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DETERGENT-DISINFECTANT AGENT BASED ON COLLOIDAL SILVER STABILIZED BY QUATERNARY AMMONIUM COMPOUNDS FOR THE DAIRY INDUSTRY

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Abstract

Within the framework of the present work, in order to obtain the basis for a detergent, a method for the synthesis of colloidal silver stabilized by a quaternary ammonium compound was developed and optimized.

The preparation of colloidal silver was obtained by the method of chemical reduction in aqueous medium using didecyldimethylammonium bromide as a stabilizer, silver nitrate as a precursor, and sodium borohydride as a reducing agent. The structure, morphology, properties and size of silver particles in the preparation were studied using photon correlation spectroscopy, atomic force and scanning electron microscopy, acoustic and electroacoustic spectroscopy. By means of neural network modelling, the process of washing away protein-fat contamination from the working surface with the developed detergent has been optimized in a model experiment. A model experiment was carried out under conditions of circulation washing at the temperatures of 50 - 55 °C. The working surfaces in the model experiment were glass, steel and aluminium plates. The effectiveness of the detergent based on colloidal silver preparation has been studied. For this purpose, during the experiment, the time of complete removal of contamination from the surface of the plates, as well as the change in the pH and conductivity of the detergents solutions with the change of the contamination concentration were registered. The corrosion rate was determined by the gravimetric method, based on the difference in the mass of the sample before and after washing, respected to the unit of surface and to the unit of time

It has been established that colloidal silver particles are spherical with an average hydrodynamic diameter of about 50 nm. The colloidal preparation shows high aggregative stability. A study of the quality of sanitization of working surfaces with the developed detergent made it possible to establish the fact of the complete absence of bacteria of the group of *Escherichia coli*, pathogenic microorganisms, including *Salmonella* spp., on the working surfaces treated with detergent.

It has been determined that the developed detergent does not possess corrosive properties, and completely washed off from the working surfaces upon completion of the washing process and therefore can compete with modern detergents used at dairy enterprises.

Key words: Colloidal silver, Detergent-disinfectant, Sanitizing, dairy industry, Washing.

1. Introduction

The high quality of dairy products directly depends on the efficiency of the washing and disinfecting processes, technological equipment and containers. Obvious, that high-quality sanitization of the working surface



prevents microbial infection of the finished products of the dairy industry, ensuring its safety for the consumers [1, 2, and 3].

The main tools used in the dairy industry for cleaning and disinfecting the working surfaces of containers and equipment are various detergent-disinfectants that must have: low toxicity, high bactericidal and fungicidal activities, and ensure good washability of contaminants from the working surfaces.

Colloidal silver may be used as a disinfectant, as shown in papers of various authors [4, 5, 6, 7, and 8]. Colloidal silver has important advantages compared with other disinfecting agents, as: no addiction to microorganisms, safety in relation to the human body, versatility of action, and high antimicrobial effect. High bactericidal activity of colloidal silver particles extends to more than 600 species of bacteria, viruses, and fungi. The unique physicochemical and biomedical properties of colloidal silver particles make it possible to use them in various fields of medicine, food industry, sanitation and other sectors of the national economy for disinfection of: water, surfaces, antimicrobial protection of clothing, footwear, household items, food preservation and much more.

Colloidal silver stabilized by quaternary ammonium compounds, possessing high bactericidal activity, has low working concentrations, which helps to reduce the cost of the disinfection process and also prevents pathogenic microorganisms resistant to traditional disinfectants from entering the finished product.

The purpose of this research is to assess the possibility of using colloidal silver, stabilized by a quaternary ammonium compound and a non-ionic surfactant, as a detergent-disinfectant at dairy industry enterprises, as well as to determine the effectiveness of washing off protein-fat contaminations and the quality of sanitary treatment of working surfaces with the developed detergent-disinfectant.

