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## CONTROL OF RESIDUAL HYDROGEN PEROXIDE AFTER DISINFECTION OF LABORATORY GLASSWARE AND EQUIPMENT

V. M. Britsun, N. L. Tarasenko, N. O. Savina, N. V. Ostanina

State Institution "O.M. Marzиеv Institute for Public Health" NAMSU, Kyiv

[britsun167@ukr.net](mailto:britsun167@ukr.net)

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### ABSTRACT

**The aim of the work.** To determine the residual concentration of hydrogen peroxide in the distilled water which meets the requirements of the State Pharmacopoeia of Ukraine. It seemed necessary to justify the amount of flushing water required for the complete removal of  $H_2O_2$ .

**Materials and Methods.** Deionized water was obtained from the "Millipore Direct-Q 3 UV" system, distilled water – from the "GFL2008" aquadistillator. Hydrogen peroxide solutions of 30 % (produced by "Carlo Erba Reagents S.A.S", "For analysis ACS-Reag.Ph.Eur.-Reag.USP-Stabilized" qualification) and 35 % (produced by "UKRHIM", "medical" qualification) were used. The experiments were carried out using the pharmacopoeial test "oxidizable substances" and conductometric measurements. The conductometric studies were performed on the "Hanna HI 2300" conductometer.

**Results and Discussion.** It was established that the use of the pharmacopoeia test "oxidizable substances" (permanganometric method) allows the identification of hydrogen peroxide at concentration of approximately  $\geq 10^{-4}\%$ .

The specific conductance (SC) of diluted ( $3.5 \times 10^{-5}$ ...3.5%) "pharmacopoeial" and "medical" hydrogen peroxide solutions was also measured. It was found that the diluted solutions of  $H_2O_2$  "pharmacopoeial" and "medical" qualification significantly differ in SC. Probably, this is due to the presence of stabilizers of different nature (organic and inorganic) and in different concentrations (in "pharmacopoeial"  $H_2O_2$  – in minimal, in "medical" – in high concentration).

**Conclusions.** It was found that the concentration of hydrogen peroxide decreases by about 50–100 times with each rinsing of laboratory glassware with distilled water. Therefore, 3–4 times washing with distilled water is needed for complete removal (to reach a concentration  $<10^{-4}\%$ ) of hydrogen peroxide from the laboratory glassware and equipment. Theoretical calculations were confirmed by experimental data.

Thus, hydrogen peroxide has a low SC, which is less than the SC of the stabilizer solutions in it, and the conductometric method is not suitable for controlling residual  $H_2O_2$  in the laboratory glassware and equipment.

**Introduction.** The State Scientific Research Laboratory for quality control of medicines of the State Institution "O.M. Marzиеv Institute for Public Health" NAMSU

checks medicines, substances, pharmaceutical raw materials and hygiene products for compliance with the requirements of regulatory documents and pharmacopoeia

(SPU, EP, BP and USP). These procedures require a significant amount of distilled water, which is obtained by distillation from drinking water and is used for the preparation of solutions, testing of medicinal samples etc. The quality of distilled water must comply with the requirements of the State Pharmacopoeia of Ukraine: the content of microorganisms for microbiological monitoring should be <100 CFU/ml [1]. Some bacteria, for example *Campylobacter spp.*, *Coxiella burnetii*, *Enterococcus faecalis*, *Salmonella choleraesuis* and others, remain viable in water at temperatures of 60–65 °C, and *Legionella spp.* – even up to 80 °C [2]. It means that equipment (receiving cube of water distiller) and ware (containers) for the distilled water should be periodically disinfected. After disinfection the equipment and glass containers for storing water must be washed from residual disinfectant.

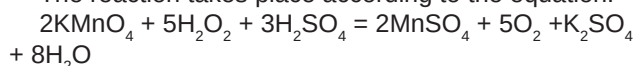
For this purpose, the optimal reagent is hydrogen peroxide (in the form of 3–30 % aqueous solutions), which is widely used in medicine and pharmaceuticals as an effective antiseptic and disinfectant against viruses, bacteria and spores [3, 4]. Compared to other disinfectants, hydrogen peroxide is characterized by low toxicity to warm-blooded animals, absence of odor, usable aggregate state, a moderate price and minimal environmental impact.

