

*The Preparation of Nitrogen Samples for Mass-spectrographic Analyses.*

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Nitrogen obtained by the Dumas procedure for examination in the mass spectrometer may contain impurities due to incomplete combustion which cause slight errors. Incomplete combustion is avoided if a high temperature of combustion is used with a nickel oxide furnace filling. The correction for air contamination is better calculated from the argon-40 peak than from the oxygen-32 peak.

WE have described (*J.*, 1953, 1666) a modified Dumas procedure which is a satisfactory alternative to Rittenberg's procedure for the preparation from isotopically labelled substances of nitrogen samples for mass-spectrographic analysis. Unlike Rittenberg's, Dumas's procedure does not necessarily produce random pairing of the atoms in the nitrogen molecules on oxidation of compounds containing two adjacent nitrogen atoms, and it is therefore useful in the study of isotope distribution in diazo-compounds. As the nitrogen is obtained from the compound in one stage, it may sometimes be preferable to Rittenberg's procedure for normal routine determinations also, particularly when dealing with multiple samples.

Errors may arise in the determination of isotope ratios owing to inclusion of impurities with a mass number identical with that of one of the isotopes measured. When nitrogen isotopes are assayed by Rittenberg's procedure, nitrous and nitric oxides may give rise to a peak at mass 30. Dr. D. H. Tomlin (personal communication) states that the correction which is made for air inadvertently introduced during the manipulation of the samples

may also be inaccurate because of oxygen derived from the hypobromite. Errors might be introduced in results derived by the Dumas procedure either by substances produced during the combustion of the sample, such as carbon monoxide and ethylene (mass 28), ethane (giving ethyl radicals, mass 29, in the mass spectrometer), and nitric oxide (mass 30), or by air present in the carbon dioxide used for sweeping out the combustion train.

Samples of nitrogen were obtained from labelled phthalimide by the method previously described but including in the combustion train a packing of iodine pentoxide kept at 130° to remove carbon monoxide. Examination of the samples in the mass spectrometer showed that a discrepancy of about 2—4% occurred in the atom % <sup>15</sup>N values according to whether they were calculated from the mass ratio 28/29 or the mass ratio 29/30 (Table 1, cols. 2 and 3, samples 1—5). This result suggested the presence of an impurity other than carbon monoxide in the nitrogen samples.

TABLE 1. *Percentage abundance of <sup>15</sup>N in nitrogen samples derived from labelled phthalimide.*

| Calc. from mass ratio<br>Sample | Air correction base on |       |         |       |
|---------------------------------|------------------------|-------|---------|-------|
|                                 | 32 peak                |       | 40 peak |       |
|                                 | 28/29                  | 29/30 | 28/29   | 29/30 |
| 1                               | 32.7                   | 33.8  | 33.7    | 33.8  |
| 2                               | 32.8                   | 33.4  | 34.2    | 33.5  |
| 3                               | 32.4                   | 33.8  | 33.8    | 33.8  |
| 4                               | 32.7                   | 33.5  | 33.4    | 33.5  |
| 5                               | 31.8                   | 33.8  | 33.7    | 33.8  |
| Mean                            | 32.5                   | 33.8  | 33.7    | 33.7  |
| 6                               | 18.7                   | 33.5  | 34.6    | 33.7  |
| 7                               | 19.9                   | 33.3  | 34.2    | 33.5  |

The presence of hydrocarbons amongst the combustion products of several organic compounds, indicating incomplete combustion of the sample, is shown both by a peak in the mass spectrum at mass 16 (Table 2, col. 2), and by a complicated spectrum above mass

TABLE 2. *Relative intensity of 16 peak [(28) + (29) + (30) taken as 100].*

| Fillings<br>Substance | CuO                       | NiO                          | CuO + NiO  |
|-----------------------|---------------------------|------------------------------|--|
| 1 Phthalimide ...     | 3.5, 0.73, 0.46, 2.8, 2.4 | 0.003, <0.001, 0.003, <0.001 | 0.004, 0.006   |
| 2 Azobenzene ...      | 0.31, 0.11, 0.97, 0.21    | 0.012, 0.001                 | 0.002, 0.06, <0.001, <0.001<br>0.04, <0.001, 0.007, <0.001 |
| 3 Tyrosine .....      | 1.2                       | <0.001                       | —  |
| 4 Serum albumen       | 3.1                       | <0.001                       | —  |

