

2. Velocity constants for the hydrolysis of hydrogen cyanide using various concentrations of hydrochloric and hydrobromic acids as catalysts have been determined at 45 and 65°. The velocity goes up very rapidly with increase in concentration of acid. The conclusion is drawn that the principal catalyst is the undissociated acid molecule.

3. The results obtained with sulfuric acid at 65° show this acid to be a poor catalyst compared with the other two acids, especially at the higher concentrations.

4. The relative difference in velocity between two acid concentrations is independent of the temperature.

5. The addition of sodium and potassium chlorides to hydrochloric acid and of sodium and potassium bromides to hydrobromic acid increases the velocity very markedly. In some cases the increase is five fold.

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The Preparation of Acetate and Phosphate Buffer Solutions of Known P_H and Ionic Strength

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A systematic study of the dissociation constant of acetic acid¹ and of the second dissociation constant of phosphoric acid² in the presence of their salts was undertaken by Cohn and by Cohn, Heyroth and Menkin in terms of recent theories of electrolyte solutions. The equilibria in mixtures of CH_3COOH and CH_3COONa up to 2 molar, and of K_2HPO_4 and KH_2PO_4 in concentrations as high as 1.2 molar phosphate, were described. It has been found advisable to extend the concentration limit of the phosphates also to two molar solutions in order to study the behavior of proteins in concentrated electrolytes.^{3,4,5,6} The electromotive force measurements in this concentrated range have been carried out both with the hydrogen electrode and with the glass electrode.

The interpolation tables and graphs presented in the earlier communications from this Laboratory have been replaced in use by a system better adapted to facilitate the preparation of solutions of known P_H , concentration and ionic strength. These are given in terms of the mole fractions of salt required to yield a given P_H , whereas in the previous theoretical treatment the activity coefficients at given mole fractions were calculated.

(1) Cohn, Heyroth and Menkin. *THIS JOURNAL*, **50**, 696 (1928).

(2) Cohn, *ibid.*, **49**, 173 (1927).

(3) Cohn and Green, *J. Biol. Chem.*, **78**, 32 (1928).

(4) Green, *ibid.*, **93**, 517 (1931).

(5) Green, *ibid.*, **93**, 495 (1931).

(6) Florkin, *ibid.*, **87**, 629 (1930).

It seems desirable to make generally available these calculations and graphical representations.

The two molar phosphate solutions were prepared by dissolving the dihydrogen salt in the requisite quantity of two molar potassium hydroxide and diluting to volume with water. The greater solubility of K_2HPO_4 than KH_2PO_4 permits more concentrated mixtures to be prepared the greater the P_H . Thus at room temperatures, at mole fraction of K_2HPO_4 , 0.15, a two molar solution is essentially saturated, whereas at mole fraction 0.5 a three molar solution can be made, and at 0.8 a four molar solution.

The hydrogen electrode measurements were made using a Simms⁷ electrode and a saturated potassium chloride bridge. Readings were taken until equilibrium was established. Although equilibrium was reached slowly in the concentrated solutions, the final readings on two different electrodes were constant, and checked satisfactorily. Any potential arising at the liquid junction has been ignored in these calculations.

The glass electrode measurements were made with a glass membrane of the MacInnes type⁸ in Stadie's electrode vessel.⁹ In calculating the P_H as determined on this electrode the $M/10$ solutions were assumed to have the P_H calculated according to equation 1 below, and the P_H of the more concentrated solutions of the same composition calculated from the difference in e. m. f. The results of both series of measurements are given in Table I.

