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the Exploration of the Sea

Interlaboratory Report

Information on Techniques and Methods

for Sea Water Analysis

No. 2

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Contents

Preface

Dear Colleagues,

  After the succesful start of our Information Sheet, I am happy to present the second series of contributions. Let us hope that an active exchange of information of this type will continue in the future.

  As usual, the contributions should not be regarded as official publications, but rather as ideas and comments by the individual investigators. The contributions, therefore, should not be cited without prior reference to the authors.

  I should like to draw the attention to the following: As stated in the preface of the first Information Sheet, we should like to see some information on current work in the various laboratories. This would make a closer contact possible between the various investigators. For the next edition we should very much appreciate if the various laboratories of the ICES countries, which are involved in work in marine chemistry, could send a very short summary of what research is going on at the time.

  Looking forward to your future contributions

<div align="right">

for the Subcommittee on
Chemical Analysis of Sea Water

K. Grasshoff

</div>

Note. The Information Sheet will be issued once or twice a year. Contributions for the next issue should be sent to the member of the Subcommittee from the country in question, or directly to the Chairman of the Subcommittee. The present members are:-

  Prof. E. Føyn (Norway)
  Mr. S. Fonselius (Sweden)
  Dr. K. Grasshoff (Germany)
  Dr. P. Jones (United Kingdom)
  Dr. F. Koroleff (Finland)
  Dr. F. Mosetti (Italy)
  Mr. V. Olsen (Denmark)
  Dr. U. Stefánsson (Iceland)
  Dr. A. Simonov (U.S.S.R.)
  Mr. S.B. Tyssen (Netherlands)

An apparatus for the filtration of sea water

by

A.R. Folkard

Fisheries Laboratory, Lowestoft

Over the past few years the Fisheries Laboratory at Lowestoft has carried out many nutrient salt surveys of the Irish and southern North Sea. These areas, especially the inshore regions, are very turbid and contain large amounts of both organic and inorganic sediment. The presence of these sediments gives rise to high turbidity blanks, but more serious errors can occur through chemical decomposition of the suspension during analysis.

Filtration of the sea water samples immediately after collection to remove the suspended matter has been our standard practice when working in these turbid waters. The apparatus shown in Figure 1 has been developed for this purpose. On board ship it may be erected over a sink and takes up very little working space.

The filter papers used are Whatman Glass Fibre GF/C and tests have shown that there is no leaching of phosphate, nitrate or even silicate during filtration.

Description of the apparatus

A 5-cm diameter Steffi-Cole filter fitted with a B24/29 cone is fitted into a vertical receiver adapter, which in turn is fitted to a pear-shaped funnel. The side arm of the adapter is connected by pressure tubing to a three-way tap via a water trap and a moisture trap containing fused calcium chloride to a small vacuum pump.

Operation

Fit a GF/C filter paper (diameter 5.5 cm) into the Steffi-Cole filter. Close tap A, turn tap B to position 1 and switch on vacuum pump. Run about 25 ml of sea water through the filter paper. Open tap A, when air will enter the funnel and thoroughly wash it with filtered sea water. Turn tap B to position 2 and run the small amount of filtered sea water to waste. Close tap A and return tap B to position 1 and resume filtration of sea water sample until sufficient volume is in the funnel. Return tap B to position 2 and run off filtered sample from A as required.

The main advantage of this apparatus is that there is no need to dismantle it once it has been set in position on board ship, and it therefore overcomes the difficulties associated with the use of a conventional Buchner filtration arrangement.
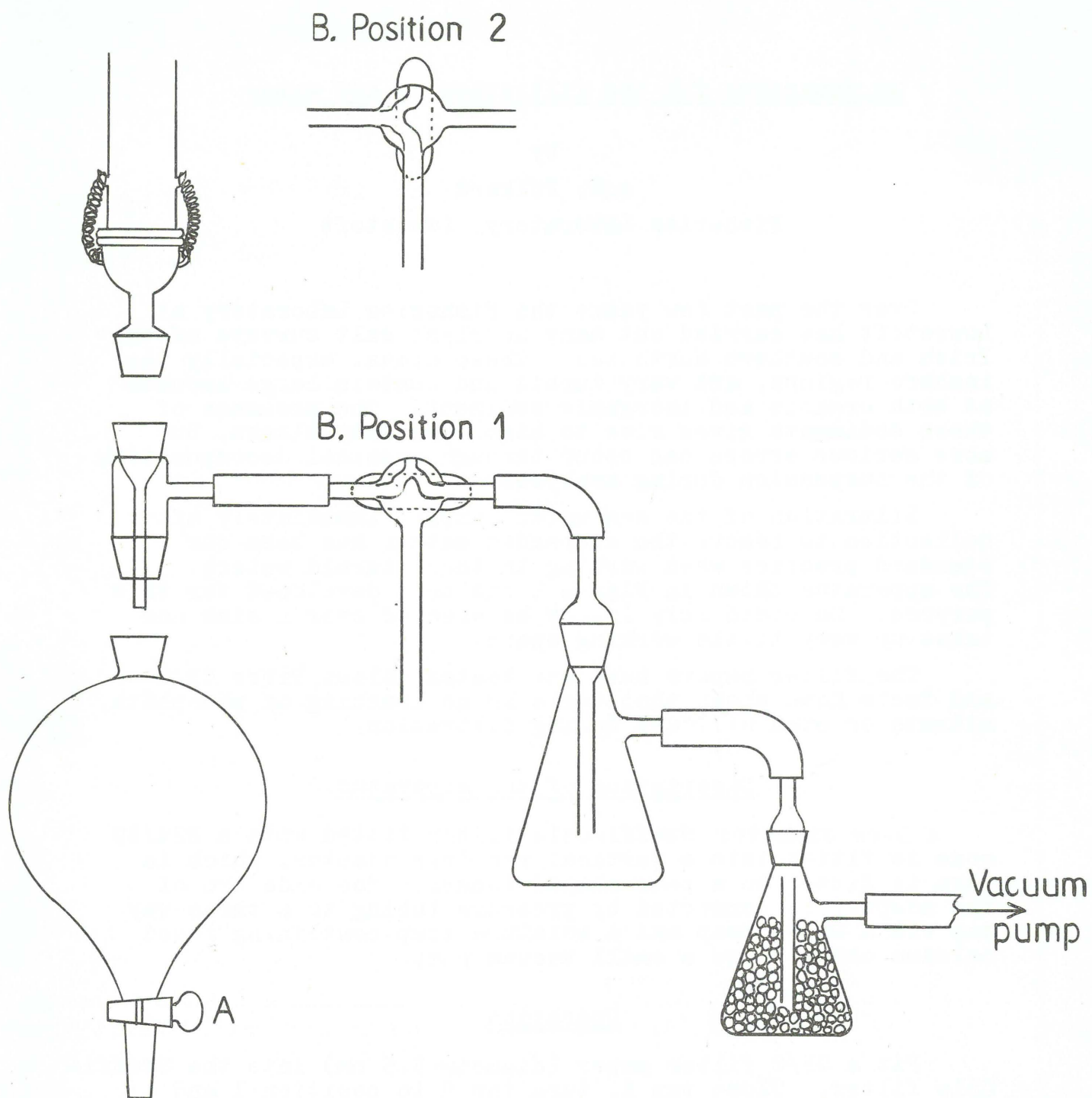
B. Position 2

B. Position 1

A

Vacuum
pump

Fig. 1. Diagrammatic representation of filtration apparatus
(funnel assembly expanded).

