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Theoretical and Practical Basics of Obtaining Bactericide Compositions Based On Cluster Silver

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Abstract:

Currently, storage issues are becoming important for the economy, especially for food products. Packaging is the main factor of the quality preservation of products. Requirements for various groups of packaging must protect the products from a variety of extraneous factors and external influences. The main reason for the spoilage of most products is the development of pathogenic microorganisms that can enter the product at any stage of the production line. To prevent economic losses, the development of a new packaging material with antimicrobial and fungicidal properties is topical today. The paper offers a formulation and technology of preparing a bactericidal composition based on cluster silver. Various substances and compounds can be used as an antimicrobial additive in the manufacture: organic acids and their salts, metals, antibiotics; microbial and plant bacteriocins. However, the prolonged action of cluster silver separates it from the known antimicrobial agents. Cluster silver provides a long service life of packaging and long-term preservation of its antimicrobial activity. The antibacterial and fungicidal properties of the bactericidal composition were experimentally confirmed, and its physicochemical parameters were also studied. An optimal method for introducing cluster silver into the structure composition and its concentration at which the composition has the maximum fungicidal activity were determined. The resulting composition can be used to create a packaging material for various groups of food, pharmaceutical and medical products. The use of packaging materials with an antimicrobial activity can significantly increase the shelf life of goods without reducing the quality indicators.

Keywords: antimicrobial packaging, biocidal composition, cluster silver, polymer, microorganism, antimicrobial activity, fungicidal activity

INTRODUCTION

Modern packaging is the final element of the chain, including packaging materials – product – equipment – packaging – sales of the packaged product. Packing in this closed system is responsible for maintaining the quality of the product and its safety; it makes a significant contribution to the ergonomic and economic feasibility of packaged products and their promotion in the market [1-2].

According to numerous studies taking into account the development trends and possible fluctuations in the packaging consumption, it can be assumed that in the next few years the following distribution will remain: polymer materials – 40-50%; paper and cardboard – 30-40%; metals – 10-15%; glass – 5-10% [3-5].

The global packaging market is the leading branch of the economy. The world expenditures on the development of packaging reached more than 580 billion dollars per year; the share of food packaging in the total production structure is 40% [4]. One of the most important factors contributing to the growth of the packaging market is an increase in the production output of industries that consume the bulk of packaging materials and products [6-7]. First of all, these are food industry branches, using from 60% to 85% of most packaging types [5].

At present, the growth dynamics in the consumption of polymer materials in Russia is close to the global one; polymeric packaging materials make up about 30%. The growth rate of polymer packaging, according to various estimates, is 7-13% higher than the growth rate for other materials [8-9]. The accelerated growth of polymer packaging using is associated with fundamentally new quality indicators of modern synthetic materials and packaging products made of them. A high share of polymer packaging is achieved, first, by increasing the demand for polyethylene, polypropylene, resins and other polymers [7].

Among the priority directions of food technologies in the 21st century there are loss prevention, preservation of quality and ensuring the biological safety of products, including food, at all stages of production and subsequent storage [2, 10-11]. Therefore, new properties, such as barrier properties, antimicrobial activity, the possibility of dosing and re-sealing the packaging, long-term resistance to the effects of cold-heat cycles, and many others are added to the classical packaging functions [12-13]. To convert used packaging materials into modern packaging of long-term functional action, their modification is necessary [1].

Antimicrobial packaging materials are very interesting for manufacturers [14], because they are able to provide aseptic conditions and, therefore, the safety of the products, and also significantly increase the shelf life of the goods packed in them [6, 15].

The use of packaging with an expanded protective action complex is especially important for the Russian market, where the transportation of products is carried out over long distances. In addition, this requires an increase in the shelf life, while observing its sanitary safety [16]. In this case, the shelf life of products can be significantly increased without reducing the quality indicators if using polymeric packaging materials with antimicrobial activity.

There is a large number of active antimicrobial effect substances. They are promising for use as additives in packaging materials. They differ in the effectiveness of the action (the spectrum of antimicrobial activity), technological properties, in particular, thermal stability, compatibility with other components, organoleptic characteristics (organic acids and their salts, antibiotics and bacteriocins of microbial and plant origin, metal ions, silver nanoparticles, zinc and others) [9, 17].

Preparations based on silver nanoparticles are particularly common [18], since they have a strong bactericidal and fungicidal action [19-20]. Moreover, the direction of silver antimicrobial action is much broader than that of many antibiotics and sulfonamides. It is established that such additives have a wide spectrum of action, and microorganisms has not developed resistance to them yet. In addition, these drugs remain stable for a long time [6, 21-22].

In addition, silver has a more potent antimicrobial effect than penicillin, biomycin and other antibiotics [23], and has a harmful effect on antibiotic-resistant strains (varieties) of bacteria. Silver exhibits a high bactericidal activity against both aerobic and anaerobic microorganisms (including antibiotic-resistant varieties) and against certain viruses and fungi [24-25].

