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THE PHOTOMETRIC DETERMINATION OF NITRATE IN SEA WATER WITH A STRYCHNIDINE REAGENT

By

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INTRODUCTION

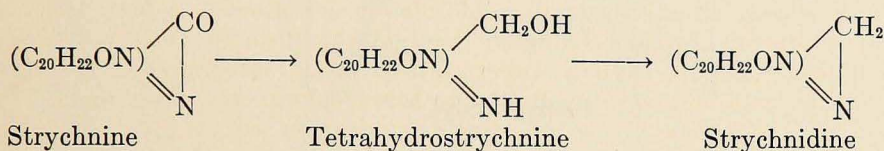
The routine determination of small concentrations of nitrate in water containing considerable chloride ion is limited to a few special procedures. Indirect reductive methods are usually avoided since natural waters contain other nitrogeneous substances. Direct estimations have been restricted to a few colorimetric procedures.

Of the several types of organic substances which give colored aqueous solutions with dilute oxidants, only one, the substituted aryl amines, has been found to have sufficient sensitivity to nitrate to be of use in the presence of significant chloride concentrations. Two substances of this class, diphenylamine (or diphenylbenzidine) and reduced strychnine, have been widely used in oceanographic work. Wattenberg (17) reported little difference between the sensitivity of Harvey's (7, 8) reduced strychnine method and Atkin's (2) diphenyl benzidine procedure. The reduced strychnine reagent, as prepared by customary methods, must be aged before being used, and therefore it is difficult to prepare reduced strychnine of reproducible sensitivity; furthermore the red oxidation color is not very stable.

The present investigation was undertaken in order to improve the reagent so that the method would be more useful in oceanographic work. The chemical nature of the active ingredients in reduced strychnine were studied, and the essential variables in the determination of nitrate in dilute solutions were investigated. The reduced strychnine reagent is shown to contain a mixture of reduction products. Of these, strychnidine is selected as having the greatest analytical possibilities. When using a strychnidine reagent the determination of nitrate is studied photometrically. In the oxidation of strychnidine, bistrychnidyl is probably first formed. The characteristics of bistrychnidyl as a reagent for nitrate are also presented.

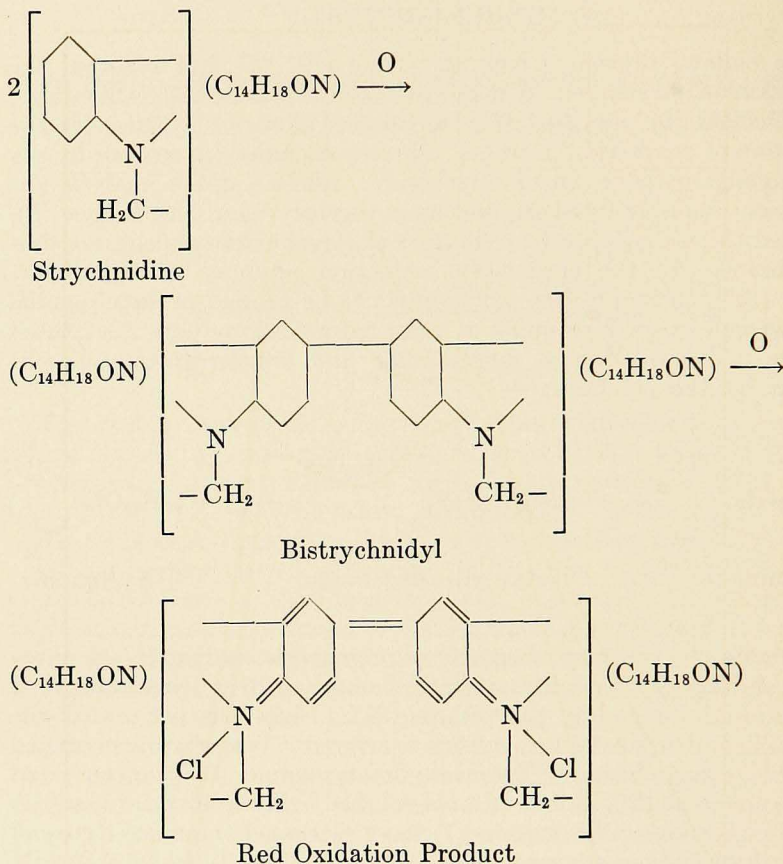
EXPERIMENTAL

The reduced strychnine reagent. Tafel (13, 14) first reported the reduction of strychnine to a mixture of oxidant-sensitive alkaloids, and Denigès (5) discussed the preparation of such a reagent for the detection of nitrites and nitrates. Harvey prepared his reagent by the zinc amalgam reduction of strychnine. While a direct analysis has not been reported, Denigès, and more recently Giral and Ibanez (6), claimed the products to be a mixture of strychnidine and tetrahydrostrychnine. In the electrolytic reduction products of strychnine, Tafel (15) had found these compounds to be present in large quantities, as well as small amounts of other reduction products of a related nature. The relation of strychnidine and tetrahydrostrychnine is shown by the equation:



A reduced strychnine reagent was prepared according to the directions of Harvey (9) and immediately analyzed by a recrystallization procedure developed by the authors (21). Eighty-six per cent of the total alkaloid originally present was recovered. This mixture consisted of: 58% strychnidine, 18% tetrahydrostrychnine, 12% unconverted strychnine and 12% brown alcohol-soluble "dihydro-derivatives."

