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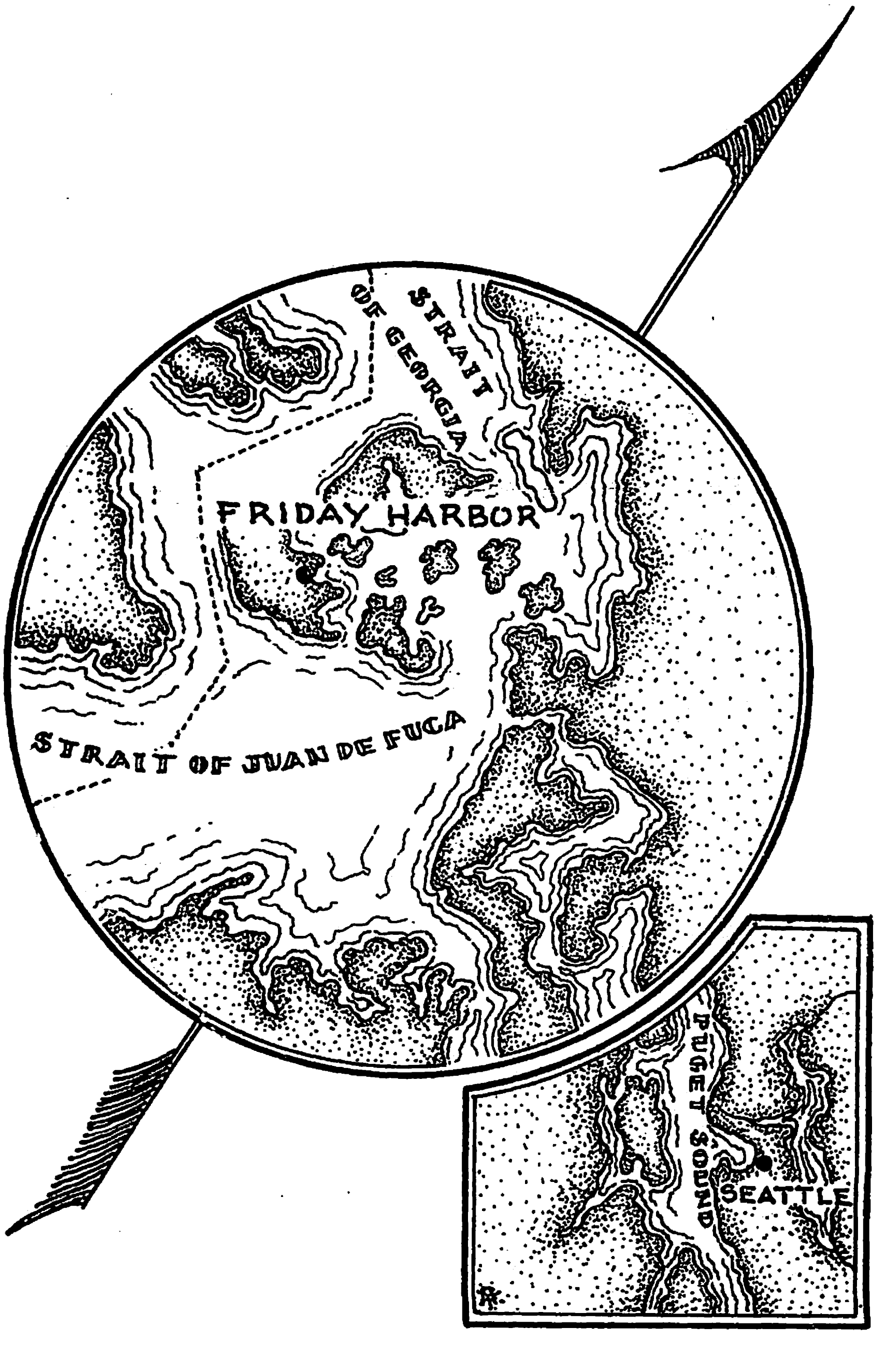
POLAROGRAPHIC DETERMINATION OF NITRATE IN SEA WATER

