Investigation of stability and disinfecting efficacy of disinfectant solutions for using in veterinary

Gulizar Shakhbanovna Shcherbakova[1,*], *Dmitry Vyacheslavovich* Gruznov[1], *Olga Aleksandrovna* Gruznova[2], *Nikolay Ivanovich* Popov[1], and *Viktor Andreevich* Pirozhikhin[1]

[1]All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology – Branch of Federal Scientific Center – K. I. Skryabin, Ya. R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine, Russian Academy of Sciences, Moscow, 123022, Russian Federation
[2]Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, 119991, Russian Federation

Abstract The stability of work solutions of domestic disinfectants "STEROX vet" and "Biolok" of different concentrations (0.1%, 0.5% and 1%) was examined by electronic absorption spectroscopy. The study was conducted between July and September 2023. When stored according to manufacturer requirements, the changes in the content of the work solution components were found to be directly dependent on the dilution degree. At a concentration of 1.0%, the level of active substance (N,N-bis(3-aminopropyl)-dodecylamine) remained virtually unchanged. Based on the data obtained, it is recommended to prepare work solutions of lower concentrations immediately before using. In addition, effective disinfection modes and efficacious concentrations for these products were determined in relation to sanitary-indicative microorganisms of different resistance groups to the chemical disinfectants.
Keywords: disinfectants, absorption spectra, microorganisms, work solutions, biological threat

1 Introduction

Veterinary sanitary is one of the factors ensuring the food safety and national security of the country. A serious biological threat associated with artificially created resistant strains of microorganisms, which are sources of highly contagious and dangerous animal diseases, including those common to humans, implies enormous economic harm [1-4]. Therefore, veterinary sanitary, as a science, faces a number of demanding challenge in developing measures for the prevention and elimination of infectious diseases [5]. The successful resolution of these problems is conditioned by the conscientious implementation of preventive measures to avert outbreaks of epizootics that, in turn, depends on the availability of highly effective, environmentally friendly broad-spectrum disinfectants, which are concurrently affordable for a wide range of consumers (from farmers with a

* Corresponding author: rabadanova2009@yandex.ru

small number of livestock to huge agricultural enterprises) in veterinary scientific and practicing activities. In recent times, manufacturers have stepped up the production of disinfectants supplemented with cleaning properties, since this is more economically profitable and optimizes the work of maintenance staff [5-7].

High sanctions pressure on Russia caused an outflow of foreign suppliers of bactericidal agents, which occupied a significant share in the domestic market of disinfectants for their using in veterinary practice. Under these circumstances the Russian chemical industry received a boost for active search and development of protection means against diseases in the home country that resulted in market appearance of disinfectants, which combine high bactericidal activity, cleaning properties and low corrosiveness to metal surfaces at a relatively affordable cost. These include, among others, the products “STEROX vet” and “Biolok” [8, 9].

Disinfectant “STEROX vet” is a concentrate, represented as a transparent, bluish-colored liquid of uniform consistency, containing the tertiary amine N,N-bis(3-aminopropyl)-dodecylamine as an active ingredient (Figure 1), as well as auxiliary components including nonionic surfactants, corrosion inhibitor, complexant, dye, fragrance and purified water.

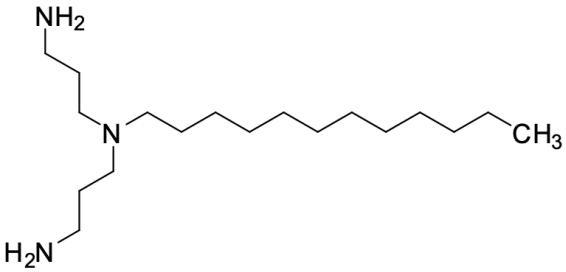

Fig. 1. Structure of N,N-bis(3-aminopropyl)-dodecylamine.

Disinfectant “Biolok” is a transparent liquid from pale blue to blue-green color with a weak specific fragrance. It contains N,N-bis-(triaminopropyl)-dodecylamine as an active ingredient. Auxiliary components include nonionic surfactants, functional additives, dye and water [10].