Taking into account the scale of H<sub>2</sub>O<sub>2</sub> use in the chemical and pharmaceutical industries, as well as in laboratory practice, the determination of residual amounts of hydrogen peroxide in washing and waste water is an actual and difficult task. The sensitivity of H<sub>2</sub>O<sub>2</sub> detection when titrated with a solution of permanganate in the presence of 3,3',5,5'-tetramethylbenzidine is 1.7x10<sup>-6</sup>% [5], in the presence of 4-aminophenazone – 5x10<sup>-5</sup>% [6], by spectrophotometric method – up to 10<sup>-6</sup>% [7–9]. Lately, a significant attention was paid to the development of reusable sensors for determining the amounts of hydrogen peroxide in aqueous media with sensitivity of up to 3x10<sup>-9</sup>% [10, 11]. However, all of these methods are non-pharmacopoeial and for use in pharmaceutical laboratories – require validation and verification.

**Materials and Methods.** In order to detect the traces of H<sub>2</sub>O<sub>2</sub> in water quickly, we can use the Pharmacopoeia test “oxidizable substances” (permanganometric determination) and quality control of distilled water for the absence of impurities by the conductometric method [1].

We set a goal to find out the sensitivity of the permanganometric determination of the hydrogen peroxide (“oxidizable substances” test), carried out in accordance with the requirements of the State Pharmacopoeia.

The reaction takes place according to the equation:



Considering that hydrogen peroxide is a weak acid (acid dissociation constant of H<sub>2</sub>O<sub>2</sub> 1.78x10<sup>-12</sup>, pKa = 11.75 at 20 °C [12]), the aim of the work was also to determine the suitability of the conductometric method for

determining of H<sub>2</sub>O<sub>2</sub> residual amounts in washing water.

The obtained data will justify the number of washing required to completely remove the residual hydrogen peroxide after disinfection of equipment, containers and laboratory glassware.

**Apparatus and reagents.** The specific conductance (SC) of deionized/distilled water and hydrogen peroxide solutions was measured on Hanna HI 2300 conductivity meter, in plastic containers (100 ml volume) at a temperature of (25.0±0.5) °C, in μS/cm.

The conductometer was pre-calibrated using solutions “Conductivity Standart 84 μS/cm” and “Conductivity Standart 1413 μS/cm” (Mettler Toledo). The measurements in each case were made in triplicate and the average value was calculated. Used solutions of 30 % H<sub>2</sub>O<sub>2</sub>, produced by “Carlo Erba Reagents S.A.S” purity “For analysis ACS-Reag.Ph.Eur.-Reag.USP-Stabilized”, and 35 % H<sub>2</sub>O<sub>2</sub>, produced by “UkrKhim” purity “medical”. Potassium permanganate – was manufactured by Sigma-Aldrich, purity “CS reagent”.

Deionized and distilled water was obtained, respectively, from the Millipore Direct-Q 3 UV system (SC ≤0.06 μS/cm at (25.0±0.5) °C) and from the GFL2008 distiller (SC <<5.1 μS/cm at (25.0±0.5) °C).

The exact concentration of the initial solutions of H<sub>2</sub>O<sub>2</sub> produced by “Carlo Erba Reagents S.A.S.” and “UkrKhim” (30.4 % and 34.7 %, respectively) was established by the permanganometric method in accordance with the requirements of the SFU [1].

**Preparation of hydrogen peroxide solutions.** 10 ml of 30 % hydrogen peroxide was taken with a Mora pipette and added to a 100 ml volumetric flask containing 50 ml of deionized (distilled) water. After stirring, water was added to the mark. Further, solutions with concentrations of 0.3...3x10<sup>-5</sup>% were obtained in volumetric flasks (first accuracy class) by the method of serial dilution by deionized (distilled) water. Similarly, solutions of H<sub>2</sub>O<sub>2</sub> with a concentration of 3.5...3.5x10<sup>-5</sup>% were obtained from 35 % hydrogen peroxide.

