

THE DETERMINATION OF MICROMOLAR CONCENTRATIONS
OF AMMONIA WITH 1-FLUORO, 2:4-DINITROBENZENE

by

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B.Sc., Hons., Sheffield University, 1963

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department of Chemistry
and
Institute of Oceanography

We accept this thesis as conforming to the
required standard

The University of British Columbia
November, 1966

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ABSTRACT

The conversion of ammonia to 2:4-dinitroaniline by reaction with 1-fluoro, 2:4-dinitrobenzene and the subsequent conversion of dinitroaniline to a diazo-dye with N-(1-naphthyl)ethylenediamine has been investigated as an analytical method for determining ammonia at the micromolar concentration level. Particular emphasis was placed upon the possibility of applying this method to the analysis of sea water. Dinitroaniline was formed under alkaline conditions (pH greater than 8) and required the presence of the fluoro-dinitrobenzene as a separate phase for initiation of the reaction. The conversion, which was light sensitive, was accelerated by increases in pH and temperature, but neither of these factors improved the final yield. The yield of dinitroaniline had a marked dependence on the amount of fluorodinitrobenzene; initially increasing with increasing fluorodinitrobenzene content, it then decreased with higher fluorodinitrobenzene concentrations suggesting further reaction between dinitroaniline and fluorodinitrobenzene. In both distilled and sea water, the maximum yield of dinitroaniline from solutions containing ammonia at the micromolar concentration level was found to be 55-58%. The absorbance of the diazo-dye in sea water of salinity 30.4 ‰ was only 42% of that observed in distilled water. Although sufficiently sensitive for application to sea water analysis, the precision of the conversion of dinitroaniline to the diazo-dye in sea water was poor compared to that achieved in distilled water.

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ACKNOWLEDGMENTS

I wish to thank my supervisor, Dr. E. V. Grill, for his advice and co-operation.

I am indebted to the Institute of Oceanography for financial support throughout the course of my studies.

INTRODUCTION

The purpose of this research was to investigate the use of 1-fluoro, 2:4-dinitrobenzene as an analytical reagent for ammonia. The method studied was the conversion of fluorodinitrobenzene to dinitroaniline and the subsequent conversion of this substance to a diazo-dye. Of primary interest was the determination of ammonia at the micromolar concentration levels found in natural waters, particularly sea water where the ammonia concentration ordinarily is between 0 and 3 micromoles/litre.

The Nessler method commonly used for the estimation of ammonia in water is not, generally, directly applicable to sea water analysis. Although the interference due to the magnesium ion can be avoided by precipitation or complexing, the method lacks precision because of the colloidal nature and instability of the coloured product (Wirth and Robinson, 1933). The methods developed for the analysis of sea water may be differentiated into two classes. In the first, ammonia is separated from the sample by distillation (Krogh, 1934; Riley, 1953; Gillbricht, 1963) or diffusion (Riley and Sinhaseni, 1957), captured in dilute acid and finally determined by a colorimetric technique. Distillation or diffusion circumvents the interferences due to other non-volatile substances present in sea water and provides a method that, because of the possibility of concentrating the ammonia, is generally more sensitive than direct techniques that do not require preliminary separation of ammonia from the sample. Unfortunately, such procedures are time consuming and require equipment not well adapted to routine use on board ship.

The second category consists of colorimetric techniques run directly on the raw sea water samples. The common indophenol-blue test for ammonia

(Riley, 1953; Riley and Sinhaseni, 1957) is not directly applicable to sea water analysis due to the precipitation of magnesium and calcium in the alkaline pH range at which the reaction occurs. Newell and Dal Pont (1964) avoided this interference by converting the ammonia to quinone chlorimide, extracting with hexanol and completing the formation of the indophenol in the organic solvent. In an alternate method devised by Roskam and de Langen (1964), magnesium and calcium precipitation was avoided by use of a chelating agent. By substituting thymol for phenol, a more stable and more sensitive dye was formed than that obtained in the indophenol-blue method of Riley and Sinhaseni (1957). The pyridine-pyrazolone method of Kruse and Mellon (1953) has been modified for sea water analysis by Atkins (1957) and Strickland and Austin (1959). Richards and Kletsch (1964) described a procedure in which ammonia is oxidised to nitrite and the nitrite is subsequently assayed as a diazo-dye. Although reported to have a precision as good as or better than distillation procedures, the method is subject to interferences since the amino nitrogen of a number of amino acids was shown to also undergo oxidation to nitrite.

1-fluoro, 2:4-dinitrobenzene (FDNB) was first employed by Sanger (1945) for the purpose of identifying the terminal groups of proteins. Its use has since been extended to the analysis of various nitrogenous compounds such as amino acids (Levy, 1954; Rapp, 1963) and amines (Lockhart, 1956). Palmork (1962) used FDNB to isolate and identify microgram quantities of amino acids from sea water.

Levy (1954) and Lockhart (1956) reported that FDNB reacts with ammonia at about pH 9. By determining the dinitroaniline formed by absorption

spectrophotometry following its isolation by paper chromatography, Bradbury (1960) successfully employed this reaction to estimate small amounts of ammonia. Such a procedure, however, would appear to be too cumbersome for routine analytical work. The possibility of converting dinitroaniline to an intensely coloured diazo-dye suggested an alternative procedure; one by which the reaction between FDNB and ammonia might be made the basis of a sensitive and interference free test for ammonia.. It was hoped that such a test would prove applicable to sea water analysis.

APPARATUS AND REAGENTS

Absorbance measurements were made with a Beckman model DU spectrophotometer, using a slit width of 0.01 mm and matched quartz cells of either 1 cm. or 5 cm. optical path length.

Ammonia free water (afterwards referred to as distilled water) was obtained by passing distilled water through a mixed bed ion exchange resin column and finally through Dowex 50 immediately before use.

Standard dinitroaniline solution was prepared by dissolving 0.020 gm of 2:4-dinitroaniline in distilled water containing 100 ml of concentrated sulphuric acid and adjusting the volume to 1 litre. Ten-fold dilution gave a working solution containing 9.11 micromoles dinitroaniline / litre.

Naphthylethylenediamine solutions were prepared by diluting a stock solution containing 0.30 gm of N-(1-naphthyl)ethylenediamine dihydrochloride in 100 ml distilled water. The stock solution was stored in an amber glass bottle in which it is stable over periods of several weeks (Bendschneider and Robinson, 1952).

Standard ammonia solution was prepared by dissolving 0.114 gm of ammonium sulphate in 1 litre of distilled water. The resultant solution contained 1.73 micromoles ammonia/ml. This solution was made up freshly each two days.

Saturated sodium borate solution was prepared from sodium borate and distilled water and was boiled prior to use to expel any ammonia that might have been present.

Sodium nitrite solution was prepared by dissolving 1 gm of sodium nitrite in 100 ml distilled water.

1-fluoro, 2:4-dinitrobenzene was added to the samples as the pure liquid (m.p. 25-27°C).

EXPERIMENTAL

The method of analysis relies on two separate steps; firstly, the conversion of ammonia to 2:4-dinitroaniline and, secondly, the quantitative estimation of dinitroaniline by the development of a diazo-dye. Before investigation of the first step could proceed, it was necessary to develop adequate methods for carrying out the second step.

The coupling agent selected was N-(1-naphthyl)ethylenediamine. This reagent had previously been employed as a coupling agent by Bendschneider and Robinson (1952) and Richards and Kletsch (1964) and was considered suitable for this work since it forms intensely coloured dyes and couples under the strongly acidic conditions required to diazotise dinitroaniline. Phenolic agents, which couple under mildly alkaline conditions, were found to be less satisfactory because of the difficulty of neutralizing the initially strongly acidic diazotisation mixture to a reproducible pH. The dye formed from dinitroaniline and naphthylethylenediamine is rose-red, is stable under strongly acidic conditions and has an absorption maximum at 525 m μ which is sufficiently intense to readily differentiate between micromolar concentrations of dinitroaniline (Fig. 1).

