A DIRECT TITRIMETRIC METHOD FOR MICRODETERMINATION OF NITROGEN IN BIOLOGICAL MATERIALS

MOHAMMAD ASHRAF

Pharmacy Department, Panjab University, Lahore

MEHBOOB ILLAHI AND M.K. BHATTY

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

AND

R. A. SHAH

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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Nitrogen in biological materials can be determined by a Kjeldahl method without distillation. The material is digested with sulphuric acid in the presence of mercuric and potassium sulphates, and the resultant ammonium sulphate is titrated with hypochlorite.

For relatively large amounts of biological nitrogen (0.5 mg. or more) the classical micro-Kjeldahl method[1] is excellent. Alternatively, the nesslerization technique is used, particularly for 5-200 μg. of nitrogen. One of the standard colorimetric methods due to Koch and McMeekin[2] has been generally employed in clinical practice. However, for various reasons a direct determination of nitrogen would be preferred.

It has already been established that the distillation step in the Kjeldahl method can be eliminated advantageously. This can be accomplished by employing either formaldehyde reaction[3] or by resorting to the oxidation of ammonia with hypochlorite.[4] Nitrogen has been determined by the hypochlorite method in organic compounds on the submicro,[5] micro, semimicro,[6,7] and macro[8] scales, and in agriculture and animal products.[9]

The same hypochlorite method has now been successfully extended to the determination of nitrogen in biological materials.

Experimental

REAGENTS AND MATERIALS

Sodium hypochlorite, 0.02 N., was prepared as follows:[10]

Sodium hydroxide (320 g.) was dissolved in 1500 ml. distilled water and 1500 g. of crushed ice was also added in a 5-litre flat bottom flask. The container was placed in an ice-salt bath, and chlorine was passed till the solution had absorbed 210-245 g. of the gas and colour of the solution had turned yellow. During the passage of chlorine the temperature of the solution remained below 0°C. The hypochlorite contents of the final solution were assayed by titration against a standard solution of arsenious oxide. The solution was then diluted with distilled water to prepare 0.02 N reagent which was kept in a dark-coloured bottle. The reagent was fairly stable, but to ensure its stability, it was standardised about twice a week.

Arsenious oxide solution, 0.02 N; tartrazine indicator solution, 0.01 N; sulphuric acid (d. 1.84) A.R.; mercuric sulphate solid A.R.; sodium bicarbonate solid A.R.; potassium bromide solid A.R.; and alundum pieces.

The biological materials for nitrogen determination were prepared according to the method of Koch and McMeekin,[2] as described by Bodansky and Fay.[11]

PROCEDURE

The material was measured out in a 100-ml. short necked round bottom flask, and 0.625 g. potassium sulphate plus 0.075 g. of mercuric sulphate catalyst and 2 ml. of sulphuric acid were added. A few alundum pieces were also added to ensure smooth boiling and digestion was carried out for 40 minutes. After having cooled the digest, the cone was detached and washed in. The solution was cooled and sodium bicarbonate was gradually added till a yellow precipitate appeared due to the formation of mercuric oxide. One g. of potassium bromide was added and the flask was stirred until the solution was clear. An excess of sodium hypochlorite solution was run in till the solution had turned pale yellow. After 5 minutes a known excess of arsenious oxide solution was added and the excess was then back titrated with the hypochlorite solution, using tartrazine indicator. A blank was also run under identical conditions.

Milk and faeces were digested with 5 ml. sulphuric acid, 2.5 g. mercuric sulphate and 0.3 g. of potassium sulphate in 250 ml. flask. Titration of the nitrogen was, however, carried out as before.

1 ml. of 0.01 N NaOCl ≡ 0.0467 mg. N

Discussion and Conclusions

For the sake of comparison nitrogen in biological materials was also determined by the Koch and McMeekin method.[2] The results for eight biological materials analysed by both the methods are recorded in Table 1. Results of the determinations were recorded on single samples of faeces and milk but for the rest of the materials three different samples from different subjects were drawn for analysis. The difference in the nitrogen contents by the two methods is not significant.

The present titrimetric method has the advantage that it does not involve any distillation or colorimetric measurement and the whole determination

TABLE 1.—DETERMINATION OF NITROGEN IN BIOLOGICAL MATERIALS USING A SINGLE VESSEL FOR DIGESTION AND TITRATION IN THE NONDISTILLATION KJELDAHL METHOD.

No.	Material		Percentage nitrogen in samples *	
			Koch-McMeekin method	This method
1.	Plasma (non-protein nitrogen) 5 ml. of plasma was deproteinised and diluted to 50 ml. 5 ml. of the diluted solution was then digested.	a.	0.056	0.051
		b.	0.048	0.045
		c.	0.043	0.042
2.	Blood urea 5 ml. of the above diluted solution was hydrolysed under pressure in the presence of sulphuric acid and nitrogen was determined while omitting digestion.	a.	0.052	0.056
		b.	0.041	0.040
		c.	0.041	0.041
3.	Serum (total nitrogen) 1 ml. of serum was diluted to 50 ml. out of which 1 ml. sample was drawn for digestion.	a.	0.930	0.952
		b.	0.978	1.000
		c.	0.947	1.200
4.	Cerebrospinal fluid (total nitrogen) 1 ml. of this material was taken for digestion without any dilution.	a.	0.027	0.032
		b.	0.025	0.035
		c.	0.026	0.032
5.	Ascitic fluid 0.2 ml. of this solution was digested.	a.	0.198	0.234
		b.	0.134	0.149
		c.	0.106	0.125
6.	Urine 5 ml. of urine was diluted to 100 ml. and then 1 ml. of the diluted solution was employed for digestion.	a.	0.009	0.015
		b.	0.009	0.010
		c.	0.011	0.012
7.	Faeces† (total nitrogen) 100 mg. of the dried material was digested.		2.400	2.490
			2.400	2.400
			2.390	2.400
8.	Milk† 1 ml. of milk was digested as such.		0.620	0.610
			0.595	0.600
			0.620	0.610

*a,b and c denote different samples of the materials of different persons.
†Because of their comparatively larger solid contents, semimicro conditions were employed for digestion.

can be carried out in the same vessel. Further, the method is throughout chemical in approach and its reproducibility and stoichiometry remain under control and beyond any doubt. Moreover, the titrimetric method in actual practice takes less time and is more expeditious as compared with the colorimetric method. Furthermore, the titrimetric method is mostly independent of the physcial conditions such as minor variation in the temperature of the final solution to be titrated. In other words, it is more accurate, direct and straightforward and no prestandardization of the method is necessary. Such standardization is, however, the prerequisite in the colorimetric method. Multiple determinations can be carried out rapidly as the distillation and the transference of the digest from the digestion to the titration flasks have been eliminated.

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