

THE PROBLEM OF DILUTION IN COLORIMETRIC H-ION MEASUREMENTS

I. ISOHYDRIC INDICATOR METHODS FOR ACCURATE DETERMINATION OF pH IN VERY DILUTE SOLUTIONS

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INTRODUCTION

Both chemical and biological literature show increasingly the need for more exact and dependable methods of colorimetric procedure for H-ion measurements (checked by the E.M.F. method when possible) on water and on very dilute solutions beyond the usual range of 0.2 to 0.02 M.

This was recognized by Birge and Acree in 1918 when they emphasized the necessity for adjusting the pH of the indicator for use with weakly buffered solutions, and of employing what we shall henceforth call the isohydric indicator method (abbreviated to iso-indicator or i. i. method) to obtain the final correct pH readings for standards and unknowns. This result is accomplished by the use of adjusted indicator solutions having nearly or exactly the same pH that the solution or water under examination shows from preliminary tests. In a study of the pH of water and solutions used in intravenous medication Williams and Swett in coöperation with Mellon, Slagle and Acree in 1920-1921 prepared excellent double distilled water showing pH values of about 6.8 to 7.1 when tested with brom thymol blue adjusted to about pH 7.0. This water gradually absorbed carbon dioxide from the air and generally reached a pH of about 5.7 to 6.0.

Brightman, Meacham and Acree in 1919-1920 combined E.M.F. and spectrophotometric methods to correct the data for the salt errors of indicators in such work.

That bacteriologists are beginning to feel the same need is shown by the statement made in the last Manual of Methods issued by the Society of American Bacteriologists (p. c. 45, Committee on Technic, 1927). In the discussion of the colorimetric procedure the committee points out that "if insufficient buffer is present or if either the distilled water or the indicator solution should be too far from the desired reaction, the result may be misleading." We also see occasional references by biological workers, who are using colorimetric methods, to the pH of the water used and to the means by which the water was prepared.

The list of problems to which the kind of technique developed in the present investigations is applicable is large and includes pH tests on plant extracts, weakly buffered culture media like corn meal agar (especially when it is necessary to dilute the latter more highly than usual to eliminate color or turbidity), tests on dilute buffer solutions, natural and purified drinking water, boiler water, sewage, factory waste liquors, solutions of sugar during the refining process, distilled water used in industries including the manufacture of beverages, and solutions for ore flotations.

In such problems workers often fail to recognize that the colorimetric technic heretofore employed is inadequate for determining the pH of water or of very dilute solutions. The data presented in this article show the latter to be true for the following several important and fundamental reasons.

1. The "salt errors" or "buffer corrections" that must be applied to the buffer-color standards and the unknown solutions of 1.0 to 0.02 M are of an entirely different order from those occurring in solutions of less than 0.001 M.

2. The adjustment of the pH value of the indicator solution itself is without practical influence on the standard buffers and unknowns to which it is added in making colorimetric tests on the usual solutions of 0.2 to 0.02 M. Such adjustment is strikingly

necessary, however, because of its marked effect, with solutions which are so dilute that the added indicator produces considerable change in the total buffer and H-ion content. A method of adjusting the indicators was therefore devised.

3. In the usual colorimetric work the effect of the carbon-dioxide and other impurities in the distilled water used for making the solutions or dilutions thereof may be neglected, but in very dilute solutions these factors are of marked influence. As shown in these investigations, widely different effects may be produced by variations in the character or condition of the water used; for example, water containing considerable CO_2 as when freshly collected from ordinary stills (usually about pH 5.0), water which has reached equilibrium with the CO_2 in the air (generally about pH 5.7) or CO_2 -free water in the neighborhood of pH 7.0, all of these values being subject to more or less variation due to the presence of small quantities of sprayed-over solids. Methods were devised for preparing experimentally and for testing all these "kinds" of water.

4. In view of the three foregoing phases of the problem it is clear that we are in great need of accurate quantitative colorimetric and E.M.F. data on the dilution of the important buffers, culture media and indicators with these different types of water in order to discover and test more fully the laws underlying the hydrolysis and ionization of buffers and indicators by water. Accordingly, the accompanying tables and curves are here presented for possible assistance to others.

HISTORICAL

This coöperative work was begun in January, 1924, in order to apply the isohydric indicator methods and explain and rectify the source of errors which Miss Fawcett had previously observed in her work (covering about 400 experiments) on the dilution of culture media. She had shown in 1923 that stock indicator solutions standing in soft glass bottles gradually become more alkaline and give readings different from those of either fresh indicator solutions or old ones readjusted with acid to the first faint color change. These differences were very pronounced and

variable in her work on certain solutions with inherently weak buffer properties and on others diluted with (a) ordinary distilled water, (b) reboiled distilled water and (c) double distilled water. Under these conditions it was impossible to determine the correct pH values. Similar results had been obtained by Acree and his coworkers. The isohydric indicator method offered the only correct means of (a) preparing, studying, keeping and using distilled and superpure water; of (b) measuring the actual pH thereof and of buffer solutions diluted therewith; of (c) studying the salt errors of indicators; of (d) making reliable comparisons of colorimetric pH values with the corresponding E.M.F. data; and of (e) studying solutions involving the carbon dioxide-bicarbonate-carbonate ($\text{CO}_2 - \text{NaHCO}_3 - \text{Na}_2\text{CO}_3$) equilibrium, especially when neutral gases are passed therethrough and into pure water to investigate the CO_2 equilibrium. This article is the first of a series of our researches on the above subjects.

Important work having a bearing on this general subject has been done by other investigators, notably Kolthoff (1925), who studied the effect of the dilution of buffers on indicator salt errors; McBain, Dubois and Hay (1926) in their paper on the "Salt Error¹ of Indicators Caused by Standard Alkaline Buffers Themselves," and the earlier work of McClendon (1917) and that of Wells (1920) on sea water. A paper dealing specifically with the adjustment of indicators for use with weakly buffered solutions has recently appeared by Pierre and Fudge (1928), who worked out both colorimetric and electrometric methods of adjusting the phenolsulphonphthaleins to their mid-points when determining the pH of soil extracts. Schlegel and Steuber (1927) recognized the effect both of CO_2 and of the reaction of the indicator on their determinations of weakly buffered sugar solutions, and attempted to adjust their brom thymol blue indicator to pH 7.0. Karraker (1923) and Marsh (1924) also published notes suggesting the adjustment of indicators to their mid-points.

¹ In certain of their experiments the so-called salt errors were actual pH changes caused by the neutralization of their alkali standards by the free indicator acids used.

Their work grew out of a need for greater accuracy in colorimetric work with soil extracts.

Because of the impracticability of publishing in one article, (a) the many parallel determinations which have been necessary to study fully the effect of CO₂ and of the indicator acids on the pH of dilute solutions from a colorimetric standpoint, and (b) the complete electrometric data necessary for calculating the *salt errors*, we have reserved this phase of the subject for another paper in which the relation of our work to all previous work on salt effects will be discussed. This paper is now nearly completed and will clarify some points left in doubt by the present paper, such as the accuracy of indicator adjustments, and of pH values assigned to water containing small amounts of impurities.

TECHNIC

Since the accuracy of colorimetric work with dilute or weakly buffered solutions depends entirely on the use of the proper technic, the methods found best suited to the needs of the experiments described in this paper must be given in detail at the risk of tedium.

1. *Glassware*

All glassware used was Pyrex, with the exception of some of the graduated pipettes. The *flasks* were prepared as follows: They were heated in the Arnold Sterilizer for about 1 hour successively with (1) N/100 alkali, (2) N/100 acid and (3) distilled water, rinsing carefully between (2) and (3). They were then emptied and drained while hot and plugged with cotton to keep out dust.

Tubes after having been cleaned by a thorough laboratory routine, were given an extra treatment as follows: They were placed in large pyrex beakers, filled and covered with distilled water which was drained off, refilled and covered with fresh distilled water, steamed for one hour in the Arnold Sterilizer, emptied and drained on removing from the steamer, dumped on a clean towel and placed quickly while very hot in clean wire baskets in an inverted position. In this way the tubes dried almost immediately without retaining much of the rinse water.

Pipettes were carefully selected, cleaned, rinsed repeatedly with distilled water and tested with an indicator to determine whether the glass changed the color of the indicator. If a small quantity of brom cresol purple adjusted to pH 5.7 (which is the pH of water at equilibrium with the CO₂ in the air) did not become bluer on standing for 5 or 10 minutes in the pipette it was considered safe for use. We recommend the use of Pyrex pipettes and burettes after our very satisfactory work with a few of them.

2. Water

*Super-pure water*² for use in the dilution experiments was made with a pH of 6.9 to 7.1 in a special commercial still and was also prepared from water from this high capacity still by redistillation in a specially designed table or desk still. The latter, with the exception of the distilling flask and condenser jacket (which were of glass), was constructed entirely of block tin. The water was distilled from a pyrex flask through tin rings in a tin column, cooled in a tin condenser, and collected in pyrex flasks. No chemicals were added to the water in the distilling flask but aeration by means of air led through a special type of soda lime jar arranged to remove all CO₂ and soda lime dust was carried on continuously both in the distilling flask and in the collecting flask during the distillation process. In this way water, entirely free or nearly free from CO₂, could be collected and tested immediately on removal of the collecting flask. Both of these stills and the soda lime jar will be described in a separate paper.

The *water was always tested* by the technic of section 5 (*d*) with a solution of brom thymol blue adjusted to pH 7.0 and the readings varied between pH 7.0 and pH 7.05 with the exception of the first portions coming over, which were sometimes more alkaline, or more acid, due to incomplete aeration as proved by further aeration. These slight variations in pH were apparently not due to temperature, since no appreciable change could be detected experimentally by means of the indicator over a range of 17° to 25°C.

² *Note:* Conductivity experiments to be published in another article show this water to be superpure.

The earlier prevalent belief (for example, Dawson, 1925) that the customary distillation of about one-third of ordinary distilled water will drive out the carbon dioxide and leave a nearly neutral or buffer-free pH 7.0 water rests upon the failure to recognize the presence of sprayed-over alkaline impurities still in the residual water. Consequently, in making pH tests of such water with the usual unadjusted mono sodium brom and thymol blue having a pH value of possibly 5.9 to 6.2, it is not always understood that the added indicator may be partially neutralized by the residual carbonate-bicarbonate buffer salts of possibly pH 7.5 to 8.5 in the water to give a reading which is close to pH 7.0 and is mistaken for that of the water itself.