To obtain quantitative conversion of dinitroaniline to its diazonium salt and ensure that the yield is independent of the nitrite concentration, diazotisation must be carried out in the presence of an excess of nitrite. Nitrite, however, interferes during the subsequent coupling reaction since naphthylethylenediamine not only reacts with diazonium salts, but also with nitrite (Benschneider and Robinson, 1952) forming, in the latter case, a mauve coloured material with an absorption peak at 565 m μ (Fig. 1). It was found that sodium sulphite reduced this mauve product to a near-colourless material with no appreciable absorbance at 525 m μ and that the

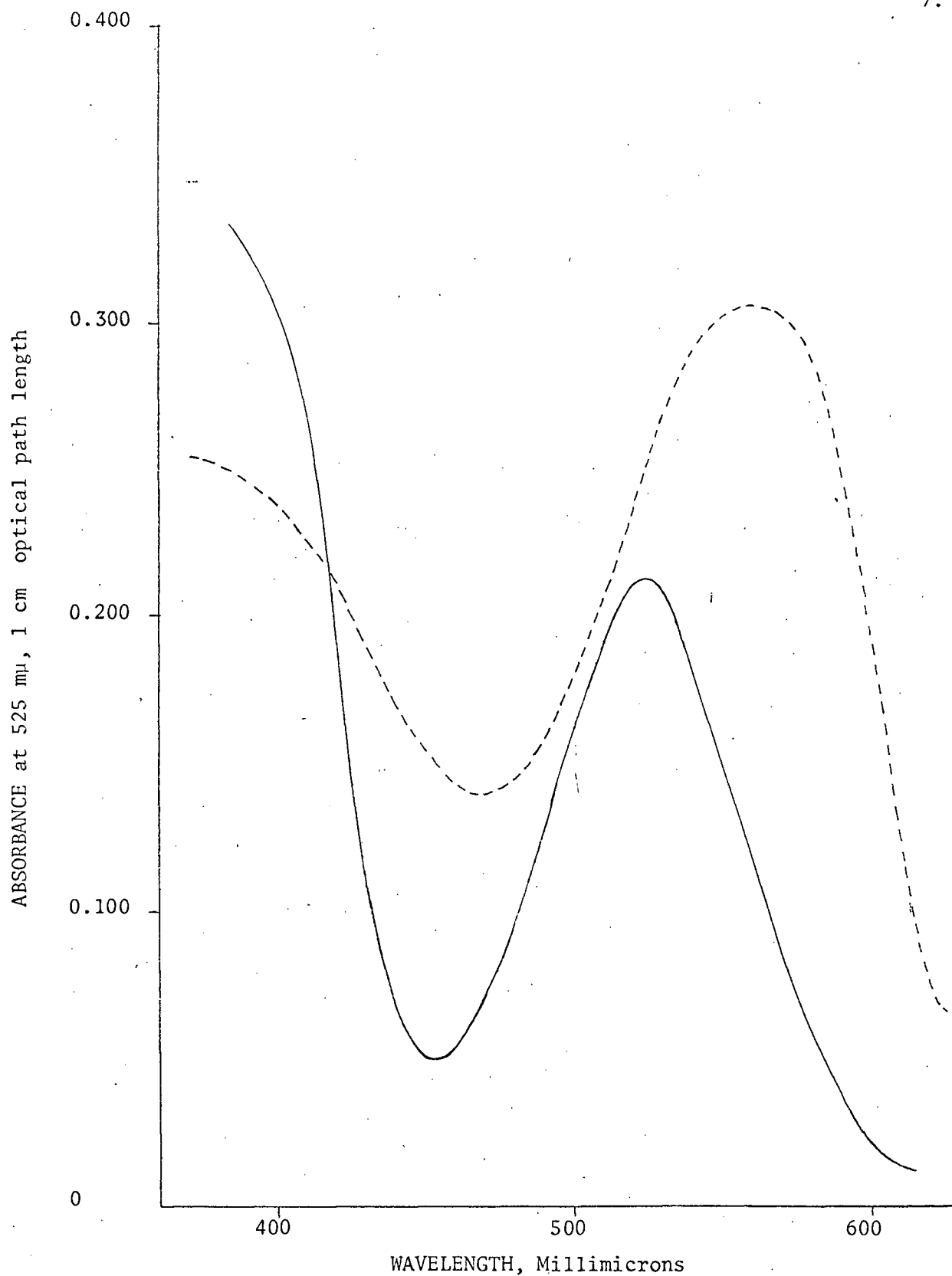


Fig. 1. The spectra of the diazo-dye formed from naphthyl-ethylenediamine and dinitroaniline (solid line) and the compound produced by the reaction of nitrite with naphthylethylenediamine (dashed line).

sulphite could be added in solution along with naphthylethylenediamine without interfering in the formation of the dizao-dye.

Before attempting to apply this procedure to sea water, the optimum conditions for carrying out the reactions in distilled water were studied.

I. Diazotisation and Coupling of Dinitroaniline in Distilled Water

(a) General Procedures

The optimum conditions for diazotisation and coupling were determined using a standard solution containing 9.11 micromoles/l dinitroaniline. Sulphuric acid- either concentrated or diluted 1:1 with distilled water- was added by pipette to 10 ml of the standard dinitroaniline solution in a glass-stoppered graduated cylinder and the final volume was brought to 20 ml with distilled water. The heat generated by the addition of the acid was dissipated by holding the samples under running tap water. Diazotisation was effected by adding 2 drops of a 1% sodium nitrite solution and coupling by the addition of 1 ml of an aqueous solution containing N-(1-naphthyl)ethylenediamine dihydrochloride and sodium sulphite.

(b) Sulphite Concentration

The optimum sulphite concentration preventing interference from the side reaction between nitrite and the coupling agent was determined by taking 15 ml of distilled water and adding 5 ml of concentrated sulphuric acid. After cooling to about 10-15°C, 2 drops of 1% sodium nitrite solution were added followed by 1 ml of a solution containing 0.01% of naphthyl-dethylenediamine and varying amounts of sodium sulphite. This second solution was added by means of a syringe pipette. The samples were mixed after each addition by inverting the glass stoppered cylinders in which they were contained.

The affect of sodium sulphite concentrations ranging between 0.1% and 5% was observed. It was found that a 2% sodium sulphite solution gave a near colourless blank which faded in a few seconds to yield a pale

green solution with no appreciable absorbance at 525 m μ . With lower sulphite concentrations the mauve coloured product faded less rapidly. In all cases, the mauve material could be regenerated through aerial oxidation when the samples were agitated, but the remaining sulphite again bleached out this colour when the samples were allowed to stand. Using a 2% sulphite solution, it was found that filling the spectrophotometer cells at least 10 minutes before readings were to be made ensured complete removal of the interference. Higher sulphite concentrations offered no improvement and were, in any case, undesirable since they might endanger the success of the method by reduction of the diazonium salt. The coupling solution was made up freshly each day from solid sodium sulphite and a stock solution of N-(1-naphthyl)ethylenediamine.

c) Naphthylethylenediamine Concentration

The naphthylethylene diamine concentration necessary to produce maximum colour development was determined by taking 10 ml aliquots of the standard dinitroaniline solution and making these up to 20 ml with sulphuric acid diluted 1:1 with distilled water. The solutions were cooled to about 10-15°C and 2 drops of a 1% sodium nitrite solution were added. After standing for 10 minutes, 1 ml of a solution containing 2% sodium sulphite and naphthylethylenediamine in concentrations ranging from 0.005% to 0.08% was admitted by means of a syringe pipette. Time series observations established that the dye had obtained maximum development after about 15 minutes and was stable for periods of at least an hour. To compare the affect of varying the naphthylethylenediamine concentrations on the final colour intensity, readings were taken against a distilled water optical

blank at 525 m μ in a 1 cm cell after the colour had been allowed to develop for 30 minutes. These readings were compared with reagent blanks prepared by substituting distilled water for the dinitroaniline. The results, depicted in Fig. 2, indicated that maximum colour intensity could be achieved when the coupling solution contained 0.03% N-(1-naphthyl) ethylenediamine dihydrochloride. This concentration, in conjunction with a 2% sodium sulphite concentration, was employed in subsequent studies.

(d) Time and conditions of diazotisation

The affect of the time of diazotisation upon the final colour intensity of the diazo-dye was studied for solutions prepared by the addition of 10 ml of 1:1 sulphuric acid to 10 ml of the 9.11 micromolar dinitroaniline solution. The samples were cooled to about 10-15°C and 2 drops of a 1% sodium nitrite solution were added. After intervals between 2 and 25 minutes, 1 ml of the coupling solution was added and the absorbance of the dye was measured at 525 m μ in cells of 1 cm optical path length after the colour had developed for 30 minutes. The results, shown in the table below, indicated that the diazonium salt was formed quickly and was stable under the experimental conditions for periods up to 20 minutes. To ensure maximum colour development, it was decided to add the coupling solution 10 minutes after the addition of the nitrite solution. It was observed that the absorbance did not increase significantly if the diazotisation and coupling were carried out at 0°C.

