SEMI-MICRO-KJELDAHL DETERMINATION OF NITRO AND AMIDO NITROGEN. I.

Selenium Catalysts.

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The Kjeldahl method has certain advantages over the Dumas method. The time required per analysis is less, because several samples may be digested simultaneously. The use of any particular apparatus or reagents is not indispensable for analysis. However, since there were recognized several limitations in applicability of the classical method, various modifications of the usual procedure have been intended by many workers to change the original binding form of nitrogen to digestible one. The applicability of this method, without the introduction of reducing agents, is limited to amines, amino acids, ureas, thio ureas and amides, provided that their molecular structure is not too complex. If the nitrogen atom is attached to either oxygen or another nitrogen atom, a special reductive pre-treatment or introduction of reducing agent such as glucose is essential before the usual digestion[1]. Certain nitrogen heterocyclic compounds show resistance to the usual method and require a prolonged digestion.[2)3)]

Various combinations of catalysts are used to reduce the digesting period. The effectiveness of copper as well as of mercury is pronounced. Catalyzing action of selenium or selenium compounds has also been reported by many workers[4)5)6)], while the loss of nitrogen was reported by other[7]. Various combinations of catalysts containing selenium or selenium compounds are now known, e. g. selenium oxychloride alone[8], mixture of selenium oxychloride and copper sulfate[9], mixture of selenium oxychloride, cupric sulfate and mercuric oxide[10], mixture of selenium and cupric sulfate or ferric sulfate[11], mixture of selenium and mercuric oxide[12) 6)] and mixture of selenium oxychloride and mercury[5].

The work of Poe[13] *et al.* indicates that the use of the combination of copper, selenium and mercury effects the greatest saving of time. Proofs to the effectiveness of selenium catalysts were given also by Shirley[5], Cole[6], Fish[2] and other investigators separately.

We have now analyzed a series of compounds such as aromatic amine, amide and nitro compound in semi-micro scale according to the modified Kjeldahl methods using selenium mixtures as digestion catalysts intending semi-micro adaptation of selenium-catalyzed digestion suitable for the estimation of nitrogen in above-mentioned compounds. Following combinations of catalysts were used: (A) mixture of selenium and mercuric oxide; (B)

mixture of selenium, mercuric oxide and cupric sulfate: (C) mixture of selenium and cupric sulfate. The analytical data are summarized in Table 1.

Table 1. Determination of nitrogen in organic compounds.

Samples	Formula	Nitrogen % (Calcd.)	Nitrogen % (Found)					
			Catalyst (A)		Catalyst (B)		Catalyst (C)	
Urethane	$C_3H_7O_2N$	15.71	15.8	15.8	—		15.8	15.8
Acetanilide	C_8H_9ON	10.36	10.4	10.4	10.4	10.4	—	—
p-Chloroacetanilide	C_8H_8ONCl	8.25	8.2	8.3	8.3	8.3	8.3	8.3
p-Chloroaniline	C_6H_6NCl	10.97	11.0	11.0	11.0	11.0	—	—
2, 4-Dichloroacetanilide	$C_8H_7ONCl_2$	6.86	6.9	6.9	7.0	6.9	—	—
2, 4-Dichloroaniline	$C_6H_5NCl_2$	8.64	8.7	8.7	8.7	8.7	8.7	8.6
N-(4-Chlorophenyl)glycine	$C_8H_8O_2NCl$	7.54	7.6	7.6	7.6	7.6	—	—
N-(2, 4-Dichlorophenyl)glycine	$C_8H_7O_2NCl_2$	6.36	6.4	6.3	6.4	6.4	6.4	6.4
N-(3, 4-Dichlorophenyl)glycine	$C_8H_7O_2NCl_2$	6.36	6.4	6.3	6.4	6.4	6.4	6.4
N-(2, 5-Dichlorophenyl)glycine	$C_8H_7O_2NCl_2$	6.36	6.4	6.4	6.4	6.4	—	—
N-(4-Chlorophenyl)glycine diethylamine salt	$C_{12}H_{19}O_2N_2Cl$	10.82	—		10.9	10.8	10.9	10.8
N-(2, 4-Dichlorophenyl)glycine sodium salt	$C_8H_6O_2NCl_2Na$	5.78	—		5.8	5.7	—	—
Sodium N-(2, 5-dichlorophenyl)-aminomethanesulfonate	$C_7H_6SO_3Cl_2Na$	5.03	—		5.0	5.1	5.0	5.0
p-Nitrophenol	$C_6H_5O_3N$	10.06	9.2	9.4	9.3	9.4	9.9	10.0
p-Nitroaniline	$C_8H_6O_2N_2$	20.27	19.8	19.9	19.8	19.7	20.4	20.4
m-Nitrobenzaldehyde	$C_7H_5O_3N$	9.26	8.7	9.0	8.3	8.4	9.3	9.4
p-Nitrotoluene	$C_7H_7O_2N$	10.21	8.9	9.3	8.9	8.9	9.8	9.7
m-Dinitrobenzene	$C_6H_4O_4N_2$	16.66	—		14.4	14.5	16.1	16.2
2, 4, 6-Trinitrophenol	$C_6H_3O_7N_3$	18.33	—		18.2	18.3	18.3	18.3
2-Nitro-4-chloroacetanilide	$C_8H_7O_3N_2Cl$	13.05	12.0	12.2	12.1	12.2	13.0 13.0	12.7
2-Nitro-4-chloroaniline	$C_6H_5O_2NCl$	16.26	14.8	14.9	14.9	14.9	15.9	15.9
3, 4-Dichloro-1-nitrobenzene	$C_6H_3O_2Cl_2$	7.29	2.7	2.2	2.3 2.6	3.0	5.7	5.4
2, 5-Dichloro-1-nitrobenzene	$C_6H_3O_2Cl_2$	7.29	2.0 2.3	2.6	2.6 2.2	2.3	5.0	5.5