Observations on variations in the sensitivity of Murphy and Riley's method of estimating phosphate in sea water

by

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The method of estimating dissolved inorganic phosphate in sea water is now usually determined by Murphy and Riley's (1962) procedure. During a recent survey off the coast of north west Africa by H.M.S. HYDRA, the sensitivity of Murphy and Riley's method was observed to vary, usually being lower than expected. The method was periodically calibrated on unfiltered sea water, low in phosphate, that had been collected and stored in a plastic bucket. To a subsample was added the equivalent of 1 µg-atom PO_4-P/l. The samples, with and without the increment, were then analysed in duplicate for phosphate.

Controlled tests were then made to investigate the nature of this phenomenon. Preliminary investigations established that the variations in sensitivity were not caused by a deterioration of any reagent. Surface sea water was then collected approximately 40 miles west of Cape Verde. Part of the sample was stored in a plastic bucket and part in a pyrex glass flask, both at room temperature. After one day the sensitivity of the method was measured in each sample. Table 1 shows that the sensitivity in the sample stored in polythene was lower than in that stored in glass.

Table 1. The sensitivity of Murphy and Riley's method in sea water stored in plastic and in glass. Extinction measured in a 10-cm cell at 882 mµ in a Unicam S.P.500 spectrophotometer

	Extinction in sea water (A)	Extinction in sea water + 1 µg-atom PO_4-P/l (B)	Sensitivity mean (B-A)
Sea water stored in plastic	0.011 0.011	0.183 0.185	0.173
Sea water stored in glass	0.021 0.021	0.209 0.208	0.187

A subsample from the plastic bucket was then filtered through a Whatman GF/C glass paper. The sensitivity of the method was estimated in the filtered water and the calibration in the unfiltered sample was repeated (Table 2).

Table 2. The sensitivity of Murphy and Riley's method using
filtered and unfiltered sea water from a plastic
bucket. Extinction measured in a lo-cm cell at
882 mμ in a Unicam S.P.500 spectrophotometer

	Extinction in sea water (A)	Extinction in sea water + 1 μg-atom PO_4-P/l (B)	Sensitivity mean (B-A)
Unfiltered sea water	0.014 0.016	0.188 0.191	0.175
Filtered sea water	0.006 0.006	0.193 0.196	0.188

Table 2 shows that the sensitivity in the unfiltered water was
lower than that in the filtered sample. The sensitivity of the
method in both the sea water stored in glass and that from the
plastic bucket after filtration was similar, and of a value to be
expected, from previous experience. In Table 2 the extinction
in the sample without the addition of phosphate was greater in
the unfiltered water than in the filtered sample. A turbidity
was visible to the eye in the unfiltered water although the
sample was clear on collection. Heron (1962) is of the opinion
that polythene encourages the growth of bacteria in fresh water.
Harvey (1948) found that a suspension of bacteria in sea water
reduced the sensitivity by 4 per cent of a method of estimating
phosphate in sea water. His procedure employed a different re-
ductant to that used by Murphy and Riley, but the two methods
are similar in that they both involve the reduction of a phospho-
molybdate complex. The reduced sensitivity of the method observ-
ed during the present investigation would therefore appear to
have been caused by microbiological growth in sea water stored
in plastic. The phenomenon was directly caused by the presence
of micro-organisms in the water, since filtration of the sample
removed the anomaly.

For the standardization of the phosphate method many
workers use sea water that has been stored in a plastic container.
The above observations indicate that the water must be filtered
before use if microbiological organisms have proliferated in
the sample.

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phosphorus in sea waters. J. mar. biol. Ass. U.K., 27:
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storage in polyethylene. Limnol. Oceanogr., 7:316-21.

Murphy, J. and Riley, J.P., 1962. A modified single solution
method for the determination of phosphate in natural
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A note on the estimation of silicate in sea water
using glass reaction vessels

by

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For a number of years dissolved silicate in sea water has
been estimated at this laboratory using the method described
by Strickland (1960).

Until recently, samples were analysed in 50 ml stoppered
graduated glass measuring cylinders supplied by E-mil. New
cylinders were first cleaned by washing in chromic acid as
prescribed by Strickland. However, it was found that the re-
producibility of the method was poor when estimations were
made in these new cylinders.

Table 1 shows a set of results where each sample was sub-
divided and analysed in new and aged cylinders. Samples used
in this test were from inshore stations off Lowestoft.

Table 1. Extinction values for silicate analyses of
Surface (S) and bottom (B) sea water samples
collected off Lowestoft and analysed in new
and old glass cylinders. Extinction measured
in a 10 cm cell at 810 mμ in a Unicam S.P.
500 spectrophotometer

Station		Extinction new cylinder	Extinction old cylinder
19C	S	0.700	1.467
	B	0.935	1.450
20C	S	1.021	1.310
	B	0.310	1.295
21C	S	0.925	1.218
	B	0.815	1.210
19D	S	1.040	1.453
	B	0.940	1.405
20D	S	0.742	1.305
	B	1.145	1.305
21D	S	0.625	1.218
	B	0.632	1.198

In this instance the analyses in the new cylinders resulted
in mainly low extinction values. On other occasions, values above
normal were recorded.

After the new cylinders had been used for approximately five sets of analyses the anomaly disappeared and values with a normal degree of reproducibility would be obtained.

The anomaly could not be caused by silica leaking from the glass into the sample since this would result in extinction values always higher than those analysed in aged cylinders. It is possible that the surface of the glass acted as a catalyst in the reduction of the silico-molybdate complex. A physical or chemical ageing of the glass surface by repeated analyses was necessary before a reproducible degree of reduction could be obtained.

During recent years, silicate analyses at this laboratory have been made using 60 ml capacity polythene bottles as reaction vessels. These bottles give satisfactory results from first use with a sensitivity factor similar to that recorded in aged glass cylinders.