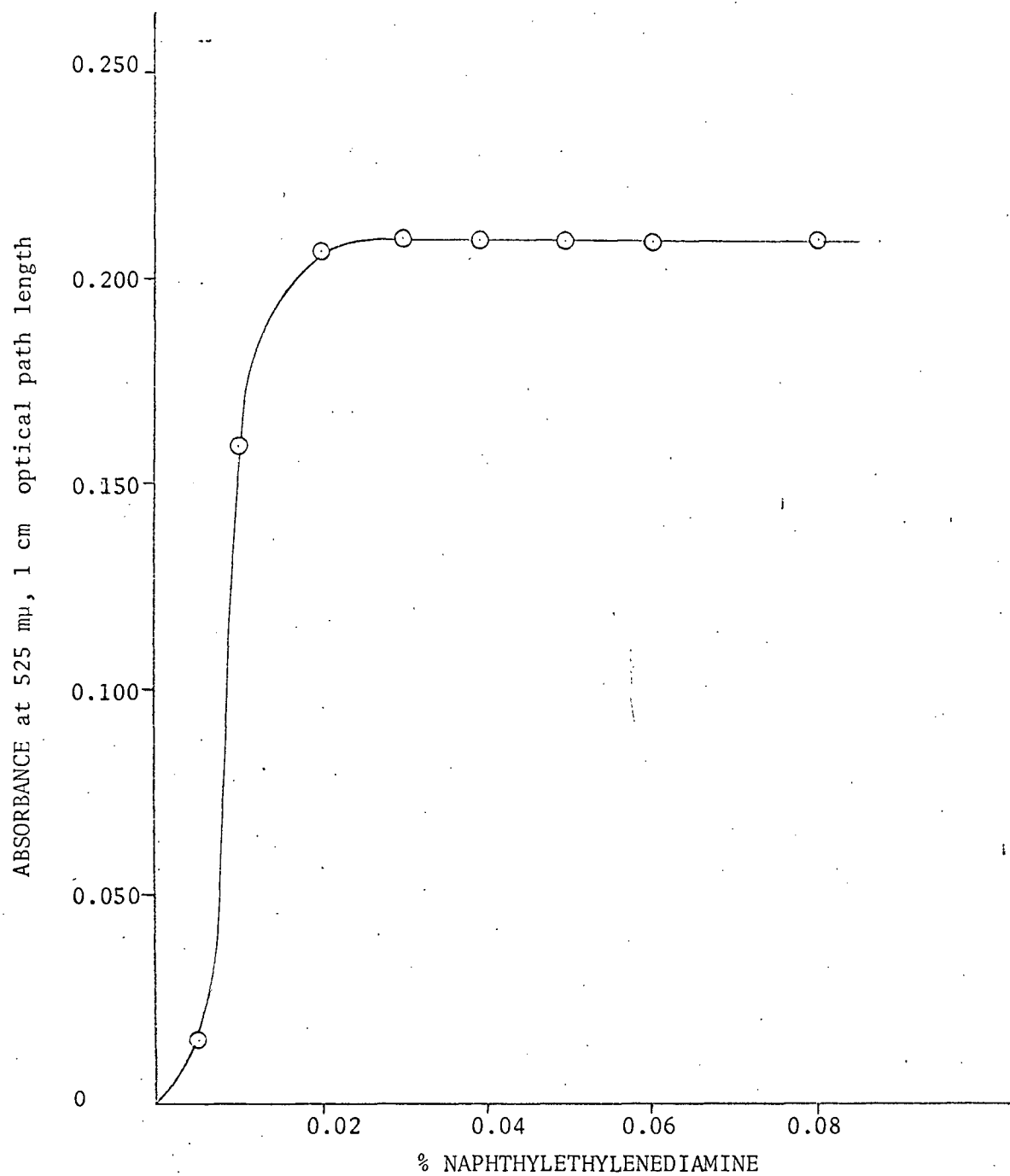


Fig. 2.

The variation in the absorbance of the diazo-dye with naphthylethylenediamine concentration. Dinitroaniline concentration, 9.11 micromoles/l.

Table I. The rate of formation and stability of the diazonium salt
of 2:4-dinitroaniline.

<u>Time of diazotisation</u> <u>(minutes)</u>	<u>Absorbance</u>
2	0.170
5	0.205
8	0.201
10	0.208
12	0.210
15	0.205
20	0.207
25	0.202

(e) Acid Concentration

The stability of the diazo-dye was found to be dependent upon the final acid concentration of the solution. Tests were conducted by adding varying amounts of concentrated sulphuric acid to 10 ml of the standard dinitroaniline solution and bringing the samples to a final volume of 20 ml with distilled water. Diazotisation was affected by the addition of 2 drops of 1% sodium nitrite solution and the coupling solution was added after 10 minutes. The development of the diazo-dye was followed with time by measuring its absorbance at 525 m μ in a 1 cm cell versus a distilled water optical blank. The results are shown in Fig. 3. When less than 3 ml of concentrated sulphuric acid were present, the absorbance of the solution rapidly achieved a maximum and then faded. As the acid

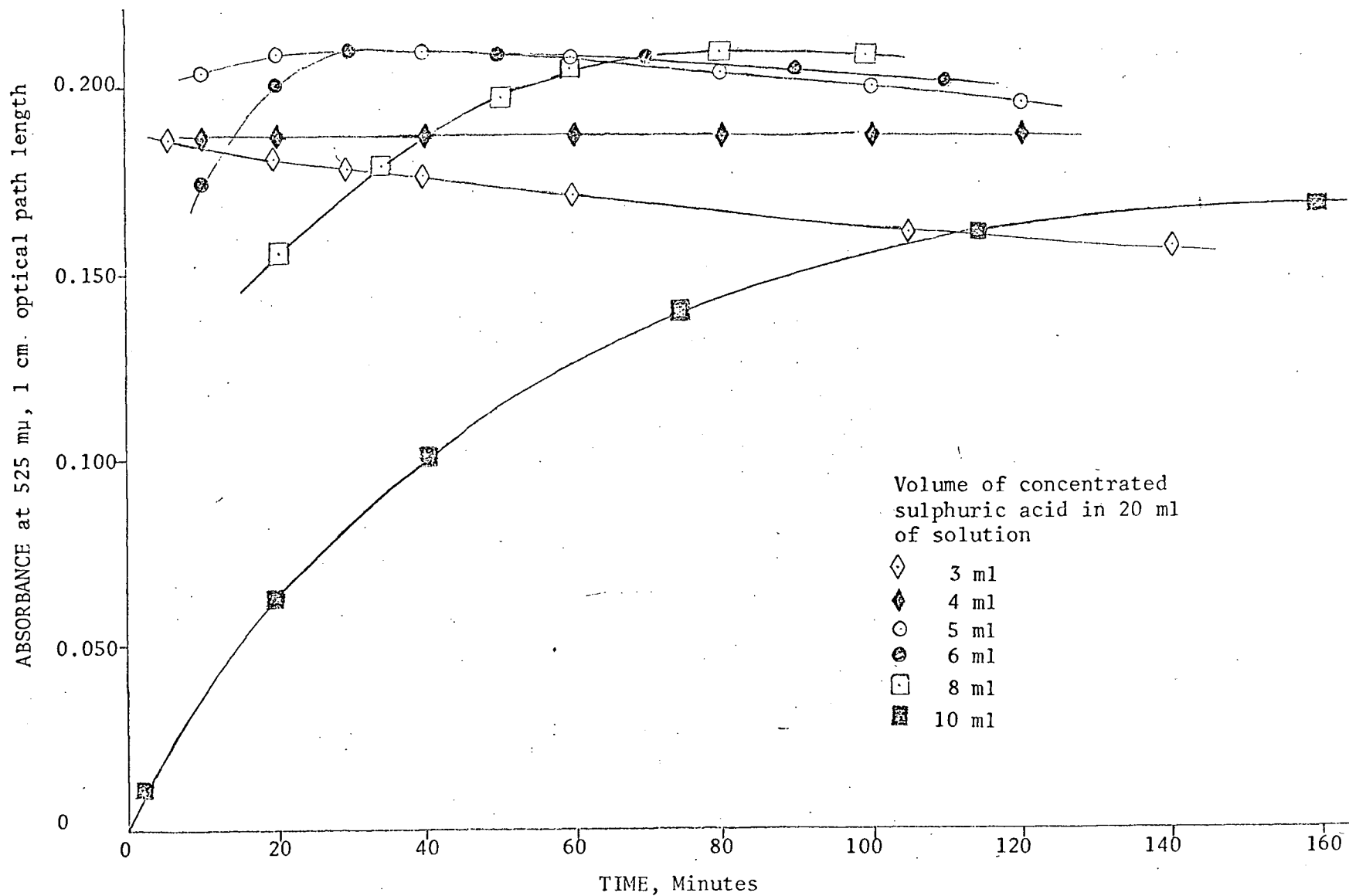


Fig. 3. The time dependence of the absorbance of the diazo-dye formed in solutions of varying sulphuric acid concentration. Dinitroaniline concentration, 9.11 micromoles/l

concentration increased, the colour was formed less rapidly, but remained more stable. In the case where 5 ml. of acid were present, maximum colour intensity was obtained in less than 20 minutes whereas with 10 ml of acid maximum development was achieved only after 160 minutes. It was verified for all acid concentrations that: a) reagent blanks in which distilled water was substituted for the dinitroaniline solution had a negligible absorbance; b) that diazotisation was accomplished within a few minutes at 10-15°C and that diazonium salt was stable for periods of up to 20 minutes. These results indicated that acid concentrations approximately 5 ml of concentrated sulphuric acid in a total volume of 20 ml would be suitable for further work provided that the absorbance was recorded 30 minutes after the addition of the coupling agent.

f) Summary

Concentrations of dinitroaniline in the range 0-10 micromoles/l can be estimated by the following procedure.

