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(71) Applicant *(for all designated States except US)*: **CLOSED STOCK COMPANY "INSTITUTE OF APPLIED NANOTECHNOLOGY"** [RU/RU]; Savelkinsky travel 4, offis 1314, Zelenograd, Moscow, 124482 (RU).

(71) Applicant: **FONDAZIONE SALVATORE MAUGERI CLINICA DEL LAVORO E DELLA RIABILITAZIONE** [IT/IT]; Via Salvatore Maugeri 4, I-27100 Pavia (IT).

(71) Applicant *(for all designated States except US)*: **SIB LABORATORIES LIMITED** [MT/MT]; 19, San Marco Street, Valletta, VLT 1362 (MT).

(71) Applicant: **MAKHONIN, Igor Ivanovich** [RU/RU]; Kuibysheva street 3, flat 15, Solnechnogorsk District, Moscow Region, Mendeleevo, 141570 (RU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ABRAMYAN, Ara Arshavirovich** [RU/RU]; Vernadskogo prospect 15, flat 237, Moscow, 117296 (RU). **BEKLEMYSHEV, Viacheslav Ivanovich** [RU/RU]; Zelenograd 1103, flat 153, Moscow, 124460 (RU). **FILIPPOV, Konstantin Vitalievich** [RU/RU]; Tarasa Shevchenko embankment 3, building 3, flat 151, Moscow, 121248 (RU). **MAUGERI, Umberto Orazio Giuseppe** [IT/IT]; Località Montesereno, I-27040 Calvignano (IT). **SOLODOVNIKOV, Vladimir Aleksandrovich** [RU/RU]; Leninscky prospect 93, flat 241, Moscow, 119313 (RU).

(74) Agents: **GERVASI, Gemma** et al.; Notarbartolo & Gervasi S.p.a., Corso di Porta Vittoria 9, I-20122 Milan (IT).

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(54) Title: PREVENTIVE BACTERICIDAL POLISH FOR NAIL TREATMENT

(57) Abstract: The invention concerns the area of medicine, hygiene, cosmetology and provides products which can be used as a preventive bactericidal enamel for nail treatment. The preventive bactericidal polishes for treatment of nails of the invention contain a water-soluble film-forming substance, a bactericidal agent and a polar solvent. Powders of bentonite are used as the bactericidal agent. Bentonite is nanostructured up to a size of the particles of no more than 150 nm and is intercalated by ions of metals $Ag^{+}$ or/and $Cu^{2+}$ and $Zn2^{+}$. The bentonite containing the metal ions is introduced in a preliminarily prepared emulsion of the film-forming substance, as 4 - 10 % hydrosol. The nail polish of the invention has the following ratio of components, in % b.w.: film-forming substance 1,0 - 10,0; bactericidal agent 0,2 - 1,5; polar solvent the rest. By realization of the invention effective protection of treated nails against a wide spectrum of bacteria and funguses is provided, with polishes with a low cost of manufacture. The polish can be used for treating nails and nail platens, for preventive avoidance and treatment of superficial mycosises and inflammatory processes.

# PREVENTIVE BACTERICIDAL POLISH FOR NAIL TREATMENT

## Technical Field

The invention concerns the area of medicine, hygiene and cosmetology and, specifically, a preventive bactericidal enamel for nail treatment.

Mycosis of nail and nail cushion (onichyte mycosis) is a widespread illness which cannot be well cured in many cases. The main activators of onichyte mycosis are dermatophytes, funguses capable to invade keratin and attack skin, nails and hair. Funguses of kind *Trichophyton, Epidermophyton, Microsporum* are among them. Besides dermatophytes superficial mycosises can be caused by others funguses, for example, Candida spp.

Candida spp. are frequently detected after dermatophytes at epidermomycosis and onichyte mycosis; these funguses cause a candidiasis of nails.

Progress of onichyte mycosis is promoted by difficulty of blood circulation, decrease in immunity, superfluous sweating, wearing of rubber gloves or footwear with a rubber sole, manicure traumas and frequent contacts with water and soap. Usually affected professional groups include sportsmen and people working in the field of medicine.

Loss of transparency, change of color (whitish, yellowish), thickening, hyponychial hyperkeratosis, crumbling or destruction up to the nail platen are characteristic symptoms for various types of fungous affections of nail plates (onichyte mycosis).