Technical Report No. 12



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POLAROGRAPHIC DETERMINATION OF NITRATE IN SEA WATER

by

Tung-whei Chow and Rex J. Robinson

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August, 1952

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POLAROGRAPHIC DETERMINATION OF NITRATE IN SEA WATER

ABSTRACT

This investigation was undertaken to develop a new, rapid and accurate method for the determination of nitrate in sea water, based upon the polarographic reduction of nitrate in the presence of uranyl ions.

It has been found that sulfate ions depress the reduction current by a definite proportion depending on the chlorinity of the water sample. For chlorinities in the range of 14 to 19 °/oo, this effect is rather constant. Fluoride interferes strongly with the reduction of nitrate, but the use of aluminum or zirconyl salts may satisfactorily eliminate this effect. Other interfering ions and the selection of preservative have also been discussed. A procedure for the determination of nitrate in sea water has been proposed and its validity tested.

It has been concluded that this method can be used to determine nitrate in sea water of various types.

POLAROGRAPHIC DETERMINATION OF NITRATE IN SEA WATER

INTRODUCTION

Nitrate plays an important role in sea water although present in very small amount. It is considered one of the nutrient salts upon which the biological activity in the sea depends. Ecological studies involving the nutrient salts necessitate extensive surveys and numerous analyses. Methods for obtaining such data need be simple, rapid, sensitive and accurate. However, such a method for nitrate has been lacking and ecological studies involving nitrate analyses of sea water have been discouraged. Previously reported data on nitrate have been obtained mainly by the colorimetric "reduced strychnine" method of Harvey (1929). This method has been investigated and revised by Zwicker and Robinson (1944) but at the best it is not convenient and the reliability of the results may be questioned, though apparently they are reasonably accurate.

In the present investigation, there has been developed a new method of determination of nitrate in sea water, based upon the polarographic reduction of nitrate using uranyl ions as catalyst. The catalytic effect of uranyl was first reported by Kolthoff, Harris and Matsuyama (1944). The polarographic determination of nitrate with molybdate as the catalyst has also been reported by Johnson and Robinson (1952) but lacked the sensitivity demanded for the determination of the small amounts of nitrate present in sea water.

APPARATUS

The polarographic data were obtained with a Heyrovsky Micropolarograph, Model X, calibrated by the method of Kolthoff and Lingane (1946). An H-shaped electrolytic cell (Lingane and Laitinen, 1939) with a saturated calomel reference electrode as one arm connected by a potassium chloride bridge was used. The capillary characteristic, $m^{2/3}\ t^{1/6}$, was 1.30 $mg^{2/3}\ sec^{-1/2}$. The electrolytic cell was immersed in a thermostat adjusted to 25.0 ± 0.1°C by a "merc-to-merc" thermo-regulator and a "merc-to-merc" relay box (Precision Scientific Co.).

REAGENTS

All chemicals used in this investigation were of reagent grade. The various solutions were prepared with distilled water.

1. Uranyl Acetate Solution. 0.8484 gram of uranyl acetate crystals, $UO_2(CH_3COO)_2 \cdot 2H_2O$, was dissolved in water to one liter. The concentration of uranyl ion in this solution was 2.00×10^{-3} M.

2. Aluminum Chloride-Hydrochloric Acid Reagent. 32.2 grams of aluminum chloride crystals, $AlCl_3 \cdot 6H_2O$, and 83.3 ml. of 12 N hydrochloric acid were added to water and diluted to one liter. This reagent was 1 M in hydrochloric acid and 0.13 M in aluminum chloride.

