ISSN 1070-3284, Russian Journal of Coordination Chemistry, 2022, Vol. 48, No. 10, pp. 641–646. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Koordinatsionnaya Khimiya, 2022, Vol. 48, No. 10, pp. 625–631.

Dedicated to Academician Yu.A. Zolotov in the year of his 90th birthday

Application of Complex Forming Impregnated Polyvinyl Alcohol for the Determination of Carbohydrates by Optical Micrometry

I. S. Shchemelev[a, *], M. A. Smirnova[a], A. V. Ivanov[a, b], and N. B. Ferapontov[a]

[a] *Moscow State University, Moscow, Russia*

[b] *Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Moscow, 119992 Russia*

**e-mail: shchemelev_93@mail.ru*

Received April 19, 2022; revised April 21, 2022; accepted April 22, 2022

Abstract—A possibility of determining concentrations of water-soluble carbohydrates by optical micrometry using granules of polyvinyl alcohol (PVA) impregnated with sodium tetraborate is shown. The influence of the acidity on the analytical signal is studied. The optimum pH range for the determination of the glucose concentration in the presence of sucrose is 8.5–10.0. A sensitive polymer granule needs about 30 min to achieve an equilibrium volume, and a change in the volume is reversible. The calibration plot for the determination of the glucose concentration in the range from 0 to 40 mmol/dm^3 is constructed. The impregnation of the PVA granules with sodium tetraborate is shown to decrease the detectability threshold to 6.9 mmol/dm^3 and to increase the selectivity, which can be varied by changing the pH of the solution.

Keywords: polyvinyl alcohol, optical micrometry, determination of glucose, ester chelates, ligand exchange

DOI: 10.1134/S1070328422100050

INTRODUCTION

One of important problems of modern analytical chemistry is the development of out-of-laboratory methods for carbohydrate determination in food products and also with purposes of clinical diagnostics. To date, sensors and test systems of various types that make it possible to determine concentrations of glucose, sucrose, fructose, and other sugars without complicated sample preparation were already developed for the solution of this problem. Among them are amperometric [1–4], optical [5–7], thermometric [8], and other analytical systems, the principle of operation of which, in the most part of cases, is based on the enzymatic cleavage of carbohydrates followed by an analytical response to the reaction products. These methods allow carbohydrate concentrations to be determined at a level of ~1 mmol/dm^3 and lower with the relative standard deviation to 10%. The main limitation of this approach is a low stability of enzyme leading to a short service life of sensors and their high cost.

Another approach to the solution of the problem is the application of sensor materials based on the polymers with covalently immobilized phenylboronic acid. In the majority of cases, similar studies were carried out for photon-crystalline [9–11] and holographic [12–14] sensors, whose principle of operation is based on a change in Bragg diffraction of light in the optical wavelength range. When an analyte is deposited on a photon-crystalline structure or its analog (e.g., holographic sensor), the degree of swelling of the sensitive unit changes inducing a change in the period of the photon crystal structure and in the effective reflective index resulting in the shift of the maximum in the reflection spectrum [14–16]. However, the cost of these materials for glucose determination increases because of the modification of the polymers with expensive phenylboronic acid derivatives.

An alternative method for the determination of the concentration of dissolved substances is optical micrometry (OM), which allows the direct determination of the degree of swelling of polymers and serves as a tool for physicochemical investigation of processes resulting in a change in this value when the pure solvent is replaced by a solution of the sample. In this case, spherical granules of hydrophilic polymers are used as a sensitive unit. Their volume changes with a change in the composition of the solution in which they are placed. The granule volume is measured by processing photographs obtained with an optical microscope equipped with a digital video camera. Owing to possibilities of a special program package for obtaining an analytical signal, the concentration of the dissolved substance can be determined by the OM

method from both measurements of the volume of the granules stored in a solution of the sample to the state of equilibrium [17, 18] and of the 3D kinetic surfaces using kinetic coefficients obtained by experimental data processing with the kinetic heterophase model of the polymer gel structure composing the database of the instrument [19–21]. The possibility of determining the concentration of dissolved carbohydrate by this method was studied to date [22–24]. It was shown that the equilibrium degree of swelling of the polymer in a solution differed slightly from that in water and the equilibration required from 2 to 5 h. All these facts made impossible to analyze solutions containing carbohydrates from equilibrium data and, hence, the kinetic surfaces with the characteristic minimum in the initial region were constructed [23]. Nevertheless, the absence of selectivity of the studied polymers to water-soluble sugars made this approach possible only for an analysis of individual model solutions and substantially restricted its application for an analysis of real objects even when determining the total content of carbohydrates.

