42. M. Cochet, F. Perrin, F. Gannon, A. Krust, P. Chambon, G. S. MacKnight, D. C. Lee, K. E. Mayo, and R. D. Palmiter, Nucl. Acids Res., 6, No. 7, 2435 (1979).
43. A. P. Ryskov, N. A. Timchenko, L. T. Timchenko, T. A. Salikhov, and V. S. Gaitskhoki, Mol. Biol., 18, No. 1 (1984).
44. D. S. Lee. G. S. MacKnight, and R. D. Palmiter, J. Biol. Chem., 255, No. 4, 1442 (1980).
45. F. Perrin, M. Cochet, P. Gerlinger, B. Cami, J. P. LePennec, and P. Chambon, Nucl. Acids. Res., 6, No. 8, 2731 (1979).
46. M. Cochet, F. Gannon, R. Hen, L. Moroteaux, F. Perrin, and P. Chambon, Nature (London), 282, No. 5739, 567 (1979).
47. A. L. Shvartsman, Usp. Sovr. Biol., 90, No. 1, 3 (1980).
48. F. Crick, Science, 204, 264 (1979).
49. H. Sakano, J. Rogers, R. Muppi, C. Brack, A. Trauneckok, R. Maki, R. Wall, and S. Tonegawa, Nature (London), 277, No. 5698, 627 (1979).
50. S. Tonegawa, A. M. Maxam, K. Tizard, O. Berhard, and W. Gilbert, Proc. Nat. Acad. Sci. USA, 75, No. 3, 1485 (1978).
51. J. Wozney, D. Hanahan, R. Morimoto, H. Boedtker, and P. Doty, Proc. Nat. Acad. Sci. USA, 78, No. 2, 712 (1981).
52. W. Wahli, J. B. Dawid, T. Wyler, R. Jaggi, and G. U. Ryffel, Cell, 16, No. 3, 535 (1979).
53. E. Bresnick, J. Levy, R. M. Hines, W. Lewin, and P. E. Thomas, Arch. Biochem. Biophys., 212, No. 2, 501 (1981).
54. D. Pribnow, J. Mol. Biol., 99, 419 (1975).
55. S. J. Tsai, M. Y. Tsai, and B. W. O'Malley, Proc. Nat. Acad. Sci. USA, 78, 879 (1981).
56. B. Wasylyk, R. Derbyshire, A. Guy, D. Molko, A. Roget, T. Teonle, and P. Chambon, Proc. Nat. Acad. Sci. USA, 77, No. 12, 7024 (1980).
57. B. Wasylyk, C. Kedinger, J. Corden, O. Brison, and P. Chambon, Nature (London), 285, No. 5764, 367 (1980).
58. B. Wasylyk and P. Chambon, Nucl. Acids Res., 9, No. 8, 1813 (1981).
59. P. Sassone-Gorsi, J. Cordon, C. Kedinger, and P. Chambon, Nucl. Acids Res., 9, No. 19, 3914 (1981).
60. R. Newman, C. Schneider, R. Sutherland, L. Vodinelich, and M. Greaves, Trends Biochem. Sci., 7, No. 11, 397 (1982).

ANALYSIS OF α-GLYCOLIC COMPOUNDS USING THE POTENTIOMETRIC DIFFERENTIATED TITRATION OF PERIODATE AND FORMALDEHYDE

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UDC 543.242.3:547.426.1+547.426.24

The conditions of the analysis of α-glycolic compoounds (ethylene glycol, glycerol, mannitol, D-glucose, D-ribose, and dihydroxyacetone) using the potentiometric differentiated titration of periodate and formaldehyde have been studied. A procedure for quantitative determination is proposed and the possibilities have been shown of using it for evaluating the stoichiometry of oxidation and the separate analysis of two-component mixtures. The performance of the determinations is distinguished by simplicity and adequate reliability.

In the quantitative analysis of compounds containing α-glycol groupings, use is frequently made of oxidation with periodate followed by the determination if its excess or of one of the reactions products – iodate, an aldehyde, or formic acid [1-3]. In the study of the structure and kinetics of the oxidation of polyhydroxy compounds, in the analysis of mixtures, and in other cases the necessity arises for performing parallel determinations both of the consumption of periodate and of the amount of formic acid, formaldehyde, and other products that have been formed. A combination of two or more methods is not infrequently used for these purposes [4, 5], but the performance of the analyses is then an extremely complicated and laborious process.