According to the GOST 12.1.007-76 parameters of acute toxicity, disinfectants “STEROX vet” and “Biolok” are classified as the 3rd class of moderately hazardous substances when introduced into the stomach; as the 4th class of low hazardous substances when applied to the skin; as the 4th class of low hazardous substances at inhalation exposure through the vapors; as the 4th class of low toxic substances according to the volatility degree (C_{20}); and as the 4th class of low toxic substances when introduced into the peritoneum according to K.K. Sidorov classification. The products have a moderate local irritant effect on the skin and a pronounced irritant effect on the conjunctiva, for that reason during work the protection of the skin and eyes is required; simultaneously they do not have skin-resorptive or sensitizing activity.

Both products have cleaning properties and are easily mixed with water in any ratio.

The purpose of this work was to examine the stability of aqueous work solutions of disinfectants “STEROX vet” and “Biolok” during storage by recording their electronic absorption spectra, as well as to determine efficacious concentrations and effective disinfection modes against microorganisms of different resistance groups to these chemical disinfectants.

2 Materials and methods

For research the following product samples were taken: "STEROX vet" manufactured in accordance with TU 20.20.14-074-46842767-2022 (LLC "INTERSEN-PLUS"), and "Biolok" manufactured in accordance with TU 9392-003-99637464-2009 (LLC "Biosphere").

Electronic absorption spectra of 0.1, 0.5 and 1.0% disinfectant solutions were recorded using a PE5400UF spectrophotometer (Ekroskhim, Russia) with a spectral range from 190 to 1000 nm. The analyzed solutions were placed in standard quartz cuvettes with 10 mm absorbing layer thickness.

The spectra were recorded for 28 days with 7 days' interval. The stability of the solutions was assessed by the identity of the spectral profiles, as well as by the absence of a decrease in the optical density value in the absorption band with λ_{max} = 275 nm. Optical density scanning in a specified wavelength range, saving and loading tables of the obtained results were carried out with the SC5400 software (version 2.1) (Ekroskhim, Russia).

The abovementioned studies were conducted between July and September 2023.

In order to assess the disinfectant efficacy, solutions of the examined products "STEROX vet" and "Biolok" of various concentrations were tested under production conditions. Production tests were carried out at various veterinary supervision facilities in the Dagestan Republic: in a room for keeping fattening bulls; in the premises for keeping replacement young laying hens, as well as in the sausage production workshops of Makhachkala Meat Processing Plant ZAO, which are naturally contaminated with sanitary-indicative microorganisms.

Before disinfection, the surfaces in the premises were subjected to thorough mechanical cleaning and washing, followed by the control sampling to determine the natural background of microorganisms by the availability of sanitary indicative microorganisms (*Escherichia coli* and *staphylococcus*).

Solutions of "STEROX vet" and "Biolok" products were tested in concentrations from 0.2 to 3.5% with 1 and 3 hour exposures. Disinfection was carried out using the wet method by fine-droplet irrigation of smooth and rough surfaces. The quality of disinfection was controlled by the isolation of microorganisms *E. coli bacteria* and *staphylococci* from naturally contaminated surfaces in accordance with the requirements of the guidelines "On the procedure for testing new disinfectants for veterinary practice" (1987) and "Rules for disinfection and disinvasion of objects of state veterinary supervision" (2002). To isolate E. coli, Koda medium and Endo agar were used; for staphylococci isolation, 6.5% saline MPB and 8.5% saline MPA were used. The criterion for disinfection efficacy was 100% death of microorganisms. When calculating concentrations, the product itself was taken as 100% substance.

The investigation of the products "STEROX vet" and "Biolok" disinfecting efficacy against *Mycobacterium* strain B-5 was not carried out under production conditions, since laboratory studies of artificial bacterial contamination and subsequent quality control of disinfection by *Mycobacterium* B-5 indications were performed in accordance with the "Methodological instructions on the procedure for testing new disinfectants for veterinary practice" (approved by the State Inspectorate of the State Agricultural Industry of the USSR on January 7, 1987) and "Rules for disinfection and disinvasion of objects of state veterinary supervision" (M., 2002).