**Washing procedure for 2.5 L bottles.** A 2.5 L glass bottle was disinfected with 100 ml of 3 % H<sub>2</sub>O<sub>2</sub> and drained for 20 seconds. A bottle was filled with 100 (200) ml of distilled water, stirred for 1 min and inverted. The content of the bottle was poured into a graduated cylinder for 20 s and the difference in the volume of water that remained in the bottle was noted.

**Test for the presence of oxidizable substances.** 10 ml of 1 M H<sub>2</sub>SO<sub>4</sub> solution and 0.1 ml of KMnO<sub>4</sub> 0.02 M solution were added to 100 ml of washing distilled water. The resulting test solution was boiled for 5 minutes. In the absence of H<sub>2</sub>O<sub>2</sub>, the solution should remain weakly pink [1].

**The calculation of the uncertainty** of the test results was carried out in accordance with the requirements of SFU (p. 5.3.N.1, p. 5.3.N.2) [7].

### Results and Discussion

We used the Pharmacopoeia test “oxidizable substances” to identify residual amounts of H<sub>2</sub>O<sub>2</sub> in labora-

tory glassware and equipment. Testing was carried out with  $H_2O_2$  solutions (on deionized or distilled water) at concentrations of 0.00003–0.35 %. The results of the research are shown in Table 1.

It was found that during the pharmacopeia test "oxidizable substances", the color change of the  $KMnO_4$  solution occurs in the range of  $H_2O_2$  concentrations 0.00006-0.00015 %. It allows to identify the presence of hydrogen peroxide in the wash water at a concentration of approximately  $\geq 10^{-4}$  % ( $\geq 1$  ppm). The results of experiments with  $H_2O_2$  solutions, prepared on deionized and distilled water, are identical.

A glass bottle made of dark glass with the capacity of 2.5 liters was used as a model for determining the number of washes. It was found that with each rinsing of the bottle with distilled water, the concentration of hydrogen

peroxide decreases by about 50 times (the volume of washing water is 100 ml) or 100 times (the volume of washing water is 200 ml) (Table 2). The presence of residual amounts of  $H_2O_2$  in the washing water was checked by the "oxidizable substances" test.

The concentration of the  $H_2O_2$  solution (Table 2, No. 3) was determined by adding 10 % KI, 1 M  $H_2SO_4$  solutions and titrating of the released iodine with 0,01 M  $Na_2S_2O_3$  solution in the presence of starch and chloroform until the blue color disappears [13]. The calculation was carried out according to the formula  $X = ((V_e - V_{st}) \cdot 0,00017 \cdot 100) / mH_2O_2$  ( $V_e$  – volume of thiosulfate solution consumed for titration of the test solution,  $V_{st}$  – volume of thiosulfate solution used for titration of blank solution,  $mH_2O_2$  – solution weight). The resulting concentration of  $H_2O_2$  was  $(1,2 \pm 0.1) \times 10^{-4}$  %, which is in sa-

Table 1

Data of test "oxidizable substances" for solutions of  $H_2O_2$

No.	$H_2O_2$ produced by "UkrKhim"			No.	$H_2O_2$ produced by "Carlo Erba Reagents S.A.S"	
	Concentr. $H_2O_2$ , %	Color of $KMnO_4/H_2SO_4$ solution (in deioniz. water)	Color of $KMnO_4/H_2SO_4$ solution (in dist. water)		Concentr. $H_2O_2$ , %	Color of $KMnO_4/H_2SO_4$ solution (in deioniz. water)
1	0.35	colorless	colorless	11	0.3	colorless
2	0.175	colorless	colorless	12	$6 \times 10^{-2}$	colorless
3	$3.5 \times 10^{-2}$	colorless	colorless	13	$3 \times 10^{-2}$	colorless
4	$3.5 \times 10^{-3}$	colorless	colorless	14	$3 \times 10^{-3}$	colorless
5	$3.5 \times 10^{-4}$	colorless	colorless	15	$6 \times 10^{-4}$	colorless
6	$1.75 \times 10^{-4}$	colorless	colorless	16	$3 \times 10^{-4}$	colorless
7	$1.06 \times 10^{-4}$	almost colorless	almost colorless	17	$1.5 \times 10^{-4}$	almost colorless
8	$7 \times 10^{-5}$	weakly weak; pink	weakly weak; pink	18	$6 \times 10^{-5}$	weakly weak; pink
9	$3.5 \times 10^{-5}$	weakly pink	weakly pink	19	$3 \times 10^{-5}$	weakly pink
10	0	weakly pink	weakly pink	20	0	weakly pink