Decrease in incidence of fungous infections requires observance of rules of personal hygiene and also use of preventive means with bactericidal action for treatment of nails and nail platens.

## State of the art

Local treatment of nails with bactericidal, antifungal (fungicidal) polishes which are deposited on the surface of nails, are used as means for preventive protection from onichyte mycosis of nails.

Preventive treatment with bactericidal polishes is more favourable in comparison with other ways of treatment and preventive protection, as it does not possess side effects and influences only locally the nails. Various bactericidal polishes for treatment of nails and nail platens are known.

For example, in the technical specification WO 99/39680, a bactericidal polish for treatment of onichyte mycosis of nails is described, which contains effective amounts of

tsiklopiroks and its pharmaceutically acceptable salts. The polish contains a film-forming polymer which protects a nail exposed to treatment by means of formation of a solid, transparent and waterproof film.

In the technical solution according to the application EP-Ç-226984, an antifungal nail polish is described containing 1-hydroxy-2-pyridone, for example tsiklopiroksolamin, a film-forming substance and physiologically compatible solvent and also usual additives for cosmetics.

The following products are also used in compositions of polishes as antifungal agents:

- imidazole derivatives and their salts, for example, clotrimazole, econazole, isoconazole, ketoconazole, miconazole, tioconazole, bifonazole, fenticonazole and oxiconazole;

- morpholine derivatives and their salts, for example amorolfin and morpholine (see US patent No 5120530);

- griseofulvin (see US patent No 5487776). Nitrocellulose and a mix of various solvents is used in the given polish as film-forming substance. The composition of the polish contains also organically modified bentonite on the basis of bentonite stearylalcony in amount up to 2% b.w. for improvement of its characteristics.

However use in nail polishes of the specified antifungal means on the basis of synthetic organic compounds does not provide efficiency of their action in respect to a wide spectrum of bacteria and funguses; they also do not provide anti-inflammatory influence on the skin of the patient in the zone of the nail bed and leads to the hereinafter specified dermatological inconveniences:

- itch, burning, reddening of the skin around of the nail bed that limits expediency of use of polishes with specified antifungal agents for patients with a sensitive skin, especially at presence of diabetes.

A bactericidal polish containing a film-forming substance, a bactericidal agent and a solvent (see for example patent RU No. 2311186) is also known.

In the known technical solution products of the metabolism of bacteria Bacillus subtilis or Bacillus licheniformis or their mix are used as a bactericidal agent. The given polish contains also a preservative with antifungal properties on the basis of paraform. However the technology of obtaining the named bactericidal agent includes suspension

cultivation of a strain of bacteria in a nutrient medium, separation of bacterial culture from the nutrient medium and then centrifugation for increase of concentration of bacteria.

The complex technology for obtaining the given agent leads to increase in expenses for manufacturing the polish. The difficulties derive from the process of polymerization of formaldehyde for obtaining paraform (paraformaldehyde, polyoximethylene). The preservative used is a derivative of formaldehyde which is the most cancerogenic, neurotoxic and allergenic among all preservatives.

Besides, the presence of a film-forming substance in the structure of the known polish, strongly increases adhesion of the polish film, what complicates the process for its removal from the surface of the nail. It also requires the use of organic solvents which negatively influence the skin.

The known technical solution according to the Russian patent No. 2245715 is chosen as the closest prior art with respect to the present invention. A bactericidal polish for treatment of nails containing a water-soluble film-forming substance, a bactericidal agent and a polar solvent is described in the given invention.

The composition of the polish described in said invention contains as the bactericidal agent:

- at least one antifungal substance or a combination of antifungal substances and, preferably, compounds of 1-hydroxy-2-pyridones and their salts;

- active substances, for example, antibiotics, anti-inflammatory means, antiseptics and/or local anesthetics.

Use of a film-forming substance on the basis of water-soluble derivatives of chitosan, basically hydroxypropylchitosans and carboxyalkylchitosans, in the known invention provides formation of a thin layer of a film permeable to moisture and air on the treated nail. That is suitable for use of the given polish for preventive purposes.

The presence of the specified film-forming substance in a composition of polish provides use of polar solvents, basically aqueous-alcoholic solutions, what avoids influence of aggressive organic solvents on nails and skin. As a result dermatological risks are reduced both at drawing and at removal of the polish film from the surface of the nail.

