Estimation of nitrogen functional groups by reaction gas chromatography — frontal technique Oximes, semicarbazones, nitroanilines, and amino acids

K. VENTURA, K. KOMÁREK, J. CHURÁČEK, J. ŠAFÁŘ, and R. KREUZIG

Department of Analytical Chemistry, Institute of Chemical Technology, CS-532 10 Pardubice

Received 12 March 1987

A method has been elaborated for nitrogen estimation in oximes, semicarbazones, nitroanilines, and amino acids which is based on a compound decomposition in a wet way with gas chromatographic estimation of the elemental nitrogen released by frontal technique. The decomposition of compounds takes place in a reaction apparatus placed before the chromatographic part of CHN-1 analyzer (Laboratorní přístroje, Prague). The method elaborated enables both selective and summary nitrogen functional group estimations, offers reliable and precise results and thus extends further applications of the apparatus.

Разработан метод определения содержания азота в оксимах, семикарбазонах, нитроанилинах и аминокислотах, основанный на мокром разложении соответствующего соединения с последующим газово-хроматографическим определением выделяющегося элементарного азота фронтальной техникой. Разложение соединений происходит в реакционном приборе, помещенном перед хроматографической частью анализатора CHN-1 (Laboratorní přístroje, Прага). Разработанный метод позволяет проводить как селективное, так и суммарное определение азота функциональных групп и дает надежные и точные результаты, таким образом расширяя возможности применения прибора.

Organic elemental analysis plays a very important role in both identification and determination of natural materials and new synthesized compounds. Recently, the classical gasometric and volumetric estimations of functional groups [1, 2] have been replaced by more modern spectroscopic and chromatographic techniques.

The connection of known chemical reactions with the instrumental methods results in a considerable simplification and acceleration of analyses with the preservation of their accuracy. One of the possible solutions is a reaction gas chromatography, *i.e.* connection of chemical reaction with chromatographic process enabling both the subsequent determination of the individual functional groups and partial automatization of the analysis [3, 4].

In contrast with the methods described above, the present work is focused on the nitrogen estimations in oximes, semicarbazones, nitroanilines, and amino acids using a frontal chromatographic method with the application of the elemental CHN-1 analyzer. It extends the methods already described, of nitrogen estimations in azo compounds [5], of hydrazine and its derivatives [6], ammonium salts, urea, nitrates, nitrites, and fertilizers [7].

Experimental

Apparatus

CHN-1 analyzer (Laboratorní přístroje, Prague) [8, 9], in which the oxidation tube has been substituted by reaction and sorption apparatuses, and heating unit, was used. Assembly of the individual parts of the apparatus and their linkages have been described [5, 7, 10] in detail together with the method of estimation and evaluation.

Compounds tested

Commercial or laboratory preparations were used. Their purity was tested by melting point determination and by elemental analysis. Compounds are unambiguously identified by the table number and the sequence number, *e.g.* 1/*I*.

Oximes: 4-dimethylaminobenzaldehyde oxime (1/*I*), 4-nitrobenzaldehyde oxime (1/*II*), benzaldehyde oxime (1/*III*), 2-hydroxybenzaldehyde oxime (1/*IV*), cinnamaldehyde oxime (1/*V*), 3-nitrobenzaldehyde oxime (1/*VI*), dimethylglyoxime (1/*VII*), benzoin oxime (1/*VIII*), 2-isatine oxime (1/*IX*), acetylbenzoyl dioxime (1/*X*).

Semicarbazones: cinnamaldehyde semicarbazone (2/*I*), 2-butanone semicarbazone (2/*II*), acetone semicarbazone (2/*III*), cyclohexanone semicarbazone (2/*IV*), benzaldehyde semicarbazone (2/*V*), methyl phenyl ketone semicarbazone (2/*VI*), butyraldehyde semicarbazone (2/*VII*), 2-hexanone semicarbazone (2/*VIII*), diethyl ketone semicarbazone (2/*IX*), 4-methylcyclohexanone semicarbazone (2/*X*), semicarbazide chloride (2/*XI*).

Nitroanilines: 1-nitroaniline (3/*I*), 2-nitroaniline (3/*II*), 3-nitroaniline (3/*III*), 2,4-dinitroaniline (3/*IV*).

