New Method of Preparation of Reduced Strychnine Reagent for the Nitrate Determination in Seawater

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The determination of nitrate is the essential work in the oceano-chemical studies. Since H. W. Harvey(1) applied the G. Deniges's reduced strychnine method(2) to the colorimetric determination of nitrate in sea water, this reagent has been used universally in such studies.(3) The writer has reported with regard to the Harvey's method applied to his oceanochemical studies of Tokyo Bay.(4) As the Harvey's method of preparation presents some trouble and inconvenience, he has devised a newly preparing reduced strychnine reagent by the use of liquid amalgam.

Inconvenient points of Harvey's method are as follows: (1) The time of reduction is so long as from several hours to more than twenty hours. (2) The extent of the reduction is not constant every time. (3) Zinc of high purity is necessary. Even if amalgamation of zinc is perfect, iron and lead are occasionally dissolved and on account of these impurities this reagent shows pink color even in the absence of nitrite or nitrate in the sample water. (4) Zinc is unnecessarily consumed in mere hydrogen generation. (5) When concentrated sulfuric acid is added after the reduction, a large quantity of zinc sulfate crystallizes out and also the generation of heat and hydrogen chloride is so violent that the operation is very troublesome. (6) Reduced strychnine is oxidized by oxygen or nitric oxide in the atmosphere.

The application of liquid amalgam to analytical chemistry was made by Prof. M. Kobayashi.(6) Of the excellent characteristics of

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(1) H. W. Harvey, *J. Mar. Biol. Assoc., N. S.*, **14**, 71-88 (1926).

(2) G. Deniges, *Bull. soc. chim.*, **9**, 544-546 (1911).

(3) D. Rchford, *Commonwealth of Australia, Council for Scientific and Industrial-Research, Bull.*, No. 220 (1947).

(4) Y. Matida, in press.

(5) Y. Miyake, "Water Analysis," Tokyo, 1949, p. 102.

(6) K. Nakazono, *J. Chem. Soc. Japan*, **42**, 526 (1921).

this application, the following points(7) are worthy of special notice: (1) Reduction velocity is accelerated by shaking. (2) Wasteful consumption of zinc is avoided. (3) By the substitution of the air in the reductor with suitable inactive gas (carbon dioxide is the most convenient) the reoxidation of reduced strychnine is avoided.

Experiment and Discussion

I. Operation.—(1) Method of reduction. Into the reductor (Fig. 1) the acid solution of strychnine sulfate (0.27 g./20 cc.) and 150 g. liquid amalgam were added, then carbon dioxide was vigorously passed from Kipp's apparatus. The reductor was heated on a water-bath for three minutes. Hereafter it was heated and shaken for two and three minutes respectively. When the sum of shaking periods attained to the definite time, zinc amalgam was taken out into a beaker, carbon dioxide having been passed slowly through the reductor. Then 120 cc. of concentrated sulfuric acid was added and thoroughly mixed.

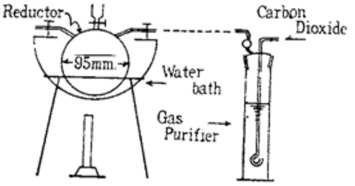

Fig. 1.

The purpose of passing carbon dioxide is to protect the reduced strychnine from reoxidation by the oxygen in the atmosphere as D. Rochford's indication, and also by this operation the contamination due to nitrate or nitrite in the laboratory air is preserved.

(2) Colorimetry. In a small test tube with glass-stopper, 5 cc. of sample water or standard solution was taken, then about 0.2 g. of purified sodium chloride or two drops of concentrated hydrchloric acid were added. After the dissolution of sodium chloride, 5 cc. of the reagent were slowly poured on the sample water along the inner side of the tube. After stirring, the tube was preserved in a dark and cold place for definite time. The extinction coefficient of the colored solution was determined with the Pulfrich's photometer or compared with the standard color solution (aqueous solution of safranine red was used). The usefulness of the addition of chloride ion is already found(8) and the writer recognized that the above mentioned quantity of sodium chloride was necessary and sufficient.

II. Preriminary Experiment.—In the first place the suitable acid and its concentration for reduction were studied. From the results, 9 *N* sulfuric acid solution was adopted.