Table 2

Test data of "oxidizable substance" for washing water containing  $H_2O_2$

No.	Bottle (volume 2.5 l) washing after disinfection with 3 % $H_2O_2$ solution				
	Volume of washing water/ residue after washing, ml	Dilution	Estimated concentration of $H_2O_2$ in wash water, %	Color of $KMnO_4/H_2SO_4$ solution	Actual concentration of $H_2O_2$ in wash water, % **
1	100/2	50	$6 \times 10^{-2}$	colorless	-
2	100/2	50	$1.2 \times 10^{-3}$	colorless	$\geq 1.75 \times 10^{-4}$
3*	100/2	50	$2.4 \times 10^{-5}$	almost colorless	$(1.06 \dots 1.5) \times 10^{-4}$
4	100/2	50	$4.8 \times 10^{-7}$	weakly pink	$\leq 3.5 \times 10^{-5}$
5	200/2	100	$3 \times 10^{-2}$	colorless	$\geq 1.75 \times 10^{-4}$
6	200/2	100	$3 \times 10^{-4}$	colorless	$\geq 1.75 \times 10^{-4}$
7	200/2	100	$3 \times 10^{-6}$	weakly pink	$\leq 3.5 \times 10^{-5}$

Notes: \* The  $H_2O_2$  concentration of the solution was also determined by titration

\*\*In accordance with the data of Table 1.

tisfactory agreement with the results of the permanganometric express test  $((1.06...1.5) \times 10^{-4} \%$ , Table 1).

Table 2 shows that if washing with a volume of water, which is 4 % of the bottle capacity, 4 washes are needed to remove  $H_2O_2$  residues completely. Significant number of washes is obviously explained by the heterogeneity of the manual washing with a small volume of water and the adsorption of  $H_2O_2$  on glass [12, 14].

With an increase of the volume of washing water to 8 % of the bottle capacity, 3 washes are sufficient to remove the  $H_2O_2$  residues completely.

A study of the specific conductance of hydrogen peroxide solutions is given in Table 3. The obtained data were processed by Excel in order to construct calibra-

tion graphs and creation of mathematical equations for the dependence of electrical conductivity on concentration (Figure 1).

To avoid a systematic error (the influence of the conductivity of "extraneous" ions), we did not use solutions SC, but the difference ( $\Delta$ ), respectively, between solutions SC and deionized water SC to plot the graph.

It was found that SC of  $H_2O_2$  solutions "pharmacopoeial" and "medical" qualifications differ significantly.

This is probably due to the presence of stabilizers of various nature (inorganic – phosphate and sodium stannate, organic – oxyquinoline) and in various concentrations (in "pharmacopoeial"  $H_2O_2$  – in minimal, in "medical"  $H_2O_2$  – in significant concentration) [12].

Table 3

Specific conductance (SC) data of  $H_2O_2$  solutions at 25 °C

No.	$H_2O_2$ produced by "UkrKhim"				No.	$H_2O_2$ produced by "Carlo Erba Reagents S.A.S"			
	Concentration, %	SC of $H_2O_2$ solutions, $\mu S/cm$	Uncertainty, $\mu S/cm$	$\Delta SC H_2O_2$ and deionized water		Concentration, %	SC of $H_2O_2$ solutions, $\mu S/cm$	Uncertainty, $\mu S/cm$	$\Delta SC H_2O_2$ and deionized water
1	3.5	74.7	0.9	74.2	8	3.0	1.42	0.13	0.88
2	0.35	8.34	0.13	7.80	9	0.3	0.98	0.12	0.44
3	0.175	4.66	0.12	4.12	10	$6 \times 10^{-2}$	0.91	0.12	0.37
4	$3.5 \times 10^{-2}$	1.34	0.12	0.80	11	$3 \times 10^{-2}$	0.88	0.12	0.34
5	$3.5 \times 10^{-3}$	0.73	0.12	0.19	12	$3 \times 10^{-3}$	0.84	0.12	0.3
6	$3.5 \times 10^{-4}$	0.63	0.12	0.09	13	$6 \times 10^{-4}$	0.83	0.12	0.29
7	$3.5 \times 10^{-5}$	0.61	0.12	0.07	14	$3 \times 10^{-4}$	0.72	0.12	0.18