3. Fluoride-free Synthetic Sea Water. This was prepared according to Robinson and Spoor (1936) by dissolving per liter 23.38 grams of sodium chloride, 3.9066 grams of anhydrous sodium sulfate, 0.7455 gram of potassium chloride, 10.676 grams of $MgCl_2 \cdot 6H_2O$ and 1.47 grams of $CaCl_2 \cdot 2H_2O$. This synthetic sea water had a chlorinity of 19 parts per mille (°/oo)

and the concentrations of the various salts were as follows: 0.40 M in sodium chloride, 0.0273 M in sodium sulfate, 0.0525 M in magnesium chloride, 0.01 M in potassium chloride and 0.01 M in calcium chloride. Synthetic sea water with lesser chlorinities was made by diluting this solution with appropriate amounts of distilled water.

4. Synthetic Sea Water containing Fluoride. This was made by dissolving in the above solution 0.0029 gram of sodium fluoride per liter. The resulting concentration of fluroide ion was 7×10^{-5} M.

5. Standard Nitrate Solution. A 0.00200 M sodium nitrate solution was used as the standard for nitrate.

EXPERIMENTAL

Polarograms were obtained after the dissolved oxygen in the various solutions had been driven off with nitrogen gas. The current due to nitrate was obtained by measuring the current at -1.2 v. vs. S.C.E. and subtracting the current of an uranium blank. All potentials reported in this work have been referred to the saturated calomel electrode, S.C.E.

I. Polarographic Reduction of Nitrate in Fluoride-free Synthetic Sea Water in the Presence of Uranyl Ions.

Kolthoff et al. (1944) investigated the polarography of uranium and found two waves in acid solution indicating reductions of uranium VI to uranium V and uranium V to uranium III. They also found that, in the presence of uranyl ions, nitrate was reduced to nitrogen gas at potentials where the second uranium wave occurred. They offered the explanation that the uranium III formed at the cathode acted as an "activator" for the reduction of nitrate. A typical polarogram was shown in their paper. They also reported that there was a linear relationship between the reduction current and nitrate concentration when the uranyl concentration was above a certain limiting value which varied depending upon the nitrate concentration.

Since in sea water the nitrate content ranges from zero to about 70 μg-atoms per liter, a uranyl concentration of 1×10^{-4} M would be needed for application of this method on the basis of the work of Kolthoff et al. The reduction current should then be directly proportional to the nitrate concentration for the range involved.

Preliminary investigations were made with solutions containing various amounts of nitrate in 0.2 M sodium chloride and also in fluoride-free synthetic sea water. To a 100 ml. portion of each solution were added 5 ml. of uranyl solution and 1.5 ml. of 1 M hydrochloric acid and the polarogram then taken. The results, recorded in Tables I and II and plotted in Figure 1, indicate that the current is proportional to the nitrate concentration in either medium. The data obtained with nitrate in 0.2 M sodium chloride solutions agree very well with those reported by Kolthoff, Harris and Matsuyama. It should be noted that the current in synthetic sea water is somewhat less than in the sodium chloride medium due to the effect of sulfate. The half-wave potential of the second uranium wave was also found to be shifted from -0.95 v. to -1.04 v. vs. S.C.E. in the presence of sulfate. The effect of sulfate on the reduction current of nitrate was investigated further in synthetic sea water media of varying chlorinities. Since there is a definite ratio between the chlorinity and sulfate concentration in sea water, the amount of sulfate

TABLE I

Diffusion Current of Nitrate in Sodium Chloride Solutions

0.015 M hydrochloric acid, 0.2 M sodium chloride, 1 x 10^{-4} M uranyl acetate, $m^{2/3}t^{1/6}$ = 1.30 $mg^{2/3}sec^{-1/2}$, residual current at -1.2 v. vs. S.C.E. = 0.734 microamperes.