To 10 ml of the dinitroaniline solution to be analyzed add 10 ml of 1:1 sulphuric acid and cool to 10-15°C. Add 2 drops of a 1% sodium nitrite solution and, after 10 minutes, 1 ml of a solution containing 0.03% N-(1-naphthyl)ethylenediamine dihydrochloride and 2% sodium sulphite. Measure the absorbance at 525 mμ in a 5 cm cell after colour development has proceeded for 30 minutes and compare with a reagent blank. The spectrophotometer cells should be filled at least 10 minutes before readings are to be made.

The selection of a 10 minute interval between the addition of the nitrite and coupling solution, respectively, was justified by the

consistency of the results obtained. The relationship between the intensity of the dye produced and the amount of dinitroaniline present was studied using the method of analysis described above (recording the absorbance in 5 cm cells) and was found to be linear (Fig. 4).

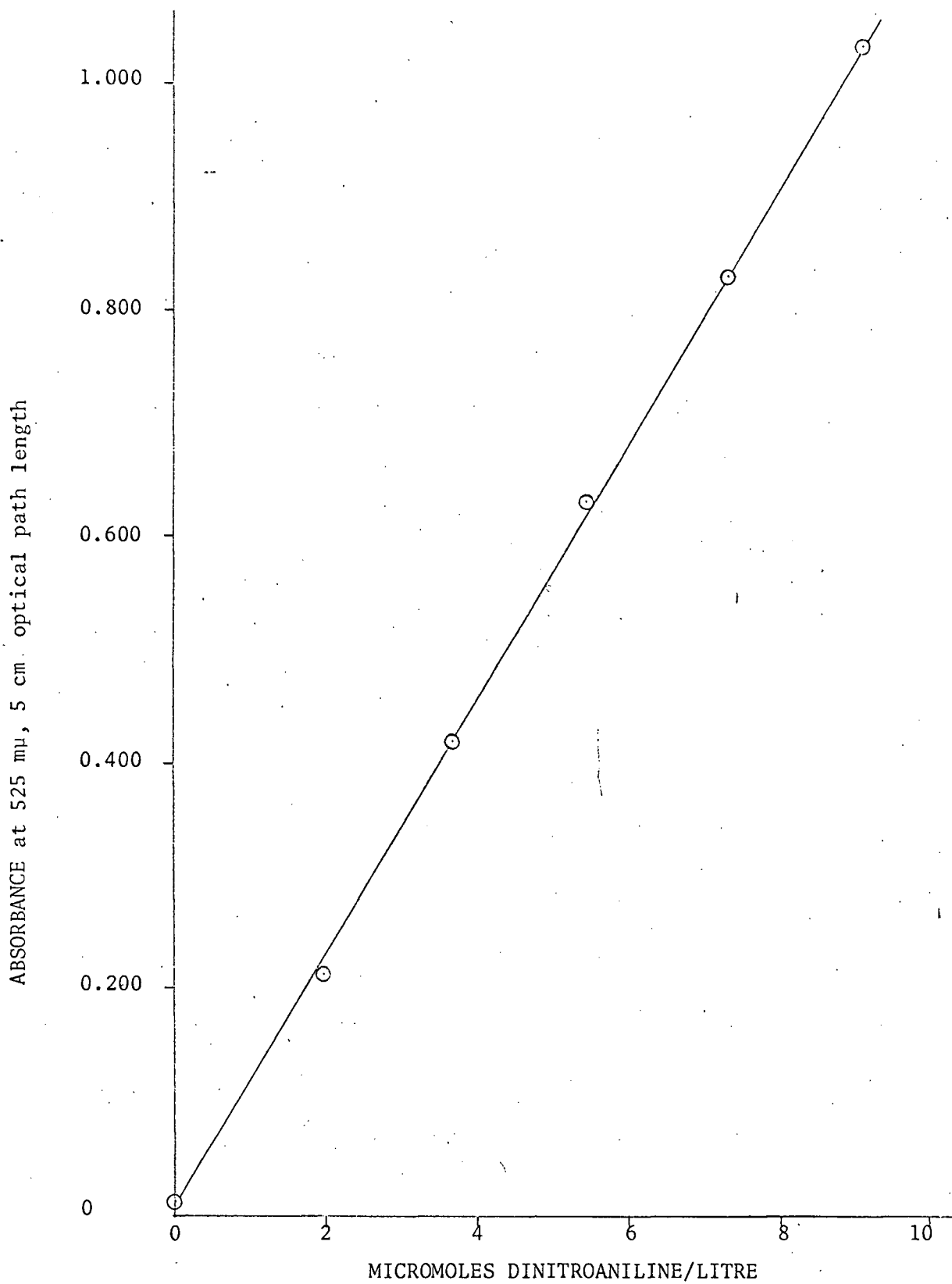


Fig. 4.

The relationship between the absorbance of the diazo-dye formed in distilled water and the dinitroaniline concentration.

II. The Formation of Dinitroaniline in Distilled Water

Previous work (Levy, 1954; Palmork, 1962) indicated that FDNB reacts with nitrogenous compounds under mildly alkaline conditions (pH 8-10). In the following experiments test samples were made up by diluting 1 ml of standard ammonia solution to 100 ml with distilled water giving a solution 17.3 micromolar in ammonia, and adding 2 ml of saturated sodium borate solution to buffer the solution to pH 9.3.

FDNB has generally been employed by the addition of either an acetone or ethanolic solution (Sanger, 1945; Bradbury, 1960). This practice was followed in the initial experiments, adding 1 ml of a 2% solution of FDNB in acetone or ethanol to 100 ml of test sample contained in tightly stoppered clear or light-tight glass bottles. The samples were agitated at room temperature either by a magnetic stirrer or a Burrel wrist action shaker and 10 ml aliquots were withdrawn at various times for dinitroaniline analysis. Using the previously described procedure, no dinitroaniline could be detected, even after the experiments had been allowed to proceed for as long as 48 hours. Further experiments in which the initial pH of the sample containing borate was adjusted over the range of 7.5 to 12 by the addition of sodium hydroxide or hydrochloric acid, respectively, indicated that no dinitroaniline was formed when the FDNB was added in solution.

When, however, pure liquid FDNB was added so that a two phase system was produced, slow conversion of ammonia to dinitroaniline was obtained. The two phase system existed for only about one quarter of the time necessary to obtain maximum development of dinitroaniline. By substituting 1 ml of a 2% aqueous dinitrophenol solution for liquid FDNB and conducting the experiment under identical conditions, no dinitroaniline was observed

even after 72 hours indicating that FDNB was necessary for the reaction to proceed. As observed in other work with this compound, the reaction was found to be sensitive to light (Peraïno and Harper, 1961; Pataki, 1964). When carried out in amber glass bottles, conversion to dinitroaniline occurred; no dinitroaniline was formed when the reaction was conducted in clear glass bottles.

