

ISSN 2518-1491 (Online),
ISSN 2224-5286 (Print)

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

Д.В. Сокольский атындағы
«Жанармай, катализ және электрохимия институты» АҚ

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РЕСПУБЛИКИ КАЗАХСТАН
АО «Институт топлива, катализа и
электрохимии им. Д.В. Сокольского»

N E W S

OF THE ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN
JSC «D.V. Sokolsky institute of fuel,
catalysis and electrochemistry»

SERIES
CHEMISTRY AND TECHNOLOGY

2 (455)

APRIL – JUNE 2023

PUBLISHED SINCE JANUARY 1947

PUBLISHED 4 TIMES A YEAR

ALMATY, NAS RK

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ISSN 2518-1491 (Online),

ISSN 2224-5286 (Print)

Меншіктенуші: «Қазақстан Республикасының Ұлттық ғылым академиясы» РҚБ (Алматы қ.) Қазақстан Республикасының Ақпарат және қоғамдық даму министрлігінің Ақпарат комитетінде 29.07.2020 ж. берілген № **KZ66VPY00025419** мерзімдік басылым тіркеуіне қойылу туралы куәлік.

Тақырыптық бағыты: *органикалық химия, бейорганикалық химия, катализ, электрохимия және коррозия, фармацевтикалық химия және технологиялар.*

Мерзімділігі: жылына 4 рет.

Тиражы: 300 дана.

Редакцияның мекен-жайы: 050010, Алматы қ., Шевченко көш., 28, 219 бөл., тел.: 272-13-19

<http://chemistry-technology.kz/index.php/en/arithv>

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Редакцияның мекенжайы: 050100, Алматы қ., Қонаев к-сі, 142, «Д.В. Сокольский атындағы отын, катализ және электрохимия институты» АҚ, каб. 310, тел. 291-62-80, факс 291-57-22, e-mail: orgcat@nursat.kz

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«Известия НАН РК. Серия химии и технологий».

ISSN 2518-1491 (Online),

ISSN 2224-5286 (Print)

Собственник: Республиканское общественное объединение «Национальная академия наук Республики Казахстан» (г. Алматы).

Свидетельство о постановке на учет периодического печатного издания в Комитете информации Министерства информации и общественного развития Республики Казахстан № KZ66VPY00025419, выданное 29.07.2020 г.

Тематическая направленность: *органическая химия, неорганическая химия, катализ, электрохимия и коррозия, фармацевтическая химия и технологии.*

Периодичность: 4 раз в год.

Тираж: 300 экземпляров.

Адрес редакции: 050010, г. Алматы, ул. Шевченко, 28, оф. 219, тел.: 272-13-19

<http://chemistry-technology.kz/index.php/en/archiv>

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News of the National Academy of Sciences of the Republic of Kazakhstan. Series of chemistry and technology.

ISSN 2518-1491 (Online),

ISSN 2224-5286 (Print)

Owner: RPA «National Academy of Sciences of the Republic of Kazakhstan» (Almaty).

The certificate of registration of a periodical printed publication in the Committee of information of the Ministry of Information and Social Development of the Republic of Kazakhstan No. **KZ66VPY00025419**, issued 29.07.2020.

Thematic scope: *organic chemistry, inorganic chemistry, catalysis, electrochemistry and corrosion, pharmaceutical chemistry and technology.*

Periodicity: 4 times a year.

Circulation: 300 copies.

Editorial address: 28, Shevchenko str., of. 219, Almaty, 050010, tel. 272-13-19

<http://chemistry-technology.kz/index.php/en/arhiv>

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Editorial address: JSC «D.V. Sokolsky institute of fuel, catalysis and electrochemistry», 142, Kunayev str., of. 310, Almaty, 050100, tel. 291-62-80, fax 291-57-22, e-mail: orgcat@nursat.kz

Address of printing house: ST «Aruna», 75, Muratbayev str, Almaty.

NEWS

OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN

SERIES CHEMISTRY AND TECHNOLOGY

ISSN 2224–5286

Volume 2. Number 455 (2023), 164–176

<https://doi.org/10.32014/2023.2518-1491.172>

UDC 556.11 (075.8)

IRSTI 31.19.00

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CHEMICAL ANALYSIS OF WATER USED IN THE PRODUCTION OF BIOLOGICAL PRODUCTS

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Abstract. This work shows a chemical analysis of various types of water used in pharmaceutical production in terms of hydrogen ion concentration (pH), electrical conductivity, anion and cation content, and content of organic and inorganic carbon compounds. Based on the results obtained, two diagrams of the dependence of pH and electrical conductivity on temperature were constructed. As the temperature rises, the pH values for tap, purified, injection and deionized water decrease and the electrical conductivity values increase. Water samples were analyzed by capillary electrophoresis (CE), and electrophoregrams (EPG) of tap water were obtained, on which the following

ions were detected: Na^+ (107.2 mg/dm³), Mg^{2+} (18.00 mg/dm³), Ca^{2+} (42.40 mg/dm³), NO_2^- (47.35 mg/dm³), NO_3^- (237.0 mg/dm³), F^- (6.980 mg/dm³), PO_4^{3-} (3.205 mg/dm³). Also, Phosphate ions were found in purified water at 0.788 mg/dm³. No ions were found in samples of deionized and injection water. Using the TOC-L analyzer, the concentration of total carbon in tap, purified, deionized and injection water was determined and amounted to 28.16 mg/l, 0.3289 mg/l, 0.1833 mg/l, 0.06446 mg/l, respectively. Thus, the chemical analysis of water samples according to these indicators shows the suitability of using these waters on the production line.

