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Zinc Oxide Nanoparticles Modified with Galactose as Potential Drug Carrier with Reduced Releasing of Zinc Ions

Jolanta Pulit-Prociak, Olga Długosz, Marcin Banach

***Abstract*—**The toxicity of bare zinc oxide nanoparticles used as drug carriers may be the result of releasing zinc ions. Thus, zinc oxide nanoparticles modified with galactose were obtained. The process of their formation was conducted in the microwave field. The physicochemical properties of the obtained products were studied. The size and electrokinetic potential were defined by using dynamic light scattering technique. The crystalline properties were assessed by X-ray diffractometry. In order to confirm the formation of the desired products, Fourier-transform infrared spectroscopy was used. Releasing of zinc ions from the prepared products when comparing to the bare oxide was analyzed. It was found out that modification of zinc oxide nanoparticles with galactose limits the releasing of zinc ions which are responsible for the toxic effect of the whole carrier-drug conjugate.

***Keywords*—**Nanomaterials, zinc oxide, drug delivery system, toxicity.

I. Introduction

AMONG the well-known and used nanometric drug carriers are: liposomes, dendrimers, polymer nanoparticles, hydrogels, carbon nano-tubes, metal nanoparticles and metal oxide nanoparticles. The latter are characterized by high mechanical strength [1]. However, the release of metal ions from the surface of metal oxide-based drug carriers can lead to undesirable processes [2]. The releasing of ions from the particles can take place to a varying degree depending on the compounds surrounding the particle (from 10 to 100%) [3], [4]. These ions contribute to the formation of reactive oxygen species (ROS), which in turn induce intracellular oxidative stress [5]. The nanoparticles captured by the cell can also dissolve inside the cell, releasing metal ions [6]. Limiting the release of ions is tantamount to reducing the toxic properties of nanoparticles [7].

The aim of the work was to develop modification processes of zinc oxide nanoparticles by depositing galactose on their surface, which in turn will contribute to limiting the release of zinc ions. The explanation of the phenomenon of limiting the toxic properties should be seen in the limitation of the release of metal ions from nanoparticles due to the attachment of surrounding particles, in particular galactose, thereto. The presence of galactose on the surface of nanoparticles also limits their penetration into the cells, as well as inhibits their dissolution.

II. Materials and Methods

A. Materials

The following compounds were used in this study: zinc sulfate (99.9%), potassium hydroxide (≥ 98.-%), and glucose (≥ 99.0%). All compounds were obtained from Sigma-Aldrich. All aqueous solutions were prepared using deionized water (Polwater, 0.18 μS).

B. Methods

The method of producing a series of modified zinc oxide nanoparticles was based on the performance of precipitation and dehydration processes. Zinc sulfate was used as the source of zinc ions. The precipitating agent was potassium hydroxide. Galactose was used to modify the surface of the forming zinc oxide nanoparticles. A series of products with different process parameters was prepared. Initially, the aqueous potassium hydroxide solution was added dropwise to the aqueous zinc sulfate solution in a 110 ml Teflon vessel and homogenized for 60 seconds (Hielscher UP400St, 40W). Then, the aqueous galactose solution was introduced into the reaction mixture and the whole was homogenized again for additional various time (depending on the design of experiment). The concentrations and volumes of the individual solutions were selected so that the theoretical weight of zinc oxide was 1.01725 g. In order to carry out the zinc hydroxide dehydration process, the vessel was placed in the Magnum II microwave reactor by Ertec Poland. The mixture was kept at 150 °C. The time of the trials was equal to 5 minutes. Upon completion of the process, the product was filtered off and washed with deionized water. The filtrate was discarded and the solid product was dried in a laboratory drier at 105 °C, and then triturated in an agate mortar to make it homogeneous. The first stage of the work was to design the experiment (Design of Experiment, DoE technique). Planning of experiments is an interdisciplinary field of science that combines elements of statistics, metrology, computer science and applied mathematics. The main goal of the preliminary stage is to plan the research carried out at the lowest possible cost, and at the same time as much of the feedback received. In the case of this work, the object of research is the technological process, which is perceived in the techniques of experiment planning as a "black box". Its characteristic features are input quantities, which are controlled by the researcher, and output quantities, which are a set of observed and measured

