IX. THE DISCREPANCY BETWEEN ELECTRO-METRIC AND COLORIMETRIC (PHENOL RED) DETERMINATIONS OF c_H ACCORDING TO THE SALT-CONTENT OF THE SOLUTION.

BY ELIZABETH HERDMAN LEPPER
AND CHARLES JAMES MARTIN.

From the Department of Experimental Pathology, Lister Institute, London.

(Received January 5th, 1926.)

THE method of Friedenthal [1904] and Salm [1904] for the approximate determination of c_H by adding various indicators to the solution was developed by Sørensen [1909] into a colorimetric method of considerable precision and great usefulness for determining p_H and is now universally employed.

Michaelis and Rona [1908, 1909] were the first to draw attention to aberrations of the colorimetric method due to the salt-content of the solution under investigation. For accurate work it is necessary to be aware of these and to correct for them. The entire blame was attributed to the indicator, until it was pointed out by Sørensen and Palitzsch [1910] that the addition of NaCl or KCl to a buffer solution of phosphates also altered the c_H , as determined electrometrically, and that the discrepancy between the two methods was the algebraic sum of the influence of the electrolyte upon the dissociation of the weak acid of the buffer mixture and the indicator respectively.

The earlier literature will be found in Sørensen's article on the measurement and significance of hydrogen ion concentration [1912].

For the purpose of their investigation into the p_H of sea-water, Sørensen and Palitzsch [1913, 1, 2] determined the discrepancy occurring with concentrations of NaCl up to 4 %, when phenolphthalein and naphtholphthalein were the indicators and the buffer mixture was a "weak" solution of phosphates. From their observations they plotted "error" against NaCl content. The standards for comparison contained $M/15$ phosphate. The colorimetric and electrometric methods agreed when about 0.7 % NaCl was added. The discrepancy without added NaCl and with 4 % salt was about 0.2 in the p_H but in opposite directions. When neutral red was used as indicator the discrepancy was within the error of observation until the concentration of NaCl exceeded 1 %.

Palitzsch [1911] had previously made similar observations on the effect of NaCl when the indicator used was methyl red. His observations are however difficult to interpret, as citrates were used in the tubes for comparison. The tint of this indicator is not quite the same with citrates as with phosphates.

Bjerrum [1914] when discussing the theory of titration with indicators, referred to their "salt error" and concluded that it must be attributed either (1) to a molecular combination of the indicator with the salt whereby its colour is changed, or (2) to the effect of the electrolyte upon the dissociation constant of the indicator.

Michaelis and Gyemant [1920] determined the discrepancy between colorimetric and electrometric observations caused by 0.5 *M* solutions of NaCl and KCl when the nitrophenols and phenolphthalein were used as indicators. The discrepancy caused by certain concentrations of CaCl_2 , K_2SO_4 and AlCl_3 can also be derived from the data in some other of the tables. They discuss the theory of "salt error" at some length.

Kolthoff [1922] published useful observations on the "salt error" of a number of indicators arrived at from electrometric and colorimetric measurements of buffer solutions, generally 0.05 *M* phosphates, with and without the addition of 0.5 *M* NaCl.

Most of the observations recorded above have been made with phosphate buffers, but sometimes borates, acetates or cacodylates were employed. Evans [1921], using a buffer of NaHCO_3 in equilibrium with CO_2 pressures, came to the conclusion that in the presence of 0.85 % NaCl there was a constant discrepancy of about 0.2 p_{H} , the colorimetric method indicating the higher p_{H} . This he attributed to a fundamental defect of the hydrogen electrode in the presence of bicarbonates. He believed this was due to the reduction of carbonic acid to formic acid at the electrode.

We have used mixtures of sodium phosphates and sodium bicarbonate at constant CO_2 pressure as buffers. The concentration of the buffering salts was varied. The indicator we employed was phenol red. As information regarding the effect of NaCl is more particularly useful for physiological experiments, the variation in the concentration of electrolytes was produced by addition of this salt.