This influence of both indicator and water on the final pH reading is shown by the fact that superpure pH 7.0 water gave the reading pH 4.6 with brom phenol blue of pH 3.4; registered pH 5.7 with brom cresol purple of pH 5.2; showed a pH 7.05 with brom thymol blue of pH 7.0; and had the pH 8.05 when mixed with phenol red of pH 8.6. This same change of the pH reading of the indicator is shown in the tables and columns for the highly diluted buffers, which may of course be considered as slightly impure water. Neutral salts like sodium chloride in such minute concentrations have no appreciable influence on the neutrality or pH of the superpure water and its solutions but are non-buffer impurities that increase the conductivity. Hence a statement should be made at this place to the effect that the only way the pH of water containing very small quantities of alkaline salts (pH 7.0 to 9.0) or of CO_2 (pH 5.0 to 7.0), or of both, can be determined accurately or even approximately is by adding 0.2 cc. of each member of a graded pH series of phenol red, brom thymol blue or brom and cresol purple solutions to 10 cc. portions of the water and finding the point or zone at which there is agreement between the pH of one of the adjusted indicator solutions and its pH after mixture with the water under test and comparison with the buffer-color standards.

The following method was used for keeping water free from CO_2 during an experiment. The pyrex flasks were fitted with double perforated thoroughly cleaned rubber stoppers. In one opening

was inserted a pyrex tube through which CO₂-free air was led into the water by means of a rubber tube connected with the soda lime jar. The air was thus allowed to bubble through the water at a fairly rapid rate and escape through the free opening of the stopper. Water which was allowed to stand without aeration for several hours in these flasks, even when tightly closed with rubber stoppers, reabsorbed CO₂ to some extent through the stoppers (the pH falling from 7.0 to 6.8 or 6.5 in eighteen to forty-eight hours) and had to be reaerated before use if CO₂-free water were desired. In the case of redistilled water which had reabsorbed CO₂ (pH 6.4 to 6.8), aeration at room temperature for one hour was found to be in most cases a safe procedure for removing all traces of CO₂, while even ten or fifteen minutes' aeration would often bring it to within 0.05 pH of its CO₂-free equilibrium point if the rate of bubbling was fairly rapid. Ordinary distilled water on the other hand, which on collection is supercharged with CO₂ and has a pH of about 5.0, can only be freed completely of carbonic acid by thorough boiling (usually about ten minutes per liter) and aerating immediately while hot or by aerating for a much longer time (five or six hours) without heating. By cooling the water in the flask in a flowing cold water bath while the aeration is being carried on, it can be brought to room temperature rather quickly.

The same general method of handling the water was used in *controlling CO₂ equilibria for experimental purposes*.

1. To obtain water in equilibrium with the CO₂ in ordinary fresh air, a flask containing tested redistilled water at room temperature was saturated with the air which had been taken from a pressure cock (after blowing it off) and had been passed through a side neck filter flask filled with absorbent cotton to rid it of condensation moisture or other impurities. When redistilled water treated in this manner was tested by the technic of Section 5 (d) with brom cresol purple adjusted to pH 5.7 the equilibrium point was generally reached in about ten minutes and found to be pH 5.7 at temperatures between 25° and 30°C.

2. Since repeated tests showed that water freshly collected from ordinary stills contained more CO₂ than it did after standing in an

open vessel and coming to equilibrium with the CO_2 in the air, and since the distilled water was found to be practically constant at pH 5.0 for the still and tap water used in our laboratory, it was chosen as one of the "kinds" of water to be used experimentally in making dilutions. Accordingly pH 5.0 water was prepared artificially by adding to 200 cc. of tested CO_2 -free redistilled water, 1 cc. of the same water which had previously been saturated with CO_2 from a tank containing the compressed gas. An experimentally constructed curve for pure water containing graded quantities of CO_2 will be given in another paper.

3. Adjustment of indicators

The adjusted series of indicators were prepared by the methods first devised and recommended by one of us and his co-workers in their introduction and development of the use of sulphon-phthalein indicators (Lubs and Acree, 1916; Acree and Slagle, 1908; White and Acree, 1918). These methods involve, first, the preparation of a stock solution of each of the mono-sodium uncolored indicator salts now adopted generally by other workers; (see Clark, 1920, p. 14) and secondly, the adjustment of several portions of each of these stock solutions to the desired hydrogen-ion values, preferably 0.2 pH apart, a procedure not yet generally adopted but absolutely necessary in accurate colorimetric work with dilute solutions.

The stock solutions of the indicators with two exceptions (methyl red and brom phenol blue) were prepared by grinding them in a mortar, or warming them, with a volume of $\text{N}/20$ alkali equivalent to 1 mol of indicator, i.e., sufficient to dissolve them or at most give a slight color change and then diluting with good distilled water to the strength desired, 0.02 or 0.04 per cent, as is the general practice. These uncolored or slightly colored stock solutions served as a starting point for further neutralization with standard alkali and graded adjustment of the pH values and colors. We have found it convenient for accurate colorimetric work with dilute solutions to have constantly on hand sufficient adjusted portions of each stock indicator to include its lowest,

TABLE I
Adjustment of indicators

pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH PER DECIGRAM ACTUALLY ADDED*	pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH PER DECIGRAM ACTUALLY ADDED*
Brom phenol blue				Brom cresol green			
		cc.	cc.			cc.	cc.
3.4	0.5	1.5	{ 1.5 1.5	(4.2)††	1.00	2.80	2.8
3.6	0.82	2.46	{ 2.46 2.50	4.2	1.23	3.44	3.17
(3.7)†	1.00	3.00	3.00	4.4	1.34	3.75	3.44
3.8	1.07	3.21	{ 3.00 3.10 3.21	4.6	1.45	4.06	{ 3.75 3.30
4.0	1.28	3.84	{ 3.50 3.55	4.8	1.58	4.42	{ 4.06 3.80
4.2	1.45	4.35	3.84	5.0	1.68	4.70	{ 4.20 4.05
4.4	1.60	4.80	4.07	5.2	1.78	4.98	4.42
4.6	1.72	5.16	4.35	5.4	1.84	5.15	{ 4.70 4.30
				5.6	1.90	5.32	
Brom thymol blue				Brom cresol purple			
(5.9)†	1.00	3.20	3.20	(5.0)†	1.00	3.70	3.70
6.2	1.13	3.61	{ 3.45 3.61 4.10	5.2	1.07	3.97	{ 3.97 3.75
6.4	1.20	3.84	{ 3.70 3.84	5.4	1.11	4.10	4.10
6.6	1.29	4.13	{ 3.95 4.13 4.30	5.6	1.17	4.33	{ 4.33 4.25 4.20
				5.8	1.24	4.59	4.59

TABLE 1—Continued

pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH		pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI PER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH	
		PER DECIGRAM (CALCULATED FROM CURVE)	PER DECIGRAM ACTUALLY ADDED*			PER DECIGRAM (CALCULATED FROM CURVE)	PER DECIGRAM ACTUALLY ADDED*
Brom thymol blue				Brom cresol purple			
		cc.	cc.			cc.	cc.
6.8	1.39	4.44	{ 4.20 4.44 4.80	6.0	1.33	4.92	{ 5.00 4.92 4.90
7.0	1.50	4.80	{ 4.65 4.80 5.00	6.2	1.45	5.36	{ 5.36 5.30 5.40
7.2	1.63	5.21	{ 4.95 5.21 5.15	6.4	1.56	5.77	5.77
7.4	1.73	5.53	{ 5.45 5.53	6.6	1.68	6.21	{ 6.21 6.0 6.0
7.6	1.81	5.79	{ 5.52 5.79				
Cresol red				Phenol red			
(6.6)††	1.00	5.3	5.3	(6.6)†	1.00	5.70	{ 5.70 5.70
7.2	1.075	5.69	{ 5.55* 5.88*	7.0	1.11	6.32	{ 6.57 6.70
7.4	1.11	5.88	{ 5.8 6.20	7.2	1.16	6.61	{ 7.01 7.20
7.6	1.17	6.20	{ 6.30 6.62	7.4	1.24	7.07	{ 7.72 7.70
7.8	1.25	6.62	7.05	7.6	1.35	7.69	8.21
8.0	1.33	7.05	{ 7.05 7.68	7.8	1.44	8.21	8.95
8.2	1.45	7.68	{ 7.55 8.27	8.0	1.57	8.95	{ 8.70 9.69

TABLE 1—*Concluded*

pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI FER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH FER DECIGRAM		pH OF ADJUSTED INDICATOR	MOLECULAR EQUIVALENT OF ALKALI FER DECIGRAM (CALCULATED FROM CURVE)	N/20 NaOH FER DECIGRAM	
		(CALCULATED FROM CURVE)	ACTUALLY ADDED*			(CALCULATED FROM CURVE)	ACTUALLY ADDED*
Cresol red				Phenol red			
		cc.	cc.			cc.	cc.
8.4	1.56	8.27	8.95	8.2	1.70	9.69	10.09
8.6	1.69	8.95	9.43	8.4	1.77	10.09	9.2-10.43
8.8	1.78	9.43		8.6	1.83	10.43	10.93
Methyl red							
4.6	Unadjusted alcoholic solution						
4.8	1.0	7.4	7.4				
5.0	1.25	8.18	{ 8.0 9.25				
5.2	1.42	9.27	{ 9.5 10.5				
5.4	1.58	10.33	{ 10.5 11.7				
5.6	1.68	10.98	12.5				
5.8	1.82	11.90	13.5				

Note: The theory of the proper adjustment of these indicators to be given more fully in a later article will explain the discrepancies in the above tables between actual and calculated amounts of alkali necessary to reach a given pH value.

* Values included in braces represent different adjustments of the same sample or of different samples of the indicator.

† Parenthetical values were determined on 1 mol stock solutions (per se) and do not necessarily correspond to the calculated molecular equivalents.

‡ Determination made on a separate lot of indicator from those represented by the values which follow.

mid-point and highest useful pH values, or still better to cover its entire color range in steps of 0.2 pH.

The *details of the adjustment* were as follows: The stock solution of each indicator was divided into 100 cc. portions and placed in 100 cc. Erlenmeyer flasks to which were added graded amounts of $N/20$ NaOH calculated from the theoretical ionization constants or titration curves of the indicators to bring the solutions to the desired pH values preferably 0.2 pH apart (table 1). The dilution error is negligible. The fact that the indicator samples were not always free from impurities made it necessary to devise a method of checking the pH values thus obtained.

