THE STANDARDIZATION OF A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF THE HYDROGEN ION CONCENTRATION, CO₂ TENSION, AND CO₂ AND O₂ CONTENT OF SEA WATER, OF ANIMAL HEAT, AND OF CO₂ OF THE AIR, WITH A SUMMARY OF SIMILAR DATA ON BICARBONATE SOLUTIONS IN GENERAL.*

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The experiments on which this paper is based were all done by the author, but some of them have already been published (McClelond and Magoon, McClelond, 1916, b, and McClelond, Gault, and Mulholland). The main object of the present paper is the extension of this previous work by means of the new and slightly more reliable apparatus herein described. Sea water is much more difficult to investigate than blood, owing to its low buffer value and low CO₂ tension. It requires great care to measure 0.01 mm. in CO₂ tension, and yet this is about 4 per cent of the CO₂ tension of the sea. After the CO₂ tension is determined it may be changed by the solution of glass. The difficulties of making determinations with the hydrogen electrode are increased as the buffer value is lowered.

A Leeds and Northrup potentiometer and a 0.1 N KCl calomel electrode were used.¹ The mercury was redistilled in a Hulett still. The hydrogen electrode is shown in Fig. 1 as set up for

* Some of the apparatus was bought with a grant from the research fund of the Graduate School, and some was borrowed from the Marine Laboratory of the Carnegie Institution.

¹ Electrolytic calomel for this was kindly sent me by Professor G. A. Hulett.
the passage of the $H_2 + CO_2$ mixture through it. It was made of a tube of 24 mm. bore with a stop-cock at each end and a gold disk welded to a platinum wire fused through the glass. The gold disk was covered with palladium black by electrolyzing a 1 per cent filtered solution of palladium chloride, using a small platinum wire as anode. A 4 volt current was used, but this had to be cut down by reducing the size of the anode, especially at the beginning. If the palladiumization proceeds too fast, the palladium black does not stick to the cathode, but breaks off and moves to the anode in a cloud. After the electrode had been used a few times the black was dissolved off by concentrated nitric acid, and deposited anew.2

The gas mixture was admitted into the electrode by a swivel joint ground so true that it could be effectively closed with a water seal. The electrode was rotated 400 revolutions per minute by means of a Tiffany motor operating on a cork wheel or pulley. If the electrode contained only 2 cc. of sea water and its $CO_2$ tension at the start did not differ from that of the gas mixture more than 0.1 mm., equilibrium was reached by the passage of a liter of the gas mixture in the course of 30 to 40 minutes.

The gas mixer is also shown in Fig. 1 and is of 1 liter capacity, with the upper portion graduated and so narrow that 0.00001 liter can be read on it with ease. The 3-way stop-cock at the top allows the connecting tubes to be washed out with the gas to be introduced. Since the apparatus holds 25 pounds of mercury, the gas mixer and leveling bulb were wound with iron wire

2 If the nitric acid is contaminated with chlorides or HCl, chlorine will be formed. If it is necessary to remove platinum black from electrodes, aqua regia is required and an abundance of chlorine is formed. The last trace of chlorine may be removed by electrolyzing a dilute solution of $H_2SO_4$, using the electrode as cathode. If, however, it is feared that a palladiumized electrode is contaminated with chlorine, it is safest to electrolyze distilled water with a higher voltage, as palladium black is attacked by mineral acids. The fact that palladium black is attacked by HCl may explain the rapid deterioration of palladiumized electrodes in gastric juice, which I have repeatedly observed. Another objection to palladium black is that it amalgamates instantaneously with mercury. The surface of hydrogen electrodes is “poisoned” by so many different substances that it often seems impossible to find the cause of the trouble. Both oxidizing and reducing gases may poison it.
Fig. 1. Gas mixer and rotating electrode for determining the pH of a solution at any desired CO₂ tension. The gas mixer at the left is of 1 liter capacity down to the mark just above the lower stop-cock, and the numbers on the upper narrow portion mark 0.0001 liter. The mixer is filled with mercury and the CO₂ generator connected through the trap that delivers CO₂ at atmospheric pressure, to the upper stop-cock, which is first turned so that the gas escapes at the top and later reversed so as to admit the desired amount of gas. These operations are repeated with H₂ and the mixer is filled with it down to the 1 liter mark at the bottom. The remaining mercury is shaken with the gas to mix it. The mixer is now turned around and connected with the rotating electrode on the right through the swivel joint closed by a water seal in the moist chamber. A similar water seal connects the right hand end of the electrode with a trap to prevent the backward diffusion of air. The electrode (containing 1 or more cc. of the solution) is rotated 400 revolutions per minute by means of the cork pulley belted to a Tiffany motor while the gas mixture is slowly passed through it by displacement with mercury in the mixer. The stop-cocks at the two ends of the electrode are closed, and the one nearest the palladiumized disk (having been left ungreased) is immersed in the KCl bath and a wire hooked in the projecting loop from the palladiumized disk. The reading is now taken in the usual manner.
Van Slyke apparatus slightly modified for determining the total CO₂ in sea water. A rubber suction tube, 1 mm. bore and 1 meter long, connects the lower end with a leveling bulb with cylindrical sides and filled with mercury. By raising the bulb the apparatus is filled with mercury. The upper stop-cock is closed and the bulb lowered so as to exhaust the apparatus. The bulb is raised without opening the upper stop-cock. The traces of air are now collected at the top and are forced out on opening the upper stop-cock. By means of careful manipulation of a pipette and the upper stop-cock, 10, 15, or 20 cc. of sea water are admitted, care being taken not to admit any air. It is permissible to leave one or two drops of this sea water in the 1 cc. cup at the top as this amount may be washed down by the introduction of 1 cc. of 2 N HCl in the same manner.

