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Two Volumetric Methods for the Determination of Glyoxal

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Two different methods have been developed for the quantitative determination of glyoxal. The first method is an alkalimetric titration and is based on the Cannizzaro reaction of glyoxal. In the second method the glyoxal-bisulphite addition reaction has been applied. The development of both methods was based on an investigation of the rates and equilibria of the corresponding reactions under conditions similar to those prevailing during the analysis. Both methods have been found to give identical results, the errors being no greater than the random titration error.

Several methods have been described previously for the quantitative determination of glyoxal. In the semiquantitative gravimetric method used by Glasstone and Hickling [1], glyoxal is precipitated as glyoxal 2,4-dinitrophenylhydrazone. Ariyama [2] has developed a colorimetric method, and Gabrielsson and Samuelsson [3] have presented a method based on the use of ion exchangers. The only volumetric method thus far developed is that described by Friedemann [4]. The method is based on the oxidation of glyoxal to oxalic acid by hydrogen peroxide in alkaline solution and the titration of the excess alkali with standard acid. This method, however, cannot be used in the presence of other oxidizable compounds. In the following, two new volumetric methods for the quantitative determination of low glyoxal concentrations are suggested which have been employed in kinetic investigations [5].

ALKALIMETRIC METHOD

In the presence of alkali, glyoxal is readily converted to glycolic acid by the Cannizzaro reaction:

$$CHO—CHO + OH^- \rightarrow CH_2OH—COO^-$$

When the alkali is present in excess, the reaction goes to completion [5] and glyoxal can be determined by back titration of the excess alkali with a standard acid solution.

For the evaluation of the time required by the reaction to go to completion under the conditions prevailing during the analysis, the results obtained in kinetic investigations [5] may be applied. It was observed that the Cannizzaro

reaction of glyoxal is of the third order, the reaction rate being proportional to the first power of the glyoxal concentration and to the square of hydroxyl ion concentration. The specific rate at 20° C, which corresponds approximately to the conditions of analysis at room temperature, is 87 l^2 $mole^{-2}$ sec^{-1}. The reaction time can be evaluated from the formula for a third order reaction:

$$t = \frac{1}{k(b-a)}\left[\frac{1}{(b-a)}\ln\frac{a(b-x)}{b(a-x)} - \left(\frac{1}{b-x} - \frac{1}{b}\right)\right] \qquad (1)$$

in which k is the specific rate, b and a the initial concentrations of alkali and glyoxal, respectively, and x the concentration of glyoxal reacted during time t. When, for instance, 20 ml of 0.05 N alkali, the excess of alkali corresponding to at least 50 % of the amount of glyoxal present, is added to give a total volume of the reaction solution not exceeding 30 ml, a reaction time of 8 minutes is found to be sufficient for the reaction to proceed nearly to completion at 20° C. After this time less than 0.1 % of the glyoxal initially present remains in the reaction solution.

The above facts lead to the following procedure for the determination of glyoxal:

To a neutral solution containing not more than 0.66 millimoles of glyoxal in a volume not exceeding 10 ml, 20 ml of 0.05 N sodium hydroxide solution are added. After 8 minutes an amount of standard hydrochloric acid equivalent to the amount of sodium hydroxide added initially is run in and the excess acid is titrated with the 0.05 N sodium hydroxide solution using phenol red as indicator. The amount of alkali consumed in the titration corresponds directly to the amount of glyoxal present. All of the operations must be performed in the absence of carbon dioxide.

In case the conditions are not the same as described above it is to be noted that a standing period four times as long is necessary if the volume of the reaction solution is twice that given above. Similarly, the use of concentrations twice as high shortens the reaction time to one fourth of that given.

BISULPHITE METHOD

This method is based on a procedure presented earlier [6,7] by the author for the determination of several other aldehydes. In this procedure the bisulphite addition reaction is carried out at pH 7.3 in a phosphate buffer in order to effect a very rapid addition reaction, after which the excess bisulphite is titrated with standard iodine in a solution acidified to below pH 4.5 in order to prevent the decomposition of the bisulphite compound during the titration. Before the method could be applied for the determination of glyoxal, it was necessary to investigate the kinetics and equilibria of the glyoxal-bisulphite reaction under the conditions of analysis, taking into account that in this case the reaction involves two successive carbonyl additions.