This drawback can be eliminated by using polymers impregnated or surface-modified with boronic acid or its salts due to the complexation of the latter with 1,2- or 1,3-diols. A similar approach was successfully applied for the photon-crystalline massif with a sensitive layer of polyvinyl alcohol [25] and in thin-layer chromatography on the plates covered with SiO_2 [26]. In this work, the approach was proposed for the determination of the concentration of soluble carbohydrates by the OM method to enhance the sensitivity and selectivity.

EXPERIMENTAL

The following reagents were used: polyvinyl alcohol (PVA) (trade mark 18/11), epichlorohydrin, sucrose (analytical grade), D-glucose (high-purity grade), NaOH, $Na_2B_4O_7$ decahydrate (analytical grade), NaCl (analytical grade), KCl (high-purity grade), $Na_2HPO_4 \cdot 12H_2O$ (high-purity grade), and KH_2PO_4 (high-purity grade).

The degree of swelling of polymer granules was studied on a setup consisting of a 48-well plate for biochemical research or a cell and an optical microscope equipped with a light source and a digital camera connected to a personal computer with a program package for obtaining and processing photographs [27].

Procedure of preparing stock solutions. A beaker (volume 300 cm^3) was loaded with NaCl (4.0 g), KCl (0.1 g), Na_2HPO_4 (1.80 g), KH_2PO_4 (0.12 g), and $Na_2B_4O_7$ (9.53 g). The salts were dissolved in water on heating and then cooled down to room temperature. The required pH was attained by the addition of concentrated solutions of HCl or NaOH monitoring the acidity with a pH meter. Then the obtained solution was quantitatively transferred to a volumetric flask (500.0 cm^3), the volume was made up with distilled water, and the solution was stirred. The obtained solutions contained 0.05 mole of sodium tetraborate in 1 dm^3. Further these solutions were used to prepare solutions containing glucose and sucrose.

Procedure of preparing spherical granules of polyvinyl alcohol. The PVA-20 granules were prepared according to the developed procedure [28, 29]. A PVA weighing sample was poured with distilled water (100 cm^3), and the solution was heated to 90–100°C attaining the complete dissolution of the polymer and removal of air bubbles. Then a solution of sodium hydroxide (10 g in 20 cm^3 of water) was poured with permanent stirring for 30 min. The obtained solution was cooled to 30–40°C, and epichlorohydrin (20 cm^3) was added. The mixture was vigorously stirred, transferred to Nujol heated to 70°C, and hold at this temperature with permanent stirring for 3 h. The formed granules were separated from Nujol and several times washed with acetone (to remove Nujol residues) and distilled water.

Procedure of studying the acidity effect on the degree of swelling of the granules. The PVA-20 granules purified from contaminating substances were transferred from water to stock solutions with different pH (from 7.5 to 10.5 with an increment of 0.5 units), and the solutions were stored in a desiccator with the tightly closed cover containing the same stock solution on the bottom to the equilibrium state for 1 h. Then the solution was poured down, and a fresh solution was added (this procedure is necessary to maintain constant concentrations of the components of the stock solution during experiment).

The granules thus equilibrated with the stock solutions were placed in wells of the plate filled with the same solution and photographed with a digital camera. Then their volume (V_0) was determined. The stock solutions were poured down from the wells and replaced with solutions of carbohydrates with a concentration of 40 mmol/dm^3 (5 granules per each solution) prepared on the corresponding stock solutions. The plates with the granules were placed in the corresponding desiccators, stored for 1 h to the state of equilibrium, and photographed again. The value of V/V_0 was calculated from the photography data. The dependence of the relative granule volume on the pH of the solution was constructed for each carbohydrate studied.