Nitrate Conc'n µg-at./l.	Diffusion Current for Nitrate, µa	K' = i/C µa/µg-at./l. (x 10^2)	K = $i/Cm^{2/3}t^{1/6}$ (x 10^2)
2.0	0.037	1.85	1.42
6.0	0.110	1.67	1.28
10.0	0.175	1.75	1.35
20.0	0.356	1.78	1.37
30.0	0.527	1.76	1.35
40.0	0.718	1.79	1.38
70.0	1.242	1.77	1.36
100.0	1.699	1.70	1.31
		------	------
	Average value	1.76	1.35

TABLE II

Diffusion Current of Nitrate in Synthetic Sea Water Solutions

0.015 M hydrochloric acid, synthetic sea water, Cl °/oo = 19, 1×10^{-4} M uranyl acetate, $m^{2/3}t^{1/6} = 1.30$ $mg^{2/3}sec^{-1/2}$, residual current at -1.2 v. vs. S.C.E. = 0.734 microamperes.

Nitrate Conc'n µg-at./l.	Diffusion Current for Nitrate, µa	$K' = i/C$ µa/µg-at./l. (x 10^2)	$K = i/Cm^{2/3}t^{1/6}$ (x 10^2)
1.0	0.022	(2.2)	(1.69)
2.0	0.029	1.45	1.12
4.0	0.060	1.50	1.15
5.0	0.081	1.62	1.25
6.0	0.093	1.55	1.19
10.0	0.154	1.54	1.19
20.0	0.304	1.52	1.17
30.0	0.458	1.53	1.18
40.0	0.602	1.51	1.16
70.0	1.026	1.47	1.13
100.0	1.495	1.50	1.15
		------	------
	Average value	1.52	1.17

