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Determination of nitrate in surface waters by ion-exchange chromatography after oxidation of total organic nitrogen to nitrate ☆

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ABSTRACT

The official method for the determination of organic nitrogen in surface water consists of a Kjeldahl distillation to reduce nitrogen to ammonia, followed by colorimetric or volumetric determination. The purpose of this work is to determine whether the Valderrama method can be used as an alternative procedure. The Valderrama method estimates total nitrogen, either organic or inorganic, after oxidation to nitrate. The first step is an oxidative digestion in an autoclave in the presence of potassium persulphate, boric acid and sodium hydroxide. Nitrate is then determined colorimetrically, after acidification by concentrated sulphuric acid, or by ion-exchange chromatography, without acidification. This last procedure takes advantage of some important features of ion-exchange chromatography: greater sensitivity than UV methods, rapid analysis, high selectivity and, thus, low matrix interferences.

INTRODUCTION

Organic nitrogen substances in surface waters arise principally from animal and vegetable proteins and from synthetic organic compounds used in agricultural practice (for example triazine herbicides), or used as dyes in various manufactures. They find their most important application in the dyeing and printing of textiles, but are also used to some extent for dyeing paper and leather, in the preparation of certain inks and in photography. Industrial wastes containing proteins include food processing and canning wastes, gelatine manufacturing wastes and slaughterhouse, dairy and tannery wastes [1].

Decomposition of nitrogen organic matter by either aerobic or anaerobic bacteria gives rise to ammonia. The oxidation of ammonia by aerobic bacteria (nitrification) produces first nitrites and then nitrates. An undesirable effect of nitrate in surface water destined for human consumption has been featured in the medical literature [2]. It has been shown that young infants receiving artificial feeds of milk diluted with water containing more than about 10–20 mg/l nitrogen as nitrate may develop "methaemoglobinaemia".

EEC Directive No. 80/778, regarding the characteristics of the water for human consumption, imposes three classes of concentrations (A_1, A_2, A_3) for Kjeldahl nitrogen: 1, 2 and 3 mg/l as nitrogen, respectively. The analytical procedures used in this work are the Kjeldahl [3] and Valderrama methods [4].

The first method determines nitrogen in the

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trinegative state. If ammonia nitrogen is not removed in the initial phase of the procedure, "organic nitrogen" can be obtained by subtracting ammonia nitrogen from Kjeldahl nitrogen. Alternatively, the Valderrama method proposes an oxidation of the nitrogen to nitrate at elevated temperature by persulphate in alkaline medium. "Organic nitrogen" can be obtained as follows: total nitrogen − (ammonia + nitrite + nitrate) nitrogen.

EXPERIMENTAL

Apparatus

For the Valderrama method, a Perkin Elmer 550S spectrophotometer was used at 220 nm, with matched silica cells of 1 cm light path. Chromatography was performed on a Dionex System 2000i/SP ion chromatograph equipped with a conductivity detector (range 30 μS) with suppression (Model AMMS). A separator column (HPIC-AS4A) with a guard column (HPIC-AG4A) was used. The volume of the sample loop was 50 μl. The integrator model was HP 3390A. The eluent solution (flow-rate 2.0 ml/min) consisted of 1.8 mM sodium carbonate and 1.7 mM sodium bicarbonate; the regenerant for AMMS was 0.0125 M sulphuric acid. For the Kjeldahl method, the digestion and distillation apparatus are described in ref. 5.

Reagents

Purified water was obtained from a Milli-Q purification system (Millipore); all other reagents (sulphuric acid, sodium carbonate and bicarbonate) were of analytical reagent grade. The oxidizing solution was prepared from potassium persulphate (50 g/l), boric acid (30 g/l) and sodium hydroxide (14 g/l). The reagents used in the Kjeldahl method are listed in ref. 5.