Colloidal silver is small silver metal particles with a size from 1 nm to several microns, ground in a liquid and forming a colloidal silver solution [25]. Colloidal silver solutions are unstable at high temperatures; after a certain time, silver particles, due to constant collision with each other, stick together and coagulate. The use of stabilizing substances in colloidal solutions, which form a protective layer in silver particles and interfere with their interaction, makes it possible to obtain solutions of colloidal silver that are stable for a long time [8, 26]. Colloidal silver in contact with air oxidizes with time; silver salts are slowly formed and turn into solution. Thus, colloidal silver particles are a kind of a "generator" of silver ions [27].

Cluster silver is a special kind of colloidal silver with a smaller size of silver particles.

There is a certain particle size distribution in both cluster and colloidal silver preparations: narrower and shifted into the region of nanoparticles (cluster preparations) or wider and displaced into the region of colloidal particles (colloidal preparations). That is, in classical colloidal preparations, clusters and silver nanoparticles are present [16, 23].

The urgency of the work is conditioned by the need to provide the food, pharmaceutical industry and medicine with safe packaging materials that have antibacterial and fungicidal properties in order to prevent the spread of diseases of a microbiological nature and to prevent the contamination of food products and pharmaceutical substances by pathogenic microorganisms.

According to the research of the World Packaging Organization (WPO), in most developing countries, about half of the final product is spoiled and does not get to the consumer due to the lack of special packaging materials. At the same time, in developed countries, where packaging technologies correspond to the current level, only 2-3% of products are affected by spoilage [28].

The purpose of this work is to develop a technology for obtaining a composition with antimicrobial properties for various industries, including medicine.

OBJECTS AND RESEARCH METHODS

Organoleptic parameters. The appearance, color and uniformity of solutions having a liquid consistency were determined by viewing tubes with liquid in transmitted or reflected daylight, or the light of an electric lamp.

Optical properties. The optical absorption spectra of cluster silver solutions and the resulting composition were determined using a UV 1800 spectrophotometer (Shimadzu, Japan).

Determination of density. The density of solutions was determined by the physical method. After measuring the mass (m) and the volume (V) of the liquid, the density of the liquid was calculated by the formula (1):

$$\rho = \frac{m}{V} \quad (1)$$

A clean flask was used; its weight was measured using a balance. Then a portion of liquid was poured into a beaker, its volume was measured. The liquid was poured into the flask. The flask together with the liquid was weighed. Knowing the mass of the dry flask, we calculated the mass of water. According to the known volume and mass of the liquid, the density was determined.

Hydrogen index. The pH value was determined by the electrometric method. The method is based on the fact that when the electrode is immersed in the solution, ions are exchanged between the electrode and the solution, so that a potential appears on the electrode, the value of which depends on the concentration of hydrogen ions in the solution. Starting the analysis, a portion of the product was taken from the prepared sample to ensure immersion of the electrodes. The electrodes were immersed into the cup with the product and after stabilization of the instrument, the pH value was determined. The measurements were carried out three times, each time taking out the electrodes and, when measuring, immersing them again in the test product.

Analysis of the distribution size of cluster silver by laser diffraction. The analysis of the granulometric composition of silver nanoparticles was carried out using a Shimadzu SALD 7101 laser diffraction particle size analyzer.

Two separate probes of each sample of colloidal solutions of cluster silver were measured to obtain particle size distribution histograms, as well as the mean particle diameter MEANV (μm), MEDIAND (μm), distribution mode MODALD (μm), and the levels of cumulative percentage at 25%, 50%, 75%. The measurements were

carried out in distilled water with constant mechanical stirring and under the influence of ultrasonic vibrations to prevent agglomeration of the particles.

Analysis of the antimicrobial activity of biocidal compositions by the diffusion method. The test strain was plated on the agar nutrient medium in a lawn and simultaneously paper discs impregnated with a biocidal composition (10 µl/disk) were placed on the lawn. The disks were disposed so that the distance between their centers was not less than 24 mm. After placing the discs on the agar, they were pressed with a sterile needle or tweezers until they completely contacted the surface of the medium. As a control, a disk with the MRS medium was used, as a reference preparation – a disk with the antibiotic ciprofloxacin (from a standard kit). The plates were incubated at 37 °C for 24 hours. The results were taken into account by the presence and size (in mm) of the transparent zone of non-growth of microorganisms around the disk.

When studying the antimicrobial activity of cluster silver solutions, the following test strains were used: *Escherichia coli B 4207*; *Pseudomonas aeruginosa B 6643*; *Staphylococcus aureus B 8171*; *Aspergillus niger F 876*; *Enterococcus hirae B 5099*; *Bacillus subtilis B 1448*; *Salmonella enteridis ATCC 13076*; *Candida albicans Y 2808*.

RESULTS AND THEIR DISCUSSION

In order to introduce cluster silver into various materials to impart antimicrobial properties, it is necessary to obtain a silver dispersion in an organic solvent. Due to the small particle size, it is difficult to separate particles from the aqueous phase, so there are methods for obtaining silver nanoparticles in organic solvents, with further drying and dispersing. During the interaction, silver complexes are formed, which, when heated, decompose to form metal nanoparticles. Organic solvents act as a complexing agent and a stabilizer.