Achmatowicz and Robinson (1) have discussed the many types of reduced strychnine compounds and their color reactions with slightly acidic ferric alum solutions. The probable structure of these red oxidation products was established by Wieland and his co-workers (18). In their work on the structure of the strychnos alkaloids, colorless bis-compounds were prepared from both strychnidine and tetrahydrostrychnine which were "doubly sensitive" to oxidation. This reaction was explained by direct analogy to the quinoline-quinoid reaction of other substituted aryl amines such as diphenylamine or indole. In the case of strychnidine this reaction would be:



Harvey (7) found that his reagent increased in sensitivity on aging six weeks. The authors observed, spectrophotometrically, that the red oxidation product from a sensitive, aged reagent gave a higher ratio of k_{5300}/k_{5000} * than that from the less sensitive, freshly prepared reagent whose analysis has been reported above. Using pure strychnidine and tetrahydrostrychnine prepared by the authors (21) from the electrolytic reduction of strychnine, the curves shown in Figure 52 were obtained from absorption measurements with a Bausch and Lomb spectrophotometer. The oxidation product of strychnidine in strong acid with either chromate or nitrate displayed a sharp maximum

* k_{5300} represents the specific extinction coefficient at the 5300 Å average spectrum cut with the S-53 filter of the Zeiss-Pulfrich photometer.

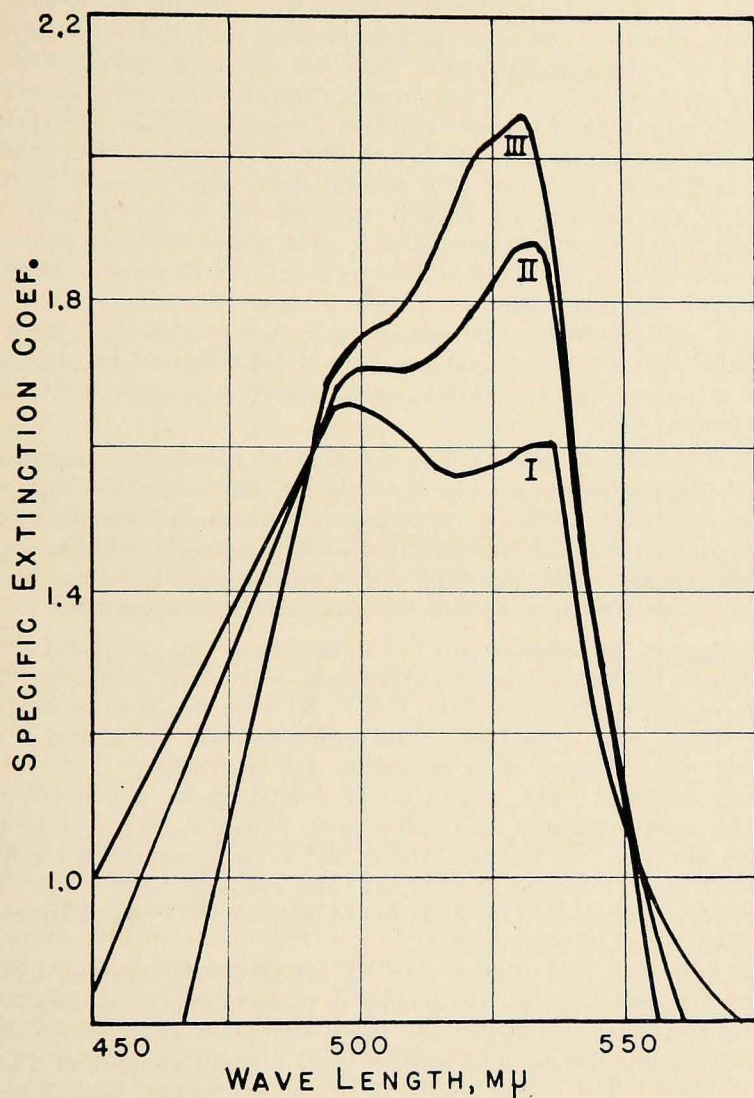


Figure 52. Absorption Spectra for Oxidized Strychnidine and Tetrahydrostrychnine. (I) Tetrahydrostrychnine, cold chromate oxidation; (II) Tetrahydrostrychnine, normal nitrate; (III) Strychnidine, normal nitrate.

at 5315 Å. When tetrahydrostrychnine was carefully oxidized with chromate in strong acid, two distinct maxima were observed, the one at 4980 Å being slightly higher than the second at about 5300 Å. By the usual oxidation with hot nitrate, variable curves were observed, lying between that of the cold reaction product and that of strychnidine. The maximum at 5300 Å was 10 to 30% greater than that at 4980 Å. Tafel (15) found that tetrahydrostrychnine could be converted to strychnidine by boiling with mineral acids, or better by treating with phosphorus oxychloride. The above evidence points to this conversion in the aging of Harvey's reduced strychnine reagent. Since even the most sensitive sample of Harvey's reagent that was available still displayed a prominent maximum at 4980 Å, it must be concluded that a complete conversion to strychnidine on standing was not obtained, and that probably other reduced strychnine derivatives were present also.

The strychnidine reagent. The selection of strychnidine as an improved reagent for nitrate was based on the above results. Not only does strychnidine produce a dye of more intense absorption maximum, (see Figure 52), but the colored oxidation product is largely limited to a single species. Also, the 5300 Å absorption band lies close to the maximum sensitivity of human vision at low light intensities.

Preparation of Strychnidine. Strychnidine was prepared from strychnine by electrolytic reduction on a mercury cathode (21). About 10 g of strychnine sulfate, U.S.P. XI were dissolved in 55 ml of 64% sulfuric acid (sp gr 1.54). The cooled solution was placed in the cathode compartment of a reduction cell containing 17.8 cm² of mercury cathode. The porous anode compartment was filled with 60% by weight sulfuric acid and a strip platinum anode of 15 cm² surface inserted. A temperature of 25° C was maintained during reduction at 0.75 ampere, 5 terminal volts and 3 cathode volts. The reduction was continued for 35 hours to increase the yield of strychnidine over tetrahydrostrychnine.