TABLE I
COMPARISON OF CALCULATED AND MEASURED VALUES OF P_H IN CONCENTRATED
PHOSPHATE SOLUTIONS

Mole fraction of total phosphate as D_2HPO_4	Total moles phosphate	P_H hydrogen electrode	P_H glass electrode	P_H calculated (equation 1)
0.9	1.6	7.958	7.926	7.945
.9	2.0	8.025	7.997	8.044
.7	1.6	7.156	7.107	7.130
.7	2.0	7.220	7.168	7.190
.5	1.6	6.577	6.548	6.570
.5	2.0	6.590	6.561	6.570
.3	1.6	5.954	5.972	5.950
.3	2.0	5.912	5.935	5.900
.15	1.6	5.447	5.434	5.380
.15	2.0	5.374	5.342	5.270

The value of the $N/10$ calomel cell has been taken as 0.3357 volt at 18°. This is in accordance with the calculations of Sørensen and Linderstrøm-Lang¹⁰ and of Scatchard,¹¹ which were previously employed in dealing with

(7) Simms, *THIS JOURNAL*, **45**, 2504 (1923).

(8) MacInnes and Dole, *ibid.*, **52**, 29 (1930).

(9) Stadie, O'Brien and Lang, *J. Biol. Chem.*, **91**, 243 (1931).

(10) Sørensen and Linderstrøm-Lang, *Compt. rend. trav. Lab. Carlsberg*, **15**, 1 (1924).

(11) Scatchard, *THIS JOURNAL*, **47**, 696 (1925).

the acetate buffers,¹ but not with the phosphates. The values of P_H calculated with this lower value for the calomel cell are 0.04 unit higher and on this basis pK_2 for phosphoric acid becomes 7.20. Bjerrum and Unmack's¹² redetermination of this quantity, in which a somewhat different extrapolation formula was employed, is 7.227 at 18° and 7.207 at 25°. The estimate of pK for acetic acid calculated with this value of the calomel electrode by Cohn, Heyroth and Menkin,¹ was 4.77. The most recent estimates by MacInnes and Shedlovsky¹³ and by Harned and Ehlers¹⁴ more accurately determine the value of pK of acetic acid at 25° as 4.756.

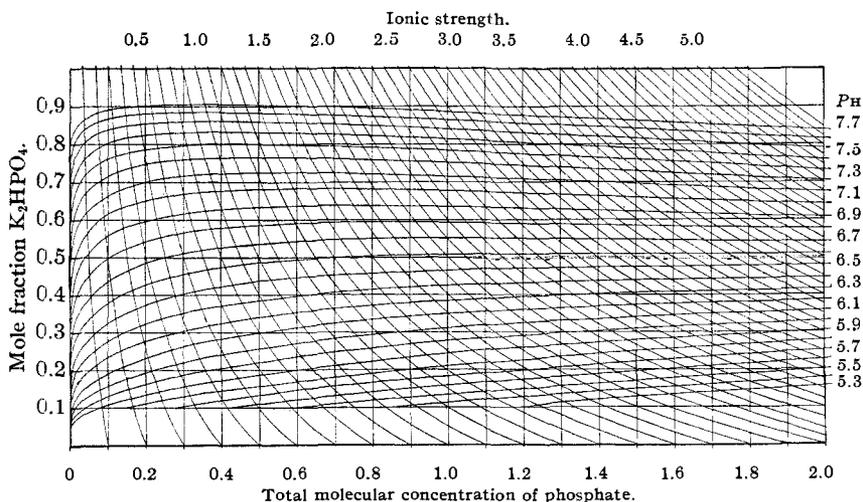


Fig. 1.

The equation previously employed² in describing the equilibria in phosphate solutions, with the new value for pK_2 , becomes

$$P_H + \log \frac{(\text{KH}_2\text{PO}_4)}{(\text{K}_2\text{HPO}_4)} = pK_2 - \frac{0.5(Z^{-2} - Z^{-2})\sqrt{\mu}}{1 + \kappa b} = 7.20 - \frac{1.5\sqrt{\mu}}{1 + 1.5\sqrt{\mu}} + K_s\mu \quad (1)$$

where μ , the ionic strength, is three times the concentration of K_2HPO_4 plus the concentration of the KH_2PO_4 ; and K_s is a constant varying with the ratio of the two salts. It was pointed out that "the assumption that b is constant, and the explanation of the observed variation . . . by a term proportional to μ instead of to $\sqrt{\mu}$, must for the present be regarded as yielding an empirical interpolation formula" (Ref. 2, p. 186). For convenience in the preparation of solutions all the calculations in this paper are in terms of the number of moles or the ionic strength per liter of solution rather than per 1000 g. of water.