Keywords: Hydrogen ion concentration, electrical conductivity, organic and inorganic carbon compounds, anions, cations

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Аннотация. Бұл жұмыста сутегі иондарының концентрациясы (рН), электр өткізгіштігі, аниондар мен катиондардың құрамы және көміртектің органикалық және бейорганикалық қосылыстарының құрамы сияқты көрсеткіштер бойынша фармацевтикалық өндірісте қолданылатын әртүрлі су түрлеріне химиялық талдау берілген. Алынған нәтижелерге сәйкес рН пен электр өткізгіштіктің

температураға тәуелділігінің екі диаграммасы жасалды. Температураның жоғарылауымен ағынды, тазартылған, инъекциялық және деионизацияланған судың рН мәні төмендейді, ал электр өткізгіштік мәні артады. Капиллярлық электрофорез (КЭ) әдісімен су сынамасына талдау жүргізілді, су құбыры суының электрофореграммалары (ЭФГ) алынды, оларда мынадай иондар табылды: Na^+ (103,3 мг/дм³), Mg^{2+} (17,80 мг/дм³), Sr^{2+} (0,807 мг/дм³), Ca^{2+} (41,52 мг/дм³) NO_3^- (237,0 мг/дм³), NO_2^- (47,35 мг/дм³), F^- (6,980 мг/дм³), PO_4^{3-} (3,205 мг/дм³). Тазартылған суда PO_4^{3-} (0,788 мг/дм³) табылды. Деионизацияланған және инъекциялық су сынамаларында иондар табылған жоқ. ТОС-Л анализаторының көмегімен ағынды, тазартылған, деионизацияланған және инъекциялық судағы жалпы көміртектің концентрациясы анықталды және сәйкесінше 28.16 мг/л, 0.3289 мг/л, 0.1833 мг/л, 0.06446 мг/л, құрады. Осылайша, осы көрсеткіштер бойынша су сынамаларына жүргізілген химиялық талдау осы суларды өндірістік желіде пайдалануға жарамдылығын көрсетеді.

Түйін сөздер: Сутегі иондарының концентрациясы, электрөткізгіштік, көміртектің органикалық және бейорганикалық қосылыстары, аниондар, катиондар

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ХИМИЧЕСКИЙ АНАЛИЗ ВОДЫ ДЛЯ ПРОИЗВОДСТВА БИОПРЕПАРАТОВ

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Аннотация. В данной работе представлен химический анализ различных типов воды, используемых в фармацевтическом производстве по таким показателям, как концентрация ионов водорода (рН), электропроводимость, содержание анионов и катионов, и содержание органических и неорганических соединений углерода. По полученным результатам были построены две диаграммы зависимости рН и электропроводимости от температуры. При повышении температуры значения рН для водопроводной, очищенной, инъекционной и деионизированной воды уменьшаются, а значения электропроводимости увеличиваются. Проведен анализ проб воды методом капиллярного электрофореза (КЭ), получены электрофореграммы (ЭФГ) водопроводной воды на которых обнаружены следующие ионы: Na^+ (103,3 мг/дм³), Mg^{2+} (17,80 мг/дм³), Sr^{2+} (0,807 мг/дм³), Ca^{2+} (41,52 мг/дм³) NO_3^- (237,0 мг/дм³), NO_2^- (47,35 мг/дм³), F^- (6,980 мг/дм³), PO_4^{3-} (3,205 мг/дм³). В очищенной воде обнаружен PO_4^{3-} (0,788 мг/дм³). В пробах деионизированной и инъекционной воды ионов не было обнаружено. С помощью ТОС-Л анализатора была определена концентрация общего углерода в водопроводной, очищенной, деионизированной и инъекционной воде и составила 28.16 мг/л, 0.3289 мг/л, 0.1833 мг/л, 0.06446 мг/л, соответственно. Таким образом, проведенный химический анализ проб воды по данным показателям показывает пригодность использования этих вод на производственной линии.

Ключевые слова: концентрация ионов водорода, электропроводимость, органические и неорганические соединения углерода, анионы, катионы

Introduction

Water is widely used as raw material, ingredient, and solvent in processing and manufacturing processes, as well as a component in drug formulations, active pharmaceutical ingredients (APIs), intermediates, and analytical reagents (Hideharu, 2016: 203–214).

The term "water" is used to refer to drinking water freshly collected directly from a public water source and suitable for drinking. The water used in the pharmaceutical industry and related industries is divided into the following types: drinking water (suitable for drinking), purified water, purified sterile water, water for injection, sterile water for injection, bacteriostatic water for injection, sterile water for irrigation and sterile water for inhalation.

The chemical composition of drinking water is diverse, and the nature and concentration of impurities in it depend on the source from which it is taken (Maria, 2016: 724–733). For pharmaceutical use, drinking water is in most cases purified by distillation, ion exchange, reverse osmosis, or other processes suitable for the production of purified water (Cornelius, 2021: 6058). For a number of purposes, water that meets pharmacopoeia requirements other than those for purified water (for example, water for injection) is required.

Water is chemically stable in all of its physical states (ice, liquid and vapor). Water that has been treated at a pharmaceutical plant and enters the storage tank must meet certain requirements. The main task in the design and operation of the water storage

and distribution system is to prevent the deviation of its quality indicators from the permissible limit values. In particular, the storage and distribution system must protect the water from contamination by ions and organic molecules, which can lead to an increase in the electrical conductivity of water and an increase in its level of total organic carbon, respectively.

The purpose of this work was to assess the quality of water for production by such chemical parameters as the concentration of hydrogen ions (pH), electrical conductivity, the content of organic and inorganic carbon compounds, as well as the determination of the content of anions and cations.

Materials and methods:

Determination of pH. This indicator is determined by the potentiometric method, in accordance with the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), v. 1, p. 41.

Determination of electrical conductivity. The electrical conductivity is measured using a conductometer while simultaneously recording the temperature in accordance with the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), v.2, p. 165.