The catholyte was diluted with 83 ml of 0.01% sulfuric acid to give 28.5% sulfuric acid. On cooling to room temperature, or colder, most of the unreduced strychnine was precipitated as the acid sulfate (20). This was filtered and washed with a small amount of 28.5% sulfuric acid. The excess acid was slowly neutralized with concentrated ammonia, 100 to 200 g of ice being added to cool the solution. The amorphous base, largely strychnidine and tetrahydrostrychnine, was digested at 40° to 50° C to remove excess ammonia and to dissolve the tetrahydrostrychnine. The strychnidine residue was filtered and purified further with two extractions of 100 ml portions of warm water.

To increase the yield the aqueous extracts were concentrated, the crude tetrahydrostrychnine extracted by chloroform and then mixed with ten times its weight of phosphorous oxychloride under an atmosphere of carbon dioxide. After refluxing 45 minutes at 60 to 70° C under reduced pressure, the excess reagent was removed by vacuum distillation. The strychnidine was obtained from the diluted brown syrup by precipitation with ammonia, or as the hydriodide.

The crude strychnidine was recrystallized from 100 ml of 95% ethyl alcohol and finally recrystallized slowly two or three times from thiophene-free benzene to give pure strychnidine, m. p. 256.6° C in vacuo.

Photometric determination of nitrate. A reagent of one millimole of strychnidine (0.320 g per liter) is recommended for nitrate concentrations up to 50 μg at.¹ $\text{NO}_3\text{-N}$ /liter. The strychnidine reagent was prepared from the colorless prisms of strychnidine. The alkaloid was weighed directly into a volumetric flask and oxidant-free sulfuric acid (sp gr 1.84) was added immediately. The solution was kept in an aged brown glass bottle. Sulfuric acid, manufactured by the contact process, is the most convenient to use. If acid of sufficient purity is not available, nitrates may be removed with ammonium sulfate according to Cooper (4) or with hydrogen sulfide according to Atkins (3).

A stock solution of 750 μg at. $\text{NO}_3\text{-N}$ /liter was prepared from recrystallized C.P. sodium nitrate that had been dried at 110° C. The stock solution was made to contain 25 parts of chloride-ion per liter with 20 ml of a saturated solution of C.P. mercuric chloride solution per liter as a preservative and twice recrystallized sodium chloride. The standard nitrate solutions were prepared from the stock solution by appropriate dilution with sodium chloride solutions containing 25‰ Cl, i. e. grams Cl per 1000 grams of solution.

Equal volumes of reagent (in concentrated sulfuric acid) and nitrate solutions were carefully mixed in well aged pyrex tubes by four careful but rapid transfers from one to the other. The hot mixtures were immediately placed in the dark for three to five hours. Measurements were made with the Zeiss-Pulfrich photometer; the 0.1–15 mm. variable depth cell was convenient for the present investigations, and a 10 mm cell containing distilled water was used for the reference standard. A blank determination for the variable cell was made with 60% by weight sulfuric acid. A preliminary check comparison was always made on the S-50 filter before the measurement with the S-53 filter; repetition of the readings was always necessary

¹ A microgram atom, μg at., is one millionth of a gram atomic weight.

unless the operator's eyes had been "conditioned" by a series of readings. Operation in a darkened room was found effective in increasing efficiency and eye sensitivity.

Concentration of the reagent. To 3.0 ml portions of standard nitrate solution of 75 μg at. per liter was added 3.0 ml of 38% sulfuric acid containing increasing amounts of strychnidine reagent. The results are shown in Table I and Figure 53. At lower concentrations of the reagent Beer's law was followed closely. Finally maximum color was obtained and further increases in the ratio of strychnidine to nitrate resulted in gradual decrease of color. Fading with time was more rapid at the higher reagent concentrations.

The increase in specific extinction coefficient per mole of strychnidine concentration in the mixture (i. e., molar extinction coefficient) is equal to the slope of the linear part of the curve, which was found to be 20,500. The intercept of this linear portion with that showing maximum color formation indicated that at least fourteen moles of the strychnidine are required for reaction with one of nitrate.

Table II and Figure 54 show standardization data for reagents of one and six millimoles of strychnidine per liter over a range of 2 to 75 μg at. $\text{NO}_3\text{-N}$ per liter. Although divergencies from a linear curve are less than 5%, at lower concentrations there is a tendency for lower absorption values with the higher reagent concentrations. The standardization curve for the best Harvey's reduced strychnine reagent is included to show its linearity at a lower sensitivity, as well as the curve for the bistrychnidyl reagent at nearly double sensitivity.

A reagent of one millimole of strychnidine per liter is recommended for nitrate concentrations between 5 and 50 μg at. per liter.

Time required for color development. The effect of time on the color development is shown in Figure 55 for three typical nitrate concentrations. The time required for maximum color formation increased with increasing nitrate concentration. The greater instability of the color with high nitrate concentration was typical. Harvey (8), Sund (12) and others have found that Beer's law failed to hold above 25 to 30 μg at. $\text{NO}_3\text{-N}$ per liter with Harvey's reagent, but they measured the color 12 to 24 hours after mixing. With pure strychnidine, Beer's law failed above 50 μg at. $\text{NO}_3\text{-N}$ per liter with a reagent concentration of 2 millimoles per liter in 24 hours.

Three to five hours were the most satisfactory for color measurements over a wide range of concentrations. Harvey's reagent required about 20% longer for the high nitrate concentrations than was noted with strychnidine.

