

Estimation of DL-glyceraldehyde, dihydroxyacetone, methylglyoxal, and their mixtures by oxidation with sodium periodate

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An analytical method for the estimation of DL-glyceraldehyde, dihydroxyacetone, methylglyoxal, and their binary and ternary mixtures by oxidation with sodium periodate in unbuffered aqueous media was worked out. Oxidations proceeded at 70°C during 1 hour. On the basis of chemical equations of oxidation of the individual compounds studied, of the unchanged sodium periodate as well as the produced acids and formaldehyde, mathematical relations were derived for calculation of the concentrations of individual compounds also in the mentioned types of mixtures. The error of estimation of individual compounds in the concentration range 5–20 mg did not exceed $\pm 2\%$. In the case of binary and ternary mixtures for 20 mg sample, when the concentration range of individual compounds was 20–80%, the relative error of their estimation did not exceed $\pm 10\%$. In this work, also mechanisms of oxidations with sodium periodate of the estimated compounds are discussed.

In our studies of acid-base catalyzed isomerizations of trioses and their dehydration to methylglyoxal [1], we worked out a quick and fairly precise analytical method for the estimation of the mentioned compounds mainly in their mixtures [2]. This polarographic method was based on different reactivity of the studied compounds with primary amines. Because the mentioned compounds are significant and frequently occur in mixtures, we looked for another, purely chemical method of their determination side by side. The most suitable method appeared to be periodate oxidation which was used with success not only in analytical chemistry but also in structural studies and syntheses of organic compounds, mainly sugars. There is a great number of original works concerning mainly the methodic side of periodate oxidations. The majority of them is summarized in monographs [3–7]. Knowing the concentration of the starting compounds, Speck [8] followed the kinetics of mutual isomerization and dehydration of glyceraldehyde and/or dihydroxyacetone to methylglyoxal on the basis of periodate consumption and formaldehyde production. Method, based on periodate oxidation, which would enable the determination of all three compounds side by side has not been worked out up to now.

The aim of this work is to work out a simple and fairly precise method for the quantitative determination of glyceraldehyde, dihydroxyacetone, and methylglyoxal as well as of their binary and ternary mixtures on the basis of sodium periodate consumption, production of formaldehyde and appropriate acids, respectively.

Experimental

Chemicals

DL-Glyceraldehyde (anal. grade, Reanal, Budapest), dihydroxyacetone (anal. grade, Light Co Ltd., Colnbrook), and dimedone (anal. grade, Lachema, Brno) were commercial products. Methylglyoxal was prepared by oxidation of acetone with selenium dioxide according to [9] and was stored in the form of its diisopropyl acetal because of its instability. Sodium carbonate (anal. grade) was dried at 250°C to constant weight. Sodium periodate (Carlo Erba, Milano) was recrystallized several times from water. Sodium arsenate(III) was prepared from resublimed arsenic(III) oxide of anal. grade. The iodine solution was prepared from resublimed iodine of anal. grade. Starch paste and indicator Tashiro (0.2% alcoholic solution of methyl red and 0.1% alcoholic solution of methylene blue in the ratio of 1 : 1) were used in iodometry and alkalimetry, respectively.

Preparation of solutions

A 0.01 M solution of glyceraldehyde and similarly that of dihydroxyacetone was prepared by dissolution of the mentioned compounds (90.08 mg) in distilled water and made up to 100 ml. A 0.01 M solution of methylglyoxal was prepared from acetal of methylglyoxal (350 mg) by hydrolysis with 1% sulfuric acid (2 ml). The reaction mixture was heated for 5 hours at 80°C on a water bath. Then it was cooled and made up to 200 ml with distilled water. Methylglyoxal was standardized according to *Friedmann* [10].

A 0.01 N solution of sodium arsenate(III) was obtained by dissolution of resublimed arsenic(III) oxide (247.27 mg) in 30% sodium hydroxide (10 ml). To the solution, neutralized with sulfuric acid (phenolphthalein), 10 g of sodium hydrogen carbonate (anal. grade) dissolved in the smallest amount of distilled water was added and the solution was made up to 500 ml.

