# A STUDY OF ANTIMICROBIAL ACTIVITY OF FOAM-WASHING AGENT SPECIMENS AT ACIDIC pH VALUES

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**Introduction.** It is well-known that any parapharmaceutical substance, in particular foam-washing agents comprising water in combination with detergents, extracts, water-soluble vitamins, viscosity regulators, pH, etc., is the ideal environment for microbial growth [1-5]. In addition, the unfavorable storage conditions when using most foam-washing agents (for example, a bathroom with the increased air humidity, long-term use of an agent, a permanent contact with the hands skin when squeezed out of a flask) do not contribute to a long-term preservation either. Therefore, it is indispensable to use preservatives to protect any foam-washing agent from possible contamination by microorganisms. The main and primary effect of the preservative is its activity against cells of bacteria, molds and yeasts. Only under this condition a preservative can perform its main task, i.e. to prevent the growth of microorganisms. The preservatives mainly act in the following way, by stages: initially they affect the cell membrane of microorganisms while changing its properties, structure and function (lipophilic acids, alcohols, phenols, quaternary ammonium compounds, etc.), and then penetrate cells and react with their nucleophilic components, disrupting the processes of cell function - protein synthesis, energy transfer, the process of cell division. These stages form the electrophilic mechanism of preservatives [6-9]. When developing a product, it is necessary to take into account that preservatives are able to show toxicity in relation to the skin cells and mucous membranes of a human, causing a number of adverse allergic reactions. Traditionally, namely preservatives, in combination with aromatizers and "aggressive" surfactants (Sodium laureth sulfate) are considered to be a major cause of allergic reactions in people with sensitive skin when foam-washing agents are being used [10]. Of course, it is not possible to renounce the use of preservatives in this group of cleaning agents.

Analyzing the above-mentioned, we can conclude that a state-of-the- art preservative must meet the following criteria:
- have a broad spectrum of activity (against fungi, bacteria, yeasts);
- be effective for the duration of its storage;
- slow development and growth of pathogenic microorganisms;
- be safe in selected concentrations under current regulatory documentation;
- be well soluble in water;
- be compatible with all components of the formulation;
- have no color or smell, does not affect the organoleptic properties of the cleaner;
- have some thermal stability;
- be compatible with packaging, because some materials can adsorb (absorb) preservatives;
- be easily recognizable by conventional methods of analysis in a product of this type;
- be effective in a wide pH range;
- be approved for use in the EU;
- hold harmless and environmental biodegradation;
be economical.

However, it should be noted that there are no preservatives which would meet all these criteria straight away. The microbiological purity of modern parapharmaceuticals for the required shelf life (no less than 2 years) is provided not just by increasing the concentration of a preservative, but thanks to the rational combination of at least two substances. (for example, “Kathon CG": methylchloroisothiazolinone and methylisothiazolinone), and in some cases such mixtures can comprise even six preservatives (e.g., «Phenonip»: methyl, ethyl, propil-, butyl-, izobutylparabeny and phenoxyethanol) [11-14].

In Ukraine and EU countries currently more than 30 mixtures of preservatives are being used. The main advantages of such mixtures are: one composition containing preservatives with antimicrobial and antifungal activities, expansion of the antimicrobial spectrum, reduction of the risk of resistance of microorganisms, they are safer due to synergy (the mass share of individual preservatives decreases in the mixture and consequently the overall toxicity gets reduced) [12-14].

We are developing a state-of-the-art foam-washing agent with a low pH level (3,5-4,0). It is well known that the antimicrobial effect of preservatives depends on the pH value. Some preservatives begin to go through hydrolysis in solutions at a pH value less than 6. So the purpose of this study is to choose a preservative that will be active for a given pH range and justify its concentration in the experimental samples. For the study, we used one mono preservative (sodium benzoate) and the rest of multicomponent mixtures.

## Materials and methods

For this study, we have made a number of samples of foam-washing bases with a number of preservatives, which are often used in developing foam-washing agents with acidic pH value, namely:
sample number 1 – foam-washing base + sodium benzoate;sample number 2 – foam-washing base + «Euxyl K300» (phenoxyethanol, methylparaben, bulylparaben, ethylparaben, propylparaben, isobutylaraben); sample number 3 – foam-washing base + «Germaben II» (polypropylene glycol, diazolium dinomovine, methylparaben, propylparaben);
sample number 4 – foam-washing base + «Nipaquard CMB» (benzyl alcohol, triethylene glycol, chloromethylisothiazoline, methylisothiazoline).