III. Criticism on Shaking Period, Reserving Time and Reproducibility by the Method of Variance Analysis.—The difference of color developing power due to change in shaking period and preserving time was tested. Also the reproducibility—whether two reagents prepared by strictly identical procedure have the same color developing power or not—was investigated. Simaltaneously by this factorial analysis the interrelations of these factors were tested.

Table 1*

	D1		D2		D3		Sum
	H1	H2	H1	H2	H1	H2	
B1	1.7, 2.3	2.8, 1.1	−0.6, −0.7	0.8, 3.2	0.4, −1.0	2.8, 3.5	16.3
B2	0.2, 1.4	0.0, 0.4	2.0, 1.7	0.2, 1.3	−0.6, −0.5	0.1, 3.1	9.3
Sum	1.9, 3.7	2.8, 1.5	1.4, 1.0	1.0, 4.5	−0.2, −1.5	2.9, 6.6	25.6

D: Reduction time (D1…24 minutes, D2…18 minutes, D3…12 minutes).
H: Preserved time (H1…Immediately after reduction, H2…after 30 days).
B: Repetition … B1 and B2 were prepared strictly by the same method.

* The values of this table were obtained as follows. The colored solution (NO_3-N 500 microgram/1.) was compared with safranine red solution (0.005 %), then the constant value deduced from the observed value.

Table 2

Factor	Variance	Dregree of freedom	Mean square
D	0.36	2	0.18
H	7.48	1	7.48*
B	2.04	1	2.04
DH	9.61	2	4.81*
HB	4.79	1	4.79*
BD	4.71	2	2.36
DBH	3.24	2	1.62
R(DHB)	11.59	12	0.97
DHBR	43.82	23	

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(7) S. Isimaru, "Experimental Studying Method of Chemistry" Analytical Chemistry, **2** (1942), Tokyo.

(8) B. M. G. Zuwicker and P. J. Robinson, *J. Mar. Res.*, **5**, 214 (1944).

In Table 1, the results of the experiment are shown. Table 2 is the results of variance analysis, in which the mark (*) shows that the factor is significant when the level of significance is 0.05. From this analysis the following conclusions have been obtained: (1) Color developing power changed in preservation. (2) The interrelation of shaking period and reproducibility, and also that of preservation and reproducibility were recognized.

VI. Characteristics of this New Reagent. — (1) Absorption Curve. The curve observed with the Pulfrich's photometer is shown in Fig. 2. From this result the filter S 53 is adopted for the colorimetric determination of nitrate in sea water. Also it was recognised that the aqueous solution of safranine red, used as an artificial standard by L. H. N. Cooper,(9) can be used.

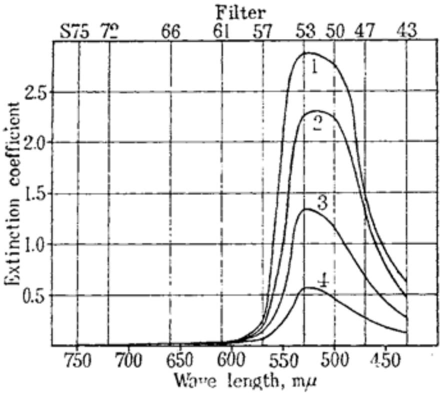

Fig. 2.—Absorption curves of the colored solutions produced by the reactions of reduced strychnine reagents and aqueous solutions of nitrate: curve 1, the product of the new reagent and the aqueous solution containing 200 γ/l. nitrate nitrogen; curve 2, 0.005% aqueous solution of safranine red; curve 3, the product of Harvey's reagent and the aqueous solution containing 200 γ/l. nitrate nitrogen; curve 4, the product of the new reagent and the aqueous solution containing 50 γ/l. nitrate nitrogen.

(2) The Time for Developing the Maximum Intensity of Coloration. After the addition of the reagent, the time for developing the maximum coloration differed with the change in the nitrate concentration. But, twelve hours' standing is enough for any concentration (Fig. 3). The colored substance was easily decomposed by the action of light, so the colorimetric observation needs rapid operation and much care should be taken to protect the colored solution from the light.

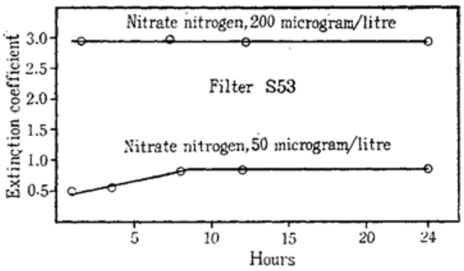

Fig. 3.—A figure to show the time of color development.