Amino acids: aminoacetic acid (4/*I*), 2-amino-3-phenylpropionic acid (4/*II*), 2-aminohexanoic acid (4/*III*), 2-amino-2-methylpropanoic acid (4/*IV*), aspartic acid (4/*V*), 2-amino-3-(4-hydroxyphenyl)propionic acid (4/*VI*), 2-aminobutyric acid (4/*VII*), glutamic acid (4/*VIII*), 2-aminopropionic acid (4/*IX*).

Results and discussion

Nitrogen estimation in oximes (isonitroso compounds)

Ten oximes were oxidatively cleaved by Fe(III) salt in acidic medium and by chromic acid. The reaction apparatus (Fig. 1) was used in both cases.

Oxidation with Fe(III) *salt*

To a solution of a sample (2—10 mg) in 2 cm^3 of H_2SO_4 (φ_r(volume ratio) = 1 : 1), a saturated solution of $Fe_2(SO_4)_3$ (2 cm^3) was added. Helium flow rate was set to 20 $cm^3\,min^{-1}$ and the reaction tube was inserted into a heating block having 150 °C. Under these conditions, the total period of one analysis is about 30 min.

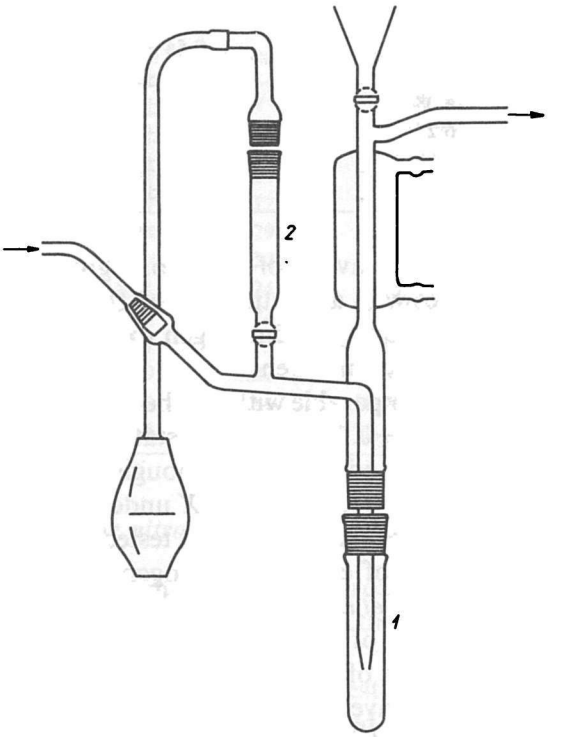

Fig. 1. Reaction apparatus for estimation of oximes and semicarbazones.
1. Ground joint reaction tube; *2.* decomposition agent container.

Oxidation with chromic acid

Decomposition was performed by a standard procedure, *i.e.* conc. H_2SO_4 (2 cm^3) and H_2CrO_4 (2 cm^3, c = 1.67 mol dm^{-3}) were added to a sample (5—15 mg) tested. The reaction time (the total period of diffusion chamber filling) was about 20 min at a flow rate of 20 $cm^3\,min^{-1}$; temperature of the heating block was 160 °C. The nitrogen content was calculated from the calibration obtained by the hydrazinium chloride decomposition with chromic acid. (Parameters of regression line and average deviation are $y = 211.40x + 1.18$; $s_{y,x} = 2.564$.)

Table 1

Results of oxime estimations given in mass %

Compound	μ	$\bar{x}$	R	IS	s	u
I	8.53	8.54	0.16	8.54 ± 0.081	0.069	0.063
II	8.43	8.56	0.25	8.56 ± 0.127	0.108	0.520
III	11.56	11.70	0.25	11.70 ± 0.127	0.108	0.560
IV	10.21	10.20	0.40	10.20 ± 0.203	0.172	0.025
V	9.53	9.54	0.51	9.54 ± 0.259	0.219	0.020
VI	8.43	8.38	0.15	8.38 ± 0.076	0.065	0.333
VII	24.13	24.08	0.25	24.08 ± 0.127	0.108	0.200
VIII	6.16	6.27	0.12	6.27 ± 0.061	0.052	0.910
IX	8.64	8.56	0.15	8.56 ± 0.076	0.065	0.530
X	15.72	15.77	0.28	15.77 ± 0.142	0.204	0.180

A quantitative hydrolytic cleavage of isonitroso group takes place in the oxidative cleavage of oximes with a solution of Fe(III) salt and hydroxylamine released is oxidized simultaneously to dinitrogen oxide, which is reduced over red hot copper to the elemental nitrogen.