An approximately 0.01 M solution of sodium periodate (500 ml) was prepared by dissolution of recrystallized sodium periodate (1.07 g) in distilled water and made up to the mark. The factor of this solution was established by titration of the liberated iodine from potassium iodide in acidic medium with 0.01 N sodium thiosulfate.

A 0.01 N solution of iodine was prepared by dissolution of potassium iodide (approximately 20 g) in 50 ml of distilled water, resublimed iodine (0.635 g) was added and the solution was made up to 500 ml. This solution was standardized with 0.01 N sodium thiosulfate.

A 0.01 N standard solution of sodium carbonate was obtained by dissolution of dried sodium carbonate (52.9 mg) in distilled water and made up to 100 ml.

The starch paste was prepared by dissolving some mg of starch in boiling distilled water (20–50 ml).

Dimedone (1.2 g) was dissolved in ethanol (15 ml) and the solution was made up to 1000 ml with buffer prepared from 0.1 M citric acid (27.5 ml) and 0.2 M sodium hydrogen phosphate (972.5 ml).

Study of the oxidation parameters

Oxidations of compounds were accomplished in 250 ml dark ground flasks at 20, 50, 70, and 80°C. To 5–20 ml of 0.01 M solutions of glyceraldehyde, dihydroxyacetone, methylglyoxal or their mixtures, 20–50% excess of sodium periodate solution was

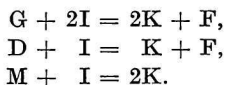
added. In the case of methylglyoxal, sulfuric acid present in the sample was neutralized first with sodium carbonate solution.

From the reaction mixture, 5-ml portions were pipetted into a titrimetric flask at chosen intervals (1/4–5 hours), 3–4 drops of indicator Tashiro were added and the produced acids were titrated with 0.01 N sodium carbonate. The unchanged sodium periodate in the solution was established after titration. Sodium arsenate(III) (0.01 N; 5 ml) was added and the excess of sodium arsenate(III) was titrated with 0.01 N iodine after 15 minutes (starch paste). In the second portion of the reaction mixture the produced formaldehyde was estimated. Into an Erlenmeyer ground flask, 5–40 ml of the reaction mixture was pipetted and 100–200 ml of dimedone solution added. The solution was allowed to stay 20–30 hours and then acidified with concentrated hydrochloric acid (25 ml) of anal. grade and stirred thoroughly. The precipitate was allowed to sink down during 1–2 hours, then filtered through a glass filter (porosity G-4), washed with distilled water and dried at 85–90°C in the air flow during 30 minutes. After cooling in desiccator, the crucible with the precipitate was weighed.

Calculations

On the basis of chemical equations of oxidation of glyceraldehyde, dihydroxyacetone, and methylglyoxal with sodium periodate as well as of moles of consumed sodium periodate, produced acids, and formaldehyde, it is possible to derive relations for the quantitative determination of the mentioned compounds also in their binary and ternary mixtures, respectively.

For these basic chemical equations expressing the course of oxidation of all three compounds studied (see Discussion) holds



G — glyceraldehyde, D — dihydroxyacetone, M — methylglyoxal, K — acids, I — sodium periodate, and F — formaldehyde.

From these chemical equations, it is possible to express mathematical relations for calculation of amounts of the estimated glyceraldehyde (g), dihydroxyacetone (d), and methylglyoxal (m) on the basis of consumed sodium periodate (i), produced acids (k), and formaldehyde (f) as follows:

$$g = \frac{i}{2} = \frac{k}{2} = f, \quad d = i = k = f, \quad m = i = \frac{k}{2}.$$

It is apparent that in the case of individual compounds, each determined quantity, *i.e.* the value of the consumed sodium periodate, produced formaldehyde, and acids can be used for the determination of the analyzed compound. This gives a manifold independent checking of quantitative determination.

Relations for the quantitative determination of individual compounds in binary and ternary mixtures can be calculated from the equations representing equivalent contributions of individual compounds estimated to the sodium periodate consumption, produced formaldehyde, and appropriate acids, respectively.

Solution of equations for binary mixtures gives expressions for calculation of the amounts of individual compounds.

