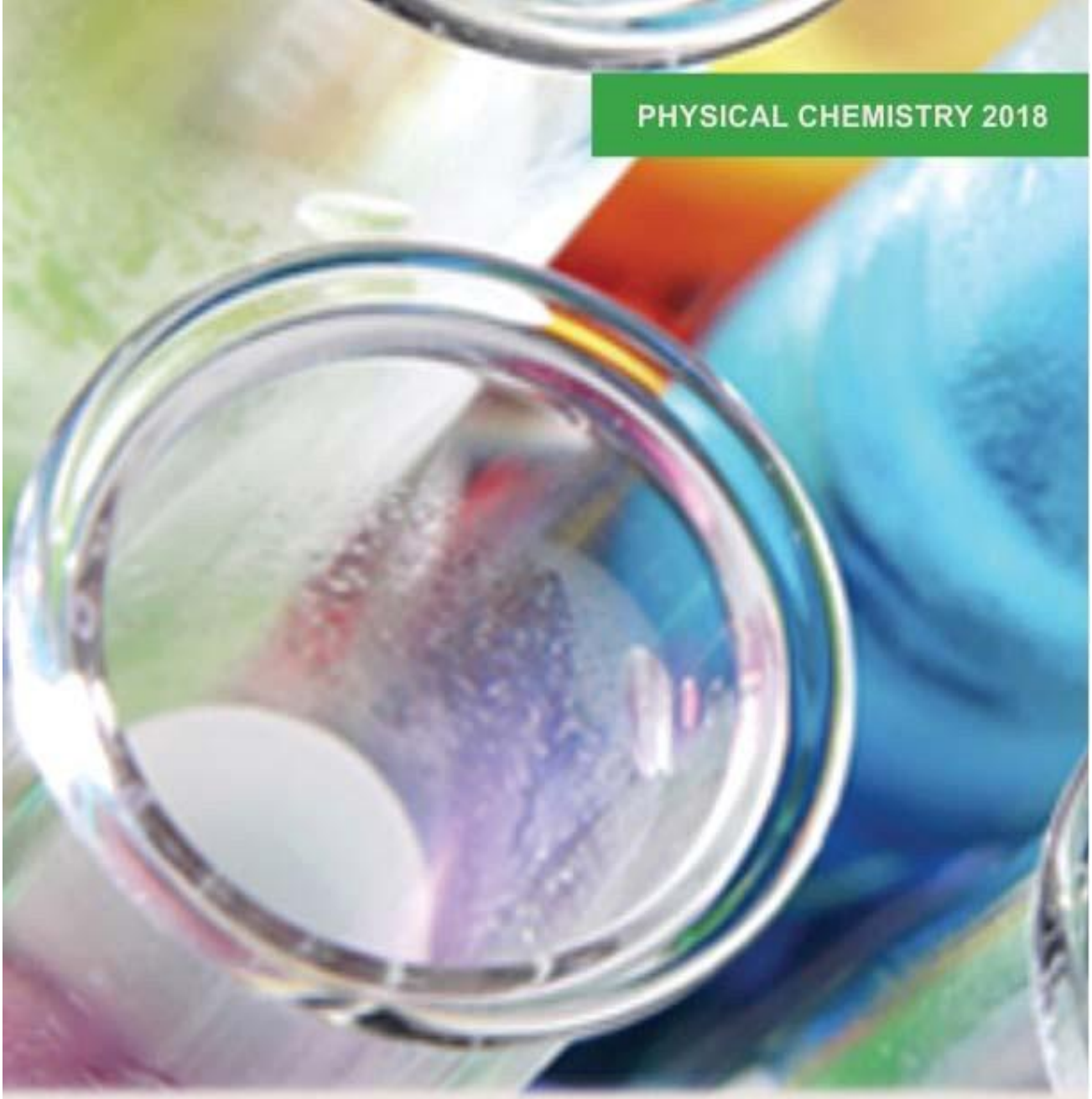

PHYSICAL CHEMISTRY 2018

6th Workshop

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 27th 2018, Vinča Institute of Nuclear Sciences, Belgrade, Serbia

