTION

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*STECIŠTE NAUKE I PRAKSE U OBLASTIMA KOROZIJE,  
ZAŠTITE MATERIJALA I ŽIVOTNE SREDINE*

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# PROCEEDINGS

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# *KNJIGA RADOVA*

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# Chemocompatibility of fluorapatite-based antibacterial nanophosphorus prepared by precipitation method for biomedical applications

## *Hemokompatibilnost antibakterijskih nanofosfora na bazi fluorapatita pripremljenog metodom precipitacije za biomedicinsku primenu*

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### **Abstract**

*In Luminescent inorganic nanomaterials (nanophosphorus) based on fluorapatite (FAP) are attracting significant interest for biomedical, biotechnological and environmental applications. Luminescent nanopowders based on fluorapatite substituted with Pr<sup>3+</sup> and CO<sub>3</sub><sup>2-</sup> ions (PrCFAP) were obtained by a simple precipitation method. Previously published results have shown that nanopowders are monophasic and composed of nanospheres, with luminescence in violet color. Also, nanopowders exhibit antibacterial behavior against E. coli. In this study the results of chemocompatibility of antibacterial luminescent nanophosphorus obtained by precipitation for various biomedical and health applications are presented. Pure FAP showed a degree of hemolysis of 5% and can be considered chemocompatible. Doped nanopowders showed hemolysis up to about 10%. Slight hemolytic behavior of the tested PrCFAP nanopowders may be a consequence of mechanical stress of the cell in the interaction with nanoparticles, as well as the toxic effect of dissolved Pr<sup>3+</sup> and F<sup>-</sup> ions from the FAP lattice. The above results indicate that the antibacterial PrCFAP nanophosphorus may be future evaluated for different biomedical applications, such as agents for cells labeling, therapy and drug delivery.*

*Keywords: fluorapatite; chemocompatibility; luminescence; biomedical nanomaterials.*

### **Izvod**

*Luminescentni neorganski nanomaterijali (nanofosfor) na bazi fluorapatita (FAP) privlače značajno interesovanje za biomedicinske, biotehnoške i ekološke primene. Jednostavnom metodom precipitacije dobijeni su luminescentni nanomaterijali na bazi fluorapatita supstituisani Pr<sup>3+</sup> i CO<sub>3</sub><sup>2-</sup> jonima (PrCFAP). Ranije objavljeni rezultati su pokazali da su nanoprahovi monofazni i sastavljeni od nanosfera, sa luminiscencijom u ljubičastoj boji. Pored toga, nanoprahovi su pokazali i antibakterijsko ponašanje protiv E. coli. U ovoj studiji prikazani su rezultati hemokompatibilnosti antibakterijskih luminescentnih nanofosfora dobijenih precipitacijom za različite biomedicinske i zdravstvene primene. Čisti FAP je pokazao stepen hemolize od 5% i može se smatrati hemokompatibilnim. Dopirani nanoprahovi su pokazali hemolizu do oko 10%. Blago hemolitičko ponašanje ispitivanih PrCFAP nanoprahova može biti posledica mehaničkog naprezanja ćelije u interakciji sa nanočesticama, kao i toksičnog dejstva rastvorenih Pr<sup>3+</sup> i F<sup>-</sup> jona iz FAP rešetke. Gore navedeni rezultati ukazuju da antibakterijski PrCFAP nanofosfori u budućnosti mogu biti procenjeni za različite biomedicinske primene, kao što su agenti za obeležavanje ćelija, terapije i isporuku lekova.*

***Ključne reči:** fluorapatit; hemokompatibilnost; luminescencija; biomedicinski nanomaterijali.*

## Introduction

In the past few years, luminescent fluorapatite (FAP) based nanomaterials have attracted significant interest in medical applications such as nanoparticles for cancer labeling, bioimaging and therapies, antibacterial agents for bone repair and treatment, as well as light emitting diodes for phototherapy and sterilization [1–4].