Notes: SC of deionized water in glassware is  $0.54 \pm 0.12 \mu S/cm$ .

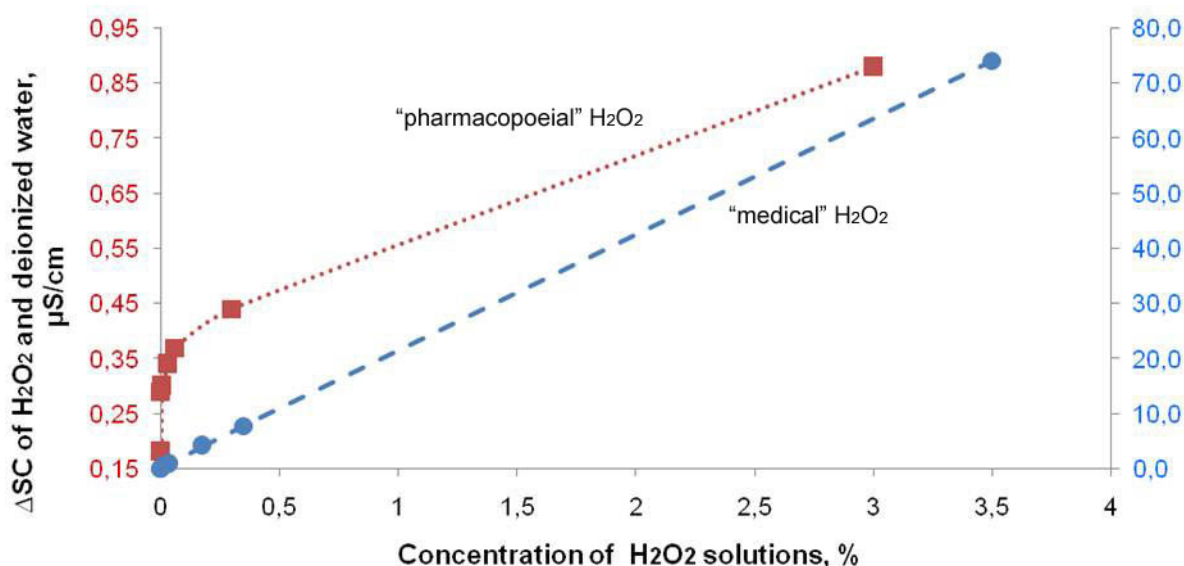


Fig. 1. Graph of the  $\Delta SC$  of  $H_2O_2$  solutions and deionized water as a function of concentration (for "pharmacopoeial"  $H_2O_2$ ,  $y = 0.194x + 0.305$ ,  $R^2 = 0.916$ ; for "medical"  $H_2O_2$ ,  $y = 21.15x + 0.184$ ,  $R^2 = 0.999$ ).

### Conclusions

Test "oxidizable substances" allows to identify  $H_2O_2$  in distilled water at the concentration of up to  $1 \times 10^{-4}\%$ . Thus, the method demonstrated high sensitivity. Application of the method made it possible to establish that at least 3–4 rinsings with distilled water are necessary (the volume of water is 4–8 % of the volume of glassware) for the complete removal of hydrogen peroxide from laboratory glassware.

Hydrogen peroxide has a low specific conductance, which is smaller than the specific conductance of stabilizer solutions in it. Therefore, the method of determination by specific electrical conductivity is not suitable for  $H_2O_2$  residual amounts monitoring in the laboratory glassware and equipment.