At room temperature, with a sample containing 0.1 ml of FDNB per 100 ml of test solution and pH adjusted to 9.3 by the addition of 2 ml saturated sodium borate, maximum conversion was achieved after a period of 32 hours. Based on the absorbance of solutions of known dinitroaniline content, 55% of the ammonia originally present had been converted to dinitroaniline. It was consistently found that 45% conversion was obtained after 24 hours.

a) Amount of FDNB

The affect of varying the FDNB content on the yield of dinitroaniline after 24 hours at pH 9.3 was studied. As shown by Fig. 5, the amount of dinitroaniline formed increased in approximately linear proportion to the FDNB up to a maximum of 0.1 ml FDNB/100 ml sample. Further increases in the amount of FDNB resulted in a decreased yield of dinitroaniline. The addition of 0.1 ml FDNB to 100 ml of the test solution consistently gave 45% conversion after 24 hours, and thus, this volume of the reagent was used for subsequent tests. Further observations indicated that the pH of the test solution decreased as the reaction proceeded, presumably due to the hydrolysis of FDNB to dinitrophenol. Tests were carried out to determine the affect of adding more sodium borate in an attempt to maintain the pH of the test solution. It was observed that the amount of dinitroaniline formed was not affected by increasing the amount of borate.

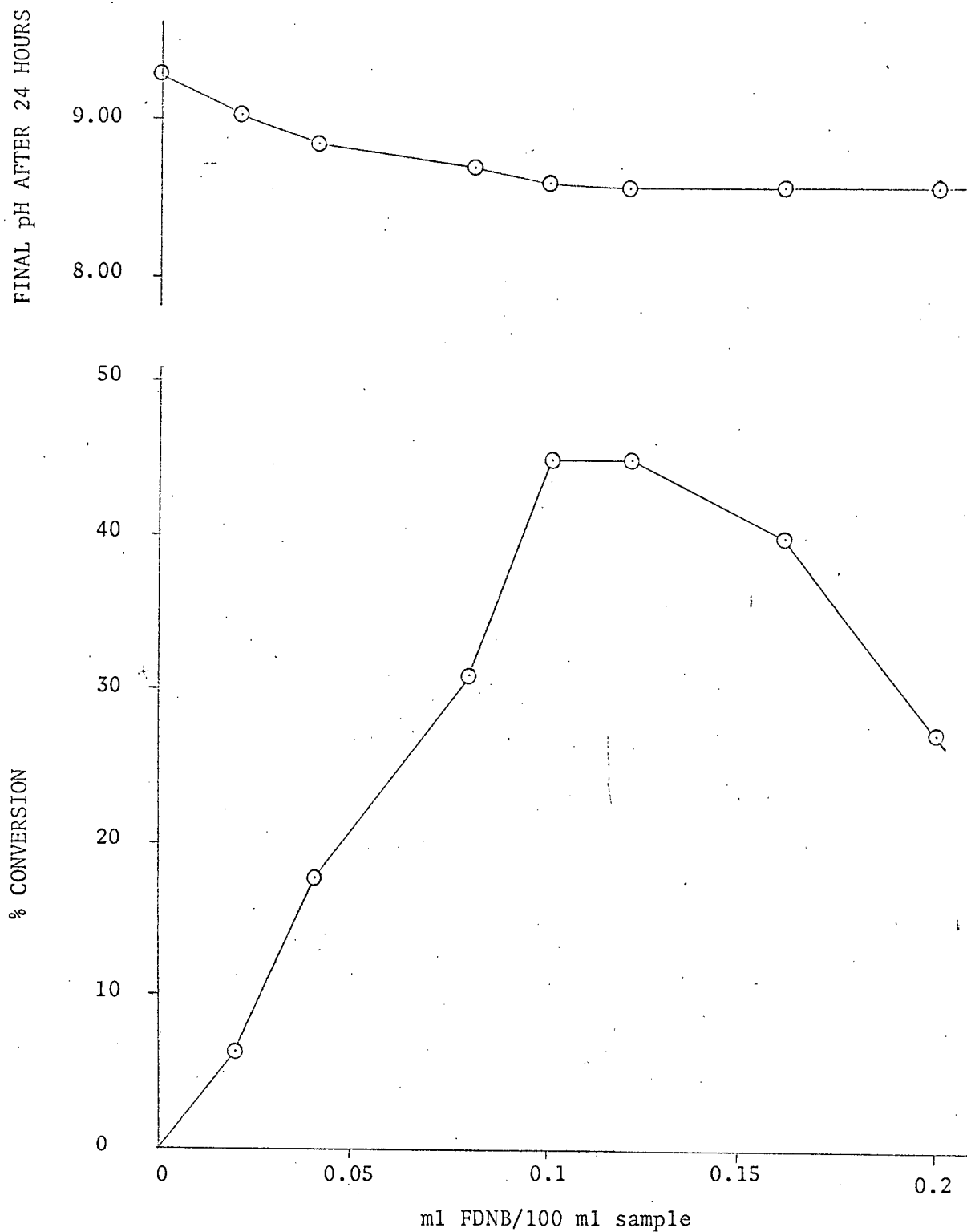


Fig. 5.

The effect of the amount of FDNB on the pH and yield of dinitroaniline after 24 hours. Ammonia concentration, 17.3 micromoles/l.

b) pH

Tests were conducted to observe the affect of pH on the reaction. 2 ml of saturated sodium borate solution were added to 100 ml of distilled water and the pH of the solution was adjusted by the addition of hydrochloric acid or sodium hydroxide, respectively. 0.1 ml of FDNB and 1 ml of the standard ammonia solution were added and the reaction was allowed to proceed for 24 hours with shaking. After this period 10 ml samples were withdrawn and analyzed for dinitroaniline. In each case a reagent blank was run. As shown in Fig. 6, below pH 8, the amount of conversion was insignificant; above pH 8, the yield increased with pH until a maximum was reached at about pH 9.5. Further increase in pH did not improve the yield. By allowing the reaction to go to completion, it was observed that 55% conversion of ammonia to dinitroaniline was achieved for all samples with an initial pH greater than 8.0. At pH 10 maximum conversion was obtained after 28 hours, 4 hours quicker than at pH 9.3.

c) Temperature

Increasing the temperature produced a marked acceleration in the rate of formation of dinitroaniline. Tests were conducted by shaking the reaction vessels in a thermostatically controlled water bath. At higher temperatures the same maximum percentage conversion, 55%, as at room temperatures was consistently obtained. At 50°C and pH 9.3 the reaction reached completion after about 8 hours; at pH 10 the period necessary to achieve maximum conversion was reduced to less than 3 hours.

d) Relationship between the Yield of Dinitroaniline and the Ammonia Concentration.