2 Materials and Methods

The preparation of colloidal silver was obtained by the method of chemical reduction in an aqueous medium using didecyldimethylammonium bromide (DDAB) and non-ionic surfactant Kolliphor HS 15 as a stabilizer, $AgNO_3$ silver nitrate as a precursor, and sodium borohydride NaBH₄ as a reducing agent. Synthesis of colloidal silver stabilized by DDAB was carried out stepwise. At the first stage of the synthesis sodium borohydride and Kolliphor HS 15 were added into the DDAB solution under vigorous stirring. The resulting reaction mixture was stirred for 15 minutes until a homogeneous, clear solution was formed. At the second stage, the solution of silver nitrate was prepared. At the third stage, an aqueous solution of silver nitrate was added dropwise

with vigorous stirring (1400 rpm) to the solution of the reducing agent and stabilizers. Stirring of the resulting solution was continued for a 1 hour. Upon completion of the synthesis, the preparation was poured into glass containers and stored at t = 5 °C.

Determination of the size, morphology, and structure of colloidal silver particles was carried out by the following methods:

- 1. Photon-correlation spectroscopy of dynamic light scattering on the "Photocor Complex" setting (LLC Antek-97, Russia). Computer processing of the data was performed using the DynaLS software.
- 2. Acoustic and electroacoustic spectroscopy was carried out on a DT 1202 spectrometer (Dispersion technology, Inc., USA) [9, 10].
- 3. Atomic force microscopy (AFM) was performed using an atomic force microscope NT-MDT Ntegra Aura (NT-MDT, Russia) [11, 12]. Before starting the measurements, the preparation was applied on glass substrates by centrifugation. Then glass substrates were dried at room temperature. Measurements were conducted in semi-contact mode.

The study of the effect of various parameters on the washability of protein-fat contamination from the working surface was carried out as part of a model experiment that simulates the actual conditions of washing and disinfecting of contaminated working surfaces of manufacturing equipment and containers in enterprises. The study of detergent properties was carried out in the mode of circulation washing at a temperature of 50 - 55 °C. Sour cream with a mass fraction of fat of 20% served as a polluter; glass plates served as a working surface.

To determine the optimal values of various factors (variable parameters) affecting the washability, a multivariate experiment was conducted. As a result, the following parameters of the washing process were identified as the most significant:

- 1. Active acidity of washing solutions (pH).
- 2. Solution temperature (t), °C.
- 3. Exposure time (τ), min.
- 4. The working concentration of the solution (C) (%).

The output parameter Y is the washability of contamination from the surface of metal plates by a solution of a developed detergent-disinfectant (%). Washability was calculated as follows:

$$Y = \frac{M_2 - M_1}{m} \cdot 100\%$$
 (1)

Where: Y is the washability of contamination, %; M_2 is the mass of the plate with the pollutant before the washing, g; M_1 is the mass of the plate after the washing, g; m is the mass of the pollutant on the plate, g.



The variation levels of all the specified variables are presented in Table 1.

Variables	Variation levels			
рН	3	6	9	12
t, ⁰C	20	40	60	80
τ, min	5	10	15	20
C (preparation), wt.%	0.001	0.01	0.1	1

To study the mutual influence of all factors with a minimum number of experiments, the planning matrix presented in Table 2 was used.

Table 2. Experiment planning matrix with an indicationof the numerical values of variable parameters for eachexperiment

Experiment N°	pН	<i>t,</i> ⁰C	τ, min	C, wt.%
1	3	20	5	0.001
2	3	40	10	0.01
3	3	60	15	0.1
4	3	80	20	1
5	6	20	10	0.1
6	6	40	5	1
7	6	60	20	0.001
8	6	80	15	0.01
9	9	20	15	1
10	9	40	20	0.1
11	9	60	5	0.01
12	9	80	10	0.001
13	12	20	20	0.01
14	12	40	15	0.001
15	12	60	10	1
16	12	80	5	0.1

Mathematical processing of the experimental data obtained was performed using the Neural Statistica Network application software package.

The study of the effectiveness of the use of the developed detergent-disinfectant was carried out in the mode of circulation washing at the temperature of the solution of 50 - 55 °C. The pollutant was sour cream with a fat content of 20%. A 0.1% solution of colloidal silver and a 0.1% solution of an alkaline detergent were used as test solutions. In the experiment, the time of complete washing of contamination from the surface of the plates, as well as the change in the active acidity and the dependence of the conductivity of the detergents solution on the concentration of contamination were recorded.