The concentration of preservative in each sample was 0.1% (average concentration that is recommended for developing foam-washing agents).
These samples were provided by a pharmaceutical

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research center "Alliance of Beauty" (c.Kyiv, Ukraine). As a pH regulator of foam-washing bases the lactic acid (Lactic Acid, «Galactic», Belgium) was used that is the best component in our opinion. Lactic acid is a part of the acid mantle of the skin, that moisturizes and improves its condition and the thickness of the epidermis, and it is also allowed to be used in foam-washing products for children (Regulation (EU) No 1223/2009).

The level of pH value of the samples was determined potentiometrically (SPU 1.2, 2.2.3) using the device "pH Meter Metrohm 744 " (Germany) [15].

The antimicrobial activity of prototype gels was studied in vitro by diffusion in agar ("wells" method). This method is based on the ability of active substances to diffuse in the agar medium, which was previously inoculated by bacterial crops. The results of the studies make it possible to characterize both the antimicrobial activity of the samples and the release of antimicrobial substances from the base, because the growth inhibition zones of microorganisms are formed as a result of the diffusion of these substances into a dense nutrient medium.

The antimicrobial activity was measured immediately after sample preparation. All the studies were performed in aseptic conditions using a laminar box (biological safety cabinet AS2-4E1 "Esco" Indonesia).

The pure cultures from the American Collection of Crops (ATCC) were used as test cultures: gram-positive bacteria *of Staphylococcus of aureus* of ATCC 25293, spore culture *of Bacillus of subtilis* of ATCC 6633, gram-negative cultures *of Escherichia of coli* of ATCC 25922 and *Proteus of vulgaris* of ATCC 4636. The antifungal effect was elucidated with respect to the yeast-like fungus of the genus Candida - *Candida albicans* ATCC 885-653 and the fungus *Aspergillus brasiliensis* ATCC 16404. In the experiments, one-day suspensions of bacterial microorganisms and a two-day crop of fungi in physiological saline were used. The microbial load was 107 colony-forming units of microorganisms in 1 ml of nutrient medium (CFU / ml).

In Petri dishes, which were installed on a horizontal surface, 10 ml of melted "hungry" agar were added. After solidification of this lower layer of agar, 3 sterile steel cylinders (inner diameter - 6.0 ± 0.1 mm, height - 10.0 ± 0.1 mm) were placed on its surface at equal distance from each other and from the edge of the dish. Around the cylinders, an upper layer was filled, consisting of 14 ml of melted and cooled to 45-48°C agar mixed with the seed dose of the test microorganism.When working with bacterial cultures, meat-peptone agar (MPA) was used for the second layer, while working with fungal crops - agar Saburo. After cooling the upper layer, the cylinders were removed with sterile forceps and the test samples were added to the resulting wells until they were completely filled. Petri dishes were held for 30-40 minutes at room temperature and placed in a thermostat - bacterial cultures at a temperature of 32.5 ± 2.5 ° C for 18-24 hours.

The results were recorded by measuring the growth inhibition zone of microorganisms, including the diameter of the wells. The measurements were carried out with an accuracy of 1 mm, while focusing on the complete absence of visible growth [16, 17].

The diameter of the growth inhibition zone of microorganisms characterized the antimicrobial activity of the experimental samples:
- the absence of growth inhibition zone of microorganisms around the well, as well as a inhibition zone with a diameter of up to 10 mm, was assessed as insensitivity of microorganisms to the sample introduced into the well;
- the growth inhibition areas 11-15 mm in diameter were assessed as a weak sensitivity of the culture to the concentration of the active antimicrobial substance that was being studied;
- growth inhibition zones with a diameter of 16-25 mm - as an indicator of the moderate sensitivity of strains of the microorganism to the test sample;
- growth inhibition zones, the diameter of which exceeded 25 mm, indicate a high sensitivity of microorganisms to the test sample.

The given researches have been carried out at the Biotechnology Department, National University of Pharmacy under the guidance of prof. Strilets A.P.

**Results.** As an outcome of the studies carried out to investigate the antimicrobial properties of preservatives in the samples of foam-washing agents for various cultures of microorganisms, the results were obtained, which are given in Table 1.