Determination of the amount of organic and inorganic carbon compounds. Works on the determination of limited and inorganic carbon compounds in the composition of water are carried out on the analyzer TOS-L according to the approved method of the manufacturer (Christian, 2002: 43–47).

Determination of the content of anions and cations. Works on the determination of anions and cations in the composition of water are carried out on capillary electrophoresis (CE) "KAPEL-105M" according to the approved method of the manufacturer (Fukushi, 2019: 1606).

Results

One of the main requirements for water, which is used in the production of biological products, is its pH, determined by the activity of hydrogen ions in the solution, which quantitatively expresses its acidity.

The analysis was carried out on different types of waters according to the SP RK, v. 1, p. 41. on a pH-meter MARK-901 with increasing temperature.

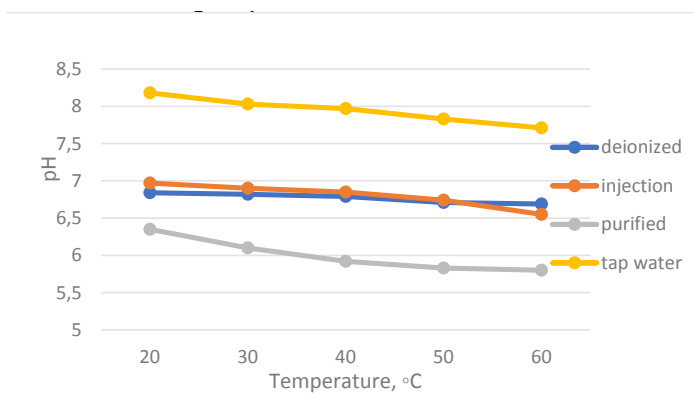


Fig. 1 - Dependence of pH on temperature

The results obtained, presented in Figure 1 in terms of pH for deionized, injection and purified water, are within the allowable values according to the State Pharmacopoeia of the Republic of Kazakhstan (SP RK). The data in the diagram shows that as the temperature rises, the pH value decreases.

The second requirement for assessing water quality is its electrical conductivity, which is determined by the presence of charged particles in it. The determination of the electrical conductivity of various types of water was carried out in accordance with the SP RK, v.2, p. 165, using an ANION 4100 conductometer with increasing temperature.

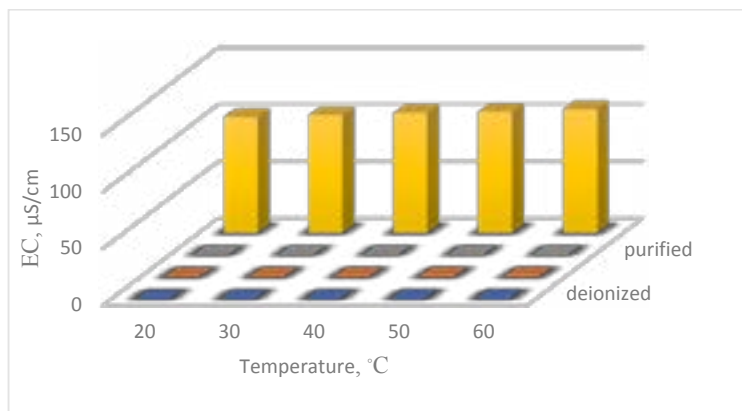
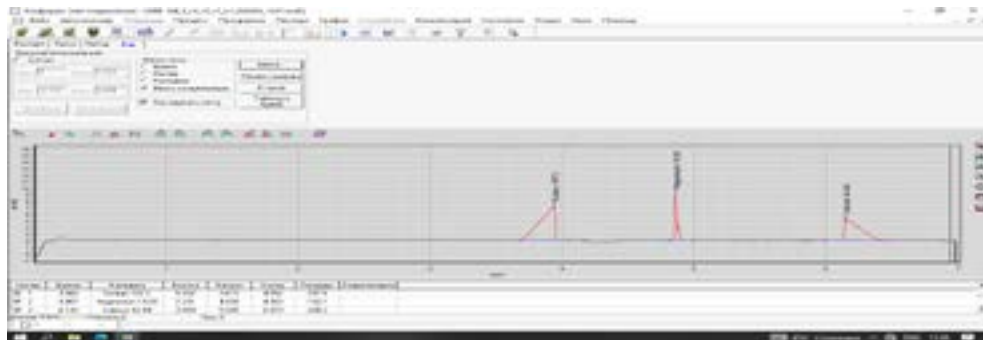


Fig. 2 - Dependence of electrical conductivity on temperature

Based on the obtained results of the conductometer, a diagram of the dependence of electrical conductivity on temperature was constructed (Fig. 2). As can be seen from Figure 2, the readings of the conductometer for all types of water are within the allowable values. For example, according to the requirement for injection water, the electrical conductivity is $1.1 \mu\text{S}/\text{cm}$ at a temperature of $T = 20.0^\circ\text{C}$, while the result of the analysis showed $1.0 \mu\text{S}/\text{cm}$. The results of the diagram clearly prove that with increasing temperature, the electrical conductivity of water increases.

The next analysis was the determination of the content of cations and anions in water using capillary electrophoresis "Kapel-105M", the results of which are presented below in the form of electrophoregrams (EPG) (Figure 3–4).



Number	Time	Component name	Height	Begin	End	Area	Concentration
1	3.965	Sodium	5.295	3.710	3.975	383.0	107.2
2	4.900	Magnesium	7.931	4.863	5.015	101.5	18.00
3	6.210	Calcium	3.395	6.192	6.565	262.6	42.40

Figure 3a - EFG of tap water (cations)

Number	Time	Component name	Height	Begin	End	Area	Concentration
1	2.320	Nitrite	6.588	2.260	2.352	69.03	47.35
2	2.493	Nitrate	28.412	2.450	2.548	295.8	237.0
3	2.617	Fluoride	2.718	2.590	2.670	36.50	6.980
4	2.845	Phosphate	0.797	2.832	2.893	6.897	3.205
5	3.110		6.395	2.980	3.253	280.3	



Figure 3b - EFG of tap water (anions)

The following cations were found on the EFG of tap water: sodium (107.2 mg/dm³), magnesium (18.00 mg/dm³), calcium (42.40 mg/dm³) (Fig. 3a) and anions: nitrite (47.35 mg/dm³), nitrate (237.0 mg/dm³), fluoride (6.980 mg/dm³), phosphate (3.205 mg/dm³) (Fig. 3b). EPG of tap water (anions) also revealed an indefinite anion with an area of 280.3.



