

Original articles

Research article

<https://doi.org/10.17308/kcmf.2023.25/11260>

A study of the local atomic structure the environment of zinc ions of different concentrations during their interaction with the arachidic acid Langmuir monolayer

V. Yu. Lysenko¹, M. A. Kremennaya¹, S. N. Yakunin², A. V. Rogachev², G. E. Yalovega¹✉

¹*Southern Federal University*

105/42 Bolshaya Sadovaya st., Rostov-on-Don 344006, Russian Federation

²*National Research Centre “Kurchatov Institute”*

1 Academician Kurchatova pl., Moscow 123182, Russian Federation

Abstract

Vital cellular processes depend on the controlled transport of metal ions across biological membranes. A biological membrane is a complex system consisting of lipids and proteins, that is why simplified systems, in particular monomolecular layers, are used to model it.

This work presents the results of a study of the interaction of zinc ions from the aqueous subphase with the Langmuir monolayer of arachidic acid. The study was carried out for the first time and used total external reflection X-ray absorption spectroscopy. It considers the influence of the concentration of a ZnCl₂ aqueous subphase solution on the local environment of zinc ions when interacting with the lipid monolayer immediately after its formation.

The theoretical analysis of experimental XANES spectra showed that one of the interaction ways of arachidic acid molecules with zinc ions immediately after the monolayer formation is an intramolecular interaction with the formation of spodium bonds between the zinc cation and the OH carboxyl group of arachidic acid.

Keywords: arachidic acid, X-ray absorption spectroscopy, total external reflection, lipid layer, Langmuir bath, subphase, thin films

Funding: The study was supported by the grant of the President of the Russian Federation (grant No. MK-2767.2021).

For citation: Lysenko V. Yu., Kremennaya M. A., Yakunin S. N., Rogachev A. V., Yalovega G. E. A study of the local atomic structure the environment of zinc ions of different concentrations during their interaction with the arachidic acid Langmuir monolayer. *Condensed Matter and Interphases*. 2023;25(3): 367–372. <https://doi.org/10.17308/kcmf.2023.25/11260>

Для цитирования: Лысенко В. Ю., Кременная М. А., Якунин С. Н., Рогачев А. В., Яловега Г. Э. Исследование локальной атомной структуры окружения ионов цинка различной концентрации при их взаимодействии с лэнгмюровским монослоем арахидиновой кислоты. *Конденсированные среды и межфазные границы*. 2023;25(3): 367–372. <https://doi.org/10.17308/kcmf.2023.25/11260>

✉ Galina E. Yalovega, e-mail: yalovega@sfedu.ru

© Lysenko V. Yu., Kremennaya M. A., Yakunin S. N., Rogachev A. V., Yalovega G. E., 2023



1. Introduction

The structure and functioning of biological membranes is still one of the most important issues in biology and medicine. Biological membranes are molecular “shells” that separate the content of cells from the external environment. They provide barrier, transport, receptor, energy, and other functions of cells. They regulate the interaction of the cell with the external environment and are the medium for many biochemical processes [1]. Such diverse functions are impossible without a complex structure, which is provided by the diversity of the main components of the membrane and their interactions. Biological membranes consist of two main components: lipids and proteins. The structural backbone of the membrane is its lipid bilayer, in which various proteins are embedded [2].

Simplified artificial models of biological membranes are used to better understand the interactions between lipids and the cellular medium at the molecular level. There are two groups of models of biological membranes: vesicular and planar. The planar model uses lipid monolayers as a model biological membrane. Despite the fact that biomembranes have a double layer, the molecular monolayer method allows modelling some processes occurring in lipid bilayers and in the lipid monolayer on the water surface [3].

The lipid monolayer model is also suitable for studying membrane binding by introducing the tested substance into an aqueous subphase under the monolayer. In particular, vital cellular processes depend on the controlled transport of metal ions across the membrane. In these cases, Langmuir monolayers at the air-water interface are the best model for studying the interaction of lipids with metal ions.

Layers of arachidic acid can be used as a model of a cell membrane. Arachidic acid is a saturated long-chain fatty acid with twenty carbon chains containing an aliphatic tail. Zinc is an essential microelement for almost all existing living forms and at high concentrations can act as a toxic agent [4]. Its high biological accumulation in organisms and plants is due, among other things, to the good solubility of zinc chlorides in water [5]. In our work, we considered the interaction of arachidic acid with zinc ions from a $ZnCl_2$ aqueous

solution as a model for the interaction of zinc ions from a body fluid with the lipid layer of the biomembrane.