(3) The Relation Between the Extinction Coefficient and the Concentration of Nitrate Nitrogen. Ordinarily in natural sea water, nitrate nitrogen concentration is about 500 microgram per litre or less. So in Fig. 4 the calibration curve between extinction coefficient and nitrate nitrogen concentration is shown in this range.

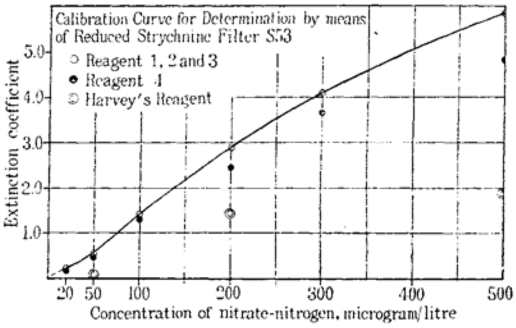

Fig. 4.—A figure to show the relation between the extinction coefficient and the concentration of nitrate. The reduction times to prepare the reagent 1, 2, 3 and 4 are 24, 18 12 and 5 minutes respectively.

(4) Application to Sea Water. To portions of sea water, various quantities of nitrate were added, then the concentrations thereof in the waters were determined. The results were additive, so there might be no considerable salt error larger than the experimental error. Nitrate nitrogen shows the same color, so the correction of this salt was made as follows: In the first place the corresponding extinction coefficient of the nitrite, obtained from the calibration curve thereof (Fig. 5), was subtracted from the measured extinction coefficient of the sample. Nitrite was determined by the use of Griess-Romijn's reagent.

(5) Sensitiveness of the New Reagent. The

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(9) L. H. N. Cooper, *J. Mar. Biol. Assoc., N. S.*, **18**, 161 (1932).

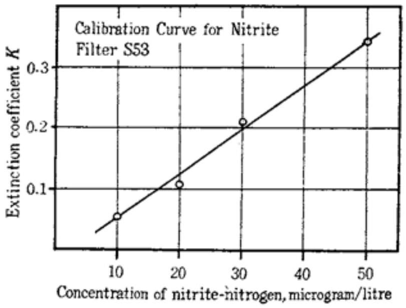

Fig. 5.—A figure to show the relation between the extinction coefficient and the concentration of nitrite.

concentration of this reagent is 1.93 g. per litre as strychnine sulfate. In B. M. G. Zuwicker's report 6 milli-molar strychnidine solution (1.92 g./l.) showed the extinction coefficient 3.48 in the presence of 280 microgram nitrate nitrogen per litre. The new reagent's extinction coefficient in the same concentration of nitrate nitrogen was higher than 3.8, so the new reagent and Zuwicker's one may be of nearly equal sensitiveness. Harvey's reagent aged thoroughly showed considerably lower value than the new one as shown in the fourth figure.

Cooper reported that 0.0008 per cent safranine red solution equaled nitrate nitrogen from 90 to 130 microgram. Also Rochford's reagent showed that 0.0025 per cent solution of the dye equaled 100 or 200 microgram of the nitrate nitrogen. The writer's showed the following relation: 0.005 per cent safranine red solution equaled 150 microgram nitrate nitrogen per litre. So the new reagent is considered to be an excellent one.

Conclusion

Preparation of the Reagent.—(1) 4 g. strychnine sulfate were dissolved in 150 cc. of 18 N sulfuric acid. (2) Into the reductor 10 cc. of the strychnine solution, 10 cc. of water and 150 g. liquid amalgam were added. Then the strychnine was reduced for 15 minutes by the method stated above. (3) After the reduction 120 cc. concentrated sulfuric acid were added and they were preserved in a glass bottle of good quality, cleaned with bromine water. It was better to let it stand in a dark and cool place.

Convenient Points of this Method.—(1) The time of preparation is shortened. (2) Reproducibility of the reagent is enhanced. (3) Zinc of high purity is not necessary. (4) Wasteful consumption of zinc is avoided completely, so the operation of adding sulfuric acid is very easy. (5) There is no need of aging. (6) By the replacement of the air in the reductor with carbon dioxide the reoxidation of the reduced strychnine is avoided.

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