However, as it follows from the description of the given technical solution, bactericidal activity of the given polish used for treatment of nails, depends both on the presence of antifungal agents in its composition and on the presence of other active

components, including antiseptic and anti-inflammatory ones. In result the compounding of the known polish becomes complicated due to the insertion of additional active components. That increases costs for its manufacturing, also due to the use of active components on the basis of synthesized organic compounds having also a limited spectrum of action, in respect to various microbes and funguses.

At the same time diffusion of bactericidal agents obtained on the basis of inorganic components (see patent RU No. 2330673, pub. 2008) results from the available art. Bactericidal agents on the basis of nanostructured powders of bentonite, intercalated by ions of metals $Ag^{+}$ or/and $Cu^{2+}$, are described in said patent. They are obtained by modifying a bentonite with solutions of inorganic salts of silver or copper. Bentonite in Na-form is preliminarly enriched with cations of sodium by processing with a water solution of an inorganic salt of sodium. Bentonite obtained at the first stage of processing is cleaned from chlorine anions and bentonite obtained at the second stage, after intercalation by ions of the specified metals, is cleaned from salts of sodium. Nanostructuring of the product is carried out till a powder of bentonite with a size of the particles of no more than 150 nm is obtained.

The bactericidal agent is obtained from mineral, ecologically safe components, and provides efficiency of action concerning a wide spectrum of bacteria and funguses; it is safe dermatologically and can be used as additive to various preparations for medical and cosmetic purposes.

However, development of compositions of a bactericidal polish, on the basis of the described bactericidal agent, for treatment of nails, is not foreseen in the given patent.

**Disclosure of the invention**

The problem of the present invention consisted in creating a bactericidal polish with preventing activity in treatment of nails, providing the technical result of expanding assortment of polishes corresponding to the technology requirements, decreasing the risks of occurrence of negative dermatological effects and providing effective bactericidal protection of treated nails while decreasing the expenses for manufacturing the polish composition.

For the solution of the put technical problem, a preventive bactericidal polish for treatment of nails is described containing a water-soluble film-forming substance, a bactericidal agent and a polar solvent. According to the present declared invention,

powders of bentonite nanostructured till the size of particles of no more than 150 nm and intercalated by ions of metals $Ag^+$ or/and $Cu^{2+}$ and $Zn^{2+}$ are used as the bactericidal agent. The bactericide is introduced in the preliminarily prepared emulsion of the film-forming substance, as 4 - 10 % hydrosol. Thus a composition of the polish has the following content of components, expressed as percentage by weight (% b.w.):

| | |
|---|---|
| film-forming substance | 1,0 - 10,0 |
| bactericidal agent | 0,2 - 1,5 |
| polar solvent | the rest |

According to the invention, nanostructured powders of bentonite, intercalated by ions of metals $Ag^+$ and $Zn^{2+}$ are used as the bactericidal agent at a ratio in the mix of 1: (0,5 ÷ 1) parts by weight.

According to the invention nanostructured powders of bentonite intercalated by ions of metals $Cu^{2+}$ and $Zn^{2+}$ are used as the bactericidal agent at a ratio in the mix of (1÷0,5): (0,5÷ 1) parts by weight.

According to the invention nanostructured powders of bentonite intercalated by ions of metals $Ag^+$, $Cu^{2+}$and $Zn^{2+}$ are used as the bactericidal agent at a ratio in the mix of 1: (0,5): (0 ÷1) parts by weight.

According to the invention nanostructured powders of bentonite intercalated by ions of metals $Ag^+$, $Cu^{2+}$and $Zn^{2+}$ are used as the bactericidal agent at a ratio in the mix of 1: (0, 5): (0,5) parts by weight.

According to the invention the following steps are implemented for obtaining nanostructured powders of bentonite intercalated by ions of the mentioned metals:

a) enrichment of bentonite in Na–form with cations of $Na^+$ by processing with 3-10 % b.w. water solution of an inorganic salt of sodium and subsequent cleaning of bentonite from anions of chlorine;

b) intercalation of the products obtained at the step a) with ions of $Ag^+$, $Cu^{2+}$ and $Zn^{2+}$ by processing them with a 10-20 % water solution of inorganic salts of silver, copper and zinc with subsequent cleaning of the obtained product from salts of sodium;

c) nanostructuring the products obtained at the step b) by grinding them up to a size of the particles of no more than 150 nm with subsequent drying.