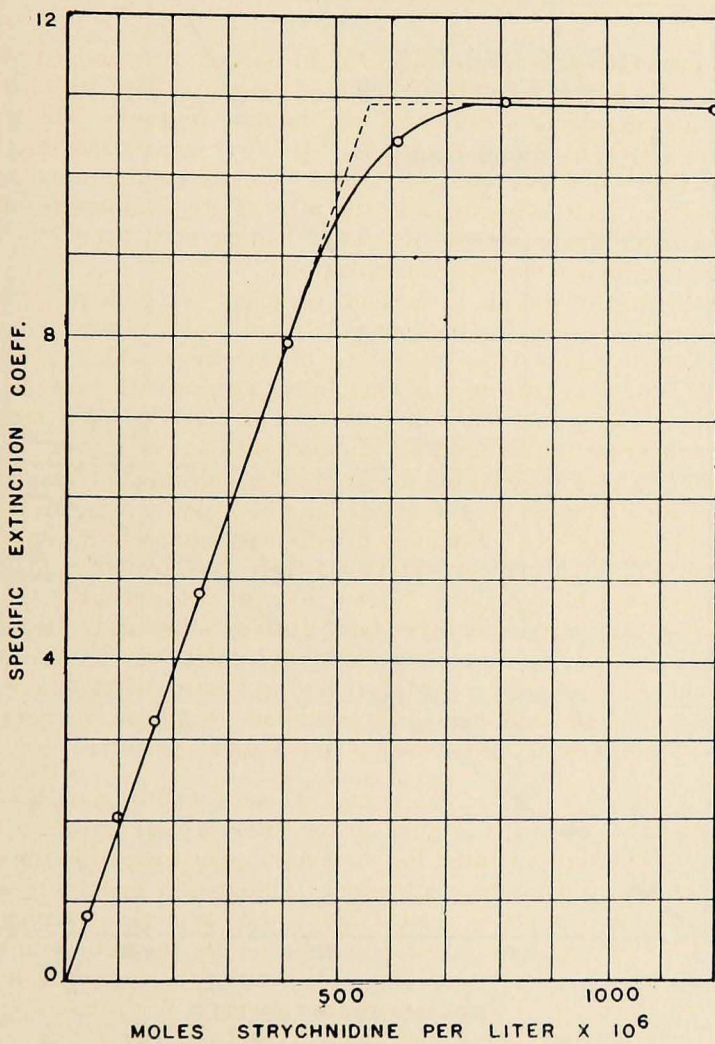


Figure 53. Strychnidine Requirements for $75 \mu\text{g at./l. NO}_2\text{-N}$.

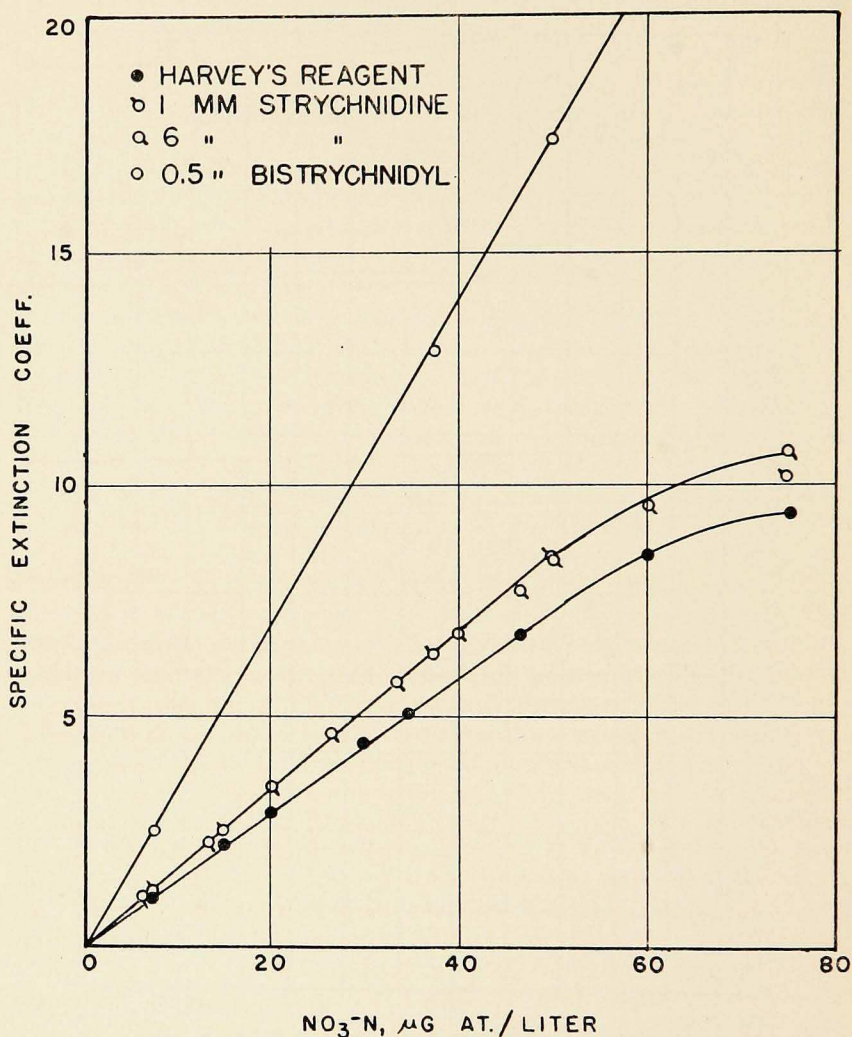


Figure 54. Standardization Curves at Four Hours Color Development.