Evaluation of the quality of sanitary treatment of the working surface using the developed detergent-disinfectant based on colloidal silver preparation was carried out on the basis of the FSBI"Stavropol Interregional Veterinary Laboratory" (FSBI "Stavropol MVL", Stavropol, Russia). For comparison, an alkaline detergent was used. A model experiment was performed under conditions of circulation washing at the temperature of the solution of 50 - 55 °C. In the experiment, washings were taken from the surface of the glass, steel, and aluminum plates. The determined microbiological indicators were: *E. coli*; total microbial count; pathogens, including *Salmonella* spp. [13].

Further, the corrosivity of the developed colloidal silver preparation with respect to the different metals was investigated. The objects of this study were samples of aluminum and steel plates with a size of 25×25 mm. Before testing, the surface of the metal plates was polished. Comparison solvents were tap and distilled water.

The essence of the experiment was as follows: the metal plates were dipped into the test medium for 100 hours, the test was carried out at temperatures of 20, 35 and 50 °C. The plates were weighed before and after testing. The corrosion rate was determined by the gravimetric method, based on the difference in sample mass before and after weighing (accurate to 0.0001 g), referred to a unit of surface (1 m²) and a unit of time (hour, year). The change in the mass of the samples was transferred to a deep indicator characterizing the decrease in the thickness of the metal (mm per year).

The corrosion rate (mm / year) was determined as follows [14]:

$$C = \frac{8760 \cdot L}{\rho \cdot 1000}$$
(2)

Where: C is the corrosion rate, mm / year; L - the loss of metal, $[g / (m^2 \cdot h)]$; $324 \cdot 24 = 8760$ - hours per year, h; ρ is the metal density, kg/m³.

The method for determining the degree of removal of the detergent-disinfectant from the glass surface was as follows. The developed detergent was applied to the glass surface and dried. Then, the transmission spectra of the obtained samples were taken. After that, the plate was subjected to standard modes of washing and rinsing, and the transmission spectra were measured again.

In order to determine the mass fraction of DDAB, upon completion of all washing steps, the washed glass plate was additionally placed in a container with distilled water. After one day, the plate was removed, and the obtained sample was subjected to two-phase titration with a mixed indicator. Preparation for the analysis included preparation of a solution of a mixed indicator of bromphenol blue and chromic dark blue, buffer solution with the pH = 11, and an aqueous solution of sodium dodecyl sulfate with a molar concentration of 0.004 mol/L.



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3. Results and Discussion

Processing the experimental data of photon correlation spectroscopy allowed us to obtain a histogram of the size distribution of colloidal silver particles in the preparation, which is shown in Figure 1.

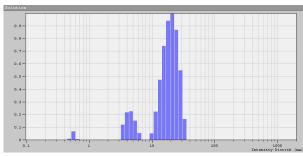
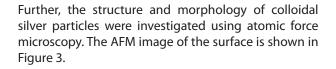


Figure 1. Histogram of the hydrodynamic radii distribution for colloidal silver particles in an optimized preparation

It was established that in the colloidal silver preparation there are two fractions of particles. The average hydrodynamic radius of the first fraction was about 4.5 nm, and the second is 20 nm.

Figure 2 shows a histogram of the colloidal silver particles size distribution, obtained using acoustic spectroscopy.



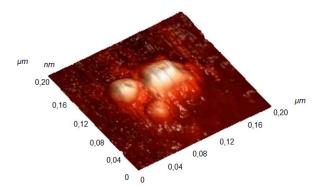
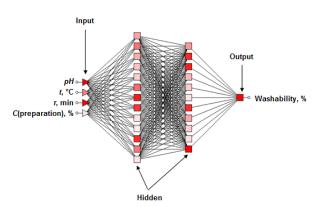


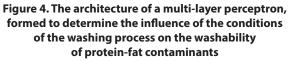
Figure 3. AFM image of colloidal silver particles

Analysis of the AFM image presented in Figure 3 showed that silver particles have a spherical shape with a diameter of about 40 - 50 nm.