Inorganic ions present in the subphase alter the arrangement of the monolayer molecules in arachidic acid at the air-water interface. Inorganic ions of different species differently affect the structural organisation of the monolayer and the separation of the surface and volume of stearic acid monolayers [6].

Currently, there is a big variety of physico-chemical methods for studying biochemical processes in cells. However, the study of the effect of microelements on the course of various cellular processes is still a challenging task due to their presence in the body in trace amounts. Unlike other methods, X-ray absorption spectroscopy in the near-edge region (XANES – X-ray Absorption Near Edge Structure) can be applied to almost any kind of atom and any concentration of the studied element. X-ray absorption spectroscopy is sensitive to the local chemical environment of a particular element, the nature of the chemical bond, the number and type of ligands, metal-ligand distances, and the degree of metal oxidation [7, 8].

XANES measurements in fluorescence mode under total external reflection (TER) conditions on the surface of the liquid subphase is an evolving method that opens up new opportunities for *in situ* monitoring of the structure formation processes in two-dimensional monolayers and the study of objects with extremely low concentrations of the absorbing atom, such as metalloproteins monolayers and lipids [9, 10].

This work is devoted to the study of the interaction of zinc ions from the aqueous subphase with the Langmuir layer of arachidic acid by X-ray absorption spectroscopy XANES. It analyses the changes in the local environment of zinc ions depending on the concentrations of a $ZnCl_2$ solution. It presents the results of the theoretical analysis of experimental spectra, which were used to study one of the ways of interaction of the monolayer with zinc ions.

2. Experimental and theoretical methods

Sample preparation. Arachidic acid was purchased from Sigma-Aldrich Co. A $ZnCl_2$ aqueous solution was used as a subphase in the

Langmuir bath. Measurements were made for three concentrations of ZnCl_2 in the solution: $6 \cdot 10^{-4}$, $6 \cdot 10^{-5}$, $3 \cdot 10^{-6}$ M. A solution of arachidic acid was applied to the subphase surface. The layers were compressed to a surface pressure of $\pi = 20$ mN/m, the pressure was constant during X-ray measurements.

The XANES measurements on the arachidic acid monolayer at the air/liquid interface were performed in the Kurchatov Center for Synchrotron Radiation on the LANGMUIR bending magnet line. The zinc K-edge XANES spectra were recorded in fluorescence mode under total external reflection conditions using a Si(111) monochromator with a spectral width of ~ 2 eV. The X-ray beam was directed to the surface of the liquid using two flat mirrors. XANES spectra were measured at a fixed incident angle of 0.8 critical angle θ_c . The X-ray penetration depth, which determines the sensitivity of the measurements, was approximately 85 Å.

Theoretical analysis of the zinc K-edge X-ray absorption spectra was carried out by the finite-difference method at full potential using the FDMNES code [11]. The size of the clusters for the calculation was selected so that all atoms of the studied model were included. Lorentzian convolution was performed for the obtained spectra to determine spreading effects.

3. Results and discussion

Arachidic acid ($\text{C}_{20}\text{H}_{40}\text{O}_2$) has a hydrophilic carboxyl group and hydrophobic hydrocarbon chains in its composition. The structure of arachidic acid determines the location of these molecules in the composition of the monolayer on the surface of the aqueous subphase of the ZnCl_2 solution in the Langmuir bath. Polar carboxyl groups are immersed in the liquid phase, while non-polar hydrocarbon chains face the air medium (Fig. 1).

The surface pressure of the lipid monolayer is an important parameter for the analysis of the dynamic and structural properties of monolayers [12]. To investigate lipid binding, the studied bioactive substance is introduced into the aqueous subphase under the lipid monolayer. Its penetration into the lipid layer can be controlled by recording changes in the surface pressure or changes in the monolayer area at certain pressure

values [13]. During our experiment, changes in the monolayer area were used to control the dynamics of the ongoing processes.

The measurements of the zinc K-edge XANES spectra in the system of a monolayer of arachidic acid on the surface of a ZnCl_2 solution (indicated in the figure as AA+ ZnCl_2) began almost immediately (about 7 minutes) after the surface pressure reached the value of 20 mN/m. The measurements were taken for 3 concentrations of ZnCl_2 in the solution: $6 \cdot 10^{-4}$, $6 \cdot 10^{-5}$, $3 \cdot 10^{-6}$ M. For all concentrations, the pressure in the monolayer was constant during the measurements.