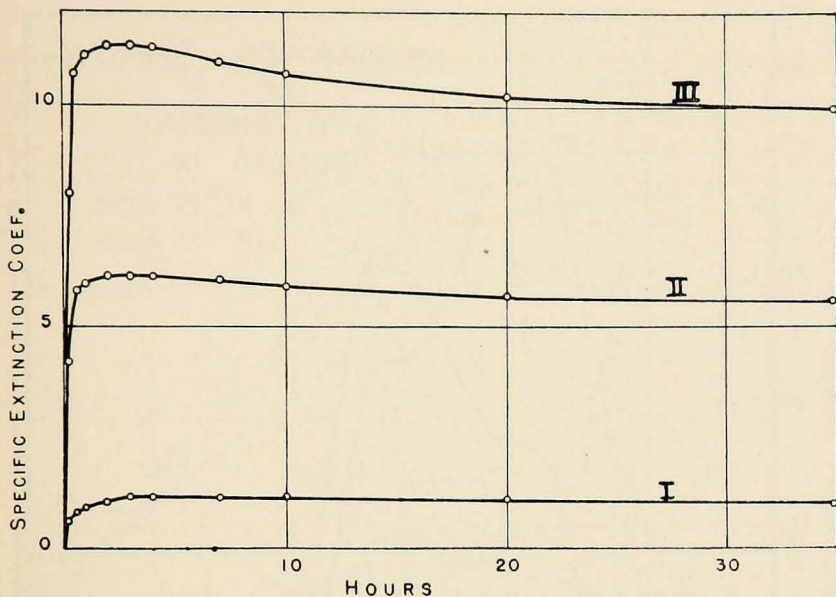


Figure 55. Development and Fading of Color with 1 millimolar Strychnidine Reagent. (I) 7.5 μg at./l. $\text{NO}_2\text{-N}$; (II) 37.5 μg at./l. $\text{NO}_2\text{-N}$; (III) 75 μg at./l. $\text{NO}_2\text{-N}$.

Characteristics of the colored oxidation product of strychnidine. Spectrophotometric absorption data were obtained for the pure strychnidine, m p 256.5° C in vacuo, with a Bausch and Lomb spectrophotometer, oxidized in dilute and concentrated acid by dilute chromic acid; the curves are shown in Figure 56. The maximum at 5315 Å was more intense in strong acid, while the maximum at 4980 Å was more pronounced in weak acid.¹ Further evidence of two oxidation products is shown in Figure 57 in which specific extinction coefficient for the 5300 Å is plotted against time for several concentrations of acid. These data were obtained with the Zeiss-Pulfrich photometer. All the solutions were mixtures of equal volumes of 0.001 molar strychnidine in sulfuric acid and 0.00005 molar ferric alum.

Part of the above data were predicted qualitatively by Achmatowicz and Robinson (1), who claimed that the color produced with ferric alum and strychnidine decreased with an increase of acidity from 0.01 to 0.1 N. However, the change in color and the increase in intensity in strongly acid solutions has not been reported.

¹ The colors were visually quite different. At low acid concentrations an initial salmon red faded to a light yellow, while in concentrated acid a bright cherry red was produced.