Luminescent inorganic materials, phosphors, are crystalline structures that emit radiation in the visible and/or ultraviolet part of the spectrum after excitation by an external energy source in the form of electrons or photons [5]. Phosphors consist of a matrix, a chemical compound of the host, in which luminescent centers in the form of rare earth ions or transition metals are doped. The first phosphors were prepared based on binary compounds of sulfides and oxides, such as zinc sulfide (ZnS), cadmium sulfide (CdS), lead sulfide (PbS), zinc oxide (ZnO), cadmium oxide (CdO) or calcium tungstate ( $\text{CaWO}_4$ ) [6, 7]. However, the main disadvantage of these phosphors was the synthesis at high temperatures from toxic compounds, but also chemical instability and the emission of toxic gases during operation. In search of more chemically and thermally stable materials, new types of phosphorus based on ternary compounds, aluminates, silicates and phosphates have been developed, such as zinc aluminate ( $\text{ZnAl}_2\text{O}_4$ ), magnesium silicate ( $\text{MgSiO}_3$ ) and calcium phosphate ( $\text{Ca}_x(\text{PO}_4)_2$ ) [6, 7].

In particular, calcium phosphates have proven to be extremely chemically stable, non-toxic and as excellent matrices for doping rare earth ions and transition metals. In addition to these suitable chemical and structural properties, in the field of biomedicine, particularly interesting properties of calcium phosphate are expressed in biocompatibility, bioactivity, and osteoconductivity [8].

Due to the possibility of a wide range of applications, fluorescent materials based on fluorapatite have attracted a lot of attention from researchers in recent decades. Synthetic haloapatites, commercially called fluorapatite and chlorapatite, co-activated  $\text{Sn}^{3+}$  and  $\text{Mn}^{2+}$  were one of the first phosphors used in fluorescent lamps [8, 9]. The most important characteristics of apatite crystal structure is the possibility of the structure to withstand many ionic substitutions. The crystal structure of FAP have the strong crystal field, and it is a potentially important host for optically active trivalent rare earths ions, that allows luminescent transitions of electrons from the *f* orbitals [9]. Due to all these properties, FAP doped with rare earth ions has found application in UV lamps, LEDs for display and plasma TVs and lasers [9, 10, 11, 12].

Fluorapatite, together with hydroxyapatite (HAP), is used as a biomaterial for bone tissue repair because it has a similar structure as the inorganic component of bones and teeth in living organisms [13, 14]. In addition to this suitable structural, fluorapatite it also shows biocompatible, bioactive and antibacterial properties [14]. Compared to HAP, it has better physicochemical properties, such as high melting point, poor solubility in acids, and corrosion resistance [13]. FAP doped with rare earth and metal ions has therefore been tested in recent years as a material for use in biomedicine for bone engineering, biomarking and bioimaging [15, 16, 17, 18]. As FAP is similar to bone tissue, research on the interaction of FAP with radioactive radiation has also been conducted in recent years for use as a dosimeter in medicine and environmental protection [19].

Luminescent FAP nanoparticles doped with rare earth elements have been extensively studied in recent years as a potential multifunctional system for detection, imaging and therapy of cancer group of diseases [1-4]. In order to perfect these materials and make breakthroughs in high technologies, it is necessary to consider the correlations between crystal structure, substitution in structure and luminescence. Also, biocompatibility, chemocompatibility and antimicrobial activity of these materials are particularly important for biomedical applications.

In the previous study, PrCFAP nanopowders with luminescent and antibacterial properties were synthesized and characterized [1]. In this paper, the hemolytic properties of these materials were additionally tested, to examine the possibility of application for biomedical purposes, but also a wider range of health care applications.

## Materials and methods

### *Synthesis of FAP nanopowders*

Fabrication of FAP nanopowders were performed by the precipitation method according to the procedure described in the previous publication [1]. The precipitation of FAP nanomaterials consists of adding an anionic solution in drops to the cation solution. The atomic ratios  $[\text{Pr}/(\text{Pr}+\text{Ca})]\times 100\%$  were 0.1, 0.5 and 1%. The resulting suspensions, after maturing for 12 h at 25 °C, were filtered, washed and dried at 105 °C. The obtained materials were ground to a powder and the samples were labeled as FAP, PrCFAP0.1, PrCFAP0.5 and PrCFAP1.

