PROMISING NANOBIOTECHNOLOGY TO INCREASE THE SHELF LIFE OF CHICKEN EGGES BASED ON NANOSILVER PREPARATION IN HYBRID CARRIERS

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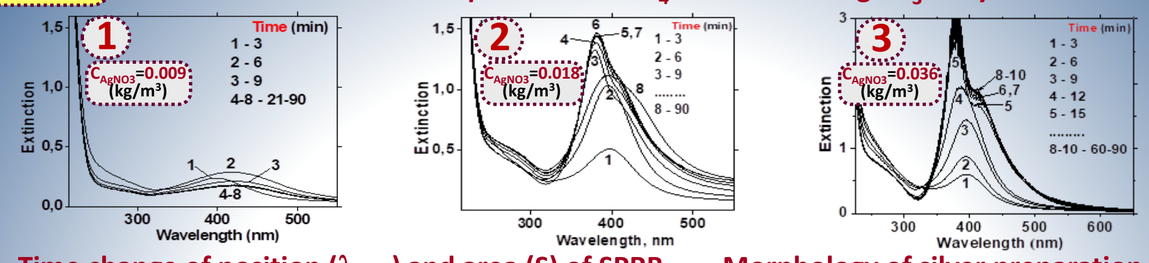
A simple and effective nanobiotechnology is proposed, aimed at reducing the endogenous and exogenous contamination of chicken eggs by opportunistic and pathogenic microflora in the processes of their formation and subsequent storage. The key element of this nanobiotechnology is a special preparation of nanosilver, stabilized in biocompatible and biodegradable silica/polyacrylamide hybrid carriers (SiO_2-*g*-PAAm), which is orally administrated to laying hens with drinking water [1,2]. In this paper, various aspects of obtaining a nanosilver preparation in hybrid solutions and possible changes in its state under the influence of factors such as the pH of the solution, the concentration of nanoparticles, the presence of NaCl as in a "physiological solution", as well as visible light are considered. Then the results of systematic studies of the biological impact of the developed nanosilver preparation on the clinical condition of laying hens, morphological and biochemical parameters of their blood, as well as morphological, microbiological and chemical parameters of chicken eggs are partially represented.

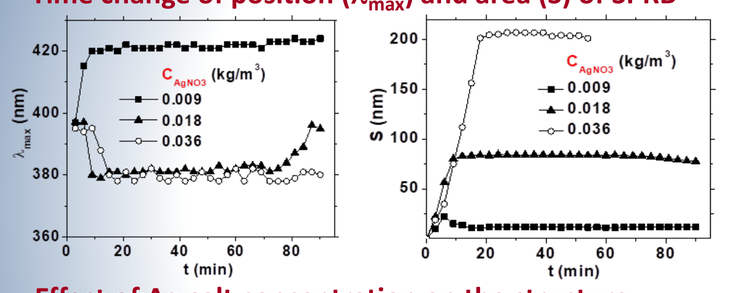
Formation of nanosilver preparation by *in situ* method

UV-Vis

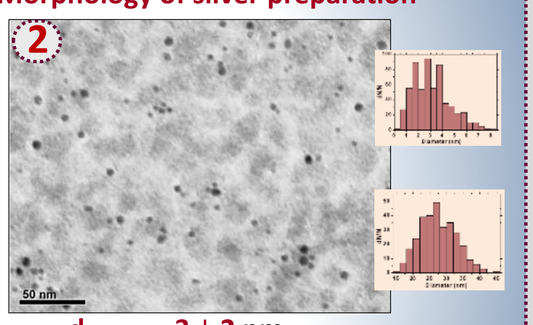
Time evolution of UV-Vis spectra at $NaBH_4$ reduction of $AgNO_3$ in hybrid carriers