According to the invention silver nitrate, copper sulfate, zinc sulfate or zinc chloride are used as 10-20 % water solutions of inorganic salts of silver, copper and zinc.

According to the invention, derivatives of chitosan, preferably hydroxypropylchitosan or carboxyalkylchitosan, in amount of 3-5% b.w., are used as the water-soluble film-forming substance.

According to the invention, 30-50 % water-alcohol solution is used as the polar solvent.

According to the invention the nail composition additionally contains from 1 up to 5% b.w. of industrially suitable additives, preferably surfactants, lanolin derivatives, stabilizers at light, odors.

By realization of the present invention enlargement of assortment of preventive bactericidal polishes for treatment of nails is provided, with decrease in expenses for their manufacturing and decrease in risks of occurrence of negative dermatological effects with simultaneous maintenance of effective bactericidal activity against a wide spectrum of gram-positive, gram-negative bacteria and fungoid microorganisms. The inventive results of the present invention are explained as follows:

- by creation of a bactericidal polish on the basis of synergically and biologically compatible natural components;

- by the use of bactericidal mineral components modified (intercalated) by the cations of metals $Ag^{+}$, $Cu^{2+}$, $Zn^{2+}$, possessing antifungal and antimicrobic properties concerning a wide spectrum of bacteria and funguses and having effective anti-inflammatory action on a zone of the skin close to nail by creating a composite structure of a polish;

- by the use of a bactericidal agent on the basis of nanostructured powders of bentonite with a size of particles of no more than 150 nm, intercalated by ions of metals $Ag^{+}$, $Cu^{2+,}$ $Zn^{2+}$ in the composite structure of a polish. That increases the effective area of interaction with the bacterial environment providing activity on pathogenic microflora concerning a wide spectrum of gram-positive, gram-negative bacteria and fungoid microorganisms;

- by the use of a nanostructured mineral component, i.e. bentonite with a size of the particles of no more than 150 nm with flow properties allowing improvement of structuring a composition of a polish creating a new composite structure of a polish;

- by the use of a film-forming substance compatible with polar solvents, that provides the possibility of removal of a polish film from nails without use of the organic solvents adversely influencing the skin;

- by the use in a composition of polish of a film-forming substance creating a film permeable to moisture and air on the surface of a nail.

Analysis of the available art did not reveal technical solutions with a set of characteristics corresponding to those declared with the present invention and realizing the above described results.

The result of the analysis of the available art testifies about conformity of the declared technical solution to criteria of "novelty" and "degree of inventiveness".

The declared invention can be industrially realized for manufacturing preventive bactericidal polishes for treatment of nails.

**Detailed description of the invention**

The invention is explained by means of the data collected in the following tables. Accordingly:

Table 1- illustrates «Bactericidal properties of polish compositions»;

Table 2 – illustrates «Fungicidal properties of polish compositions».

For realization of the invention medical, labware and commodity products are used including the following:

water-soluble film-forming substances on the basis of derivatives of chitosan which pertains to the class of biological polymers. Hydroxypropylchitosan and carboxyalkylchotosan are preferably used according to the present invention. Hydroxypropylchitosans are derivatives of chitosans with hydroxy-groups. Carboxyalkylchotosans are derivatives of chitosan with carboxy-groups.

According to the present invention the named water-soluble film-forming substances are used in amount from 1,0 up to 10,0% b.w. on the total mass of a composition of a polish; they provide effective formation of a film on the surface of a nail. Decrease in the content of the specified film-forming substances in the composition of the polish reduces hiding power of the work surface of a nail and decreases technical time of protection of nails with preventive treatment. The increase in the quantitative content of film-forming substance in the composition of the polish, increases the charge of the given component and worsens quality of the formed film.

The bactericidal agent possessing antimicrobic and antifungal properties described in the patent RU No. 2330673 was used for realization of the present invention.

The given preparation was obtained as follows:

**1st step.** Obtaining semi-finished products of bentonite powders preliminarily enriched with cations of sodium $Na^+$.

Bentonite (montmorillonite) in Na-form was saturated with 5% water solution of NaCl. There was at time interval of retention of bentonite in the given solution what caused enrichment of bentonite with ions of sodium. Then repeated washing for removal of anions of chlorine, with subsequent filtration and drying, was carried out.