All-Union Scientific-Research Institute of Applied Biochemistry. "Biokhimreaktiv" Scientific-Production Association, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 305-309. May-June, 1985. Original article submitted July 3, 1984.

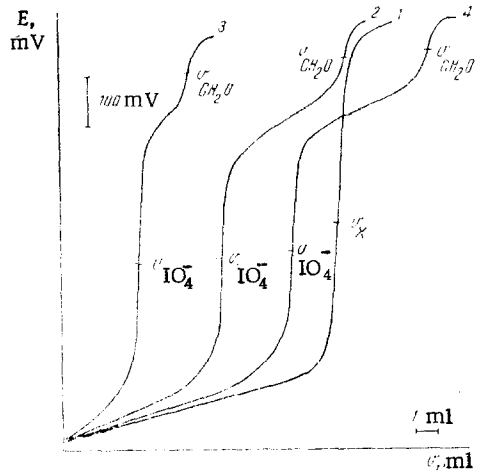

Fig. 1. Potentiometric curves of titration in a mixture of aqueous sodium tetraborate solution and dimethyl sulfoxide with a 0.1 N solution of hydroxylamine hydrochloride of: 1) potassium periodate in a "blank" experiment; 2-4) mixtures of potassium periodate and formaldehyde after the oxidation of glycerol (2), of D-glucose (3), and of ethylene glycol (4).

Possibilities for simplifying the separate analysis of mixtures are possessed by methods of potentiometric differentiated titration, which permit the successive determinations of two or more components without their preliminary separation. We have investigated the conditions for the potentiometric differentiated titration of periodate and formaldehyde [6]. To solve the problem posed, we selected the hydroxylamine method, since it is known that hydroxylamine is capable of reacting both with aldehyde and with strong oxidizing agents. Potentiometric titration with hydroxylamine has been proposed previously for determining potassium and sodium periodates [7] and formaldehyde [8] in alkaline solutions. As our investigations have shown, on such titration in media with pH values of 11-12 a combined determination of the components of a mixture of periodate and formaldehyde is observed. In aqueous solutions with lower pH values (for example, in buffer solutions of sodium acetate, carbonate, or tetraborate) only the periodate titrates, and there is no potential jump on the curve of the potentiometric titration of formaldehyde. It has been established experimentally that the optimum medium for the successive titration of periodate and formaldehyde is a mixture of an aqueous tetraborate solution with a protophilic organic solvent – dimethyl sulfoxide (DMSO).

For the selective determination of formaldehyde in the presence of periodate a mixture of a saturated aqueous solution of sodium tetraborate with DMSO in a volume ratio of 1:1 has been used [9]. It is true, as quantitative determinations show, that on titration in this medium low results of the analyses for the periodate content are obtained, which is explained by the partial precipitation of the latter under the influence of the organic solvent. For this reason, DMSO must be added to the solution being analyzed gradually as the periodate is reduced. For this purpose it is recommended to use as titrant a standard solution of hydroxylamine hydrochloride in DMSO [10]. Since the organic solvent is necessary for creating the medium for the titration of formaldehyde, the required amount of DMSO may also be introduced after the periodate has been titrated (i.e., after the first potential jump on the curve).

In the present investigation we have shown the possibilities of analyzing a number of glycolic compounds by using the potentiometric differentiated titration of periodate and formaldehyde. A feature of the performance of the determinations is the necessity for performing a "blank" experiment. For this, a solution of periodate prepared as in the analysis of the α-glycolic compounds but without the sample substance being determined is determined potentiometrically. On the titration curve (Fig. 1, curve 1) a well-defined potential jump appears that corresponds to the complete reduction of the periodate by the hydroxylamine (V_X) with a strict observance of a 1:1 stoichiometric molar ratio. When the compounds being analyzed are treated with an excess (in relation to the weight of the sample but taking the

TABLE 1. Results of the Quantitative Determination of Some α-Glycolic Compounds (at n = 7, α = 0.95).

