PHYSICAL CHEMISTRY 2021

15th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume II

The Conference is dedicated to the

30th Anniversary of the founding of the Society of Physical Chemists of Serbia

and

100th Anniversary of Bray-Liebhafsky reaction

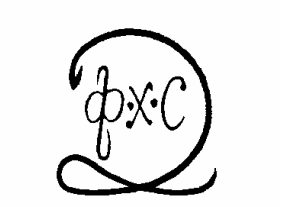
September 20-24, 2021
Belgrade, Serbia

Title: Physical Chemistry 2021 (Proceedings) **ISBN** 978-86-82475-40-8
Volume II: ISBN 978-86-82475-39-2
Editors: Željko Čupić and Slobodan Anić
Published by: Society of Physical Chemists of Serbia, Studentski Trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: ″Jovan″, <Printing and Publishing Company, 200 Copies
Number of pages: 6+388, Format A4, printing finished in December 2021

Text and Layout: ″Jovan″

200 - *Copy printing*

CONTENT



PHYSICAL CHEMISTRY 2021

15th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Organized by

The Society of Physical Chemists of Serbia

in co-operation with

Institute of Catalysis Bulgarian Academy of Sciences

and

Boreskov Institute of Catalysis Siberian Branch of Russian Academy of Sciences

and

University of Belgrade, Serbia:

Faculty of Physical Chemistry
Institute of Chemistry, Technology and Metallurgy
Vinča Institute of Nuclear Sciences
Faculty of Pharmacy

and

Institute of General and Physical Chemistry, Belgrade, Serbia

International Organizing Committee

Chairman: S. Anić (Serbia)
Vice-chairman: M. Gabrovska (Bulgaria)
A. A. Vedyagin (Russia)
S. N. Blagojević (Serbia)

Members: N. Cvjetićanin (Serbia), S. M. Blagojević (Serbia), M. Daković (Serbia), J. Dimitrić Marković (Serbia), T. Grozdić (Serbia), Lj. Ignjatović (Serbia), D. Jovanović (Serbia), M. Kuzmanović (Serbia), D. Marković (Serbia), B. Milosavljević (USA), M. Mojović (Serbia), N. Pejić (Serbia), M. Petković (Serbia), A. Popović-Bjelić (Serbia), B. Simonović (Serbia), M.Stanković (Serbia), B. Šljukić (Serbia), G. Tasić (Serbia), S. Veličković (Serbia), N. Vukelić (Serbia)

International Scientific Committee

Chairman: Ž. Čupić (Serbia)
Vice-chairman: V. Bukhtiyarov (Russia)
S. Todorova (Bulgaria)
B. Adnađević (Serbia)

Members: S. Anić (Serbia), A. Antić-Jovanović (Serbia), A. Azizoğlu (Turky), R. Cervellati (Italy), G. Ćirić-Marjanović (Serbia), V. Dondur (Serbia), I. I. Grinvald (Russia), R. Jerala (Slovenia), M. Jeremić (Serbia), G. N. Kaluđerović (Germany), E. Kiš (Serbia), A.V. Knyazev (Russia), Lj. Kolar-Anić (Serbia), U. Kortz (Germany), T. Kowalska (Poljska), A. Lemarchand (France), G. Lente (Hungary), Z. Marković (Serbia), S. Mentus (Serbia), K. Novaković (UK), N. Ostrovski (Serbia), V. Parmon (Russia), Z. Petkova Cherkezova-Zheleva (Bulgary), M. Plavšić (Serbia), J. Savović (Serbia), G. Schmitz (Belgium), I. Schreiber (Czech), L. Schreiberova (Czech), D. Stanisavljev (Serbia), N. Stepanov (Russia), M. Stojanović (USA), E. Szabó (Slovakia), Zs. Szakacs (Romania), Z. Šaponjić (Serbia), Á. Tóth (Hungary), M. Trtica (Serbia), V. Vasić (Serbia), D. Veselinović (Serbia), V. Vukojević (Sweden)

Local Executive Committee

Chairman: S. N. Blagojević
Vice-chairman: A. Ivanović-Šašić
N. Jović-Jovičić
A. Stanojević

Members: M. Ajduković, I. N. Bubanja, A. Dobrota, J. Dostanić, D. Dimić, S. Jovanović, Z. Jovanović, D. Lončarević, M. Kragović, J. Krstić, B. Marković, S. Maćešić, J. Maksimović, S. Marinović, D. Milenković, T. Mudrinić, M. Pagnacco, N. Potkonjak, B. Stanković, I. Stefanović, G. Stevanović, A. Stoiljković, M. Vasić

SPONSORS

Ministry of Education, Science and Technological Development of the Republic Serbia
Institute of General and Physical Chemistry, Belgrade
Belgrade Analysis d.o.o.

