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Publisher *Taylor & Francis*

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International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713640455>

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To cite this Article Kopáček, J. and Procházková, L.(1993) 'Semi-Micro Determination of Ammonia in Water by the Rubazoic Acid Method', International Journal of Environmental Analytical Chemistry, 53: 3, 243 – 248

To link to this Article: DOI: 10.1080/03067319308045993

URL: <http://dx.doi.org/10.1080/03067319308045993>

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SEMI-MICRO DETERMINATION OF AMMONIA IN WATER BY THE RUBAZOIC ACID METHOD

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(Received 19 August 1992; in final form 29 January 1993)

A simplified rubazoic acid method for the determination of ammonia on a semi-micro scale is described. The absorbance of a pink-violet aqueous solution of rubazoic acid is measured at 550 nm. The method can be directly applied to fresh waters with $\text{NH}_4^+\text{-N}$ concentrations of between 20 and 1000 $\mu\text{g. l}^{-1}$. The semi-micro method was compared with the original extraction rubazoic acid method of Procházková (1964) and the results showed good agreement ($R^2 = 0.98$, $n = 25$). The direct nesslerization method gave significant overestimates.

KEY WORDS: Ammonia determination, rubazoic acid, fresh water.

INTRODUCTION

The use of bispyrazolone in combination with pyridine and monopyrrolone for the determination of ammonia was first reported by Kruse and Mellon¹ and later applied for sea water by Strickland and Austin² and Strickland and Parsons³. Procházková⁴, in a detailed study of the reactions involved, eliminated the use of pyridine and defined the individual reaction steps and the final coloured reaction product as rubazoic acid (RA). The specificity of the reaction follows from the reaction mechanism and was experimentally confirmed (neither amines nor amino acids contribute to the final coloured product). Using optimum conditions for the formation of RA, she developed a sensitive extraction method for the determination of ammonia in fresh waters. The procedure by Procházková was further modified for sea water by Johnston⁵. Slawyk and Mac Isaac⁶ reported good agreement between the determination of ammonia as indophenol blue and the RA method in non-polluted sea water ($R^2 = 0.96$). Despite the specificity and high sensitivity, the RA method was not widely used, mainly due to its time-consuming extraction steps.

This paper describes a simplified RA method on a semi-micro scale. The proposed method causes a 5-fold reduction in the use of reagents and eliminates the tedious extraction steps and transfer of samples. The absorbance of the pink-violet aqueous solution of RA is measured at 550 nm.

MATERIALS AND METHODS

The semi-micro method was performed in test tubes (18 × 180 mm) fitted with a ground glass stopper.

The water for sample dilution, standard and reagent solutions, and for glassware rinsing was prepared by redistilling distilled water after the addition of KMnO_4 and H_2SO_4 (RdW). It may be replaced by distilled water passed through a column of a strong cation-exchange resin in the hydrogen form⁷.

Reagents

Citrate-phosphate buffer ($\text{pH} = 5.8$). Mix 400 ml of 0.1 M citric acid ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$, 21.01 $\text{g} \cdot \text{l}^{-1}$) and 600 ml of 0.2 M Na_2HPO_4 and stabilize by 0.5 ml of CHCl_3 . Store at 5°C. Reagent 1.

Chloramine T (8 $\text{g} \cdot \text{l}^{-1}$). Dissolve 0.8 g of chloramine T in RdW and dilute to 100 ml. The solution may be stored at 5°C for at least 2 weeks. Reagent 2.

Bispyrazolone (2 $\text{g} \cdot \text{l}^{-1}$). Dissolve 0.2 g of 3,3'-dimethyl-5,5'-dioxo-1,1'-diphenyl-(4,4'-bi-2-pyrazoline) in 100 ml of 0.25 M Na_2CO_3 at 90°C and allow to cool. Prepare daily. Reagent 3.

Pyrazolone (2 $\text{g} \cdot \text{l}^{-1}$). Dissolve 1 g of 3-methyl-5-oxo-1-phenyl-2-pyrazoline in 500 ml of hot RdW. The solution is stable for at least 2 months. Reagent 4.

Standard solution (1 $\text{mg} \cdot \text{l}^{-1}$ $\text{NH}_4^+ - \text{N}$). Prepare daily from a 100-fold concentrated solution of NH_4Cl . Reagent 5.