A number of solutions of the buffer containing NaCl in concentrations varying from 0.001 to 1.0 *M* were prepared and the p_{H} determined electrometrically and colorimetrically. The results are expressed in Tables I to V. From them graphs were drawn with the p_{H} plotted against the $-\log$ of the concentration of total Na. The figures for the discrepancy between electrometric and colorimetric observations given in the tables are derived from the smoothed curves in the corresponding graphs. The scale of all the graphs is the same.

METHODS.

Equilibration of the bicarbonate solutions with constant CO_2 pressure.

A supply of H_2 with a constant percentage of CO_2 was obtained in the following way. A small steel gas-cylinder was washed out with H_2 . It was then charged with CO_2 until the pressure was, say, 5 atmospheres. Without undoing any of the connections, which had been previously washed out with H_2 , H_2 was run in from a large cylinder until the pressure was 100 atmospheres.

Some of the CO_2 disappeared rather quickly from the first cylinder filled. At the partial pressure of about 5 atmospheres it attacked the steel, forming $FeCO_3$ on the walls, but after the cylinder had been used once, the loss of CO_2 became so slow as to be unimportant. The disappearance of CO_2 from our cylinder is now so gradual that, over a period of one week, it does not exceed the error of analysis.

In our earlier experiments, equilibration was accomplished in a hard glass round-bottomed flask of about 250 cc. capacity. 20 cc. of the solution of bicarbonate was swirled round the wall of the flask mechanically, whilst a mixture of CO_2 and H_2 of known composition, previously saturated with H_2O at 18° , streamed through the flask. It was found that half an hour was sufficient to attain equilibrium. The temperature of the room was maintained at $18^\circ \pm 0.5^\circ$. At the end of an hour, samples were withdrawn from the flask into the hydrogen electrode and also into the pots of the colorimeter, avoiding contact with air during the transfer.

In the later experiments the indicator was added to the solution before equilibration with CO_2 . This was carried out in a rotating bottle inside the thermostat containing the electrode vessels. It was previously ascertained that indicator, in the amount added, did not interfere with the electrometric determinations.

Electrometric measurements.

In the earlier series of experiments (Tables I, II and IV) the hydrogen electrode vessel used was of the U-tube type formerly employed by Michaelis. This was subsequently abandoned for a modification we devised of that used by Walpole [1913] for electrometric titration. Walpole's electrode vessel has an all-glass syringe attached to the H_2 inlet tube by means of which the liquid can be drawn up over the electrode or displaced at will. The handle of the syringe was outside the thermostat so it was not necessary to open the thermostat during the observations.

Our electrodes contained about 4 cc. of liquid. They permitted complete de-oxygenation of the liquid and thorough washing of the electrode with it. By efficient agitation, false equilibria were avoided and once the electrode was charged with hydrogen the final reading was obtained in a few minutes. We found agitation of the liquid and frequent washing of the electrode to be essential when the buffer consisted of bicarbonates and CO_2 . The hydrogen

CO₂ mixture supplied to the electrode in this case was the same as that with which the equilibrium with CO₂ was obtained. It was previously saturated with H₂O at 18°.

A 3.5 *N* KCl calomel electrode was used as the other half cell. For the π_0 value of this electrode at 18° 0.2548 volt [Sørensen, 1924] was used. It was tested before each day's observations against "standard acetate" (0.1 *N* $\text{H}\bar{\text{A}}$ + 0.1 *M* $\text{Na}\bar{\text{A}}$) and any small discrepancy between the E.M.F. found and 5.218 volts was allowed for in calculating the p_{H} . The values assigned to the electrometric measurements are those calculated for dry H₂ at 760 mm. pressure.

Colorimetric measurements.

In all but the last series of experiments, the colorimetric determinations were made by the method used by Dale and Evans [1920] for determining the p_{H} of dialysates from blood. We used a large colorimeter box into which we could get our heads, instead of the small one figured in their paper. The solutions were run into the colorimeter pots under paraffin and precautions were taken to avoid loss or gain of CO₂ during the transfer. The method proved satisfactory when the buffer was a solution of phosphates, but when bicarbonate and CO₂ were used the discrepancy between experiments was more than we liked, sometimes amounting to 0.06 p_{H} . Therefore, many observations had to be made and the averages taken (see Table IV).