The following procedure was found to be quite simple and satisfactory. This experimental application of the isohydric indicator method was developed by Miss Fawcett and is much better than the use of drops on white test plates or in shallow pyrex dishes (Brown, 1924) or than the usual comparisons in 16 to 18 mm. test tubes or in a Duboscq colorimeter or by Gillespie's (1920-1921) drop ratio method. (1) A volume of 0.2 cc. of the adjusted indicator to be tested for pH was introduced into the bottom of a tube test. (2) The same volume was placed in a second test tube to which was also added 0.2 cc. (even 0.05 cc. is sufficient) of a $M/20$ (or even $M/1000$) buffer having the same desired pH value to form the buffer-color standard. The two test tubes were then stoppered and quickly brought to a horizontal position over white paper or porcelain to cause the solutions to spread out in thin layers of substantially equal area.*

* Although the volumes in (1) and (2) are different, according to Beer's law and in actual practice the colors match at the same pH. In the equation $H \times \text{In}/H\text{In} = K_{\text{ind}}$ expressing the concentration of the colored ions In and molecules HIn and H-ions in terms of the indicator ionization constant K_{ind} , the H is the same for both solutions at the same pH value. Therefore the fraction $\text{In}/H\text{In}$ is also constant at this H value, and for equal areas viewed the colors of the two solutions are the same regardless of the difference in the volume and depth. Conversely, if the colors are alike, the pH value of the indicator solution may be considered as accurately adjusted to that of the standard buffer-indicator mixture, which however may not be free from salt errors. Since the hue of the indicator also changes greatly with pH, this isohydric indicator method gives a remarkably easy way of adjusting the pH and colors of these very dilute indicator solutions to those of known reproducible buffer-color standards.

In this way the colors were readily compared. If the indicator solution under test did not match the same solution when mixed with buffer, it was further corrected by adding more alkali or acid, cautiously, to the flask and retesting until the colors were exactly the same. If only part of the series is adjusted theoretically before beginning testing, enough of the stock solution may be reserved for making the corrections and completing the series as desired. The adjustments may be made entirely in an empirical way with success but it is a help to have the theoretical values from the titration curves or our table 1 as a guide.

As shown in table 1, the stock solutions made with one mol-equivalent of alkali were usually found to be near the acid end of the range. Brom phenol blue and methyl red were exceptions to this rule. In the case of the former it was found that a quantity of alkali equivalent to only 0.5 mol of the indicator was sufficient to bring the solution to pH 3.4 which falls a little short of being the acid end of its range. In the case of methyl red hydrochloride the unadjusted indicator matched the indicator-buffer mixture pH 4.6 and was used as number 1 in that series. Where the amounts of alkali actually added in adjusting the indicators differed from the theoretical quantities they are given in parallel series.

When adjusting the indicators for alkaline ranges, viz., brom thymol blue, phenol red and cresol red, it was necessary to do the matching rapidly unless the test tubes were first filled with CO₂ free air, because the indicator solution containing no buffer absorbed CO₂ readily from the air when spread out in a thin layer. With practice, however, this can be done successfully. We shall later report on the use of graded amounts of sodium bicarbonate in buffers and indicators to keep them at air-CO₂ equilibrium.

Unfortunately some of the solutions were found to be very unstable for reasons not fully understood, especially in the alkaline ranges above pH 7.0. The changes which took place were, in the cases of brom thymol blue, phenol red and cresol red, nearly always in the acid direction but their occasional irregularity seemed to point to some cause or causes other than absorption of

CO₂, though it is possible that a gradual action of the latter in conjunction with uneven distribution of colloidal or particulate impurities from the indicators or accidental introduction of particles of dust, bits of cork or occasional slight fungus growth may be responsible for this instability. These alkaline solutions required continual readjustment by the addition of more alkali, or of acid in a few cases. One sample of brom cresol purple always gave solutions which became more alkaline on standing.

In practice the series of indicators being used in any given experiment was checked by the method described on page 175 and readjusted when necessary. Results did not seem to indicate that the increase in buffer content or the dilution of the solutions thus readjusted was sufficient to affect the readings. This point was checked with care and indicator solutions to which a double portion of alkali had been added gave identical readings in pure CO₂-free water with those having the same pH values, but containing only the original calculated amounts of NaOH. Uniformity of readings made on redistilled water under similar conditions with different lots of adjusted and readjusted indicators furnish ample proof of the accuracy of the method.

4. Technic for making dilution series

The following procedure was used in making the dilution series. The pH of the undiluted medium or buffer was always determined electrometrically. A double hydrogen electrode was used in making all pH determinations together with a M/10 sodium acid phthalate solution (pH 3.95 to 3.97 as determined by different workers) as the standard. The same standard solution was used in making electrometric determinations throughout the experiments covered in this publication.

Clark and Lub's buffers were used in the colorimetric standards and these were also checked by means of the hydrogen electrode. Accuracy to within 0.03 pH was attempted and care was taken that slight errors in value were all in the same direction in order to keep the intervals even. The pH 7.0 buffer actually did not vary more than ± 0.01 pH during the entire period of experimentation.

Buffer-color standards were made up at frequent intervals by adding 0.2 cc. of the indicator to 10 cc. of buffer. The color standards used in any given set of experiments were always made with one, usually the middle one, of the adjusted series of indicators used in the unknown solutions under test. This takes care of the variation in the purity of commercial indicators.

In the method of making dilutions, a large flask of redistilled water (1) was kept aerated with CO₂-free air from the soda lime scrubber at pH 7.0. Two more flasks, (2) and (3), were prepared at pH 5.7 and pH 5.0 respectively from the same redistilled water, as described under *Technic, Sec. 2, Water*. It was found that water containing sufficient CO₂ to register pH 5.0 would not lose its acidity to any appreciable extent during the progress of an experiment if kept in a closed flask. Having prepared these three "kinds" of water, the lower dilutions for each series could be made directly and the higher ones prepared from a 1:10 or 1:100 dilution with water which was kept aerated with (1) CO₂-free air or (2) ordinary air, as the case might be, or (3) simply kept in a closed flask when pH 5.0 water was needed.

5. *Procedure for making the colorimetric tests and calculating the pH values*

a. A volume of 0.2 cc. of the indicator was introduced from a graduated pipette into the bottom of the tube.

b. The required amount of buffer or medium or dilution of the same or of water was added to the indicator by holding the tip of the pipette always just below the surface while adding the liquid to prevent loss or reabsorption of CO₂. By introducing the indicator first and filling carefully, as described, the color becomes evenly distributed without shaking or inverting the tube and in this way chances for changing the CO₂ equilibrium are lessened. Dilutions and comparisons with the usual buffer-color standards were made singly or in groups of two or three as quickly as possible to minimize changes in CO₂ content. The test tubes may be first filled with CO₂-free air when working in alkaline ranges and with super pure water.

c. A free circulation of fresh air in the room is essential when

carrying on an experiment with CO₂-free water. Reabsorption of CO₂ in the tubes is so rapid under any other condition (as when the air is close and impure or when there are gas flames in the room) that it is often impossible to obtain good readings. The rather rapid action of CO₂ entering the liquid from the atmosphere can be readily detected by the appearance of the "acid" color of the indicator at the top of the liquid in contrast to the color below.

d. The tables show that in studying the mutual effect of the water, buffer and indicator in giving a resultant final hydrogen-ion concentration, pH readings were made on each buffer dilution, and on the water, mixed with each and every adjusted solution of the indicator or indicators falling in the pH range measured. Since all these pH readings differ, we must analyze the data to find the actual pH of the diluted buffer or the water containing no indicator. According to the isohydric principle, buffers and indicators unlike in pH change each other into a resultant intermediate pH; the correct pH, barring salt errors, is found only when the pH of one of the indicators is the same before and after the addition of the water or solution under test. But to estimate this correct pH point or zone, the unknown solution need not be treated with *all* of the different pH steps of the given indicator. For ordinary practical work it will be found sufficient to have a short series of three solutions of each indicator adjusted at or near the lowest, midpoint and highest useful pH points of its range, say at pH 6.0, 7.0 and 7.6 for brom thymol blue. For more precise work the complete 9-point series with intermediate steps 0.2 pH apart should also be made. The convenience and relative usefulness of the short and the complete series depend greatly on the concentration of the buffer in the unknown solution and can well be illustrated by table 5, giving the tests of the pH values of dilute phosphate buffers with phenol red and cresol red.

The electrometric pH of the M/20 phosphate was adjusted to 7.82. The first colorimetric test of the unknown should always be made with the midpoint indicator solution, or pH 7.8 phenol red in this case, to see whether the solution shows a pH below, at or above the midpoint pH. Table 5 shows that the low, high and

all intermediate pH points of phenol red and cresol red gave the reading pH 7.8+ with the 1:5 and 1:10 dilutions. We conclude then that it is practically immaterial what the pH adjustment of the indicator is for measuring phosphates more concentrated than $m/200$. At a dilution of about 1:100 ($m/2000$) the pH 7.0 and 8.6 indicators slightly lower or raise the pH of the phosphate and give the readings pH 7.8 and 7.85. A dilution of 1:1000 ($m/20,000$) is typical of cases where the short or 3-point or 3-step or 3-pH phenol red series is both necessary and sufficient. The midpoint or pH 7.8 phenol red gives a resultant reading 7.7, thus showing that the pH 7.82 of the $m/20$ phosphate buffer has been decreased by the pH 7.0 water on dilution, and is below 7.7. The lowest or pH 7.0 phenol red gives the reading 7.5, thus proving that the pH of the diluted phosphate buffer is above 7.5. As the diluted phosphate itself is above pH 7.5 and below pH 7.7, or about three-fourths way from the pH 7.0 to 7.8 indicator, we use three-fourths as a factor on the difference of 0.2 pH between the pH 7.5 and 7.7 readings and choose 7.65 as the pH of the 1:1000 ($m/20,000$) phosphate. We would choose pH 7.6 if this were midway between the pH 7.0 and 7.8 indicators. In fact, we note that the pH 7.65 chosen is also the midpoint of the readings pH 7.5, 7.65 and 7.8 given by the equally spaced pH 7.4, 7.6 and 7.8 indicators. Hence a reading of pH 7.65 or possibly 7.67 is given accurately and quickly by the use of two of the shorter 3-pH phenol red series. The 1:10,000 phosphate dilution ($m/200,000$) is brought still closer to pH 7.0 by the pH 7.0 superpure water and gives the readings pH 7.2 and 7.6 with the pH 7.0 and 7.8 phenol and cresol reds. The average of the pH 7.2 and 7.6 is the pH 7.4 chosen which is also midway between the pH 7.0 and 7.8 indicators used and remains unchanged (isohydric) by the pH 7.4 indicator of the 9-point phenol and cresol red series. It must therefore be nearly correct. When the unknown solution is very dilute and the pH readings given by two of the 3-pH indicator series differ more than 0.2 to 0.4 pH or so much that the pH "chosen" as correct is in doubt, the two indicator solutions may be mixed in proper proportions from table 1 to give the "chosen" pH and this indicator added to the unknown solution should give

a final very accurate pH reading. It is not safe to make linear interpolations to 0.1 pH on pH differences greater than about 0.4 pH because the pH scale is logarithmic, not linear, as Snyder (1928) has emphasized. But if the research work involves constant tests of extremely dilute solutions or superpure water, it is much more convenient and accurate to make up the longer 9-pH indicator series and make the final pH measurement with that indicator solution most nearly isohydric with the pH given by the 3-pH series. Tables 3, 4 and 5 contain a few heavily typed pH values "chosen" by the isohydric method as illustrations.