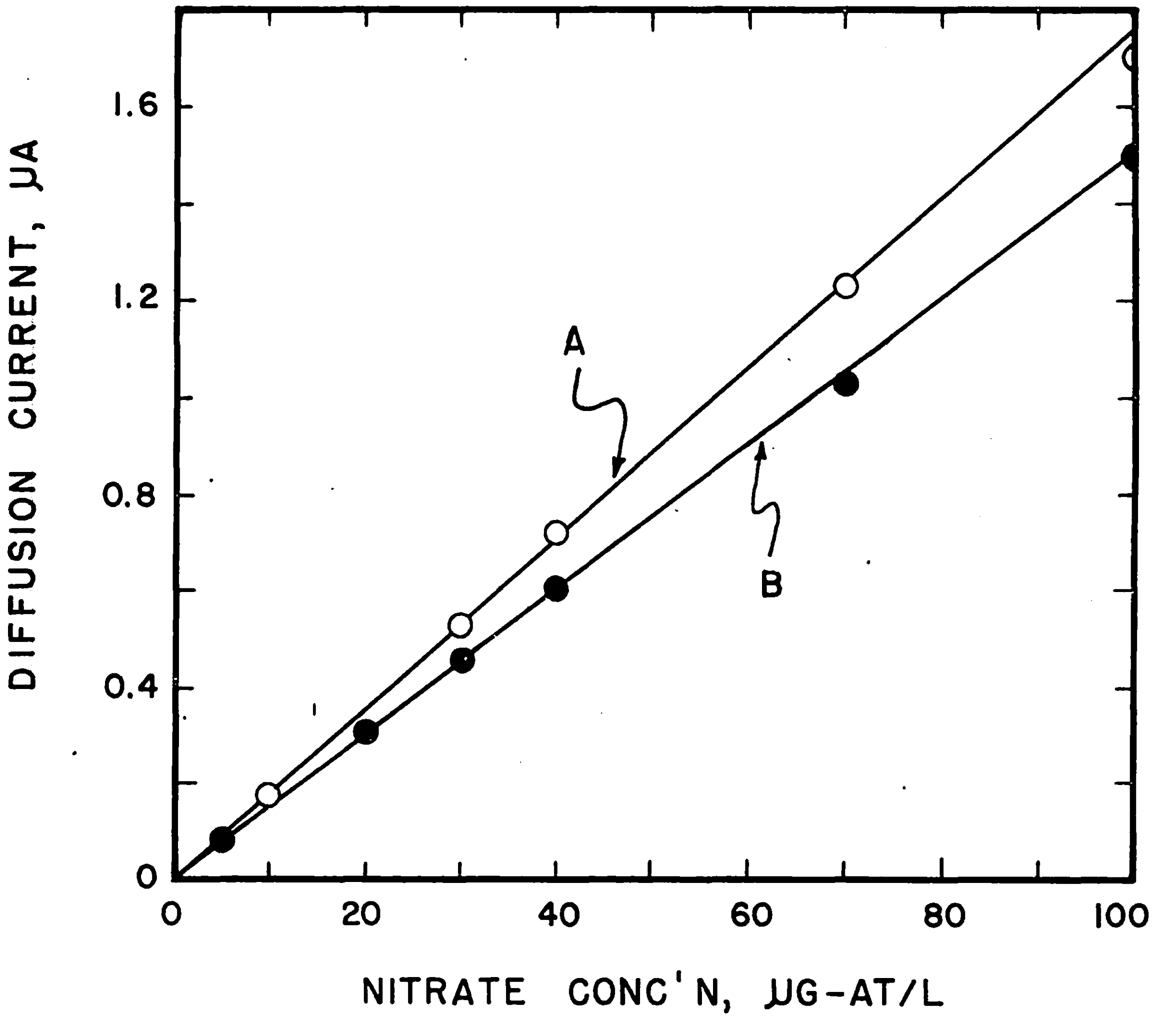

Figure 1. Calibration Curve for Nitrate in Presence of Uranyl in Sodium Chloride and in Synthetic Sea Water Solutions.

[illegible] A: 0.015 M hydrochloric acid, 0.2 M sodium chloride, 1×10^{-4} M uranyl acetate.

Curve B: 0.015 M hydrochloric acid, synthetic sea water, Cl °/oo = 19, 1×10^{-4} M uranyl acetate.

Current measured at -1.2 v. vs. S.C.E.

was varied accordingly. It was found that variations of chlorinity in the range of 14 to 19 °/oo had very little effect on the current. When the chlorinity became less than 14 °/oo, the current increased as the chlorinity decreased. Since in natural sea water the chlorinity is normally within the range of 14 to 19 °/oo, it was considered feasible to obtain calibration data using a synthetic sea water of chlorinity of 19 °/oo. The nitrate concentration in actual sea water may be obtained by using the calibration curve, the lower curve in Figure 1, or by using the following equation derived from the Ilkovic equation (Ilkovic, 1934):

$$i = (1.17 \times 10^{-2})(m^{2/3}t^{1/6})(C),$$

where i is the observed diffusion current due to nitrate in microamperes; $m^{2/3}t^{1/6}$, the capillary characteristics; and C, the concentration of nitrate in the sample in microgram-atoms per liter. The constant of 1.17×10^{-2} has been obtained by taking the ratio of current in microamperes to the product of the capillary characteristics and the nitrate concentration in microgram-atoms per liter as given in the last column in Table II.

The effect of hydrogen ion concentration on the current was also studied. In neutral solutions neither the second uranium wave nor the nitrate wave occurred. When the hydrogen ion concentration was too high, the reduction of hydrogen took place at about -1.2 v vs. S.C.E., making the method impractical. It was found that the hydrogen ion concentration range of 0.005 to 0.02 M gave the best results and the current remained constant in this range for a given amount of nitrate. One and a half milliliters of 1 M hydrochloric acid per 100 ml. of sample gave the desired hydrogen ion concentration.