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quantities. The experiment was planned using the $3^{(n-1)}$ fractional design. The variable parameters were: the molar ratio of galactose to zinc oxide (0.02, 0.11 or 0.2), the fold ratio of the stoichiometric potassium hydroxide to zinc nitrate (1, 2 or 3) and the homogenization time (0, 60 or 120 min). Table I shows the process parameters. A reference product without modifier (galactose) was also prepared.

TABLE I
PROCESSES PARAMETERS

Sample	n GAL: n ZnO	fold of KOH vs. stoichiometric amount	homogenization time, sec
E1	0.02	1	0
E2	0.02	2	120
E3	0.02	3	60
E4	0.11	1	120
E5	0.11	2	60
E6	0.11	3	0
E7	0.20	1	60
E8	0.20	2	0
E9	0.20	3	120
E/Base	0	1	0

The obtained products were tested for their physical and chemical properties. XRD analyzes were performed. The surface topography of the products was examined with elemental analysis (TEM-EDS). Also, in order to assess the size and stability of obtained nanoparticles, the DLS technique was applied. Specific surface area and both pore volume and size have been measured by Brunauer–Emmett–Teller (BET) technique.

Zinc ion release from the obtained products was analyzed. After the fixed time of mixing the powders in water (0.5% w/v, 37°C), the concentration of zinc (ASA) in this acceptor fluid was tested. Based on these results, for each product the mean value of the difference of zinc releasing from modified samples against base sample was calculated for all time intervals (Δ_{Zn2+}).

C. Statistical Analysis

The obtained numerical results were subjected to statistical analysis. It was performed in order to identify the variables that had a significant impact on the initial parameters. The obtained results of mean values of Δ_{Zn2+} were subjected to the analysis of variance (ANOVA). Standardized effects were presented graphically in PARETO charts, maintaining the significance level of $\alpha = 0.05$. Approximation profiles were created to determine the best output parameters to obtain the desired result which was the highest value of Δ_{Zn2+}.

III. RESULTS

Based on the XRD analysis, it was confirmed that pure zinc oxide nanoparticles were obtained (Fig. 1). The results of the TEM-EDS analysis indicate the obtaining of ring-like zinc oxide particles with a galactose coating their surface, as evidenced by the presence of carbon in the EDS spectrum (Fig. 2).

Based on the BET (Fig. 3) analysis, it was found out that modified zinc oxide nanoparticles have greater specific area than not modified product. This, both size and volume of pores have been larger.

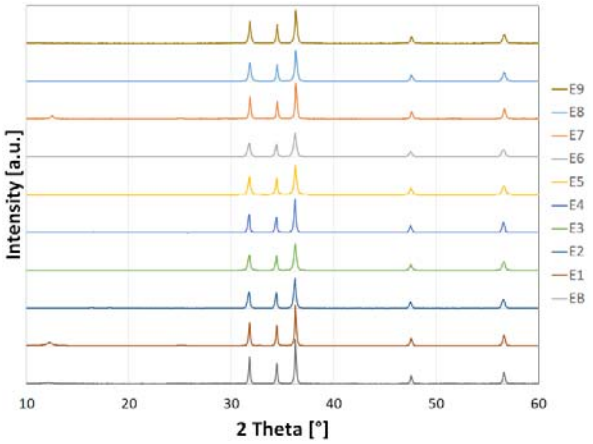


Fig. 1 XRD diffractograms indicating obtaining ZnO in all cases

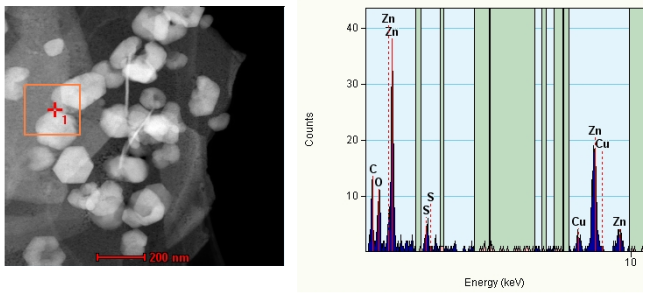


Fig. 2 HR-TEM microphotography of zinc oxide nanoparticles modified with galactose (E4)