DEFINITIONS

Before presenting or discussing the actual data it will be necessary to define certain terms which will appear in the text.

In discussing the data presented in the tables and curves, two terms will be used which may need definitions, viz., "*water-indicator effect*" and "*buffer-dilution effect*." The first term is used to mean the combined effects of the water used (controlled mainly by the presence or the absence of CO_2) and of the indicator ions. These effects are, obviously, to be seen only in the higher dilutions. The second term is used to mean the effect produced by changes in ionization due to dilution of the buffer substances forming the basis of the solution or medium. This effect is important in the lower dilutions and may be either in the acid or in the alkaline direction. The chemical basis of buffer dilution is not yet well systematized with accurate data and need not be discussed here.⁴

When diluting with water containing CO_2 these two effects may be in opposite directions, as will be shown. When, however, CO_2 -free water is used, buffer-dilution and water-indicator effects may merge imperceptibly as we find ourselves dealing either primarily with the ionizing substances of the medium in the first part of the curve (lower dilutions) or with the ionization of the indicator itself as an important source of H or OH ions in the

⁴ See Sörensön's table of pH values for different dilutions of glycocoll and asparagine given in Clark's Determination of H-ions, 1920 Ed., p. 30, and similar data on aniline hydrochloride given by Loomis and Acree, 1911.

TABLE 2
Dilution of acid potassium phthalate-HCl buffer from phenol blue

pH OF INDICATOR	ELECTROMETRIC pH OF UNDI-LUTED BUFFER 0.05 M‡	DILUTION FACTORS							CO ₂ -FREE WATER*
		1:5 0.01 M	1:10 0.005 M	1:50 0.001 M	1:100 0.0005 M	1:200 0.00025 M	1:500 0.0001 M	1:1,000 0.00005 M	
Colorimetric pH of diluted buffer									
3.4	3.75	3.77	3.80	4.0	4.1	4.2	4.35	4.45	4.6
3.6	3.75	3.77	3.80					4.45	4.6+
3.8	3.75	3.77	3.80					4.55	4.8+
4.0	3.75	3.77	3.80		4.15	4.3	4.5	4.60	4.8+
4.2	3.75	3.77	3.80					4.60	4.8+
4.4	3.75	3.77	3.80					4.70	4.8+
4.6	3.75	3.77	3.80	4.0	4.2	4.35	4.6	4.80	4.8+
AIR-CO ₂ EQUILIBRIUM WATER†									
3.4	3.75	3.77	3.8	4.0	4.1	4.2	4.35	4.45	4.6
3.6	3.75	3.77	3.8	4.0	4.1	4.25	4.4	4.50	4.6+
3.8	3.75	3.77	3.8	4.0	4.1	4.25	4.42	4.52	4.8
4.0	3.75	3.77	3.8	4.0	4.1	4.25	4.45	4.55	4.8+
4.2	3.75	3.77	3.8	4.0	4.1	4.25	4.50	4.55	4.8+
4.4	3.75	3.77	3.8	4.0	4.1	4.30	4.55	4.60	4.8+
4.6	3.75	3.77	3.8	4.0	4.15	4.35	4.60	4.6+	4.8+
pH 5.0 WATER DIRECT FROM STILL									
3.4	3.75	3.77	3.8-	4.0	4.1	4.25	4.35	4.45	4.6
3.6	3.75	3.77							4.6+†
3.8	3.75	3.77							4.8
4.0	3.75	3.77			4.15	4.35	4.45	4.6	4.8+
4.2	3.75	3.77							4.8+
4.4	3.75	3.77							4.8+
4.6	3.75	3.77	3.8-	4.0	4.2	4.4	4.6	4.7	4.8+

* pH 7.0 with brom thymol blue (7.0).

† pH 5.65 with brom cresol purple (5.7).

‡ In this and all succeeding tables the electrometric readings were made on the buffer without the presence of an indicator. Where the colorimetric value is repeated throughout an entire column actual readings were made only at the beginning and end. This applies to all succeeding tables as well.

last part (covering the higher dilutions). In either case the dilution effect is probably of the same nature from a chemical viewpoint; that is, it is due to ionization and hydrolysis of systems of weak acids or bases and their salts.

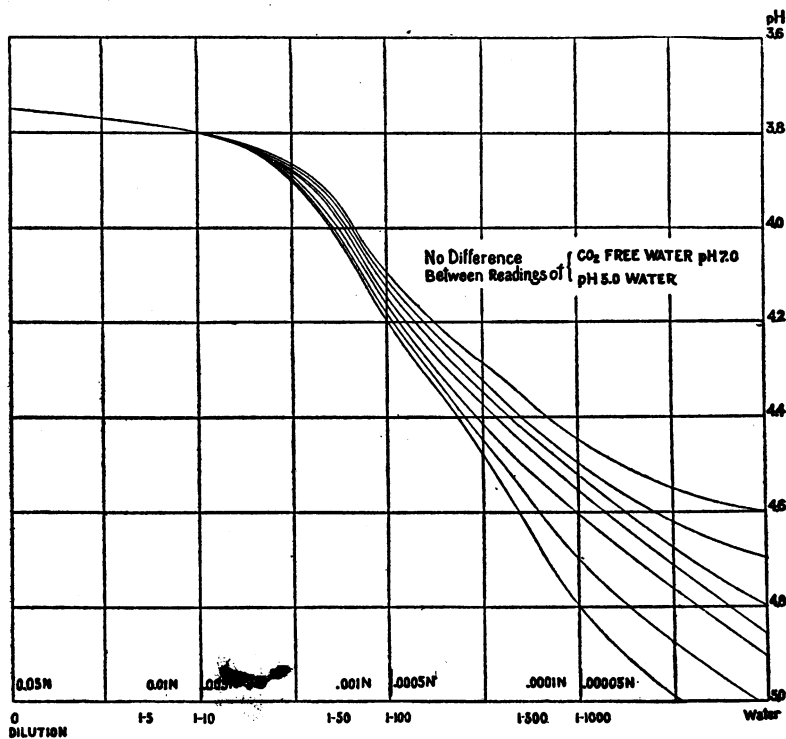


FIG. 1. RELATION OF pH VALUES TO DILUTION OF PHTHALATE-HCl BUFFER (ELEC. pH 3.75) WHEN TESTED WITH BROM PHENOL BLUE IN ADJUSTED SERIES (pH 3.4 TO 4.6 IN INTERVALS OF 0.2 pH)

In studying the tables and charts the reader must keep in mind that the buffer-dilution curves would be slightly more alkaline if the points were determined electrometrically as will be demonstrated in the paper on *salt errors* now under way. Hence in all previous work the errors due to CO₂ and indicators are even more misleading than they appear to be from the colorimetric data.

DISCUSSION OF TABLES AND CURVES

*Dilution of acid potassium phthalate-HCl buffer of pH 3.75
(brom phenol blue)*

In table 2 (fig. 1) are presented the data obtained on dilution of an acid-potassium phthalate-HCl buffer mixture (0.05M).

Differences in pH due to dilution are apparent throughout, grading evenly from 1:10 where they are first appreciable to 1:1000 where the readings are barely distinguishable from those of water itself. This gradual increase in pH in the upper part of the curves as far as 1 part in 50, falls without doubt under the category of buffer-dilution effect. In about that region of the curves representing a dilution of 1 part in 100 (0.0005 M) the buffer dilution and water-indicator effects merge and we have as a result smooth rapidly descending curves. It will be seen that all the readings obtained when the indicator, whatever its adjustment, was introduced into water alone (see last column) are either near the extreme alkaline end of the range of brom phenol blue (pH 4.8) or beyond it.

That practically no differences in pH are found between readings made with pH 7.0, pH 5.7 and pH 5.0 water, provided the adjustment of the indicator solution is the same, is due no doubt to the fact that formation of hydrogen ions from the ionization of the indicator acid is of a greater order than that from the ionization of the acid formed by the amount of dissolved CO₂ present, even in pH 5.0 water.

Differences due to the adjustment of the indicator are slight and do not become apparent until a dilution of 1 part in 100 is reached. Even in the higher dilutions the range of values obtained with the adjusted series of indicator solutions is much more restricted than that of the readings made with other indicator series at the same dilutions (see other charts). This is due to the fact that the medium being diluted is a comparatively strong acid buffer which over-balances the lower acidities and concentrations of this indicator at all its pH values.