Procedure of studying the swelling kinetics of the granules in carbohydrate solutions. The swelling kinetics for the PVA-20 granules was studied according to a described procedure [19, 20]. A PVA-20 granule equilibrated with the stock solution at a certain pH was placed in a measurement cell and photographed 5 times. The value of V_0 was determined from the obtained photographs. Then the stock solution in the cell was replaced with a solution of carbohydrate of a

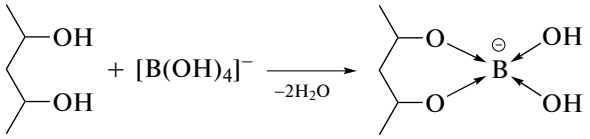
certain concentration, and the photography of the granule in the cell with a frequency of 1 frame/s was started immediately. After 5 min, the photography frequency was changed by 1 frame per 10 s. The time of experiment was ~1 h. The consequent processing of the photographs was performed in the same way as that for V_0 determination. The kinetic curves in the coordinates V/V_0–$f(t)$ were constructed from the obtained data for solutions with various concentrations of carbohydrates in order to estimate the time required for equilibration.

The PVA-20 granules purified from contaminants were transferred from water to the stock solution with the pH optimum for this carbohydrate, and the solution was stored for 1 h in a desiccator (containing the same stock solution on the bottom) with the tightly closed cover to the state of equilibrium. Then the solution was poured down, and a fresh solution was poured (this procedure is necessary to maintain constant concentrations of the stock solution components during experiment).

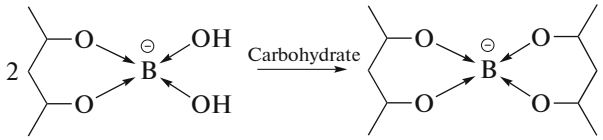
The granules thus equilibrated with the stock solution were placed in wells of the plate filled with the same solution and were photographed. The granule volume V_0 was determined. Then the stock solution was poured down from the wells and replaced with carbohydrate solutions with the concentrations 2, 4, 6, 10, and 20 mmol/dm^3 (5 granules per each solution). The plate with the granules was placed in a desiccator with the stock solution on the bottom, stored for 1 h to the state of equilibrium, and photographed again. The value of V/V_0 was calculated from the photography data. The calibrated plots were constructed from the averaged data obtained and were used to determine the carbohydrate concentrations in the analyzed solutions.

RESULTS AND DISCUSSION

Reaction of polyvinyl alcohol with sodium tetraborate. It is known [30, 31] that boronic acid and its derivatives are capable of reversibly reacting with 1,2- and 1,3-diols to form chelate esters. They were named in such a way, since they contain five- or six-membered cycles (chelates) with the boron atom as the central atom and polyatomic alcohols as ligands. In particular, the following reaction occurs when polyvinyl alcohol is treated with a sodium tetraborate excess in a weakly alkaline medium:



The following process can occur upon the interaction of thus treated PVA with a weakly alkaline solution containing a water-soluble carbohydrate:



Additional cross-linkages are formed in the polymer network during this process resulting in a decrease in the degree of swelling of the polymer gel. An increase in the carbohydrate concentration can induce the further desorption of boron-containing groups from the PVA surface, which results in its complete regeneration [22].

The response of the polymers covalently modified by phenylboronic acid or its derivatives is based on the same principle. The main distinction is that the carbohydrates determined in the solution are directly bound to the functional groups of the polymer; i.e., the chemisorption of the carbohydrates occurs [9]. However, as mentioned previously, the cost of phenylboronic acid and its derivatives is fairly high, and the modification of the known polymers by organic reagents of this class proceeds sometimes in several stages each of which is associated with the necessity to remove excesses of participants of the reaction [32], which results in still higher increasing cost of the produced sensitive units. The application of polyalcohols impregnated with boronic acid or its salts, which are more available and cheaper reagents, and simplicity of the impregnation stage make it possible to use the procedure proposed for carbohydrate determination in almost all laboratories and mobile stations engaged in out-of-laboratory analysis.