Reference

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Some experiences in the Danish Fisheries Laboratory

by
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Nitrate Method

Some experiments were carried out to investigate the improvement of the reduction of nitrate to nitrite in a cadmium column. We tried variations in length and also in the temperature of the column. Both amalgamated and copper treated cadmium were used. Unfortunately, no non-amalgamated cadmium was available, so that the copper treated cadmium was also amalgamated. The reduction of the nitrate with amalgamated cadmium was too slow (less than 2 ml/min. at 22°C on a 35 cm column). Raising the temperature to 50°C enhanced reduction sufficiently, but this temperature is not very convenient in routine work. A copper treated column of 35 cm length caused very rapid reduction, but with slower passage there was a tendency to low figures, which might be due to over-reduction. An 18 cm copper treated column caused complete reduction at a flow rate of 25 ml/min., and the over-reduction was negligible.

Silicates

The blue α method of Grasshoff was used, with the following modifications:

(1). The concentration of the molybdate was decreased by 80%. This leads to no change in the extinction at 6400 Å, but the pH of the sample changes with about 1.3 pH units.

(2). The monochloro acetic acid is substituted by $0.9N\ H_2SO_4$. This leads to no significant change in the pH.

(3). Na_2SiF_6 is used as a standard. Especially on board a ship this is a much more convenient standard than molten SiO_2.

Some samples from the Øresund were analysed for silicates, both on filtered and on unfiltered samples, with no significant differences.

Storage

Experiments with freeze storage showed that

(1). The phosphate content decreased when using PVC bottles.

(2). Iodine treated PVC bottles show less of this effect.

(3). Glass bottles are best. These can stand freezing quite well, when only half filled.

Notes on the determination of nitrite, nitrate and ammonia

by
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Nitrite

The procedure for the determination of nitrite is based on the classical Griess' reaction, in which nitrous acid is converted into a highly colored azo dye. The diazotising reaction with sulphanilamide requires a pH < 2. The amount of acid used in the reagent given below is sufficient to give this pH, also in samples buffered for determination of nitrate by means of the hydrazine and the Cd-Cu reduction. The azo dye is developed with the reagent suggested by Shinn (1), which is in common use in chemical oceanographic work.

Reagents

A. Sulphanilamide Solution: 14 g. of the reagent is dissolved in diluted hydrochloric acid (200 ml of HCl, 1.19, diluted to one liter with distilled water).

B. N-(1-naphtyl)-ethylenediamine dihydrochloride Solution: 0.70 g. of the reagent is dissolved in one liter of distilled water. The solution is stored cold in a dark bottle and renewed when a brownish color develops (final concentration only half of that given by Shinn).

Procedure

To 35 ml of a sample (minimum amount needed for a common 10 cm cell) is added 0.5 ml of solution A and after 5 ± 1 minutes 0.5 ml of solution B. The absorbance is measured in a 10 cm cell at 5450 Å after 30 ± 10 minutes.

Nitrate (hydrazine reduction)

In our laboratory and at sea nitrate has been determined by means of the hydrazine reduction, first suggested by Mullin and Riley (2). At an early stage the unstable phenate buffer solution was replaced by a borax-NaOH-system, and the reduction was performed for one hour at 70°C. The same temperature has later been applied by Henriksen (3) for an autoanalyzer technique. Further studies have shown that the reduction is not at all so sensitive to temperature changes as stated by Strickland and Parsons (4). Consequently, we now work at room temperature (15°-25°), and no thermostat is needed.

It has been found that the copper ions necessary for catalyzing the reduction can be added to the buffer solution. A soluble borax - metal - complex is formed. All the reagent solutions needed for the determination are thus fairly stable. The concentrations for optimum reduction rate have been re-checked and slightly modified.

As has been observed by Johnston (5) the addition of acetone in order to destroy excess of hydrazine is unnecessary.

Reagents

C. Buffer - Copper - Solution: 45 g. of borax is dissolved in 950 mlof distilled water and 1 N NaOH added until the pH is 10.0 (pH meter). To 965 ml of this solution is added 35 ml of a copper sulphate solution containing 400 micrograms of Cu /ml.

D. Reducing Solution: 3.70 g. of hydrazine sulphate is dissolved in one liter of distilled water.

Procedure

To 35 ml of sample (filtered through quarz wool if necessary) are added 1.0 ml of the buffer solution and 0.5 ml of the reducing solution. After thorough mixing the reaction is allowed to proceed for 20 ± 4 hours in a dark place. The determination is then continued with the nitrite reagents as described before.

Remarks

1. All reaction flasks must have the same size, because a certain amount of air has a small decreasing effect (ca. 10% / 50 ml of air) on the reduction rate. On the other hand, without air present no reduction takes place.

2. During the reduction the flasks should not be rocked, a moderate sea, however, has no effect !

3. The rate of reduction depends on salinity and pH. The "salt" effect is of the same order as given by Strickland and Parsons. Consequently, standard curves or F factors must be determined in sea water having a salinity within $\pm$ 3‰ from that of the sample.

4. If nitrite is present in significant amounts multiply the measured amount of nitrite with 0.44, and subtract the product from the calculated nitrate value.

Nitrate   (Cadmium - Copper reduction)

Morris and Riley 1963 (6) have shown that nitrate in a water sample can be reduced to nitrite by percolating it through a column of amalgamated cadmium filings. This method and the modifications by Grasshoff 1964 and Strickland - Parsons 1965 have been studied, but for various reasons, none of these procedures have been applied to routine work.

Cadmium filings treated with copper instead of mercury were first used by Armstrong and La Fond 1966 and a detailed study is given by Wood, Armstrong and Richards 1967 (7). A column prepared according to their description has been used on board R/V "Aranda" during her cruises this summer. The experiences have been good and parallel analyses of samples by means of the hydrazine reduction have been satisfactory.

According to personal information other marine chemists have had bad results with this type of columns. The author assumes, that they have not observed the fact, that a new column must be treated with about 3 liters of a solution containing ca. 60 microgram atoms of nitrate nitrogen per liter. First after passing this amount through the column the reduction rate will be constant and equal to ca. 99 per cent.

The only negative fact hitherto observed is the great volume of sample needed (min. 50 ml) to flush out the previous sample. Further studies of the connection between column size, reduction rate, flow rate, etc., are needed.

Ammonia

In our laboratory ammonia has been determined according to the method of Koroleff (ICES 1960 and ref. 8). Ammonia reacts with hypobromite in an alkaline medium and the excess of the reagent is determined with an azo dye, Bordeaux B. The influence of other compounds reacting with hypobromite are estimated by adding the reagents to the sample in reverse order, and the absorbance thus obtained is subtracted from the absorbance measured after the alkaline reaction.

When, last year, the procedure was applied on more or less yellow colored river water samples it was observed, that the reaction between hypobromite and these organic substances depended on time, the reaction being quantitative first after about 3 minutes. The procedure was modified accordingly, and is now as follows:

Reagents

A. Sodium Hypobromite Solution: Stock solution 0.1 N and working solution 0.003 N in 0.3 N NaOH (details in ref. 8).

B. Acetic Acid 10 N.

C. Bordeaux B dye Solution: 150 mg of the dye is dissolved in one liter of 0.01 N acetic acid. (The dye is manufactured by Edward Gurr Ltd. London S.W. 14.)