3 Results and discussion

Studies on the stability of work solutions of disinfectants "STEROX vet" and "Biolok" were carried out when stored according to requirements. For this purpose, electronic absorption spectra of aqueous solutions at concentrations of 0.1, 0.5 and 1.0% (vol.%) were

recorded when stored according to the instructions in containers with closed lids in a dark place.

As one can see from the data presented in Figures 2-4, the parameters of the 1.0% solution of the product "STEROX vet" remained unchanged throughout the entire study period (Figure 2) taking into account the measurement error. The change in the optical density of its 0.5% solution over a similar time period did not exceed 10% of the original value (Figure 3). More significant changes (about 14%) in the product "STEROX vet" occurred in a solution of a minimal 0.1% concentration (Figure 4).

Figures 5-7 show the spectra of Biolok solutions. The 1% solution demonstrated maximum stability (Figure 5). The parameters of the 0.5% solution were changed by 6.3% (Figure 6). The most significant changes (38.5%) were noted in the Biolok solution of a minimal 0.1% concentration (Figure 7).

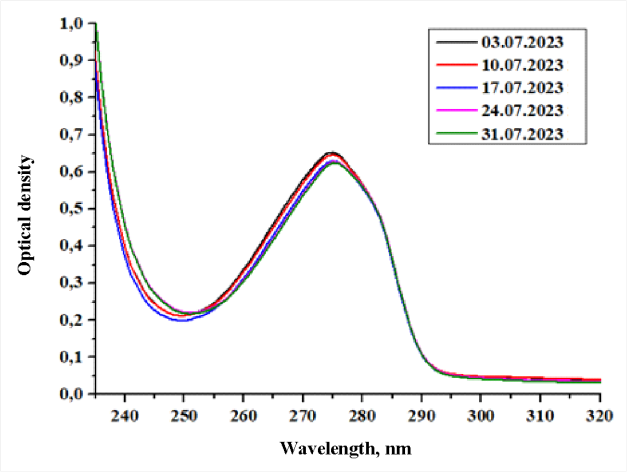

Fig. 2. Stability of 1.0% "STEROX vet"solution. (X axis – wavelength, nm; Y axis – optical density).

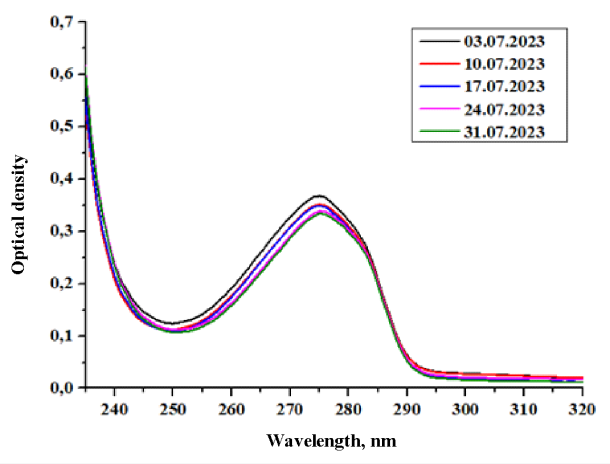

Fig. 3. Stability of 0.5% "STEROX vet" solution.

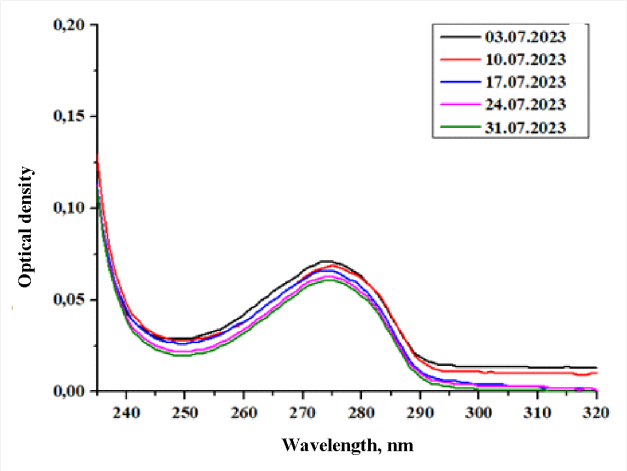

Fig. 4. Stability of 0.1% “STEROX vet”solution.