In the series of experiments detailed in Table V the technique was modified as follows. The indicator was added to the solutions of bicarbonate before they were brought into equilibrium with the mixture of H₂ and CO₂. When equilibrium had been obtained the solution was drawn into the electrode vessel without contact with air and the E.M.F. measured. This being accomplished, samples were withdrawn *from the electrode vessel* into small hard glass tubes drawn out at each end, and the ends immediately sealed in a flame. The sampling tubes were filled with the mixture of H₂ and CO₂ before the liquid from the electrode was allowed to flow into them. Subsequently, the apparent p_{H} of the solution was determined by matching the colour against freshly made standard solutions of *M*/15 phosphates containing the same amount of indicator in similar tubes. With the adoption of this technique, our troubles disappeared and duplicate observations agreed.

THE EFFECT OF DILUTION ON THE p_{H} OF PHOSPHATE SOLUTIONS AS DETERMINED ELECTROMETRICALLY AND COLORIMETRICALLY.

Diluting a phosphate mixture considerably increases its p_{H} . As may be seen from Table I or Fig. 1 the p_{H} increases 0.47 in going from 0.3 *M* to 0.001 *M*. Michaelis and Kruger [1921] pointed out this fact, but it is not generally appreciated. Under the same conditions the apparent p_{H} from colorimetric measurements changes much less. At a concentration of *M*/15 the electrometric and colorimetric estimations agree because this is the

ERRATUM

Page 49, Table I at head of first column:

for Dilution *read* Dilution
NaCl PO₄

concentration of phosphates in the standards with which they are compared. Dilution of the phosphates affects the dissociation of the indicator as well as the c_H of the solution or there would be no discrepancy. The former effect to some extent compensates the latter. Consequently, when the electrometric and colorimetric observations are plotted against the concentration of phosphates in solution, as in Fig. 1, the curves cross at $M/15$. If the colour comparison had been made with standards containing $M/1000$ phosphates instead

Table I. *Effect of dilution on the c_H of a phosphate mixture (8.5 parts Na_2HPO_4 + 1.5 parts NaH_2PO_4) when determined electrometrically and colorimetrically with phenol red. Temperature 18° .*

(The standards for colorimetric comparison contained $M/15$ phosphates.)

Dilution NaCl	Dilution Na	p_H electrometric	p_H colorimetric*	Discrepancy (from smoothed curve)
$M/3$	$M/1.62$	7.36	7.44	+0.07
$M/6$	$M/3.24$	7.45	7.48	+0.04
$M/12$	$M/6.48$	—	7.53	+0.01
$M/15$	$M/8.1$	7.55	7.55	0
$M/25$	$M/13.5$	—	7.57	-0.02
$M/30$	$M/16.2$	—	7.58	-0.03
$M/40$	$M/21.6$	7.65	7.61	-0.045
$M/100$	$M/54$	—	7.64	-0.09
$M/150$	$M/81$	7.74	7.66	-0.11
$M/225$	$M/102$	7.78	—	—
$M/400$	$M/216$	7.81	7.66	-0.15
$M/1000$	$M/540$	7.83	7.65	-0.16

When the figures under "discrepancy" have a + sign it signifies that the colorimetric is higher than the electrometric.

* The dilution of the solutions for the colorimetric determinations was 10 % greater than that for the electrometric, due to solution of indicator added.

Fig. 1

of $M/15$ the two curves would agree at this point and not cross, but the colorimetric curve would fall less rapidly than the electrometric as the concentration of phosphates increases.

In all the graphs p_H is plotted as ordinate against the logarithm of the dilution of Na as abscissa, i.e. the negative logarithm of c_H is plotted against the negative logarithm of c_{Na} . This method was chosen so that all the graphs should be comparable. It is also more instructive, for, phenol red having a coloured anion, it is more particularly the concentration of kations which modifies its dissociation. If 0.25 be added to any value along the abscissa it will be equal to the negative logarithm of the concentration of the phosphates.

EFFECT OF NaCl ON THE p_H OF PHOSPHATE SOLUTIONS AS DETERMINED ELECTROMETRICALLY AND COLORIMETRICALLY. (Tables II and III and graphs in Figs. 2 and 3.)