The method of Friedrich[14], which consists of reductive pre-treatment with hydriodic acid followed by catalyzed digestion, is probably the most widely accepted for compounds containing nitrogen atom to be reduced, but a somewhat tedious work to eliminate iodine would not permit the economy of time. The method of Elek and Sobotka, later modified by Harte for semi-micro scale analysis[9], seems to be as well satisfactory for use with those compounds. Accordingly, glucose was used also in our experiment as reducing agent if necessary. Samples of 2,5-dichloro-1-nitrobenzene and 2-nitro-4-chloroacetanilide were digested with the catalyst (B). The results are shown in Table 2. It may be seen that an afterboil for more than 2 hours

Table 2. The change in analytical values during the course of digesting time.

Time (hr.)	1.0		1.5		2.0		2.5		Nitrogen % (Calcd.)
2, 5-Dichloro-1-nitrobenzene	1.9,	2.0	2.0,	2.1	2.6,	2.6	2.2,	2.3	7.29
2-Nitro-4-chloroacetanilide	12.0,	12.0	12.0,	12.1	12.1,	12.2	12.0,	11.8	13.05

is unavailing even for these refractory compounds. The 1.5 hours afterboil is enough to give satisfactory results with most samples employed.

The results of the present experiments indicate that the compounds, which contain only amino or imino groups in the molecule, including their acylated derivatives, were converted to ammonium sulfate quantitatively. Hence the use of these selenium catalysts probably did not cause the loss of nitrogen at the experimental condition. The use of selenium catalysts in combination with mercuric oxide, on the contrary, gave unsatisfactory results in the estimation of nitrogen in nitro compounds. Similar catalyzing mixture was reported by Poe, but the analytical data concerning nitro compounds lacked. There is another example concerning the digestion catalyzed by mixture of selenium and mercuric oxide[2]. The method obtained almost quantitative results with a series of 2,4-dinitrophenylhydrazones of aromatic carbonyl compounds but was preceded by reduction with zinc and hydrochloric acid instead. A few nitro compounds were analyzed by means of catalyst (A) with or without the addition of glucose. The results are listed in Table 3. The reduction with glucose is probably too mild for these compounds.

Table 3. Determination of nitrogen in nitro compounds with and without the introduction of glucose.

Samples	Formula	Nitrogen % (Calcd.)	Nitrogen % (Found)					
			without the introduction of glucose			with the introduction of glucose		
p-Nitrotoluene	$C_7H_7O_2N$	10.21	8.8	8.6		8.9	9.3	8.9
p-Nitrophenol	$C_6H_5O_3N$	10.06	8.0	8.1		9.2	9.4	
m-Nitrobenzaldehyde	$C_7H_5O_3N$	9.26	6.0	6.1	6.0	8.7	9.0	

The catalyst (C), which consists of selenium and cupric sulfate, gave quantitative results with a certain nitro compounds but not with more refractory compounds like halogeno-nitrobenzene.

It is known that the ease, with which aromatic nitro compounds are decomposed to ammonium sulfate, is correlated to the character and position of substituents. Both stability and volatility of the substances during digestion may be leading factors to cause a loss of nitrogen. It is notable that even the introduction of glucose as a reducing agent gives less than quantitative results with 3,4-dichloro-1-nitrobenzene or 2,5-dichloro-1-nitrobenzene. It is conceivable that such compounds as halogeno-nitrobenzene are comparatively stable in digesting reagents, hence the more effective reducing pretreatment is necessary to be employed before the usual digestion.

Reagents

The catalyst used in one experiment consists of (A) 1 g. of anhydrous potassium sulfate, 15 mg. of metallic selenium and 70 mg. of mercuric oxide yellow; (B) 1 g. of anhydrous potassium sulfate, 15 mg. of metallic selenium, 70 mg. of mercuric oxide yellow and 20 mg. of cupric sulfate pentahydrate; (C) 1 g. of anhydrous potassium sulfate, 15 mg. of metallic selenium and 20 mg. of cupric sulfate pentahydrate, respectively. Approximately 1 g. of each catalyst is used in one experiment.

Procedure

A sample containing about 5 mg. of nitrogen is weighed into a 100-ml. Kjeldahl flask. Approximately 1 g. of catalyzing mixture is then introduced to the flask. Three hundred milligrams of glucose is added if a reducing agent is necessary for the analysis. The neck of the flask is then washed with 4 ml. of concentrated sulfuric acid. The mixture is then digested over a 500-w. electric heater. Heating is continued for additional 1.5 hrs. after the liquid cleared. The content of the flask is diluted and washed into the distilling apparatus with 30 ml. of distilled water quantitatively. The distillation and the titration are performed in the usual manner. To the solution, 20 ml. of a 30 per cent sodium hydroxide solution is added just before distillation. The distillate is received in 25 ml. of 0.02 *N* hydrochloric acid, and the resulted acid solution is titrated with 0.02 *N* sodium hydroxide solution using methyl red as indicator. Blank values varying from 0.03 ml. to 0.05 ml. are observed on the reagents. If mercuric oxide is used as catalyst, sodium thiosulfate is added to the 30 per cent sodium hydroxide solution.

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