At the next stage, the effect of various parameters on the washability of protein-fat contamination from the working surface was investigated in a model experiment.

As a result of the mathematical processing of the experimental data in the Neural Statistica Network software package, a neural network was formed, which architecture is shown in Figure 4.





As a result of mathematical processing of experimental data, a regression dependence (3) was obtained, which most adequately describes the effect of parameters on the washability of protein-fat contamination by a detergent-disinfectant from the working surface:

signed to be a set of the colloidal silver particles size distribution

An analysis of the results of the acoustic spectroscopy also showed that colloidal Ag particles have a narrow log-normal size distribution with an average diameter of about 35 nm.

$$Y = f(pH, t, \tau, C) = 54,1246 - 24,7194 \cdot pH$$

- 0,1870·t + 2,9752·t + 8,3588·C - 0,0371·pH·t
- 0,2128·pH·t +0.2381·pH·C + 0.0093·t·t
+0.0194·t·C - 1,2710·t·C + 2,2282·pH²
+0.0043·t² - 0,0532·t² + 0,5730·C²

Where: pH is active acidity; t is the solution temperature, $^{o}C;\,\tau$ is the exposure time, min; C - concentration of the solution, wt %.

The adequacy of the resulting equation was tested according to Fisher criteria - the probability was 0.96 at a significance level of 0.05.

Then the effect of washing parameters on the process of washability of protein-fat contamination from the working surface in a model experiment. Figure 5 shows the response of the output parameter Y (contamination washability) depending on the active acidity and the temperature of the solution, under other optimal conditions.

Analysis of the surface shown in Figure 5 shows that the washability of protein-fat contamination increases linearly with increasing the temperature of the detergent-disinfectant solution over the entire pH range. The dependence of contamination washability on the active acidity of the solution is a parabolic function. As shown by the analysis of Figure 5, the extremum of this function is at pH = 7. High washability in an alkaline medium (pH ~ 12) is due to alkaline hydrolysis of milk contamination. The minimum detergency was observed in a neutral medium (pH = 7) when acid or alkaline hydrolysis could not occur. Good washability at pH ~ 3 is due to acid hydrolysis of the protein-fat contaminant. The process of hydrolysis in an acidic environment leads to the splitting of triglycerides (fats) into glycerol and fatty acids, and the milk protein into peptides and amino acids. The process of hydrolysis in an alkaline medium leads to the splitting of triglycerides into glycerol and fatty acids salts, and milk protein is split into salts of amino acids. The temperature dependence is almost linear, which is explained by an increase in the degree of hydrolysis with increasing temperature.

Next, Figure 6 shows the response of the output parameter Y (contamination washability) depending on the active acidity of the solution and the exposure time under other optimal conditions.

The analysis of the surface depicted in Figure 6 also confirms the previously made conclusions about the effect of the active acidity of the solution on the process of washability of protein-fat contamination from metal surfaces in a model experiment. The surface also shows a linear increase in the washability of contamination with an increase in exposure time.

Figure 7 shows the response surface of the output parameter Y (contamination washability) depending on the solution temperature and the exposure time, under other optimal conditions.

Analysis of the surface shown in Figure 7 shows that the washability of contamination linearly depends on the temperature of the solution and on the time of washing, that is, the higher the temperature and the longer the exposure time, the more contaminant is washed off from the working surface. Washability reaches the maximum possible value at a temperature of 80 °C and an exposure time of 20 minutes.

Figure 8 shows the response of the output parameter Y (contamination washability) depending on the concentration of colloidal silver preparation and the time of washing, under other optimal conditions.