The size of obtained particles measured by DLS technique was different and it was in the range of 217-764 nm (Fig. 4). What is more, the values of electrokinetic potential were greater than 20 mV in all cases, which is satisfactory result.

The results of zinc releasing are presented in Fig. 5. It was found that all modified zinc oxide nanoparticles are less soluble and have limited zinc releasing. The difference of zinc releasing from modified samples against base sample was calculated for all time intervals. For this purpose, (1) was used. Next, for each product the mean value of Δ_{Zn2+} was calculated. The results are presented in Table II.

TABLE II
MEAN VALUES OF Δ_{ZN2+}

Sample	$\Delta_{Zn2+\ (mean\ value)}$, %
E1	22.46
E2	77.28
E3	97.68
E4	11.59
E5	82.59
E6	90.97
E7	10.25
E8	75.41
E9	79.73

$$\Delta_{Zn^{2+}} = 100 - \left(\frac{C_{Zn^{2+}(\text{mod.sample})}}{C_{Zn^{2+}(Base)}} \right) \cdot 100\% \quad (1)$$

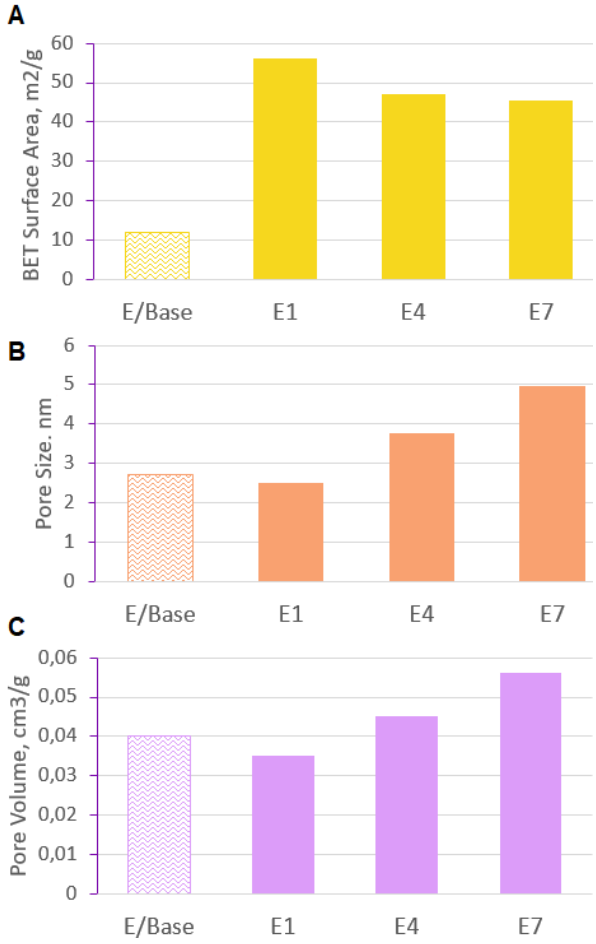


Fig. 3 Results of BET analysis: (A) Surface area, (B) Pore size, (C) Pore volume

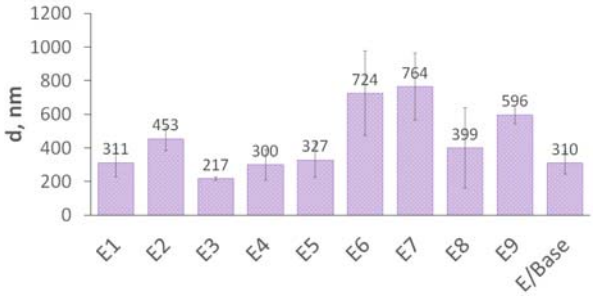


Fig. 4 Results of DLS analysis

The greatest difference between base sample and the modified one is observed for product E3. In this case the releasing of zinc was almost completely inhibited. The preparation of this product was performed by applying galactose in the ratio of 0.02:1.00. The fold of KOH vs. stoichiometric amount was equal to 3 and the homogenization time was equal to 60 seconds. The rest of the samples also exhibited the limited releasing of zinc ions.