Procedure

Alkaline reaction: To a suitable volume of sample, 25 to 35 ml, is added 1.0 ml of reagent A. After 3 minutes $\pm$ 10 sec. 3 drops of solution B are added, immediately followed by 5 ml of solution C.

Acid reaction: To the same volume of sample are added 3 drops of B, immediately followed by 1.0 ml of reagent A. After 3 min. $\pm$ 10 sec., 5 ml of the dye solution are added. The absorbance is measured at 5250 Å after 30 $\pm$ 10 minutes. The "salt" effect is rather small. However, it is recommended to determine the standard curve in sea water having a salinity within $\pm$ 5‰ from that of the samples.

Baltic sea water samples with varying salinities have been analyzed according to the present procedure and with the method developed by Grasshoff 1966 (9). The results were almost identical. We also intended a comparison with the method by Johnston 1966 (10). His procedure, however, cannot be applied without modifications on samples with varying salinities. The studies will be continued.

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     method for the automatic and manual determination
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Note. The storage of samples for the determination of total
phosphorus will be discussed in a paper of the Hydro-
graphy Committee, 1968.

Some remarks on the determination of nitrate and
ammonia in sea water

by

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For the investigation of the nutrient cycle in sea water
the exact analysis of the inorganic nitrogen compounds is very
important. Accurate determinations of nitrate and ammonium
ions in sea water have been difficult, especially under ship-
board conditions, whereas the analysis of nitrite by the
method of BENTSCHNEIDER and ROBINSON (1952) does not cause
any trouble.

In our laboratory the nitrate content is determined by
reduction to nitrite in a reductor containing amalgamated
cadmium filings as described by MORRIS and RILEY (1963) or by
GRASSHOFF (1964). First of all, I should like to report on
our modifications of this method.

Special care must be taken in the preparation of the
cadmium amalgam. We found that reactivated filings, which
had been treated with acids and amalgamated several times,
have a higher efficiency than freshly prepared filings. One
reason for this may be that the often used filings have a
larger surface.

We obtained amalgamated cadmium filings of high activity
in the following way: Before activating the cadmium with
acids we washed the filings with acetone in order to remove
traces of fatty oils. Contrary to the above authors the
cadmium was amalgated with a 3 - 5% mercury chloride solution.
The excess of mercury was removed by washing with destilled
water. The acid activation and the amalgamation of the
cadmium was repeated until the filings had assumed a dark
colour. These filings are of very high efficiency.

The reductors we use are 300 mm high and have an inside
diameter of 10 mm. To prevent the cadmium filings from
running dry the reductors contain standpipes with 3 mm inside
diameter. The current resistance of the whole system is so
high that it is only passed by 100 ml sea water in one hour.
In contrast to GRASSHOFF no ammonium chloride/ammonia reagent
is added to the water sample, because we found that this
reagent often contains traces of nitrate. For one determin-
ation three 25 ml aliquots are put on to the column, the
first for washing, the second for the determination, and the
third to displace the second charge. Under these conditions
the reduction was quantitative and no salt effect could be ob-
served. The standard deviation was smaller than $\pm$ 0.5% at the
4 µg-at./l level. More than 50 samples (4 l sea water) per
column were investigated without any loss of reduction effi-
ciency.

For the determination of ammonia in sea water KOROLEFF and VOIPIO (1958) used a method which depends on the action of ammonia with alkaline sodium hypobromite. After 1 min. the excess of hypobromite is estimated colorimetrically by adding bordeaux-red B and decoloration of the dye solution. GILLBRICHT (1961) describes a modification of this method, taking a reaction time of 10 min. Using this method during the spring outburst of the phytoplankton we obtained values which were, I think, too high, though they were very reproducible. One reason for these high values may be the content of organic matter, which also reacts with hypobromite. At the Congress of the Baltic Oceanographers in Sopot 1968 KOROLEFF reported some improvements of his method, which will give better results.

We also tested the method of GRASSHOFF (1968), in which the first intermediate reaction product of ammonia and hypobromite reacts with iodide and starch between a pH range of 7.5 - 8.5. In the course of this procedure a blue iodine starch complex is formed. The method seems to be highly sensitive, but we had troubles owing to impurities of the reagents. Furthermore we found that the reaction was influenced by air.

Good results in the determination of ammonia in sea water were obtained with the method of SAGI (1966). In this method a blue indophenol dye is formed in a 50 ml water sample by the reaction of ammonia with phenol, sodium hypochlorite, and sodium nitroprusside (as a catalyst). The reaction is very slow. SAGI proposed a reaction time of at least 5 hours. But in my opinion, we had better wait 15 - 24 hours at least, mainly with regard to the deposition of the precipitate. The standard deviation in the above investigations was about ± 3% at the 2 µg-at./l level. At low salt concentrations we observed strong salt effects.

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indophenol method and its application to coastal and
off-shore waters. Oceanogr.Magazine 18:43-51.

Some experiences with the analysis of ammonia in sea water

by

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During a year of studies of the variation of micro-nutri-
ents in the Oslofjord, where one person had to do both the
sampling and the laboratory work, two main problems arose:

1. long storage of the samples should be avoided, and
2. some methods had to be simplified to make the laboratory
   work possible.

The method for ammonia described by Richards and Kletsch
(1964) and advocated by Strickland and Parsons (1965), recom-
mends that samples not be stored longer than 2 hours, not even
in a refrigerator. It also states that higher accuracy can be
obtained by acidifying the solution in order to adjust the
pH value.

It was found possible to add the oxidizing reagent immedi-
ately after the sea water had been collected from the Nansen
bottles.This avoided storage of the samples. Usually, further
analysis of the samples was delayed for about 2 days.

100 ml brown glass bottles marked at the 50 m level were
used. 50 ml of sea water were collected, and 10 ml of the
oxidizing reagent, prepared immediately before use, were added
from an automatic pipette. It also proved convenient to make
the samples for the calibration curve at sea. Standard ammonia
solution was added, with micro pipettes, to sea water collected
from a depth at which a low NH_4-concentration could be expected.

An advantage of adding the oxidizing reagent at sea is
that there scarcely will be any contamination of the samples by
ammonia in the nearly open air laboratory on board.

In the Richards and Kletsch method HCl is used as the
acidifying reagent. This requires great accuracy both in the
volume and the normality of the HCl added. It is known that the
rate of diazotization is increased by high acidities, and the
coupling rate is decreased, and that low acidities have the
reverse affect. Small variations in the volume and the normality
of the HCl added, will change the pH markedly and this will hence
interfere both with the colour development and the colour itself.

When handling a large amount of samples it proved to be
convenient to use concentrated acetic acid, which has a large
buffering capacity. Therefore, small variations in the acid
added will have no significant effect on the pH.