**2nd step.** Obtaining nanostructured powders of bentonite intercalated by ions of metals $Ag^+$, $Zn^{2+}$ and $Cu^{2+}$.

Nanostructured bentonite powders (nanoparticles of bentonite), intercalated by ions of the specified metals and not containing salts of sodium, are obtained from semifinished products of bentonite made at the 1st step, according to the following examples:

Example 1.

A semifinished product cleaned from acid anions was dried up and modified by 10-20 % water solution of silver nitrate (at red illumination). 15 % water solution of silver nitrate (AgNO3) was preferably used. The obtained modified semifinished product was repeatedly washed out for removal of salts of sodium, filtered, grinded and dried.

The amount of water solution for processing 5 g of semifinished product (1st step) was the following: bentonite : water solution as 1:20 parts by weight.

As a result bentonite intercalated by ions of $Ag^+$, and not containing salts of sodium, was obtained. Yield was 4,8 g.

Example 2.

The same materials and technical methods as in the Example 1 were used, but 15% water solution of copper sulfate (CuSO4) was used to modify the bentonite enriched with ions of sodium $Na^+$.

A bentonite powder intercalated by ions of $Cu^{2+}$ and not containing salts of sodium was obtained. Yield was 4,8 g.

Example 3.

The same materials and technical methods as in the Example 1 were used, but 15% water solution of an inorganic salt of zinc, preferably zinc chloride ($ZnCl_2$), was used to

modify bentonite enriched with ions of sodium $Na^+$. It is possible to use zinc sulfate ($ZnSO_4$).

A bentonite powder intercalated by ions of $Zn^{2+}$, and not containing salts of sodium, was obtained. Yield was 4,8 g.

Deionized water is used for realization of technical steps 1 and 2 mentioned above.

Process of grinding of the products obtained according to Examples 1-3, till the sizes of particles of bentonite powder specified in the invention, was carried out in all examples as follows:

the products obtained according to the Examples 1-3 were added in deionized water, at the ratio product: solvent as 1:10 in parts by weight.

Nanostructuring (superdispersion) up to the size of particles of no more than 150 nm was carried out with the help of an ultrasonic dispersant, accelerating nanostructuring of the bentonite powders.

Said process was carried out with use of the dispersant Bandelin Sonoplus HD2070 at power 40 Wt, within 5 minutes.

The colloidal systems obtained were powered on a sublayer and, after drying, the sizes of the particles of the nanostructured powder of bentonite were measured by methods of microscopy. The tests performed have shown that nanostructured powders of bentonite have sizes of the particles of no more than 150 nm.

Nanostructured powders of bentonite (Examples 1-3) were analyzed to define the content of silver, copper and zinc (in % b.w.) with the method of titrimetric analysis.

The titrimetric analysis was carried out by using indicators, fixing a point of equivalence of titration.

As a result of the analysis carried out it has been established that powders of bentonite, intercalated by ions of the named metals, can contain from 2,0 up to 8,0% b.w. of silver or copper or zinc, depending on the amount of activating and modifying (intercalating) reagents.

The specified contents of metals in an intercalated (modified) bentonite powder are the optimum. Increase of the percentage of the contents of the metals leads to increase in costs for the reagents used for obtaining bentonite powders. Decrease of the percentage of the contents of the named metals in bentonite powders leads to a decrease of their antimicrobic and fungicidal activity.

According to the results of the titrimetric analyses the quantitative contents of metals (Ag, Cu, Zn) in nanostructured powders of bentonite (products of Examples 1-3) was about 2,0-8,0% b.w..

Bactericidal agents used according to the present invention have been obtained by mixing products according to the Examples 1-3, in a ratio in parts b.w. as hereinafter specified.

Example 4.

A bactericidal agent containing nanostructured powders of bentonite, intercalated by ions of metals $Ag^+$ and $Zn^{2+}$ (Example 1 and Example 3), at a ratio in the mix as 1: 0,7 .

Example 5.

A bactericidal agent containing nanostructured powders of bentonite, intercalated by ions of metals $Cu^{2+}$ and $Zn^{2+}$ (Example 2 and Example 3), at a ratio in the mix as 1: 0,5.

Example 6.

A bactericidal agent containing nanostructured powders of bentonite, intercalated by ions of metals $Ag^+$, $Cu^{2+}$ and $Zn^{2+}$ (Examples 1-3) at a ratio in the mix as 1: (0,5): (0,5).