We used 0.0044 and 0.01 M solutions of phosphates. The concentration of NaCl varied between 0.001 and 1.0 M . The effect upon the p_H was greater with the less buffered solutions (Table II and Fig. 2). This fact was noticed by Palitzsch [1911]. The influence of increasing the concentration of Na^+ by adding NaCl is more pronounced than when it is increased by augmenting the concentration of the phosphate mixture as in the experiment detailed in Table I and illustrated in Fig. 1. This is presumably because in Exp. 1 the concentration of the buffer is increased *pari passu* with the increase in concentration of kations.

The effect of increasing the salt concentration on the p_H of the solution is similar to that found by Michaelis and Kruger [1921].

In both Exps. 2 and 3 the colorimetric estimation coincides with the electrometric when the concentration of kations is about equal to that of the $M/15$ phosphate solution used for making the colorimetric comparisons.

Table II. *Effect of NaCl on the c_H of a 0.0044 M solution of phosphates (Na_2HPO_4 8.4 parts, NaH_2PO_4 1.6 parts) of concentration 0.0044 M when determined electrometrically and colorimetrically with phenol red. Temperature 18°.*

(The standards for colorimetric comparison contained $M/15$ phosphates.)

Dilution NaCl	Dilution Na	p_H electrometric	p_H colorimetric*	Discrepancy (from smoothed curve)
$M/1$	$M/0.99$	6.82	7.22	+0.36
$M/2$	$M/1.93$	7.13	7.37	+0.24
$M/4$	$M/3.88$	7.32	7.49	+0.10
$M/8$	$M/7.52$	7.53	7.53	0
$M/16$	$M/14.08$	7.61	7.56	-0.06
$M/32$	$M/25.64$	7.70	7.58	-0.10
$M/100$	$M/55.56$	7.74	7.61	-0.14
$M/1000$	$M/111.1$	7.76	7.62	-0.14

When the figures under "discrepancy" have a + sign it signifies that the colorimetric is higher than the electrometric.

* The dilution of the solutions for the colorimetric determinations was 10 % greater than that for the electrometric, due to solution of indicator added.

Fig. 2.

Table III. Effect of NaCl on the c_H of a 0.01 M solution of phosphates (Na_2HPO_4 7.75 parts, NaH_2PO_4 2.25 parts) when determined electrometrically and colorimetrically with phenol red. Temperature 18°.

(The standards for colorimetric comparison contained M/15 phosphates.)

Dilution NaCl	Dilution Na	p_H electrometric	p_H colorimetric*	Discrepancy (from smoothed curve)
M/1	M/ 0.98	6.75	6.98	+0.20
M/2	M/ 1.93	6.95	7.10	+0.14
M/4	M/ 3.7	7.10	7.20	+0.07
M/8	M/ 7	7.215	7.22	+0.01
M/16	M/12.4	7.31	7.27	-0.04
M/32	M/20.3	7.38	7.31	-0.08
M/128	M/38.7	7.45	7.33	-0.12
M/1000	M/52.6	7.50	7.36	-0.15
M/ ∞	M/55.5	7.505	7.36	-0.15

When the figures under "discrepancy" have a + sign it signifies that the colorimetric is higher than the electrometric.

* The total Na in the colorimetric observations was 10/11 of that present in the electrometric.

Fig. 3.

EFFECT OF NaCl ON THE p_H OF SOLUTIONS BUFFERED WITH $NaHCO_3$ IN EQUILIBRIUM WITH A CONSTANT PRESSURE OF CO_2 . (Tables IV and V, Figs. 4 and 5.)

0.02 and 0.04 M solutions of $NaHCO_3$ were used. These were brought into equilibrium with H_2 containing 3.17 % and 5.85 % CO_2 respectively. In the first series of experiments our colorimetric observations of p_H varied as much as 0.05 in duplicate experiments so we had to do a good many and take the means. This involved another error as the barometer was not the same over the period. The maximum error in p_H from this cause was, however, not more than 0.02. In the second experiment as explained above our technique was improved and the observations were all carried out in two days, during which time the barometer varied only a few millimetres.

