The Determination of Oxime Nitrogen

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The acid hydrolysis method which is usually employed for oxime determination does not give quantitative results as reported by Novak and Wilson¹. One cause of error is that the reaction is reversible and the hydroxylamine formed recombines with the keto compound. Moreover, this method does not give satisfactory results in the presence of ascorbic acid². Yamafuji *et al.*³ developed a quantitative method for oxime determination under alkaline conditions. This method is suitable for oximes of some keto compounds. It is, however, not applicable to acetoxime or glucosoxime. I, therefore, developed a modification of the acid hydrolysis method involving the addition of 2,4-dinitrophenylhydrazine and can confirm that this procedure gives good results with various oximes even in the presence of interfering substances.

The determinations were carried out according to the following procedure.

Reagents: 2 % 2,4-dinitrophenylhydrazine in 6 N H₂SO₄, 10 % (v/v) ethyl acetate in benzene and benzene and hydroxylamine reagents, prepared according to Csáky's method⁴, *i. e.* 35 % Na-acetate, sulphanilic acid (30 % acetic acid solution), I₂ in glacial acetic acid, α -naphthylamine (30 % acetic acid solution) and Na-arsenate.

Conditions of hydrolysis: 1 ml of 2,4-dinitrophenylhydrazine in 6 N H₂SO₄ and 2 ml of the sample (containing 0.1 ~ 0.4 μ mole of oxime) were mixed and boiled for 2 hours on a water bath.

Extraction of 2,4-dinitrophenylhydrazine and hydrazone: After cooling the appropriate amount of water was added to the hydrolyzed mixture*. Usually the total volume was 7 ml. Hydrazone together with hydrazine were extracted three times with 3 ml of 10 % ethyl acetate (in benzene) and then twice with 3 ml of benzene. After separating the water phase

from the benzene 5 ml of the water phase was transferred to another test tube and mixed with 2 ml of Na-acetate solution to neutralize excess H₂SO₄ and then extracted again with 3 ml benzene three times. The 10 % ethyl acetate was used because it had a stronger extracting capacity than benzene only for 2,4-dinitrophenylhydrazine and especially the hydrazone. However, it also extracts free hydroxylamine in the absence of 2,4-dinitrophenylhydrazine as shown in Table 1. It is not, therefore, desirable to use this solvent more than is necessary.

Table 1. Effect of extraction with 10 % ethyl acetate in the absence of 2,4-dinitrohydrazine.

Number of extractions		Extinction
in acid phase with 10 % Et—Ac	in alkaline phase with benzene	
0	0	38.3
1	3	35.6
3	3	32.2
5	3	27.8
0	0	38.0
1	4	39.7
3	3	38.7
5	3	36.8

Colourimetry: 5 ml of the water phase thus obtained was taken out as the aliquot for hydroxylamine determination according to Csáky and measured with Fischer's photoelectric colourimeter using 525 m μ filter. A solution, treated similarly to the test solutions without oxime, was used as reference solution.

The experimental results obtained with several oximes are shown in Table 2. The standard solutions of pyruvic oxime and glucosoxime were prepared from solid samples and those of α -ketoglutaric oxime and acetoxime were obtained by diluting a mixture of 1 part of 4 $\times$ 10⁻⁴ M hydroxylamine and 1 part of the corresponding keto compounds (0.2 mg/ml) after a reaction time of 3 hours. The recovery in using this method was always more than 95 %. With the alkaline method, however, pyruvic oxime and α -ketoglutaric oxime are determined quantitatively. As the latter method is unsuitable for acetoxime and glucosoxime the new method may be said to have a wider application, being at the

* The suitable amount depends on the kind of colourimeter.

Table 2. Recovery of hydroxylamine from various oximes.