Example 7.

A bactericidal agent containing nanostructured powders of bentonite, intercalated by ions of metals $Ag^+$ and $Cu^{2+}$ (Example1 and Example 2), at a ratio in the mix as 1: (0,5).

The amount of the bactericidal agents specified in the present invention, when included in a composition of a polish, is the optimum for maintaining antimicrobic and antifungal protection of nails and integuments adjoining to them, if used as a preventive treatment.

Antimicrobic and antifungal protection of nails, and integuments adjoining to them, worsens at decrease in amount of the bactericidal agent in the composition of the polish. Influence of bentonite, as a rheological additive and a thickener, during production of a polish composition worsens as well.

The increase in amount of the bactericidal agent in the composition of a polish leads to increase in costs and to deterioration of the structure of the polish composition, resulting in formation of unaesthetic polish films on the surface of nails.

The ratio of mixes of nanostructured powders of bentonite intercalated by ions of metals $Ag^+$, $Cu^{2+}$ and $Zn^{2+}$ specified in the present invention and used in the bactericidal agent is the optimum for preparing a polish composition intended for preventive protection of nails from a wide spectrum of gram-positive and gram-negative bacteria and fungoid microorganisms.

Changes in the amount specified in the invention for bentonite powders intercalated by ions of $Cu^{2+}$ and $Zn^{2+}$ in the bactericidal agent, will result both to worsening antifungal and anti-inflammatory activity of the polish composition or to increase in expenses for its obtaining.

A polar solvent, preferably 30-50 % water-alcohol solution, is used in compounding a polish composition according to the present invention. Ethanol or isopropyl alcohol are used as the alcohol. The presence of an alcohol in the polish provides a time of drying of the formed film of no more than 10 minutes, when a polish composition according to the invention is used. What corresponds to the consumer requirements for the given type of products (GOST R 52701-2006).

Additives, which are traditional for the given kind of products, can be used to improve the properties required by the consumers for a polish composition according to the present invention. In particular various scents, fat components, for example derivatives of lanolin, surfactants, stabilizers to light, etc.

The bactericidal agents obtained in Examples 4-7 as 4-10% hydrosols, preferably 5% hydrosols, have been added in preliminarily prepared water-alcohol emulsions of the film-forming substance. Hydroxypropylchitosan is used as the film-forming substance.

Use of hydrosols, for adding in the emulsion of the film-forming substance, provides uniformity of distribution of the bactericidal agent in said film-forming substance.

The amount of the bactericidal agent in a polar solvent, specified in the present invention, is the optimum for formation of a stable dispersed system of hydrosols. Decrease or increase in % b.w. of the contents of a bentonite powder in the polar solvent worsens stability, self-organizing of dispersed systems and uniformity of distribution of hydrosols in the emulsion of the film-forming substance.

The sizes of the particles of a bentonite powder specified in the invention are optimum both for costs for their obtaining and for stability of the obtained hydrosol.

Polishing compositions according to the following examples have been prepared:

Example 1.4.

Hydroxypropylchitosan - 5% b.w.; bactericidal agent (Example 4) - 0,2% b.w.;

40% water-alcohol solution – the rest. In total -100 g.

Example 1.5.

Hydroxypropylchitosan - 5% b.w.; bactericidal agent (Example 5) – 0,5% b.w.;

40% water-alcohol solution – the rest. In total -100 g.

Example 1.6.

Hydroxypropylchitosan - 5% b.w.; bactericidal agent (Example 6) - 1,0% b.w.;

40% water-alcohol solution – the rest. In total -100 g.

Example 1.7.

Carboxyalkylchitosan - 3% b.w.; bactericidal agent (Example 7) - 1,5% b.w.;

40% water-alcohol solution – the rest. In total -100 g.

Estimation of bactericidal and antifungal (fungicidal) activity of polish compositions according to Examples 1.4-1.7 was carried out on the basis of the disco-diffusion method with use of the instructions (MUK 4.2.1890-04) «Definition of sensitivity of microorganisms to antibacterial preparations», authorized by the Main state health officer of the Russian Federation (Department of Health of the Russian Federation) on March 4, 2004.