The graphs on the left hand of Figs. 4 and 5 represent the observations as made. The effect of the addition of electrolyte on the colorimetric p_H has

in these experiments more than compensated for the actual fall in p_H . Before they can be compared with the previous experiments both curves should, however, be made steeper, for, although the CO_2 pressure was constant

Table IV. *Effect of NaCl on the c_H of a 0.02 M solution of NaHCO_3 in equilibrium with CO_2 at 24.1 mm. pressure, when determined electrometrically and colorimetrically with phenol red. Temperature 18°.*

(The standards for colorimetric comparison contained M/15 phosphates.)

Dilution NaCl	Dilution Na	p_H electrometric			p_H colorimetric*			Dis- crepancy (from smoothed curve)
		Observa- tions	Mean	Corrected for CO_2 solubility	Observa- tions	Mean	Corrected for CO_2 solubility	
M/ 1	M/ 0.99	7.225 7.23 7.23	7.23	7.10	7.44 7.43 7.42	7.43	7.30	+0.2
M/ 2	M/ 1.9	7.33 7.315	7.32	7.24	7.43 7.44 7.45	7.44	7.36	+0.1
M/ 3.7	M/ 3.36	7.39	7.39	7.35	7.42 7.44	7.43	7.39	+0.04
M/ 6.9	M/ 6.1	7.43 7.40	7.415	7.39	7.43 7.38	7.41	7.40	0.0
M/16.6	M/10.35	7.44 7.44	7.44	7.44	7.35 7.41	7.38	7.38	-0.05
M/50	M/25	7.47 7.475 7.48	7.475	7.475	7.36 7.39 7.39	7.38	7.38	-0.11
M/1000	M/47.6	7.50 7.49	7.495	7.495	7.39 7.36 7.38 7.37 7.36	7.37	7.37	-0.12

When the figures under "discrepancy" have a + sign it signifies that the colorimetric is higher than the electrometric.

* The dilution of the solutions for the colorimetric determinations was 10 % greater than that for the electrometric.

Table V. *The effect of NaCl on the c_H of a 0.04 M solution of NaHCO_3 in equilibrium with CO_2 at 44.7 mm. pressure, when determined electrometrically and colorimetrically with phenol red. Temperature 18°.*

(The standards for colorimetric comparison contained M/15 phosphates.)

Dilution NaCl	Dilution Na	p_H electrometric		p_H colorimetric		Dis- crepancy (from smoothed curve)
		Observed	Corrected for CO_2 solubility	Observed	Corrected for CO_2 solubility	
M/1	M/ 0.96	7.267	7.142	7.42	7.295	+0.15
M/2	M/ 1.85	7.33	7.251	7.445	7.365	+0.11
M/4	M/ 3.45	7.38	7.34	7.437	7.397	+0.06
M/10	M/ 7.14	7.437	7.417	7.432	7.412	0.00
M/30	M/13.7	7.473	7.463	7.427	7.417	-0.04
	M/25	7.507	—	7.41	—	-0.09

The bicarbonate solution contained the required amount of phenol red.

When the figures under "discrepancy" have a + sign it signifies that the colorimetric is higher than the electrometric.

throughout the observations, the concentration of CO_2 in the solutions varied owing to the diminishing solubility of this gas as the salt in the solution increased. In the graphs on the right-hand side of the figures the observations have been corrected for this by calculating by Hasselbalch's formula what effect the diminished solubility of CO_2 would make upon the p_{H} and subtracting it.

For these calculations Bohr's [1899] data for the solubility of CO_2 in NaCl solutions of different strengths were used.

It will be seen from Tables IV and V and Figs. 4 and 5 that, even after correcting for the solubility of CO_2 , the influence of NaCl upon the p_{H} of

Table VI. *The effect of NaCl on the c_{H} of phosphate mixtures containing KH_2PO_4 1.6 parts and Na_2HPO_4 8.4 parts in molecular concentration (a) $M/15$, (b) $M/100$.*

Phosphate	NaCl concentration molecular	Total Na concentration	p_{H}
$M/100$	0	0.0184 N	7.743
	0.1	0.1184 „	7.49
	0.333	0.3515 „	7.28
	1	1.0184 „	6.93
	—	—	6.94
$M/15$	0	0.1214 „	7.533
	0.1	0.221 „	7.433
	0.333	0.454 „	7.26
	1	1.1214 „	6.969
	—	—	—

Fig. 4.