Substance analyzed	$nIO_4^- : nCH_2O$	$\Delta VIO_4^- / VCH_2O$	Taken mg	Found from periodate			Found from formaldehyde		
				mg	relative error, %	$\pm s_r$	mg	relative error, %	$\pm s_r$
Ethylene glycol	1:2	0.51	41.2	40.5	−0.7	0.9	40.8	−1.0	0.6
Glycerol	2:2	1.02	31.6	31.2	−1.3	1.1	31.2	−1.3	0.8
Mannitol	5:2	2.53	21.5	21.3	−0.9	0.6	21.6	+1.4	1.0
D-glucose	5:1	4.97	20.4	20.1	−1.5	0.9	20.6	+1.0	1.6
D-ribose	4:1	3.95	24.2	23.9	−1.2	0.6	24.4	+0.8	1.4
Dihydroxy-acetone	1:1	1.01	56.6	56.2	−0.7	0.8	56.3	−0.5	0.7

stoichiometry of oxidation into account) of periodate, mixtures of them with formaldehyde are formed which titrate with two potential jumps on the curves (Fig. 1, curves 2-4). The first of them corresponds to the titration of the excess of periodate (VIO_4^-), and the second to the complete binding of formaldehyde in the oximation reaction of the hydroxylamine (V_{CH_2O}). Thus, the results of the potentiometric differentiated titration permit the determination to be made with respect to parameters: from the consumption of periodate in oxidation by using the difference in the volumes of the titrant $\Delta V_{IO_4^-} = V_X - V_{IO_4^-}$, and also from the direct titration of the formaldehyde liberated V_{CH_2O}. Table 1 gives the results of quantitative determinations of α-glycolic compounds. It can be seen that the results calculated with the aid of $\Delta V_{IO_4^-}$ and V_{CH_2O} agree fairly well with one another. The relative standard deviation in the analysis with respect to the consumption of periodate does not exceed ±1.1% and with respect to the titration of formaldehyde it amounts to ±1.6%.

It is known that the stoichiometry of the oxidation of α-glycolic compounds under otherwise identical conditions depends on their structure and the number of hydroxy groups in the molecule [5]. When constant conditions of oxidation are observed, the stoichiometry of the reaction and the purity of the substance under investigation can be judged by comparing the quantitative results of the determination of the consumption of periodate and the amounts of products formed. As such a criterion it is possible to use, for example, the ratio of the number of molecules of periodate reduced (nIO_4^-) and the number of molecules of formaldehyde liberated (nCH_2O) in the oxidation of a single molecule of an α-glycolic compound. We hve established that for these purposes it is also possible to use the results of potentiometric differentiated titration. It can be seen from Table 1 that the ratios of the volumes of titrant $\Delta VIO_4^-/VCH_2O$ agree with the ratios $nIO_4^-:nCH_2O$ known in the literature.

Differences in the stoichiometry of oxidation can be judged by comparing the total consumption of titrant in the analysis and in the "blank" experiment. For example, in the determination of glycerol (see Fig. 1, curve 2) the equality $V_X = V_{IO_4^-} + V_{CH_2O}$ is observed, which corresponds to the equality $nIO_4^- = nCH_2O$. At $nIO_4^- > nCH_2O$ the volumes of titrant consumed become unequal $V_X > V_{IO_4^-} + V_{CH_2O}$ (in the analysis of mannitol, ribose, and glucose; Fig. 1, curve 3). Where $nIO_4^- < nCH_2O$ (in the oxidation of ethylene glycol; Fig. 1, curve 4), again an inequality is observed, but here $V_X < V_{IO_4^-} + V_{CH_2O}$.

Potentiometric differentiated titration permits a simplification of the performance of the separate analysis of two-component mixtures of α-glycolic compounds differing in the stoichiometry of their oxidation by periodate. Table 2 gives an examples the results of analyses of mixtures of glycerol with glycol at various ratios of the components. The figures presented indicate an adequate reliability of the determinations.

EXPERIMENTAL

For the potentiometric titration we used a Radiometer apparatus consisting of a pHM-26 pH-meter, a ABU-12 automatic burette, a TTT-11 titrator, a SBR-2C recorder, and a platinum–calomel pair of electrodes. The platinum electrode (P 101) was immersed before use in a hot solution of concentrated nitric adid for 10-15 min, and was washed with water immediately before the titration. A similar tretment of the indicator electrode was repeated after evey 4-5 titrations.