The samples were acidified by 5 ml of concentrated acetic
acid, and then 0.5 ml of the sulfanilamide reagent, as recom-
mended by Strickland and Parsons for nitrate determinations,
were added. This gave the samples a pH of 4.5

Although the rate of diazotization at this pH is low and the reaction probably is incomplete, the calibration curve shown in Fig. 1 has a slope steep enough to determine small variations in the ammonia content.

The optical density was recorded with a Zeiss PMQ II spectrophotometer using 4 cm cells. The colour of the reaction depends on the pH value. Fig. 2 shows the maximum optical density at different pH values obtained by adding different volumes of acetic acid. In accurate addition of acid at a wave-length of 505 mμ will cause only very small errors.

When the treated sample is stored in a brown bottle, the colour remains stable for at least one day. Even samples read one week after the first reading, showed no variations in the optical density.

The modifications of the Richards and Kletsch method for ammonia determinations mentioned above, proved to be a convenient way of making routine analyses during our studies in which a large amount of ammonia determinations had to be carried out.

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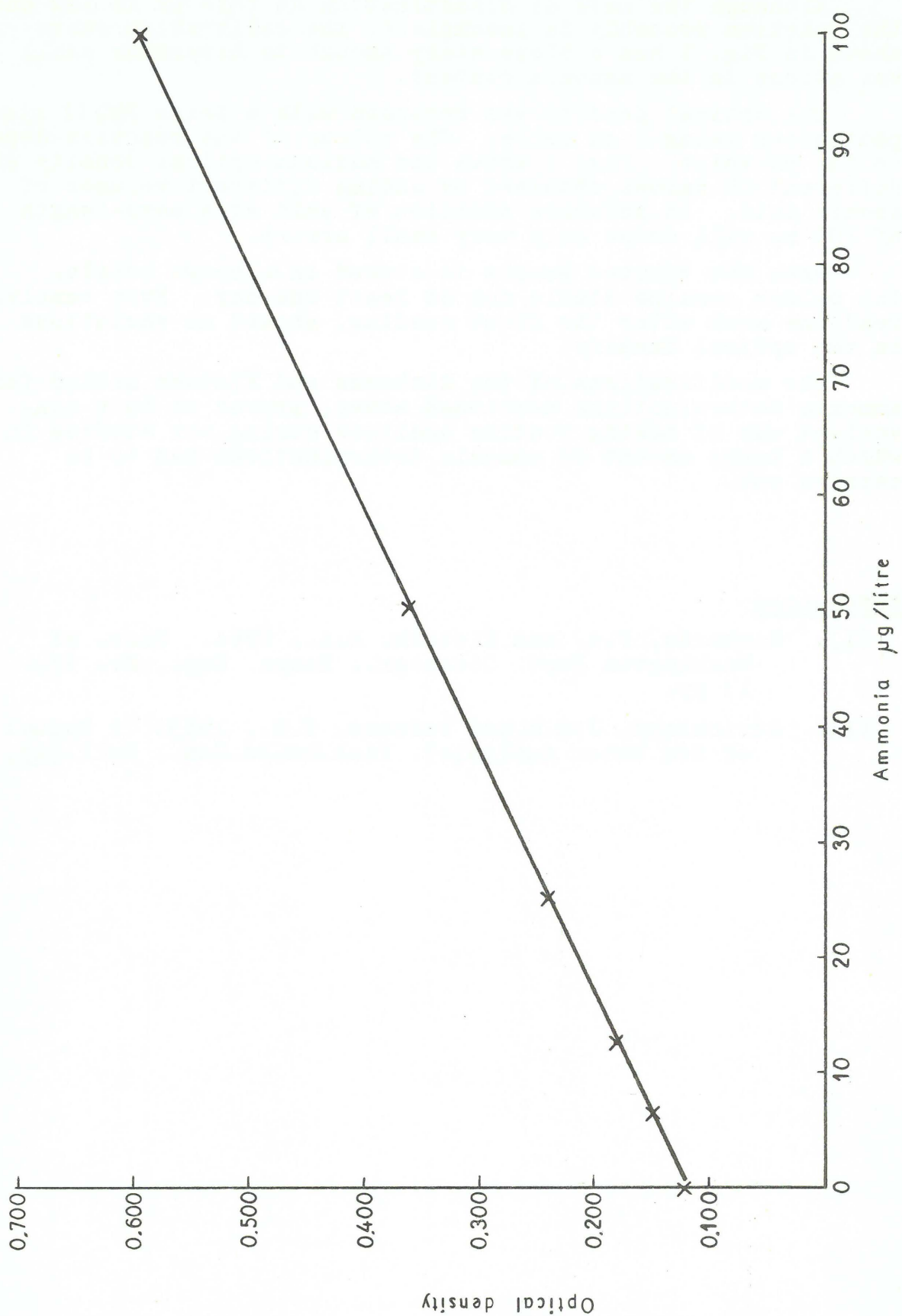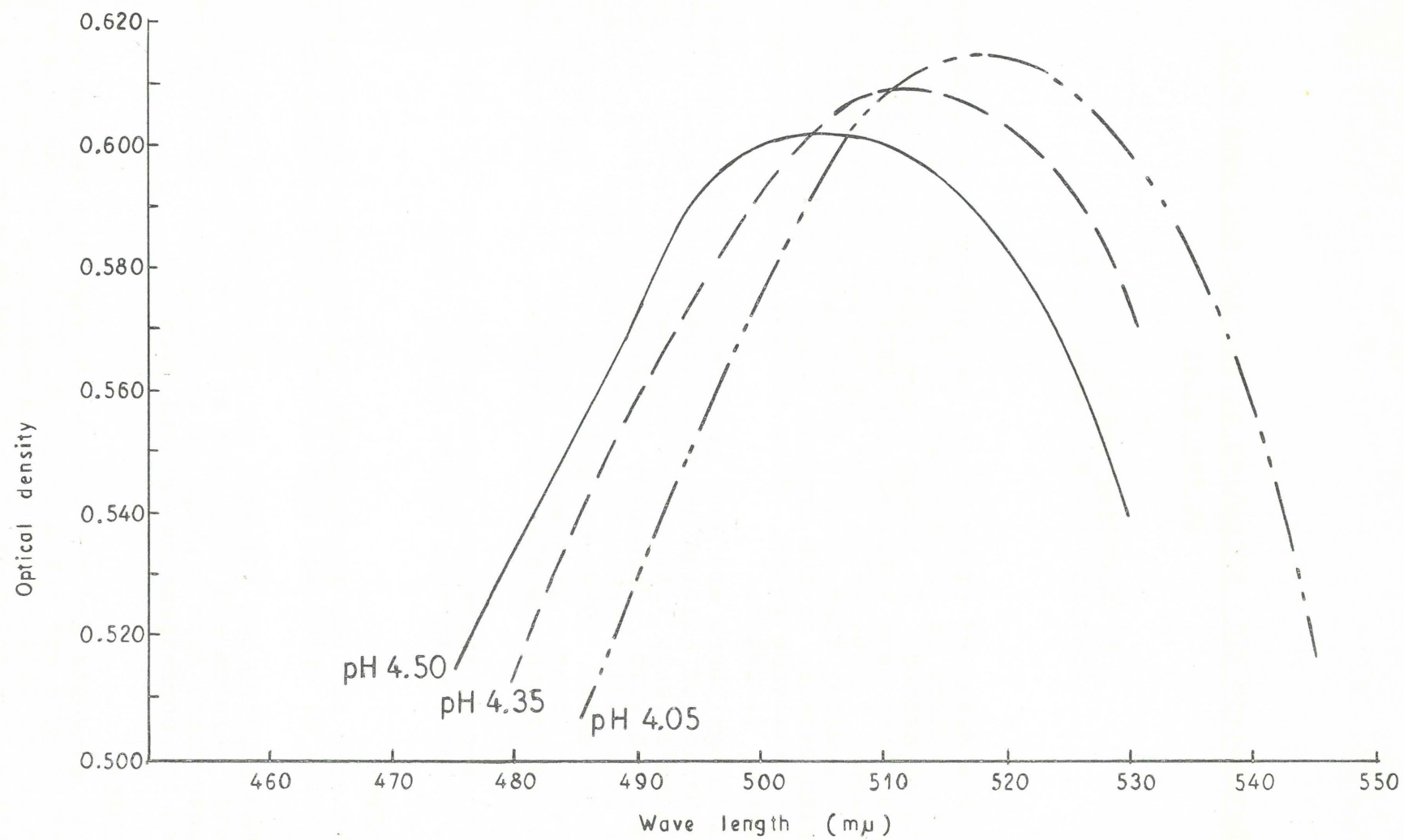
FIG. 1