In this research, the AGM synthesis and AGL synthesis methods were used to prepare cluster silver solutions.

AGM synthesis is a modified high-molecular process, which involves the reduction of silver nitrate with ethylene glycol in the presence of a stabilizer (polyvinylpyrrolidone).

An aqueous medium with polyvinylpyrrolidone and ethanol (concentration $30 \cdot 10^{-6}$ mol/dm^3), and a 1 N solution of $AgNO_3$ was used in experiment. The mole ratio of polyvinylpyrrolidone/nitrate varied from 0.2 to 4.0.

The silver nitrate reduction reaction was carried out in the "dropwise synthesis" regime with intensive mixing at a temperature of 70 °C. Under these conditions, polyvinylpyrrolidone with ethanol is simultaneously a reducer of Ag^+ ions and a stabilizer (ligand) of the Ag particles formed.

This method allowed obtaining a cluster silver solution with a concentration of 1% (10,000 ppm). The results of determining the size distribution of silver nanoparticles in polyvinylpyrrolidone are shown in Fig. 1.

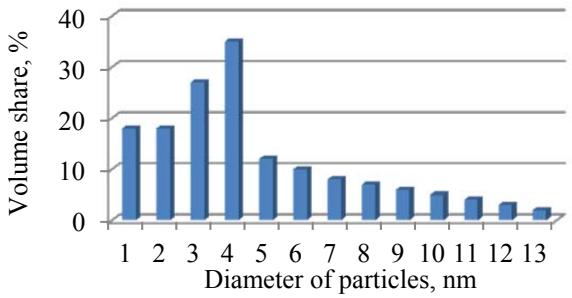

Fig. 1. Cluster silver particles distribution in a colloidal solution (synthesis into polyvinylpyrrolidone)

The data in Fig. 1 indicate that the maximum size of silver nanoparticles is 10-13 nm, while the average particle size is 3-4 nm.

22.5 mg of silver nitrate ($AgNO_3$) was dissolved in 250 ml of distilled water in the AGL synthesis procedure. Then the solution was brought to a boil with vigorous stirring, after which 4.5 ml of a 1% aqueous solution of sodium citrate ($Na_3C_6H_5O_7 \cdot H_2O$) was rapidly added dropwise, which corresponds to a molar ratio of $AgNO_3/Na_3C_6H_5O_7 \cdot H_2O$ equal to 1:1.029.

Since silver particles were obtained with a certain excess of a reducing agent, it can be assumed that all the original silver is reduced to a metallic state. As a rule, the particle size of silver after such synthesis is 2-20 nm.

However, the particle size is also determined by the temperature in the reactor and the composition of the reaction medium. It was possible to synthesize stable silver particles in a cluster state (1-2 nm in size using a controlled combination of ethanol and glycol compounds as a reducing agent.

Cluster silver, obtained by the AGL synthesis method, is a colorless liquid with a metal content of 1% (10,000 ppm). Fig. 2 shows the distribution curve of cluster silver particles by size; it follows that the most probable size of silver nanoparticles is 1-2 nm.

The appearance, color, opalescence, uniformity, optical properties, pH, density, viscosity, as well as the stability of the solution over time (coagulation and precipitation) were studied for silver nanoparticles synthesized by two techniques. The results of investigation of the properties of cluster silver solutions are shown in Table 1.

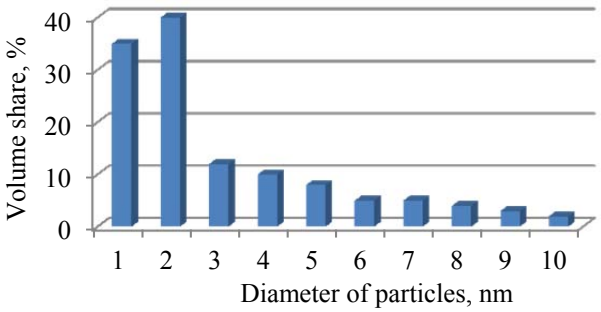

Fig. 2. Cluster silver particles distribution in a colloidal solution (citrate synthesis)

Table 1 – Generalized properties of cluster silver solutions obtained by different methods

Characteristic	Cluster silver solution	
	AGM	AGL
Appearance, color, uniformity	transparent colloid, slightly yellowish with light opalescence	transparent, colorless colloid with light opalescence
Optical properties	light scattering	light scattering
pH (hydrogen index)	4.6	4.8
Eh (redox potential), mV	149.2	141.8
Density at 20 °C, kg/dm^3	1.4	1.3
Viscosity at 20 °C, mm^2/s	11	10
Average particle size of silver, nm	3-4	1-2
Solution stability in time	Stable colloid, precipitate does not drop out	Metastable colloid, precipitate does not drop out

As the obtained cluster silver solutions are used to obtain antimicrobial compositions, it is necessary to study their bactericidal properties: antibacterial and fungicidal.