II. Polarographic Reduction of Nitrate in Synthetic Sea Water containing Fluoride.

When this method was first applied to actual sea water, only uranyl and hydrochloric acid reagents were added. It was observed that the nitrate wave was distorted in such a manner that the current could not be measured due to the lack of the flat diffusion current region. The half-wave potential was also shifted toward the more negative side, being approximately -1.1 v. vs. S.C.E., while the first uranium wave was not affected at all. The distortion of the nitrate wave was found to be due to the presence of fluoride ions in the sea water. Since the first uranium wave was not affected it would seem that the uranium III ions, forming at the dropping mercury electrode, must have been reacting with fluoride ions. The catalytic behavior of uranium upon the reduction of nitrate, thus, was greatly reduced, causing the wave to become ill-defined and the wave height to be much lowered.

If this method was to be applied successfully to sea water analyses it would seem necessary to first remove interference by fluoride ion. Conversion of the fluoride to a stable complex ion appeared to be the simplest means although it seemed conceivable that precipitation methods might be successfully used. The efficiencies of a number of cations complexing with fluoride were studied, using a fluoride-containing synthetic sea water medium. Ferric ions were first tried but were found to be unsatisfactory. When the concentration of ferric ion was small, the effect of fluoride was not eliminated completely. When concentration of ferric ion was more than needed for complexing the fluoride ions, the ferric ions themselves were reduced, thus masking the nitrate wave. Aluminum and zirconium salts were next tried and found to give satisfactory results. When the fluoride was complexed with either aluminum or zirconium the nitrate wave was restored and the wave height became the same as in the absence of fluoride. The minimum amounts of aluminum chloride and zirconyl chloride required to prevent the interference of the fluoride in sea water, Cl = 19 °/oo, were determined to be 8×10^{-4} M and 4×10^{-5} M respectively. An excess of these reagents did not affect the reduction current. Aluminum chloride was selected for use with sea water because of its cost and easy availability. In practice an aluminum concentration of 2×10^{-3} M was used to assure the complete complex formation of all the fluoride ions.

III. Polarographic Determination of Nitrate in Actual Sea Water

A) Discussion of Interferences

Nitrite is reduced in the same manner as nitrate and consequently interferes, but its current is only about three-fifths of that due to nitrate (Keilin and Otvos, 1946). In sea water the nitrite concentration is usually less than 0.4 μg-at./l. which is too small to exert an appreciable effect. If and when the nitrite content in sea water is greater than 10 μg-at./l., though this is very rare, a correction should be made. The concentration of nitrite can be readily determined by the usual colorimetric method (Robinson and Thompson, 1948).

Phosphate if present in sufficient amount interferes by precipitating the uranyl ions. In sea water the amount of phosphate was so small that no interference was observed.

Theoretically, ferric ions would interfere with this determination, since they give a reduction current starting at zero applied potential. But in sea water no current due to ferric ions was observed, apparently due to the fact that they were in some complexed form.

Sulfate decreases the nitrate current as discussed previously. In the sulfate concentration range corresponding to chlorinity values of 14 to 19 $^{o}/oo$, the current was decreased by a definite proportion for the different nitrate concentrations observed, being approximately 86 per cent of that in the absence of sulfate, as seen from Tables I and II. Within this chlorinity range, the calibration curve, the lower curve in Figure 1, or the equation given previously may be used for the determination of nitrate. When the chlorinity is less than 14 $^{o}/oo$, different calibration curves should be used. For sea water with a low chlorinity, it is suggested that calibration curves at different chlorinities be obtained for use, or sulfate may be precipitated out with strontium salts. In the latter case, the interference of sulfate is completely eliminated and the concentration of nitrate may be obtained from the upper curve in Figure 1, or by using the Ilkovic equation which has been simplified to the following form:

$$i = (1.35 \times 10^{-2})(m^{2/3}t^{1/6})(C),$$

where i is the observed diffusion current due to nitrate in microamperes; $m^{2/3}t^{1/6}$, the capillary characteristics; and C, the concentration of nitrate in the sample in microgram-atoms per liter.