FIG. 2

Photometric EDTA titration of calcium and magnesium in sea water

by

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For a detailed study of the calcium and magnesium content in Baltic waters it is necessary to dispose of a more accurate analytical method. In recent years calcium and magnesium in sea water have been titrated with EDTA by the method of Pate and Robinson (1958,1961). But the visual titration gave no greater accuracy than about ± 1% for calcium, and ±0.45% for magnesium because of the gradual colour change in the vicinity of the equivalence point. This trouble can be diminished by photometric titration.

Although there are many publications on the photometric determination of calcium and magnesium in biological materials and minerals, only little work has been done in this respect in water analysis. Carpenter (1957) has determined calcium in sea water after separating it from magnesium. Malmstadt and Hadjiioannou (1959) determined calcium and magnesium in fresh water using a photometric titration with EDTA and with calcon and eriochrome black as indicators, just as Pate and Robinson did.

It seemed, therefore, quite useful to us, to make some further studies with this method in sea water.

Apparatus:

We used for our measurements the Beckmann spectophotometer Model B, which was fitted with a 150 ml beaker and a stirrer.

The calcium was titrated at a wavelength of 620 mμ, the total calcium and magnesium at 640 mμ. The endpoints were determined from a graph of the amount of titrated EDTA against the corresponding optical density.

Procedure:

The reagents and the indicators were the same as described by Pate and Robinson (1958,1961). For the standardization of the EDTA-solution it is necessary to determine the molarity with a solution of calcium and magnesium (ratio 1:3), analogous to titration of sea water.

The titrations were carried out with weighed volumes of about 50 ml of sea water for the calcium determination, and of 20 ml for the total titration.

The best results were obtained after the addition of 97 - 98% EDTA solution prior to the photometric titration.

Results:

Precision and accuracy.

In order to assess the reproducibility of the method seven replicate titrations were carried out on Baltic water ($S\permil = 15.39\permil$).

These showed an average concentration of 0.20220 ± 0.00087 g/kg Ca^{2+}, i.e., with a coefficient of variation of 0.43%, respectively an average concentration of 0.57045 ± 0.00078 g/kg Mg^{2+}, i.e., with a coefficient of variation of 0.14%.

The accuracy of the method was investigated by carrying out 5 replicate analyses of the same water, which was spiked with 5.611 mg Ca^{3+} and 7.296 mg Mg^{2+}. The recoveries of added calcium and magnesium were 100.43% and 100.13% respectively, and hence of the same order of magnitude as the precision.

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Some remarks on the influence of pH and nutrients on the electrolytic conductance of sea water

by

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Introduction

With the universal acceptance of the electrolytic conductivity meter as salinometer, we now have a very precise way to estimate salinity provided we have adequate knowledge of chemical variances that are produced by the biogeochemical processes in sea water. pH is an important parameter to consider, for it indicates the extent of some of the chemical variances such as photosynthesis, respiration, and calcium carbonate dissolution and precipitation.

Before I make an assessment of the collective dependence of the chemical processes on pH, let us examine the extent of the conductivity change by the addition or removal of certain chemical species to and from sea water. At a salinity of 35‰, near 20°C, the specific conductance of sea water is about 0.48 ohm^{-1}cm^{-1} (Thomas, Thompson, and Utterback, 1934). We will take this as 100% sea water conductance. From the work of Bradshaw and others we can then construct Table 1.

In order to construct Table 1 I assumed that KNO_3 and KH_2PO_4 are actively taken up by phytoplankton. My assumption satisfies the electroneutrality. It also is based on higher K than Na concentrations in phytoplankton as well as on $H_2PO_4^-$ being a predominant inorganic ortho-phosphate species in sea water. Silicate, that we measure by a colorimetric technique, exists as H_4SiO_4 undissociated species. I assumed that the partial molal volume of H_4SiO_4 is twice that of CO_2; I further assumed that from the volumetric point of view the conductance effect is proportional to the molal volume. We will now examine the conductometrically measured "salinity change" due to in situ processes between surface and deep waters and also during the sea water storage.

A. <u>Comparison of the surface and deep waters</u>.

In the case of the Pacific Ocean off Oregon, the vertical distributions of various chemical parameters, with respect to the surface, show as follows:

Table 2. Difference in the various chemical species between the surface and the deep (4 km) waters off Oregon. Increases in phosphate and molecular carbon dioxide concentrations are ignored.

Reaction	Magnitude	Conductometric Salinity Effect
O_2 decrease	4 ml	+0.002 (‰S)
$CaCO_3$ dissolution	0.1 mM	+0.005
Carbonate → Bicarbonate	0.2 mM	+0.003
KNO_3 release by phyto-plankton	0.04 mM	+0.003
Undissociated H_4SiO_4 release by phytoplankton	0.15 mM	−0.002
		+0.011‰

Approximately 0.011‰ S equivalent is added to the deep water salinity by the difference in the biogeochemical processes between the surface and the deep. If we are to normalize all the salinity to a surface water, then we ought to subtract 0.011‰ S from the deep salinity values that are measured conductometrically. Since various different depths and locations differ in the extent of the chemical variance, it appears mandatory to dispose of a local 3-dimensional correction chart.