Time change of position (λ_{max}) and area (S) of SPRB



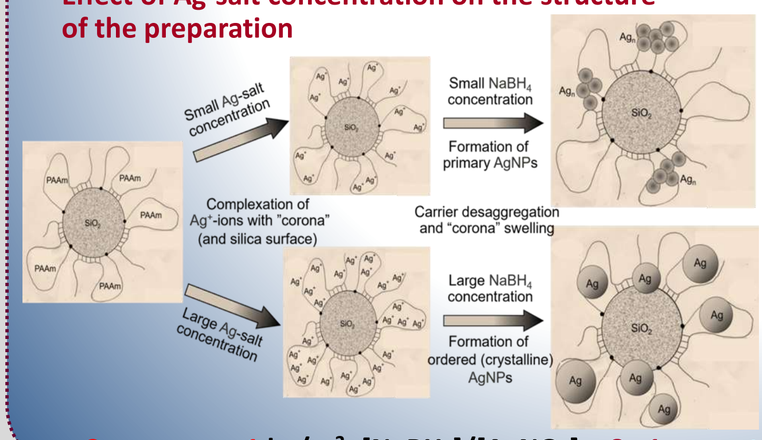
Morphology of silver preparation

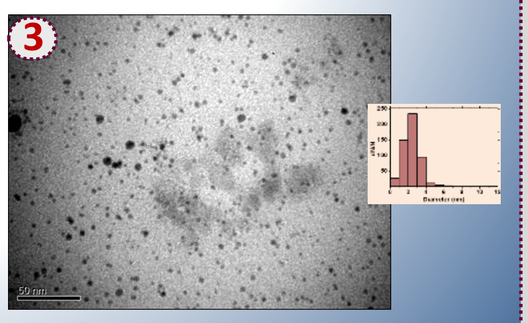


$d_{avAgNPs}=3 \pm 2$ nm
$d_{avSiO2\text{-}g\text{-}PAAm}=28 \pm 5$ nm

TEM

Effect of Ag-salt concentration on the structure of the preparation





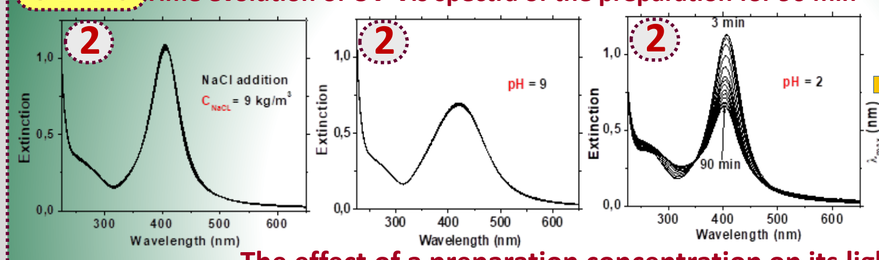
$d_{avAgNPs}=2.4 \pm 1.0$ nm

$C_{SiO2\text{-}g\text{-}PAAm}$ = 1 kg/m³; $[NaBH_4]/[AgNO_3]$ = 8 : 1 = const

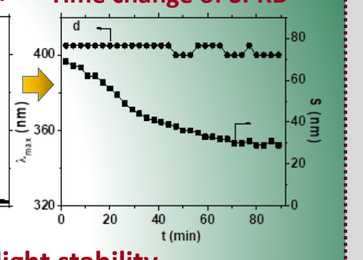
Behavior of nanosilver preparation under various conditions

UV-Vis

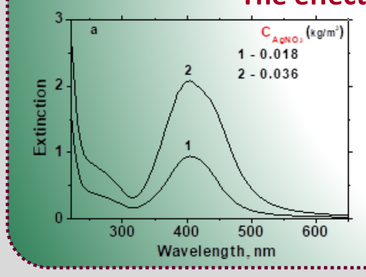
Time evolution of UV-Vis spectra of the preparation for 90 min



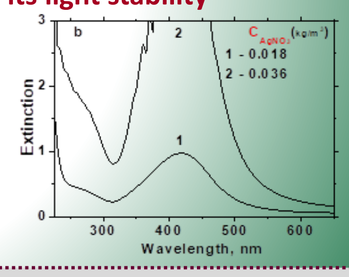
Time change of SPRB



The effect of a preparation concentration on its light stability



Extinction spectra of preparations obtained at different concentrations of Ag-salt after storage: (a) within 3 months in a dark box and (b) within the next 4 months in the light.



Biological experiments with laying hens

For the experiment, 45 laying hens of the High Line W36 cross at the age of 38 weeks were selected. Three groups of laying hens (n=15) such as Control and Research 1-2 were created. The research groups were additionally drunk 3 times a month with an interval of 10 days with an aqueous dispersion of a nanosilver preparation with a concentration of C_{AgNPs}=0.001 (Research 1) and 0.002 kg/m³ (Research 2), which corresponded to doses of nanosilver $2 \cdot 10^{-7}$ and $4 \cdot 10^{-7}$ kg per hen, respectively. The laying hens were fed from drinkers, in which the volume of consumed solution of the nanosilver preparation or water was recorded. Chickens were kept in cages of 5 heads in a room with controlled ventilation, a temperature in the range of 21-22 °C and a relative humidity of 60-62%. On the 10th, 20th and 30th days, 5 freshly laid eggs were taken from the hens of each group to study the content of various metals in the shell, white and yolk of eggs. The biochemical parameters of blood serum of chickens of different groups were also determined on the 10th, 20th and 30th days of the experiment.