**Conflicts of interest:** authors have no conflict of interest to declare.

**Конфлікт інтересів:** відсутній.

## КОНТРОЛЬ ЗАЛИШКОВИХ КІЛЬКОСТЕЙ ПЕРОКСИДУ ВОДНЮ ПІСЛЯ ДЕЗИНФЕКЦІЇ ЛАБОРАТОРНОГО ПОСУДУ ТА ОБЛАДНАННЯ

В. М. Брицун, Н. Л. Тарасенко, Н. О. Савіна, Н. В. Останіна

*ДУ «Інститут громадського здоров'я імені О. М. Марзєєва Національної академії медичних наук України», Київ*

*britsun167@ukr.net*

**Мета роботи.** Визначення залишкової концентрації пероксиду водню в дистильованій воді, яка відповідає вимогам ДФУ, й обґрунтування кількості промивної води для повного видалення  $H_2O_2$  після дезінфекції лабораторного посуду та обладнання.

**Матеріали і методи.** Воду деіонізовану отримано з системи Millipore Direct-Q 3 UV, воду дистильовану – з аквадистилятора GFL2008. Використовували розчини пероксиду водню 30 % виробництва «Carlo Erba Reagents S.A.S» чистоти «For analysis ACS-Reag.Ph.Eur.-Reag.USP-Stabilized» і 35 % кваліфікації «медичний» виробництва «УКРХІМ». Досліди виконували з використанням фармакопейного тесту «речовини, що окиснюються» і кондуктометричних вимірювань. Кондуктометричні дослідження проводили на кондуктометрі Hanna HI 2300.

**Результати й обговорення.** Фармакопейне випробовування «речовини, що окиснюються» (перманганатометричний метод) дає можливість ідентифікувати перекис водню при концентрації приблизно  $\geq 10^{-4}\%$ .

Визначали також питому електропровідність (ПЕ) розведених ( $3.5 \times 10^{-5} \dots 3.5\%$ ) «фармакопейного» і «медичного» розчинів пероксиду водню. Встановлено, що ПЕ розведених розчинів  $H_2O_2$  «фармакопейної» і «медичної» кваліфікації суттєво відрізняється. Вірогідно, це зумовлено наявністю стабілізаторів різної природи (органічних і неорганічних) і в різних концентраціях (у «фармакопейній»  $H_2O_2$  – у мінімальній, у «медичній» – в значній концентрації).

**Висновки.** При кожному ополіскуванні лабораторного посуду дистильованою водою концентрація пероксиду водню знижується приблизно в 50–100 разів. Тому для повного видалення пероксиду водню з лабораторного посуду та обладнання (досягнення концентрації  $< 10^{-4}\%$ ) необхідне 3–4-кратне промивання дистильованою водою. Теоретичні розрахунки підтверджено експериментальними даними.

Встановлено, що пероксид водню має низьку ПЕ, яка нижча від ПЕ розчинів стабілізаторів у ньому, і кондуктометричний метод не придатний для контролю залишкових кількостей  $H_2O_2$  у лабораторному посуді та обладнанні.

**Ключові слова:** пероксид водню; залишкові кількості; перманганатометрія; чутливість реакції; речовини, що окиснюються; кондуктометрія; питома електропровідність.

## КОНТРОЛЬ ОСТАТОЧНЫХ КОЛИЧЕСТВ ПЕРОКСИДА ВОДОРОДА ПОСЛЕ ДЕЗИНФЕКЦИИ ЛАБОРАТОРНОЙ ПОСУДЫ И ОБОРУДОВАНИЯ

В. Н. Брицун, Н. Л. Тарасенко, Н. А. Савина, Н. В. Останина

*ГУ «Институт общественного здоровья имени А. Н. Марзеева Национальной академии медицинских наук Украины», Киев*

*britsun167@ukr.net*

**Цель работы.** Определение остаточной концентрации пероксида водорода в дистиллированной воде, соответствующей требованиям ГФУ, и обоснование количества промывной воды, необходимой для полного удаления  $H_2O_2$  после дезинфекции лабораторной посуды и оборудования.