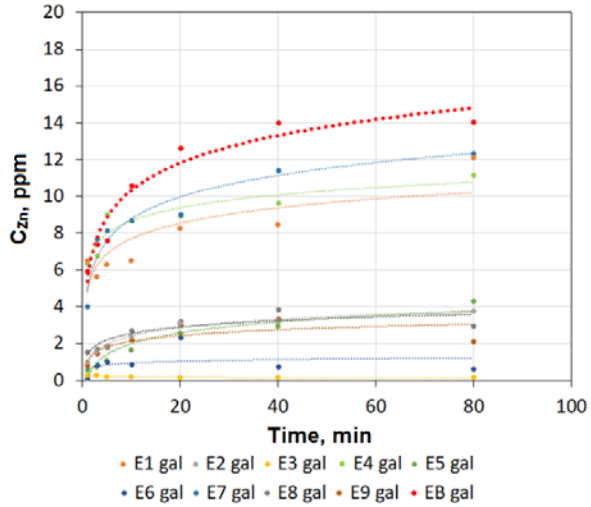
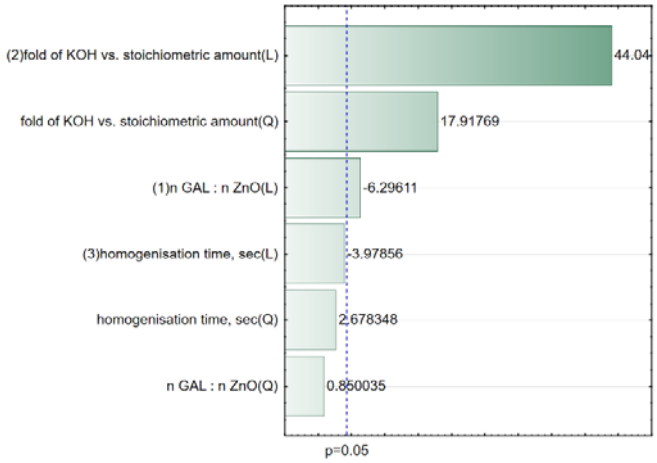


Fig. 5 Results of DLS analysis

Fig. 6 presents a Pareto chart of standardized effects showing the influence of process parameters (input variables) on the Δ_{Zn2+} value.



L – linear dependence, Q – quadratic dependence

Fig. 6 Pareto chart of standardized effects showing the influence of process parameters (input variables) on the Δ_{Zn2+} value

It can be seen that, at the assumed level of significance ($p \leq 0.05$), the Δ_{Zn2+} value was dependent on fold of KOH vs. stoichiometric amount in both linear and quadratic functions.

Table III summarizes the statistically significant parameters that determine the Δ_{Zn2+} values with the adopted significance level of 5%. Parameters for which the value of the probability level p is less than or equal to 0.05 are considered parameters that have a statistically significant influence on the tested output quantity.

Table IV shows the results of the regression analysis describing the parameters of the model. These results are the basis for determining the coefficients of the model equations.

Model equation:

$$y = 142.6x - 26.313x^2 - 93.966 \quad (2)$$
$$R^2 = 0.99914$$

y - Δ_{Zn2+}; x - fold of KOH vs. stoichiometric amount. For example: when x = 1.5, then y = 60.729%.