The results obtained are comparable with the theory and errors do not exceed in the individual estimations ±0.3 % abs. The statistical evaluation shown in Table 1 indicates that the method is reliable, though some results of the Lord test indicate that compounds 1/*II*, *III*, *VIII*, *IX* undergo a systematic error.

Chromic acid was the further oxidation agent tested. The results have shown that only a small percentage of elemental nitrogen was formed. This corresponds to the results of *Jureček et al.* [11], who claim that application of chromic acid results in the formation of several oxidation products — nitric acid, ammonia, and in a lesser extent of nitrogen oxides. The results obtained by the chromic acid decomposition have not been evaluated statistically.

It can be stated that the oxidation with Fe(III) salt in a diluted solution of sulfuric acid is the precise method and can be applied in the selective estimation of isonitroso group in the presence of other nitrogen functional groups as under the oxidation conditions given above, the estimation is not influenced by their presence.

Estimation of semicarbazide and semicarbazones

The oxidative mineralization of semicarbazide chloride and ten semicarbazones was accomplished with chromic acid in the standard way using the apparatus shown in Fig. 1.

The nitrogen content was calculated from the calibration obtained by the semicarbazide chloride analysis ($y = 209.66x - 2.29$; $s_{y,x} = 1.089$).

The results of semicarbazide and semicarbazone estimations are given in Table 2. They are in a good agreement with the theory; the errors of individual determinations do not exceed ± 0.3 % abs. The intervals of reliability calculated, values of standard deviation and the results of the Lord test show that the method is reliable. In this way, application of the analytical system for the method has been confirmed.

Table 2

Results of semicarbazide and semicarbazones estimations given in mass %

Compound	μ	$\bar{x}$	R	IS	s	u
I	14.81	14.83	0.37	14.83 ± 0.188	0.159	0.054
II	21.69	21.61	0.19	21.61 ± 0.096	0.082	0.421
III	24.33	24.31	0.12	24.31 ± 0.061	0.052	0.166
IV	18.05	18.04	0.29	18.04 ± 0.147	0.125	0.035
V	17.17	17.27	0.22	17.27 ± 0.112	0.095	0.454
VI	15.81	15.71	0.24	15.71 ± 0.122	0.103	0.416
VII	21.69	21.73	0.24	21.73 ± 0.122	0.103	0.167
VIII	17.82	17.87	0.13	17.87 ± 0.066	0.056	0.385
IX	19.57	19.59	0.27	19.59 ± 0.137	0.116	0.074
X	16.55	16.64	0.18	16.64 ± 0.091	0.077	0.500
XI	25.12	25.16	0.30	25.16 ± 0.152	0.129	0.133

Selective nitrogen estimation in nitroanilines

Under the standard conditions, four nitroanilines were subjected to the oxidative mineralization by chromic acid with the aim to estimate simultaneously nitro and amino nitrogens.

Prior to mineralization, the nitroanilines tested were converted to the corresponding acetyl derivatives [12]. Nonacetylated nitroanilines exhibit a negative error in the nitrogen estimation caused by the formation of elemental nitrogen. The nitro group is cleaved first as nitrous acid. The final oxidation product is nitric acid. However, nitrous acid may diazotate the primary amino group. If the rate of diazotization is higher than the rate of nitrous acid oxidation the diazonium salt will be formed, which can be decomposed with the formation of elemental nitrogen. A higher amount of chromic acid is necessary as usual, because a part of it is consumed in the decomposition of unreacted acetic anhydride, which is not removed after acetylation. A further step, *i.e.* work-up of ammonium and nitrate ions, is the same as described elsewhere [7]. Nitrates have to be reduced first, *e.g.* by Devard alloy in alkaline medium and ammonia has to be distilled into sulfuric acid. After pH adjustment to 11.5—12.5 with disodium tetraborate, the elemental nitrogen was released by oxidation with

alkaline hypobromite. A solution of KNO_3 was used for the construction of calibration curve ($y = 13.15x - 0.052$; $s_{y,x} = 0.480$). Sample weights were in the range of 1.4 to 22.1 mg for mononitroanilines and 0.9 to 28.8 mg for dinitroanilines.