PROCEEDINGS

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

6th WORKSHOP: SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 27th, 2018, Belgrade, Serbia

is a satellite event of

PHYSICAL CHEMISTRY 2018

14th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Organized by

VINČA INSTITUTE OF NUCLEAR SCIENCES

Vinča – Belgrade, Serbia

in co-operation with

THE SOCIETY OF PHYSICAL CHEMISTS OF SERBIA

and

FACULTY OF PHYSICAL CHEMISTRY

UNIVERSITY OF BELGRADE, SERBIA

Held under the auspices of the

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGICAL DEVELOPMENT

Organizing Committee

Chairman
Branislav Nastasijević (Serbia)

Members
Mirjana Čolović (Serbia)
Milovan Stoiljković (Serbia)
Aleksandra Bondžić (Serbia)
Ana Vujačić Nikezić (Serbia)
Jasmina Dimitrić Marković (Serbia)

International Scientific Committee

Chairman
Vesna Vasić (Serbia)

Members
Giovanna Marazza (Italy)
Enisa Omanović-Mikličanin (Bosnia and Herzegovina)
Polonca Trebše (Slovenia)
Robert Săndulescu (Romania)
Claudia Nunes Pinho (Portugal)
Salette Reis (Portugal)
Raul D. Rodriguez (Russia)
Evgeniya Sheremet (Russia)
Tatjana Momić (Serbia)
Andreja Leskovac (Serbia)
Sandra Petrović (Serbia)
Tamara Lazarević-Pašti (Serbia)

PHYSICAL CHEMISTRY 2018

14th International Conference on Fundamental and Applied Aspects of Physical Chemistry

6th Workshop

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 27th, 2018, Vinča Institute of Nuclear Sciences, Belgrade, Serbia

PROCEEDINGS

BELGRADE, SERBIA 2018

6th WORKSHOP: SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

PROCEEDINGS

Publisher
VINČA INSTITUTE OF NUCLEAR SCIENCES
Vinča - Belgrade, Serbia

Editor
Dr Vesna Vasić

Reviewers
Dr Sandra Petrović
Dr Andreja Leskovac
Dr Tamara Lazarević-Pašti
Dr Tatjana Momić

Design
Milica Ševkušić

Printed by
Skripta Internacional, Beograd

Print run
70 copies

ISBN
978-86-7306-148-1

BELGRADE, SERBIA 2018

P C4

SYNTHESIS AND CHARACTERIZATION OF LUMINESCENT FLUORAPATITE NANORODS FOR LABELLING OF *Saccharomyces cerevisiae* CELLS

D. V. Milojkov[1,2], B. J. Nastasijević[2],V. Živković-Radovanović[3], G. V. Janjić[4], S. D. Dimović[2], D. R. Mutavdžić[5] and V. Dj. Stanić[2]

[1]*University of Novi Sad, Faculty of Technology, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia. (d.v.milojkov@gmail.com)*
[2]*University of Belgrade, Vinča Institute of Nuclear Science, P. O. Box 522, 11001 Belgrade, Serbia.*
[3]*University of Belgrade, Faculty of Chemistry, P. O. Box 51, 11158 Belgrade, Serbia.*
[4]*University of Belgrade, ICTM, Njegoševa 12, 11000 Belgrade, Serbia*
[5]*University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11030 Belgrade, Serbia.*

ABSTRACT

In this study, the preparation and characterization of luminescent fluorapatite (FAP) nanoparticles (50 and 80 nm rod-size), and their use for labeling of *Saccharomyces cerevisae* cells were investigated. Fluorapatite nanoparticles were obtained by neutralization method. X-ray diffraction (XRD), scanning electron microscopy (SEM) and photoluminescence (PL) methods have confirmed a rod-like material of nanometer size with broadband emission within 380-600 nm under UVA light excitation. Fluorescence microscopy showed that fluorapatite nano-fluorophors successfully labeled *S. cerevisiae* cells, by attaching the proteins on the cell wall. Obtained luminescent fluorapatite nanorods could be used as a probe for visualization and detection of yeast cells.