A drop of mercury is placed in the cup to seal the stop-cock and the bulb lowered until the mercury falls to the etched mark near the bottom of the 100 cc. chamber. The lower stop-cock is closed and the apparatus is shaken laterally in the vertical position as vigorously as possible for 2 minutes (in case 20 cc. of sea water was used, 4 minutes). The sea water is now trapped off in the trap, T, and the mercury allowed to rise in the 100 cc. compartment until atmospheric pressure is attained in it and the lower stop-cock closed. At the end of 1 minute the amount of sea water above the mercury in the 1 cc. gas burette is measured, calculation being made for the two menisci. The apparatus is laid on its left side on a cushion and agitated 1 minute after the air is in the wide portion of the 100 cc. chamber. The apparatus is then clamped upright, the lower stop-cock opened, and the mercury levels are adjusted by means of a screw. The air volume in the 1 cc. burette is carefully measured. 1 cc. of 0.5 to 1.0 N NaOH is admitted from the cup into the gas burette to absorb the CO₂. In leveling the bulb to measure the gas residue, one-thirteenth of the height of the NaOH is reckoned as mercury. The total CO₂ that was in the original sea water is that absorbed plus that in the sea water above the mercury plus that in the sea water trapped in the trap, T. After these are calculated by means of the volumes, absorption coefficient, and CO₂ tension, they are added together and reduced to 0° and 760 mm. To do this it is necessary to know the barometric pressure and the capillary depression in the gas burette, which latter is subtracted from the former.

In the following example on 10 cc. of 0.576 N sea water at 20°, the absorption coefficient for CO₂ was 0.757, the barometric pressure 754 mm., and the capillary depression 2 mm. The CO₂ absorbed was 0.405 cc. and the gas volume before absorption 0.59 cc., making the CO₂ tension 0.687 (unity = barometric pressure). The sea water above the mercury was read as 0.075, to which was added the volume of menisci (0.0133) making 0.0883. The CO₂ in this sea water was 0.0883 × 0.687 × 0.757 = 0.0469, which added to the absorbed CO₂ makes 0.4509. This occupied a space of 89 cc. over 11 cc. of acidulated sea water; hence there is in the trap 11 × 0.4509 × 0.737 = 0.0422, which, added to that already calculated, makes a total of 0.4931 cc., and which, reduced to 0° and 760 mm., is 0.442 per 10 cc., or 44.2 cc. per liter. Since the pH was 8.2 and the alkaline reserve 25, the total CO₂ agrees with Fig. 4.
that was run through boiling sealing wax as it was wound on. After the gases were measured into the mixer, they were thoroughly mixed by shaking up the little mercury remaining in it.

The hydrogen was generated from zinc and H$_2$SO$_4$ and passed through a large and a small wash bottle of HgCl$_2$ solution, and a small one of NaOH solution and one of H$_2$O. When the small wash bottle of HgCl$_2$ showed the first trace of discoloration with arsenic, all the wash bottles were refilled. The CO$_2$ was generated from marble and HCl and washed with NaHCO$_3$ and H$_2$O. The gases were led to the gas mixer through traps (0, 0) that delivered them at atmospheric pressure, and a barometer in the same room was read during each experiment.

When a liter of the gas mixture had been passed through the revolving electrode, the stop-cocks were closed. The ungreased stop-cock was immersed in the usual KCl bath, a wire hooked into the platinum loop, and the electric potential determined. An identical gas mixture was made and passed through the electrode as before. If the potential remained the same, it was assumed that equilibrium with the gas mixture had been reached. In case it was desired to determine the total CO$_2$ on the same sample it was necessary to have 10 cc. of sea water in the electrode. The total CO$_2$ was determined by means of a modified Van Slyke apparatus for determining the CO$_2$ in serum (Fig. 2).