The antimicrobial activity of silver and its preparations is associated with the complexing, biochemical and catalytic action of silver ions on bacterial enzymes, proteins and membrane structures.

In the study, the quantitative *in vitro* method was used to assess the sensitivity of microbial strains to cluster silver solutions obtained by AGM synthesis and AGL synthesis, taken in different concentrations: 10 ppm (0.001%), 1 ppm (0.0001%), 0.1 ppm (0.00001%), 0.01 ppm (0.000001%).

The antimicrobial activity of cluster silver was determined by analyzing the survival of microbes using the colony-forming unit count (CFU) method; the results are shown in Figs. 3-4.

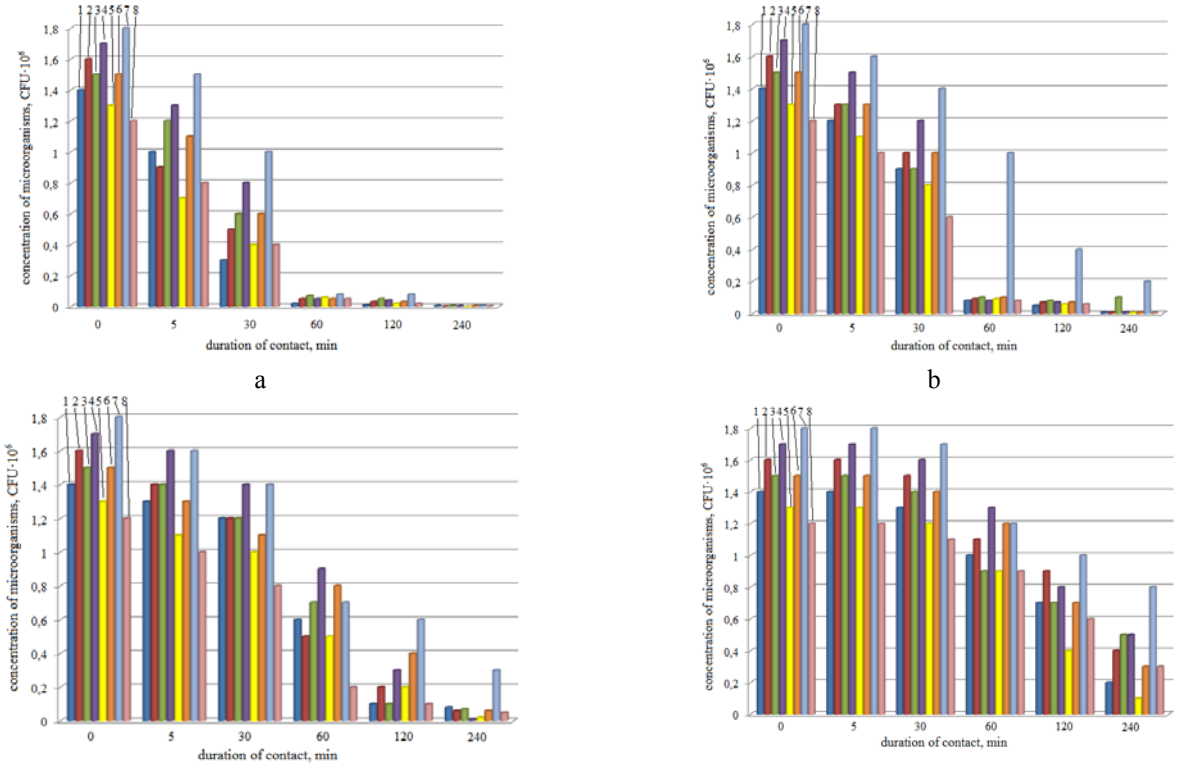

Fig. 3. Results of the antibacterial activity study of cluster silver solution obtained by AGM synthesis

1 – *Escherichia coli* B 4207; 2 – *Pseudomonas aeruginosa* B 6643;
3 – *Staphylococcus aureus* B 8171; 4 – *Aspergillus niger* F 876; 5 – *Enterococcus hirae* B 5099;
6 – *Bacillus subtilis* B 1448; 7 – *Salmonella enteridis* ATCC 13076; 8 – *Candida albicans* Y 2808;
a – concentration 10 ppm; b – concentration 1 ppm;
c – concentration 0.1 ppm; d – concentration 0.01 ppm