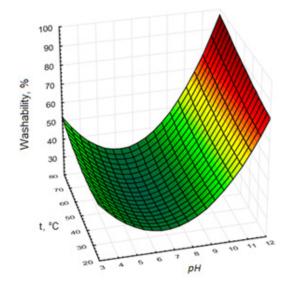


Figure 5. The response of the output parameter Y (contamination washability) depending on the active acidity and the temperature of the solution, under other optimal conditions

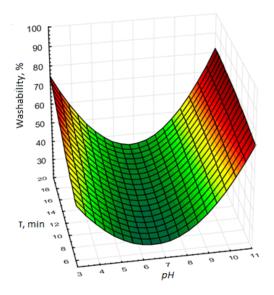
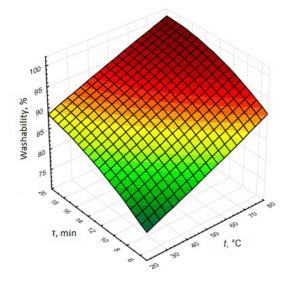
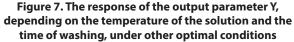


Figure 6. The response of the output parameter Y, depending on the active acidity of the medium and the time of washing





The analysis of the surface presented in Figure 8 shows that the highest washability of contamination was achieved at an exposure time of 20 minutes at a concentration of colloidal silver preparation from 0.1 to 0.5 wt %. A further increase in the concentration of the preparation leads to abundant foaming and, consequently, to the deterioration of detergent properties.

Figure 9 shows the response of the output parameter Y (contamination washability) depending on the solution temperature and the concentration of colloidal silver under other optimal conditions.

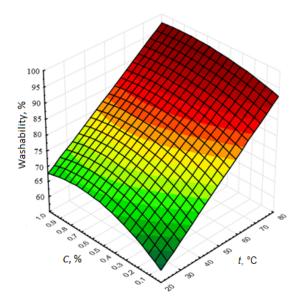


Figure 9. The response of the output parameter Y, depending on the solution temperature and the concentration of colloidal silver, under other optimal conditions

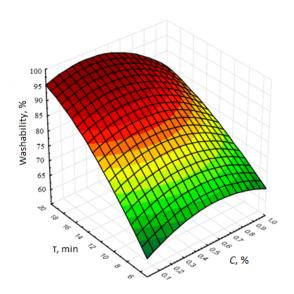


Figure 8. The response of the output parameter Y depending on the concentration of colloidal silver and the time of washing, under other optimal conditions

Analyzing the surface presented in Figure 9, we can conclude that the detergency directly depends on the temperature of the solution and nonlinearly on the concentration of the colloidal silver preparation, under other optimal conditions. At the maximum temperature of the solution, the washing ability changes slightly, with an increase in the concentration of colloidal silver, the temperature is the limiting factor, since the resulting foam is destroyed, which determines a high degree of hydrolysis of the pollutant. At the minimum temperature, the degree of hydrolysis is low and the washability mainly depends on the concentration of the preparation.

As a result of studies on the washability of protein-fat contaminants in a model experiment, the optimal values of the parameters significantly affecting the washing process were established: C (preparation) = 0.1 wt.%, pH = 12, t = 60 °C, τ = 10 min.

At the next stage of the study, the effectiveness of contamination washability by the developed detergent-disinfectant under optimal conditions was studied. As is known, the efficiency of using the detergent disinfectant shows how many times the same washing solution can be used for "perfect" washing and wetting of equipment. As a result, dependencies presented in Figures 10 - 12 were obtained.

The analysis of dependencies presented in Figure 10 shows that the active acidity of the working solutions of both the developed detergent-disinfectant and the reference solution at the initial stage of the experiment is the same (pH = 12.4 ± 0.2). With increasing concentration of contamination to C(contamination) = 15 ± 5 g / l, the active acidity of the working solutions does not change, then there is a sharp decrease in the active

acidity of both the developed detergent-disinfectant and the reference solutions. The decrease in the active acidity of the working solutions in the process of washing and removal of contamination is associated with a decrease in the concentration of alkaline agents involved in the hydrolysis of milk fat and proteins. At pH = 10.7 \pm 0.5, there was no washability of contamination by the reference solution. It is important to note that upon reaching this value of the pH in the solutions of colloidal silver preparation, the contamination concentration was 35 \pm 2 g / l, which is 20% more than in the reference solution (C (contamination) = 27 \pm 2 g / l). This fact indirectly indicates a better removal efficiency of protein-fat contaminants by the developed detergent-disinfectant.