The metal content in the shell of chicken eggs

Metal	Metal content ·10⁶, kg/kg		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.006 ± 0.002	0.008 ± 0.001	0.008 ± 0.001
Cu	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.1
Zn	2.0 ± 0.2	1.5 ± 0.6	2.8 ± 0.9
Fe	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.2
Pb	0.009 ± 0.004	0.004 ± 0.001	0.008 ± 0.004
	After 20 days		
Ag	0.007 ± 0.004	0.063 ± 0.020	0.080 ± 0.006
Cu	2.7 ± 0.9	0.8 ± 0.2	1.1 ± 0.4
Zn	3.0 ± 1.0	2.0 ± 0.2	3.0 ± 2.0
Fe	0.5 ± 0.2	0.7 ± 0.1	0.9 ± 0.2
Pb	0.024 ± 0.009	0.007 ± 0.001	0.010 ± 0.002
	After 30 days		
Ag	0.006 ± 0.006	0.038 ± 0.007	0.060 ± 0.020
Cu	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Zn	2.3 ± 0.5	1.1 ± 0.3	2.5 ± 0.9
Fe	0.2 ± 0.1	0.17 ± 0.03	0.1 ± 0.1
Pb	0.006 ± 0.002	0.005 ± 0.001	0.006 ± 0.001

Plasma-optical emission spectrometry

The metal content in the protein of chicken eggs

Metal	Metal content ·10⁶, kg/kg		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.0006 ± 0.0001	0.0008 ± 0.0001	0.0007 ± 0.0001
Cu	0.30 ± 0.10	0.28 ± 0.08	0.30 ± 0.05
Zn	0.0020 ± 0.0010	0.0018 ± 0.0004	0.0020 ± 0.0003
Fe	0.027 ± 0.004	0.030 ± 0.007	0.030 ± 0.008
Pb	0.033 ± 0.003	0.049 ± 0.004	0.061 ± 0.005
	After 20 days		
Ag	0.0006 ± 0.0001	0.0006 ± 0.0003	0.0009 ± 0.0002
Cu	0.15 ± 0.01	0.21 ± 0.07	0.19 ± 0.02
Zn	0.0020 ± 0.0003	0.0017 ± 0.0004	0.0019 ± 0.0001
Fe	0.010 ± 0.010	0.014 ± 0.009	0.010 ± 0.002
Pb	0.040 ± 0.010	0.039 ± 0.008	0.036 ± 0.003
	After 30 days		
Ag	0.0005 ± 0.0001	0.0011 ± 0.0001	0.0011 ± 0.0001
Cu	0.30 ± 0.10	0.30 ± 0.10	0.20 ± 0.07
Zn	0.0020 ± 0.0001	0.0017 ± 0.0004	0.0021 ± 0.0002
Fe	0.025 ± 0.002	0.030 ± 0.003	0.030 ± 0.010
Pb	0.088 ± 0.008	0.083 ± 0.004	0.093 ± 0.003

Plasma-optical emission spectrometry

The metal content in the yolk of chicken eggs

Metal	Metal content ·10⁶, kg/kg		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.0007 ± 0.0001	0.0040 ± 0.0020	0.0040 ± 0.0040
Cu	1.4 ± 0.1	0.9 ± 0.4	1.0 ± 0.3
Zn	22 ± 8	20 ± 8	19 ± 5
Fe	27 ± 8	35 ± 10	23 ± 10
Pb	0.035 ± 0.020	0.053 ± 0.030	0.025 ± 0.010
	After 20 days		
Ag	0.0007 ± 0.0001	0.0010 ± 0.0001	0.0040 ± 0.0001
Cu	1.6 ± 0.1	1.4 ± 0.5	1.2 ± 0.5
Zn	29 ± 3	26 ± 10	26 ± 10
Fe	45 ± 4	39 ± 20	40 ± 20
Pb	0.013 ± 0.004	0.020 ± 0.020	0.017 ± 0.003
	After 30 days		
Ag	0.0006 ± 0.0001	0.0011 ± 0.0002	0.0013 ± 0.0001
Cu	1.4 ± 0.4	1.6 ± 0.3	1.2 ± 0.5
Zn	26 ± 10	28 ± 5	17 ± 6
Fe	38 ± 20	41 ± 10	29 ± 10
Pb	0.029 ± 0.010	0.047 ± 0.009	0.030 ± 0.002