Number	Time	Component name	Height	Begin	End	Area	Concentration
1	2,972	Phosphate	0.309	2.950	2.998	1.697	0.7886

Based on the EPG data, no cations were found in purified water, while the presence of phosphate ions with a concentration of 0.7886 mg/dm³ is observed (Figure 4).

When receiving injection water by distillation of purified water, the phosphate ion found on the EPG of purified water and cations were absent.

When analyzing deionized water, ions (anions and cations) were also not detected.

The next parameter in assessing water quality was the determination of the amount of organic and inorganic carbons in water using the TOC-L analyzer, the results of which are presented in the form of graphs (Figure 5).

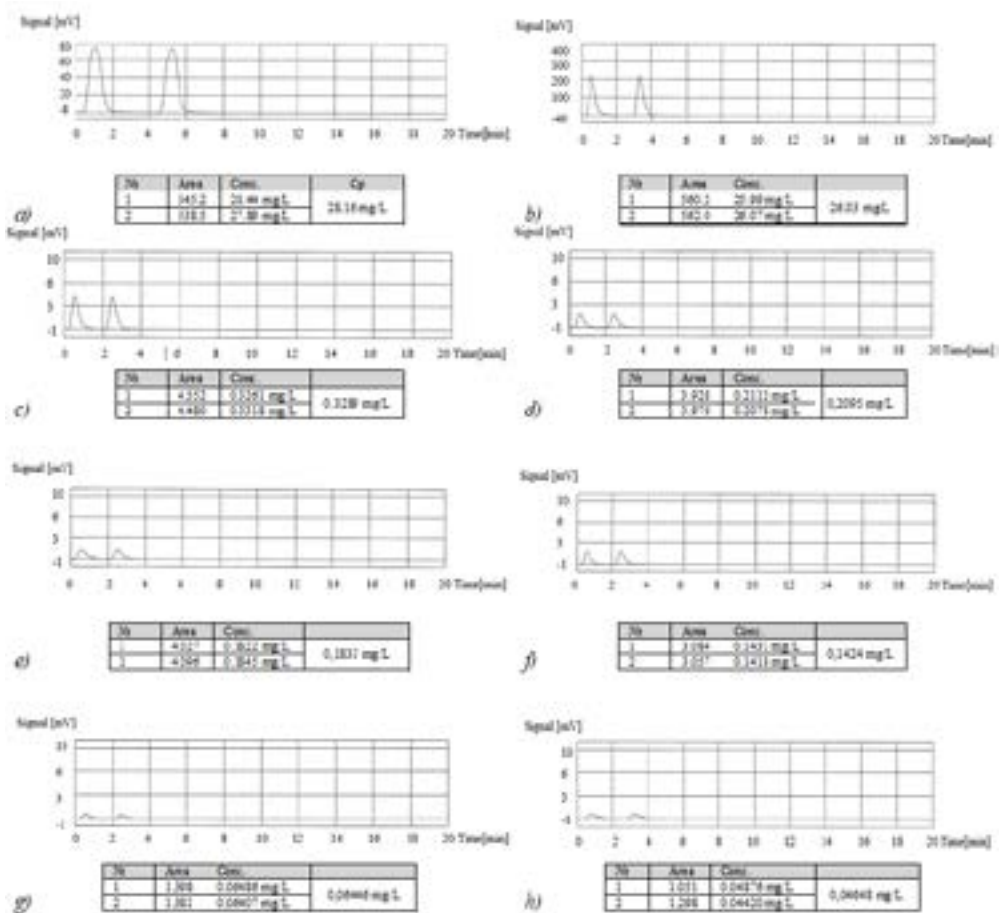


Fig. 5 - Results of determining the content of organic and inorganic carbons
 Note: a, c, e, g - total carbon content; b,d,f,h - content of inorganic carbons.

Figure 5 shows graphs of total carbon content and inorganic carbon content. In tap, purified, deionized and injection water, the total carbon content (a, c, e, g) was 28.16 mg/l, 0.3289 mg/l, 0.1833 mg/l, 0.06446 mg/l, respectively, while the content of inorganic carbon compounds (b, d, f, h), represented by carbonate ions and dissolved dioxide, for the same types of water was 26.03 mg/l, 0.2095 mg/l, 0.1424 mg/l, 0.04648

mg/l, respectively. The concentrations of organic carbon associated with other atoms, usually hydrogen and oxygen atoms, in the studied water samples are determined by the difference between the concentration of the total carbon content and the content of inorganic carbon compounds and amounted to 2.13 mg/l, 0.1194 mg/l, 0.0409 mg/l, 0.01798 mg/l, respectively.

Discussion

This paper presents the results of analyzes to assess the chemical parameters of different types of water used in the production of biological products. The waters used in the production of biological products must meet the special requirements described in the State Pharmacopoeia, in terms of such parameters as pH, electrical conductivity, the content of cations and anions, and the content of organic and inorganic carbon compounds.

The main factors determining the quality of water are its electrical conductivity and the concentration of hydrogen ions. According to the results of this work, the electrical conductivity of water increases with increasing temperature. This is due to the speed of ions, a decrease in their solvation, and a decrease in viscosity (Masaki, 2004:119–128). Whereas the concentration of hydrogen ions (pH) decreases with increasing temperature. This, in turn, is associated with different dissociation of hydrogen ions (H^+) (Iman, 2018: 23–24).