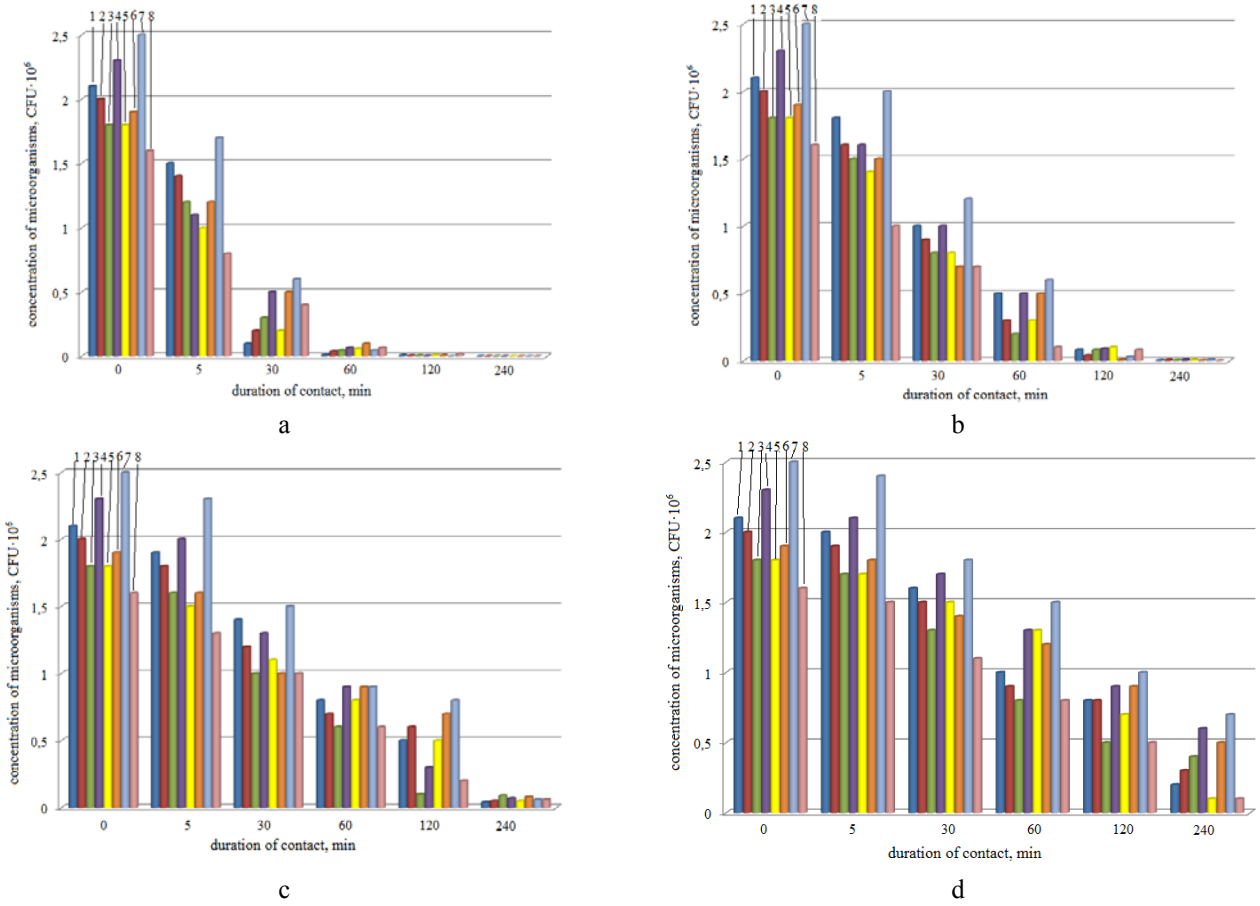

Fig. 4. Results of antibacterial activity study of cluster silver solution obtained by AGL synthesis:
1 – *Escherichia coli* B 4207; 2 – *Pseudomonas aeruginosa* B 6643;
3 – *Staphylococcus aureus* B 8171; 4 – *Aspergillus niger* F 876; 5 – *Enterococcus hirae* B 5099;
6 – *Bacillus subtilis* B 1448; 7 – *Salmonella enteridis* ATCC 13076; 8 – *Candida albicans* Y 2808;
a – concentration 10 ppm; b – concentration 1 ppm;
c – concentration 0.1 ppm; d – concentration 0.01 ppm

The indices of the ability or inability of the tested microorganisms to reproduce *in vitro* in cluster silver solutions of different concentrations were classified as follows:

- stability: the microorganism is not suppressed completely by the indicated concentration of nanosilver;
- sensitivity: the microorganism is suppressed by the indicated concentration of nanosilver;
- average sensitivity: the microorganism is partially suppressed by the indicated concentration of nanosilver;
- the lack of microorganisms growth, which indicates the antimicrobial effect of nanosilver.

The analysis of Fig. 4 indicates that the cluster silver solution obtained by AGM synthesis shows an antimicrobial effect starting at a concentration of 0.01 ppm (0.00001% silver content). It is also clear from Fig. 4 that the antimicrobial effect of the cluster silver solution is directly proportional to the concentration of silver. It can be seen that all tested strains are equally sensitive to the action of the cluster silver solution.

Similar results were obtained for a solution of cluster silver synthesized by the AGL method (Fig. 6): antimicrobial action is manifested at silver concentrations ranging from 0.01 ppm; all tested strains are equally sensitive to the action of cluster silver solution.

A comparative analysis of Figs. 3-4 showed that the cluster silver solution obtained by AGL synthesis has the most pronounced antimicrobial effect in comparison with the solution obtained by AGM synthesis.