All the determinations were made in a constant temperature room automatically controlled within 0.2° by means of the apparatus previously described (McClendon, 1916, a). A damping vane in glycerol was added to the bimetallic thermoregulator. Some trouble was caused by arcing between the relay contacts carrying the 1,500 watt heating current. A lump of solder was placed on them so that it fell and rang a bell before a hot arc had time to form. Since a man's body heats 1 cubic meter of air about 0.5° per minute, rapid stirring of the air is needed. This was accomplished by means of a one-sixth horse power electric fan and two smaller fans. As the outdoor temperature varied from 0° to $-30^\circ$, no special arrangement for cooling the room was required, but the electric heat regulation was later found sufficient even with a window slightly raised and a vertical fan set in front of it.
Great care had to be taken in the manipulation of the Van Slyke apparatus and the reading of the menisci in order to reduce the error below 1 per cent. The greatest difficulty was experienced in greasing the stop-cocks so that they would hold a vacuum without soiling the interior of the apparatus with grease, but this difficulty disappeared on reducing the bore of the stop-cocks to 1 mm. A mixture of equal parts of soft paraffin, vaseline, and chicle, melted together and thoroughly stirred was found to be the best stop-cock grease. It was thinned with vaseline for lower temperatures. A source of error, the magnitude of which has not been determined, is the holding of some CO₂ by the thin film of sea water between the mercury and the glass, when the mercury is readmitted into the large compartment. This source of error is smaller in the modified apparatus. This error makes the values obtained too low, but in the standardization of the apparatus with Na₂CO₃ solution in CO₂-free distilled water, this error was so small as to be overcompensated by the absorption of CO₂ from the air by the solution in introducing it into the apparatus. In the case of sea water, some CO₂ is rarely lost and never gained.

The titration alkalinity or alkaline reserve was determined by titrating 100 cc. of sea water while boiling in an Erlenmeyer flask of resistance glass with 0.01 N HCl, using dibrom-o-cresol-sulfophthalein as indicator. It usually required 1 cc. more than by the usual method of titration with phenolphthalein. The latter indicator was discarded since it was thought to be affected by some of the weak, non-volatile acids in sea water, although not by boric acid. The 0.01 N HCl is affected by the solution of glass about ten times as rapidly as 0.1 N acid (being noticeably changed in a month), and hence the stock solution was made of the latter and the former made from time to time by dilution. If a liberal supply of sea water is at hand, it is very desirable to make the titration on a liter of it with 0.1 N acid, as the end-point is not very sharp in any case. The water should remain yellow after vigorous boiling for 5 minutes after the last acid has been added.

In the experiments on which this paper is based, all liquid volumes were determined at 20° and the solutions used at 10°, 20°, and 30° without correction for volume change. The density
at 20° compared with distilled water at 4° is given. The concentration of the sea water is indicated by the normality of the chloride titration with silver nitrate and may be reduced to gm. per liter by multiplying by 35.46 and gm. per kilo by dividing the product by the density. The alkaline reserve is indicated by the number of cc. of 0.1 N HCl required to titrate a liter of the sea water. The total CO₂ is expressed as the number of cc. of dry CO₂ at 0° and 760 mm. that may be evolved from a liter of sea water by adding acid and boiling (but as stated above, the results are within the limits of accuracy of the micro method). The CO₂ tension is expressed as mm. of mercury, and may be reduced to atmospheres by dividing by 760. The pH was determined by the hydrogen electrode at the same temperature at which the CO₂ tension was regulated, but it was also found that the pH is not perceptibly affected by temperature provided there is no gain or loss of CO₂. The temperature change in hydrolysis of alkaline carbonates is compensated by the change in the dissociation of water.

Besides the Tortugas sea water previously investigated, determinations were made on 0.5366 N sea water from near San Diego (alkaline reserve = 23.5, determined density = 1.0238), on 0.513 N sea water from Woods Hole, Massachusetts (alkaline reserve = 24, determined density = 1.0225), and especially on 0.576 N sea water (alkaline reserve = 25, determined density = 1.0254) taken from the Gulf Stream off Miami, Florida, and examined immediately on arrival by express. No difference was detected between these sea waters in the relation of CO₂ tension to pH. The relation of the total CO₂ to pH was affected only by the alkaline reserve (within the limit of error of the micro method).

It was found that the pH plotted against the logarithm of the CO₂ tension made a very gentle curve at higher CO₂ tensions. Within the limits given in Fig. 3 it was indistinguishable from a straight line (i.e., the curvature is within the limits of error of the determinations). If the CO₂ tension remains constant the pH varies directly with temperature, 1° corresponding to 0.01


pH. Fig. 3 may be used as a conversion table for finding the CO₂ tension of sea water from the pH and temperature. These values compare favorably with those of Henderson and Cohn in so far as comparison may be made.

When the pH is plotted against the total CO₂, the curvature is possibly greater, but the limits of error are greater, and the graph shown in Fig. 4 is as accurate as it has been possible to make from the data so far accumulated. This figure may be used as a conversion table for finding the total CO₂ from the pH and the alkaline reserve. The lines are not straight if extended. In one determination at pH 7.4 and alkaline reserve 25, the total CO₂ was 54 cc., whereas by the extended chart it would be 57 cc.