In addition to the above interferences in sea water, it was also found that water samples which had been stored in polyethylene plastic bottles gave a rapidly increasing current at the potential of about -1.1 v. vs. S.C.E., thus masking the nitrate wave completely. This was possibly due to the fact that reducible substances in the plastic material had been dissolved in sea water, resulting in the appearance of this current. Thus plastic bottles could not be used to keep the water samples. Glass bottles were found suitable for this purpose.

B) Preservation of Water Samples

In the sea, plankton utilizes nitrate as a nutrient and bacteria may decompose organic nitrogen compounds forming nitrate ions. In order to obtain the amount of nitrate in sea water samples at the time they are collected, the analyses should be made as soon as possible. But since this method is impractical for use aboard the ship, possible change in the nitrate content may result from the effects of plankton and bacteria during the time interval between the collection of the samples and their determination. Consequently it is necessary to add preservatives to the samples to prevent the above actions from taking place.

Mercuric chloride solution, though frequently used for preservation of sea water samples, is not considered desirable for use with samples to be analyzed polarographically, because mercuric salts give a reduction current starting at zero applied potential. Although the currents due to nitrate and mercuric ions are additive and with addition of definite amounts of mercuric salts it would be possible to apply a correction, the use of mercuric salts greatly reduces the sensitivity of this method making it unsuitable.

Chloroform is also used as a preservative for water samples. It was found to be very convenient for use in this determination. Dissolved chloroform is driven off by nitrogen gas when freeing the water samples from dissolved oxygen. Any remaining chloroform in the solution does not interfere because it is not reduced at the dropping mercury electrode. Determinations showed that samples to which chloroform had been added several days before gave the same nitrate results as just before its addition. The addition of 1 to 2 ml. of chloroform to each 300 ml. of sample was found satisfactory.

C) Proposed Procedure for the Determination of Nitrate in Sea Water

In view of the previous discussions, the following procedure for the polarographic determination of nitrate in sea water is proposed:

As soon as the water sample is brought aboard the ship, 2 ml. of chloroform are added to each 300 ml. of sample and the samples are stored in glass bottles in a dark and cool place. They should be analyzed for nitrate at the earliest possible time.

One hundred milliliters of water sample are measured into the electrolytic cell with a pipet, 5 ml. of uranyl acetate solution and 1.5 ml. of the aluminum chloride-hydrochloric acid reagent are added. The dissolved oxygen is removed by washing with nitrogen gas and the polarograms taken immediately. The current at -1.2 v. vs. S.C.E. is measured and the nitrate concentration is obtained from a previously determined calibration curve or from the equation:

$$i = (1.17 \times 10^{-2})(m^{2/3}t^{1/6})(C),$$

after correction for the residual current of uranium has been made.

D) Validity of the Method

The validity of this method was tested by determining the nitrate contents of sea water samples before and after the addition of known amounts of nitrate. The results are shown in Table III.

TABLE III.

Test of the Validity of the Method

Chlorinity	Nitrate Concentration, µg-at./l.			
‰	Originally Present	Added	Found	Difference
16.40	0.0	10.0	9.8	-0.2
16.20	5.2	10.0	15.1	-0.1
15.47	12.5	20.0	32.0	-0.5
16.47	14.5	20.0	34.5	0.0
15.95	14.7	40.0	54.7	0.0
14.34	17.0	20.0	37.0	0.0
16.40	18.5	10.0	28.7	+0.2
16.79	22.3	20.0	42.4	+0.1
16.60	23.5	10.0	33.2	-0.3
16.48	23.5	20.0	43.5	0.0
16.71	24.4	20.0	44.7	+0.3
16.62	25.5	20.0	45.5	0.0
16.14	26.5	40.0	66.5	0.0
16.78	27.2	20.0	48.0	+0.8
16.69	28.0	20.0	48.0	0.0
16.44	29.2	40.0	69.0	-0.2
16.89	29.4	10.0	39.4	0.0
16.75	29.4	40.0	70.0	+0.6
16.91	29.8	10.0	39.6	-0.2
16.74	31.0	20.0	50.3	-0.7
19.05	46.1	10.0	56.0	-0.1
19.08	47.2	20.0	67.0	-0.2
		Average deviation		±0.20