The next stage of research is development of the optimal antimicrobial compositions based on cluster silver in order to obtain a composition with bactericidal properties. The following substances with bactericidal action have been studied in the developed biocidal compositions: citric acid, acetic acid, sorbic acid, sodium benzoate, hydrogen peroxide, flaxseed oil, glycerin, and urotropine. The compositions of the tested biocidal compositions are shown in Table 2.

For biocidal compositions, which are presented in Table 2, antibacterial and fungicidal properties were studied using the disc-diffusion method. The results are shown in Table 3.

Table 2 – Biocidal compositions

Number of the biocidal composition	Amount of ingredient, wt%										
	AGM 1 ppm	AGL 1 ppm	LA	AA	SA	SB	HP	LO	GR	UT	H_2O
1	0.5	-	5.0	-	-	-	-	-	-	-	94.5
2	-	0.5	5.0	-	-	-	-	-	-	-	94.5
3	0.5	-	3.0	-	-	-	2.5	-	-	-	94.0
4	-	0.5	3.0	-	-	-	2.5	-	-	-	94.0
5	0.5	-	-	5.0	-	-	-	5.0	-	-	89.5
6	-	0.5	-	5.0	-	-	-	5.0	-	-	89.5
7	0.5	-	-	-	10.0	-	-	5.0	-	-	84.5
8	-	0.5	-	-	10.0	-	-	5.0	-	-	84.5
9	0.5	-	4.0	-	-	-	-	2.5	1.0	-	92.0
10	-	0.5	4.0	-	-	-	-	2.5	1.0	-	92.0
11	0.5	-	3.0	-	-	2.0	3.0	-	2.0	-	89.5
12	-	0.5	3.0	-	-	2.0	3.0	-	2.0	-	89.5
13	0.5	-	-	-	-	-	-	-	-	3.5	96.0
14	-	0.5	-	-	-	-	-	-	-	3.5	96.0
15	0.5	-	-	-	-	-	5.0	-	-	-	94.5
16	-	0.5	-	-	-	-	5.0	-	-	-	94.5

Note: LA – lemon acid, AA – acetic acid, SA – sorbic acid, SB – sodium benzoate, HP – hydrogen peroxide, LO – linseed oil, GR – glycerol, UT – urotropine

Table 3 – Results of studying the antibacterial and fungicidal properties of biocidal compositions based on colloidal silver solutions

Number of the biocidal composition	Diameter of the inhibition zone for different test strains, mm							
	Escherichia coli B 4207	*Pseudomonas aeruginosa* B 6643	*Staphylococcus aureus* B 8171	*Aspergillus niger* F 876	*Enterococcus hirae* B 5099	*Bacillus subtilis* B 1448	*Salmonella enteridis* ATCC 13076	*Candida albicans* Y 2808
1	9.0±0.9	3.5±0.4	4.0±0.4	10.0±1.0	7.5±0.8	6.0±0.6	5.0±0.5	0
2	0	1.5±0.2	3.0±0.3	0	6.0±0.6	2.0±0.2	0	1.0±0.1
3	15.0±1.5	22.0±2.2	17.0±1.7	20.0±2.0	26.0±2.6	23.0±2.3	25.0±2.5	19.0±1.9
4	8.5±0.9	5.0±0.5	7.0±0.7	11.0±1.1	13.5±1.4	6.0±0.6	4.0±0.4	3.0±0.3
5	0	0	2.5±0.3	4.0±0.4	3.0±0.3	1.5±0.2	2.0±0.2	0
6	24.0±2.4	18.0±1.8	26.0±2.6	18.0±1.8	22.0±2.2	15.0±1.5	24.0±2.4	27.0±2.7
7	5.5±0.6	7.0±0.7	2.0±0.2	0	1.0±0.1	3.0±0.3	4.5±0.5	1.5±0.2
8	0	5.0±0.5	8.0±0.8	10.0±1.0	5.5±0.6	3.0±0.3	2.0±0.2	4.0±0.4
9	24.5±2.5	26.0±2.6	30.0±3.0	18.0±1.8	17.5±1.8	25.0±2.5	27.0±2.7	25.5±2.6
10	3.0±0.3	5.0±0.5	0	7.5±0.8	0	0	2.0±0.2	5.5±0.6
11	21.0±2.1	19.0±1.9	18.0±1.8	25.0±2.5	23.5±2.4	17.5±1.8	26.0±2.6	25.0±2.5
12	0	5.0±0.5	0	0	4.0±0.4	2.0±0.2	1.0±0.1	3.5±0.4
13	11.0±1.1	8.0±0.8	6.0±0.6	4.0±0.4	2.5±0.3	7.5±0.8	0	0
14	5.0±0.5	4.0±0.4	3.0±0.3	2.5±0.3	0	1.5±0.2	4.0±0.4	6.0±0.6
15	8.0±0.8	5.5±0.6	2.0±0.2	3.0±0.3	4.5±0.5	0	0	0
16	5.0±0.5	3.0±0.3	0	4.0±0.4	0	2.0±0.2	0	0

From the data analysis in Table 3, it is clear that the bactericidal compositions No. 3, No. 6, No. 9 and No. 11 based on cluster silver solutions are characterized as the maximum antimicrobial (antibacterial and fungicidal) properties. These biocidal compositions will be used in further research.