Since there is from twenty to thirty times as much CO₂ in the sea as in the air, the small surface of contact of these two cannot locally affect the CO₂ content of the sea water very much. The oxygen content of the sea water is more significantly affected, since it varies with the climatic zones, but the exchange of O₂ between sea and air is probably very slow. To the extent that the sea is a closed system, O₂ varies inversely to CO₂, due to the action of organisms, the possible error being 30 per cent. We may therefore use Fig. 4 for finding the oxygen content of sea water, provided the pH and alkaline reserve are known, and on the assumption that the respiratory quotient is unity and that a kilo calorie will raise the temperature of a liter of sea water 1°. It is obviously impossible to use these data in any attempt to determine the respiratory quotient of a marine animal in a sealed jar of sea water, but the data are valuable in indicating the limits of the oxygen supply, since the respiratory quotient of animals has been found to vary within narrow limits (0.7 to 1.0). The animal heat per cc. of CO₂ produced by the burning of carbohydrates is about 5 gm. calories, of proteins about 5.9, and of fats about 6.6. The error in estimating the total animal heat with the aid of Fig. 4 will be great only in case the respiratory quotient varies greatly from unity; i.e., when a large proportion of fats and proteins is burned. It seems to be a fact that no gill-breathing animal has a temperature more than 2–3° above the surrounding water. After an inspection of Fig. 4 it seems incredible that even the recorded temperatures of aquatic animals could be maintained. The oxygen necessary for the generation
A simple calculation may be made for any other temperature since, if the CO₂ tension is kept constant, the pH varies directly with temperature. The CO₂ tension is marked on the ordinates and the hydrogen ion concentration is marked on the abscissa. At the bottom is a scale for measuring the pH on the abscissa. The light diagonal lines denote pure bicarbonate solutions, the normality of which is indicated. The heavy diagonals denote blood and sea water.
Fig. 4. Conversion table for finding the total CO$_2$ of sea water from the pH and alkaline reserve (cc. of 0.1 N HCl to titrate 1 liter of boiling sea water). The total CO$_2$ is measured on the ordinates and the pH on the abscissa. Each diagonal is for sea water of the alkaline reserve indicated. At the left is given the expected O$_2$ per liter for tropical and again for temperate oceans. If the change in CO$_2$ is due to animal oxidations, the temperature change caused thereby is indicated in hundredths of a degree.
of heat must be carried from the gills to the interior by an aqueous fluid of high specific heat, and it would appear that the heat transfer must be nearly as effective as the oxygen transfer. The possession of hemoglobin, thus facilitating the oxygen transfer, should, however, allow a slightly higher body temperature to be maintained. A parallel case is indicated by Dallwig, Kolls, and Loevenhart in the comparison of the oxygen necessary to support a flame and maintain the temperature of a mammal. The flame can be maintained in 20 mm. \(O_2\) tension made by rarefying the air, because the decreased oxygen content of the air is compensated by the decreased conduction of heat. But the flame is extinguished at 116 mm. \(O_2\) tension made by diluting the air with nitrogen, because its specific heat is maintained. The mammal is unaffected by either process down to 40 mm. \(O_2\) tension and perhaps farther, because the heat conductance depends largely on the specific heat of the blood, which is constant.

It follows from Figs. 3 and 4 that the total \(CO_2\) plotted against the logarithm of the \(CO_2\) tension forms a straight line. If Fox’s data are plotted in this way, a straight line is approximated only for low \(CO_2\) tensions. He used 500 cc. samples and hence his determinations were probably far more accurate than mine. He determined the \(CO_2\) tension by titrating the \(CO_2\) in air shaken with the sea water (and the sea water left in the bottle) by the Pettenkofer method. Perhaps the limits of error of this method could include the differences between Fox’s data and mine. Or perhaps the air Fox analyzed had not come to equilibrium with the sea water. Fox discarded whole series of his data, and those remaining closely followed an empirical formula. I am not attempting to overthrow the determinations Fox made with his elaborate gas analysis apparatus by means of a micro method designed for other purposes than that for which it was used. I can only say that the two sets of data are for the present uncertain. It may be significant that his \(pH\) data are unreconcilable with mine. He determined these with the hydrogen electrode and also calculated them from the law of mass action and found the \(pH\) of sea water to be about 6, whereas I find it to be about 8. In this my results are in harmony with those of Palitzsch, Henderson and Cohn, and others.
Since Figs. 3 and 4 may be used as conversion tables provided the two titrations are made and the temperature and pH are determined, it seems desirable that a method be perfected for determining the pH on shipboard or in poorly equipped laboratories. The colorimetric method is the only one adaptable, and phenolphthalein is objectionable because errors in concentration simulate changes in pH. The buffer mixtures in use have a very different salt concentration from sea water, and the salt error has to be determined for every new indicator. For these reasons it seemed desirable to use the new indicators recommended by Lubs and Clark with buffer mixtures having the same salt concentration as sea water. Sea water varies in concentration, but the range from 0.4 to 0.6 N is perhaps great enough for all purposes. It was also desirable to increase the concentration of the buffers so that they will be less affected by the solution of glass. Since the solubility of buffers is reduced by the increase in salt concentration, the latter was made to correspond to 0.4 N sea water and a slight correction applied for 0.5 N sea water and a greater one for 0.6 N sea water. In actual practice the correction for 0.5 N sea water has already been made on the labels on the sealed tubes of buffers. A correction of 0.05 pH is then indicated for 0.6 N sea water and a similar correction but in the reverse direction for 0.4 N sea water. The salt error between the buffer mixtures and 0.5 N sea water was redetermined with the hydrogen electrode a large number of times at different temperatures and for both thymolsulfophthalein and o-cresolsulfophthalein, and likewise with 0.6 N sea water, but I do not believe this absolutely necessary. A moderate dilution of sea water with distilled water does not appreciably change the pH if the CO₂ tension is near that of the atmosphere and it is not agitated with or exposed to the air. It is then only necessary to dilute the sea water to determine the salt error with any new indicator, taking the 0.4 N sea water as the standard for the calculation of the correction in the pH of 0.5 and 0.6 N sea water. The salt error for the above indicators holds for phenolsulfophthalein and probably for all sulfophthalein indicators.