The general trend for changing spectra for the system (AA+ ZnCl_2) with an increase in the concentration of ZnCl_2 in the solution was the shift of the absorption edge by ~ 1.1 eV, a decrease in the intensities of peak A and C with a simultaneous increase in the energy distances between them, as well as an increase in the intensity of peak B (Fig. 2, the direction of changes is shown by arrows). The observed differences in spectra reflect changes in the local environment of zinc ions in the presence of arachidic acid depending on the concentration of the ZnCl_2 solution. As can be seen from Fig. 2, the spectra for the AA+ ZnCl_2 system coincided in shape (the same set of maxima and redistribution of intensities between peaks) with the spectrum of the ZnCl_2 solution (in the absence of arachidic acid) [10], however, they differed in intensity and energy distances between the peaks. This indicates differences in the local environment of zinc ions in the pure solution and in the presence of arachidic acid. The spectrum of the

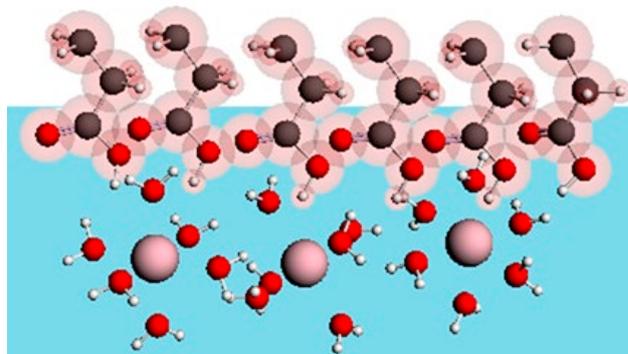


Fig. 1. Schematic representation of the arrangement of the molecules of arachidic acid on the surface of a ZnCl_2 solution (blue plane)

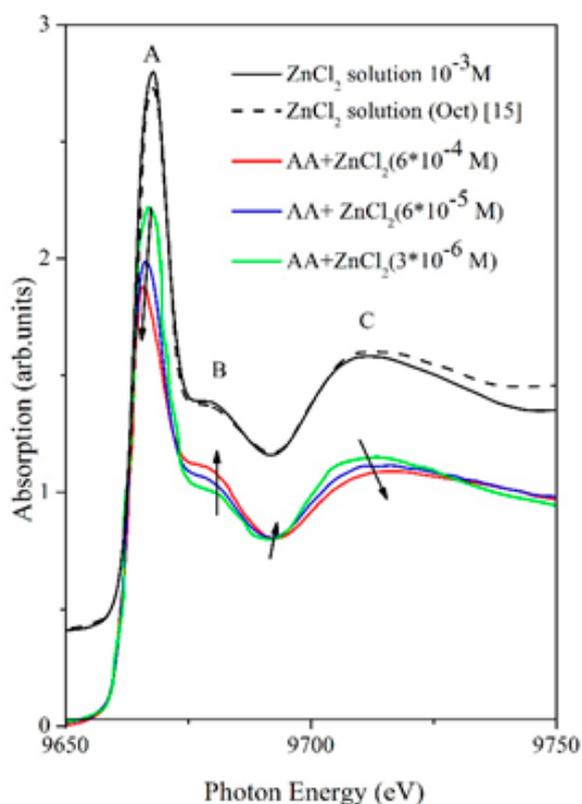


Fig. 2. Experimental zinc K-edge XANES spectra: (a) of a ZnCl_2 solution with a concentration of 10^{-3} M as compared with the spectrum from work [15]; (b) monolayer of arachidic acid (AA) on the surface of ZnCl_2 solutions with concentrations of $6 \cdot 10^{-4}$, $6 \cdot 10^{-5}$, $3 \cdot 10^{-6}$ M

solution with a concentration of 10^{-5} M almost completely coincided with the spectrum from the work [14] (Fig. 2, dotted line), where Zn^{2+} was in the octahedral environment. It should be noted that the spectra of the solution were obtained by the same registering method as in the AA+ ZnCl_2 system, however, from the subphase volume, i.e. at the X-ray beam incidence angle greater than critical angle θ_c .