For the selected antimicrobial compositions based on cluster silver solutions, such physicochemical properties as appearance, optical properties, pH, redox potential, density, viscosity, stability of the solution over time were studied (Table 4).

Table 4 – Physicochemical properties of antimicrobial compositions based on cluster silver solutions

Characteristic	Number of biocidal composition			
	3	**6**	**9**	**11**
Appearance, color, uniformity	Transparent colloid, colorless, homogeneous	White color emulsion, heterogeneous	Transparent colloid, colorless, homogeneous	Transparent colloid, colorless, homogeneous
Optical properties	light scattering	light scattering	light scattering	light scattering
pH (hydrogen index)	4.1	5.5	5.9	7.6
Eh (redox potential), mV	184.5	141.8	77.6	-44.5
Density at 20 °C, kg/dm^3	1.2	1.4	1.4	1.4
Viscosity at 20 °C, mm^2/s	10	10	12	12
Solution stability in time	Stable colloid, precipitate does not drop out	Stable colloid, precipitate does not drop out	Stable colloid, precipitate does not drop out	Stable colloid, precipitate does not drop out

The studying of the optical properties of biocidal compositions based on cluster silver solutions has a special meaning. The optical absorption spectra of the tested biocidal compositions are shown in Fig. 5.

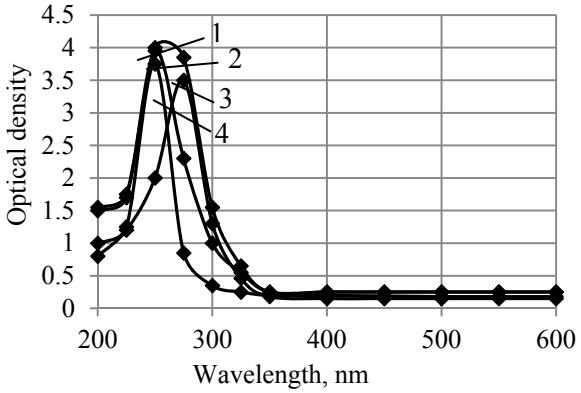

Fig. 5. Optical absorption spectra of biocidal compositions based on cluster silver solutions:
1 – composition No. 3; 2 – composition No. 6; 3 – composition No. 9; 4 – composition No. 11

As a result of the analysis of Fig. 5, it was concluded that the maximum optical absorption of the biocidal composition No. 3 falls at a wavelength of 250.55 nm, biocidal composition No. 6 – at a wavelength of 250.65 nm, biocidal composition No. 9 – at a wavelength of 266, 75 nm, biocidal composition No. 11 – at a wavelength of 279.25 nm.

The obtained biocidal compositions are expediently used in the technology of obtaining a packaging material with antimicrobial properties.

An important condition for the applicability of antimicrobial compositions based on cluster silver solutions for the manufacture of packaging material is their ability to anchor on the surface or in the bulk of the substrate. This condition can be ensured by using various variants of introducing bactericidal compositions into the structure of the packaging material.

Various methods for introducing the cluster silver solution into the package structure are investigated:

1. Spraying the bactericidal composition onto the surface of the packaging material;
2. Immersing the packaging material in a container with a bactericidal composition and holding for a certain period;
3. The introduction of the bactericidal composition into the suspension of the resulting mass of the semi-finished product in the manufacturing process of the packaging material.

In each of the obtaining methods, bactericidal compositions No. 3, 6, 9 and 11 were used in different concentrations (0.05 wt%, 0.15 wt%, 0.25 wt%). The antimicrobial activity of the packaging material with respect to the four test strains (*Escherichia coli B 4207*, *Staphylococcus aureus B 8171*, *Aspergillus niger F 876*, *Candida albicans Y 2808*) was analyzed by diffusion to agar. The results are shown in Table 5.

From Table 5 it follows that the most effective way of introducing into the matrix structure is to introduce the bactericidal composition into the slurry of the mass of the semi-finished product in the manufacturing process of the packaging material. In this case, the maximum antibacterial activity against strains of *Escherichia coli B 4207* and *Staphylococcus aureus B 8171* and the maximum fungicidal activity with respect to *Aspergillus niger F 876* and *Candida albicans Y 2808* are observed. Table 5 also shows that the least effective a method of manufacturing a packaging material with antimicrobial properties is spraying the bactericidal composition onto the surface of the packaging material.

The analysis of the data presented in Table 5 revealed that 0.15 wt% is the optimal concentration of bactericidal compositions introduced into the structure of the packaging material. A further increase in the concentration of biocidal compositions is not advisable, since it does not lead to a significant increase in the antimicrobial activity of the packaging material.