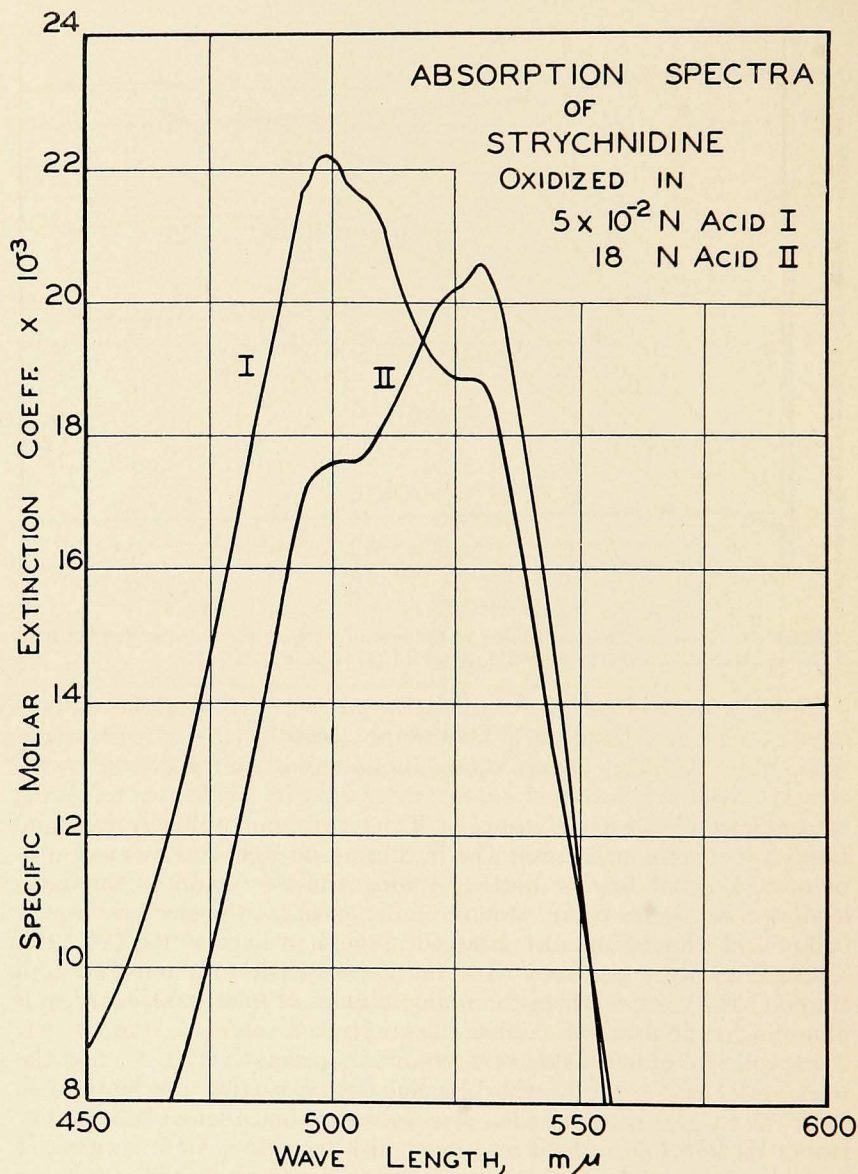


Figure 56. Effect of Acidity on Absorption Spectra of Strychnidine.

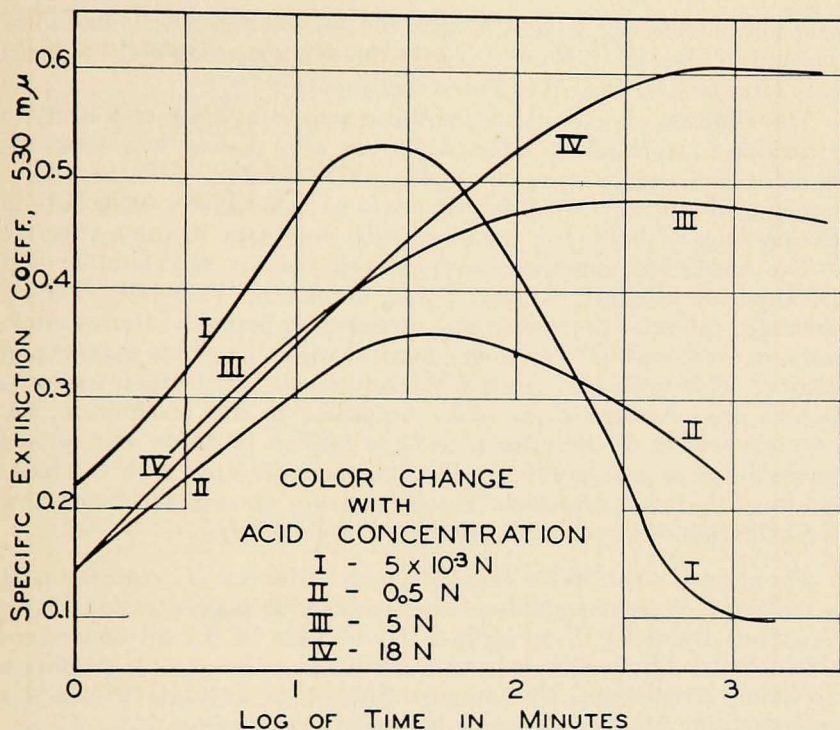


Figure 57. Effect of Acidity on Color Change with Time.

Contrary to the conclusions of Tafel (14) and Achmatowicz and Robinson (1), the color produced in dilute acid solutions was found to be quite heat labile. In dilute solutions it faded rapidly in the dark, even at room temperature, and in more concentrated solutions, a pure yellow to buff solution was obtained in one-half hour at 100° C. The solutions used by the above authors were considerably more concentrated than those in which the fading was so strikingly displayed, but photometric analysis of similarly concentrated solutions showed that there was a decided decrease in color in an hour at 100° C, and the mixture became quite brownish in hue as the heating was continued.

In strong acid solution the more brilliant crimson color was considerably more stable to heat and light, although three to four hours at 100° C. was sufficient to convert this to a brown or buff. Further evidence of the more stable nature of this oxidation product formed in concentrated acid solution was obtained by the addition of aliquots of sulfurous acid solution to equivalent quantities of dye at several

acid concentrations. In 0.01 N acid, the red solution was immediately decolorized, but in 15 N sulfuric acid the dye was completely reduced only after several days of repeated treatments.

The addition of concentrated sulfuric acid to a dilute acid solution of oxidized strychnidine, either before or after fading had occurred, resulted in the rapid formation of the stable red dye. Dilution of the strong acid solution of the dye caused it to fade rapidly, especially in the presence of light, but the absorption spectrum was not affected.

The spectrophotometric absorption curves for strychnidine and tetrahydrostrychnine oxidized with chromate, vanadate, iodate, bromate, chlorate, permanganate, manganate, peroxide, ferricyanide, ferric or ceric ions, proved to be identical with those from nitrate and nitrite. This evidence supports Wieland et al. (18) in their explanation of the structure of the dye. According to this mechanism, the easy conversion of the salmon red dye formed in dilute acid to the crimson dye in concentrated acid might be associated with the basic nature of the indole nitrogen. The previous reactions given above show the oxidation of strychnidine to the merquinoid dye.

The effect of chlorides on color formation. Harvey (7) reported that a well aged reagent was insensitive to chloride concentration above 6‰, but Riddell (10) prescribed the addition of 0.5 ml of concentrated hydrochloric acid to each 7 ml of sea water sample to obtain maximum sensitivity. Preliminary work of the authors (19) on this subject, using Harvey's reagent, indicated that at least 25‰ Cl was necessary for concentrations of nitrate greater than 20 µg at. NO₃-N per liter to obtain maximum sensitivity.

