1091. *Submicro-methods for the Analysis of Organic Compounds. Part XVII.* Determination of the Carbonyl Group.*

By R. Belcher and B. Fleet.

Carbonyl groups in only 30—50 μg. of organic material can be determined by oximation in a non-aqueous medium; the excess of reagent is titrated with perchloric acid. The accuracy of the method varies from ±0·5% absolute for most aldehydes to ±1·4% for the less reactive ketones. The method has also been applied to a limited range of carbohydrate samples. A wide range of aldehydes and ketones has been analysed successfully.

The most attractive reaction for the determination of the carbonyl group is that involving hydroxylamine. The use of phenylhydrazine and other substituted hydrazines requires separation of the solid derivative, followed by an analysis of either the excess of reagent or the solid derivative. The latter type of procedure is to be avoided on the submicro-scale, for it is both tedious and inaccurate. Several variations of the oximation have been used on the macro-scale; the procedure chosen for the present study was a modification of that recommended by Fritz, Yamamura, and Bradford.[1] A known quantity of free hydroxylamine is liberated by the addition of an exactly known amount of organic base (2-dimethylaminoethanol) to a solution of hydroxylammonium chloride; after the addition of the carbonyl compound and the completion of oximation, the excess of hydroxylamine is titrated with perchloric acid. The reaction proceeds in a non-aqueous medium, the hydroxylammonium salt and the organic base being dissolved in propan-2-ol, and the perchloric acid titrant in 2-methoxyethanol.

The precision of other titrimetric oximation methods suffers because the oxime is appreciably basic and buffers the end-point. In this procedure, however, where the oximation and subsequent titration are done in a non-aqueous medium, the interference from the basicity of the oxime is almost entirely eliminated.

Fritz *et al.* applied this method to a wide range of compounds; for some of the less reactive ketones a reaction temperature of 70° was used. The use of elevated temperatures on the submicro-scale is not possible, for the losses by volatility would be too great. In order to obtain a rough guide to the reaction time required by a compound it is necessary to have an approximate measure of its reactivity. It is often possible to do this by examining related reactions, particularly semicarbazone formation, which has been the subject of much detailed study.[2] Although the oximation has been studied extensively, only a limited range of compounds has been examined.

Reaction times can be predicted roughly from structural details of the compound and a knowledge of the electronic nature of any substituent groups. Vanillin reacts in 3½—4 hours, whereas *p*-dimethylaminobenzaldehyde requires 24 hours, as do the majority of ketones.

In the development of the submicro-method several factors were examined. The reagent concentrations were arranged to give the maximum accuracy in the measurement of the 2-dimethylaminoethanol, whilst the total volume was kept as low as possible. Oximation is subject to general acid-catalysis, but in the present case a ten-fold increase in the concentration of hydroxylammonium ion increased the rate of reaction only very slightly.

The titration was first examined potentiometrically. This was necessary because any small variation in the colour of the visual end-point between sample and blank causes significant errors on the submicro-scale, although it is almost negligible on the macro-scale.

* Part XVI, *J.*, 1963, 531.

[1] Fritz, Yamamura, and Bradford, *Analyt. Chem.*, 1959, **31**, 260.
[2] Conant and Bartlett, *J. Amer. Chem. Soc.*, 1932, **54**, 2881.

Hence, the effect on the end-point of variations in the volume and ionic strength of the medium were examined, as well as the correlation between the potentiometric and the visual end-point. Earlier work [3] on the adaptation of non-aqueous titrations to the submicro-scale had shown that significant variations could be obtained unless the blank titration (giving the amount of titrant required to bring the solution to a predetermined pH) was carefully controlled. Hence the 2-methoxyethanol used as dilution solvent was adjusted to the same pH as that occurring at the end-point. Several workers [4,5] have noted that it is possible for the shade of the indicator colour to vary at the end-point; two factors responsible for this are base strength and the ionic strength of the medium. The present studies showed slight variation in the potentiometric end-points of sample and blank titrations, probably owing to the slight buffering effect of the oxime (Fig. 1).

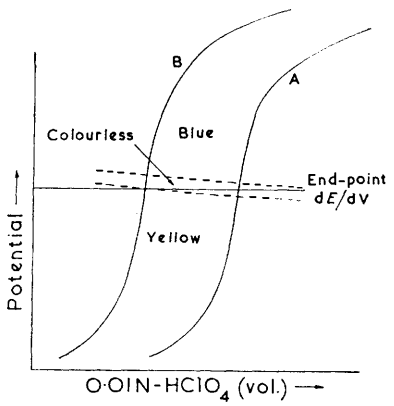

FIG. 1. Potentiometric study of the end-point.
(A) Sample. (B) Blank.