Theoretical modelling of XANES experimental spectra was used to study the changes in the local structure of Zn^{2+} when zinc ions came into contact with the monolayer. Zinc chlorides in an aqueous solution can form complexes of different types with the different number of bound water molecules in different geometric environments [15]. At low concentrations of < 1 M, one of the dominant roles in the solution is played by $\text{Zn}(\text{H}_2\text{O})_6^{2+}$ complexes [16]. The theoretical zinc K-edge XANES spectra were calculated for this complex (Fig. 3a). According to various sources,

Zn-O interatomic distances in the $\text{Zn}(\text{H}_2\text{O})_6^{2+}$ complex range from 2.05 to 2.14 Å. Figure 3 shows the sensitivity of the XANES spectra to changes in the closest symmetry of the Zn^{2+} environment. The deviation from the octahedral geometry of the zinc environment with H_2O ligands towards the distorted octahedron resulted in a more pronounced peak B in the spectrum and a change in the shape of the main peak. The distortions in the octahedron included elongation of the axial distances to 2.7 Å as compared to distances typical of the $\text{Zn}(\text{H}_2\text{O})_6^{2+}$ aqueous complex. Next, calculations were made for a structural model in which the OH hydroxyl group of the carboxyl group of arachidic acid replaced the water molecule at the octahedron's vertex (Fig. 3b). In this case, the Zn–OH interatomic distances were also 2.7 Å. This model was used to consider the possibility of forming intramolecular Zn...O spodium bonds between the zinc cation and the OH group of arachidic acid when the interatomic distances exceed the sum of the covalent radii. This type of interaction is an alternative to hydrogen bonding and was observed in Zn^{2+} complexes [17]. This model was in good agreement with the experiment. It should be noted that a longer interaction of arachidic acid with a ZnCl_2 solution resulted in an increased trend of changing XANES spectra in the AA+ ZnCl_2 system, which may indicate the presence of other types of binding in this system [18].

4. Conclusions

For the first time, zinc K-edge total external reflection X-ray absorption spectra were obtained for the system of a arachidic acid monolayer on the ZnCl_2 solution surface of various concentrations ($6 \cdot 10^{-4}$, $6 \cdot 10^{-5}$, $3 \cdot 10^{-6}$ M). The analysis of the XANES spectra indicated that the local environment of zinc ions changed immediately after the formation of the Langmuir monolayer and depended even on minor changes in concentration. The analysis of theoretical XANES spectra allowed assuming that one of the ways of interaction of arachidic acid with aqueous Zn^{2+} complexes is an intramolecular interaction resulting in the formation of spodium bonds between the zinc cation and the OH carboxyl group of arachidic acid. In this case, the symmetry of the zinc environment changes from an octahedron to a distorted octahedron with an