Oxime	Added conc. ($10^{-7}M$)	Found conc. ($10^{-7}M$)	Recovery %
Pyruvic-oxime	3.98	3.76	94
	3.64	3.57	98
	2.99	2.92	98
	2.73	2.53	93
	1.99	1.88	95
	1.82	1.78	98
	1.00	1.02	102
	0.91	0.90	99
Glucos-oxime	4.40	4.34	99
	2.20	2.26	103
α -Ketoglutaric oxime	4.14	3.96	95
	2.07	1.88	95
Acetoxime	4.14	4.06	98

Table 3. Effect of coexisting substances on the pyruvic oxime determination.
Amount of oxime: 1.8×10^{-7} mole.

Coexisting substance		2,4-Dinitrophenyl- hydrazine ml 2 % solution	Extinction
Substance	Amount		
None		1	34.5
Pyruvate	0.2 mg	1	34.5
	1 mg	1	30.8
	1 mg	2	34.5
α -Ketoglutarate	0.2 mg	1	34.5
	1 mg	1	33.7
	1 mg	2	34.5
Ascorbic acid	0.2 mg	2	34.5

Table 4. Effect of nitrite and hydroxylamine. Nitrite was decomposed by adding a sufficient amount of urea.

Added (10^{-7} mole)			Found NO_2 (10^{-7} mole) after I_2 -oxidation (total NH_2OH)	Found NH_2OH $\frac{\text{NH}_2\text{OH} + \text{oxime}}{\%}$
NO_2	NH_2OH	Pyruvic oxime		
10	0	1.82	1.74	95
1.0	1.0	1.99	2.91	97
1.0	1.0	0.99	1.97	99
0.5	0.5	0.80	1.34	103

same time more accurate than the ordinary acidic methods.

When oximes of ketoacids were hydrolysed ordinarily in an acidic medium, the liberated hydroxylamine was decomposed by various substances, including the liberated ketoacids. This interfering effect was eliminated by addition of 2,4-dinitrophenylhydrazine as well as by using the alkaline method (Table 3).

As the coexisting nitrite tends to decompose hydroxylamine and is colourized simultaneously, it is desirable to remove the nitrite beforehand. This was achieved when the test solution containing nitrite was kept for 20 min. with urea in an acidic solution before addition of 2,4-dinitrophenylhydrazine. In this case, it was necessary to increase the amount of Na-acetate to neutralize excess of acid. The results are shown in Table 4. This method is more convenient because oxime can be determined independently.

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Electrophoretic Studies of Vitamin B₁₂-factors. III

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Bernhauer *et al.* have recently isolated five different vitamin B₁₂-factors from digested sewage sludge^{1,2}. One of these factors, provisionally called "Factor III", has practically the same microbiological activity towards *E. coli* and *Lb. leichmanii* in the tube-assay as cyanocobalamin (vitamin B₁₂) and has strong activity towards *Ochromonas malthamensis*. The anti-

Fig. 1.

pernicious anemia activity of Factor III is similar to that of cyanocobalamin³. Factor III, however, differs from other vitamin B₁₂-factors with regard to *R_F*-values and distribution coefficients³. The absorption spectrum of Factor III closely resembles that of cyanocobalamin with the difference that the narrow band at 278 mμ is only faintly marked while a wide band at 295 mμ appears. Such a band has never been noticed before in any other vitamin B₁₂-factor. Factor III like cyanocobalamin itself is able to form aquo- and hydroxo-complexes as well as a cyano-complex containing more than one CN-group per molecule of Factor III.

A comparison between the effect of pH on the electrophoretic mobility of Factor III and of cyanocobalamin has been carried out in our laboratories.⁴ It was concluded at first that the electrophoretic properties of Factor III closely resembled those of cyanocobalamin, except at pH values between 1 and 2. On reinvestigating thoroughly the electrophoretic mobility of cyanocobalamin in this pH-region, we found that the isoelectric point of this compound is somewhat lower than pH 1.9, as was first stated, and occurs at pH 1.5. Factor III has its isoelectric point at the same pH-value. The variation of