The buffer mixtures are made from two stock solutions "boric" and "borax," kept in Squibb's automatic burettes provided with soda-lime tubes. The distilled water used in making them is
boiled 15 minutes to rid it of CO$_2$ and cooled in a tightly stoppered, narrow necked flask or by means of a stream of CO$_2$-free air. The boric acid is recrystallized and dried in a desiccator (not by heat). The borax is recrystallized and dried in dry air short of efflorescence. The NaCl should be pure, but drying by heat is unnecessary because the error due to occluded moisture in the crystals is too small to cause a noticeable difference in the color of the indicators.

The "boric" contains 18.6 gm. of boric acid and 22.5 gm. of NaCl to the liter.

The "borax" contains 28.67 gm. of borax and 19 gm. of NaCl to the liter, and has the same salt action as the "boric" on the indicators. If kept at a low temperature, borax crystallizes out and must be made uniform in solution before mixtures are made. The same applies to the mixtures. The desired indicator is added to the stock solutions to the extent of 10 mg. to the liter, or added to the mixtures in the same proportion. In order to avoid dilution of the buffers and also to avoid the necessity of weighing the indicator for each solution, it was made up in 0.1 per cent solution in alcohol redistilled over sodium. Thymol-sulfophthalein requires the addition of a little NaOH to get it into solution at this concentration and hence the solution becomes less sensitive if allowed to absorb CO$_2$. If 0.01 per cent aqueous solution of the indicator is used for addition to sea water, the concentration of the sea water after the addition is used in calculating the salt error, since this dilution of 10 per cent corresponds to 0.025 pH in the salt error.

30 cc. of each mixture containing the indicator were sealed up (by fusing the glass) in a "Nonso" test-tube of exactly 24 mm. bore without introducing CO$_2$ from the blast lamp. Since these indicators are impure, it is necessary to test each lot, both as to concentration and range. The concentration may be tested by comparison with a sealed tube of the original indicator in distilled water, the concentration being the same as in the mixtures. In order to test the range, it is necessary to save samples of the stock solutions without indicator. This may be done by making mixtures, one near the middle or one near each end of the range of the indicator and sealing them in "Nonso" tubes. It is then only necessary to cut off the tip of the seal and introduce the
new sample of indicator in order to test it. The "boric" allows
the growth of mold, but the "borax" is antiseptic. The tubes
containing a large proportion of "boric" should be sterilized by
immersion in water up to the air space and boiling the water.

To facilitate comparison of the tubes, a colorimeter was made
by placing stereoscope prisms together in a sharp line and placing
two of the tubes at such a distance behind them that the centers
of the images were brought together in a sharp line. A thin
milk-white (opal) glass was placed immediately behind the tubes
to disperse the direct sunlight or mazda light passed through
"daylite" glass.4

The mixtures are given in the table on the following page.

The salt action on the indicators is approximately directly
proportional to the salt concentration over the range from 0.1 to
0.6 N, the difference in salt error of two sea waters being one-half
to three-fifths the difference in normality, and increase in salinity
causing the same color change as increase in alkalinity (increase
in pH).

The useful range of o-cresolsulfophthalein is from the first of
the series to pH 8.3 and of thymolsulfophthalein from 7.9 to the
last of the series. For the surface water of the open sea one
indicator is about as good as the other, except in the tropics,
where thymolsulfophthalein is the best. In the study of the
respiration of marine animals o-cresolsulfophthalein or α-naph-
tholsulfophthalein should be used.

A more or less definite relation between the pH and the solubility
of calcium salts in the sea water seems to exist. Dittmar showed that there
is less calcium in the surface waters than in the deep waters of the ocean,
and Sørensen and Palitzsch showed that the pH is higher in the surface
waters. It is difficult to study this question in vitro, owing to the relative
stability of the supersaturated solutions of CaCO3, and the existence of it
in solid form as aragonite, calcite, lublinite, and vaterite, with different
solubilities. Presumably the surface water over lime mud flats in the
tropics is saturated with calcite, or nearly so. If CaCl2 is added to this
water, in the form of a concentrated solution, no precipitate occurs, but
if the pH is only slightly increased, CaCO3 begins to deposit on the glass,
and it takes relatively little increase in the pH to cause a precipitate

4 The sealed tubes and colorimeter may be obtained from Hynson,
Westcott and Dunning, Baltimore. The tubes are labeled for 0.5 N sea
water.
pH and CO₂ Tension of Bicarbonates

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<td>8</td>
<td>92</td>
<td>9.00</td>
</tr>
<tr>
<td>4.5</td>
<td>95.5</td>
<td>9.05</td>
</tr>
</tbody>
</table>

throughout the solution. The size of grain makes a difference in the solubility of the precipitate, but if time for equilibrium is allowed, the small grains will change into less soluble crystals. According to Irvine and Young, sea water will dissolve 125 parts per million of crystallized CaCO₃. Rona and Takahashi determined the total Ca and the pH in mixtures of Na and Ca carbonates, bicarbonates, and chlorides, in contact with pre-
The concentration of the chlorides varied, but the alkaline reserve was about 160 in each. The following table gives the pH and the normality of 4 Ca.