It is evident from the values given in Table 3 that the nitrogen release was in all cases quantitative. The method enables to estimate in the compounds containing simultaneously amino and nitro groups one group in the presence of the other one or both nitrogen forms from one sample. The results are in agreement with the theory and an average error of estimations does not exceed ± 0.3 % abs. The statistical evaluation has confirmed that the method is reliable, easily reproducible and does not involve a systematic error. The theoretical value of nitrogen content lies mostly in the middle of the interval of reliability. The experimental Lord coefficient is always lower than the critical value. Therefore, it can be stated that the values tested differ only negligibly and the method can be considered as analytically applicable. The total period of analysis of one sample comprises 75 min. The simultaneous performance of operations enables to increase the number of daily performed estimations.

Table 3

Results of nitroaniline estimations given in mass %

Compound	μ	$\bar{x}$	R	IS	s	u
NH_2 group						
I	10.14	10.30	0.44	10.30 ± 0.223	0.189	0.364
II	10.14	9.92	0.53	9.92 ± 0.269	0.228	0.415
III	10.14	10.08	0.30	10.08 ± 0.152	0.129	0.200
IV	7.65	7.50	0.38	7.50 ± 0.193	0.163	0.395
NO_2 group						
I	10.14	10.10	0.28	10.10 ± 0.142	0.120	0.143
II	10.14	10.17	0.46	10.17 ± 0.283	0.198	0.065
III	10.14	10.13	0.21	10.13 ± 0.106	0.090	0.048
IV	15.30	15.28	0.29	15.28 ± 0.147	0.125	0.069

Estimation of primary amino groups in amino acids

Nine amino acids were decomposed by nitrosyl bromide. The estimation was performed by the modified Kainz method with the difference that the nitrometer was replaced by the modified CHN-1 analyzer. Like with nitroanilines, the calibration dependence obtained by KNO_3 analysis was used for calculation. Weights of samples were selected in such a way that they could correspond to the calibration curve range of 0.14—2.24 mg of nitrogen. Deamination was accomplished in a reaction apparatus given in Fig. 2.

To an amino acid tested in a reaction tube, acetic acid (0.5 cm^3), formic acid (1 cm^3), and sodium formate (0.1 cm^3) in formic acid ($\varphi_r = 1 : 1$) were added. The tube was inserted into the reaction apparatus (Fig. 2) and after 5 min of bubbling through with helium, the agent (3 cm^3) was added from a container and a flow rate was adjusted to 15—10 $cm^3 min^{-1}$ (reaction time 30—40 min). The tube was cooled with water so that the temperature of reaction mixture did not exceed 30 °C. The apparatus was washed with the concentrated acetic acid after finishing the reaction. The period of one analysis was about 45 min.

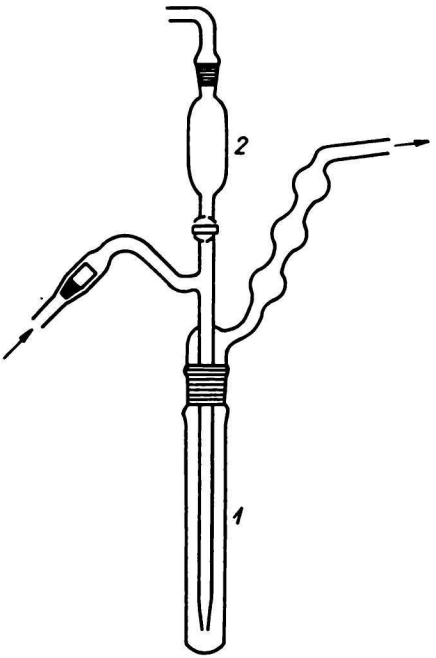

Fig. 2. Reaction apparatus for estimation of amino acids.
1. Ground joint reaction tube; *2.* decomposition agent container.

In the determination of amino groups, the preparation of nitrosyl bromide causes several problems. The stability of the agent prepared according to *Kainz et al.* [13] is not sufficient (Table 4); they succeeded in a quantitative release of nitrogen from the primary amino group only after 1—2 subsequent analyses. Stability of the agent was improved (4—6 analyses) by solubilization of nitrite and bromine in a buffered medium (5 g of sodium acetate to 30 cm^3 of acetic acid). However, the value of blanc increased simultaneously by four times. Addition of acetic anhydride was not efficient — low stability, high blanc. In the case of sodium nitrite precipitation after addition of the agent to the reaction tube, the efficiency of the agent was lowered and the nitrogen liberation was not