The disco-diffusion method (DDM) of definition of sensitivity of microorganisms (bacteria and funguses), is based on ability of the bactericidal agents, included in polish compositions, to diffuse in nutrient mediums from the cardboard (paper) disks impregnated with them thus stopping growth of the microorganisms sown on a surface of nutrient mediums.

A dense nutrient medium, i.e. Trypcase-soy agar, was used for the estimation of the bactericidal activity of polish compositions.

A dense nutrient medium, i.e. Sabouraud aga and Czapek-Dox agar, was used for estimation of the antifungal activity of the polish compositions.

Preparation of Petri dishes with a dense nutrient medium was carried out according to the instructions for the manufacturer of nutrient medium and according to the methodical instructions of MUK 4.2.1890-04.

Standard disks in diameter of 6 mm, from a special filtering cardboard (paper), were used as diagnostic disks.

The disks were impregnated with the tested polish compositions (Examples 1.4-1.7).

Strains of kinds of bacteria Staphylococcus aureus and Pseudomonas aeruginosa were used as test-microorganisms for estimation of bactericidal activity of polish compositions.

Bacteria of the kind Staphylococcus aureus are the most resistant representatives of gram-positive microflora of a human being. They are responsible for pustular infections of skin, including furuncles and abscesses.

Bacteria of kind Pseudomonas aeruginosa are one of the most resistant representatives of gram-negative microflora. They possess high resistance to physical and chemical factors. They often display resistance to many drugs and disinfectants. Bacteria of this kind are known as activators of infectious complications of wounds from burning, bacteremias, septicemias with a fatal outcome and other complications of infectious etiology.

Strains of kinds of the funguses Trichophyton rubrum, Trichophyton mentagrophytes interdigital and Candida albicans were used as test-microorganisms for an estimation of antifungal (fungicidal) activity of polish compositions.

Funguses of kind Trichophyton are dermatophytes and are also one of the basic activators of superficial mycosis (epidermomycosis) on nails, skin and hair.

Funguses of the kind Candida spp. are the second, after dermatophytes, for frequency of release at epidermomycosis and onychomycosis. Candida albicans cause a candidiasis of nails.

Suspensions (inoculums) of each test-culture of bacteria and test-culture of funguses in sterile physiological solutions, were prepared with a density (turbidity) of 0,5-0,6, according to the standard of Mc Farland. Suspensions contained approximately 1,5-2,0x$10^8$ CFU/ml, i.e. colony-forming units /ml.

Inoculums (within 15 minutes after preparation) were used for inoculation of the dense nutrient mediums in Petri dishes mentioned above.

The inoculum was placed by a pipette on the surface of a nutrient medium in a volume of 1-3 ml and was distributed on the surface at regular intervals. Slightly opened Petri dishes were dried at room temperature during 15-20 minutes.

The disks processed with tested polish compositions (Examples 1.4-1.7) were placed on the surface of a nutrient medium (with the help of a sterile tweezers) after inoculation. Uniform and close contact of disks to a surface of environment was provided.

Petri dishes were placed in the thermostat and were incubated at temperatures of 25-35°C over 18-72 hours (depending on the kind of tested microorganism) directly after application of disks.

Measurement of diameter of the zones of growth inhibition of test-microorganisms (around the disks), accurate within 1 mm, was made after termination of the incubation. Zones of full suppression of growth were taken as landmarks at measurements.

Testing of each disk with a tested polish composition (Examples 1.4-1.7) and each test-microorganism was repeated three times to increase reliability of the tests. Average value of diameter of a zone of growth inhibition was estimated.

Results of the tests with polish compositions (Examples 1.4-1.7) are submitted in Table 1- «Bactericidal properties of polish compositions» and Table 2- «Fungicidal properties of polish compositions».

Results of the tests testify the presence of zones of full suppression of visible growth of test-microorganisms (bacteria and funguses) around of disks with the tested polish compositions containing bactericidal agents.

Presence and size of the zones of growth inhibition of test-microorganisms, testifies the stable and essential bactericidal and fungicidal effect in the tested polish compositions, intended for treatment of nails and nail platens for preventive avoidance and treatment of superficial mycosis.