Table 3. Maximum allowable values of hydrogen ions (pH) and electrical conductivity for different types of water.

Name	Hydrogen ion concentration (pH)	Electrical conductivity
Purified water	5,0–7,0	From 2.4 μ S/cm at 0°C to 10.2 μ S/cm at 100°C
Injection water		From 0.6 μ S/cm at 0°C to 3.1 μ S/cm at 100°C
Deionized water		0.055 μ S/cm
Tap water		From 100 to 2000 μ S/cm

Based on the maximum permissible concentration (MPC) presented in Table 3, we can conclude that the values of the electrical conductivity of tap, purified, deionized and injection water are within acceptable values. The results of the concentration of hydrogen ions (pH) of purified, deionized and injection water also meet the requirements. Whereas, the result of the pH of tap water is 8.18 ± 0.00019 , therefore, it exceeds the MPC.

There is a work in the literature where an analysis was carried out to assess the effectiveness of treatment facilities and the quality of drinking water. Using a portable digital multi-parameter probe, results were obtained on such indicators as pH and electrical conductivity of tap water, where pH showed a value of 6.88 ± 0.05 and electrical conductivity of 170.6 ± 0.1 μ S/cm (Belay, 2021: 1–8). If we compare the values obtained by the author with the required values of the SP RK, it can be argued that these readings do not exceed the MPC. Another example is the work to assess the quality of surface water from the city of Skardu in Pakistan using a water quality index. In this study, the pH results ranged from 7.26 to 7.98, with an average value

of 7.67. Whereas the electrical conductivity readings varied from 150 to 780 $\mu\text{S}/\text{cm}$. The results obtained in this literature in terms of electrical conductivity do not exceed MPC. However, the pH values exceed the MPC, which is associated with a higher concentration of acid ions and salts in spring water (Wazir, 2021: 20537–20548).

In recent years, the use of capillary electrophoresis for the analysis of various types of water has expanded significantly, due to higher resolution, shorter analysis time, less reagent consumption, and greater ease of operation. The literature reports numerous applications of CE for the determination of alkali and alkaline earth cations (Foret, 2015: 780; Weston, 2015: 593; Koberda, 2014: 235–240) and common inorganic anions (Bowser, 2013: 257–264; Jones, 2014: 445; Wildman, 2012: 459; Vera, 2012: 169–180) in various aqueous media. However, since CE separation is based on the difference in the electrophoretic mobility of the analytes, the determination of fast anions and cations in the same run under normal CE conditions was practically impossible. But due to the improvement of analyzers for the determination of ions, to date, the simultaneous determination of cations and anions has become possible. So, in the literature there is a work of simultaneous determination of inorganic anions and cations in tap water by capillary electrophoresis with a non-contact conductivity detector with capacitive coupling (C^4D) of the system, and the following ions were obtained on the electrophoregram: Cl^- , SO_4^{2-} , Na^+ , Ca^{2+} , Mg^{2+} [Michel A. (2000): 89-100]. Another example is a new approach for the simultaneous CE separation of inorganic anions and cations based on sample injection by electromigration from both ends of the capillary and indirect UV detection. So, after analyzing tap water, the author found the following ions: Cl^- (21,4), SO_4^{2-} (39,0), HCO_3^- (186,0), Na^+ (17,9), Ca^{2+} (66,3), Mg^{2+} (19,8) [Tamisier-Karolak S.L. (2000): 487-498]. As a result of our work, the presence of the following ions in tap water was also found: Na^+ (107,2), Mg^{2+} (18,00), Ca^{2+} (42,40), NO_2^- (47,35), NO_3^- (237,0), F^- (6,980), PO_4^{3-} (3,205). Along with the above ions, an unidentified anion was also found with a peak area of 280.3. Phosphate ion PO_4^{3-} (0.7886) was found in purified water, which indicates insufficient purification of tap water, but no ions were found in deionized and injection water.

The concentration of total organic carbon in samples is widely used to characterize the organic pollution of water bodies (Han-Saem, 2020: 3901). In particular, the content of organic carbon is commonly used to assess the quality of environmental aquatic systems, to check sources of pollution of aquatic environments, and to monitor and evaluate organic micro-pollution of drinking water. In the literature there is a work (Yiming, 2010: 789–795) based on the study of the effect of physical sterilization on the determination of organic carbon content. In this study, a new method for sterilizing water samples was developed - microfiltration of 0.45 μm in combination with UV radiation. The results showed that this method is able to have a bactericidal effect on the water sample while significantly reducing the negative impact on the analysis of organic carbon content. The following results were obtained by the author: the total carbon content in tap water is 58.99 ± 0.23 , the content of inorganic carbon is 31.81 ± 0.79 , the content of organic carbon is determined by subtracting the content of inorganic carbon from the total carbon content and amounted to 27.18 ± 0.56 . As a result of our work, the

total carbon content in tap water is 28.16 mg/l, the content of inorganic carbon is 26.03 mg/l, based on this, the content of organic carbon was 2.13 mg / l. Another example is the work on monitoring dissolved organic carbon in surface and drinking waters. In this literature, analysis to determine the concentration of organic substances in rivers, sludge, filter and wastewater from plants was measured using a portable analyzer (TOC). The results on the content of organic carbon compounds in river water ranged from 2.15 to 11.90 mg/l, the average value was 4.00 mg/l. In addition, water samples were regularly sent to the laboratory and analyzed using a high-temperature analyzer (TOC Shimadzu analyzer). The results ranged from 2.51 to 10.1 mg/l. Data comparison showed good agreement between the two analyzers (Christian, 2002: 43–47).