The experiments indicated that in the case of glyoxal the addition to the first carbonyl group takes place almost quantitatively as compared with the addition to the second carbonyl group. If Gl denotes glyoxal, B sulphite present in the solution in any form *cf.* [6], GlB the monobisulphite compound and GlB_2 the dibisulphite compound of glyoxal we have the following equilibria:

$$\frac{[GlB][B]}{[GlB_2]} = K_1 \qquad \frac{[Gl][B]}{[GlB]} = K_2 \qquad (2)$$

in which K_1 and K_2 are, respectively, the first and the second dissociation constants of the glyoxal-dibisulphite compound. When the dissociation of the glyoxal-dibisulphite compound was investigated, it was observed that $K_1 \gg K_2$ and therefore the second dissociation causes no error in analysis. Table 1 contains examples of the experiments. The dissociation constant K_1 has been calculated from the first of the foregoing equations by taking into account that in the present case [GlB] = [B].

Table 1. The dissociation of glyoxal-dibisulphite compound in phosphate buffer at pH 7.3 at 20° C. Analyses of equilibrium solutions.

$[GlB_2]1^{-1}$ mole^1	$[B]1^{-1}$ mole^1	$10^4 \times K_1 1^{-1}$ mole^1
0.01637	0.002095	2.68
0.01216	0.001817	2.71
0.00795	0.001511	2.87
0.00385	0.001009	2.64
		Average K_1 = 0.000272 1^{-1} mole^1.

The value of the equilibrium constant K_1 is approximately the same as that of benzaldehyde [7] under similar conditions. From the analytical viewpoint, the equilibrium is not favourable enough for the formation of the dibisulphite compound and therefore a small correction due to the unreacted monobisulphite compound should be applied when accurate results are required.

The correction may be evaluated as follows. Let x denote the concentration of glyoxal that has reacted with bisulphite and y the concentration of glyoxal-dibisulphite compound present at equilibrium, and b the initial total concentration of sulphite. The first of eqns. (2) can then be written in the form $(x - y)(b - x - y)/y = K_1$, from which it follows that the relative error is approximately $(x - y)/2y = K_1/2\ (b - x - y) = K_1/2\ c$, in which c is the concentration of excess sulphite in the reaction solution. Using the value given above for K_1, 0.000272 1^{-1}mole^1, the correction, which is to be added to the amount of glyoxal found, is thus $0.0136/c$ per cent. Table 2 contains some sample

Table 2. The evaluation of the correction due to unreacted glyoxal-monobisulphite compound in the determination of glyoxal by the bisulphite method. v = ml of glyoxal stock solution (approximately 0.053 M) taken for analysis. V = approximate volume of the reaction solution in ml. ml I = ml of 0.0752 N iodine solution consumed in the titration of excess bisulphite. ml I_g = ml of 0.0752 N iodine equivalent to the amount of glyoxal reacted. p = correction in per cent.

v	ml I	ml I_g	V	p	ml I_g (corr.)	ml I_g (corr.) / v
—	26.82	—	30	—	—	—
1.75	21.87	4.95	32	0.5	4.97	2.84
2.50	19.75	7.07	32	0.6	7.11	2.84
3.25	17.56	9.26	33	0.7	9.32	2.87
4.00	15.61	11.21	34	0.8	11.30	2.82
4.75	13.52	13.30	35	0.9	13.42	2.83
5.50	11.36	15.46	36	1.1	15.63	2.84
6.25	9.40	17.42	36	1.4	17.66	2.83
7.00	7.39	19.43	37	1.8	19.78	2.83
7.50	6.00	20.82	38	2.3	21.30	2.84
8.00	4.70	22.12	38	2.9	22.76	2.84

analyses. The constancy of the values given in the last column of the table shows that conforming analytical results are obtained in experiments with various intial concentrations when the correction is taken into account.