### *In vitro hemolysis tests*

In order to determine the biocompatibility of nanomaterials that come into contact with blood, it is necessary to perform *in vitro* hemolysis tests. The hemolytic activity of fabricated nanophosphorus was investigated by the method described in the literature [20]. 100 mg of each sterile sample and 10 mL of sterile saline was added to the tubes. Fresh blood obtained from healthy human volunteers (8 mL) was first diluted with 10 mL of sterile saline, and then 0.2 mL of this diluted blood was added to a tube with nanopowder. The tubes with the mixture were equilibrated at 37 °C for 30 min and then incubated at the same temperature in a water bath shaker for 60 min. The mixture was then centrifuged (700 rpm for 10 min) and the amount of free hemoglobin was determined by spectrophotometric analysis of the supernatant at 540 nm with a Perkin Elmer Lambda 35 UV–VIS spectrophotometer. The hemolysis rate (HR) was calculated by Eq. (1):

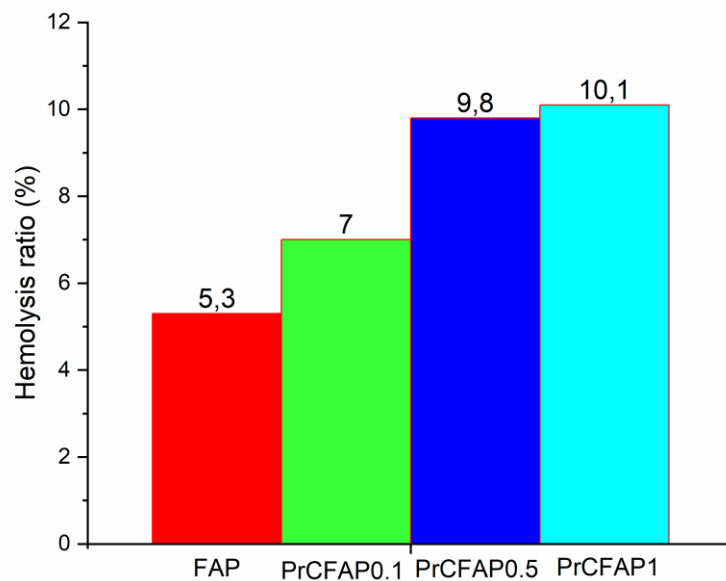
$$\text{HR} = (\text{Dt} - \text{Dnc})/(\text{Dpc} - \text{Dnc}) \times 100\% \quad (1)$$

where Dt is the absorbance of the sample, Dnc and Dpc are the absorbance of the negative and positive control which were also included. The experiments were run in triplicate and the results represent their average values with deviation.

## Results and discussion

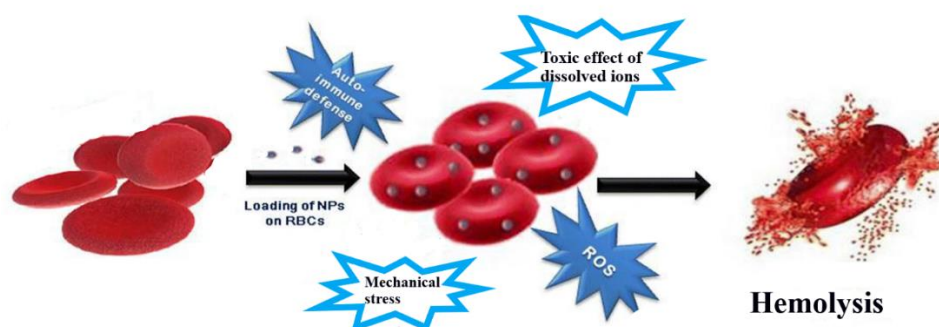
Fluorapatite based nanophosphorus substituted with praseodymium ( $\text{Pr}^{3+}$ ) and carbonate ( $\text{CO}_3^{2-}$ ) ions were successfully fabricated by precipitation method at room temperature (25 °C) [1]. In the previous work, structural, morphological, chemical and optical properties of single-phase material were characterized by XRD, FTIR–FAR, SEM, TEM and PL methods [1]. SEM analysis determined that the synthesized nanopowders were composed of irregular spheres, and TEM confirmed the nanometer particle size [1]. The fluorescence of FAP nanoparticles was in the violet-blue part of the visible part of the spectrum with re-absorption in blue-green when  $\text{Pr}^{3+}$  was doped in the lattice [1]. In addition, luminescent nanopowders have shown significant antibacterial activity against *E. coli* [1]. Obtained optically active nanomaterials showed good absorption of UVA light and reabsorption of blue-green luminescence, and can be promising additives for the development of multifunctional cosmetic and health products.