Table 4

Time dependence of the nitrosyl bromide content in the agent (estimation of 2-aminohexane acid)

t/min	w(N)/%	$\Delta(w(N))$/%
0	10.83	+0.15
35	10.73	+0.05
65	9.10	−1.58
115	6.25	−4.43
148	5.62	−5.06
368	2.26	−8.42

Table 5

Results of primary amino group estimations in amino acids given in mass %

Compound	μ	$\bar{x}$	R	IS	s	u
I	18.68	19.48	0.96	19.48 ± 0.487	0.413	0.833
II	8.48	9.50	0.89	9.50 ± 0.451	0.383	1.146
III	10.68	10.78	0.34	10.78 ± 0.172	0.146	0.294
IV	11.96	12.03	0.94	12.03 ± 0.477	0.404	0.074
V	10.53	10.76	0.92	10.76 ± 0.466	0.396	0.250
VI	7.73	7.98	0.85	7.98 ± 0.431	0.366	0.294
VII	13.59	13.60	0.59	13.60 ± 0.299	0.254	0.017
VIII	9.52	9.64	0.75	9.64 ± 0.380	0.323	0.160
IX	15.73	15.60	0.97	15.60 ± 0.492	0.417	0.134

Number of estimations $n = 5$; μ, $\bar{x}$ — theoretical and mean value of nitrogen content; R — variation range ($R = x_{max} - x_{min}$); IS — interval of reliability ($IS = \bar{x} \pm K_n R$); s — standard deviation ($s = k_n R$); u — Lord coefficient ($u = |\mu - \bar{x}|/R$). Values of coefficients given in tables ($\alpha = 0.05$) $K_5 = 0.507$; $k_5 = 0.430$; $\mu_0 = 0.507$.

quantitative. This phenomenon was suppressed by the addition of formic acid. The sorption apparatus filled, in all other cases by silica gel or ascarite, had to be replaced by a small washing bottle containing 50 % potassium hydroxide (to absorb the acidic products of agent decomposition).

It is evident from Table 5 that in seven out of nine amino acids the average value of determination lies in the interval required of ±0.3 % abs. Aminoacetic and 2-amino-3-phenylpropionic acids gave higher nitrogen values than those corresponding to the theory. In seven cases, the statistical evaluation has shown that the differences between the mean and theoretical values are not statistically important and thus, the method does not comprise a systematic error.

However, the results obtained by this method could not be compared with the results published thus far.

References

1. Jureček, M., *Organická analýza II.* (Organic Analysis II.) Nakladatelství Československé akademie věd (Publishing House of the Czechoslovak Academy of Sciences), Prague, 1957.
2. Veibel, S., *Praktická příručka organické analýzy.* (A Handbook of Organic Analysis.) Nakladatelství technické literatury (Publishing House of Technical Literature), Prague, 1963.
3. Komárková, H., Churáček, J., Komárek, K., and Křížek, F., *J. Chromatogr. 119,* 243 (1976).
4. Komárková, H., Komárek, K., Churáček, J., and Krajčíková, L., *Sborník vědeckých prací VŠCHT, Pardubice* (Collection of Communications, Institute of Chemical Technology, Pardubice) *45,* 165 (1982).
5. Komárek, K., Ventura, K., Smrček, Z., Churáček, J., and Janák, J., *J. Chromatogr. 154,* 191 (1978).
6. Komárek, K., Ventura, K., Smrček, Z., Churáček, J., and Janák, J., *Collect. Czechoslov. Chem. Commun. 43,* 1863 (1978).
7. Ventura, K., Komárek, K., Churáček, J., Langhans, J., Šafář, J., and Šalamoun, J., *Chem. Prum. 35,* 580 (1985).
8. Rezl, V., *Microchem. J. 15,* 381 (1970).
9. Laboratorní přístroje, Prague, Original technical documentation; Elemental analyzer CHN-1.
10. Ventura, K., Komárek, K., and Churáček, J., *Czechoslov.* 211473 (1984).
11. Kozák, P. and Jureček, M., *Sborník vědeckých prací VŠCHT, Pardubice* (Collection of Communications, Institute of Chemical Technology, Pardubice) *21,* 43 (1969).
12. Jureček, M., Kozák, P., and Novák, V., *Sborník vědeckých prací VŠCHT, Pardubice* (Collection of Communications, Institute of Chemical Technology, Pardubice) *13,* 81 (1966).
13. Kainz, G., Huber, H., and Kasler, F., *Microchim. Acta 1957,* 744.

Translated by R. Toman