<table>
<thead>
<tr>
<th>pH</th>
<th>4 Ca N</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.65</td>
<td>0.00985</td>
</tr>
<tr>
<td>6.80</td>
<td>0.0064</td>
</tr>
<tr>
<td>6.89</td>
<td>0.00492</td>
</tr>
<tr>
<td>6.97</td>
<td>0.00455</td>
</tr>
<tr>
<td>7.03</td>
<td>0.00405</td>
</tr>
</tbody>
</table>

In sea water the pH is about 8, the alkaline reserve is about 25, and the normality of 4 Ca about 0.1. The greater solubility of Ca in sea water notwithstanding the greater pH is due to decreased concentration of total CO2 and possibly to the increased concentration of chlorides. Since the chlorides are nearly constant and the alkaline reserve, total CO2, and Ca are interdependent, any change in the pH must cause a change in the solubility of Ca. When the pH is sufficiently raised, CaCO3 is precipitated and the Ca content and alkaline reserve are lowered. At present the law of mass action cannot be applied to such complex mixtures containing divalent salts whose second dissociation constant is unknown. Harkins and Pearce found that the addition of a salt with a common polyvalent ion may increase rather than decrease the solubility of a polyvalent salt. It is possible that the presence of CaCl2 may increase rather than decrease the solubility of CaCO3 in sea water. The solubility product law as applied to univalent salts does not apply without modification to polyvalent salts of certain types.

In a previous paper (McClendon, 1916, b) I described an experiment in which the CO2 tension of the air was determined by drawing it through sea water and then determining the pH of the sea water. A considerable time was required to reach equilibrium, and therefore special precautions had to be taken to prevent contamination or evaporation of the sea water. In order to avoid these precautions, I have experimented with bicarbonate solutions of such low alkaline reserve that equilibrium is reached quickly. In the meantime Higgins and Marriott published similar experiments, but their method was not sensitive enough for my purposes. I found that the pH of a 0.0003 N NaHCO3 solution as measured colorimetrically with phenolsulfophthalein and Sörensen’s phosphate mixtures changed with low CO2 tensions as shown in Fig. 3, for 20°. The pH varied directly with the temperature, 1° corresponding to 0.01 pH. Hence the 0.0003 N line in Fig. 3 may be used as a conversion table to find the CO2 tension of the air from the pH and temperature. If the temperature is 19°, 0.01 is added to the pH before using the conversion table, and if the temperature is 27°, 0.07 is subtracted from the pH before using the conversion table.
The phenolsulfophthalein was made up in 0.04 per cent solution in absolute alcohol and 5 cc. of it were added to 300 cc. of the phosphate solutions. The latter, m/15 solutions of KH$_2$PO$_4$ and Na$_2$HPO$_4$, were mixed in the following proportions:

<table>
<thead>
<tr>
<th>pH</th>
<th>Na$_2$HPO$_4$</th>
<th>KH$_2$PO$_4$</th>
<th>pH</th>
<th>Na$_2$HPO$_4$</th>
<th>KH$_2$PO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.00</td>
<td>18.2</td>
<td>11.8</td>
<td>7.40</td>
<td>24.2</td>
<td>5.8</td>
</tr>
<tr>
<td>7.05</td>
<td>19.0</td>
<td>11.6</td>
<td>7.45</td>
<td>24.7</td>
<td>5.3</td>
</tr>
<tr>
<td>7.10</td>
<td>19.8</td>
<td>10.2</td>
<td>7.50</td>
<td>25.2</td>
<td>4.8</td>
</tr>
<tr>
<td>7.15</td>
<td>20.6</td>
<td>9.4</td>
<td>7.55</td>
<td>25.6</td>
<td>4.4</td>
</tr>
<tr>
<td>7.20</td>
<td>21.4</td>
<td>8.6</td>
<td>7.60</td>
<td>26.0</td>
<td>4.0</td>
</tr>
<tr>
<td>7.25</td>
<td>22.2</td>
<td>7.8</td>
<td>7.65</td>
<td>26.4</td>
<td>3.6</td>
</tr>
<tr>
<td>7.30</td>
<td>23.0</td>
<td>7.0</td>
<td>7.70</td>
<td>26.7</td>
<td>3.3</td>
</tr>
<tr>
<td>7.35</td>
<td>23.6</td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These mixtures were sealed in "Nonsol" test-tubes of exactly 24 mm. bore. The apparatus for testing the air was shaped similar to a Dutchman's pipe. The bowl consisted of a short piece of "Nonsol" test-tube of exactly 24 mm. bore, and the stem of a piece of "Nonsol" tubing of about 7 mm. bore and a meter long. About 10 cc. of 0.0003 N NaHCO$_3$ solution, containing the same concentration of phenolsulfophthalein as the phosphate mixtures contain, was placed in the bowl of the pipe and suction applied to the mouthpiece by means of the mouth of the operator, so that the air passed up through the solution in bubbles in the pipe-stem. This process was continued until no further color change occurred in the solution, which was then allowed to run back into the bowl and its color compared with that of the sealed tubes. A thermometer was then placed in the bowl and the temperature of the solution determined.