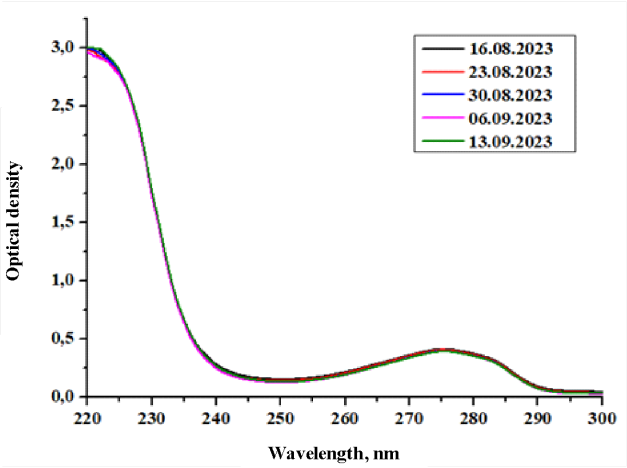

Fig. 5. Stability of 1.0% Biolok solution.

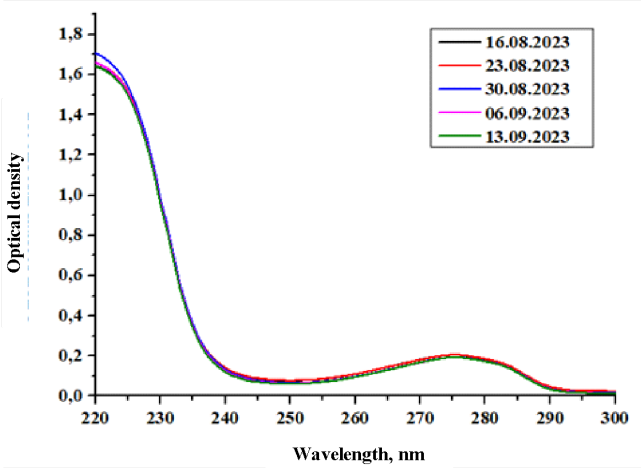

Fig. 6. Stability of 0.5% Biolok solution.

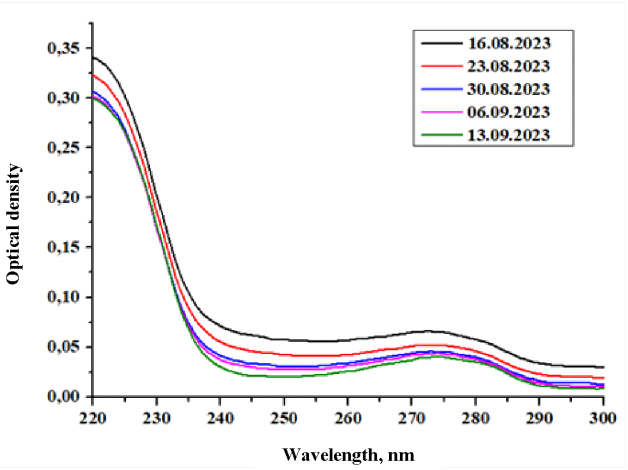

Fig. 7. Stability of 0.1% "Biolok" solution.

From the data obtained, we can conclude that the lower the concentration of the solution, namely, the concentration of the active substance N,N-bis(3-aminopropyl)-dodecylamine, the faster its activity decreases over time; that can be explained by the dissociation of the components in solutions at low concentrations.

Considering the abovementioned data, it should be recommended to prepare work solutions of disinfectants with low concentrations immediately before using or to store them for no more than two weeks.

In addition, to determine efficacy of disinfection modes, tests on the disinfecting activity of the examined disinfectants were carried out, and the efficacious product concentrations are presented in Tables 1 and 2.

Disinfection of surfaces against microorganisms of various resistance groups with the product "STEROX vet" was achieved as follows: against low resistance microorganisms it was done at a product consumption rate of 0.25-0.3 l/m^2 on smooth surfaces and 0.5 l/m^2 on rough surfaces; against stable ones it was done at a product consumption rate of 0.25-0.3 l/m^2 on smooth surfaces and 0.5 l/m^2 on rough surfaces; against highly resistant microorganisms it required double product application with an hour interval at a consumption rate of 0.5 l/m^2 for each irrigation.