A series of standard ammonia solutions was prepared in distilled

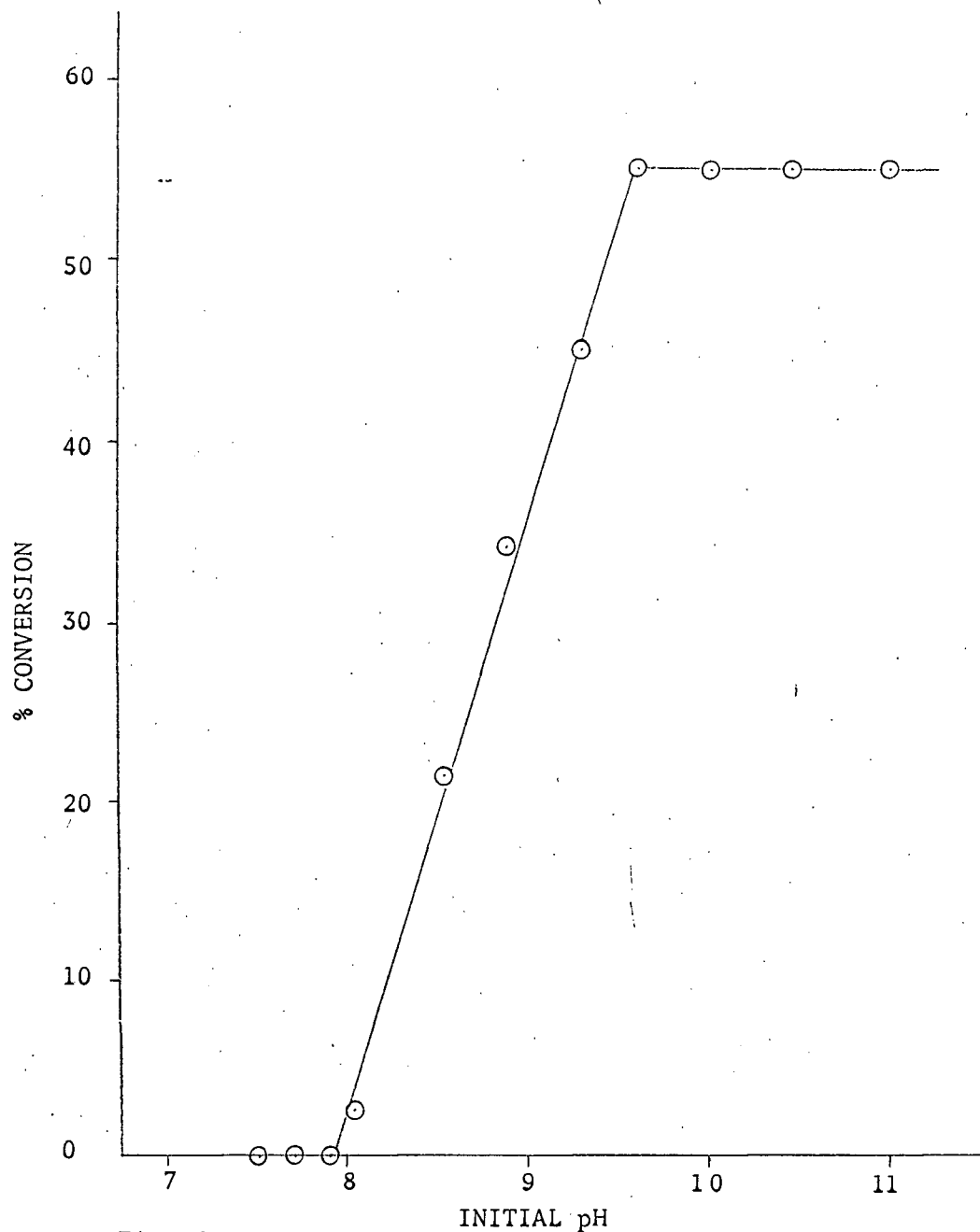


Fig. 6.

The dependence of the yield of dinitroaniline in distilled water after 24 hours on the initial pH. Ammonia concentration, 17.3 micromoles/l.

water and 100 ml samples were treated at 50°C with 2 ml of saturated sodium borate and 0.1 ml FDNB. After 10 hours, 10 ml aliquots were withdrawn for dinitroaniline analysis. The results are depicted in Fig. 7. Absorbancies were measured in 5 cm cells at 525 mμ. The linear relationship between concentration and absorbance indicated that the yield of dinitroaniline was independent of ammonia concentration for solutions up to at least 12 micromolar in ammonia concentration and, thus, that small quantities of ammonia can be detected and differentiated by this procedure. Moreover, the method appeared to be sufficiently sensitive for application to sea water.

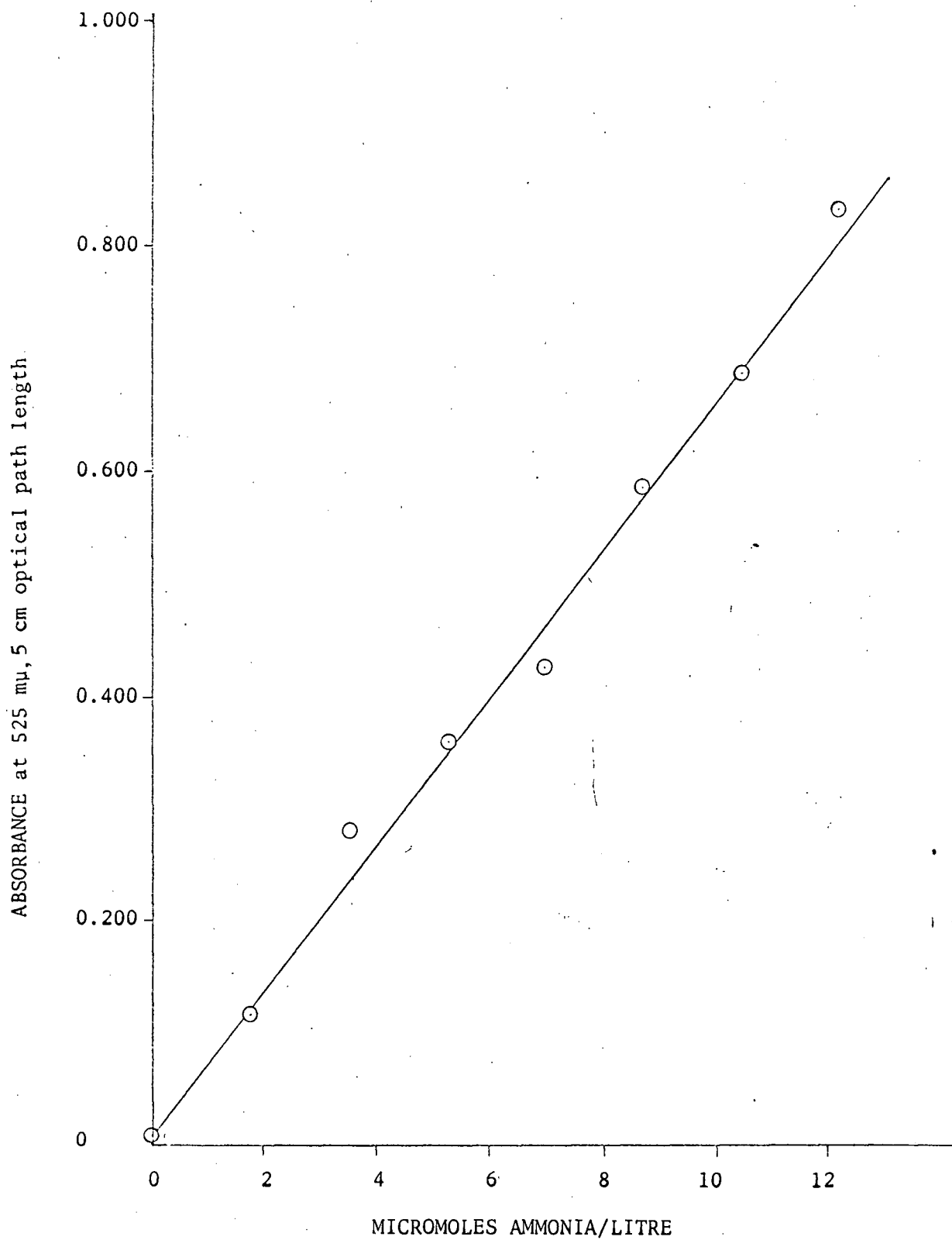


Fig. 7.

The relationship between the absorbance of the diazo-dye in distilled water and the ammonia concentration.

III. Application to Sea Water

Tests were conducted to determine the applicability of the procedures developed to sea water systems. In order to investigate the effect of sea water upon the diazotisation and coupling steps, stock dinitroaniline solution was diluted ten-fold with Pacific Ocean water of salinity 33.6‰ which had been passed through a 0.45 μ Millipore filter. The resultant solution had a dinitroaniline concentration of 9.11 micromole/l and a salinity of 30.4‰. Blanks were prepared by substituting distilled water for the dinitroaniline solution. 10 ml of 1:1 sulphuric acid were added to 10 ml of these solutions. Tests with the blank indicated that the colour resulting from the reaction of the naphthylethylenediamine with nitrite was dissipated much more slowly in sea water than in distilled water. This colour was dispersed within 35 minutes using a 2% sulphite solution and within 20 minutes when the sulphite concentration was increased to 6%. The absorbance of the blank at 525 m μ was small and consistent when the coupling solution contained 6% sulphite and the spectrophotometer cells were filled 25 minutes before reading. Diazotisation of the dinitroaniline occurred quickly in water of this salinity and the diazonium salt was found to be stable over a period similar to that in distilled water. Satisfactory results were obtained by adding 1 ml of a solution containing 0.03% naphthylethylenediamine and 6% sodium sulphite 10 minutes after the addition of 2 drops of 1% sodium nitrite to samples cooled to about 10-15°C. The absorbance of the dye was read at 525 m μ after the colour had developed for 30 minutes. Parallel tests conducted in distilled water indicated that coupling was not affected by the increase in sulphite concentration, but the presence of sea water salts considerably reduced the colour intensity of the diazo-dye (Fig. 8).