Colorimetric readings agree with the electrometric reading on the undiluted buffer within an error of 0.05 pH when the buffer is

TABLE 3
Dilution of 1 per cent peptone beef infusion bouillon
 Brom cresol purple

pH OF INDICATOR	ELECTRO-METRIC pH OF UNDILUTED MEDIUM	COLORIMETRIC pH OF UNDILUTED MEDIUM	DILUTION FACTORS						CO ₂ -FREE WATER*
			1:5	1:10	1:50	1:100	1:500	1:1,000	
			Colorimetric pH of diluted medium						
5.2	6.05	6.0+	6.0+	6.0	6.0	5.95	5.85	5.80	5.70
5.4	6.05	6.0+	6.0+	6.0			5.85	5.80	5.75
5.6	6.05	6.0+	6.0+	6.0			5.95	5.90	5.9-
5.8	6.05	6.0+	6.0+	6.0			6.0-	5.95	6.0
6.0	6.05	6.0+	6.0+	6.0			6.05	6.00	6.05
6.2	6.05	6.0+	6.0+	6.0			6.10	6.10	6.3-
6.4	6.05	6.0+	6.0+	6.0			6.2-	6.20	6.4
6.6	6.05	6.0+	6.0+	6.0	6.05	6.1	6.25	6.30	6.6-
AIR-CO ₂ EQUILIBRIUM WATER†									
5.2	6.05	6.0+	6.0	6.0	5.95	5.90	5.75	5.6	5.45
5.4	6.05	6.0+	6.0	6.0			5.8-	5.6+	5.50
5.6	6.05	6.0+	6.0	6.0			5.80	5.7	5.60
5.8	6.05	6.0+	6.0	6.0			5.85	5.75	5.65
6.0	6.05	6.0+	6.0	6.0			5.9	5.8-	5.75
6.2	6.05	6.0+	6.0	6.0			5.95	5.85	5.85
6.4	6.05	6.0+	6.0	6.0			6.00	5.9	5.90
6.6	6.05	6.0+	6.0	6.0	6.0	6.05	6.05	6.0	6.0
SAME WATER CO ₂ FROM TANK‡									
5.2	6.05	6.0+	6.0	5.9	5.6	5.4	5.2	5.2-	5.0
5.4	6.05	6.0+	6.0	5.9					5.0
5.6	6.05	6.0+	6.0	5.9					5.0
5.8	6.05	6.0+	6.0	5.9					5.0
6.0	6.05	6.0+	6.0	5.9					5.0
6.2	6.05	6.0+	6.0	5.9					5.0
6.4	6.05	6.0+	6.0	5.9					5.0
6.6	6.05	6.0+	6.0	5.9	5.6	5.4	5.2+	5.1	5.0

* pH 7.0 with brom thymol blue (7.0).

† Note that the point of agreement between the pH of adjusted indicators and the series of pH values for water in the last column is at 5.6 instead of 5.7. This may have been due to impurity of the air in the room since the fresh air was not always kept bubbling through the dilution water.

‡ pH 5.0 brom cresol purple (5.2); pH 5.0 methyl red (5.0). CO₂ was not measured in this case and the water was made a little too acid when introducing CO₂ directly from the tank. The actual pH was probably 4.8 instead of 5.0.

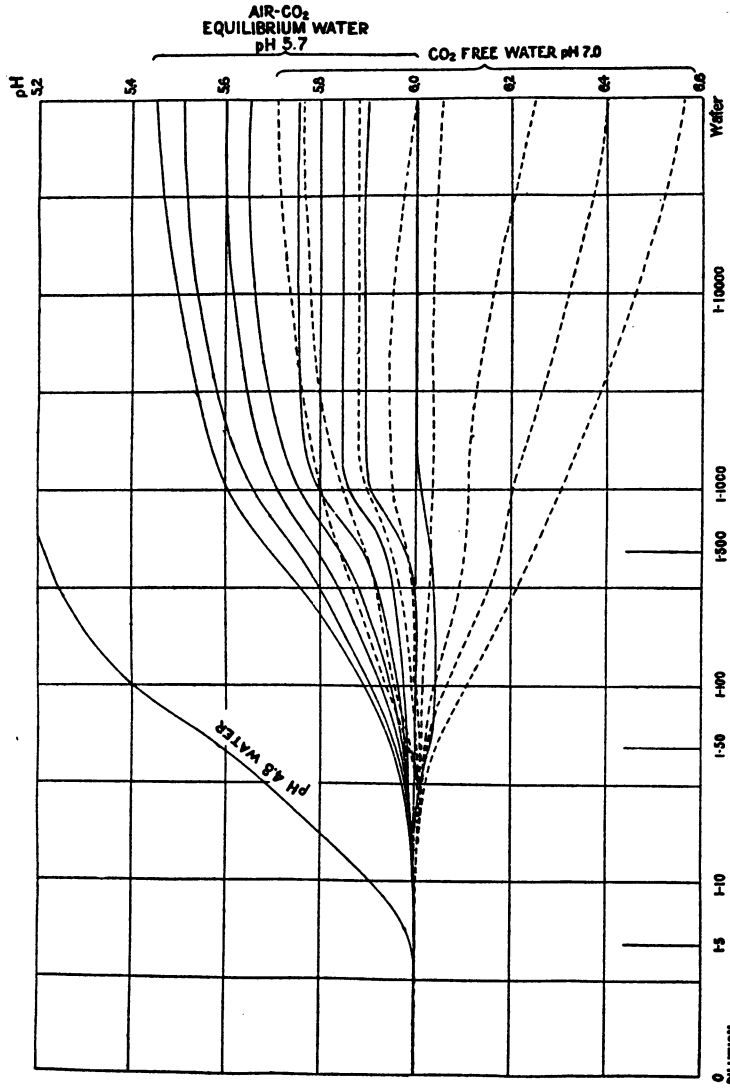


FIG. 2. RELATION OF pH VALUES TO DILUTION OF 1 PER CENT PEPTONE BEEF INFUSION BOULLON (ELEC. pH 6.05) WHEN TESTED WITH BROM CRESOL PURPLE IN ADJUSTED SERIES (pH 5.2 TO 6.6 IN INTERVALS OF 0.2 pH)

not diluted further than 1 part in 10. At 1 part in 50 the difference reaches 0.25 pH and rapidly increases as dilution proceeds.

Dilution of 1 per cent peptone, beef infusion bouillon (brom cresol purple)

In table 3 (fig. 2) are presented dilution data for 1 per cent peptone beef infusion bouillon.

There are no *changes in pH due to dilution* worthy of note until 1 part in 50 is reached and then these are very slight except with pH 4.8 water in which case the high CO_2 content deflects the curves to the extent of 0.1 pH at a dilution of 1:10 and causes all the curves to fall into a single line, a very good illustration of the buffering effect of CO_2 in water. Likewise, *the differences due to indicator adjustment* seen when the other waters were used are here wiped out. In contrast to the brom phenol blue series in table 2 (fig. 1), where the indicator acid is a stronger factor than the CO_2 effect, here the effect of CO_2 out-balances the effect of the brom cresol purple indicator regardless of its pH; in other words, the CO_2 in solution forms a stronger acid than the indicator acid in any part of the adjusted series.

On the other hand *the effect of CO_2* is scarcely appreciable in readings made with pH 5.7 water until a dilution of 1 part in 500 is reached where the greatest difference between readings made with pH 5.7 and CO_2 -free (pH 7.0) water was 0.2 pH and even at a dilution of 1:1000 the error is only 0.3 pH at the extreme alkaline end of the indicator range (pH 6.6). At the mid-point of the indicator series this type of error does not exceed 0.15 pH at a dilution of 1:500 or 0.2 pH at 1:1000.

There is almost exact *agreement between colorimetric readings and the electrometric reading* on the undiluted medium when the dilution does not exceed 1:10 with the exception of those made with pH 4.8 water. In the case of CO_2 -free water, errors are not greater than 0.1 pH even at a dilution of 1:100, and then only at the extremes of the indicator series. At its mid-point (pH 6.0) the values recorded are practically identical throughout the

dilution series and agree with the electrometric reading; this is obviously because the pH of the indicator used (6.0) almost exactly coincides with that of the medium under test (6.05) and because the pH of a brom cresol purple solution adjusted to pH 6.0 was changed very slightly (6.0 to 6.05) when introduced into CO₂-free water (see last column on the right).

Were the solution being measured of a H-ion concentration near the acid end of the range of brom cresol purple, for example pH 5.2 to 5.4, readings made with pH 5.7 or even pH 5.0 water would agree more nearly with the true pH than those made with pH 7.0 water. Seldom in ordinary practice would every condition be found ideal, as in the case just cited where dilution did not greatly effect the pH value in any part of the series from the undiluted medium all the way down to water itself. The same condition may be observed at some one point in all of the other charts.

Dilution of Fermi's solution⁵ (brom thymol blue)

Table 4 (fig. 3) embodies dilution data for Fermi's solution—a much used, highly buffered, synthetic medium—and exemplifies in a striking way all the principles previously discussed.

In addition figure 3 illustrates well the fact that when a medium, the reaction of which is in the region of neutrality, is diluted with water containing CO₂, the initial changes may be in the opposite direction from those which occur on further dilution. Thus it will be seen that the curves dip to the extent of 0.15 pH at 1 part in 5 due to *buffer-dilution* and that later most of them rise again but at markedly different rates according to the amount of CO₂ in the water or the pH of the indicator used.

Another fact which stands out in this table is the extreme to which dilution may be carried (1 part in 100,000) and still show

⁵ Distilled water.....	1,000 cc.
MgSO ₄	0.2 gram
KH ₂ PO ₄	1.0 gram
(NH ₄) ₂ HPO ₄	10.0 grams
Glycerine.....	45.0 cc.

This solution is 0.083 M with respect to the phosphate buffer. Since the MgSO₄ does not affect the buffer action above pH 7.0 it was ignored in the calculation.