**Table 1 - Results for antimicrobial activity of samples (n=5)**

| **Sample** | **Cultures of microorganisms** | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus* ATCC 25293** | ***B.subtilis* ATCC 6633** | ***E.coli* ATCC 25922** | ***Pr.vulgaris* ATCC 4636** | ***C.albicans* of ATCC 885-653** | ***Asp. brasiliensis* of ATCC 16404** |
| | **Diameters of growth inhibition zones of microorganisms, mm** | | | | | |
| №1 | 17,8±0,4 | 21,0±0,7 | 19,2±0,8 | 15,4±0,5 | 21,4±0,5 | 24,0±0,7 |
| №2 | 19,0±0,7 | 22,4±0,5 | 18,2±0,4 | 15,2±0,4 | 22,2±0,4 | 24,2±0,4 |
| №3 | 19,2±0,4 | 21,6±0,5 | 19,6±0,5 | 15,2±0,4 | 21,6±0,5 | 25,2±0,4 |
| №4 | 30,4±0,5 | 29,4±0,8 | 34,8±0,4 | 29,0±0,7 | 34,8±0,4 | 32,2±0,4 |

The data obtained experimentally and presented in Table 1 indicate that all of the test samples No. 1-4 have a broad spectrum of antimicrobial effect and antimicrobial activity against all the test strains used, namely, gram-positive (*Staphylococcus aureus* ATCC 25293 and *Bacillus subtilis* spore culture ATCC 6633) and gram-negative (*Escherichia coli* ATCC 25922, *Pr. vulgaris* ATCC 4636) bacterial cultures and antifungal activity against fungi - *Candida albicans* ATCC 885-653 and *Aspergillus brasiliensis* ATCC 16,404. It was, however, noted that the test sample No.4 (preservative "Nipaquard CMB") shows a higher activity with respect to all bacterial cultures of microorganisms used, than in samples No.1-3 (the diameter of the growth inhibition zones of cultures (mm) is: *Staphylococcus of aureus* - 30,4±0,5; *Bacillus of subtilis* - 29,4±0,8; *Escherichia of coli* - 34,8±0,4; *Pr. vulgaris* - 29,0±0,7). In relation to the effect on fungal cultures - yeast-like *Candida albicans* and mold *Aspergillus brasiliensis*, sample No.4 also showed the greatest activity (diameter of growth inhibition zones (mm) - Candida *of albicans* 34,8±0,4; Aspergillus *of brasiliensis* - 32,2±0,4). It should be noted that all used cultures showed high sensitivity to the antimicrobial activity of the sample No.4.

Therefore, the next step of our research was to study the antimicrobial activity of the foam-washing agent samples with different concentrations of the antimicrobial preservative «Nipaquard CMB». For this purpose, we have made samples: №5 (foam-washing base + «Nipaquard CMB» 0,08%), №6 (foam- washing base + «Nipaquard CMB» 0,12%) and №7 (foam-washing base + «Nipaquard CMB» 0,14%). For the study, the diffusion method in agar and microorganism cultures were used, which were used in previous experiments, namely: gram-positive microorganisms *of Staphylococcus of aureus* of ATCC 25293, spore culture *of Bacillus of subtilis* of ATCC 6633, gram-negative cultures *of Escherichia of coli* of ATCC 25922 and *Proteus of vulgaris* of ATCC 4636. The antifungal effect was elucidated with respect to yeast-like fungi of the genus Candida - *Candida albicans* ATCC 885-653 and fungus *Aspergillus brasiliensis* ATCC 16404. The results are presented in table 2.

**Table 2. Results of antimicrobial activity of samples (n=5)**

| **Sample** | **Cultures of microorganisms** | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus* ATCC 25293** | ***B. subtilis* ATCC 6633** | ***E. coli* ATCC 25922** | ***Pr.vulgaris* ATCC 4636** | ***C.albicans* of ATCC 885-653** | ***Asp. brasiliensis* of ATCC 16404** |
| | **Diameters of growth inhibition zones of microorganisms, mm** | | | | | |
| №4 (0,1) | 30,4±0,5 | 29,4±0,8 | 34,8±0,4 | 29,0±0,7 | 34,8±0,4 | 32,2±0,4 |
| №5 (0,08) | 24,6±0,5 | 25,0±0,7 | 23,4±0,5 | 22,4±0,5 | 27,8±0,4 | 27,4±0,5 |
| №6 (0,12) | 30,2±0,4 | 29,6±0,5 | 34,8±0,8 | 28,8±0,8 | 35,0±0,7 | 32,0±0,7 |
| №7 (0,14) | 30,8±0,4 | 29,8±0,4 | 35,4±0,5 | 29,6±0,5 | 35,2±0,4 | 32,8±0,4 |