CYTOGENOTOXICITY ASSESSMENT OF POLYOXOPALLADATES(II) AS PROMISING ANTILEUKEMIC DRUG CANDIDATES

M. B. Čolović[1], G. Gajski[2], M. Gerić[2], T. Ma[3], X. Ma[3], U. Kortz[3] and D. Z. Krstic[4]

[1]*Department of Physical Chemistry, "Vinča" Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Serbia* (colovicm@vin.bg.ac.rs)
[2]*Institute for Medical Research and Occupational Health, Zagreb, Croatia*
[3]*Department of Life Sciences and Chemistry, Jacobs University, Bremen, Germany*
[4]*Institute of Medical Chemistry, Faculty of Medicine, University of Belgrade, Serbia*

ABSTRACT

Polyoxopalladates(II) (POPs) are discrete, anionic palladium(II)- oxo nanoclusters that possess features of both conventional polyoxometalates (POMs) and palladium(II), which were shown to exhibit promising antitumor properties. In this study, *in vitro* cyto- and genotoxicity evaluation was performed on normal non-target human blood cells using two isostructural POPs with tetravalent metal ions (Sn^{IV}, Pb^{IV}) encapsulated in the cuboid Pd_{12}-oxo host, $Na_{12}[SnO_8Pd_{12}(PO_4)_8]\cdot 43H_2O$ (**SnPd$_{12}$**) and $Na_{12}[PbO_8Pd_{12}(PO_4)_8]\cdot 38H_2O$ (**PbPd$_{12}$**), with confirmed *in vitro* antileukemic actions against HL-60 cell line. For this purpose, whole blood samples were exposed to the POPs, at concentrations of ≈ IC_{50} (24 h) values, resulting in cytotoxicity in HL-60 cells for 24 h at 37 °C. The cytotoxicity studies were performed on human peripheral blood mononuclear cells which were stained with acridine orange and ethidium bromide, and then viewed under a fluorescence microscope. The genotoxicity was tested in whole blood by the alkaline comet assay (microgel electrophoresis). The results of the cytotoxicity evaluation and the comet assay demonstrated that none of the tested POPs, within the investigated concentration range 12.5 – 50 μM, resulted in a statistically significant modulation of blood cell viability as well as DNA damage, expressed as % of tail DNA (relative increase of tail DNA), compared to the untreated controls. Therefore, the promising antileukemic drug candidates, **SnPd$_{12}$** and **PbPd$_{12}$**, can be considered as selective and safe from a cytogenotoxicity point of view.

INTRODUCTION

Numerous palladium(II) complexes have been synthesized and tested against various tumor cells *in vitro* and *in vivo* in order to find an appropriate alternative to platinum(II)-based antitumor drugs as most frequently prescribed chemotherapeutics [1]. Polyoxopalladates (POPs) are another group of palladium(II)-based compounds, which are discrete, anionic palladium-oxo clusters and hence belong to the class of polyoxometalates (POMs)[2]. POMs were also reported to exhibit different biological activities including significant cytotoxic action against various types of malignant cells *in vitro* and *in vivo* [3]. However, toxicity studies revealed adverse effects after *in vivo* POM application, which could limit their potential application in biomedicine [4].

Taking into account that the development of novel drugs requires an assessment of their safety, the aim of this study was to evaluate the cytogenotoxicity of two POPs containing tetravalent metal ion guests (Sn^{IV} and Pb^{IV}), $Na_{12}[SnO_8Pd_{12}(PO_4)_8]\cdot 43H_2O$ (**SnPd$_{12}$**) and $Na_{12}[PbO_8Pd_{12}(PO_4)_8]\cdot 38H_2O$ (**PbPd$_{12}$**) (Figure 1), towards normal non-target cells by employing cell viability and the comet assays on human peripheral blood cells (HPBCs). **SnPd$_{12}$** and **PbPd$_{12}$** were reported in our previous study [5] to exhibit cytotoxicity against human acute promyelocytic cell line HL-60 (IC_{50} (24h) = 37.1 ± 4.4 and 34.7 ± 9.2 μM for **SnPd$_{12}$** and **PbPd$_{12}$**, respectively), and can therefore be regarded as potential antileukemic metallodrugs.