Table 5 – Antimicrobial properties testing results of packaging material treated with biocidal compositions based on cluster silver solutions

Number of the biocidal composition/concentration	Inhibition zone diameter for different treatment methods and different test strains, mm											
	Spraying				Immersion				Technological process			
	Escherichia coli B 4207	*Staphylococcus aureus* B 8171	*Aspergillus niger* F 876	*Candida albicans* Y2808	*Escherichia coli* B 4207	*Staphylococcus aureus* B 8171	*Aspergillus niger* F 876	*Candida albicans* Y2808	*Escherichia coli* B 4207	*Staphylococcus aureus* B 8171	*Aspergillus niger* F 876	*Candida albicans* Y2808
No. 3 0.05 wt%	5.0± 0.5	3.0± 0.3	2.0± 0.2	4.0± 0.4	7.5± 0.8	5.5± 0.6	4.0± 0.4	6.0± 0.6	15.0± 1.5	18.5±1.9	16.0±1.6	17.0±1.7
0.15 wt%	10.0±1.0	7.0± 0.7	5.5± 0.6	8.0± 0.8	12.5±1.3	9.0± 0.9	7.0± 0.7	10.5±1.1	23.0± 2.3	25.0±2.5	24.5±2.5	26.0±2.6
0.25 wt%	11.0±1.1	7.0± 0.7	6.0± 0.6	8.5± 0.9	12.0±1.2	9.5± 1.0	7.5± 0.8	11.0±1.1	22.5± 2.3	25.5±2.6	25.0±2.5	26.5±2.7
No. 6 0.05 wt%	0	2.0± 0.2	4.0± 0.4	3.0± 0.3	5.5± 0.6	7.0± 0.7	10.0±1.0	6.0± 0.6	17.0± 1.7	22.0±2.2	19.0±1.9	20.5±2.0
0.15 wt%	2.5± 0.3	4.0± 0.4	6.0± 0.6	5.0± 0.5	11.0±1.1	13.5±1.4	12.0±1.2	9.0± 0.9	25.0± 2.5	28.0±2.8	23.5±2.4	24.0±2.4
0.25 wt%	3.0± 0.3	4.0± 0.4	6.5± 0.7	5.5± 0.6	11.5±1.2	14.0±1.4	12.5±1.3	9.0± 0.9	25.5± 2.6	28.0±2.8	24.0±2.4	24.5±2.5
No. 9 0.05 wt%	7.0± 0.7	5.5± 0.6	4.5± 0.5	3.0± 0.3	11.0±1.1	10.0±1.0	9.5± 1.0	6.5± 0.7	18.0± 1.8	19.5±2.0	17.0±1.7	15.0±1.5
0.15 wt%	14.0±1.4	12.5±1.3	10.0±1.0	7.5± 0.8	16.5±1.7	13.0±1.3	12.5±1.3	10.0±1.0	26.0± 2.6	25.5±2.6	23.0±2.3	20.0±2.0
0.25 wt%	14.5±1.5	13.0±1.3	10.0±1.0	8.0± 0.8	17.0±1.7	13.0±1.3	13.0±1.3	10.0±1.0	25.5± 2.6	26.0±2.6	23.0±2.3	21.0±2.1
No. 11 0.05 wt%	5.5± 0.6	6.0± 0.6	7.0± 0.7	4.0± 0.4	8.0± 0.8	13.5±1.4	16.0±1.6	9.0± 0.9	20.0± 2.0	23.0±2.3	21.5±2.2	18.0±1.8
0.15 wt%	9.0± 0.9	11.0±1.1	15.0±1.5	8.5± 0.9	15.5±1.6	17.0±1.7	18.0±1.8	14.5±1.5	24.5± 2.5	28.0±2.8	25.0±2.5	24.5±2.5
0.25 wt%	9.5± 1.0	12.0±1.2	15.5±1.6	9.0± 0.9	16.0±1.6	17.5±1.8	18.0±1.8	15.0±1.5	25.0± 2.5	28.0±2.8	25.0±2.5	25.0±2.5

CONCLUSION

Four optimal antimicrobial compositions based on cluster silver firmly fixed with a natural polymer were developed:

1. 0.5 wt% AGM (1 ppm) + 3.0 wt% citric acid + 2.5 wt% hydrogen peroxide + 94.0 wt% water;
2. 0.5 wt% AGL (1 ppm) + 5.0 wt% acetic acid + 5.0 wt% of flax oil + 89.5 wt% water;
3. 0.5 wt% AGM (1 ppm) + 4.0 wt% citric acid + 2.5 wt% linseed oil + 1.0 wt% of glycerin + 92.0 wt% water;
4. 0.5 wt% AGM (1 ppm) + 3.0 wt% citric acid + 2.0 wt% sodium benzoate + 2.0 wt% of glycerin + 89.5 wt% water.

The results of the performed studies of the quality indices of antimicrobial compositions based on cluster silver solutions are presented. The physicochemical, antibacterial and fungicidal properties determining the quality of such a composition have been determined, and an optimal concentration of bactericidal compositions insertion into the structure of the packaging material has been established.

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