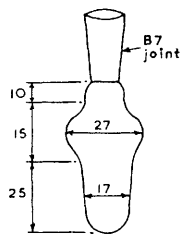

FIG. 2. Submicro-titration vessel (dimensions in mm.).

The main source of error was due to the decomposition of the free hydroxylamine. This loss could be due to several factors: volatilisation or spontaneous decomposition, or atmospheric oxidation. Experiment showed both to operate in this case. However, when a nitrogen atmosphere was used, the blank determinations were far more reproducible, so this was adopted as standard procedure.

It was thought that differing amounts of hydroxylamine in the sample and the blank determination might be a source of error. In the previous experiment, however, it was found that there was rapid decomposition in the first 15—30 minutes, followed by a slow steady loss. During this first half-hour the extent of oximation is not very great, so that the discrepancy between loss of hydroxylamine from sample and blank solutions should not be significant.

Some of the results obtained for aldehydes and ketones by this method are shown in Tables 1 and 2. The time required for the analysis of six samples, together with six blank determinations including a four-hour reaction period, is 8—9 hours.

The method was also applied to several carbohydrates (see Table 3). Solubility was effected by occasional gentle swirling as oximation proceeded, and unless this was done results were low.

An attempt was made [6] to develop a method for the determination of aldehyde groups, so that ketone groups could be determined by difference. It was based on oxidation of

[3] Belcher, Berger, and West, *J.*, 1959, 2877, 2882.
[4] Conant and Werner, *J. Amer. Chem. Soc.*, 1930, **52**, 1.
[5] Seaman and Allen, *Analyt. Chem.*, 1955, **23**, 592.
[6] Fleet, M.Sc. Thesis, Birmingham, 1962.

TABLE 1.

Aldehydes.

Compound	CO (%), found
Vanillin (theor.: CO, 19·07%) (error ± 0·5% absolute), 50—90 μg.; 2½—20 hr.	18·97, 19·13, 18·53, 19·07, 18·97, 18·99, 18·91, 18·67, 18·84
o-Nitrobenzaldehyde (theor: CO, 19·20%) (error ± 0·7%), 40—80 μg.; 4 hr.	19·20, 19·22, 18·53, 18·83
m-Nitrobenzaldehyde (error ± 0·6%), 40—80 μg.; 4 hr.	19·12, 19·01, 19·14, 19·78
p-Nitrobenzaldehyde (error ± 0·6%), 60—70 μg.; 3—5 hr.	19·68, 18·89, 18·85, 18·59
m-Hydroxybenzaldehyde (theor.: CO, 23·76%) (error ± 0·3%), 60—90 μg., 5 hr.	23·74, 23·45
p-Hydroxybenzaldehyde (error ± 1·5%), 35—90 μg.; 5½ hr.	24·38, 24·50, 25·26, 22·51, 25·17, 23·41, 22·69
o-Methoxybenzaldehyde (theor.: CO, 24·16%) (impure), 45—150 μg.; 6—24 hr.	21·38, 21·07, 20·87, 22·06, 20·37, 20·80, 19·67, 21·24, 20·83
p-Dimethylaminobenzaldehyde (theor.: CO, 19·45%) (error ±0·5%), 40—100 μg.; 6—18 hr.	18·20,* 18·90, 19·34, 19·00, 19·98
3,4-Dihydroxybenzaldehyde (theor.: CO, 21·01%) (error ±1%), 40—80 μg.; 5 hr.	21·78, 19·98, 20·41, 21·84, 21·01
2-Hydroxy-1-naphthaldehyde (theor.: CO, 16·84%) (error ±0·5%), 50—170 μg.; 18 hr.	16·32, 16·74, 17·13
Piperonaldehyde (theor.: CO, 19·34%) (error ±0·35%), 60—120 μg.; 18 hr.	19·54, 18·99, 19·30, 19·64

* Reaction time, 6 hr.

TABLE 2.

Ketones.

Compound	CO (%), found
Dibenzyl ketone (theor.: CO, 13·32%) (error ±1·1%), 50—100 μg.; 18 hr.	13·80, 12·30, 12·33, 12·19, 13·69
Methyl β-naphthyl ketone (theor: CO, 16·45%) (error ±0·25%), 70—100 μg.; 24 hr.	16·38, 16·20
Benzoin (theor: CO, 13·20%) (error ±0·35%), 40—50 μg.; 24 hr.	13·75, 12·97
ω-Chloroacetophenone (theor.: CO, 18·12%) (error ±1·4%), 40—50 μg.; 18 hr.	16·71, 17·83
2,2-Pyridoin (theor: CO, 13·07%) (error +0·4%), 58·33 μg.; 24 hr.	13·46

The following reacted incompletely or not at all in 24 hr.; benzophenone, *p*-hydroxyacetophenone, 3-methylcyclopentane-1,2-dione, α-furoin.