**Table 1. Bactericidal properties of polish compositions.**

| № | Tested compositions (intercalated by ions of metals) | Kind of bacteria | |
|---|---|---|---|
| | | Staphylococcus aureus | Pseudomonas aeruginosa |
| | | Diameters of zones of growth inhibition of test-microorganisms around the disks processed with tested compositions, mm | |
| 1 | **Example 1.4** ($Ag^+ + Zn^{2+}$) | 16 | 14 |
| 2 | **Example 1.5** ($Cu^{2+} + Zn^{2+}$) | 13 | 11 |
| 3 | **Example 1.6** ($Ag^+ + Cu^{2+} + Zn^{2+}$) | 19 | 17 |
| 4 | **Example 1.7** ($Ag^+ + Cu^{2+}$) | 17 | 16 |

**Table 2. Fungicidal properties of polish compositions.**

| № | Tested compositions (intercalated by ions of metals) | Kind of funguses | | |
|---|---|---|---|---|
| | | Trichophyton rubrum | Trichophyton mentagrophytes | Candida albicans |
| | | Diameters of zones of growth inhibition of test-microorganisms around of the disks processed with tested compositions, mm | | |
| 1 | **Example 1.4** ($Ag^+ + Zn^{2+}$) | 12 | 11 | 14 |
| 2 | **Example 1.5** ($Cu^{2+} + Zn^{2+}$) | 11 | 10 | 9 |
| 3 | **Example 1.6** ($Ag^+ + Cu^{2+} + Zn^{2+}$) | 13 | 12 | 14 |
| 4 | **Example 1.7** ($Ag^+ + Cu^{2+}$) | 13 | 11 | 13 |

## CLAIMS

1. Preventive bactericidal polish for treatment of nails comprising, as essential components, a water-soluble film-forming substance, a bactericidal agent and a polar solvent, wherein the bactericidal agent is a nanostructured bentonite intercalated by ions of metals selected in the group consisting of $Ag^{+}$, $Cu^{2+}$, $Zn^{2+}$, with a size of the particles of no more than 150 nm.
2. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the film-forming substance is a chitosan derivative, preferably hydroxypropylchitosan or carboxyalkylchitosan.
3. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the polar solvent is a 30-50% water-alcohol solution.
4. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the nanostructured bentonite intercalated by ions of metals is present in the amount of 0,2-1,5% b.w..
5. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the film-forming substance is present in the amount of 1,0-10,0% b.w..
6. Preventive bactericidal polish for treatment of nails according to claim 2 wherein the film-forming chitosan derivative is used in an amount of 3-5% b.w.
7. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the bactericidal agent is a nanostructured powder of bentonite intercalated by ions of the metals $Ag^{+}$, and $Zn^{2+}$ in a ratio between them of 1: (0,5÷ 1) in parts by weight.
8. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the bactericidal agent is a nanotructured powder of bentonite intercalated by ions of the metals $Cu^{2+}$ and $Zn^{2+}$ in a ratio between them of (1÷0,5) : (0,5÷ 1) in parts by weight.
9. Preventive bactericidal polish for treatment of nails according to claim 1, wherein the bactericidal agent is a nanostructured powder of bentonite intercalated by ions of metals $Ag^{+}$, $Cu^{2+}$and $Zn^{2+}$ in a ratio between them of 1: (0,5): (0 ÷1) in parts by weight.

10. Preventive bactericidal polish for treatment of nails according to claim 9, wherein the bactericidal agent is a nanostructured powder of bentonite intercalated by ions of metals $Ag^{+}$, $Cu^{2+}$and $Zn^{2+}$ in a ratio between them of 1: (0,5): (0,5).
11. Preventive bactericidal polish for treatment of nails according to claim 1 further containing from 1 to 5% b.w. of usual additives, such as surfactants, light stabilizers, perfumes and the like.
12. Process for the preparation of nanostructured powders of bentonite, intercalated by ions of metals, suitable as bactericidal agent for preventive bactericidal polishes for treatment of nails, comprising:
    a) enrichment of bentonite in Na-form with cations of $Na^{+}$ by processing with 3-10% water solution of an inorganic salt of sodium and subsequent cleaning from anions of chlorine;
    b) intercalation of products obtained at the step a) with ions of $Ag^{+}$, $Cu^{2+}$ and $Zn^{2+}$ by processing with 10-20 % water solution of inorganic salts of silver, copper and zinc and subsequent cleaning of the obtained products from salts of sodium;
    c) nanostructuring the products obtained at the step b) by grinding up to a size of the particles of no more than 150 nm and subsequent drying.