By making the bowl of the pipe 10 mm. bore, it was found possible to use Hynson and Westcott phenolsulfophthalein tubes made for determination of pH of blood dialysate, but with less accuracy.

The main difficulty experienced has been in keeping a 0.0003 N solution of NaHCO$_3$. A gold flask would be too expensive and a pure silver flask hard to procure. It seems unwise to invest in a gold-plated flask without the guarantee that the plate will remain perfect. Ceresin bottles may be used, but ceresin and
rubber lacquer peeled off. I silvered the interior of a flask of resistance glass. The silvering peeled off in the upper part of the neck, and was affected by sulfur from the rubber stopper. If gold is burned on the glass in a layer thick enough to be opaque, it peels off. If the layer is thinner, it may stick for a long time. I gilded one resistance flask as follows: Campana's bright liquid gold was thinned with banana oil and poured into the flask and poured out, and dried thoroughly in an inverted position by means of a current of air. It was then heated in the blast lamp. Perhaps better results might be obtained by substituting regular thinner made for china painter's gold, and a china kiln. Irving Langmuir has described a process of gilding by volatilizing gold on an electrically heated tungsten filament in high vacua. Ceresin bottles do not absorb the indicator and are perhaps the best for holding the bicarbonate solution colored with the indicator. The indicator undergoes some change in absolute alcohol.

The time required for reaching equilibrium is lessened if air is passed through the stock solution of bicarbonate so as to remove some of the CO₂. The stock solution may be made by passing CO₂ or the breath through a NaOH or Na₂CO₃ solution. The more CO₂ the solution contains, the less it attacks glass, silica, and other flask materials, and rubber stoppers. Sulfur-free rubber stoppers are very desirable for closing the flasks. On a trip from San Francisco to Samoa and return, Dr. A. G. Mayer found the CO₂ tension of air over the Pacific to vary from about 0.23 to 0.3 mm. by this method, whereas the variation of the CO₂ tension of the sea surface was much greater and not correlated with that of the air. This indicates that diffusion of CO₂ between air and sea is very slow.

When the CO₂ tension is known, the per cent of CO₂ in the air may be found by multiplying by 100 and dividing by the barometric pressure in mm.

In the determination of the CO₂ of the air and in other studies, a number of NaHCO₃ solutions were investigated, as shown in Fig. 3. These were preliminary determinations, and no special accuracy is claimed for them. The chief source of error probably lies in failure to reach equilibrium, as shown by the follow-

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5 Paper presented before the American Physical Society, 1916.
ing example on sea water. A rapid stream of CO₂-free hydrogen was passed through a few cc. of sea water and at the end of 270 minutes the alkali had been reduced to carbonate, but only a small fraction to NaOH, and yet CO₂ was being continuously eliminated at the end of the experiment. The experiments were accurate enough, however, to indicate, at least within certain limits, that at constant temperature the pH of these solutions is proportional to the logarithm of the alkaline reserve and inversely proportional to the logarithm of the CO₂ tension. If the CO₂ tension remains constant, a rise of 1° in temperature causes an increase of 0.01 pH, whereas if the total CO₂ content remains constant, the pH is not affected by temperature. At constant CO₂ tension and temperature, as shown by Fox, the total CO₂ is directly proportional to the alkaline reserve within a slight error equal to the CO₂ absorbed by distilled water under the same conditions.

My experiments indicate, at least within certain limits, that the slope of the curves, as in Fig. 3, is the same for sea water, blood, and other biological media (except those exceptionally rich in phosphates) as it is for bicarbonate solutions. Neutral salts slightly decrease the hydrolysis of bicarbonate and decrease the pH. The chief buffer in these media is bicarbonate, and other buffers are not present, even in blood, in sufficient concentration to change the slope of the curve greatly, at least in its upper regions, but the buffer action of proteins in blood comes into play when the blood is made strongly alkaline by the elimination of CO₂; the curve for blood in Fig. 3 being slightly incorrect in the lower CO₂ tensions.

It would be of interest in this connection to know the concentration of bicarbonate in normal blood, but the presence of phosphates and organic matter makes titration very uncertain. (The compensation dialysis method might yield somewhat more accurate results.) The bicarbonate concentration of different bloods is practically proportional to the total CO₂ content at the same CO₂ tension, and hence Van Slyke's method of determining the alkaline reserve of plasma might be used to determine the bicarbonate, provided the conversion factors from his units to the titration units were known. The bicarbonate content of blood does not often exceed 0.04 N, as will be shown in a later paper.
Since the concentration of neutral salts is only about a fourth as
great as in sea water, their effect in reducing the hydrolysis is slight.
The bicarbonate concentration is probably but little more than
that of a pure bicarbonate solution that has the same pH at the
same CO₂ tension and temperature, and hence could be estimated
by means of a chart constructed on the same principles as Fig. 3
but somewhat more accurate in this region.