It is seen that the concentrations of nitrate obtained after the addition of known amounts agree very well with the sum of the added and the actual nitrate contents in the samples. This not only indicates that the proposed method accurately determines nitrate, but also that the calibration curve obtained with synthetic sea water with a chlorinity of 19 $^{0}/_{00}$ is applicable equally well to the analyses of actual sea water samples over the chlorinity range of 14 to 19 $^{0}/_{00}$.

E) Results of the Analyses for Nitrate in Sea Water

Several series of sea water samples were collected from East Sound and San Juan Channel in the San Juan Islands and also from the Pacific Ocean. They were analyzed following the procedure outlined previously. The stations selected offered for analysis waters of different types and of the maximum range of nitrate concentrations which would normally be encountered. The results are shown in Table IV.

East Sound is essentially an enclosed basin with but little exchange of waters with the adjoining water bodies. The water in East Sound is also stratified. At the season of the year when the samples were collected, its water supported an abundant diatom population which rapidly depleted the water of nitrate.

The waters of San Juan Channel are rather uniform in distribution because of vertical mixing. The water itself shows the effect of dilution by the Fraser River.

The ocean station offered depth samples rich in nitrate and surface samples with reduced nitrate concentrations.

TABLE IV.

Analyses of Sea Water Samples for Nitrate

East Sound, San Juan Islands, 48° 40.3' N, 122° 53.7' W, July 11, 1952.

Depth, meters	Nitrate Conc'n, µg-at./l.	Chlorinity, °/oo
0	0.0	16.50
5	0.0	16.49
12	0.0	------
18	0.0	16.38
23	0.5	16.66

San Juan Channel, San Juan Islands, 48° 35' N, 123° 01' W, July 11, 1952.

0	9.6	
5	11.7	
10	14.5	
20	14.8	
50	13.5	
100	13.4	

Pacific Ocean, 48° 30' N, 136° 30' W, July 7, 1952.

0	6.5	17.99
5	5.5	17.99
10	5.5	17.99
20	7.8	17.99
30	3.2	18.01
50	5.3	18.05
100	5.2	18.06
200	22.0	18.65

TABLE IV. (Continued)

300	30.5	18.73
500	38.2	18.85
750	41.5	18.93
1000	46.6	19.01
1500	42.2	19.05
2000	47.2	19.08

The results shown in Table IV for nitrate are in general agreement with the previously reported data for these waters (Phifer and Thompson, 1937; Sverdrup, Johnson and Fleming, 1946). It may be concluded from these results that the method yields satisfactory results when applied to the analysis of sea waters.

IV. Applications

This method may also be applied to the determination of nitrate in fresh waters. Since no interfering ions are present in sufficient amount in fresh water, the determination of nitrate may be performed simply by adding a supporting electrolyte, such as sodium chloride to make a 0.1 M solution, to the water samples and then following the same procedure as outlined previously. It should also be applicable to other saline waters similar to sea water.

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1	Allen Hancock Foundation University of Southern California Los Angeles 7, California
1	U.S. Fish & Wildlife Service Fort Crockett Galveston, Texas
1	U.S. Fish & Wildlife Service 450 B. Jordan Hall Stanford University Stanford, California
2	Director Scripps Institution of Oceanography La Jolla, California
1	Director Hawaii Marine Laboratory University of Hawaii Honolulu, T.H.
1	Director Marine Laboratory University of Miami Coral Gables, Florida
1	Head, Department of Oceanography Texas A. & M. College College Station, Texas
1	Head, Department of Oceanography Brown University Providence, Rhode Island
1	Department of Zoology Rutgers University New Brunswick, New Jersey ATTN: Dr. H. Haskins