TABLE III
ANOVA DISTINGUISHING INDEPENDENT PARAMETERS SIGNIFICANTLY INFLUENCING THE Δ_{Zn2+} VALUE

Parameter	Sum of squares	Degree of freedom	Test-F	Probability level, p
(1)n GAL: n ZnO(L)	**170.99**	**1**	**39.641**	**0.024310**
n GAL: n ZnO(Q)	3.12	1	0.723	0.484833
(2) fold of KOH vs. stoichiometric amount(L)	**8368.64**	**1**	**1940.160**	**0.000515**
fold of KOH vs. stoichiometric amount(Q)	**1384.78**	**1**	**321.044**	**0.003100**
(3) homogenization time, sec(L)	68.28	1	15.829	0.057756
homogenization time, sec (Q)	30.94	1	7.174	0.115703
Error	8.63	2		

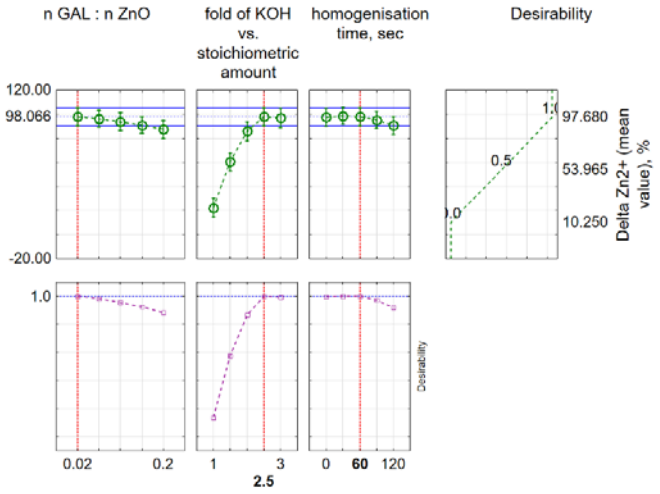
Statistically significant parameters are marked in **bold** ($p < 0.05$)

TABLE IV
REGRESSION COEFFICIENTS FOR THE Δ_{Zn2+} VALUE

Parameter	Regression coefficient	Standard error	Test-T	Probability level, p
Mean	**-93,966**	**5,5521**	**-16,9243**	**0,003473**
(1)n GAL: n ZnO(L)	-25,409	40,9845	-0,6200	0,598497
n GAL: n ZnO(Q)	-154,115	181,3046	-0,8500	0,484833
(2) fold of KOH vs. stoichiometric amount(L)	**142,600**	**5,9351**	**24,0264**	**0,001728**
fold of KOH vs. stoichiometric amount(Q)	**-26,313**	**1,4686**	**-17,9177**	**0,003100**
(3) homogenization time, sec(L)	0,075	0,0510	1,4698	0,279394
homogenization time, sec(Q)	-0,001	0,0004	-2,6783	0,115703

Statistically significant parameters are marked in **bold** ($p < 0.05$)

Fig. 7 shows the approximation profile for the Δ_{Zn2+} value and Fig. 8 presents a saddle diagram showing a three-dimensional interpretation of the results.