Using a millimolar solution of strychnidine, the results shown in Figure 58 were obtained. The low nitrate concentration required more chloride for the maximum value than the higher concentrations, but at the same time the higher concentrations were affected more critically by the excess chloride concentrations. For the region of nitrate concentration around 7.5 µg at. per liter, a molar Cl/NO₃ ratio of at least 5,000 (Cl = 1.5‰) was necessary, but it could be as large as 19,000 (Cl = 6‰) without affecting color formation. The range that was usable for 37.5 µg at. NO₃-N per liter was found to be between Cl/NO₃ = 1,900 (Cl = 2.5‰) and Cl/NO₃ = 18,800 (Cl = 25‰). The usable range for 75 µg at. NO₃-N per liter was found to be between Cl/NO₃ = 13.3 (Cl = less than 1) and Cl/NO₃ = 5,600 (Cl = 15‰). This accounts for the apparent failure of the reagent in determinations using the usual nitrate procedure. The increase of the concentration of the strychnidine to 5 millimolar failed to change the standardization curve up to 75 µg at. NO₃-N per liter, for chlorinities between 5

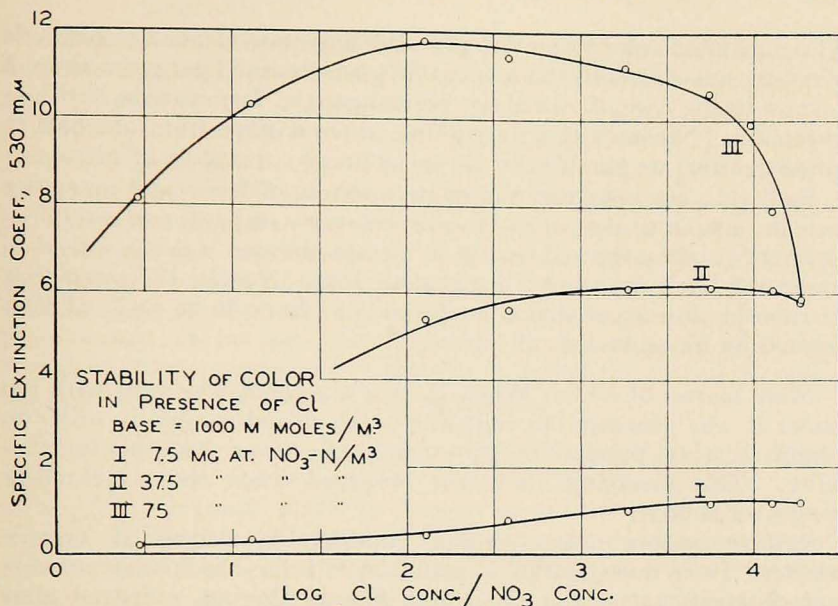


Figure 58. Effect of Chlorinity on Color Stability.

and 25‰. Since the normal nitrate concentrations in sea water vary between 5 and 40 μg at. per liter with chlorinities of 16 to 20‰, there appears to be no need for added chlorides when a pure strychnidine reagent is used, even in quite brackish waters. For fresh water, the addition of some chloride is essential.

Stoichiometry of the reaction. The data presented in Table I and Figure 53 indicate an abnormal reaction with nitrate in strong acid solution. If Wieland's reaction is correct, the oxidation of the merquinoid compound would require two oxidizing equivalents per mole of strychnidine. The indicated reaction of about sixteen moles of strychnidine with each nitrate is not consistent with the known oxidizing power of the nitrate alone, even assuming reduction to ammonia and complete oxidation to the red dye.

The action of other oxidants on strychnidine in various acid concentrations was studied with inconclusive results.† The concentration of both the strychnidine and the oxidant affected the color formation. The presence of chlorides reduced the color formation in many instances and the acid concentration also affected the results. In all cases the spectrophotometric absorption curves of the strong acid oxidation products were identical with those obtained by nitrate.

The maximum color formation per oxidizing equivalent in strong acid was only one-sixteenth the amount of color obtained per gram mole of nitrate in the case of vanadate, permanganate, ferricyanide, ferric or chromate. Nitrite was an exception, since it gave from one-half to three-quarters as much color as an equimolar quantity of nitrate.

Stoll (11) has discussed the relative effects of ferric and manganic ions in relation to that of nitrates on Harvey's reagent, and a recalculation of his data showed that each nitrate-nitrogen was equivalent to about 4.5 ferric ions and 2.6 manganic ions. Harvey (8) noted that nitrites produced variable color formation, from 50 to 80% of that formed by its equivalent of nitrate.

Some sources of error. When Harvey's reagent was used with sea water it was necessary to centrifuge each mixed sample in order to remove a white precipitate before comparison was made photometrically. This precipitate was not observed when the strychnidine reagent was used.

Surface waters often contain considerable suspended organic matter. In an investigation of plankton cultures, check analyses were not obtained unless the water was filtered through a fritted glass crucible of fine porosity (plate pores 5 to 10 microns).

The presence of dissolved organic matter and inorganic reducing agents, especially in brackish and polluted waters, probably will result in erroneously low nitrate values. The addition of some easily oxidized compounds such as oxalic acid, glucose or sulfites to standard nitrate samples lowered the color formation materially. Sund (12) in discussing the problem, advised the addition of two different quantities of barium nitrate to brackish water, whereby the effect of reducing substances could be approximately determined by calculation.

Strychnidine reacts in acid solution with nearly all common oxidants to form the red dye which is spectrophotometrically identical to that formed with nitrate. Harvey (7, 8), Thompson and Johnson (16), Cooper (4) and others have considered the presence of oxidants other than nitrates in sea water. They have agreed that nitrite-nitrogen is the only one that is usually present in sufficient quantities to be effective. Since its concentration is normally less than 5% of that of nitrate-nitrogen, its presence is not a serious matter.