TABLE 4
Dilution of Fermi's solution
Brom thymol blue

pH OF INDICATOR	ELECTRO-METRIC pH OF UNDI-LUTED BUFFER	COLORI-METRIC pH OF UNDI-LUTED BUFFER	DILUTION FACTORS									CO ₂ -FREE WATER*
			1:5 0.0166 M	1:10 0.0083 M	1:50 0.00166 M	1:100 0.00083 M	1:500 0.000166 M	1:1,000 0.000083 M	1:10,000 0.0000083 M	1:100,000 0.00000083 M		
			Colorimetric pH of diluted medium									
6.2	7.00	7.0+	7.15	7.15	7.2	7.15	7.10	7.02	6.95	6.6	6.4	
6.4	7.00	7.0+	7.15	7.15	7.2	7.15			7.00	6.7	6.5+	
6.6	7.00	7.0+	7.15	7.15	7.2	7.15			7.02	6.75	6.75	
6.8	7.00	7.0+	7.15	7.15	7.2	7.15			7.05	6.85	6.85	
7.0	7.00	7.0+	7.15	7.15	7.2	7.15			7.10	7.05	7.05	
7.2	7.00	7.0+	7.15	7.15	7.2	7.15			7.15	7.2	7.2+	
7.4	7.00	7.0+	7.15	7.15	7.2	7.15			7.17	7.4-	7.4-	
7.6	7.00	7.0+	7.15	7.15	7.2	7.15	7.15	7.15	7.20	7.4	7.5	
AIR-CO ₂ EQUILIBRIUM WATER†												
6.2	7.00	7.0+	7.15	7.15	7.15	7.15	7.05	6.85	6.1	6.0-	6.0-	
6.4	7.00	7.0+	7.15	7.15	7.15	7.15			6.1	6.0-	6.0-	
6.6	7.00	7.0+	7.15	7.15	7.15	7.15			6.2	6.0	6.0	
6.8	7.00	7.0+	7.15	7.15	7.15	7.15			6.25	6.0	6.0	
7.0	7.00	7.0+	7.15	7.15	7.15	7.15			6.3	6.1	6.1	
7.2	7.00	7.0+	7.15	7.15	7.15	7.15			6.4	6.2	6.2	
7.4	7.00	7.0+	7.15	7.15	7.15	7.15			6.5	6.3+	6.3+	
7.6	7.00	7.0+	7.15	7.15	7.15	7.15	7.15	7.00	6.6	6.35	6.35	
pH 5.0 WATER‡												
6.2	7.00	7.0+	7.15	7.15	7.15	7.05	6.5	6.0	6.0	Full acid color	Full acid color	
6.4	7.00	7.0+	7.15	7.15	7.15	7.05				Full acid color	Full acid color	
6.6	7.00	7.0+	7.15	7.15	7.15	7.05				Full acid color	Full acid color	
6.8	7.00	7.0+	7.15	7.15	7.15	7.05				Full acid color	Full acid color	
7.0	7.00	7.0+	7.15	7.15	7.17	7.05				Full acid color	Full acid color	
7.2	7.00	7.0+	7.15	7.15	7.15	7.05				Full acid color	Full acid color	
7.4	7.00	7.0+	7.15	7.15	7.15	7.05				Full acid color	Full acid color	
7.6	7.00	7.0+	7.15	7.15	7.15	7.10	6.6	6.2	6.0	Full acid color	Full acid color	

* pH 7.05 with B.T.B. (7.0).

† pH 5.7 with B.C.P. (5.7).

‡ Direct from still.

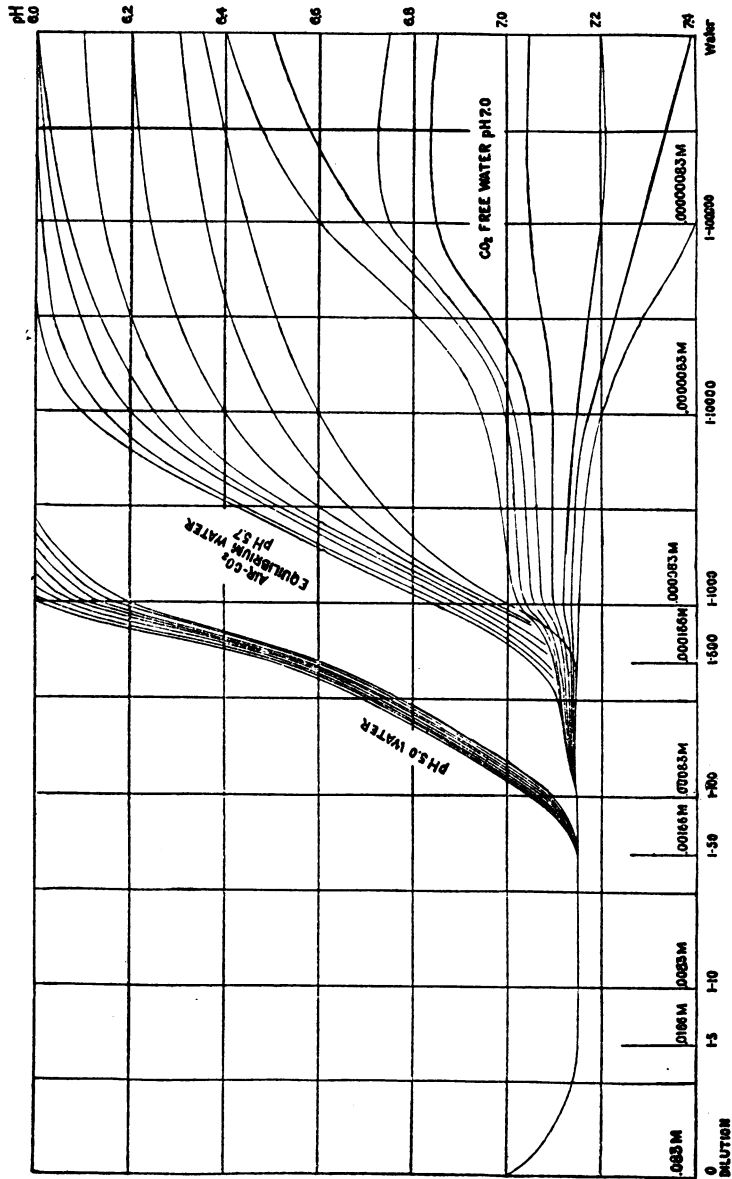


FIG. 3. RELATION OF pH VALUES TO DILUTION OF FERMI'S SOLUTION (ELEC. pH 7.0) WHEN TESTED WITH BROM THYMOL BLUE IN ADJUSTED SERIES (pH 6.2 TO 7.6 IN INTERVALS OF 0.2 pH)

evidence in the readings of the presence of the medium. This is true especially where CO₂-free water is used, and to a slightly

TABLE 5
Dilution of acid potassium phosphate-NaOH buffer
 Phenol red, cresol red. Data with CO₂-free water

INDICATOR	pH OF INDICATOR	ELEC. pH OF UN-DILUTED BUFFER	DILUTION FACTORS					CO ₂ FREE WATER
			1:5 0.01M	1:10 0.005M	1:100 0.0005M	1:1000 0.00005M	1:10,000 0.000005M	
			Colorimetric pH of diluted buffers					
Phenol red.....	7.0	7.82	7.8+	7.8+	7.8	7.5	7.2	7.0
Cresol red.....		7.82	7.8+	7.8+	7.8	7.5	7.25	7.0-
Phenol red.....	7.2	7.82	7.8+	7.8+	7.8	(7.55)*	7.45	7.2
Cresol red.....		7.82	7.8+	7.8+	7.8	(7.55)	7.35	7.2-
Phenol red.....	7.4	7.82	7.8+	7.8+	7.8	7.6	7.4	7.35
Cresol red.....		7.82	7.8+	7.8+	7.8	7.6	7.4	7.3
Phenol red.....	7.6	7.82	7.8+	7.8+	7.8	(7.65)	7.5	7.45
Cresol red.....		7.82	7.8+	7.8+	7.8	(7.65)	7.5	7.4
Phenol red.....	7.8	7.82	7.8+	7.8+	7.8+	7.7	7.6-	7.6
Cresol red.....		7.82	7.8+	7.8+	7.8	7.7	7.6	7.6
Phenol red.....	8.0	7.82	7.8+	7.8+	7.8	(7.75)	(7.7)	7.8
Cresol red.....		7.82	7.8+	7.8+	7.8+	(7.75)	7.8	7.8
Phenol red.....	8.2	7.82	7.8+	7.8+	7.8	7.8	7.8	7.9
Cresol red.....		7.82	7.8+	7.8+	7.8	7.8	(7.8)	7.9
Phenol red.....	8.4	7.82	7.8+	7.8+	7.8	(7.85)	7.9	8.0
Cresol red.....		7.82	7.8+	7.8+	7.8	(7.85)	7.85	7.95
Phenol red.....	8.6	7.82	7.8+	7.8+	7.85	7.9	7.95	8.1
Cresol red.....		7.82	7.8+	7.8+	7.8+	7.9	7.9	8.0

* Figures in parentheses were interpolated.

less degree with air-CO₂ equilibrium water. In the case of pH 5.0 water the buffering power of the CO₂ practically obliterates the effect of the medium in the highest dilutions.

TABLE 6
Dilution of acid potassium phosphate-NaOH buffer
 Phenol red, cresol red

INDICATOR	pH OF INDICATOR	ELEC. pH OF UNDILUTED BUFFER	DILUTION FACTORS					AIR-CO ₂ EQUILIBRIUM WATER*	
			1:5	1:10	1:50	1:100	1:500		1:1,000
Colorimetric pH of diluted buffer									
Data with air-CO ₂ equilibrium water									
Phenol red.....	7.0	7.82	7.8	7.8	7.75	7.6	7.2	7.0—5 minutes	Full acid color
					7.65	7.55	7.15	6.8—30 minutes	
					7.65	7.55	7.1	6.8—1 hour	
Cresol red.....	7.0	7.82	7.8	7.8	7.75	7.6	7.05	6.7—1½ hours	
							7.2	7.0—5 minutes	
							7.3	7.1—5 minutes	
Phenol red.....	7.8	7.82	7.8	7.8			7.2	30 minutes	Full acid color
							7.15	1 hour	
							7.15	1½ hours	
Cresol red.....	7.8	7.82	7.8	7.8			7.4	7.1—5 minutes	Full acid color
					7.8	7.65	7.25	7.2—5 minutes	
Phenol red.....	8.6	7.82	7.8	7.8	7.7	7.6	7.25	7.0—30 minutes	
					7.7	7.6	7.2	7.0—1 hour	
					7.7	7.6—	7.2—	6.9—1½ hours	
Cresol red.....	8.6	7.82	7.8	7.8	7.8	7.65	7.4	7.2—5 minutes	

Data with pH 5.0 water

									pH 5.0 water†
Phenol red.....	7.0	7.8	7.8	7.50	7.25			Full acid color	Full acid color
Cresol red.....		7.82	7.8	7.50	7.25			Full acid color	Full acid color
Phenol red.....	7.8	7.8	7.8	7.57	7.3			Full acid color	Full acid color
Cresol red.....		7.82	7.8	7.55	7.3			Full acid color	Full acid color
Phenol red.....	8.6	7.8	7.8	7.6	7.35			Full acid color	Full acid color
Cresol red.....		7.82	7.8	7.6	7.35			Full acid color	Full acid color

* pH 5.7 with B.C.P. 5.7.

† One cubic centimeter water saturated with CO₂ per 200 cc. CO₂-free water. pH 5.0 B.C.P. (5.0). pH 5.2 M.R. (5.0).