If all the constants be assumed to have the same values in the more concentrated solutions that have now been measured, the P_H may be

(12) Bjerrum and Unmack, *Det Kgl. Danske Videnskabernes Selskab. Math.-fys. Medd.*, **9**, 1 (1929).

(13) MacInnes and Shedlovsky, *THIS JOURNAL*, **54**, 1429 (1932).

(14) Harned and Ehlers, *ibid.*, **54**, 1350 (1932).

calculated. These values are recorded in Table I. In general the calculated values fall between those determined with the hydrogen and with the glass electrodes, except in the most acid solutions.

The preparation of buffers of known P_{H} and ionic strength has been greatly facilitated by the graphical method presented in Fig. 1, in which the curves are calculated by means of equation 1. Four fundamental variables are represented on the chart: P_{H} , ionic strength, total molar concentration of phosphate and mole fraction of total phosphate as K_2HPO_4 . If any two of these variables are fixed, the values of the other two are uniquely determined. The total molecular concentration of phosphate and the mole fraction of the total phosphate as K_2HPO_4 are the rectangular coordinates. Curves at constant P_{H} at intervals of 0.1 have been plotted as contour lines, as have also curves giving the ionic strength. One can read directly from the chart the molar concentration of phosphate and the mole fraction of K_2HPO_4 for a series of buffers at either constant ionic strength or constant P_{H} . The values from which the curves at constant P_{H} are drawn are given in Table II, so that comparable figures on a larger scale such as are employed for interpolation in this Laboratory, could readily be constructed.

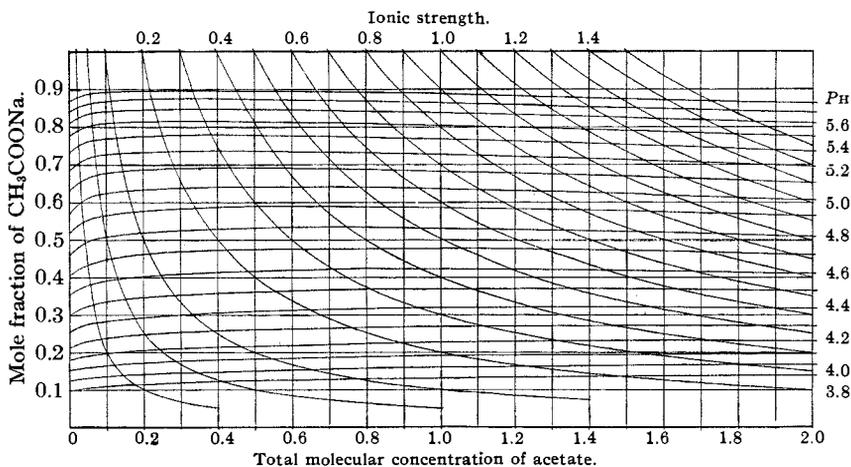


Fig. 2.

The preparation of acetate buffers is facilitated by the use of Fig. 2 and Table III calculated according to the equation

$$P_{\text{H}} + \log \frac{(\text{CH}_3\text{COO}^-)}{(\text{CH}_3\text{COOH})} = 4.77 - \frac{0.5 \sqrt{\mu}}{1 - \kappa b} - 0.16 \mu \quad (2)$$

where μ is put equal to the concentration of sodium acetate and b is, for purposes of interpolation, assumed to vary with the mole fraction of total acetate as the sodium salt, and to have the values calculated by Cohn, Heyroth and Menkin (Ref. 1, p. 708).