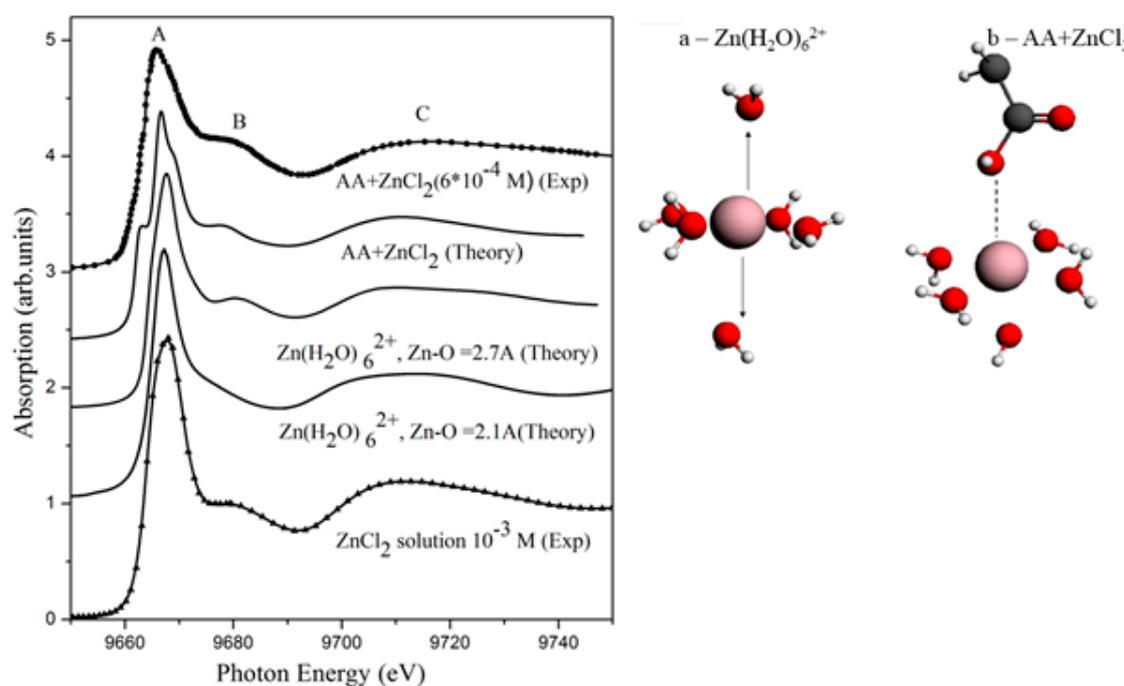


Fig. 3. Comparison of the theoretical Zn K-edge XANES (solid line) for the model $\text{Zn}(\text{H}_2\text{O})_6^{2+}$ (a) and $\text{AA}+\text{ZnCl}_2$ (b) with the experimental spectra (symbol) of a solution of ZnCl_2 and $\text{AA}+\text{ZnCl}_2$ ($6 \cdot 10^{-4}$ M)

increased Zn–OH axial distance. Differences in the degree of changes in the local structure of zinc in the system “the monolayer of arachidic acid - a ZnCl_2 solution” at different concentrations can be due to the number of the interacted molecules of arachidic acid or to another type of interaction.

Contribution of the authors

V. Yu. Lysenko – theoretical modelling of XANES X-ray absorption spectra, study of literature. M. A. Kremennaya – construction and substantiation of structural models. S. N. Yakunin – development of methods, measuring of XANES X-ray absorption spectra in fluorescence mode under total external reflection using synchrotron radiation. A. V. Rogachev - measuring XANES X-ray absorption spectra in fluorescent mode under total external reflection using synchrotron radiation. G. E. Yalovega – analysis of literary sources, analysis and interpretation of experimental and theoretical XANES spectra, substantiation of results and conclusions.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Watson H. Biological membranes. *Essays in Biochemistry*. 2015;59: 43–69. <https://doi.org/10.1042/bse0590043>
2. Mukhomedzyanova S., Pivovarov Y., Bogdanova O., Dmitrieva L., Shulunov A. The lipids of biological membranes (Literature review). *Acta Biomedica Scientifica*. 2017;2(5(1)): 43–49. https://doi.org/10.12737/article_59e8bcd3d6fcb1.49315019
3. Wiśniewska-Becker A., Gruszecki W. I. 2 – Biomembrane models. In: *Drug – biomembrane interaction studies*. Woodhead Publishing. 2013: 47–59. <https://doi.org/10.1533/9781908818348.47>
4. Sandstead H. H. *Handbook on the Toxicology of Metals, 4th ed.* Elsevier. 2014: 1369–1386.
5. Pipan-Tkalec Z., Drobne D., Jemec A., Romih T., Zidar P., Bele M. Zinc bioaccumulation in a terrestrial invertebrate fed a diet treated with particulate ZnO or ZnCl_2 solution. *Toxicology*. 2010;269(2-3): 198–203. <https://doi.org/10.1016/j.tox.2009.08.004>
6. Li S., Du L., Wei Z., Wang W. Aqueous-phase aerosols on the air-water interface: Response of fatty acid Langmuir monolayers to atmospheric inorganic ions. *Science of the Total Environment*. 2017;580: 1155–1161. <https://doi.org/10.1016/j.scitotenv.2016.12.072>
7. Bokhoven J. A., Lamberti C. (eds.). *X-Ray absorption and X-Ray emission spectroscopy: Theory and Applications*. John Wiley & Sons. 2016. <https://doi.org/10.1002/9781118844243>