$B = A$ corrected for varying CO_2 concentration.

0.02 and 0.04 M NaHCO_3 solutions is much less than upon 0.01 M phosphate solution. The smaller molecular strength of the phosphate buffer is not enough to account for this. Nearly the same drop in p_H is produced by NaCl upon 0.067 M as upon 0.01 M phosphate solution, as shown in Table VI. The curves cross at a point where the $-\log$ of the concentration of the Na is $0.85 = 0.142 N$ Na which is about the concentration in 0.85 % NaCl or serum. At this point there was no discrepancy between the colorimetric and electrometric estimations.

The curves drawn from experiments in which the buffer was a phosphate mixture (Figs. 1, 2 and 3) appeared to intersect when the concentration of sodium was a little less, viz. 0.123 N . We are uncertain whether there is a real difference in the concentration of Na^+ at the point of intersection in the two cases. A small error in drawing the curve or one of 0.02 p_H in the observations would be more than sufficient to account for this.

Fig. 5.

$B = A$ corrected for varying CO_2 concentration.

DISCUSSION.

We have found the data derived from the experiments described above of service for correcting our colorimetric observations with phenol red and may be they will be useful to others. Unfortunately, however, these corrections for NaCl content with phosphate or carbonate buffers cannot be applied if another indicator is used, or in the case of other buffers or if other electrolytes are present in considerable concentration. Even with phosphate and carbonate buffers they are only valid for phenol red and only accurate for the concentrations of buffer stated.

From the observations of Michaelis and Kakinuma [1923] the effect of NaCl upon the p_H of a mixture of 0.01 M $H\bar{A}$, and 0.01 M $Na\bar{A}$ is considerably less than Michaelis and Kruger [1921] found upon phosphates "much diluted." They made the interesting discovery that the influence of the kations of the univalent alkali metals upon both phosphate and acetate solutions varies in the inverse order of their atomic weights. Bivalent kations were more potent. It is probable that, similarly, the effect of different kations upon the dissociation of indicators will be found to vary. At present, therefore, it seems to be necessary to ascertain the discrepancy of the colorimetric method for the particular conditions under which one is working.

As far as the influence of NaCl on phosphate and bicarbonate buffers is concerned, it is clear, from our experiments, that with phenol red coincidence of colorimetric with electrometric observation only occurs when the concentration of Na^+ is about the same as that of the phosphate solutions used for colorimetric comparison. If it is more, the colorimetric gives too high results, if less, too low results.

Our observations with bicarbonate buffers lend no support to Evans' [1921] conclusion that the hydrogen electrode is incapable of affording the correct E.M.F. in solutions of 0.02 M bicarbonate. We think the systematic discrepancy of 0.2 p_H found by him between the electrometric and colorimetric p_H of 0.2 M $NaHCO_3$ dissolved in 0.18 M NaCl must be explained in some other way. According to our experiments (Table IV, Fig. 4 and Table V, Fig. 5) the p_H of 0.02 M $NaHCO_3$ in 0.18 M NaCl solution, determined by the two methods, would agree within the error of experiment.

Cullen and Hastings [1922] made electrometric and colorimetric observations of the p_H of 0.06 M $NaHCO_3$ solution and three mixtures of $NaHCO_3$ and phosphates. The solutions were equilibrated with CO_2 at constant pressure. Phenol red was the indicator used and Sørensen's phosphate solutions were the standards for colorimetric comparison.

They found good agreement notwithstanding that the total kations in the solutions varied from 0.06 M to 0.13 M . In view of our experiments detailed above, we should not expect such coincidence except in their third experiment in which the concentration of Na was 0.13 M . The conditions of Cullen and Hastings' experiment were, however, different from ours. Their solutions were strongly buffered and therefore not so sensitive to concentration of kations. Moreover, the variation in kation concentration between the different solutions was brought about by altering the amount of buffer, which tends to obliterate the effect.