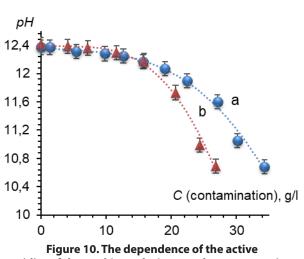
A similar dependence is observed in the case of studying the specific conductivity of washing solutions when the concentration of contamination changed. This is reflected in Figure 11.

It was established that at the initial stage of the experiment, the electrical conductivity of both the developed detergent-disinfectant and the reference solution was approximately the same $(23 \pm 2 \text{ mS/cm and})$ 26 ± 2 mS/cm respectively). With an increase in the contamination concentration, the specific electrical conductivity of the solutions decreases, which is associated with a decrease in the electrokinetic mobility of the charged detergent particles due to the process of solubilizing the contamination particles into the surfactant micelles. When reaching the concentration of contamination in the reference solution is higher than 20 ± 2 g/L, the electrical conductivity of the solution remained constant (about 11 ± 2 mS/cm). A similar situation was observed in the solution of the developed detergent-disinfectant; however, it happened when the contamination concentration was above 27 \pm 2 g/L, which also indirectly indicates a better washing efficiency of protein-fat contamination by the developed preparation.

As was shown above, the concentration of contamination in the detergent solution significantly affects the efficiency of washing the working surface from the pollutant. It should be noted that when the detergent-disinfectant solution is saturated with a pollutant above the critical value, the process of adsorption of the pollutant on the working surface begins to take place.

From our point of view, it is advisable to introduce the concept of "washout rate" ($\upsilon = f(C)$) - that is, the amount of protein-fat contamination washed per unit of time per unit of surface (g/min·cm²). The dependencies are presented in Figure 12.

The analysis of the dependencies presented in Figure 12 shows that the washout rate of contamination by both the developed detergent-disinfectant and the



acidity of the working solutions on the concentration of protein-fat contamination: a) In the developed detergent-disinfectant; b) In the reference solution

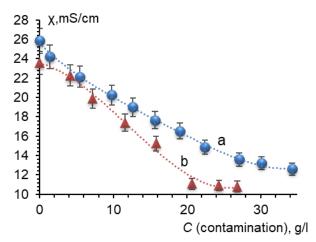
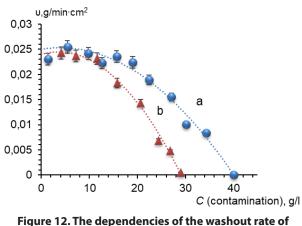


Figure 11. Dependence of the specific electrical conductivity of working solutions on the concentration of protein-fat contamination: a) In the developed detergent-disinfectant; b) In the reference solution



a) In the developed detergent-disinfectant; b) In the reference solution

reference solution until the concentration of the pollutant in the working solution about C = 12 - 15 g/L is almost the same. With further increase in the concentration of the pollutant in the working solutions, a decrease in the washout rate was observed. It is important to note that the developed detergent-disinfectant is able to wash away the contamination at higher concentrations of the pollutant in the working solution the washout rate reaches zero at a contamination concentration of about 40 g/L, which is 30% higher than in the reference solution.

Based on the obtained data, it can be concluded that the developed detergent-disinfectant based on colloidal silver has excellent washability of contamination and can make good competition to modern detergent-disinfectant agents used at dairy enterprises.

At the next stage of research, sanitary-microbiological studies of the colloidal silver detergent-disinfectant were carried out. The results of the study are presented in Table 3. Data analysis showed that *E. coli* bacteria were not detected in any of the samples. Microbial bodies were also not detected on either the glass or steel surfaces treated by all the detergents studied. When determining the total microbial number (TMN) on the aluminum surface in the case of using the reference detergent TMN = 30 CFU/mL, using the developed detergent-disinfectant TMN = 10 CFU/mL. However, this indicator is not standardized. Pathogens, including salmonella, were not detected on any of the samples.

At the next stage, the corrosion properties of the developed detergent-disinfectant with various solvents were investigated. The results are shown in Table 4.