Methods

The macro-Kjeldahl method was applied to 500 ml sample in an 800-ml Kjeldahl flask. A 50-ml aliquot of digestion reagent (sulphuric acid, potassium sulphate and mercuric sulphate catalyst) was carefully added to the distillation flask [6]. The solution was heated until the volume was reduced to about 25–50 ml, and

copious white fumes were observed. The digestion was continued for an additional 30 min, then the solution was cooled, diluted to 300 ml with water, mixed, and distilled. A distillate volume of 200 ml was collected below the surface of 50 ml of a 0.02 M sulphuric acid solution and the ammonia was determined spectrophotometrically using the phenate method [7].

In the Valderrama method the water sample (50 ml) was pipetted into a glass bottle and potassium persulphate, dissolved in sodium hydroxide solution, was added. The bottle was closed, heated in an autoclave at 120°C for 1 h, and then cooled. The oxygen available for the oxidation appears from the equation:

$$K_2S_2O_8 + H_2O \rightleftharpoons 2KHSO_4 + 1/2O_2$$
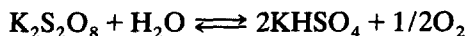
The heating time must be long enough to decompose the excess persulphate. This requisite excess is dependent not so much on the amount of nitrogen in the sample, but rather on the total amount of oxidizable substances, primarily organic matter. The chemical oxygen demand (COD) value of the sample should not appreciably exceed 10% of the oxygen available from the persulphate [8]. In our river samples, the COD value is 15–30 mg/l as oxygen, therefore 0.09 mmol of $K_2S_2O_8$ in 50 ml of sample were used. In this way, the presence of sulphate due to persulphate decomposition does not interfere in the determination of nitrate, because a nitrate–sulphate concentration ratio of 1:500 is maintained [9,10]. The obtained nitrates are determined by ion-exchange chromatography (IEC) or spectrophotometrically at 220 nm, after acidification by concentrated sulphuric acid.

RESULTS AND DISCUSSION

In samples from the river Po, collected at Pontelagoscuro (Ferrara), organic nitrogen was determined by using the Kjeldahl, Valderrama UV and Valderrama IEC methods. It has been shown by many authors that ammonia, EDTA, urea, carbamates, pyridine, glycine, proteins and their partial decomposition products are quantitatively oxidized to nitrate, whereas the oxidation of compounds containing nitrogen-to-nitro-

TABLE I

COMPARISON OF RECOVERY PERCENTAGE FOR AMMONIUM SULPHATE, GLYCINE AND UREA STANDARDS ADDED TO SURFACE WATER SAMPLES

Method	Recovery (%)					
	Ammonium sulphate spike		Glycine spike		Urea spike	
	0.05 mg/l N	0.5 mg/l N	0.05 mg/l N	0.5 mg/l N	0.05 mg/l N	0.5 mg/l N
Kjeldahl	ND	97	ND	98	ND	100
Valderrama UV	107	110	97	104	106	102
Valderrama IEC	101	100	99	106	100	99

ND = Not detected.

gen bonds seems to be incomplete [11]. In this paper experimental trials were performed to determine the recoveries by oxidation of the following nitrogen compounds: ammonium sulphate, glycine and urea added to surface water samples. For every compound tested, the fortified sample matrix technique was used. In the laboratory, a known quantity of each analyte was added to an aliquot of Po river sample. The spiked field sample matrix was analysed exactly as a field sample. In the recoveries from spiked river Po water samples, the organic nitrogen amount already present in the water sample was subtracted, after having determined it by means of the average values obtained by the IEC

Valderrama method. The obtained mean recovery percentages for two spike concentrations are reported in Table I. The comparison between the organic-free water and surface water recoveries was made to establish whether the sample matrix affected the analytical results, but no different recoveries was obtained. In Table II the relative error percentage obtained by the Valderrama UV and Valderrama IEC methods for organic-free water spiked with ammonium sulphate, glycine and urea standards at different concentrations are reported. The mean recovery percentages in Table I and the relative error percentages in Table II are the results of five replicate determinations.