One interesting point we see here is that the nutrient effects (nitrate, phosphate, and silicate) appear to compensate each other, and no significant contribution is made to the overall conductivity. In the case of marine plant growth that does not need silicate, the primary production will accompany a lowering of the conductance by KNO_3 uptake.

With respect to the pH dependence, the transformation of carbonate into bicarbonate is the only parameter that directly affects the sea water pH. Oxygen decrease must be taken as the addition of carbon dioxide, which in turn affects the pH of sea water. In order to assess these two processes realistically, it is necessary to know the vertical variation of total CO_2. Calcium carbonate dissolution affects pH also; its effect is to increase the pH. A convenient way to examine calcium carbonate dissolution is by determining the specific alkalinity.

Since organic matter decomposition favors the lowering of pH while calcium carbonate dissolution elevates it, the net pH change is somewhat reduced. Yet, these two processes increase the sea water conductance. Therefore, pH alone does not suffice to estimate the conductance change. We have to have the total CO_2 and the specific alkalinity data in addition to the pH. Since it is possible to calculate the total CO_2 from alkalinity and pH, we at least need both alkalinity and pH data for the conductivity correction.

B. Conductance change during sea water storage.

When salinity samples are stored, the pH of sea water frequently decreases. My pH measurements on the international standard sea water (Park, 1964c) show a pH range of 7.70 to 8.09 with an average value of 7.90. The major cause of this range could be the organic matter oxidation within the sea water samples. In addition we cannot exclude the possibility of carbonate precipitation or of contamination arising from the bottle itself.

If we assume that the pH decrease is from the organic matter decomposition only, then the pH decrease is essentially the transformation of carbonate into bicarbonate ions. From the alkalinity and pH data, we can calculate the extent of the transformation by employing the following two equations:

$$pH = pK_2' + \log \frac{M_{CO_3^{--}}}{M_{HCO_3^-}} \qquad (1)$$

$$Carbonate\ Alkalinity = M_{HCO_3^-} + 2\ M_{CO_3^{--}} \qquad (2)$$

where pK_2' is -log of the apparent second dissociation constant of carbonic acid in sea water, and M denotes stoichiometric concentration. The chemical data needed to calculate the magnitude of the transformation are the initial pH and alkalinity and the final pH.

In summary, it is essential to have both the pH and the alkalinity data to make a proper correction on the conductometrically measured salinity.

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Table 1. Effect of various biogeochemical processes on the sea water conductance.

Chemical species added or involved (one mM)	% Conductance change	Variance observed in the ocean	Expected conductance change	Expected change in conductometrically measured salinity	pH dependent	Reference
Molecular CO_2	−0.013%	0.007 mM at pH 8.4 to 0.08 mM at pH 7.5	0.00009% ∼ 0.001%	−0.0003‰	yes	Park et al. (1964)
Dissolved O_2 (one mM)	−0.022%	0 ml/l ∼ 7 ml/l	−0.007%	−0.003‰	Indirect-ly yes	Bradshaw (cited in Park and Burt,1965)
Calcium carbonate dissolution (one mM) as $Ca(HCO_3)_2$	+0.12%	0.1 mM	+0.012%	+0.005‰	yes	Revised data of Park,(1964b)
Replacement of bicarbonate into carbonate (one mM) by removal of CO_2	−0.04%	0.4 mM	−0.016%	−0.006‰	yes	Revised data of Park et al.(1964)
Uptake of KNO_3 by phytoplankton	−0.16%	0.045mM	−0.007%	−0.003‰	?	Bradshaw (personal communication)
Uptake of KH_2PO_4 by phytoplankton	−0.10%	0.003mM	−0.0003%	−0.0001‰	?	Revised data of Park, (1964a)
Uptake of silicate as H_4SiO_4 by phytoplankton (one mM)	+0.03%	0.15 mM	+0.005%	+0.002‰	?	Estimated, assuming partial molal volume of H_4SiO_4 is twice the partial molal volume of CO_2

Some remarks on the significance of the third decimal place in the salinity determinations

by

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To day the most used method for the determination of the salinity and of the specific gravity of sea water is the measurement of the relative conductivity with various types of salinometers. Copenhagen standard sea water serves as the primary standard. The value of the relative conductivity is converted into salinity and into density (specific gravity) by means of internationally accepted conversion tables.

In many instances the salinity data are sent to and stored in the data centers to three decimal place.

With the normally used precision instruments for the determination of the relative conductivity a reproducibility of the measurements of $\pm 0.07 - \pm 0.10$ parts per thousand can be obtained easily. This rather good reproducibility leads to the conclusion that the accuracy of the salinity may be of the same order of magnitude if the conversion tables are accurate enough. Because the data from the conversion tables have been adopted to define salinity no further error will be introduced by this conversion.

If, however, we do not consider the "normal" sources of error in the determination of the salinity (rinsing errors, degassing errors, sampling errors), the variance in the chemical composition of the sea water as caused by the synthesis and the deteoriation of organic material does influence the conductivity to a degree which is <u>not</u> negligible[*] Therefore, the significance of the third decimal place in the reported salinity figures is considered to be doubtful unless certain corrections are applied.

To understand the meaning of the third decimal place a clear distinction should be made between the reproducibility, expressed by the standard deviation of the measurements, and the accuracy which depends on the difference between the measured mean value and the true value.

The measurement of the conductivity is made relative to Copenhagen Stanadard Sea Water. This means that actually the application of the conversion tables is only allowed, if the stoechiometric concentrations of all components of sea water, electrolytes as well as non electrolytes, are exactly in the same relation to chlorinity as in the Standard Sea Water. If this is not the case, and usually it is not, an estimate of the influence of the deviation of the individual components from the normal relation must be made in order to understand the significance of the measured conductivity and salinity.

[*] See also the paper by K. Park

The most striking indicator for such deviations from the "normal" composition of sea water is the pH of the sample. The pH together with the carbonate alkalinity is an indicator for the state of the carbonate system. This influence has been studied first by Park, Weyl, and Bradshaw (1964), and has been confirmed by Miyake (1965) and Grasshoff (1964). The correlation of the apparent pH with the conductivity of sea water of various salinities expressed in terms of salinity is shown in Fig. 1.

The initial alkalinity was 2.6 mequiv/l. The pH was changed by addition of very small amounts of NaOH or by passing moist CO_2 through the sample. Thus the carbonate alkalinity remained unchanged through a set of measurements at one salinity.

The dashed lines in the figure are the outer limits of the range of the apparent pH in sea water, including conditions of stagnant waters.

Recent measurements at 35‰ S confirmed again the earlier measurements of the authors cited above.