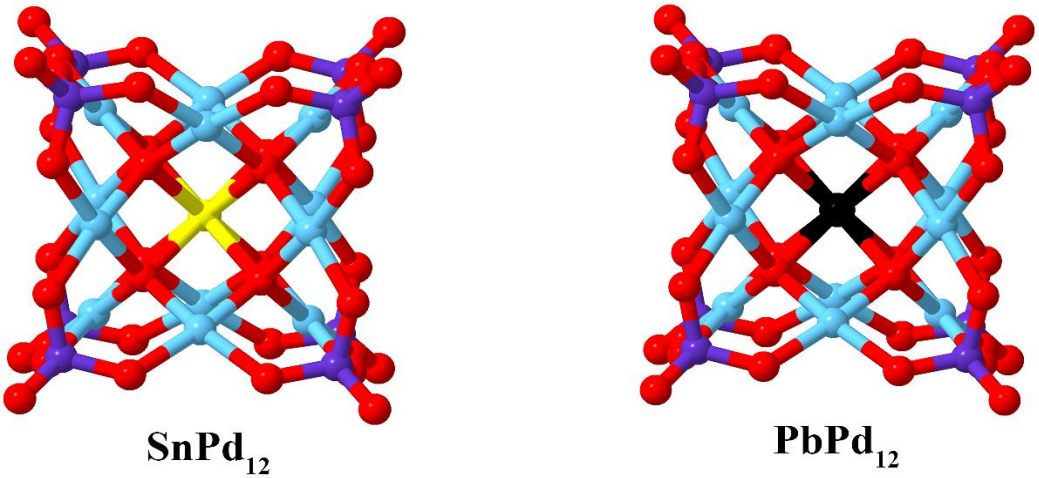


Figure 1. Structure of the investigated POPs **SnPd12** and **PbPd12**. Color codes: light blue, Pd; yellow, Sn; black, Pb; violet, P and red, O.

EXPERIMENTAL

SnPd12 and **PbPd12** were prepared according to the published procedures [5]. The cytotoxicity of the POPs in peripheral blood mononuclear cells (PBMCs) was determined by differential staining with acridine orange and ethidium bromide and by fluorescence microscopy [6]. Briefly, whole blood samples obtained from a healthy female donor were treated with the POPs, and then incubated at 37 °C for 24 h. The PBMCs were isolated by the histopaque density gradient centrifugation method, and the slides were prepared using 200 µL of PBMCs and 2 µL of stain (acridine orange and ethidium bromide). A total of 100 cells per repetition were examined with an epifluorescence microscope (Olympus BX51, Tokyo, Japan). The cells were divided in two categories: live cells with a functional membrane and with uniform green staining of the nucleus and dead cells with uniform red staining of the nucleus. Alkaline comet assay for genotoxicity evaluation was essentially carried out as described by Singh *et al*. [7]. After the exposure to different POP concentrations for 24 h, 5 µL of whole blood was embedded into an agarose matrix and subsequently lysed (2.5 M NaCl, 100 mM $EDTANa_2$, 10 mM Tris, 1% sodium sarcosinate, 1% Triton X-100, 10% DMSO, pH 10) overnight at 4 °C. After the lysis, the slides were placed into an alkaline solution (300 mM NaOH, 1 mM $EDTANa_2$, pH 13) for 20 min at 4 °C to allow DNA unwinding and subsequently electrophoresed for 20 min at 1 V/cm. Finally, the slides were neutralized in 0.4 M Tris buffer (pH 7.5) for 5 min 3 times, stained with ethidium bromide (10 µg/mL) and analyzed at 250× magnification using an epifluorescence microscope (Zeiss, Göttingen, Germany) connected through a camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd., Haverhill, Suffolk, UK). One hundred randomly captured comets from each slide were examined.

RESULTS AND DISCUSSION

For the cytogenotoxicity evaluation of **SnPd12** and **PbPd12** whole blood samples were treated with three different POP concentrations (12.5, 25, and 50 µM) inducing cytotoxic effects against HL-60 cells during 24 h treatment [5].