Dilution of acid potassium phosphate-NaOH buffer (Phenol red, cresol red)

The data given in tables 5 and 6 (fig. 4), show more strikingly than do any of the other experiments the effect of CO_2 on the readings because of the alkaline range covered. The errors are nearly all in the acid direction and increase in direct proportion to

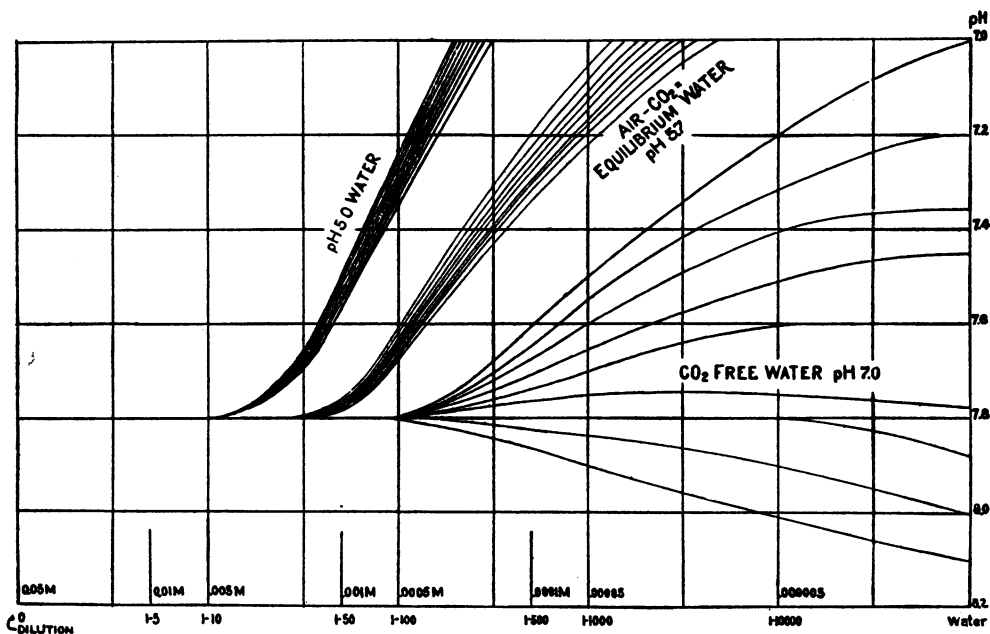


FIG. 4. RELATION OF pH VALUES TO DILUTION OF PHOSPHATE-NaOH BUFFER (ELEC. pH 7.82) WHEN TESTED WITH PHENOL RED VS. CRESOL RED IN ADJUSTED SERIES (pH 7.0 TO 8.6 IN INTERVALS OF 0.2 pH)

the amount of CO_2 present. In table 5 where the readings were made under CO_2 -free conditions the deflections from the true pH due to dilution are slight even at 1:1000, the greatest error being 0.3 pH at the extreme acid end of the indicator series, while in table 6 where pH 5.7 water was used they begin to be noticeable at 1:100, and in the case of pH 5.0 water at 1:50. Even with regard to the alkaline buffer, however, the rule holds that errors

due either to CO_2 or to adjustment of the indicator are negligible at a dilution of 1 part in 10 or less.

The outstanding point in table 6 is the *slow action of CO_2 on the readings made with pH 5.7 water*. The change in the tubes was so apparent that it was necessary to wait several minutes before taking the first readings. After five minutes the change was more gradual. A study of the figures shows that the greatest change occurred in the 1:1000 dilution where the difference between the five-minute and one-and-one-half-hour readings was 0.3 pH. At 1:500 it was 0.15 to 0.25 pH in the same length of time, and at 1:50 only 0.1. It is also noteworthy that equilibrium was reached sooner in the lower dilutions. The experiment shows how unsatisfactory it is to work with very dilute solutions of an alkaline medium in the presence of CO_2 . When pH 5.0 water was used the CO_2 , present in larger amount, acted so rapidly that it was not necessary to wait longer than a minute before taking readings.

The differences due to indicator adjustment are not great, except in the highest dilutions of the CO_2 -free series.

Good agreement was obtained between the readings made with the two indicators used, phenol red and cresol red, as also between colorimetric readings and the electrometric reading on the undiluted medium, under all conditions, at dilutions lower than and including 1 part in 10, and even at a dilution of 1 part in 100 under CO_2 -free conditions; the last mentioned fact shows the strong buffering power of the phosphate mixture.

Dilution of 1 per cent peptone, 1 per cent glucose medium with redistilled water vs. ordinary distilled water (brom thymol blue)

Table 7 (fig. 5) records an experiment which was devised to show what influence, if any, the *presence of small quantities of sprayed-over alkaline salts in the ordinary distilled water* would have on the colorimetric pH of diluted media and what weight this influence would have as compared with the effect of varying amounts of CO_2 in the water. Accordingly redistilled water (pH 7.05 under CO_2 -free conditions) was used in comparison with

TABLE 7
Dilution of 1 per cent peptone-1 per cent glucose medium
 Brom thymol-blue

pH OF INDICATOR	COLORIMETRIC pH OF UNDILUTED MEDIUM	DILUTION FACTORS					REDISTILLED WATER CO ₂ -FREE
		1:5	1:10	1:50	1:100	1:500	
		Colorimetric pH of diluted medium					
6.2	6.5	6.5	6.4	6.4	6.4	6.4	6.4
7.0	(6.5)	(6.5)	(6.5)	6.6	6.7	6.9	7.05
7.6	6.5	6.6-	6.6	6.7	6.9	7.3	7.6
ORDINARY DISTILLED WATER CO ₂ -FREE							
6.2	6.5	6.5	6.4+	6.5	6.6	6.8	7.0
7.0	(6.5)	(6.5)	(6.5)	6.65	6.85	7.2	7.55
7.6	6.5	6.6-	6.6	6.8	7.0	7.45	7.6+
REDISTILLED WATER: AIR CO ₂ EQUILIBRIUM*							
6.2	(6.5)	6.4	6.3	6.0			6.0-
7.0	(6.5)	6.4	6.4-	6.15			6.0-
7.6	(6.5)	6.4+	6.4	6.3			6.1
ORDINARY DISTILLED WATER: AIR-CO ₂ EQUILIBRIUM†							
6.2	(6.5)	(6.4)	6.3	6.0+			6.0-
7.0	(6.5)	(6.4)	6.3	6.2			6.1
7.6	(6.5)	(6.4+)	6.4	6.3+			6.3
REDISTILLED WATER (pH 5.0)‡							
6.2	6.5	6.2	6.0				6.0-
7.0	(6.5)						
7.6	6.5	6.2+	6.1				6.0-
ORDINARY DISTILLED WATER (pH 5.1)‡							
6.2	6.5	6.2	6.0				6.0-
7.0	(6.5)						
7.6	6.5	6.2+	6.1				6.0

* pH 5.5 with B.C.P. (5.6). The CO₂ content was a little high because this equilibrium was obtained by running ordinary air through pH 5.0 water without heating. It would require a long time to entirely drive out the extra quantity of CO₂ in this way.

† pH 5.8 with B.C.P. (5.6). The difference between 5.5 and 5.8 was due to salt content.

‡ pH 5.0 to 5.1 with brom cresol purple (5.0) prepared in each case by adding 1 cc. of redistilled water saturated with CO₂ to 200 cc. of the dilution water.

Figures in parentheses interpolated,—all others actual readings.

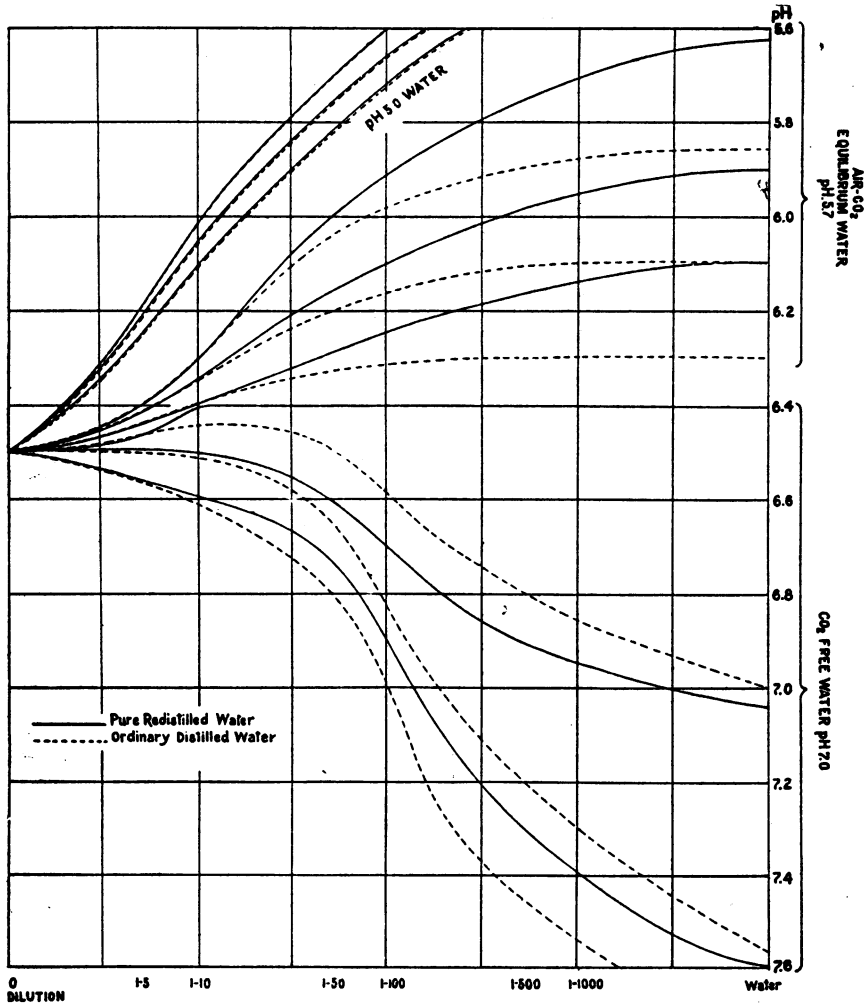


FIG. 5. RELATION OF pH VALUES TO DILUTION OF 1 PER CENT PEPTONE-1 PER CENT GLUCOSE MEDIUM WHEN TESTED WITH BROM THYMOL BLUE IN ADJUSTED SERIES (pH 6.2, 7.0, 7.6)

a sample of ordinary distilled water from a commercial still, testing pH 7.55 with brom thymol blue (7.0) and pH 8.0 with phenol red (pH 8.0) when aerated with CO₂-free air, and testing pH 5.1 with brom cresol purple (5.0) as collected. The medium chosen was a comparatively weakly buffered one, viz., 1 per cent peptone, 1 per cent glucose solution.