When biomaterials are used *in vivo* for bone repairing or drug delivery, they can interact with a several cellular systems and cause cell damage [20]. In order to determine the chemocompatibility of the obtained luminescent nanoparticles, *in vitro* erythrocyte hemolysis tests were performed. A lower rate of hemolysis correlates with better blood compatibility. Figure 1 shows the degree of hemolysis of the investigated samples.



**Figure 1.** Hemolysis ratio of investigated FAP nanopowders

The pure FAP sample showed the lowest level of hemolysis. The increase of  $\text{Pr}^{3+}$  content in a FAP caused the increase in hemolysis rate. A similar effect can be observed with the smaller size of nanoparticles. Slight hemolytic behavior of the tested PrCFAP nanopowders may be a consequence of mechanical stress of the cell in the interaction with nanoparticles, as well as the toxic effect of dissolved  $\text{Pr}^{3+}$  and  $\text{F}^-$  ions from the FAP lattice. Complete hemolysis of erythrocytes occurs due to oxidative stress due to the action of nanoparticles, as well as due to the immune response (Figure 2.). Based on literature data, the hemolysis rate induced by HAP and  $\text{Ag}^+$  doped HAP nanoparticles was less than 5% [20, 14]. Lanthanide ions in human blood can interact with hemoglobin, which affects the oxygen-binding, and can also cause reversible cell deformability. Toxic effect of lanthanide ions depends on ion radius and concentration [21, 22]. In earlier studies, neither pure HAP nor FAP in contact with blood did show cytotoxic effects on erythrocytes [23]. But in another report, which investigates the cytotoxicity of various calcium phosphate ceramics, FAP showed high cytotoxicity [24]. Due to these results, FAP and FHAP toxicity on leukemia cells was investigated [25]. Results showed that FAP and FHAP inhibit the growth of leukemia cells and induce programmed cell death through the mitochondrial/caspase-9/caspase-3-dependent pathway [25]. Thus, synthesized FAP and PrCFAP could be examined in the further studies for their possible application in the treatment of leukemia. A challenge in medicine would be the development of luminescent biomaterial with good mechanical and biological properties, which could be used simultaneously as a bio-probe and for drug delivery.



**Figure 2.** Schematic presentation of hemolytic behavior of FAP nanoparticles (NPs). Hemolysis of red blood cells (RBCs) may occur due to mechanical stress, oxidative stress, as well as due to dissolved toxic ions. Nanoparticles in erythrocytes can also encounter autoimmune defense. All these factors cumulatively lead to hemolysis of erythrocytes (modified from [26]).

## Conclusion

The precipitation method has been successfully used for the synthesis of monophase  $\text{Pr}^{3+}$  and  $\text{CO}_3^{2-}$ -substituted fluorapatite nanoparticles with fluorescence properties. An earlier characterization showed that the particles of PrCFAP are nanosized and homogenous in composition. Photoluminescence studies show emissions from the FAP lattice in the violet color of visible light, with the effect of re-absorption into the green color region with increasing  $\text{Pr}^{3+}$  concentration. Luminescence of these nanoparticles, which is in the non-invasive region of the visible part of the spectrum, opens the possibilities for their applications in various biological researches. The results of hemolysis tests show that the degree of hemolysis increases with the increase of dopant concentration, and decrease of crystallite size. The obtained results suggest that the synthesized nanoparticles could be useful for further biomedical research, such as cell and tissue labeling, cancer therapy and drug delivery. This can be beneficial in addition to their primary use as materials for the repair of bone defects.

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