INTRODUCTION

Saccharomyces cerevisae cells in biosensors have proven to be successful sensing element for detection of a large range of molecules such as sugars, alcohols, odorants, metals, intracellular metabolites and carcinogens [1]. Most of these analytes yeast enzymes converts to the signal that is easily measured as redox potential, dissolved oxygen, etc. [1,2]. In recent years, transcription-dependent biosensors based on fluorescence (typically green fluorescent protein), bioluminescence (luciferase), and colorimetry (beta-

galactosidase) were develop for pathogens detection [2]. In order to increase the sensitivity of biosensor, nanotechnology has been successfully used for preparation of nanodetectors and transducers. We synthesized luminescent fluorapatite nanoparticles and labeled them with *S. cerevisae* cells.

EXPERIMENTAL

Fluorapatite nanopowder is prepared at room temperature by neutralization method, by the same procedure used for the preparation of Ag^{+}– doped fluorapatite nanomaterials [3]. First water solution containing required amount of HF and $(NH_4)F$, was added dropwise to water suspension of $Ca(NO_3)_2 \cdot 4H_2O$. Starting atomic ratio Ca/P fixed at 1.67. Suspension was maturated for 16 h and the obtained precipitate was filtered and washed by distilled water, and then dried at $110^{o}C$ for 12 h. The resulting material pulverized into powder and characterized.

X-ray diffractogram of powder were recorded using Siemens D500 automated diffractometer. The morphology of the powder was characterized by JOEL JSM-6390LV scanning electron microscop. The excitation and emission spectra of nanopowder was measured with photoluminescence spectrophotometer Horiba JovinYvon Fluoromax 4 TCSPC at room temperature.

S. cerevisiae was purchased in local stores, and was suspended in saline solution. The yeast suspension was added to 1 mg of the FAP sample. The resulting suspension was mixed and then incubated at room temperature for 1 h without mixing. After treatment, cells were harvested, fixed, and processed for microscopy.

RESULTS AND DISCUSSION

The XRD diffractogram and micrographs of FAP powder are presented in Figure 1. All of diffraction peaks is of synthetic fluoroapatite, indicated that obtained phase of FAP is pure and with poor crystallnity. The micrography shows that FAP particles are rod-like with average particle sizes around 50 and 80 nm. These nanoparticles easily form agglomerates.

To identify luminescence properties of nanoparticles, their PL characterization was performed. Excitation and emission spectra are presented in Figure 2. Excitation spectrum shows the curve maxima at about 350 nm in the UVA part of the spectrum. Nanomaterial has the broad band emission within 380-600 nm in the visible part of spectrum under 350 nm excitation.

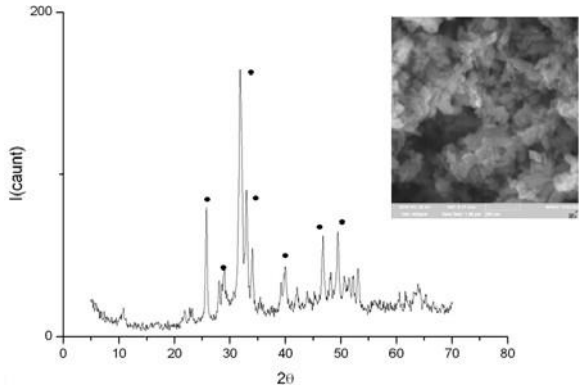

Figure 1. Difractogram and micrography of the nano-FAP powder.

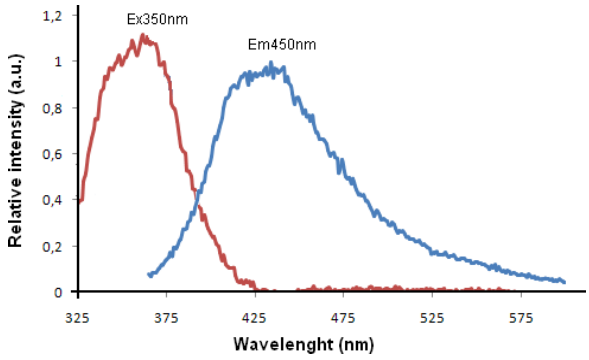

Figure 2. Excitation and emission spectra of nano-FAP powder.