Conclusions

Water is extensively used in the pharmaceutical, biopharmaceutical and life sciences industries for a variety of purposes, such as raw material, ingredient, and solvent in the processing, formulation, and production of a wide range of finished products, as APIs and intermediates, and analytical reagents. Therefore, regulatory requirements for quality control of various types of water throughout the entire production, storage and distribution process, including microbiological and chemical quality, are very high. Lack of control over any of the parameters of drinking, purified or water for injection can lead to the loss of expensive biological products or medicinal substances.

As a result of our work, we can draw the following conclusions:

– pH values in all water samples, except for tap water, do not exceed MPC and fully comply with the requirements of the SP RK.

- the values of electrical conductivity in all the studied water samples do not exceed the MPC and fully comply with the requirements of the SP RK.

– according to the content of cations and anions in tap water, the following ions were found: Na^+ (107.2 mg/dm³), Mg^{2+} (18.00 mg/dm³), Ca^{2+} (42.40 mg/dm³), NO_2^- (47.35 mg/dm³), NO_3^- (237.0 mg/dm³), F^- (6.980 mg/dm³), PO_4^{3-} (3.205 mg/dm³). In purified water, a phosphate ion with a concentration of 0.788 mg/dm³ was observed. No ions were found in injection and deionized water.

– the following results were obtained for the content of organic and inorganic carbon: in tap, purified, deionized and injection water, the total carbon content was 28.16 mg/l, 0.3289 mg/l, 0.1833 mg/l, 0.06446 mg / l, the content of inorganic carbon compounds was 26.03 mg/l, 0.2095 mg/l, 0.1424 mg/l, 0.04648 mg/l, respectively. The concentration of organic carbon compounds was 2.13 mg/l, 0.1194 mg/l, 0.0409 mg/l, 0.01798 mg/l, respectively.

Based on the above results, we can conclude that the waters used on the production line fully comply with the requirements for the above indicators and comply with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan.

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МАЗМҰНЫ

И. Акмалова, В. Меркулов ТҮРЛІ МАЙ ШИКІЗАТТАРЫНЫҢ НЕГІЗІНДЕГІ БЕТТІК-АКТИВДІ ЗАТТАРДЫ АЛУ ӘДІС.....5	5
М.Б. Ахтаева, Г.Е. Азимбаева, Ж.С. Мукагаева ЕКІҮЙЛІ ҚАЛАҚАЙ (<i>URTICA DIOCA L.</i>) ҚҰРАМЫНДАҒЫ ПОЛИФЕНОЛДЫ ҚОСЫЛЫСТАРДЫ, ФЛАВОНОИДТАРДЫ, КАРОТИНОИДТАРДЫ ЗЕРТТЕУ.....15	15
К.Б. Бажықова, Т.С. Бекежанова, Қ.Д. Рахимов СЕСКВИТЕРПЕНОИДТАР ҚАТАРЫНАН ХИМИЯЛЫҚ МОДИФИКАЦИЯЛАУ НЕГІЗІНДЕ ВИРУСҚА ҚАРСЫ ББЗ ІЗДЕСТІРУ.....24	24
М.Д. Даулетова, А.К. Үмбетова, Г.Ш. Бурашева, М.И. Чаудхари <i>ATRAPHAXIS</i> ТҰҚЫМДАС ҚАЗАҚСТАНДЫҚ ӨСІМДІК ТҮРЛЕРІНІҢ ҚЫШҚЫЛДЫҚ ҚҰРАМЫН САЛЫСТЫРМАЛЫ ЗЕРТТЕУ.....33	33
М.Ә. Дәуренбек СИНТЕЗ-ГАЗ ӨНДІРІСІНДЕ ФОТОКАТАЛИЗАТОР РЕТІНДЕ ZnIn КҮРДЕЛІ СУЛЬФИДІН ШЕТЕЛДІК ЗЕРТТЕУЛЕР ТУРАЛЫ (жағдайы мен тенденциялары).....43	43
Б.С. Гайсина, Л.К. Оразжанова, Б.Х. Мұсабаева, А.Н. Сабитова, Б.Б. Баяхметова ХИТОЗАН- НАТРИЙ АЛГИНАТЫ НЕГІЗІНДЕГІ БИОҮЙЛЕСІМДІ КРИОҚҰРЫЛЫМДЫ АЛУ ЖӘНЕ ҚАСИЕТТЕРІН ЗЕРТТЕУ.....53	53
Н. Жаникулов, А. Абдуллин, Б. Таймасов, М. Кенжехан МЫРЫШ-ФОСФАТТЫ КОМПОЗИЦИЯЛЫҚ ЦЕМЕНТ АЛУ ҮШІН ФОСФОР ШЛАГЫН ЗЕРТТЕУ.....63	63
М.Ж. Жұрынов, Т.С. Бекежанова, К.Б. Бажықова, К.Д. Рахимов, З.М. Зиятбек ДӘРМЕНЕ ЖУСАНЫ (<i>ARTEMISIA CINA BERG.</i>) ӨСІМДІК ШИКІЗАТЫНАН ЭФИР МАЙЛАРЫН БӨЛІП АЛУ ӘДІСТЕРІ ЖӘНЕ ОЛАРДЫ СТАНДАРТТАУ75	75
Б. Имангалиева, Б. Торсыкбаева, Г. Рахметова, Т. Нұрдаулетова, Б. Досанова ХИМИЯДАН "ТҮЗДАР ГИДРОЛИЗИ" ТАҚЫРЫБЫН ОҚЫТУДЫҢ ТИІМДІ ТЕХНОЛОГИЯСЫ.....85	85
А.Г. Исмаилова, Г.Ж. Аканова, Д.Х. Камысбаев, С. Исабекова НИТРАТТЫ ОРТАДАН ДИСПРОЗИЙДІ ДЭГФҚ-МЕН ЭКСТРАКЦИЯЛАУ.....98	98
Ж.А. Караев, Ж.У. Кобдиқова, Б.Б. Торсыкбаева, Б.С. Имангалиева, Н.Р. Рахым ЖОҒАРҒЫ ОҚУ ОРЫНДАРЫНДА КРИТЕРИАЛДЫ ӘДІЛ БАҒАЛАУ.....111	111
М.К. Касымова, Р.С. Алибеков, З.И. Кобжасарова, Г.Э. Орымбетова, К.А. Уразбаева ҰЫТ ҚОЛДАНАТЫН ХАЛАЛ ШҰЖЫҚ ӨНІМДЕРІ.....124	124