Table 1. Effective disinfection modes with "STEROX vet" product.

Microorganism resistance group	**Exposure, h**	**Efficacious product concentration, %**		
		On a smooth surface	**On a rough surface**	**On metlakh tiles**
Low resistant	1	0,5	2,5	1,0
	3	0,3	-	-
Resistant	1	-	3,5	-
	3	0,5	3,0	1,0
Highly resistant	24 (double irrigation with an hour interval)	X	5,0	X

Note: (X) – studies have not been conducted.

Effective disinfection with the Biolok disinfectant was achieved as follows: against low resistant microorganisms it was done at a product consumption rate of 0.25-0.3 l/m^2 on smooth surfaces and 0.5 l/m^2 on rough surfaces; against stable ones it was done at a product consumption rate of 0.25-0.3 l/m^2 on smooth surfaces and 0.5 l/m^2 on rough surfaces.

Table 2. Effective disinfection modes with "Biolok" product.

Microorganism resistance group	Exposure, h	Efficacious product concentration, %	
		On a smooth surface	On a rough surface
Low-resistant	1	1,0	-
	3	0,5	3,0
Resistant	3	3,0	4,0
Highly resistant	24 (double irrigation with an hour interval)	X	5,0

Note: (X) studies have not been conducted.

4 Conclusion

Based on the data resulted from these studies, it can be assumed that work solutions of "STEROX vet" and "Biolok" at concentrations of 1.0% maintain the level of active substance (N,N-bis(3-aminopropyl)-dodecylamine) practically unchanged. Work solutions of the specified concentration can be stored for more than 14 days as recommended by the developers of the instructions for using the products if the storage conditions are observed. The decrease in the content of components in work solutions of 0.5% concentration was insignificant. The degradation of solutions at 0.1% concentration was significantly higher that is apparently explained by the dissociation of the substances included in the product. In this regard, it can be recommended to prepare work solutions of lower concentrations immediately before using.

References

1. Ch. Tong, H. Hu, G. Chen, Zh. Li, A. Li, J. Zhang, Environ. Res. **195**, 110897 (2021). https://doi.org/10.1016/j.envres.2021.110897
2. M. Zachary, W. Joelle, S. Alexander, Viruses **10**, 205 (2018). https://doi.org/10.3390/v10040205
3. B. Bharti, H. Li, Zh. Ren, R. Zhu, Zh. Zhu, Chemosphere **308(3)**, 136404 (2022). https://doi.org/10.1016/j.chemosphere.2022.136404
4. W.A. Rutala, D.J. Weber, Clin. Infect. Dis. **39(5)**, 702-709 (2004). https://doi.org/10.1086/423182
5. A.M. Smirnov, Probl. Vet. Sanit., Hyg. Ecol. **1(1)**, 7-19 (2009)
6. Yu.I. Bochenin, D.V. Gruznov, Probl. Vet. Sanit., Hyg. Ecol. **2(4)**, 7 (2010)
7. V.I. Dorozhkin, A.M. Smirnov, A.A. Suvorov, N.K. Gunenkova, Probl. Vet. Sanit., Hyg. Ecol. **3(23)**, 6-10 (2017)
8. N.I. Popov, G.Sh. Shcherbakova, Vet. Sci. **9**, 57-64 (2022). https://doi.org/10.30896/0042-4846.2022.25.9.57-66

9. N.I. Popov, G.Sh. Shcherbakova, Bul. All-Rus. Res. Inst. Experim. Vet. Med. **83**, 206-211 (2023)
10. N.I. Popov, G.Sh. Shcherbakova, S.A. Michko, Z.E. Alieva, M.S. Saipullaev, A.U. Koychuev, Z.T. Gadzhimuradova, Probl. Vet. Sanit., Hyg. Ecol. **2(38)**, 145-151 (2021). https://doi.org/10.36871/vet.san.hyg.ecol.202102007