Biochemical markers of blood serum of laying hens

Parameters	Value		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 30 days		
Total protein, kg/m³	44 ± 3	46 ± 4	59 ± 3
Albumin, kg/m³	7.2 ± 0.5	7.1 ± 0.4	6.8 ± 0.6
Cholesterol, kg/m³	1.9 ± 0.3	2.2 ± 0.9	1.3 ± 0.6
Creatinine, mol/m³	0.070 ± 0.006	0.076 ± 0.003	0.064 ± 0.004
Glucose, mol/m³	9.9 ± 1.0	11.9 ± 0.5	8.7 ± 0.7
ALT·10³, U/m³	12 ± 2	14 ± 1	11 ± 1
AST·10³, U/m³	130 ± 20	109 ± 8	140 ± 10
ALP·10³, U/m³	190 ± 20	205 ± 5	190 ± 20
GGT·10³, U/m³	5.8 ± 0.9	6.5 ± 0.5	3.7 ± 0.5
Ca, mol/m³	2.8 ± 0.5	3.2 ± 0.4	3.0 ± 0.2
P, mol/m³	1.8 ± 0.5	1.2 ± 0.1	2.3 ± 0.3
K, mol/m³	4.4 ± 0.3	5.2 ± 0.2	4.3 ± 0.4
Mg, mol/m³	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.2

Conclusions

1. The formation of silver nanoparticles (AgNPs) in hybrid SiO_2-*g*-PAAm carriers by borohydride reduction of $AgNO_3$ was confirmed by the appearance of a surface plasmon resonance band (SPRB) in UV-Vis spectra. Kinetic studies of this process have shown that the structure and yield of AgNPs in carriers depend on the Ag-salt concentration (see the scheme). At a low concentration of Ag-salt (<0.018 kg/m³), the resulting AgNPs had a primary (disordered) structure and a low yield. In the concentration region ≥ 0.018 kg/m³, ordered (crystalline) AgNPs were formed in hybrid "coronas".
2. The developed preparation was resistant to dilution, to the addition of NaCL, as in "physiological solution", to pH=9, as in the intestines of a living organism, but showed a gradual dissolution of AgNPs at pH=2. However, the dissolution rate was relatively low, indicating that the preparation could successfully overcome the stomach of laying hens without significant dissolution of AgNPs. The silver preparation was highly stable over time in the absence of light and in the presence of light (in the latter case, at a relatively low concentration of AgNPs).
3. During the biological experiment, the safety of chickens in all groups was 100%, and their egg productivity did not differ between groups. Three times feeding of laying hens with a solution of the drug in both doses did not affect the morphological parameters of chicken eggs: the mass of the eggs themselves and their individual components (proteins, yolks and shells). At the same time, a striking effect of the selective accumulation of nanosilver in the eggshell was found in both research groups after 20 days of the experiment.
4. The results indicate the absence of a toxic effect in the organisms of laying hens after 3 oral administration of the preparation at doses of $2 \cdot 10^{-7}$ and $4 \cdot 10^{-7}$ kg/hen, which is obviously a manifestation of their high adaptive ability in relation to this drug.

[1] L.V. Shevchenko, Y.Y. Dovbnia, T.B. Zheltonozhskaya, N.M. Permyakova, L.M. Vygovska L.M., Ushkalov V.O. Influence of nanosilver preparation in carriers based on polymer/inorganic hybrids on the quality and safety of chicken eggs. Regul. Mech. Biosyst. 2021, *12* (3), 391-395. DOI: 10.15421/022153.
[2] L.V, Shevchenko, Y.Y. Dovbnia, N.M. Permyakova, T.B. Zheltonozhskaya, S.V. Shulyak, D.O. Klymchuk. Influence of nanosilver in hybrid carriers on morphological and biochemical blood parameters of laying hens. Regul. Mech. Biosyst. 2022, *13* (1), 15-22. DOI:10.15421/022203.