TABLE II

COMPARISON BETWEEN VALDERRAMA UV AND VALDERRAMA IEC METHOD OF RELATIVE ERROR PERCENTAGE FOR AMMONIUM SULPHATE, GLYCINE AND UREA STANDARDS, ADDED TO ORGANIC-FREE WATER

Theor. conc. (mg/l N)	RE (%)					
	Ammonium sulphate		Glycine		Urea	
	UV	IEC	UV	IEC	UV	IEC
0.50	+4	+6	+10	+3	+14	+3
1.00	+8	+5	+12	+4	+20	+3
2.00	+8	+3	+2	+4	−11	+1
3.00	−10	+4	−4	+3	−3	−2
5.00	−9	+6	−3	+2	−4	−5

TABLE III

COMPARISON OF MINIMUM DETECTABLE CONCENTRATION (MDC)

Method	MDC (mg/l N)
Kjeldahl	0.5
Valderrama UV	0.5
Valderrama IEC	0.05

A comparison of minimum detectable concentration in the three examined procedures is shown in Table III. In Fig. 1 a chromatogram of river Po water, after digestion, is shown.

The Valderrama method for "total nitrogen" determination is relatively simple and rapid and allows the estimation of low concentrations of organic nitrogen by subtracting the other nitrogen forms. It has been found experimentally that nitrate determination by IEC is more accurate than by the UV method as the relative errors (RE) in Table II show, and that the minimum detectable concentration is the 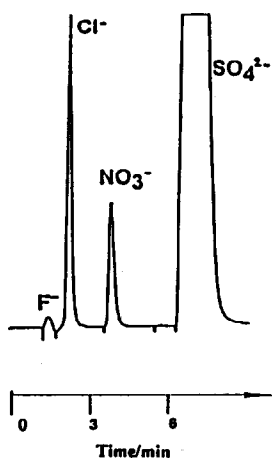lowest among the three described procedures. However, the accuracy of the analysis will be dependent on the nature of nitrogen compounds and on their relative concentrations. Unlike the spectrophotometric method, dissolved, coloured compounds do not interfere with the IEC determination. Recoveries of nitrogen substances added to natural water samples, obtained by Valderrama method, are very satisfactory, independent of their chemical nature (inorganic salt, amide, amino acid). For each kind of sample, it is advisable to adjust the amount of peroxo reagent, depending on the organic matter content of the sample. In this case, if it is not possible to dilute the oxidized solution, the sulphate must be eliminated before the IEC injection.

The results obtained in this paper show that the Valderrama method, modified by IEC, can be successfully used in place of the Kjeldahl method, to analyse surface water.

Fig. 1. Chromatogram of river Po water after digestion.

REFERENCES

1 L. Klein, River Pollution, Vol. II, Butterworths, London, 1962, pp. 26, 32–33, 202–203.
2 G. Gilli, Igiene dell'Ambiente e del Territorio, Edizioni Medico Scientifiche, 1989, pp. 294–296.
3 J. Kjeldahl, Anal. Chem., 22 (1883) 366.
4 J.C. Valderrama, Mar. Chem., 10 (1981) 109.
5 L.S. Clesceri, A.E. Greenberg and R.R. Trussell (Editors), Standard Methods for the Examination of Water and Wastewater, 17th ed., Washington, 1989, Section 4500-N org B, pp. 4.144–4.147.
6 G.B. Morgan, J.B. Lackey and F.W. Gilcreas, Anal. Chem., 29 (1957) 833.
7 L.S. Clesceri, A.E. Greenberg and R.R. Trussell (Editors), Standard Methods for the Examination of Water and Wastewater, 17th ed., Washington, 1989, Section 4500-NH₃ D, pp. 4.120–4.121.
8 F. Nydahl, Water Res., 12 (1978) 1123.
9 Method 38405, DIN, Germany, 1989.
10 Method 876, UNICHIM, Milan, 1990.
11 J. Ebina, T. Tsutsui and T. Shirai, Water Res., 17 (1983) 1721.