40. The peak at mass 16 was too large to be caused by O<sup>+</sup> or O<sub>2</sub><sup>++</sup> ions and was probably due to methane. Ravenswaay and Schweitzer (*Rec. Trav. chim.*, 1938, **57**, 688) reported that methane is produced in Dumas analyses, and Kirsten (*Mikrochem. Mikrochim. Acta*, 1952, **40**, 122) that copper oxide is ineffective in removing methane at temperatures below 800°. Kirsten used nickel oxide at 1050°. Nitrogen samples prepared from labelled phthalimide by Kirsten's procedure were found to be virtually free from methane and gave no complex spectrum above mass 40 (Table 2, col. 3). When nitrogen samples which contained a high abundance of <sup>15</sup>N were obtained by Kirsten's method, no peak at mass 31 could be detected, indicating that <sup>15</sup>NO was absent and that nitric oxide was not formed.

In computing the results of mass-spectrographic analysis, a correction is normally applied for traces of air which may contaminate the sample. This correction is based on the value of the 32 peak, and it is assumed that the amount of atmospheric nitrogen which has contaminated the sample bears the same ratio to the measured amount of oxygen as the abundance of nitrogen to the abundance of oxygen in the air. It is possible, however, that oxygen introduced from the atmosphere or from the carbon dioxide may be removed during the combustion, thus falsifying the correction. In Table 1, cols. 4 and 5, the results of previous analyses are recalculated, the corrections for contaminating air being based on the abundance of argon (mass 40) in the samples. It is seen that the discrepancy

between the atom %  $^{15}\text{N}$  values as derived from the 28/29 and the 29/30 ratios disappears. Two heavily contaminated samples, numbers 6 and 7, which would normally be discarded, gave widely divergent results when corrected by the value for the 32 peak but the two results differed by only 3% when corrected by the 40 peak value. A disadvantage of using the argon peak in correcting for air contamination is that it is always very small and errors in measurement are subsequently magnified by a factor of approximately 65. Nevertheless, we have obtained more consistent results by using the argon rather than the oxygen peak for this correction.

In most experimental work which involves the use of isotopic nitrogen, only the relative abundance of the two isotopes in the samples is of interest. For this purpose it would seem that Kirsten's high-temperature combustion procedure (*loc. cit.*) using a nickel oxide filling is to be preferred.

In studying the reactions of diazo-compounds it is sometimes necessary to obtain nitrogen from labelled samples in which the pairing of at least some of the nitrogen atoms is identical with that in the original substance. For this purpose the high-temperature combustion is less satisfactory since the degree of randomization of the atoms is variable. Four consecutive combustions carried out on samples of [ $^{15}\text{N}_1$ ]azobenzene at 1050° showed an increasing degree of random pairing in the later samples, the fractions of the N-N bonds which remained intact being 0.74, 0.71, 0.60, and 0.36. If random pairing of the nitrogen atoms takes place by a reaction catalyzed at the surface of the silica combustion tube, this effect may be due to increased roughness of the surface of the tube caused by the high temperature. When the retention of the type of pairing present in a diazo-compound is desirable, our low-temperature combustion procedure (*loc. cit.*) is preferable.

The elimination of both hydrocarbons and nitric oxide is ensured if the combustion products from the copper oxide filling at 470° are carried through a nickel oxide filling at 1050° (Table 2, col. 4).

The following values for the normal abundance of  $^{15}\text{N}$  have been determined by examining nitrogen samples obtained by high-temperature combustion: nitrogen from azobenzene, 0.369%; from tyrosine, 0.367%; and from serum albumen, 0.369%.

#### EXPERIMENTAL

*Combustion of Samples.*—(1) *Low-temperature procedure.* The combustion train and nitrometer were identical with those previously described (*loc. cit.*) except that a U-tube packed with iodine pentoxide and heated in a paraffin-bath kept electrically at 130° was inserted between the combustion tube and the nitrometer.

(2) *High-temperature procedures.* A similar apparatus was used but the copper oxide filling was replaced by nickel oxide, as in Kirsten's procedure (*loc. cit.*), and this was kept electrically at 1050°. A filling of "Hopcalite," inserted between the combustion tube and the nitrometer to ensure the recombination of any dissociated carbon dioxide, was kept at 150° to prevent the condensation of water vapour. The sample was placed in the combustion tube clear of the nickel oxide and was heated in a constant stream of carbon dioxide by a movable furnace.

Other combustions were carried out using copper oxide, as in the low-temperature procedure, except that the layer of metallic copper was omitted. The main combustion tube was followed by a subsidiary tube containing nickel oxide heated to 1050°, succeeded in turn by a filling of "Hopcalite."

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