Procedure

Add 1 ml of RdW and 1 ml of buffer (reagent 1) to 10 ml of the sample in a test-tube (rinsed by RdW just before analysis) and mix. Add 0.5 ml of chloramine T (reagent 2) and mix. Allow to stand for 5 min ± 5 s, add 1 ml of bispyrazolone (reagent 3) and mix. Allow to stand for again 5 min ± 5 s, add 2 ml of pyrazolone (reagent 4) and mix. Allow to stand for about 20 min, mix the sample again and measure the absorbance at 550 nm in a 2-cm cell against RdW.

At least 2 reaction blanks per each series should be carried through the entire procedure with fresh RdW.

Standardize the method against 1 ml of the standard solution of NH_4Cl (reagent 5) added to the another blank (external standard) or another portion of sample (internal standard) instead of 1 ml of RdW and carry them through the whole procedure.

For coloured samples or unfiltered samples containing suspended particles, following procedure is recommended. Take 2 aliquots of the sample, add 0.5 ml of chloramine T to one of them and 0.5 ml of RdW instead of chloramine T to the other one, and continue as described above. Calculate the concentration of ammonia using the corrected absorbance:

$$A = A_I - A_{I0} - (A_{II} - A_{II0})$$

where A_I and A_{I0} are the absorbances of the sample and the blank, respectively, with chloramine T, and A_{II} and A_{II0} are the absorbances of the sample and the blank, respectively, without chloramine T.

RESULTS AND DISCUSSION

The semi-micro method can be directly applied to fresh water samples with concentrations of ammonia ranging from 20 to 1000 $\mu\text{g.l}^{-1}$ of N.

The detection limit of the semi-micro method calculated as three times of the mean value of the standard deviation (SD) of the blanks was 18 $\mu\text{g.l}^{-1}$ $\text{NH}_4^+\text{-N}$. The mean value of the blank was $26 \pm 6 \mu\text{g.l}^{-1}$ $\text{NH}_4^+\text{-N}$ (\pm SD), calculated from 30 duplicates each in different series of samples. The blank value changes from laboratory to laboratory and depends on purity of chemicals, RdW, and atmosphere of the laboratory.

The precision (the average relative standard deviation)⁸ was 1.6% for the concentrations of $\text{NH}_4^+\text{-N}$ from 172 to 200 $\mu\text{g.l}^{-1}$ ($n=6$) and 7% in the range of 24 to 53 $\mu\text{g.l}^{-1}$ $\text{NH}_4^+\text{-N}$ ($n=4$). Each sample was analyzed in 5 parallels.

The accuracy of the semi-micro method was tested by adding a known quantity of ammonia (100 $\mu\text{g.l}^{-1}$ $\text{NH}_4^+\text{-N}$) to 23 different samples. The average per cent recovery was 99.3 ± 5.3 (\pm SD).

The molar absorptivity was determined to be $10850 \pm 850 \text{ l.mol}^{-1}.\text{cm}^{-1}$ ($n=23$, \pm SD) at 550 nm. The sensitivity of the extraction (CHCl_3) RA method⁴ is nearly 2 times higher (the molar absorptivity at 450 nm was $20700 \text{ l.mol}^{-1}.\text{cm}^{-1}$).

Interferences

Similarly, as with the extraction method, alkalinity or acidity of the sample greater than 2 mmol.l^{-1} , temperature of the sample out of the range of 15 – 20°C, reducing compounds, and amino acids, amines, CN^- , and CNS^- in the molar ratio of $[\text{N}]/[\text{NH}_4^+\text{-N}]$ greater than 1, interfere with the semi-micro method. Errors can be eliminated by following the guidelines of Procházková⁴ or Golterman and Clymo⁹.

The turbidity of the sample caused by particulate substances usually present in some unfiltered samples increases the absorbance. The compensation for this error is described under Procedure. The capability of this step was tested with dispersion of diatomite and clay added to standard solution of NH_4Cl (see Table 1).

The colour of the sample increases the absorbance. This error can be compensated for as described under Procedure.