Б.К. Масалимова, Г.Д. Джетписбаева, Е.В. Доқуцич, В.А. Садыков ОРГАНИКАЛЫҚ ТОТЫҚТЫРҒЫШТАР ҚАТЫСЫНДА ПЕРОВСКИТ ҚҰРЫЛЫМДЫ КҮРДЕЛІ ОКСИД LaCoO_3 АЛУ.....	143
Г.Э. Орымбетова, Р.С. Алибеков, Э.А. Габрильянц, К.А. Уразбаева, М.К. Касымова, З.И. Кобжасарова ЕТ-КӨКӨНІС ПАШТЕТТІ ӨНДІРУДЕ ХАССП ЖҮЙЕСІН ҚОЛДАНУ.....	151
С.О. Садикалиева, С.Д. Сатыбалдинова, З.Д. Ершебулов, Е.В. Фокина, К.А. Шораева БИОПРЕПАРАТТАР ӨНДІРУ ҮШІН СУДЫ ХИМИЯЛЫҚ ТАЛДАУ.....	164

СОДЕРЖАНИЕ

И. Акмалова, В. Меркулов МЕТОД ПОЛУЧЕНИЯ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ НА ОСНОВЕ РАЗЛИЧНОГО ЖИРОВОГО СЫРЬЯ.....	5
М.Б. Ахтаева, Г.Е. Азимбаева, Ж.С. Мукатаева ИССЛЕДОВАНИЕ ПОЛИФЕНОЛЬНЫХ СОЕДИНЕНИЙ, ФЛАВОНОИДОВ, КАРОТИНОИДОВ КРАПИВЫ ДВУДОМНОЙ (<i>URTICA DIOCAL</i>).....	15
К.Б. Бажыкова, Т.С. Бекежанова, К.Д. Рахимов ПОИСК БАВ ПРОТИВ ВИРУСА ИЗ РЯДА СЕСКВИТЕРПЕНОИДОВ НА ОСНОВЕ ХИМИЧЕСКОЙ МОДИФИКАЦИИ.....	24
М.Д. Даулетова, А.К. Умбетова, Г.Ш. Бурашева, М.И. Чаудхари ОБРАЗОВАНИЕ СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ КИСЛОТНОГО СОСТАВА КАЗАХСТАНСКИХ ВИДОВ РАСТЕНИЙ РОДА <i>ATRAPHAXIS</i>	33
М.А. Дауренбек О ЗАРУБЕЖНЫХ ИССЛЕДОВАНИЯХ СЛОЖНОГО СУЛЬФИДА ZnIn В КАЧЕСТВЕ ФОТОКАТАЛИЗАТОРОВ В ПРОИЗВОДСТВЕ СИНТЕЗ-ГАЗА (состояние и тенденции).....	43
Б.С. Гайсина, Л.К. Оразжанова, Б.Х. Мұсабаева, А.Н. Сабитова, Б.Б. Баяхметова ПОЛУЧЕНИЕ И ИЗУЧЕНИЕ СВОЙСТВ БИОСОВМЕСТИМОЙ КРИОСТРУКТУРЫ НА ОСНОВЕ ХИТОЗАН-АЛБГИНАТА НАТРИЯ.....	53
Н. Жаникулов, А. Абдуллин, Б. Таймасов, М. Кенжехан ИССЛЕДОВАНИЕ ФОСФОРНОГО ШЛАГА ДЛЯ ПОЛУЧЕНИЯ ЦИНК-ФОСФАТНОГО КОМПОЗИЦИОННОГО ЦЕМЕНТА.....	63
М.Ж. Жұрынов, Т.С. Бекежанова*, К.Б. Бажыкова, К.Д. Рахимов, З.М. Зиятбек СПОСОБЫ ВЫДЕЛЕНИЯ ЭФИРНЫХ МАСЕЛ ИЗ РАСТИТЕЛЬНОГО СЫРЬЯ <i>ARTEMISIA</i> <i>SINA BERG.</i> И ИХ СТАНДАРТИЗАЦИЯ.....	75
Б. Имангалиева, Б. Торсыкбаева, Г. Рахметова, Т. Нурдаулетова, Б. Досанова ЭФФЕКТИВНАЯ ТЕХНОЛОГИЯ ПРЕПОДАВАНИЯ ТЕМЫ "ГИДРОЛИЗ СОЛЕЙ" ПО ХИМИИ.....	85
А.Г. Исмаилова, Г.Ж. Аканова, Д.Х. Камысбаев, С. Исабекова ЭКСТРАКЦИЯ ДИСПРОЗИЯ С ДЭЭГФК ИЗ НИТРАТНОЙ СРЕДЫ.....	98
Ж.А. Караев, Ж.У. Кобдикова, Б.Б. Торсыкбаева, Б.С. Имангалиева, Н.Р. Рахым СПРАВЕДЛИВОЕ КРИТЕРИАЛЬНОЕ ОЦЕНИВАНИЕ В ВЫСШИХ УЧЕБНЫХ ЗАВЕДЕНИЯХ.....	111
М.К. Касымова, Р.С. Алибеков, З.И. Кобжасарова, Г.Э. Орымбетова*, К.А. Уразбаева ХАЛЯЛНЫЕ КОЛБАСНЫЕ ИЗДЕЛИЯ ИЗ ГОВЯДИНЫ С ИСПОЛЬЗОВАНИЕМ СОЛОДА.....	124