Fig, 7 Approximation profile for the Δ_{Zn2+} value

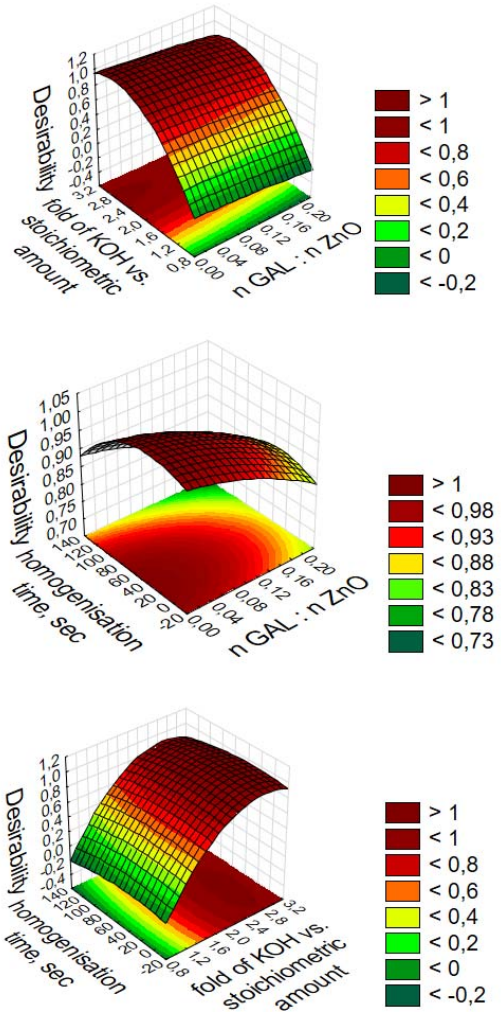


Fig. 8. Saddle chart showing the influence of independent parameters on the Δ_{Zn2+} value

On the basis of Figs. 7 and 8, it can be seen that the increase in the n GAL: n ZnO ratio causes a decrease in the Δ_{Zn2+} value, while an increase in the fold of KOH vs. stoichiometric amount causes an increase in the studies parameter. The increase in homogenization time causes in slight decrease in the Δ_{Zn2+} value.

In order to achieve the optimal effect (the highest Δ_{Zn2+} value), the process should be carried out with the following input parameters: n GAL: n ZnO = 0.02; fold of KOH vs. stoichiometric amount = 2.5 and homogenization time = 60 sec.

IV. CONCLUSION

It was confirmed that it was possible to obtain stable zinc oxide nanoparticles modified with glucose which have limited releasing of zinc ions. Thanks to that such products may find an application in safe drug delivery systems.

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REFERENCES

[1] J. Kumar Patra, G. Das, L.F. Fraceto, E. Vangelie Ramos Campos, M. del Pilar Rodriguez-Torres, L.S. Acosta-Torres, L.A. Diaz-Torres, R. Grillo, M. Kumara Swamy, S. Sharma, S. Habtemariam, H.S. Shin, "Nano based drug delivery systems: recent developments and future prospects", *Journal of Nanobiotechnology*, vol. 16, pp. 1-33, 2018.

[2] S.J. Soenen, W.J. Parak, J., Rejman, B. Manshian, "(Intra)Cellular stability of inorganic nanoparticles: Effects on cytotoxicity, particle functionality, and biomedical applications", *Chemical Reviews*, vol. 115, pp. 2109-2135, 2015.

[3] H.L. Karlsson, M.S. Toprak, B. Fadeel, "Chapter 4 - Toxicity of Metal and Metal Oxide Nanoparticles", in: H*andbook on the Toxicology of Metals* (Fourth Edition), vol. I, pp. 75-112, 2015.

[4] I-L. Hsiao, Y.-J. Huang, "Titanium oxide shell coatings decrease the cytotoxicity of ZnO nanoparticles", *Chemical Research in Toxicology*, vol. 24, pp. 303–313, 2011.

[5] T. Xia, Y. Zhao, T. Sager, S. George, S. Pokhrel, N. Li, D. Schoenfeld, H. Meng, S. Lin, X. Wang, M. Wang, Z. Ji, J.I. Zink, L. Mädler, V. Castranova, S. Lin, A.E. Nel, "Decreased dissolution of ZnO by iron doping yields nanoparticles with reduced toxicity in the rodent lung and zebrafish embryos", *ACS Nano*, vol. 22, pp. 1223-1235, 2011.

[6] B. Wang, Y. Zhang, Z. Mao, D. Yu, C. Gao, "Toxicity of ZnO nanoparticles to macrophages due to cell uptake and intracellular release of zinc ions", *Journal of Nanoscience and Nanotechnology*, vol. 14, pp. 5688-5696, 2014.

[7] H. Papavlassopoulos, Y.K. Mishra, S. Kaps, I. Paulowicz, R. Abdelaziz, M. Elbahri, E. Maser, R. Adelung, C. Röhl, "Toxicity of Functional Nano-Micro Zinc Oxide Tetrapods: Impact of Cell Culture Conditions, Cellular Age and Material Properties", *PlosOne*, vol. 13, e84983, 2014.