8. Shmatko V. A., Mysoedova T. N., Mikhailova T. A., Yalovega G. E. Features of the electronic structure and chemical bonds of polyaniline-based composites obtained by acid-free synthesis. *Condensed Matter and Interphases*. 2019;4(4): 567–578. <https://doi.org/10.17308/kcmf.2019.21/2367>
9. Konovalov O. V., Novikova N. N., Kovalchuk M. V., ... Yakunin S. N. XANES measurements for studies of adsorbed protein layers at liquid interfaces. *Materials*. 2020;13(20): 4635. <https://doi.org/10.3390/ma13204635>
10. Novikova N. N., Yakunin S. N., Koval'chuk M. V., ... Topunov A. F. Possibilities of X-ray absorption spectroscopy in the total external reflection geometry for studying protein films on liquids. *Crystallography Reports*. 2019;64(6): 952–957. <https://doi.org/10.1134/S1063774519060130>
11. Joly Y. X-ray absorption near-edge structure calculations beyond the muffin-tin approximation. *Physical Review*. 2001;63: 125120. <https://doi.org/10.1103/physrevb.63.125120>
12. Sujak A., Gagos M., Serra M. D., Gruszecki W. I. Organization of two-component monomolecular layers formed with dipalmitoylphosphatidylcholine and the carotenoid pigment, canthaxanthin. *Molecular Membrane Biology*. 2007;24(5-6): 431–41. <https://doi.org/10.1080/09687860701243899>
13. Hereć M., Gagoś M., Kulma M., Kwiatkowska K., Sobota A., Gruszecki W. I. Secondary structure and orientation of the pore-forming toxin lysenin in a sphingomyelin-containing membrane. *Biochim Biophys Acta*. 2008;1778(4): 872–9. <https://doi.org/10.1016/j.bbamem.2007.12.004>
14. Alloteau F., Valbi V., Majérus O., Biron I., Lehuede P., Caurant D., Seyeux A. Study of a surface treatment based on zinc salts to protect glasses from atmospheric alteration: Mechanisms and application to ancient glass objects in museum. In: *Glass Atmospheric Alteration: Cultural Heritage, Industrial and Nuclear Glasses*. Paris (France): Hermann edition, 2019. pp. 192–202.
15. Silber H. B., Simon D., Gaizer F. Octahedral-tetrahedral geometry changes for zinc(II) in the presence of chloride ions. *Inorganic Chemistry*. 1984;23(18): 2844–2848. <https://doi.org/10.1021/ic00186a026>
16. Parchment O. G., Vincent M. A., Hillier I. H. Speciation in aqueous zinc chloride. An *ab initio* hybrid microsolvation/continuum approach. *The Journal of Physical Chemistry A*. 1996;100(23): 9689–9693. <https://doi.org/10.1021/jp960123z>
17. Karmakar M., Frontera A., Chattopadhyay S., Mooibroek T., Bauzá A. Intramolecular spodium bonds in Zn(II) complexes: insights from theory and experiment. *International Journal of Molecular Sciences*. 2020;21(19): 7091. <https://doi.org/10.3390/ijms21197091>
18. Kremennaya M. A., Lysenko V. Y., Novikova N. N., Yakunin S. N., Rogachev A. V., Yalovega G. E. X-ray spectral diagnostics of the local environment of zinc in the arachidic acid layers. *Journal of Physics: Conference Series*. 2021;2103: 012171. <https://doi.org/10.1088/1742-6596/2103/1/012171>

Information about the authors

Victoria Yu. Lysenko, 2nd year master student of the Faculty of Physics, Southern Federal University (Rostov-on-Don, Russian Federation).

<https://orcid.org/0000-0002-0538-7772>
viktoriya.250699@mail.ru

Maria A. Kremennaya, Cand. Sci. (Phys.–Math.), Senior Lecturer, Faculty of Physics, Southern Federal University (Rostov-on-Don, Russian Federation).

<https://orcid.org/0000-0002-0894-5733>
kremennayamariya@gmail.com

Sergey N. Yakunin, Cand. Sci. (Phys.–Math.), First Deputy Head of the Kurchatov Complex for Synchrotron-Neutron Research of the National Research Center “Kurchatov Institute” (Moscow, Russian Federation).

s.n.yakunin@gmail.com

Alexander V. Rogachev, Researcher, National Research Center “Kurchatov Institute” (Moscow, Russian Federation).

<https://orcid.org/0000-0001-6026-1534>
a.v.rogachev@ya.ru

Galina E. Yalovega, Dr. Sci. (Phys.–Math.), Head of the Department of Physics of Nanosystems and Spectroscopy, Faculty of Physics, Southern Federal University (Rostov-on-Don, Russian Federation).

<https://orcid.org/0000-0002-0157-6955>
yalovega@sfedu.ru

Received 14.12.2022; approved after reviewing 22.12.2022; accepted for publication 26.12.2022; published online 25.09.2023.

Translated by Irina Charychanskaya