TABLE II

INTERPOLATED VALUES FOR THE MOLE FRACTION OF TOTAL PHOSPHATE AS K_2HPO_4 OF SOLUTIONS OF GIVEN CONCENTRATION AND P_H
 Calculated by means of the equation

$$P_H + \log \frac{(KH_2PO_4)}{(K_2HPO_4)} = pK_2 - \frac{0.5(Z^{-2} - Z^{-2}) \sqrt{\mu}}{1 + kb} + K_s\mu = 7.20 - \frac{1.5 \sqrt{\mu}}{1 + 1.5 \sqrt{\mu}} + K_s\mu$$

The value of pK_2 , 7.20, results from the measurements of Cohn made with the hydrogen electrode at room temperature, uncorrected for liquid junction potentials, and recalculated using the appropriate values for the $N/10$ calomel half cell between 0.3357 volt at 18° and 0.3353 volt at 25°. The activity coefficients calculated by Cohn's equation are assumed to be independent of temperature over this range.

P_H	Total concentration of phosphate in moles per liter															
	0	0.01	0.04	0.10	0.20	0.30	0.40	0.50	0.60	0.80	1.0	1.2	1.4	1.6	1.8	2.0
	Mole fraction of total phosphate as K_2HPO_4															
5.3												0.110	0.122	0.133	0.146	0.158
5.4										0.115	.129	.142	.154	.168	.180	
5.5									0.099	0.119	.134	.150	.164	.177	.191	.202
5.6								0.109	.121	.141	.157	.172	.188	.203	.216	.228
5.7						0.104	0.121	.132	.145	.165	.182	.198	.216	.230	.242	.254
5.8				0.085	0.110	.129	.146	.158	.171	.192	.212	.227	.244	.258	.269	.280
5.9	0.048	0.065	0.083	.106	.135	.155	.173	.186	.200	.224	.244	.259	.274	.286	.295	.306
6.0	.059	.081	.103	.132	.163	.185	.203	.219	.236	.259	.277	.292	.305	.316	.323	.330
6.1	.074	.100	.126	.160	.195	.220	.239	.256	.273	.295	.312	.325	.336	.345	.351	.358
6.2	.091	.122	.155	.192	.232	.261	.281	.298	.312	.333	.349	.360	.369	.376	.381	.386
6.3	.112	.150	.190	.232	.276	.305	.326	.341	.354	.372	.386	.395	.401	.407	.412	.416
6.4	.137	.183	.230	.278	.325	.353	.373	.385	.398	.414	.424	.432	.436	.440	.443	.446
6.5	.166	.222	.274	.328	.376	.403	.421	.435	.444	.458	.466	.471	.474	.476	.477	.478
6.6	.201	.266	.325	.381	.429	.457	.473	.484	.493	.503	.508	.510	.510	.510	.510	.510
6.7	.240	.315	.380	.438	.486	.511	.526	.535	.543	.549	.551	.550	.548	.546	.544	.541
6.8	.285	.369	.440	.497	.543	.565	.578	.586	.590	.594	.594	.590	.586	.582	.578	.574
6.9	.334	.425	.498	.557	.598	.617	.629	.634	.637	.638	.636	.631	.624	.618	.612	.607
7.0	.387	.484	.556	.615	.651	.669	.677	.681	.683	.681	.676	.671	.661	.655	.648	.641
7.1	.443	.544	.614	.668	.701	.716	.722	.724	.725	.721	.715	.707	.699	.690	.681	.673
7.2	.500	.604	.670	.717	.747	.758	.764	.763	.762	.758	.751	.742	.732	.723	.713	.704
7.3	.557	.659	.720	.762	.785	.796	.801	.800	.797	.790	.784	.774	.764	.754	.743	.733
7.4	.613	.710	.763	.802	.822	.829	.832	.832	.828	.821	.814	.803	.793	.782	.771	.761
7.5	.666	.756	.805	.837	.854	.860	.860	.859	.855	.848	.840	.830	.820	.809	.798	.788
7.6	.715	.796	.840	.866	.880	.883	.884	.883	.879	.872	.864	.855	.844	.835	.824	.813
7.7	.759	.831	.869	.890	.902	.905	.905	.904	.901	.894	.885	.876	.867	.858	.849	.837