Acidity effect on the degree of swelling of PVA-20 in carbohydrate solutions. As in any case of complexation involving the ligand representing a weak acid, the acidity of the solution exerts a substantial effect on the formation of ester chelates. We obtained the dependence of the equilibrium volume of the PVA granules on the pH of a phosphate buffer containing glucose and sucrose in a concentration of 40 mmol/dm^3 in the presence of 0.05 mol/dm^3 $Na_2B_4O_7$ (Fig. 1). It is seen from the plot that the volume of the granules stored in glucose solutions decreases with increasing pH from 7.5 to 8.5 and remains approximately constant in the range from 8.5 to 10.0. This effect can be related to an increase in the fraction of deprotonated alcohol groups of glucose and, as a consequence, to an easier ligand exchange process. The formation of a boron complex with PVA is more favorable at the pH higher than 10.0 due to an increase in the fraction of deprotonated hydroxyl groups of the polymer, which results in a reverse increase in the granule volume. It should be mentioned that no response to sucrose is observed in the pH range from 8.0 to 10.5. This can be associated with a weak ability of sucrose to form complexes with boron because of the absence of *cis*-diol fragments in the carbohydrate molecule. For the further

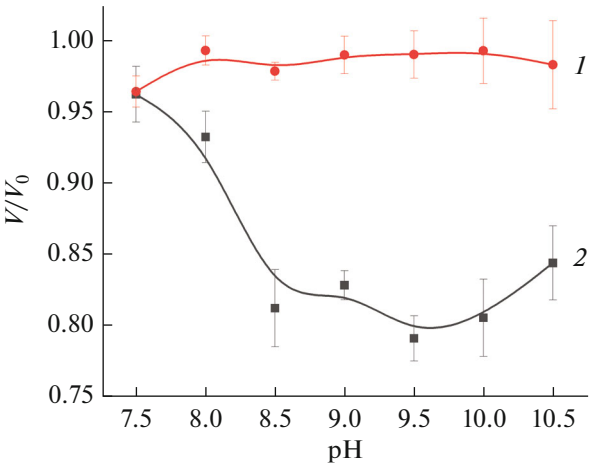


Fig. 1. Relative volume of the PVA-20 granules in solutions of (*1*) sucrose and (*2*) glucose as a function of the acidity of the medium; phosphate buffer, $C_{carbohydrate}$ = 0.04 mol/dm^3, $C_{Na_2B_4O_7}$ = 0.05 mol/dm^3.

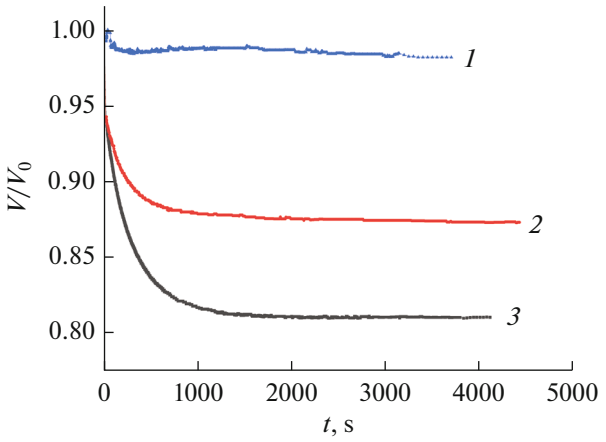


Fig. 2. Kinetic curves of the change in the volume of the PVA granules upon their dislocation from the stock solution to solutions of glucose: $C_{glucose}$ = (*1*) 1, (*2*) 8, and (*3*) 40 mmol/dm^3.

study of the behavior of the PVA granules in glucose solutions, we chose pH 8.5 for the buffer solution.

Swelling kinetics of PVA-20 in glucose solutions. When determining the concentration of the dissolved substances using sensitive polymers, it is necessary to store them in a solution of the sample to the state of equilibrium. This is often accompanied by a loss in express analysis, since for a series of polymers the time of achieving the equilibrium degree of swelling is more than 30 min and, in some cases, several hours. Therefore, it is proposed to use data on the kinetics of changing the polymer gel volume with analytical purposes.

The swelling kinetics of the PVA-20 granules during their transfer from the stock solution to a glucose-containing solution was studied in this work. The obtained kinetic data are shown in Fig. 2. The dependences represent the descending curves reaching equilibrium, which confirms the formation of additional transversal cross-linkages in the granules through the boron atom. Thus, the compression rate of the granules is defined by the desorption rate of boron from the PVA surface (from the viewpoint of the quasi-homogeneous model of the polymer gel structure). This means that the curves cannot be described in the framework of the classical heterophase model of the polymer gel structure [20], since this model ignores the chemisorption or chemidesorption of the substances dissolved in water. Note that this is imperceptible on the curve corresponding to a glucose concentration of 1 mmol/dm^3, because this concentration is lower than the detectability threshold for glucose. It is seen that about 30 min are needed to achieve the equilibrium degree of swelling of the granules, which is substantially lower than that of unmodified PVA. Probably, the replacement of PVA by the matrix that forms less stable complexes with boron would additionally decrease this parameter.