The above views seem to differ somewhat from those of Hen-
derson and Cohn on sea water. Henderson and Cohn found it
necessary to add 0.0015 M H₃BO₃ to a liter of alkaline NaCl
solution in order to make it behave like sea water in regard to pH.
My experiments were at variance to this, but since CO₂ tension is
one of the most difficult factors to determine exactly, other methods
seemed necessary to determine the concentration of non-volatile
buffers in sea water. A serviceable method was found to be the
titrination of CO₂-free sea water with CO₂-free NaOH in the hydro-
gen electrode. It is difficult to maintain the sea water and NaOH
absolutely CO₂-free, and the first trace of CO₂ is immediately
titrated as non-volatile buffer. By titrating directly into the
electrode shown in Fig. 5 (after removal of the trap at the top)
the results could be closely duplicated, and are shown in Fig. 6.
The titration must be done rapidly and not carried beyond
pH = 10 owing to the precipitation of (carbonates if present)
phosphates (borates?) and finally hydroxides of Ca and Mg.
Sea water has hardly more non-volatile buffer than artificial sea
water previously described (McClendon, 1916, b). The con-
centration of non-volatile buffer in Atlantic, Pacific, and Gulf
Stream water is practically identical. On the contrary, the non-
volatile buffer in the solution used by Henderson and Cohn is
very much higher in concentration. Boric acid was detected in
all samples of sea water, but it is evidently in less concentration
than 0.0015 M. The phosphoric acid quantitatively recovered
from sea water is negligible.

In attempting to confirm these findings by plotting the pH
against the CO₂ tension, as was done by Henderson and Cohn,
the first experiments were apparently vitiated by the presence
of some air in the CO₂. At any rate very irregular results were
obtained. In an attempt to clear up the doubt aroused by these
results, a large number of determinations were made on a varied
Fig. 5. Hydrogen electrode for CO₂-free titrations, for standardizing buffer mixtures, and determining the salt action on indicators (for which purpose its diameter is made the same as the colorimetric tubes). The tube admitting hydrogen at the bottom is ground to fit the openings at each end of the electrode, so that the latter may be inverted when it is desired to change the height of the palladiumized disk. In making the electrometric titration the trap at the top is discarded, and the burette tip inserted in its place. Glass wool moistened with distilled water and wrapped around the burette tip serves as a trap to keep out O₂ from the air above. The lower ground joint is ungreased because it is immersed in the KCl bath for electrolytic connection with the calomel electrode.

Fig. 6. Electrometric titration of CO₂-free sea water and artificial sea waters. The pH is measured on the abscissa and the number of cc. of CO₂-free NaOH on the ordinates. The left hand ends of all the curves practically coincide, the right hand ends are marked as follows: A = sea water. B = artificial sea water of formula given by McClendon (1916, b). C = the same + 0.00017 M H₃PO₄. D = the same + 0.001 M H₃BO₃. E = the same + 0.0015 M H₃BO₃.
series of solutions of alkaline reserve = 25, given in the following list:

A. 0.0025 \text{ N} \text{NaHCO}_3.
B. 0.0025 " " + 0.025 \text{ N} \text{NaCl}.
C. 0.0025 " " + 0.0025 " \text{CaCl}_2.
D. 0.0025 " " + 0.025 " "
E. 0.0025 " " + 0.025 " \text{MgCl}_2.
F. Artificial sea water, alkaline reserve = 25 (for formula see McClendon, 1916, b).
G. Artificial sea water + 0.0015 \text{ M} \text{H}_3\text{BO}_3.
H. " " " + 0.001 " "
I. " " " + 0.0008 " "
J. " " " + 0.00017 " \text{H}_2\text{PO}_4.

Some of these experiments indicate that \( \frac{1}{2} \text{Ca}^{++} \) reduces the hydrolysis of the bicarbonate more than \text{Na}^{+} does, but this note is made merely as a suggestion for further research. The experiments in general indicate that small amounts of neutral salts or non-volatile buffers have little effect on the pH at constant CO$_2$ tension. Some deviations from this rule were ascribed to impurities in the salts. The CaCl$_2$ used in the last experiments was dissolved in absolute alcohol that had been redistilled over sodium, evaporated, fused in a platinum dish, dissolved in distilled water, and carefully neutralized.

Henderson and Cohn, using the indicator method of Palitzsch, record an effect of salinity on the pH of sea water at constant CO$_2$ tension. It is not clear whether they mean a simple change in the concentration of neutral salts or whether the alkaline reserve was also changed. If the neutral salts alone were changed, the change in pH was in the wrong direction for the effect of salts on the hydrolysis of bicarbonate, but was in the right direction for the salt effect on phthalein indicators. According to my experiments, neither the salinity nor the alkaline reserve in sea water of the tropical or temperate oceans change sufficiently to change noticeably the relation of pH to CO$_2$ tension, although the alkaline reserve does change sufficiently to affect the total CO$_2$ greatly.
pH and CO₂ Tension of Bicarbonates

BIBLIOGRAPHY.

THE STANDARDIZATION OF A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF THE HYDROGEN ION CONCENTRATION, CO₂ TENSION, AND CO₂ AND O₂ CONTENT OF SEA WATER, OF ANIMAL HEAT, AND OF CO₂ OF THE AIR, WITH A SUMMARY OF SIMILAR DATA ON BICARBONATE SOLUTIONS IN GENERAL

J. F. McClendon


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