The effect of **SnPd12** and **PbPd12** on the viability in human PBMCs after 24 h exposure is presented in Figure 2. It can be seen that the cell viability, expressed as a percentage of untreated control, was not significantly affected with respect to the corresponding control at all investigated concentrations for both POPs. Even the highest concentration applied (50 µM ≈ 1.5 × IC_{50} (24 h) for HL-60) did not result in cytotoxic action.

The degree of DNA damage, a genotoxicity indicator expressed as % of tail DNA, dependent on **$SnPd_{12}$** and **$PbPd_{12}$** concentrations is presented in Figure 3. Similar to cytotoxicity, no statistically significant difference in the amount of DNA strand breaks compared to the corresponding control samples was observed for both **$SnPd_{12}$** and **$PbPd_{12}$**, regardless of the concentrations tested.

Since both **$SnPd_{12}$** and **$PbPd_{12}$** did not induce cytogenotoxic effect on normal non-target HPBCs at the concentrations ($\approx IC_{50}$ values) inducing considerable antileukemic effects, they should be considered in further research as promising, low toxic, and selective antileukemic drug candidates.

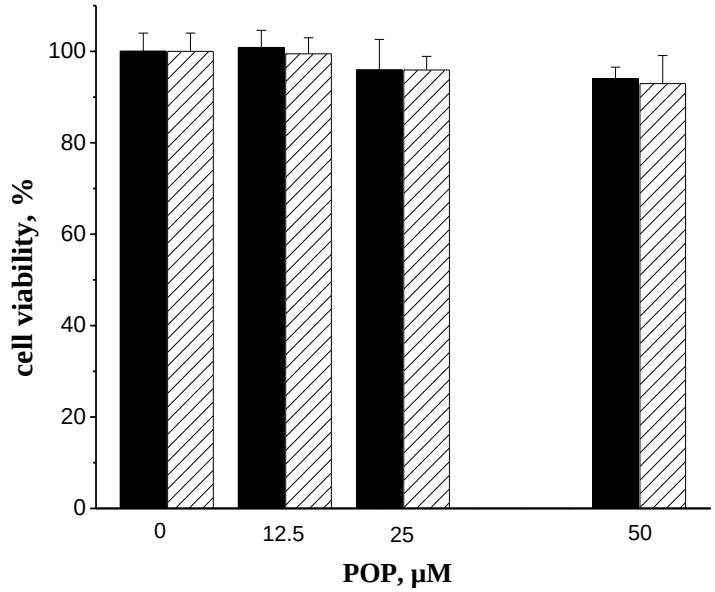


Figure 2. The effects of **$SnPd_{12}$** (solid symbol) and **$PbPd_{12}$** (sparse pattern) on the viability in human peripheral blood mononuclear cells (PBMCs) after 24 h treatment. *Statistically significant compared to the corresponding control ($p < 0.05$).

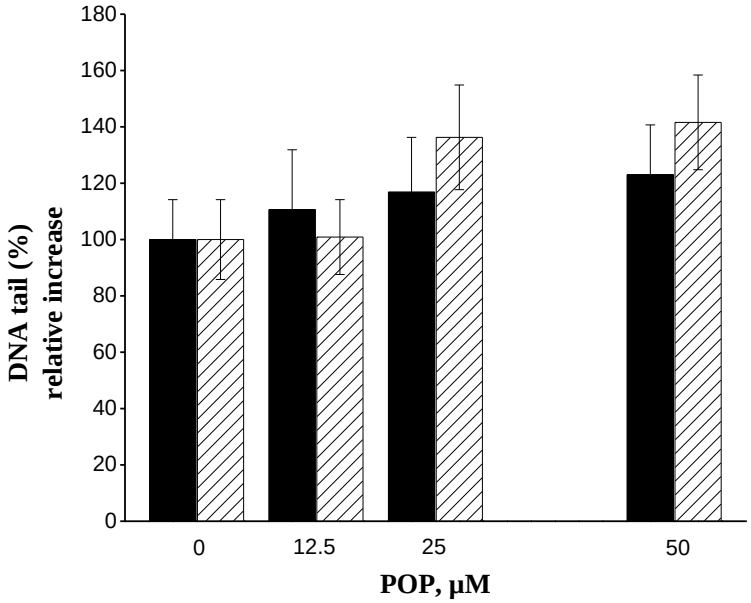


Figure 3. The effects of **$SnPd_{12}$** (solid symbol) and **$PbPd_{12}$** (sparse pattern) on DNA damage in human peripheral blood cells (HPBCs) after 24 h treatment. *Statistically significant compared to the corresponding control ($p < 0.05$).