One of the important factors of using granules as sensitive units is reversibility of the swelling when transferring from the solvent to solution and back. The kinetic curves of the direct and backward experiments are presented in Fig. 3 and show that a PVA-20–tetraborate system is reversible. This is especially significant for manufacturing sensor devices based on the PVA–tetraborate interaction and designed for multiple use, which allows researchers to perform measurements under the repetition conditions.

When analyzing real objects, another useful application of the swelling kinetics of polymer gels is a possibility to determine several simultaneously present analytes [18]. In this case, the kinetic curves are considered as multidimensional data, and the use of mathematical algorithms, such as multiple linear

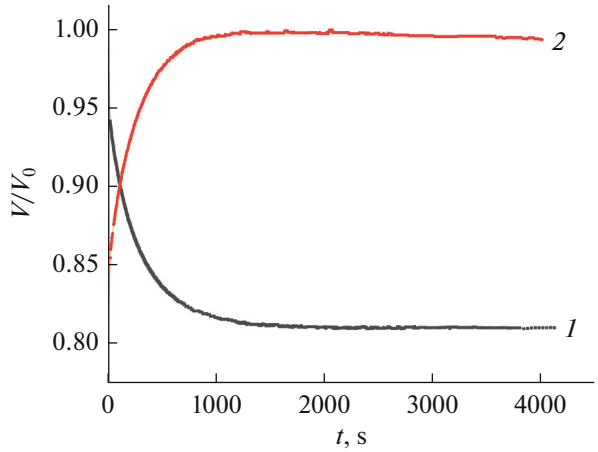


Fig. 3. Kinetic curves obtained in the (*1*) direct and (*2*) backward experiments; $C_{glucose}$ = 40 mmol/dm^3.

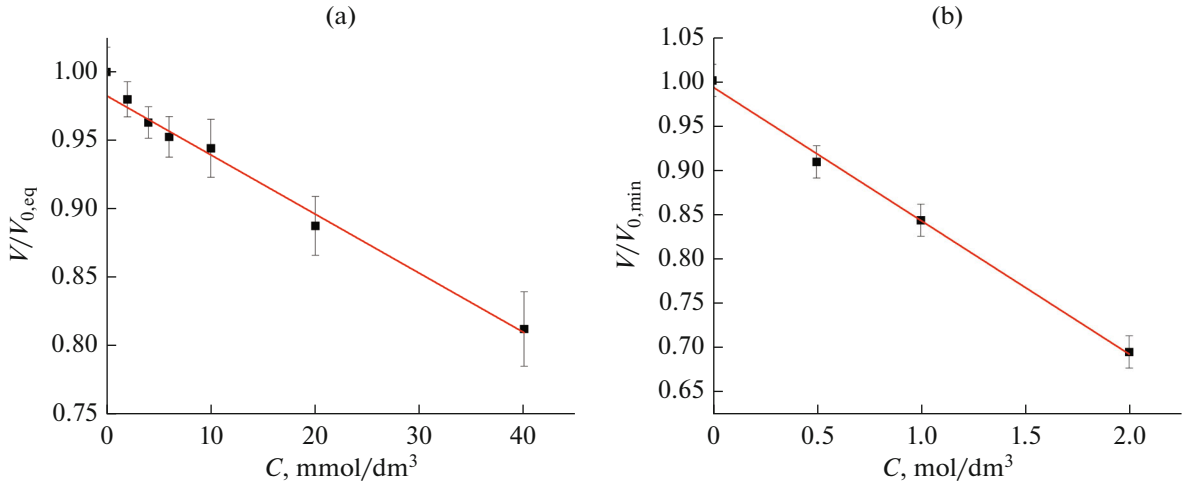


Fig. 4. Relative volume of the PVA-20 granules as a function of the glucose concentration: (a) $C_{Na_2B_4O_7}$ = 0.05 mol/dm^3 and (b) aqueous solutions of glucose; phosphate buffer, pH 8.5.

regression, regression on principal components, and the fractional least-squares method, would make it possible to solve similar problems.