To further identify the FAP nanoparticles fluorophores performance, *S. cerevisiae* cells were labeled and observed on a Leica DMIL inverted and luminescent fluorescence microscope. Figures 3a and 3b refer to blue luminescent nanoparticles attached on the cells, and agglomerate around of the cells, respectively. *S. cerevisiae* cells were successfully labeled with nanoparticles, as consequence of their binding to proteins on the cell wall [4,5]. The similar interactions were observed with magnetic nanoparticles and *S. cerevisiae* cells using magnetic resonance [6]. Future investigation will focus on preparation of bio-nano system for detection of luminescence in different color of electromagnetic spectrum in order to increase the sensitivity of biosensors.

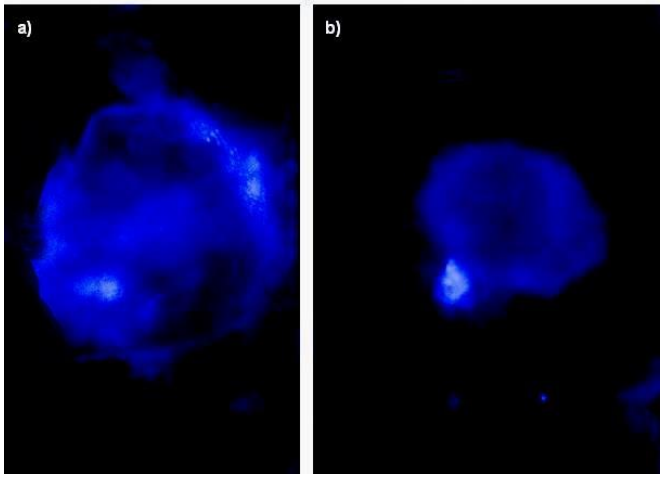

Figure 3. *S. cerevisiae* cells labeled with blue luminescent nano-FAP (a), nano-FAP agglomerate around the cells (b).

CONCLUSION

Luminescent nano-particles of FAP were synthesized by neutralization at low temperature condition. Obtained monophase nanomaterial has fluorescence in violet region under UV excitation. *Saccharomyces cerevisiae* cells were successfully labelled with nano-fluorapatite fluorophors. Luminescent FAP nanorods could be used as a part of bio-nano system for preparation of microbial biosensor.

REFERENCES

[1] A. Adeniran, M. Sherer, K.E.J. Tyo, FEMS Yeast Research, 2015, **15**, 1–15.

[2] M. Gonchar, O. Smutok, M. Karkovska, N. Stasyuk, G. Gayda, Yeast-based biosensors for clinical diagnostics and food control, A.A. Sibirny (ed.), Biotechnology of Yeasts and Filamentous Fungi, Springer International Publishing AG, 2017.

[3] V. Stanić, A.S. Radosavljević–Mihajlović, V. Zivković–Radovanović, B. Nastasijević, M. Marinović–Cincović, J. P. Marković, M. D. Budimir, Appl. Surf. Sci., 2015, **33**, 772–780.

[4] K. Wang, C. Zhou, Y. hong, X. Zhang, Interface focus, 2012, **2**, 259-277.

[5] P.-H. Hsu, P.-C. Chiang, C.-H. Liu, Y.-W. Chang, PLoS ONE, 2015, **10**(8): e0135174.

[6] J.P.M. Godoy Morais, R.B. Azevedo, L.P. Silva, Z.G.M. Lacava, S.N. Bao, O. Silva, F. Pelegrini, C. Gansau, N. Buske, I. Safarik, M. Safarikova, P.C. Morais, J. Magn. Magn. Mater., 2004, **272–276**, 2400–2401.