Verney and Bayliss [1923] have also compared electrometric and colorimetric measurements of the p_H of 0.02 M $NaHCO_3$ in equilibrium with different pressures of CO_2 . The indicators used were phenol red, cresol red and neutral red. They satisfied themselves that there was no systematic difference between the results by the two methods. We do not understand how they found agreement when phenol red was the indicator used and the

colorimetric standard contained $M/15$ phosphates, unless they dissolved the bicarbonate in 0.85 % NaCl. According to the observations in Table IV and Fig. 4, the colorimetric gives results 0.1 p_H too low with 0.02 M NaHCO_3 in water. It is evidently difficult for different observers to attain concordant results.

Our observations explain why, as found by Cullen [1922] and confirmed by us [1925], serum diluted 1 in 30 with 0.85 % NaCl is found to have the same p_H by the colorimetric as that obtained by the electrometric method in the undiluted serum, for, notwithstanding the dilution, the colorimetric comparison is made in a solution containing approximately the same concentration of Na^+ as the standard phosphate solution used for comparison.

As to the cause of the fall in the p_H of the solutions and the increased dissociation of the phenol red produced by NaCl we have little to contribute. In the light of Bjerrum's "activity" theory, Michaelis and Gyemant [1920] attribute the effects to diminished activity of the anions both of the buffer salt and the indicator, due to the added cations. This theory interprets the fundamental facts by simple reference to the mass law. It is also capable of explaining an old observation of Arrhenius [1899] that the hydrolytic action of acetic and formic acids was increased by the addition of KCl or NaCl. But there are other facts, such as the increase in c_H of $M/500$ HCl [Michaelis and Kakinuma, 1923; Sørensen, 1924] when salt is added which cannot be explained so simply. For the explanation of these it seems necessary to assume, as Arrhenius did, that salt increases the dielectric constant of water.

Our observations show that the fall in p_H produced by a given concentration of cations depends upon the nature of the buffer, and that the quantitative relationship between the electrolyte added and the concentration of the buffer salt also influences the result. Why the discrepancy between the colorimetric and electrometric values should be greater, the weaker the buffer solution, is not clear, for the concentration of indicator remains constant throughout.

REFERENCES.

- Arrhenius (1899). *Z. physikal. Chem.* **31**, 197.
Bjerrum (1914). *Sammlung chem. und chem. tech. Vorträge*, **21**.
Bohr (1899). *Wiedemann's Ann.* **68**, 502.
Cullen (1922). *J. Biol. chem.* **52**, 501.
Cullen and Hastings (1922). *J. Biol. Chem.* **52**, 517.
Dale and Evans (1920). *J. Physiol.* **54**, 167.
Evans (1921). *J. Physiol.* **54**, 353.
Friedenthal (1904). *Z. Elektrochem.* **10**, 114.
Kolthoff (1922). *Rec. Trav. Chim. Pays Bas*, **41**, 54.
Lepper and Martin (1925). *Biochem. J.* **19**, 573.
Michaelis and Gyemant (1920). *Biochem. Z.* **109**, 165.
Michaelis and Kakinuma (1923). *Biochem. Z.* **141**, 394.
Michaelis and Kruger (1921). *Biochem. Z.* **119**, 307.
Michaelis and Rona (1908). *Z. Elektrochem.* **14**, 349.
— (1909). *Biochem. Z.* **23**, 61.

- Palitzsch (1911). *Compt. Rend. Lab. Carlsberg*, **10**, 162.
Salm (1904). *Z. Elektrochem.* **10**, 342.
Sørensen (1909). *Compt. Rend. Lab. Carlsberg*, **8**, 1.
—— (1912). *Ergeb. Physiol.* **12**, 393.
—— (1924). *Compt. Rend. Lab. Carlsberg*, **15**, 1.
Sørensen and Palitzsch (1910). *Compt. Rend. Lab. Carlsberg*, **9**, 8.
—— — (1913, 1). *Biochem. Z.* **51**, 307.
—— — (1913, 2). *Compt. Rend. Lab. Carlsberg*, **10**, 252.
Verney and Bayliss (1923). *J. Physiol.* **58**, 101.
Walpole (1913). *Biochem. J.* **7**, 410.