TABLE III

INTERPOLATED VALUES FOR THE MOLE FRACTION OF TOTAL ACETATE AS CH_3COONa OF SOLUTIONS OF GIVEN CONCENTRATION AND P_{H}

Calculated by means of the equation

$$P_{\text{H}} + \log \frac{(\text{CH}_3\text{COO}^-)}{(\text{CH}_3\text{COOH})} = pK - \frac{0.5Z^2 \sqrt{\mu}}{1 + \kappa b} + K_{s\mu} = 4.77 - \frac{0.5 \sqrt{\mu}}{1 + \kappa b} - 0.16 \mu$$

The value of pK , 4.77, results from the measurements of Cohn made with the hydrogen electrode at room temperature, uncorrected for liquid junction potential, calculated by means of the appropriate values for the $N/10$ calomel half cell between 0.3357 volt at 18° and 0.3353 volt at 25°. The activity coefficients calculated by Cohn's equation are assumed to be independent of temperature over this range.

P_{H}	Total concentration of acetate in moles per liter												
	0.0	0.05	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
Mole fraction of total acetate as CH_3COONa													
3.8	0.097	0.105	0.109	0.115	0.121	0.125	0.128	0.131	0.133	0.138	0.139	0.140	0.139
3.9	.124	.132	.136	.141	.149	.153	.156	.159	.161	.166	.167	.168	.167
4.0	.152	.160	.166	.171	.180	.184	.188	.190	.192	.195	.196	.198	.197
4.1	.179	.193	.197	.205	.215	.220	.223	.228	.228	.229	.230	.231	.230
4.2	.211	.230	.239	.248	.259	.264	.268	.270	.270	.270	.270	.270	.271
4.3	.252	.276	.284	.295	.306	.313	.317	.320	.320	.320	.319	.317	.316
4.4	.299	.328	.335	.347	.358	.365	.369	.370	.370	.370	.368	.365	.362
4.5	.351	.382	.390	.403	.414	.420	.423	.421	.420	.419	.417	.413	.411
4.6	.404	.436	.449	.462	.473	.477	.480	.478	.476	.471	.468	.464	.461
4.7	.459	.496	.508	.519	.530	.534	.533	.530	.527	.522	.520	.515	.509
4.8	.518	.556	.566	.578	.588	.589	.589	.587	.584	.580	.575	.571	.565
4.9	.566	.613	.624	.634	.641	.642	.639	.636	.632	.626	.618	.613	.607
5.0	.629	.666	.678	.686	.691	.692	.689	.684	.679	.672	.664	.657	.652
5.1	.681	.716	.724	.733	.738	.734	.732	.727	.722	.716	.710	.703	.695
5.2	.730	.762	.768	.775	.780	.776	.773	.768	.762	.756	.750	.744	.736
5.3	.772	.801	.807	.812	.815	.813	.810	.807	.799	.793	.787	.780	.774
5.4	.809	.835	.840	.844	.846	.846	.841	.838	.832	.826	.820	.814	.807
5.5	.840	.862	.868	.873	.873	.874	.869	.866	.860	.855	.848	.843	.837
5.6	.867	.888	.893	.895	.895	.895	.891	.889	.881	.877	.870	.867	.862

These two series of buffers extend from P_{H} 3.8 to 7.7 and to such high electrolyte concentrations that their use has proved invaluable in this Laboratory in various investigations, especially in the preparation and study of proteins in solutions of known P_{H} and ionic strength.

Summary

1. Interpolation tables and graphs have been prepared for both acetate and phosphate buffers, giving the mole fractions which yield solutions of known molecular concentration and P_{H} .

2. Measurements are reported both with the hydrogen and glass electrodes upon more concentrated phosphate buffers than had previously been studied. The interpolation equation previously employed by Cohn suffices also to describe these more concentrated solutions.

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