Similarly as in the case of other aldehydes [6,7] the necessary reaction times for the glyoxal-bisulphite addition reaction were evaluated on the basis of results obtained in rate measurements. It was found that the glyoxal-bisulphite reaction is of the second order, the rate at constant pH being proportional to the product of the concentrations of glyoxal and total sulphite. The bisulphite addition to the second carbonyl group was found to take place immeasurably rapidly as compared with the addition to the first carbonyl group which is the rate-determining reaction. In a phosphate buffer at pH 7.3, the second order specific rate of the glyoxal-bisulphite reaction was 6.0 $l^1 mole^{-1}$ sec^{-1} at 20° C, and 4.9 l^1 $mole^{-1}$ sec^{-1} at 18° C. The rate of the glyoxal-bisulphite reaction differs thus only slightly from that of the formaldehyde-bisulphite reaction under similar conditions [6], the specific rate of the latter reaction being 5.2 l^1 $mole^{-1}$ sec^{-1} at pH 7.3 at 18° C. Thus the standing periods evaluated for the formation of the formaldehyde-bisulphite compound are nearly the same in the case of glyoxal.

On the basis of the foregoing discussion, the following procedure is suggested for the determination of glyoxal by the bisulphite method:

To a solution containing not more than 0.75 millimoles of glyoxal 10 ml of phosphate buffer (0.5 mole/l Na_2HPO_4, 0.125 mole/l KH_2PO_4) and 20 ml of approximately 0.1 M sodium bisulphite solution are added. If the total volume of the reaction solution does not exceed 40 ml, a reaction time of three minutes is sufficient, after which 5 ml of 1 N hydrochloric acid are added and the excess of bisulphite is titrated with an approximately 0.1 N standard iodine solution. The titration should be carried out rapidly in order to avoid the decomposition of the bisulphite compound near the end point. The glyoxal content is calculated from the difference between the iodine consumptions corresponding to the amount of bisulphite initially added and to that present after the addition reaction. To the result obtained a correction is added which is $0.0136/c$ % the value directly obtained, in which c is the molar concentration of excess bisulphite in the solution.

When the volumes of the reaction solution or the concentrations of the standard solutions deviate from those proposed above, the reaction time must be changed correspondingly.

EXPERIMENTAL

Glyoxal and glyoxal disodium bisulphite were prepared according to Ronzio and Waugh [8]. For further purification the latter compound was crystallized twice from 40 % ethanol and dried in air. All water used in the experiments was carbon dioxide-free distilled water. The water used in the experiments in which the kinetics and equilibria of glyoxal-bisulphite reaction were investigated, was also freed from dissolved oxygen by passing a stream of nitrogen through it during a period of 24 hours. The water was stored in a nitrogen atmosphere. The kinetic and equilibrium measurements required in the development of the analytical methods were performed as described elsewhere [5,6,7].

Table 3 contains some examples of experiments in which both methods described above were compared with each other. The values given in the table are averages of 3—5 determinations. The values calculated for the concentration of the glyoxal stock solution show that both methods give conforming results, the deviations being no greater than the ordinary titration errors. In cases in which relatively low glyoxal concentrations are analysed, the bisulphite method is more accurate and should be preferred, because the iodine equivalent of glyoxal is one fourth of its sodium hydroxide equivalent.

Table 3. A comparison between the bisulphite and alkalimetric methods for the determination of glyoxal. v = ml of glyoxal stock solution taken for analysis. V = approximate volume of the reaction solution in ml. ml I = ml of 0.0703 N iodine solution consumed in the titration of excess bisulphite. ml I_g = ml of 0.0703 N iodine equivalent to the amount of glyoxal reacted. ml NaOH = ml of 0.0522 N sodium hydroxide equivalent to the amount of glyoxal. [Gl] = calculated glyoxal concentration of the stock solution in moles per liter.

a) Bisulphite method

v	ml I	ml I_g	*V*	ml I_g (corr.)	[Gl]
—	36.72	—	28	—	
5.00	22.62	14.10	33	14.18	0.0498
10.00	8.83	27.89	38	28.37	0.0499

b) Alkalimetric method

v	ml NaOH	[Gl]	*v*	ml NaOH	[Gl]
5.00	4.71	0.0492	15.00	14.25	0.0496
10.00	9.65	0.0504	20.00	19.12	0.0499

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