Б.К. Масалимова, Г.Д. Джетписбаева, Е.В. Докунич, В.А. Садыков ПОЛУЧЕНИЕ СЛОЖНОГО ОКСИДА СО СТРУКТУРОЙ ПЕРОВСКИТА $LaCOO_3$ В ПРИ СУТСТВИИ ОРГАНИЧЕСКИХ ВОССТАНОВИТЕЛЕЙ.....	143
Г.Э. Орымбетова, Р.С. Алибеков, Э.А. Габрильянц, К.А. Уразбаева, М.К. Касымова, З.И. Кобжасарова ПРИМЕНЕНИЕ ХАССП СИСТЕМЫ В ПРОИЗВОДСТВЕ МЯСОРАСТИТЕЛЬНОГО ПАШТЕТА.....	151
С.О. Садикалиева, С.Д. Сатыбалдинова, З.Д. Ершебулов, Е.В. Фокина, К.А. Шораева ХИМИЧЕСКИЙ АНАЛИЗ ВОДЫ ДЛЯ ПРОИЗВОДСТВА БИОПРЕПАРАТОВ.....	164

CONTENTS

I. Akmalova, V. Merkulov METHOD OF OBTAINING SURFACTANTS BASED ON VARIOUS FATTY RAW MATERIALS.....	5
M.B. Akhtayeva, G.E. Azimbayeva, J.S. Mukataeva STUDY OF CARATINOID, FLAVONOID, POLYPHENOL COMPOUNDS OF DICOTYLEDONOUS NETTLE (<i>URTICA DIOCA L.</i>).....	15
K.B. Bazhykova, T.S. Bekezhanova, K.D. Rakhimov SEARCH FOR BAS AGAINST A VIRUS FROM A NUMBER OF SESQUITERPENOIDS BASED ON CHEMICAL MODIFICATION.....	24
M.D. Dauletova, A.K. Umbetova, G.S. Burasheva, M.I. Chaudhari COMPARATIVE STUDY OF THE ACID COMPOSITION OF KAZAKH PLANT SPECIES OF THE GENUS <i>ATRAPHAXIS</i>	33
M.A. Daurenbek ABOUT FOREIGN STUDIES OF ZnIn COMPOUND SULFIDE AS PHOTOCATALYSTS IN THE SYNTHESIS GAS PRODUCTION (status and tendencies).....	43
B.S. Gaisina, L.K. Orazzhanova, B.H. Musabayeva, A.N. Sabitova, B.B. Bayakhmetova OBTAINING AND STUDYING THE PROPERTIES OF A BIOCOMPATIBLE CRYOSTRUCTURE BASED ON CHITOSAN-SODIUM ALGINATE.....	53
N. Zhanikulov, A. Abdullin, B. Taimasov, M. Kenzhehan INVESTIGATION OF PHOSPHORIC SLAG FOR OBTAINING OF ZINC-PHOSPHATE COMPOSITE CEMENT.....	63
M.Zh. Zhurinov, T.S. Bekezhanova, K.B. Bazhykova, K.D. Rakhimov, Z.M. Ziyatbek METHODS OF EXTRACTING ESSENTIAL OILS FROM <i>ARTEMISIA CINA</i> BERG. PLANT RAW MATERIALS AND THEIR STANDARDIZATION.....	75
B. Imangaliyeva, B. Torsykbayeva, B. Dossanova, T. Nurdauletova, G. Rakhmetova EFFECTIVE TECHNOLOGY OF TEACHING "SALTS HYDROLYSIS" IN CHEMISTRY.....	85
A.G. Ismailova, G.Zh. Akanova, D.Kh. Kamysbayev, S. Isabekova EXTRACTION OF DYSPROSIUM BY D2EHPA FROM NITRATE MEDIUM.....	98
Zh. Karaev, Zh. Kobdikova, B. Torsykbaeva, B. Imangaliyeva, N. Rakhym FAIR CRITERIA EVALUATION IN HIGHER EDUCATIONAL INSTITUTIONS.....	111
M.K. Kassymova, R.S. Alibekov, Z.I. Kobzhasarova, G.E. Orymbetova, K.A. Urazbayeva HALAL BEEF SAUSAGE PRODUCTS USING MALT.....	124

B.K. Massalimova, G.D. Jetpisbayeva, E.V. Docuchits, V.A. Sadykov OBTAINING A COMPLEX OXIDE WITH THE PEROVSKITE STRUCTURE LaCoO_3 IN THE PRESENCE OF ORGANIC REDUCING AGENTS.....	143
G.E. Orymbetova, R.S. Alibekov, E.A. Gabrilyants, K.A. Urazbayeva, M.K. Kassymova, Z.I. Kobzhasarova APPLICATION OF HACCP SYSTEM FOR THE MEAT-PLANT PASTE PRODUCTION.....	151
S.O. Sadikaliyeva, S.D. Satybaldinova, Z.D. Yershebulov, E.V. Fokina, K.A. Shorayeva CHEMICAL ANALYSIS OF WATER USED IN THE PRODUCTION OF BIOLOGICAL PRODUCTS.....	16

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<http://chemistry-technology.kz/index.php/en/arhiv> ISSN 2518-1491 (Online), ISSN 2224-5286 (Print)

Заместитель директор отдела издания научных журналов НАН РК *Р. Жәліқызы*

Редакторы: *М.С. Ахметова, Д.С. Аленов*

Верстка на компьютере *Г.Д. Жадырановой*

Подписано в печать 05.07.2023.

Формат 60x88¹/₈. Бумага офсетная. Печать – ризограф. 11,0 п.л. Тираж 300. Заказ 2.