So, for the mixture of glyceraldehyde and dihydroxyacetone holds

$$2g + d = i, \quad 2g + d = k, \quad g + d = f.$$

From this $g = i - f = k - f$,
 $d = 2f - i = 2f - k$.

Similarly for the mixture of glyceraldehyde and methylglyoxal holds

$$2g + m = i, \quad 2g + 2m = k, \quad g = f.$$

From this $g = f = i - \frac{k}{2}$,
 $m = k - i$.

For the mixture of dihydroxyacetone and methylglyoxal holds

$$d + m = i, \quad d + 2m = k, \quad d = f.$$

From this $d = f = 2i - k$,
 $m = k - i$.

As in the case of binary mixtures there are three determined quantities, we have more possibilities to calculate the amounts of determined compounds. It is clear that all relations are mathematically equivalent but we choose the combination of relations with more precisely determined quantities.

For ternary system, *i.e.* in the case when all three compounds are present in the mixture, the following equations and relations are valid

$$2g + d + m = i, \quad 2g + d + 2m = k, \quad g + d = f.$$

From this $g = 2i - f - k$, $d = 2f + k - 2i$, $m = k - i$.

Procedure

To 20 mg of glyceraldehyde, dihydroxyacetone, methylglyoxal, their binary and ternary mixtures, respectively in a dark ground joint flask, 0.01 M solution of sodium periodate (50 ml) was added and heated at 70°C during one hour on a water bath. From the mixture, cooled to the laboratory temperature, 10 ml of solution was pipetted and the produced acids titrated with 0.01 N sodium carbonate (indicator Tashiro). Straight into this neutralized solution, 0.01 N sodium arsenate(III) (10 ml) was added and the solution was allowed to react 15 minutes. The unaltered sodium arsenate(III) was determined by titration with 0.01 N iodine (starch paste).

From the oxidized mixture, another 40 ml was pipetted, dimedone solution (150 ml) was added and allowed to stay for 24 hours at laboratory temperature. The sample was then acidified with 25 ml of concentrated hydrochloric acid and after 2 hours the precipitate was filtered through a fritted glass filter (porosity G-4) and dried at 85–90°C for 30 minutes. Molal concentrations of the estimated compounds were calculated from the derived relations.

Results and discussion

Glyceraldehyde, dihydroxyacetone, and methylglyoxal were oxidized with sodium periodate in aqueous unbuffered medium.

Different methods were applied to determine the unchanged sodium periodate. Reduction of the unaltered sodium periodate to sodium iodate with sodium arsenate(III) in the

medium of sodium hydrogen carbonate gave more reliable results than methods based on reduction of both sodium periodate and sodium iodate to iodine in acidic medium [11–13].

Acids produced during oxidation were successfully determined by solutions of sodium hydroxide as well as of sodium carbonate. Sodium carbonate was used in further experiments because its solutions were stable and had not to be standardized. From many indicators examined the best one seemed to be Tashiro which had a sharp change in equivalent point.

Gravimetric method is the most advantageous method for determining formaldehyde. Precipitation with dimedone in sodium acetate medium gave lower results but precise results were obtained when McIlvain buffer (pH 8) was used as medium.

Oxidation of dihydroxyacetone and methylglyoxal was quantitative in 45 minutes at laboratory temperature. Glyceraldehyde consumed two moles of sodium periodate (determined by the mentioned titration) at laboratory temperature quickly, while the production of acids was quantitative practically after 24 hours. Similar behaviours in periodate oxidations are known and are attributed to slow hydrolysis of esters split off from cyclic forms of saccharides [7, 14]. This, however, could not happen in our case. We found that sodium periodate consumption in the given reaction determined spectrophotometrically ($\mu = 223$ nm) proceeded slowly, *i.e.* similarly as the liberation of acids. This disagreement we explain by catalytic effect of sodium arsenate(III) on the decomposition of cyclic ester which determines the degree of reaction rate.

An example of quantitative analysis of ternary mixture on the basis of sodium periodate consumption, liberated acids as well as formaldehyde using the derived relations is in Table 1. Although it was proved that the estimation error in the case of individual compounds did not exceed $\pm 2\%$, in the case of binary and ternary mixtures it was greater.