Calibration plot for glucose determination and estimation of the detectability threshold. The calibration plot (Fig. 4a) was constructed for the quantitative determination of glucose and estimation of its detectability threshold. The plot represents the dependence of the relative equilibrium volume of the PVA granules on the glucose concentration in the presence of 0.05 mol/dm^3 $Na_2B_4O_7$ at pH 8.5 in the range of glucose concentrations from 0 to 40 mmol/dm^3. The earlier obtained data [20] were used to construct the dependence of the minimum depth on the kinetic curves on the glucose concentration in an aqueous solution in the range from 0 to 2 mol/dm^3 (Fig. 4b).

Both plots shown in Fig. 4 are linear concentration dependences of the analytical signal in the chosen concentration ranges. The detectability threshold of glucose was found to be 6.9 mmol/dm^3 in the presence of sodium tetraborate in the solution and 0.2 mol/dm^3 in the absence of sodium tetraborate. In other words, the sensitivity of glucose determination due to the use of PVA complexation with boron in a weakly alkaline medium increases by nearly 30 times, which makes it possible to perform analyses in such a concentration range where the activities of water of the stock solution and that of the sample solution are almost equal. Owing to this, the polymer would not react to other substances dissolved in water. This implies a high selectivity to carbohydrates. In addition, unmodified PVA responds to both glucose and sucrose, and the sensitivity to sucrose is twice as high as that to glucose, whereas the selectivity to one carbohydrate can be achieved by the variation of the pH of the solution as shown previously when PVA modified with sodium tetraborate is used. Therefore, the use of the complexation of boron with PVA followed by the ligand exchange results in a significant increase in the selectivity and sensitivity of the polymer to carbohydrates and allows the determination from equilibria data in the corresponding concentration range to be performed for such glucose-containing food products as honey, fruits, vegetables, and vitamin complexes and medical syrups for children.

Thus, we studied the possibility of application of polyvinyl alcohol impregnated with a sodium tetraborate solution for the determination of concentrations of carbohydrates (glucose and sucrose) dissolved in water. The material demonstrated a high selectivity for the determination of the glucose concentration in a weakly alkaline medium in the presence of sucrose and a sensitivity sufficient for an analysis of such food products as honey, vegetables, fruits, confectionery, etc. The selectivity is achieved by the variation of the pH of the solution in the range from 8.5 to 10.0. Further we are planning to study swelling of PVA granules with boron complexes formed on the surface in solutions of other carbohydrates. The main restriction of the optical micrometry method is a broad confidence interval for the measured analytical signal, which can be associated with the nonuniformity of the cross-linking reagent distribution inside the granules of one set or their nonideal sphericity. The most probable area of application of this polymer would be manufacturing from it photon-crystalline and holographic sensors and the development of approaches to an analysis of food products using the PVA granules.

FUNDING

This work was supported in part by the Ministry of Science and Higher Education of the Russian Federation in terms of state assignment of the Kurnakov Institute of the