The data presented in Table 2 show that the samples Nos.4, 6 and 7 with the preservative "Nipaquard CMB" (concentrations of 0.1, 0.12 and 0.14%, respectively) have high antimicrobial activity (the inhibition zones diameter of culture growth is more than 25 Mm) with respect to bacterial cultures (*Staphylococcus aureus* 30.4 ± 0.5, 30.2 ± 0.4, 30.8 ± 0.4, respectively, *Bacillus subtilis* - 29.4 ± 0.8, 29 6 ± 0.5, 29.8 ± 0.4, *Escherichia coli* - 34.8 ± 0.4, 34.8 ± 0.8, 35.4 ± 0.5, respectively, *Pr vulgaris* -29.0 ± 0, 7, 28.8 ± 0.8, 29.6 ± 0.5). In relation to the effect on fungal cultures, the high activity of samples Nos. 4, 6 and 7 was also obtained by *Candida albicans* (34.8 ± 0.4, 35.0 ± 0.7, 35.2 ± 0.4); *Aspergillus brasiliensis* - 32.2 ± 0.4; 32.0 ± 0.7; 32.8 ± 0.4, respectively. Sample No. 5 with the concentration of preservative "Nipaquard CMB" 0.08% showed moderate antimicrobial activity (diameter of the growth inhibition zones of microorganisms 16-25 mm) with respect to *Staphylococcus aureus* cultures - 24.6 ± 0.5; *Escherichia coli* - 23,8 ± 0,5; *Pr. Vulgaris* -22.4 ± 0.5) and a high antimicrobial effect with respect to *Bacillus subtilis* - 25.0 ± 0.7; *Candida albicans* 27.8 ± 0.4; *Aspergillus brasiliensis* - 27.4 ± 0.5.

**Conclusions.** The results of studies on the antimicrobial effect of experimental samples with the selected preservatives (sodium benzoate, Euxyl K300, Germaben II and Nipaquard CMB) at a concentration of 0.1% relative to all the test strains used, showed that all the investigated foam-washing agent samples have a broad spectrum of antimicrobial effect and antimicrobial activity. It had been proven that the best result was given by the sample of «Nipaquard CMB» at a concentration of 0.1%, which had a high antimicrobial activity against the bacterial cultures - *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pr. Vulgaris*, and in relation to the impact on fungal cultures - *Candida albicans*, *Aspergillus brasiliensis*.

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**Introduction**. It is well-known that any parapharmaceutical substance, in particular, foam-washing agents comprising water in combination with detergents, extracts, water-soluble vitamins, viscosity regulators, pH, etc., is the ideal environment for microbial growth. Therefore, it is indispensable to use preservatives to protect any foam-washing agent from possible contamination by microorganisms. Their main advantages are: presence of a single antimicrobial and antifungal effect, expanded range of effects, decrease in the risk of resistance of microorganisms and decrease in the toxicity and concentration of the preserving mixture. In this regard, the shelf life of parapharmaceutical substances is not provided through the use of large quantities of preservatives, but thanks to their rational combination. **Materials and Methods.** For this study, we have made a number of samples of foam washing bases with a number of preservatives, which are often used in developing foam-washing agents with acidic pH value, namely: sample number 1 – foam washing base + sodium benzoate; sample number 2 – foam washing base + «Euxyl K300» (phenoxyethanol, methylparaben, bulylparaben, ethylparaben, propylparaben, isobutylaraben); sample number 3 – foam washing base + «Germaben II» (polypropylene glycol, diazolium dinomovine, methylparaben, propylparaben); sample number 4 – foam washing base + «Nipaquard CMB» (benzyl alcohol, triethylene glycol, chloromethylisothiazoline, methylisothiazoline). The concentration of preservative in each sample was 0.1% (average concentration that is recommended for developing foam-washing agents). The antimicrobial activity of prototype gels was studied in vitro by diffusion in agar (“wells” method). The antimicrobial activity was measured immediately after sample preparation. All the studies were performed in aseptic conditions using a laminar box (biological safety cabinet AS2-4E1 "Esco" Indonesia). **Results.** According to the study, it was found that among the selected preservatives “Nipaquard CMB” was just the most active. When studying the antimicrobial activity of foam-washing agent samples with different concentrations of the preservative “Nipaquard CMB”, it was found that namely the sample with the concentration of “Nipaquard CMB” of 0,1% showed satisfactory results due to its antimicrobial activity against all cultures such as bacteria and fungi. **Conclusions.** On the basis of microbiological studies it has been demonstrated that all the selected preservatives such as sodium benzoate, “Euxyl K300”, “Germaben II” and “Nipaquard CMB” at a concentration of 0.1% have a broad spectrum of antimicrobial action and antimicrobial activity against all test strains used. We just chose «Nipaquard CMB» as a preservative at a concentration of 0.1% according to the results of experimental research, because it had the best results and a very high antimicrobial activity both against the bacterial cultures - *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Pr. Vulgaris* and in relation to the effect on fungal cultures – *Candida albicans*, *Aspergillus brasiliensis*. **Keywords:** biological researches, preservative, antimicrobial activity, foam- washing agent, pH value.

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