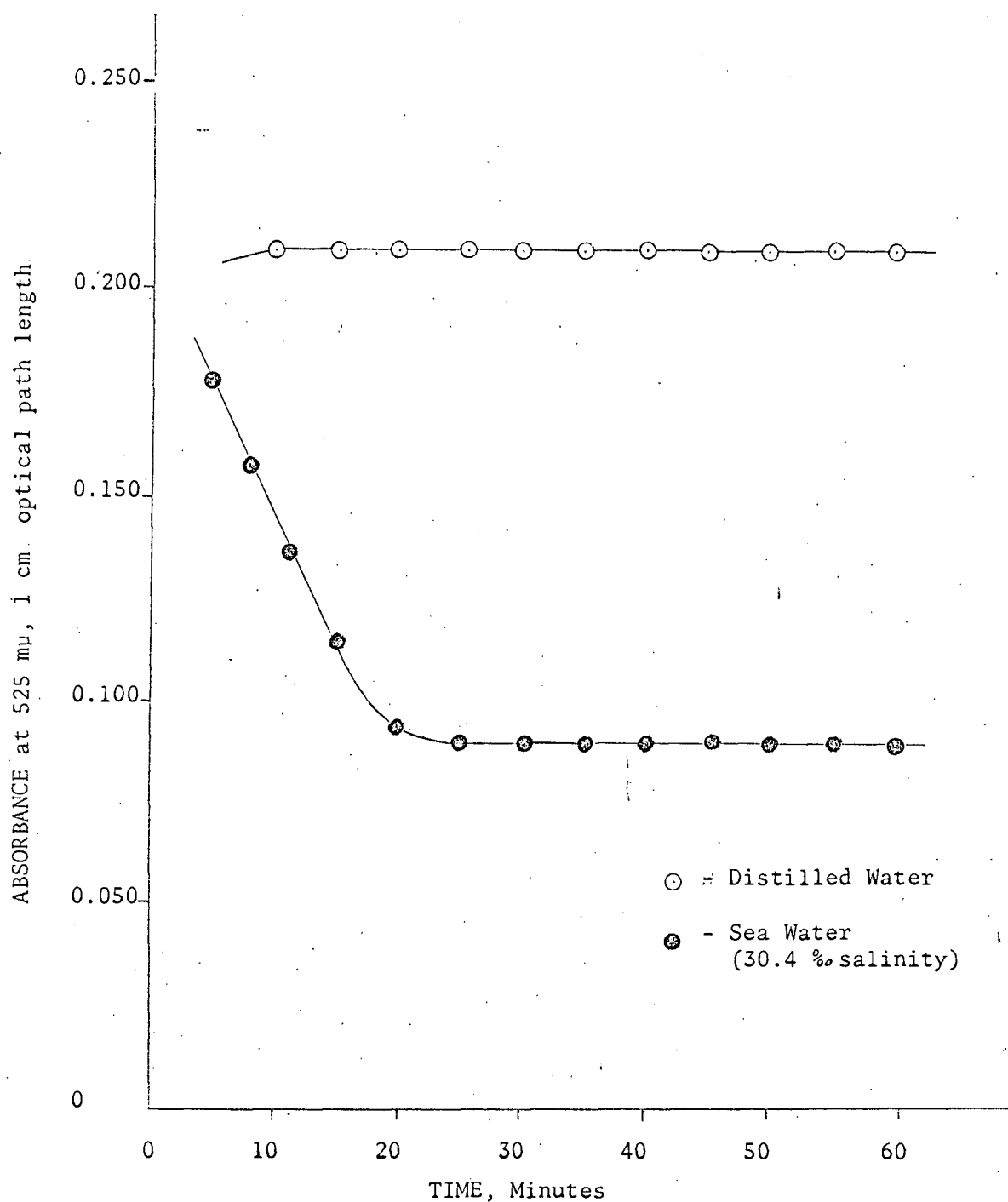


Fig. 8.

The time dependence of the absorbance of the diazo-dye in distilled water and sea water (salinity, 30.4‰). Dinitro-aniline concentration, 9.11 micromoles/l.

The absorbance of the sample was not increased either by increasing the proportion of sulphuric acid in the sample or by the amount of naphthylethylenediamine in the coupling solution.

Although the colour intensity of the dye in sea water of salinity 30.4‰ was reduced to only about 42% of that in distilled water, sensible results could still be obtained in sea water systems by using optical cells of 10 cm path length. A series of dinitroaniline solutions in the concentration range 0-17 micromoles/l was prepared in sea water so as to have a final salinity of 30.4‰. When analyzed as described above, considerable scatter occurred about the best straight line through the points (Fig. 9) suggesting that, under the conditions used, the procedure would not be reliable in sea water. Reductions in the amount of nitrite in the diazotisation step and the addition of sulphite after the coupling agent did not resolve these difficulties.

Tentative results were obtained concerning the conversion of FDNB to dinitroaniline in sea water of salinity 30.4‰. 100 ml of sea water to which 17.3 micromoles/l ammonia had been added was treated with 0.1 ml of FDNB. The solution was buffered by the addition of 2 ml of a saturated sodium borate solution and sodium hydroxide. At room temperature conversion was more rapid at pH 10 than at pH 9 and appeared to reach completion after 24 hours. By comparing the absorbance corrected for a sea water blank with that of a solution 17.3 micromolar in dinitroaniline in water of the same salinity, it was found that there was approximately 58% conversion of ammonia to dinitroaniline.

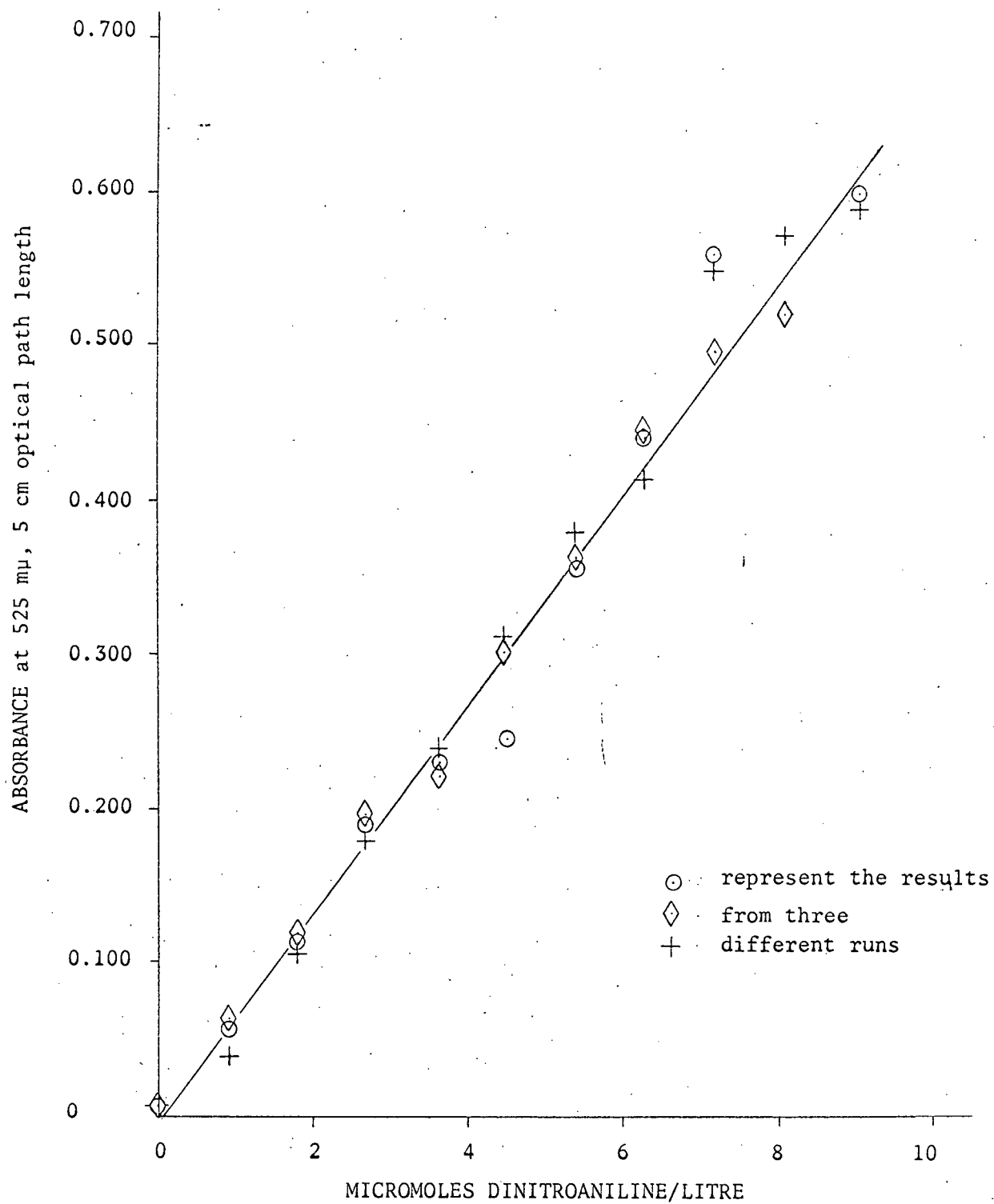


Fig. 9.

The relationship between the absorbance of the diazo-dye formed in sea water (salinity, 30.4‰) and the dinitroaniline concentration.