TABLE 2. Results of the Quantitative Determination of Ethylene Glycol and Glycerol in Mixtures (at n = 5, α = 0.95).

Ethylene glycol					Glycerol				
taken		found		$\pm s_r$	taken		found		$\pm s_r$
mg	$\bar{X}$%	mg	$\bar{X}$%		mg	$\bar{X}$%	mg	$\bar{X}$%	
30,6	71,2	30,2	70,4	0,8	12,4	28,8	12,7	29,6	1,4
20,3	52,3	20,0	51,5	0,9	18,5	47,7	18,8	48,5	1,1
10,5	29,1	10,6	29,5	1,1	25,6	70,9	25,3	70,5	1,3
5,3	14,8	5,7	15,9	1,8	30,4	85,2	30,2	84,1	1,2

The titrant was prepared by dissolving approximately 7-8 g of hydroxylamine hydrochloride (ch.d.a. ["pure for analysis"]) in one liter of DMSO (kh.ch. ["chemically pure"] grade). The titrant was standardized by the titration of accurately weighed samples of potassium hexacyanoferrate.

For the analyses samples of α-glycolic compounds weighing 20-60 mg were taken, and these were then treated with 15.0 ml of a 0.1 M aqueous solution of potassium periodate. The vessels with the solutions being analyzed were closed with stoppers and kept at room temperature in a dark place for the time necessary for the complete oxidation of the substance. The time necessary for the complete oxidation of each substance was determined beforehand by methods known in the literature [1-3]. In no case did the time of keeping the solutions exceed 90 min. Then the solutions being analyzed were diluted with 10 ml of distilled water and ~1 g of crystalline sodium tetrborate (kh.ch. grade) was added to each of them. They were then stirred for 1-2 min and subjected to potentiometric titration with a 0.1 N standard solution of hydroxylamine hydrochloride in DMSO until two potential jumps had appeared on the curves.

In the analysis of the ribose, mannitol, and glucose, after the first potential jump titration was stopped and 10 ml of DMSO was added to the solution being analyzed, after which titration was continued until the end jump. A "blank" experiment was carried out in parallel – a solution was prepared and titrated without the addition of the α-glycolic compound. From the potentiometric titration curve the equivalence points and the volumes of titrant for the performance of the calculations – V_X, $V_{IO_4^-}$, and V_{CH_2O} – were determined graphically (see Fig. 1). The time of performing a determination and a blank experiment (taking the time of oxidation into account) amounts to 25-30 min.

SUMMARY

A procedure is proposed for the quantitative determination of α-glycolic compounds that is based on potentiometric differentiated titration of periodate and formaldehyde with hydroxylamine. The possibilities of using potentiometric titration for evaluating the stoichiometry of oxidation and for the separate analysis of two-component mixtures has been shown. The performance of the determinations is distinguished by simplicty and adequate relibility.

LITERATURE CITED

1. S. V. Isai and V. E. Vas'kovskii, Khim. Prir. Soedin., 465 (1973).
2. V. Ya. Zakharans, Izv. Akad. Nauk Latv. SSR, Ser. Khim., No. 1, 3-18 (1982).
3. V. Ya. Zakharans, I. U. Lipsbergs, and A. T. Valdnietse, Khim. Prir. Soedin., 226 (1983).
4. A. Ya. Bochkov, I. G. Kozlovskii, and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 8, 1812 (1968).
5. A. Berka, Ya. Vulterin, and Ya. Zyka, New Redox Methods in Analytical Chemistry [in Russian], Moscow (1968), p. 124.
6. V. A. Spintse and A. Ya. Veveris, Abstracts of Lectures at the IIIrd Conference on Methods of Obtaining and Analyzing Biochemical Reagents [in Russian], Olaine (1979), p. 67.
7. P. K. Norkus and Yu. Yu. Yankauskas, Zh. Anal. Khim., 27, No. 12, 2421 (1972).
8. J. Vulterin, Collection Czech Chem. Commun., 36, 293 (1971).
9. B. A. Spintse and A. Ya. Veveris, Zh. Anal. Chem., 37, No. 4, 769 (1982).
10. A. Ya. Veveris and B. A. Spintse, "Method for the quantitative determination of polyhydroxy compounds," USSR Inventors' Certificate No. 732,738, Byull. Izobret., No. 17, 13 (1980).