The chart brings out two important points (1) that the pH of the diluted medium is affected to a far greater extent by the CO₂ content of the water than by its salt content and (2) that differences in pH due to variation in CO₂ content and also to a slight extent to indicator adjustment occur even in the lowest dilutions in contrast to the more heavily buffered media.

When both of the waters are supercharged with CO₂ (pH 5.0 to 5.1) the CO₂ effect so outweighs the salt content effect that the latter is obliterated. Note that the solid lines and the dotted lines follow the same path. At air-CO₂ equilibrium the differences between readings made with redistilled and ordinary distilled water are barely apparent at a dilution of 1 part in 50 and do not exceed 0.2 pH even when there is no buffer present (see readings of indicators in water alone). On the other hand, under CO₂-free conditions, the differences in pH due to the salt content of the ordinary distilled water are considerable in the higher dilutions (0.1 to 0.40 pH) and very marked (as high as 0.5 to 0.6 pH) when the two waters are compared alone without the presence of buffer. It is reassuring however to find that water with a high enough buffer salt content (pH 8.0) to register pH 7.55 with brom thymol blue adjusted to pH 7.0, does not give readings different from those obtained with pH 7.0 water at dilutions of 1 part in 5 or 1 part in 10.

It is noteworthy that a reading of pH 7.0 was obtained on this water (see last column on the right) with an indicator solution adjusted to pH 6.2, whereas a reading of pH 7.55 was obtained with the indicator adjusted to pH 7.0. This well illustrates the point that a reading of pH 7.0 on water can not be accepted as the true one unless the indicator with which it was obtained was adjusted to pH 7.0. The reading of pH 7.0 with the pH 6.2 indicator can easily be explained as being due to a partial neutrali-

zation of the indicator acid by the sprayed-over alkaline buffer salts in the CO₂-freed ordinary distilled water (pH 8.0). Hence ordinary distilled waters containing enough CO₂ to test pH 5.0 may be treated with pure air to remove the CO₂ just sufficiently to neutralize the sprayed-over alkaline salts and give pH 7.0 with pH 7.0 from thymol blue. But such waters are variable and unstable, become alkaline when boiled or aerated with CO₂-free air, exert a noticeable buffer effect when used to dilute media and buffers, give pH readings with adjusted bromthymol blue which are misleading regarding the purity of the water, and generally have specific conductivities above 2×10^{-6} arising from the sprayed-over buffer and neutral salts. But double distilled CO₂-free water of pH 7.0 and a specific conductivity less than 0.5×10^{-6} is easily prepared, stored and used by means of the stills and methods described herein, is free from all these objections, and is earnestly recommended for general use with the isohydric indicator methods in all colorimetric H-ion investigations.

RECOMMENDATIONS

The following *recommendations* are offered as a result of these investigations:

1. All *glass ware* used must be Pyrex or non-sol in character and scrupulously cleaned by such methods as are described in the early part of this paper under *Technic Sec. 1, Glassware*.

2. The *method of filling tubes* for tests and of introducing the indicator must be uniform, and designed to prevent or minimize changes in CO₂ equilibria. The method found most satisfactory by the authors is described *under Technic, Secs. 5-6*.

3. In colorimetric work with very dilute solutions the *purity of the water* used and hence its preparation by a reliable method, designed to prevent the absorption of *solids* from the apparatus as well as the carrying over of *buffer sprays*, is extremely important. The main features of our table still (to be further described in a separate paper) are indicated *under Technic, Sec. 2, Water*.

The presence of varying quantities of CO₂ in distilled water has been found to be a far more important factor affecting pH

measurements than the presence of other impurities usually found, although the latter must not be ignored. Hence measures must be taken either to *control the amount of CO₂ in the dilution water or to eliminate* it entirely.

a. Consistent, though not always accurate results may be obtained in dilute solutions by using water which has reached equilibrium with the CO₂ in the air (pH 5.7) but water containing even so small a quantity of CO₂ will measurably affect readings when working in the ranges more alkaline than pH 5.7 (air-CO₂ equilibrium). Generally speaking, however, air-equilibrium water will not seriously vitiate determinations made within the range of brom cresol purple (pH 5.0 to pH 6.6).

b. Water freshly collected from ordinary stills is commonly supercharged with CO₂, having a pH value of approximately 5.0 and cannot be used for making determinations in very dilute solutions more alkaline than pH 5.0 without affecting the readings.

c. In the ranges covered by the indicators brom thymol blue (pH 6.2 to 7.6), phenol red (pH 6.8 to 8.4) and cresol red (pH 7.2 to 8.8) it is essential to work under CO₂-free conditions in order to prevent serious errors in determining colorimetrically the pH of weakly buffered solutions, especially toward the alkaline ends of their ranges. A simple method of removing CO₂ from water is described *under Technic, Sec. 2, Water*.

4. For accurate work with solutions of the usual unknown concentrations (0.1 to 0.001 M), it is advisable to *adjust the indicator to the mid-point* of its useful range. When only one stock solution of each indicator is made up for *general tests*, the usual practice of today, we have used since January, 1924, and recommend 1.5 mols of alkali with the exceptions noted, or specifically such amount of acid or alkali as is shown in table 1 to be necessary to bring the pH value of the single stock indicator solution close to the mid-point. This procedure must on the average, except in the most dilute solutions, balance up the small effect of the indicator itself on the pH of the mixture measured. The slight error which may still occur in very weakly buffered solutions at the ends of the useful indicator range is

annulled by taking the average of the slightly too high and too low readings found by using overlapping indicator ranges in a test.

5. When dealing with *an extremely dilute solution* the concentration of which is of an order only slightly greater or less than that of the indicator itself (0.0001 M and less), approximate results may be obtained by testing the unknown with three solutions of the indicator adjusted at the lowest, midpoint and highest useful pH values, or still better with a series of solutions of the same indicator graded in steps of 0.1 to 0.2 pH, and determining the point or range at which there is coincidence between the pH as read and that of the indicator solution used. A simple practical *method of adjusting indicators to graded pH values* is described under *Technic, Sec. 3*. No successful method has yet been found for stabilizing these delicately adjusted solutions but it is essential that they be made up in scrupulously clean containers and stored in pyrex or non-sol bottles or flasks to prevent change due to contact with soft glass as has been noted also by other workers. A more detailed account of the iso-indicator method and its applications will be given in another paper now in preparation by the authors entitled, "A new method of determining the purity of water by means of indicators." This paper will also contain a complete study of CO₂ equilibria in water and methods of determining the pH of water under all laboratory conditions.

GENERAL CONCLUSIONS

1. A study of the dilution curves for the different buffers and media investigated reveals three types of variation from the true pH value as electrometrically determined for each solution in the undiluted state: (1) buffer-dilution effects; (2) changes caused by the varying CO₂ content and alkaline impurities of the water used; and (3) variations caused by the differences in the pH values of the indicator solutions. *Buffer dilution* and *water-indicator* effects may be in the same direction, as in figure 1, or in opposite directions, as in figure 3 where the initial dilutions cause an alkaline change followed by more or

less marked increases in acidity upon further dilution due to varying amounts of CO_2 in the water and the pH of the indicators used.

2. *The effect of CO_2 in the water is not marked as a rule until a dilution of 1 part in 50 is reached. As dilution is increased the effect of CO_2 rapidly becomes more marked, especially in the case of pH 5.0 water often carrying the curves beyond the range of the indicators used. The curves made with air- CO_2 equilibrium (pH 5.7) water naturally fall in a position intermediate between those made with pH 5.0 water and with CO_2 -free (pH 7.0) water.*

3. In all the charts with the exception of Chart 1, it is very noticeable that CO_2 acts not only as an *acid*, lowering the pH values markedly in the more dilute solutions, but that it also acts as a *buffer*, causing the readings made with indicator solutions adjusted in graded series to *fall closer together* than those made under CO_2 -free conditions. This phenomenon increases with the amount of CO_2 present. The effect produced by the curves is like that of the opening or closing of a fan and sometimes of a considerable shifting of the fan to a different position on the chart (figures 3 and 4 for the buffers of higher pH ranges).

4. *The adjustment of the indicator solution itself does not begin to cause noticeable variations in readings until a dilution of 1 part in 100 is reached in the organic media (figs. 2 and 5) or molalities of 0.0001 to 0.000083 in the synthetic media (figs. 3 and 4) and it was necessary to dilute the latter solutions to 0.00005 M or 0.0000083 M before the adjustment of the indicator became a very important factor in controlling the pH.*

5. In all the charts one general principle stands out, viz., that barring the comparatively slight initial variations due to buffer dilution, the readings obtained with an indicator solution adjusted to a given pH value approach closer and closer, as dilution proceeds, to the value registered by that indicator solution in the same water without the presence of the buffer (see values recorded at the extreme right of every chart).

6. A paper now in preparation dealing with *salt errors* in relation to dilution will bring out more clearly the value of the technic

described in the present article in making it possible to separate CO_2 and indicator effects from true salt effects. The statement should be made here that the *electrometric* data show rather marked increases in pH due to buffer dilution even in the first dilutions (1:5 and 1:10) in both buffers and media. This same change in pH measured *colorimetrically* is greatly lessened or entirely annulled by the fact that the salt errors of the indicators are nearly always in the acid direction; hence *colorimetrically* there often appears to be little or no change in pH in the initial dilutions, a fact which has proved to be useful in practice rather than a detriment when turbid or colored solutions are diluted to get the pH values.

7. Although the dilutions showing variations due to CO_2 content of water and adjustment of indicator solutions are higher than those recommended for ordinary colorimetric work on media, it is valuable to know just how far dilution may be carried without encountering these difficulties. It is the hope of the authors that these researches may prove helpful to those who are dealing with the colorimetric determination of H-ion concentration in very dilute or weakly buffered solutions, and who will find it of vital importance to regulate the pH of the indicator solutions and that of the water used by controlling its CO_2 content.

8. The isohydric indicator method makes possible colorimetric salt error corrections and pH measurements to about 0.1 pH by the use of indicators (*a*) adjusted at their midpoint pH for testing buffer solutions of the usual concentrations and up to about 50 fold dilutions thereof; (*b*) adjusted at their lowest, midpoint and highest useful pH values for studying such buffers diluted about 50 to 1000 fold; and (*c*) adjusted at 0.1 or 0.2 pH intervals over their useful ranges for measuring highly diluted buffers or water. The correct pH point or range is shown by agreement between the pH as read and that of the indicator solution used. This method now gives us a new technique for measuring colorimetrically the pH values of very dilute buffer solutions or water, in which cases E.M.F. methods are either inaccurate or entirely useless.

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