It has been established that the corrosivity of the developed detergent-disinfectant is at the level of low-corrosive substances, but increases with increasing temperature of the washing process.

The final stage of washing, after the process of removing contamination from the surface of the equipment, is rinsing. This stage is of paramount importance since the components of the washing solutions can remain on the surface of the equipment, which, if they enter the dairy products, can cause changes in its organoleptic and physicochemical properties. The rinse quality determines the degree of removal of detergent components from the working surfaces of the process equipment.

Since the developed detergent-disinfectant consists of two main components - colloidal silver and quaternary ammonium compound (didecyldimethylammonium bromide), model experiments to determine the degree of removal of these components from the glass surface were carried out.

It is known that colloidal silver particles have unique optical properties - the inherent absorption at 390 - 420 nm due to surface plasmon resonance [14]; then the presence of colloidal silver particles on the working surface can be determined by the presence of a characteristic absorption band in the transmission or

		Name of the microbiological indicator			
Test object	Type of surface	<i>E. coli</i> per 100 cm ²	TMN, CFU/cm ²	Pathogens, including Salmonella spp.	
Reference solution	Glass	Not detected	Not detected	Not detected	
	Steel	Not detected	Not detected	Not detected	
	Aluminum	Not detected	30	Not detected	
Developed detergent- disinfectant solution	Glass	Not detected	Not detected	Not detected	
	Steel	Not detected	Not detected	Not detected	
disinfectant solution	Aluminum	Not detected	10	Not detected	
Standard	Glass				
	Steel	Not allowed	Not standardized	Not allowed	
	Aluminum				

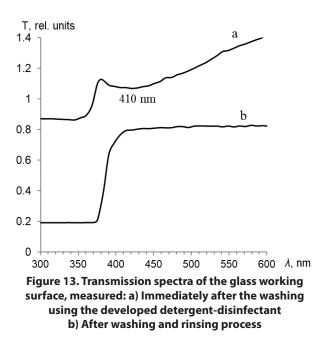
Table 3. Investigation of the quality of sanitary treatment

Table 4. Investigation of the corrosivity of the developed detergent-disinfectant

	Steel		Aluminum		
Solvent	Temperature,	Corrosion rate	Temperature,	Corrosion rate	
	٥C	mm / year	°C	mm/year	
	20	0,0041	20	0.0012	
Tap water	35	0,0052	35	0.0021	
	50	0,0074	50	0.0032	
	20	0,0035	20	0.0015	
Distilled water	35	0,0040	35	0.0018	
	50	0,0066	50	0.0022	



reflection spectra. In this regard, an experiment on optical properties measuring was conducted. The results are presented in Figure 13.



Analysis of the spectrophotometry data showed that before the washing process a high-intensity absorption band at 410 nm was in presence in the specter, which indicates the presence of colloidal silver particles on the surface. After washing and rinsing processes, absorption band was not observed which indicates complete removal of colloidal silver particles.

As a result of the titration, the equivalence point was not reliably detected, that is, the concentration of DDAB in the analyzed sample was less than 0.001 g/L, which indicates its complete removal from the surface during the rinsing process.

As a result of the study, we can conclude that the main components of the developed detergent-disinfectant were completely removed from the working surfaces in the process of standard rinsing modes.

4. Conclusions

- The method for the synthesis of a colloidal silver preparation stabilized by a quaternary ammonium compound has been developed and optimized. It was found that the colloidal silver particles in the developed preparation were spherical. The average hydrodynamic diameter of particles was about 50 nm.

- The developed colloidal silver preparation exhibits bactericidal and fungicidal activity and can be used as a disinfectant base in an alkaline detergent-disinfectant, actively suppressing the vital activity of fungal and bacterial cultures. - The process of washing away protein-fat contamination by the developed detergent-disinfectant was optimized. The optimized parameters are: C (preparation) = 0.1 wt %, pH = 12, t = 60 °C, τ = 10 min.

- It was found that the developed detergent-disinfectant based on colloidal silver has a "good" washability to protein-fat contaminations. It has been established that the developed detergent - disinfectant does not possess any corrosive properties.

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