High concentrations of organic matter decrease the recovery of ammonia and cause a negative error. The error can be eliminated by calculating the concentration of ammonia in the sample from the recovery of the internal standard. The capability of the elimination of this error was tested with mixtures of a standard solution of NH_4Cl with humic acid (n. 2366120, Roth, FRG) and with ligninsulphonic acid (Marasperse N, Marathon, Neenah, USA) (see Table 2).

Table 1 Interference of turbidity of dispersion of diatomite and clay added to the standard solution of NH_4Cl ($100 \mu\text{g.l}^{-1}$ of N) on determination of ammonia.

TURBIDITY NTU	ABSORBANCE 550nm, 2-cm cell		Recovery of $\text{NH}_4^+\text{-N}$ %
	$(A_{I1} - A_{I0})^a$	$(A_{II1} - A_{II0})^b$	
0	100	0	100
8	118	19	99
16	128	29	99
24	153	43	110
32	167	59	108
40	184	80	104

^a A_{I1} , A_{I0} is the absorbance of the sample and the blank, respectively; with chloramine T.

^b A_{II1} , A_{II0} is the absorbance of the sample and the blank, respectively; without chloramine T.

The concentration of ammonia was calculated using the absorbance of the sample (after compensation for the error of its colour) and its internal standard. The obtained results agreed with the real concentrations of ammonia in the mixed samples if the recovery of the internal standard was not less than 50%. However, at high concentration of organic matter, we recommend to dilute and reanalyse the sample.

Calcium reacts with CO_3^{2-} (reagent 3) and turbidity of CaCO_3 appears at concentrations greater than 30 mg.l^{-1} of Ca^{2+} . Up to 50 mg.l^{-1} of Ca^{2+} the error of CaCO_3 can be compensated using the same procedure as that used for turbidity. At Ca^{2+} concentrations exceeding 50 mg.l^{-1} the sample should be diluted.

Table 2 Interference of dissolved organic carbon (DOC) added as ligninsulphonic acid (LA) or humic acid (HA) on determination of ammonia*.

Organic matter	Concentration of DOC (mg.l^{-1})		Found concentration of $\text{NH}_4^+\text{-N}$ (mg.l^{-1})		Recovery of INT (%)
	$\text{NH}_4^+\text{-N}$ (mg.l^{-1})		EXT	INT	
LA	20	0.10	0.09	0.11	75
	40	0.20	0.14	0.20	70
	60	0.30	0.16	0.31	59
	80	0.40	0.17	0.42	45
	100	0.50	0.17	0.43	40
HA	10	0.10	0.11	0.12	92
	15	0.15	0.13	0.15	83
	20	0.20	0.18	0.20	77
	30	0.30	0.21	0.34	67
	50	0.50	0.24	0.51	46

*Concentrations of ammonia calculated using the external (EXT) or internal (INT) standard.

Table 3 Comparison of the extraction (EX), proposed semi-micro (SM) and direct nesslerization (DN) methods for water samples (n=5).

Sample		NH ₄ ⁺ -N (µg/l)		
		EX	SM	DN
Reservoir	.. 1	47	53	130
	2	29	24	96
	3	178	177	-
	4	111	139	207
River water	.. 1	300	335	481
	2	214	200	-
	3	57	58	74
	4	35	38	-
	5	470	455	615
	6	320	307	400
	7	446	375	1270
	8	470	490	1240
Fish-pond	.. 1	124	107	194
	2	650	670	1010
	3	350	345	1220
	4	448	489	-
Tap water	.. 1	65	58	-
	2	55	51	-
	3	208	199	-
	4	2	3	-
Precipitation ^a	.. 1	365	340	395
	2	525	470	645
	3	146	172	-
Waste water ^b	.. 1	226	197	286

^aAfter 10-fold dilution.^bAfter 50-fold dilution.

Comparison of semi-micro, extraction and direct nesslerization methods

The comparison was performed on 25 samples of water of various types (Table 3).

The results demonstrate that the semi-micro and the extraction RA methods concurred with each other ($R^2 = 0.98$, $n = 24$; paired t-test showed no statistically significant difference at the 5% level) whilst the direct nesslerization (DN) method⁹ gave significant overestimates. The average ratio of the results obtained by the DN and RA methods was 1.88 ± 0.77 ($n = 16$, \pm SD). The average percent NH₄⁺-N recovery of the DN method was 127 ± 39 ($n = 16$, \pm SD).

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