**Материалы и методы.** Вода деионизированная была получена из системы Millipore Direct-Q 3 UV, вода дистиллированная – из аквадистиллятора GFL2008. Использовались растворы пероксида водорода 30 % производства «Carlo Erba Reagents S.A.S» чистоты «For analysis ACS-Reag.Ph.Eur.-Reag.USP-Stabilized» и 35 % квалификации «медицинский» производства «УКРХИМ». Опыты проводили с использованием фармакопейного теста «окисляемые вещества» и кондуктометрических измерений. Кондуктометрические исследования осуществляли на кондуктометре Hanna HI 2300.

**Результаты и обсуждение.** Фармакопейный тест «окисляемые вещества» (перманганатометрический метод) позволяет идентифицировать перекись водорода при концентрации примерно  $\geq 10^{-4}$  %.

Определяли также удельную электропроводность (УЭ) разведенных ( $3.5 \times 10^{-5}$ ... 3.5 %) «фармакопейного» и «медицинского» растворов перекиси водорода. Установлено, что УЭ разведенных растворов  $H_2O_2$  «фармакопейной» и «медицинской» квалификации существенно отличаются. Вероятно, это обусловлено наличием стабилизаторов различной природы (органических и неорганических) и в различных концентрациях (у «фармакопейной»  $H_2O_2$  – в минимальной, в «медицинской» – в значительной концентрации).

**Выводы.** При каждом ополаскивании лабораторной посуды дистиллированной водой концентрация пероксида водорода уменьшается примерно в 50–100 раз. Поэтому для полного удаления пероксида водорода из лабораторной посуды и оборудования (достижения концентрации  $<10^{-4}$  %) нужна 3–4-кратная промывка дистиллированной водой. Теоретические расчеты подтверждены экспериментальными данными.

Определено, что пероксид водорода имеет низкую УЭ, которая меньше УЭ растворов стабилизаторов в нем, и кондуктометрический метод не пригоден для контроля остаточных количеств  $H_2O_2$  в лабораторной посуде и оборудовании.

**Ключевые слова:** пероксид водорода; остаточные количества; перманганатометрия; чувствительность реакции; окисляемые вещества; кондуктометрия; удельная электропроводность.

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## Information about the authors

**Britsun V. M.** – DSc (Chemistry), chief researcher, State Institution "O. M. Marzeyev Institute for Public Health of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine. E-mail: britsun167@ukr.net.

**Tarassenko N. L.** – lead engineer, State Institution "O. M. Marzeyev Institute for Public Health of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine. E-mail: parab2009@ukr.net.

**Savina N. O.** – scientific researcher, State Institution "O. M. Marzeyev Institute for Public Health of the National Academy of Medical Sciences of Ukraine" Kyiv, Ukraine. E-mail: katrinsavina@ukr.net.

**Ostanina N. V.** – PhD (Economics), scientific researcher, Head of the State Scientific Research Laboratory for Quality Control of Medicines, State Institution "O. M. Marzeyev Institute for Public Health of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine. E-mail: 3526309@ukr.net

## Відомості про авторів

**Брицун В. М.** – д. хім. наук, головний науковий співробітник, ДУ «Інститут громадського здоров'я імені О. М. Марзєєва Національної академії медичних наук України», Київ, Україна. E-mail: britsun167@ukr.net.

**Тарасенко Н. Л.** – провідний інженер, ДУ «Інститут громадського здоров'я імені О. М. Марзєєва Національної академії медичних наук України», Київ, Україна. E-mail: parab2009@ukr.net.

**Савіна Н. О.** – науковий співробітник, ДУ «Інститут громадського здоров'я імені О. М. Марзєєва Національної академії медичних наук України», Київ, Україна. E-mail: katrinsavina@ukr.net.

**Останіна Н. В.** – канд. екон. наук, науковий співробітник, завідувач Державною науково-дослідною лабораторією з контролю якості лікарських засобів, ДУ «Інститут громадського здоров'я імені О. М. Марзєєва Національної академії медичних наук України», Київ, Україна. E-mail: 3526309@ukr.net.