Sensitivity and accuracy. The ultimate sensitivity of the strychnidine reagent has been determined to be about 0.2 μg at. $\text{NO}_3\text{-N}$ per liter with standard nitrate solutions. However, this sensitivity is not practical, since the blank determination is usually materially greater than this value, and large volumes of solutions would be required for

the photometric analysis. The practical limit of sensitivity may be considered to be 0.5 to 1 μg at. $\text{NO}_3\text{-N}$ per liter with the Zeiss-Pulfrich photometer.

As little as 1 ml of sample has been used with success; if samples of smaller volume are desired, dilution with 60% sulfuric acid is recommended. Blank determinations are required, of course.

Using the procedure described above, an experienced operator has been able to duplicate his results consistently within the accuracy of the Zeiss-Pulfrich photometer. Under the optimum conditions of measurement an accuracy of $\pm 1.5\%$ may be expected. Table III summarizes the authors' recommendations for analysis of various ranges of nitrate concentration in order to utilize the optimum operating conditions of the reagent and instrument.

Bistrychnidyl reagent. From a study of the reactions summarized in the first part of the paper it is evident that the disadvantages of the strychnidine reagent might be largely eliminated by the use of bistrychnidyl as the active reagent. Attempts to prepare a pure sample of bistrychnidyl for such a purpose were not successful. Its crystalline form was poor and the samples were invariably contaminated with brownish-red oxidation products.

A reagent was prepared by the following procedure. This product was nearly colorless and exhibited the desired properties of sensitivity (see Figure 54).

Dissolved in a small volume of 0.5 N hydrochloric acid at 0 C° in an atmosphere of carbon dioxide was 0.160 g of pure strychnidine. To this were added slowly over a period of 10 minutes 1.1 milliequivalents of chromic acid (11.0 ml of a 0.10 N H_2CrO_4 solution). Dilute sodium bisulfite solution was then added dropwise at 0° C until a light green color was obtained. Sulfuric acid (sp gr 1.84) was added slowly, an ice bath maintaining the temperature below 5° C, until the solution had a volume of 500 ml. Very dilute chromic acid was then added until the complete removal of the excess sulfite was indicated by the appearance of a very faint pink tinge. The reagent had to be stored in an aged brown glass bottle under an atmosphere of carbon dioxide, otherwise its blank slowly increased.

The colors obtained with bistrychnidyl formed more rapidly than with strychnidine; comparison could be made after an hour in the dark. The effect of chloride was as marked as in the case of strychnidine, but fading in the dark was slow with this reagent. An increase in color was noted with low nitrate concentrations upon standing in the laboratory for 24 hours, indicating a slow air oxidation. This extreme sensitivity should make possible a material reduction in the sulfuric acid concentration necessary for the determination.

SUMMARY

A reagent prepared from strychnidine has been examined for the photometric determination of $\text{NO}_3\text{-N}$ in sea water. It was found to be more stable, reproducible and sensitive than Harvey's reduced strychnine reagent, which was shown to be a mixture of reduction products. The effects of reagent concentration, time, temperature, light, acidity and chlorinity on the color production have been studied. Recommendations have been made concerning the technique of the method for use with a Zeiss-Pulfrich photometer. The errors, sensitivity and accuracy of the procedure have been discussed. A reagent prepared from bistrychnidyl was found to be even more sensitive than the strychnidine reagent, but somewhat difficult to prepare and use.

TABLE I—EFFECT OF REAGENT CONCENTRATION

<i>Strychnidine</i> Concentration Molarity $\times 10^6$	<i>Strychnidine</i> Nitrate	<i>Specific Extinction Coefficient</i> with S-53 Zeiss filter
4010	95.0	9.87
3010	71.0	10.15
2005	47.5	10.71
1600	38.0	10.97
1200	28.4	10.85
800	19.0	10.95
600	14.3	10.46
400	7.60	7.91
240	5.65	4.81
160	3.80	3.22
99	2.35	2.04
40	0.95	0.81
7	0.18	0.04

TABLE II—STANDARDIZATION OF REAGENTS (25% Cl; AFTER 4 HOURS)

<i>Nitrate</i> $\mu\text{g. at.}$ <i>per l.</i>	<i>Specific Extinction Coefficient, S-53</i>			
	<i>Harvey's</i>	<i>Strychnidine</i>		<i>Bistrychnidyl</i>
		<i>1 millimolar</i>	<i>6 millimolar</i>	<i>0.5 millimolar</i>
6.7	1.11
7.5	1.10	1.25	2.55
13.3	2.27
15.0	2.25	2.55
20.0	2.90	3.48
26.7	4.64
30.0	4.39
33.3	5.75
35.0	5.04
37.5	6.35	12.9
40.0	6.75
46.7	6.75	7.70
50.0	8.55	8.40	17.4
60.0	8.50	9.55
75.0	9.40	10.2	10.7	24.6
100.0	11.6

TABLE III—RECOMMENDED PROCEDURE FOR THE PHOTOMETER

<i>NO₃</i> Range μg at. per l.	<i>Strychnidine</i> Millimoles per l.	<i>Sample</i> in ml.	<i>Size of</i> <i>Test tube</i> in mm.	<i>Time of</i> <i>Standing</i> in hours	<i>Thickness of</i> <i>Cell stratum</i> in mm.
0.2 to 4	0.10*	14	20 x 155	4-24	50
2.5 to 10	0.10*	3	13 x 100	2- 8	10
5 to 15	0.50	3	13 x 100	2- 5	5
12 to 40	1.0	3	13 x 100	2- 5	2
25 to 75**	5.0	3	13 x 100	2- 4	1

* Bistrychnidyl reagent is recommended for this range (see below).

** Samples above 50 μg at. *NO₃/N* per liter should be diluted for best results.

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