On the basis of kinetic spectroscopic data and use of ^{18}O , it was assumed in the literature [15–17] that cyclic ester as an intermediate was formed on oxidative cleavage of 1,2-diols with periodic acid. In accordance with these findings and on the basis of periodate consumption (2 moles $\text{IO}_4^-/\text{glyceraldehyde}$, 1 mole $\text{IO}_4^-/\text{dihydroxyacetone}$, and 1 mole $\text{IO}_4^-/\text{methylglyoxal}$), the following oxidation mechanisms of the studied compounds were suggested

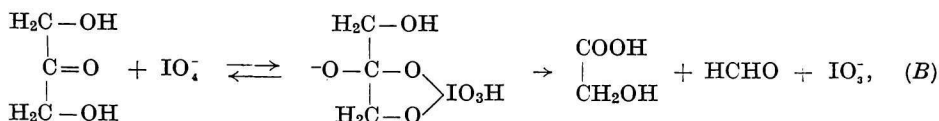
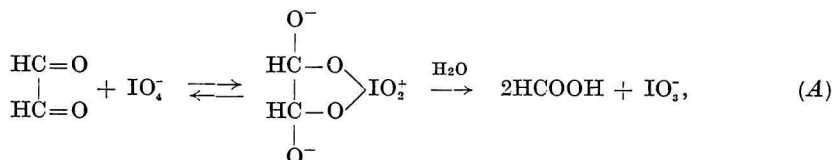
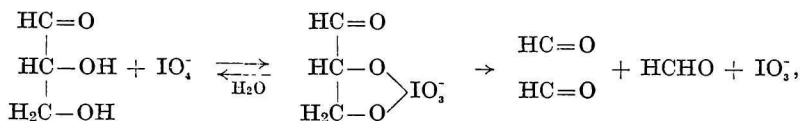
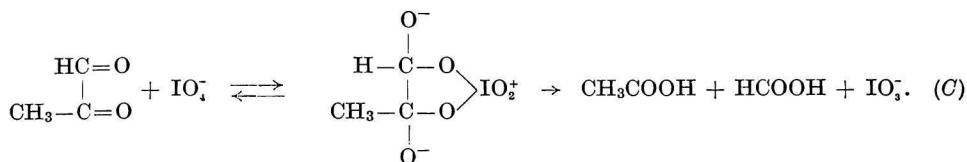


Table 1

Determination of glyceraldehyde, dihydroxyacetone, and methylglyoxal by oxidation with sodium periodate at 70°C during 1 hour side by side

Compounds									Error of determination					
analyzed			calculated			determined			absolute			relative		
G	D	M	NaIO ₄	acids	HCHO	NaIO ₄	acids	HCHO	G	D	M	G	D	M
10	5		30	35	15	29.9	34.8	14.8	+0.2	-0.4	-0.1	2	8	2
5	10	5	25	30	15	24.9	29.6	14.7	+0.5	-0.8	-0.3	10	8	6
	5	10	25	35	10	24.5	34.6	9.6	-0.2	-0.2	+0.1	4	4	1

The analyzed, calculated as well as determined amounts of compounds are given in ml of 10⁻²M solutions.



It is assumed that in the oxidation of glyceraldehyde (A), in the first step, cyclic ester is formed by usual esterification. The excluded water contains oxygen coming from periodate so that oxygens in the formed carbonyl compounds come from the diol systems [18]. On the contrary, in decomposition of α -dicarbonyl compounds as glyoxal (A) and methylglyoxal (C), oxygens in cyclic ester come from periodate, *i.e.* it is a nucleophilic addition of oxygens from periodate upon the carbonyl carbon atoms [18, 19]. In the case of dihydroxyacetone, the formation of cyclic ester is a result of the nucleophilic attack of the periodate oxygen upon the carbonyl carbon atom and of the electrophilic attack of the periodate iodine upon the oxygen of the hydroxyl group.

The formation of cyclic esters is a reversible reaction, while the further intramolecular disproportionations giving splitting products are irreversible and determine the degree of the reaction rate. The studied carbonyl compounds as well as the periodate anion are presented in free, non-hydrated forms because it is not known whether the appropriate hydrates play any, and if so, what role in the mentioned mechanisms.

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