DISCUSSION

The sensitivity of a spectrophotometric method of analysis is defined by Sandell (1950) as:

"...the number of micrograms of the element, converted to the coloured product, which in a column of solution having a cross section of one square centimetre shows an extinction of 0.001."

The sensitivity of the method under investigation was compared with other techniques devised for the estimation of small quantities of ammonia in aqueous solutions in the table below.

Table II. Comparison of the sensitivity of methods applied to the analysis of ammonia in sea water.

<u>Method</u>	<u>Sensitivity x 10³</u>
FDNB - distilled water	1.04
FDNB - sea water	2.48
Richards and Kletsch (1964)	0.504
Roskam and de Langen (1964)	1.3
Newell and Dal Pont (1964) - distilled water	0.321
Newell and Dal Pont (1964) - sea water	0.512
Riley and Sinhaseni (1957)	2.91

Although not as sensitive as the methods of Richards and Kletsch (1964), Newell and Dal Pont (1964) and Roskam and de Langen (1964), the FDNB technique, because of its simplicity, would have application to sea water analysis if the precision of the dinitroaniline determination could be improved. The procedure of Newell and Dal Pont (1964), although very

sensitive, appears to be too elaborate for routine shipboard use. The methods devised by Roskam and de Langen (1964) and Richards and Kletsch (1964) require strongly basic conditions - pH greater than 11- to oxidise the ammonia. Richards and Kletsch (1964), by running comparisons of this method on natural sea water samples against the distillation method devised by Riley (1953), showed that under these conditions there was a significant error in the ammonia estimated due, presumably, to the degradation of amino acids and amines. Further, in the Roskam and de Langen (1964) method careful preparation of the sample is required in order that all the magnesium and calcium ions present should be chelated with cyclo-hexyl trans 1:2 diaminetetraacetic acid. If even slight precipitation is allowed to occur, low results are obtained.

The possibility of interference from nitrogen containing compounds such as amino acids and amines on the FDNB procedure for ammonia determination was not specifically investigated. It is unlikely, however, that these would significantly contribute to the amount of dinitroaniline recovered. Levy (1954) and Lockhart (1956) indicated that virtually quantitative yields were obtained with primary and secondary amines and amino acids suggesting that little contribution of dinitroaniline would be likely from this source. Sanger (1945) and Bradbury (1960) found that there was no appreciable breakdown of the dinitrophenyl derivatives of amino acids and amines even when refluxed with 20% hydrochloric acid for 8 hours; with histidine, however, the imidazole group reacted slowly with FDNB so that, under the conditions of hydrolysis described above, some 2:4-dinitroaniline was obtained. Under the experimental conditions employed in this study this is an unlikely source of error.

The addition of sodium sulphite to the solution of naphthylethylenediamine used for coupling appeared to successfully remove the interference from the side reaction between nitrite and the coupling agent. Low and consistent absorbancies were obtained for reagent blanks in both distilled water and sea water and, thus, the lack of precision in the determination of dinitroaniline in sea water would not appear to be caused by interference from this side reaction. Furthermore, the results shown in Fig. 8 indicate that the diazo-dye formed stable solutions in sea water as well as in distilled water. The reason for the poor precision of the dinitroaniline determination in sea water, therefore, is obscure. The apparent affect of sea salts on the reaction could possibly be avoided, however, by extracting the dinitroaniline from sea water into an organic solvent, re-extracting into acidified distilled water and conducting the diazotisation and coupling under conditions that were found to give reliable results.

Increases in both temperature and pH significantly accelerated the reaction between FDNB and ammonia, but neither of these factors was found to influence the ultimate yield of dinitroaniline. The yield compares favourably with that obtained by other workers especially when the extreme dilution of the ammonia solution is considered. Levy (1954) found that at 40°C aqueous solutions of amines gave 100% conversion to the dinitrophenyl derivatives within 80 minutes, but could only obtain 25% conversion of ammonia to dinitroaniline even by heating for 7 hours at elevated temperatures. Lockhart (1956) has obtained 50% conversion to dinitroaniline by allowing the reaction to proceed at 105°C for 2 hours and Bradbury (1960) has reported 90% yields by evaporating an ammonium chloride solution, adding potassium

bicarbonate and FDNB and heating the mixture for 2 hours at 50°C. The inability to achieve greater than 58% conversion of ammonia to dinitroaniline could perhaps be explained by the subsequent reaction between the excess FDNB and the dinitroaniline to form a secondary amine. This could also account for the reduced yields of dinitroaniline with increasing amounts of FDNB.

The mechanism of the reaction between FDNB and ammonia is unclear. It was evident that a two phase system was required for initiation since no conversion was observed when the FDNB was added as a solution in ethanol or acetone, although both of these are commonly adopted for the analysis of biological fluids (e.g. Sanger, 1945; Levy, 1954; Bradbury, 1960). Although necessary for the initiation of the reaction, the two separate phases existed for only approximately one quarter of the time necessary to achieve maximum conversion of ammonia to dinitroaniline. At the time of disappearance of the separate phases, very little dinitroaniline had been formed suggesting the production of a relatively long-lived reaction intermediate. The participation of dinitrophenol in the reaction cannot be excluded, but the presence of FDNB is necessary since it was found that dinitroaniline could not be formed from dinitrophenol and ammonia.

Rapp (1963) reported that the reaction of FDNB and amino acids was dependent upon the hydroxyl ion concentration. The effect of the hydroxyl ion was noted in the present case. In the range pH 8.0-9.8 the conversion increased linearly with the increase in pH. In all probability this is due to the effect of pH as the equilibrium between ammonia and the ammonium ion. For this equilibrium

$$K_a = \frac{[H^+][NH_3]}{[NH_4^+]}$$

$$\text{Thus } \frac{[NH_3]}{[NH_3] + [NH_4^+]} = \frac{K_a[OH^-]}{K_w + K_a[OH^-]}$$

$$\begin{aligned} K_w &= 10^{-14} \\ K_b &= 1.76 \times 10^{-5} \\ K_a &= \frac{K_w}{K_b} = 5.68 \times 10^{-8} \end{aligned} \qquad = \frac{5.68 \times 10^{-8} \times [OH^-]}{10^{-14} + 5.68 \times 10^{-8} \times [OH^-]}$$

From this ratio it is evident that there is negligible ammonia present at pH less than 8 and the proportion of molecular ammonia to the total ammonia species increases rapidly with the increase in hydroxide ion concentration, making more molecular ammonia available for nucleophilic reaction with FDNB. Increases in pH to values greater than 9.8 apparently had no further effect upon the rate of formation of dinitroaniline.

CONCLUSION

The investigation showed that FDNB reacted with solutions of ammonia in the micromolar concentration range in both distilled and sea water to form 2:4-dinitroaniline in 55-58% yield. It was found that by converting ammonia to dinitroaniline, followed by the formation of a diazo-dye with N-(1-naphthyl)ethylenediamine, it was possible to determine ammonia in the concentration range 0-12 micromoles/l in distilled water. The scatter observed in attempts to produce a Beers's Law plot for the analysis of dinitroaniline in sea water of 30.4‰ salinity indicated that, as such, the method would be unreliable for the accurate determination of the small quantities of ammonia found in sea water.

Diazotisation of the dinitroaniline occurred quickly in both distilled and sea water and the diazonium salt formed was found to be stable for periods longer than 20 minutes under the conditions of acidity examined. The dye produced by coupling with N-(1-naphthyl)ethylenediamine was highly coloured. It formed quickly, was stable for periods of over 60 minutes and had maximum absorbance when an equal volume of 1:1 sulphuric acid had been added to the sample. In performing the coupling reaction, more consistent results were obtained when the coupling solution was added by means of a syringe pipette than by a gravity pipette. This was accounted for by the more rapid mixing achieved by introducing the coupling solution in the former manner. Reproducible results were obtained in distilled water by cooling the acidified samples to about 10-15°C: the formation of the dye was not enhanced by maintaining the temperature at 0-5°C.

To quantitatively convert dinitroaniline to the diazonium salt, an excess of nitrite was required. The interference resulting from a coloured compound produced by a side reaction between the excess nitrite and the coupling agent was successfully suppressed by adding sodium sulphite to the naphthylethylenediamine solution. Bleaching occurred readily in distilled water, but the interference was more persistent in sea water. In the latter case, however, complete bleaching was achieved by increasing the sulphite concentration. The spectrophotometer cells were filled 25 minutes before readings were to be taken; this period of time allowed complete bleaching of the interference from the sample, including that regenerated by aerial oxidation on filling the cells. The absorbance of the diazo-dye in sea water of salinity 30.4 ‰ was found to be about 42% of that in distilled water.

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