TABLE 3.

Carbohydrates.

Compound	CO (%), found
D-(+)-Glucose (theor.: CO, 14·64%) (error ±0·15%), 40—50 μg.; 24 hr.	13·17,* 14·55, 14·78
2,3,4,5,6-Penta-*O*-acetyl-*aldehydo*-glucose (theor.: CO, 7·44%) (error ±0·15%), 25—55 μg.; 18 hr.	7·46, 7·57
D-(+)-Galactose (theor.: CO, 16·01%) (error ±0·4%), 30—90 μg.; 18 hr.	15·67, 15·92
D-(−)-Arabinose (theor.: CO, 19·34%) (error ±0·7%), 35—60 μg.; 24 hr.	18·63, 20·41

* Reaction time 18 hr.

The following did not react because they were insoluble: D-(+)-glucosamine hydrochloride; maltose.

the aldehyde group to the corresponding carboxylic acid with an alkaline solution of potassium mercuri-iodide. The elementary mercury formed in the reaction was determined by iodometry. Although this method was successful for the more reactive aldehydes, such as formaldehyde and acetaldehyde, complicating side reactions occurred with less reactive aldehydes.

Experimental

Reagents.—Hydroxylammonium chloride: 0·4N; 27·8 g. of "AnalaR" reagent, dissolved in 300 ml. of carbonyl-free methanol and diluted to 1 l. with propan-2-ol.

2-Dimethylaminoethanol: 0·025M; prepared by dilution with propan-2-ol from a 0·25M-solution (6·258 ml. of 2-dimethylaminoethanol diluted to 250 ml. with propan-2-ol).

Propan-2-ol: "AnalaR." Martius Yellow indicator: 0·667 g., dissolved in 100 ml. of propan-2-ol; 0·004 g. of Methyl Violet added as a screen.

2-Methoxyethanol: Reagent grade.

Perchloric acid: 0·01N; prepared by dilution with 2-methoxyethanol from a 0·01N-solution (2·05 ml. of 72% "AnalaR" acid diluted to 250 ml. with 2-methoxyethanol). The solution was standardised by titration of tri(hydroxymethyl)aminomethane (3 times recrystallised) against Bromocresol Green.

Dilution reagent: 250 ml. of 2-methoxyethanol and 2·5 ml. of indicator solution, titrated to a colourless end-point with 0·1N-perchloric acid.

Carbonyl samples: with the exception of vanillin, samples were recrystallised from benzene or ethanol. Dihydroxy-compounds were filtered through activated charcoal, as they usually contained a considerable amount of coloured impurities.

Carbonyl-free methanol: 2 l. of methanol, refluxed with 5 g. of 2,4-dinitrophenylhydrazine and 1·5 ml. of concentrated hydrochloric acid for 4 hr.; the methanol was then distilled off, the fraction boiling over 64·8° being rejected.

Apparatus.—The microgram balance and the titration apparatus were as previously described.[7]

Titration Vessel.—This is sketched in Fig. 2.

Procedure.—The titration vessel was flushed with nitrogen. 0·025M-2-Dimethylaminoethanol (130 μl.) was added, and the tip of the burette rinsed with 3 drops of the dilution reagent; 0·4M-hydroxylammonium chloride (10 μl.) was then added, and the tip of the burette rinsed in a similar manner. During the addition of these reagents a slow stream of nitrogen was passed through the flask, care being taken not to allow the tip of the nitrogen delivery tube to come close to the surface of the solution. The carbonyl sample, which had been weighed immediately before the measurement of the solution, was then added. The flask was stoppered after the stopper had been moistened with 2 drops of dilution reagent, and dissolution of the sample was effected by placing the flask in a smooth surface and rotating it slowly. After sufficient time had been allowed for oximation, 0·3 ml. of dilution solvent containing the indicator was added. The glass-encased rotor was then added and the solution titrated with 0·01N-perchloric acid, the tip of the burette protruding just under the surface of the solution. The perchloric acid must be added at a slow and steady rate, the blank samples which were allowed to stand for an equal length of time being titrated to a colourless end-point, and the carbonyl samples to the first appearance of a blue colour.

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Edgbaston, Birmingham 15. [*Received, May 27th,* 1963.]

[7] Belcher, Gouverneur, and Macdonald, *J.*, 1962, 1938.