General and Inorganic Chemistry (Russian Academy of Sciences) in the area of basic research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Karpova, E.V., Shcherbacheva, E.V., Galushin, A.A., et al., *Anal. Chem.*, 2019, vol. 91, no. 7, p. 3778.
2. Zhuang, N., Ma, J., and Yang, L., *Microchem J.*, 2021, vol. 164, p. 106075.
3. Conzuelo, F., Gamella, M., Campuzano, S., et al., *J. Agric. Food Chem.*, 2010, vol. 58, no. 12, p. 7141.
4. Antiochia, R., Vinci, G., and Gorton, L., *Food Chem.*, 2013, vol. 40, no. 4, p. 742.
5. Adeniyi, O., Sicwetsha, S., and Mashazi, Ph., *ACS Appl. Mater. Interfaces*, 2020, vol. 12, no. 2, p. 1973.
6. Zhou, C., Shi, Ya., Ding, X., et al., *Anal. Chem.*, 2013, vol. 85, no. 2, p. 1171.
7. Aksorn, J. and Teepoo, S., *Talanta*, 2020, vol. 207, p. 120302.
8. Tao, Yi., Luo, F., Lin, Yi., et al., *Talanta*, 2021, vol. 230, p. 122364.
9. Chen, Ch., Dong, Zh.-Q., Shen, J.-H., et al., *ACS Omega*, 2018, vol. 3, p. 3211.
10. Lan, Yu., Xue, M., Qiu, L., and Meng, Z., *Chem. Select.*, 2019, vol. 4, no. 21, p. 6547.
11. Yan, Z., Xue, M., He, Q., et al., *Anal. Bioanal. Chem.*, 2016, vol. 408, no. 29, p. 8317.
12. Moghaddam, G.K., Margerison, H., Suzuki, J., et al., *Sens. Actuators*, vol. 267, p. 1.
13. Kraiskii, A.V., Postnikov, V.A., Sultanov, T.T., and Khamidulin, A.V., *Kvantovaya Elektron.*, 2010, vol. 40, no. 2, p. 178.
14. Postnikov, V.A., Kraiskii, A.V., and Sergienko, V.I., *Holography—Basic Principles and Contemporary Applications*, London: IntechOpen, 2013.
15. Chen, C., Zhu, Y., Bao, H., et al., *Soft Matter.*, 2011, vol. 7, p. 915.
16. Jiang, H., Zhu, Y., Chen, C., et al., *New J. Chem.*, 2012, vol. 36, no. 4, p. 1051.
17. Kovaleva, S.S. and Ferapontov, N.B., *Sorbts. Khromat. Protsessy*, 2007, vol. 7, no. 7, p. 883.
18. Babayan, I.I., Tokmachev, M.G., Ivanov, A.V., and Ferapontov, N.B., *J. Anal. Chem.*, 2019, vol. 74, no. 8, p. 834. https://doi.org/10.1134/S0044450219080036
19. Gagarin, A.N., Ferapontov, N.B., and Tokmachev, M.G., *Colloid J.*, 2017, vol. 79, no. 6, p. 740. https://doi.org/10.1134/S1061933X17060096
20. Tokmachev, M.G., Ferapontov, N.B., Trobov, Kh.T., and Gavlina, O.T., *Uchen. Zap. Fiz. Fak-ta Mosk. Un-ta*, 2018, no. 5. 1850303.
21. Tikhanova, O.A., Gagarin, A.N., Tokmachev, M.G., et al., *Sorbts. Khromat. Protsesy*, 2021, vol. 21, no. 6, p. 860.
22. Ivanov, A.V., Smirnova, M.A., Tikhanova, O.A., et al., *Theor. Found. Chem. Technol.*, 2021, vol. 55, no. 5, p. 1009.
23. Shchemelev, I.S., Ferapontov, N.B., and Ivanov, A.V., *Nauch. Vestn. SamGU*, 2021, no. 3(127), p. 14.
24. Shchemelev, I.S., Staroverova, A.V., Ivanov, A.V., and Ferapontov, N.B., *Sorbts. Khromat. Protsessy,* 2021, vol. 21, no. 6, p. 841.
25. Cui, Q., Muscatello, M.M.V., and Asher, S.A., *Analyst*, 2009, vol. 134, no. 5, p. 875.
26. Panahi, H.A., Moniri, E., Izadi, A., et al., *J. Appl. Chem. Res.*, 2009, vol. 9, p. 52.
27. Ferapontov, N.B., Kovaleva, S.S., and Rubin, F.F., *J. Anal. Chem.*, 2007, vol. 62, no. 10, p. 924. https://doi.org/10.1134/S1061934807100048
28. Yamskov, I.A., Budanov, M.V., and Davankov, V.A., *Bioorgan. Khim.*, 1979, vol. 5, no. 11, p. 1728.
29. Gruzdeva, A.N., Gorshkov, V.I., Gagarin, A.N., and Ferapontov, N.B., *Russ. J. Phys. Chem. A*, 2005, vol. 79, no. 7, p. 1150.
30. Van Duin, M., Peters, J.A., Kieboom, A.P.G., and van Bekkum, H., *Tetrahedron*, 1984, vol. 40, no. 15, p. 2901.
31. Van Duin, M., Peters, J.A., Kieboom, A.P.G., and van Bekkum, H., *Tetrahedron*, 1985, vol. 41, no. 16, p. 3411.
32. Wu, Zh., Zhang, Sh., Zhang, X., et al., *J. Pharm. Sci.*, 2011, vol. 100, no. 6, p. 2278.

Translated by E. Yablonskaya