To understand the meaning of these measurements one must recall that it is not the "in situ state" of the carbonate system which influences the conductivity measurement but the state when the sample is at room temperature, and after a more or less long period of storage. This makes the problem more difficult because the reference water (primary or secondary standard) has been stored a long time. After a certain period of storage (how long depends on the water) the pH reaches a relatively constant value of 7.8 ± 0.1 regardless the initial pH of the sample. This approximation of pH 7.8 is due to the deterioration of organic material, equilibration with air, and perhaps reaction with the material of the bottles.

However, after a shorter period of storage in aged, closed salinity bottles the apparent pH remains almost unchanged for several days.

Therefore, in practice the sample is measured against a standard water having a different state of the carbonate system. The conversion tables, however, are based on measurements of aged samples, which have been equilibrated with air and hence were in almost the same state with respect to the carbonate system.

This means that a systematic error is introduced in the salinity determination. How large this error may be is indicated by Fig. 1. The largest deviations are found just in the "normal range" of the apparent (not in situ) pH in oceanic salinity samples.

This systematic deviation, which is "normal" for large parts of the world ocean, is as high as 0.011 in terms of salinity. Because the pH of the primary standard and of the basic measurements for the conversion tables were about 7.8, the estimated salinities of one cast may be too low by 0.008‰ or too high by 0.003‰. These figures will become more extreme in stagnant water. Furthermore, the systematic error caused by the carbonate system will be the larger the sooner the determination of the conductivity is made after the sampling.

It is, therefore, rather difficult to make a recommendation for a sufficient treatment of the salinity sample and for a correction. Certainly a longer period of storage (2-3 months) would decrease the systematic deviation between the state of the carbonate system in the standard water and in the sample. Because of the fairly constant specific alkalinity of most oceanic waters the apparent pH of the standard water and of the sample could serve as an indicator for the carbonate system. But this means, that in addition to the conductivity measurement a determination of the pH must be carried out. (This determination should be accurate to ± 0.05 pH units.)

Another possibility to decrease the deviation between the "true" salinity and the measured one would perhaps be a measurement within 1-2 days after sampling and an application of empirically developed three dimensional correction values.

A third way out of this dilemma is to cancel the third decimal place in the salinity determination and make it quite clear to everybody that the accuracy of the salinity determination by means of a conductivity measurement is normally not better than ± 0.01‰, but may be improved by application of certain correction values.

This does not exclude, that in many areas of the world ocean the third decimal place may be used for relative comparisons. This is, however, only then possible if the determinations are made with the same instrument, by the same people, during the same time after the sampling and with the same procedure. The relative salinity and density is then and only then as good as the precision of the instruments and the technique.

It must be pointed out, that apart from the carbonate system as a source of error in the salinity determination other components of sea water likewise can cause errors. Kilho Park made a contribution to the problem of the influence of certain nutrients on the conductivity of sea water.

These remarks should be considered as a contribution to a discussion on this actual problem. Comments are highly appreciated.

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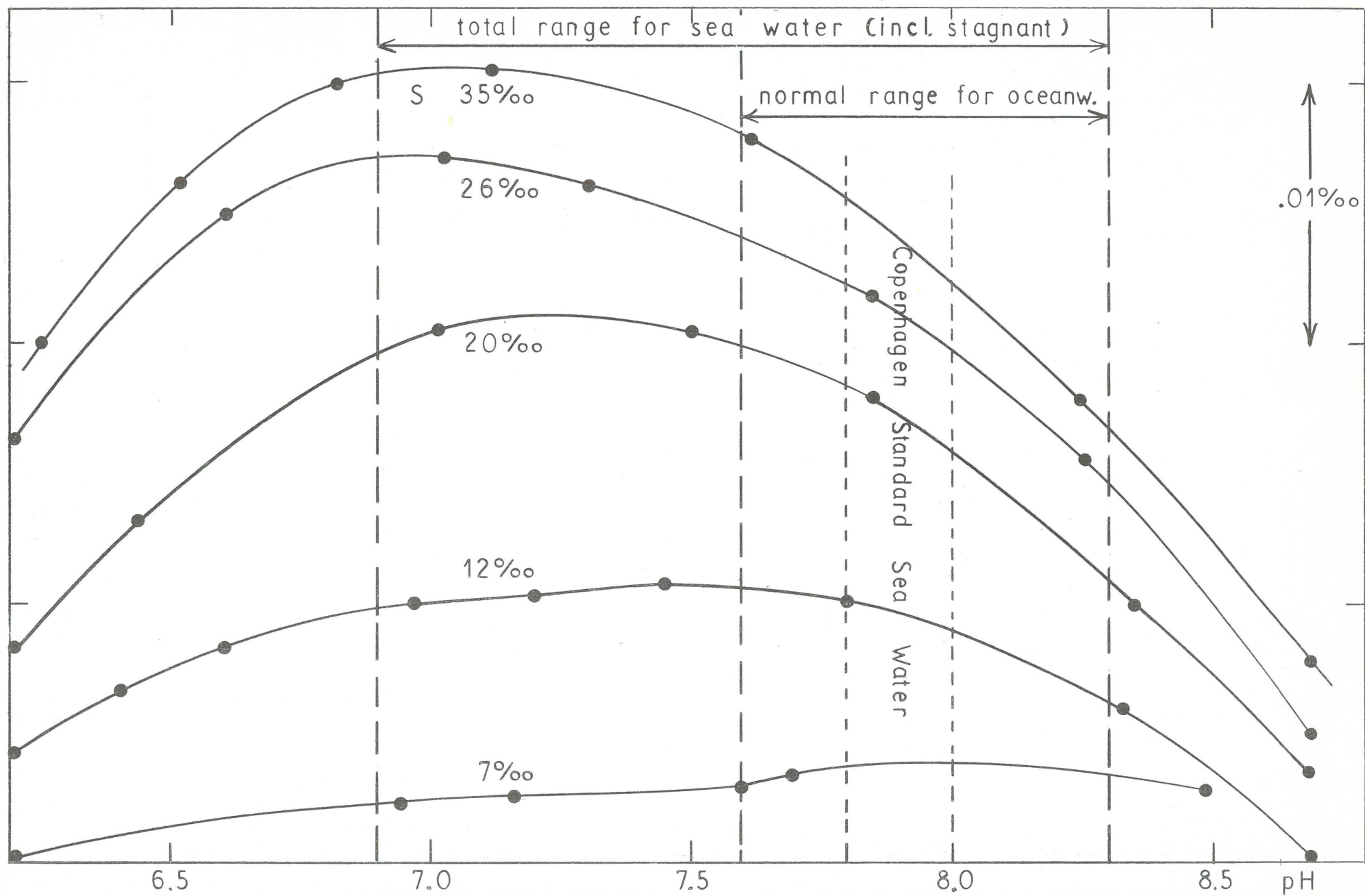
Fig. 1.   Apparent change of salinity at different pH (const. alkalinity)