CONCLUSION

The obtained cytotoxicity results indicated that neither **SnPd$_{12}$** nor **PbPd$_{12}$**, within the concentration range 12.5 – 50 μM, induced statistically significant alterations of cell viability compared to the control. Moreover, the results of the comet assay showed that none of the tested POPs at concentrations ≤ 50 μM resulted in a statistically significant relative increase of tail DNA. Accordingly, both **SnPd$_{12}$** and **PbPd$_{12}$** could be considered as promising, selective, and safe antileukemic drug candidates from a cytogenotoxicity point of view.

Acknowledgement

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract no. 451-03-9/2021-14/200017) and the Institute for Medical Research and Occupational Health, Zagreb, Croatia. The authors also gratefully acknowledge the bilateral project Serbia-Germany (no. 451-03-01038/2015-09/16, DAAD-PPP) the CMST COST Action CM1203 (PoCheMoN), the bilateral project Serbia-Croatia (no. 337-00-205/2019-09/19), and the COST Action CA16113 (CliniMARK). Figure 1 was generated with Diamond, version 3.2 (Crystal Impact GbR).

REFERENCES

[1] Alam, M.N.; Huq, F. Comprehensive review on tumour active palladium compounds and structure–activity relationships. *Coord. Chem. Rev*., **2016**, *316*, 36–67.

[2] Izarova, N.V.; Pope, M.T.; Kortz, U. Noble metals in polyoxometalates. *Angew. Chem. Int. Edit.*, **2012**, *51(38)*, 9492–9510.

[3] Čolović, M.B.; Lacković, M.; Lalatović, J.; Mougharbel, A.S.; Kortz, U.; Krstić, D. Polyoxometalates in biomedicine: update and overview. *Curr. Med. Chem*., **2020**, *27*, 362–379.

[4] Čolović, M.B.; Medić, B.; Ćetković, M.; Kravić Stevović, T.; Stojanović, M.; Ayass, W.W.; Mougharbel, A.S.; Radenković, M.; Prostran, M.; Kortz, U.; Krstić, Z. Toxicity evaluation of two polyoxotungstates with anti-acetylcholinesterase activity. *Toxicol. Appl. Pharm.*, **2017**, *333*, 68–75.

[5] Yang, P.; Ma, T.; Lang, Z.; Misirlic-Dencic, S.; Isakovic, A.M.; Bényei, A.; Čolović, M.B.; Markovic, I.; Krstić, D.Z.; Poblet, J.M.; Lin, Z.; Kortz, U. Tetravalent metal ion guests in polyoxopalladate chemistry: synthesis and anticancer activity of $[MO_8Pd_{12}(PO_4)_8]^{12-}$ ($M = Sn^{IV}$, Pb^{IV}). *Inorg. Chem.*, **2019**, *58(17)*, 11294–11299.

[6] Atale, N.; Gupta, S.; Yadav, U.; Rani, V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. Microscopy-JPN, **2014**, *255(1)*, 7–19.

[7] Singh, NP.; McCoy, M.T.; Tice, R.R.; Schneider, E.L. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell. Res*., **1988**, *175(1)*, 184–191.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

544(082)
66.017/.018(082)
502/504(082)
343.98(082)

INTERNATIONAL Conference on Fundamental and Applied Aspects of Physical Chemistry (15; 2021; Beograd)

Physical Chemistry 2021: proceedings: the Conference is dedicated to the 30th Anniversary of the founding of the Society of Physical Chemists of Serbia and 100th Anniversary of Bray-Liebhafsky reaction. Vol. 2 / 15th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 20-24, 2021, Belgrade, Serbia; [organized by The Society of Physical Chemists of Serbia in co-operation with Institute of Catalysis Bulgarian Academy of Sciences ... [et al.]]; [editors Željko Čupić and Slobodan Anić]. - Belgrade: Society of Physical Chemists of Serbia, 2021 (Belgrade: Jovan). - VI str., str. 347-732: ilustr.; 30 cm

Tiraž 200. - Bibliografija uz svaki rad. - Registar.

ISBN 978-86-82475-39-2
ISBN 978-86-82475-40-8 (niz)

а) Физичка хемија -- Зборници б) Наука о материјалима -- Зборници в) Животна средина -- Зборници г) Форензика -- Зборници

COBISS.SR-ID 53325065