present as a contaminant. Coprecipitated potassium or sodium bisulfate also effect a similar but incomplete recrystallization. It is not apparent, as yet, why such a recrystallization process should take place upon ignition of barium sulfate contaminated with the alkali metal ions, nor why the effect should be so pronounced in the case of coprecipitated lithium.

Experimental

Methods of Analysis.—The methods of chemical, X-ray, and density analysis employed were the same as described in another paper.[8] Density measurements were made between 24 and 30°; their average deviation is given in Table II. Density calculations for the several possible types of structures were based on the methods given in Clark[16]; the precision measures given for the calculated density values in Table II take into account the experimental precision measures of the X-ray, water content and SO_4^{--}/Ba^{++} mole ratio analyses.

Results of Analysis.—Analytical results for total sulfate and total barium, expressed as the mole ratio, are given in Table I. Analytical results for the water content are also included in the table both as weight and mole per cent.

Values for the lattice parameters of the precipitates are given graphically in Fig. 2, the individual parameters and the unit cell volume being plotted against water content. The circles represent the probable errors of the individual parameters, as determined from the least squares solution of the film data. Unit cell volumes and their probable errors are given numerically in Table I.

Results of density measurements on four of the precipitates are given in Table II, measurements having been taken on two of them (21, 204) both before and after ignition. For comparison, the density values calculated from the water content and X-ray data, assuming that the water is present as a substitutional solid solution in which $(H_2O)_3$ groups replace barium sulfate groups and leave "holes" upon their removal, are given in the third column (Substit.). The densities calculated on the assumption of interstitial solid solution, where the water would occupy the interstices of the lattice without displacing either barium or sulfate ions, are given in the fourth column (Interst.). Values for the densities calculated on the assumption of a mechanical mixture of barium sulfate and water, where the space occupied by the water is accessible to the hydrostatic liquid after the water is removed, are given in the fifth column (Mech.).

Summary

1. X-Ray measurements show that the variable water of hydration of barium sulfate precipitated from aqueous solution is present as a solid solution.

2. Density measurements on the precipitates indicate that the solid solution formed is complex substitutional, a group of three water molecules replacing one barium sulfate group in the lattice.

3. The removal of the water from these hydrated precipitates leaves cavities to which the hydrostatic liquid does not have access. This indicates formation of a stable "subtraction" lattice.

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(16) G. L. Clark, "Applied X-Rays," McGraw-Hill Book Co., New York, N. Y., 1932, p. 289.

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE]

The Measurement and Calculation of Hydrogen Ion Concentrations in Saline Acetate and Phosphate Buffers[1]

BY DAVID I. HITCHCOCK AND ROZANNE PETERS[2]

In spite of recent revisions of the *p*H scale[3,4,5] it is still true that *p*H is an arbitrarily defined quantity for solutions in general. The *p*H values of the widely used phosphate and acetate buffers, as shown by Brönsted[6] and by Cohn,[7] cannot be calculated from mass action equations, even when the ionic strength is kept constant, without the introduction of variable activity coefficients. It was pointed out by Guggenheim[8] that either C_H or $C_H f_{\pm}^2$ would be a more useful measure of acidity than $C_H f_H$, even if the latter could be measured with certainty, because one of the former quantities is often the determining factor in an acid-base equilibrium.

The object of the present work was to find a series of buffers, of ionic strength comparable to that of physiological fluids, having hydrogen ion concentrations which could be measured by means of a cell without liquid junction and calculated by simple mass law equations without the introduction of activity coefficients. These conditions were fulfilled by acetate and phosphate buffers, 0.025

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(1) A brief report of this work was presented before the American Society of Biological Chemists and published in *Federation Proc.*, **1**, 115 (1942).

(2) Aided by a grant from the Fluid Research Funds of the Yale University School of Medicine, 1940–1941.

(3) MacInnes, Belcher and Shedlovsky, THIS JOURNAL, **60**, 1094 (1938).

(4) Hitchcock and Taylor, *ibid.*, **59**, 1812 (1937); **60**, 2710 (1938).

(5) Hamer and Acree, *J. Research Natl. Bur. Standards*, **23**, 647 (1939); Hamer, Pinching and Acree, *ibid.*, **36**, 47 (1946).

(6) Brönsted, *J. Chem. Soc.*, **119**, 574 (1921).

(7) Cohn, THIS JOURNAL, **49**, 173 (1927); Cohn, Heyroth and Menkin, *ibid.*, **50**, 696 (1928).

(8) Guggenheim, *J. Phys. Chem.*, **34**, 1758 (1930).

M, in the presence of enough sodium chloride to keep the ionic strength constant at 0.16.

Experimental

Solutions.—Sodium acetate, disodium phosphate and sodium chloride were recrystallized. The acetate was dried in a vacuum oven at 50° and in air at 110°. The phosphate was air-dried to the dihydrate, and the chloride was ground and then dried at 110°. The purity of the acetate and chloride was confirmed by quantitative conversion to the sulfate, and that of the phosphate by ignition to the pyrophosphate. Hydrochloric acid was distilled, diluted and standardized against sodium carbonate and borax. The buffer solutions were made up by volume at 21–23°. In each case 100 ml. contained 25 ml. of 0.1 *M* sodium acetate or phosphate, with 2.5 to 22.5 ml. of 0.1 *M* hydrochloric acid and enough sodium chloride to bring the ionic strength to 0.16.

Apparatus.—Silver–silver chloride electrodes were prepared by electroplating according to Brown.[9] It was possible to use a single electrode in many different solutions without replating. Glass electrodes of the bulb type were tested in the buffer solutions used by Hitchcock and Taylor[4]; a glass electrode was considered satisfactory if its response to changes in *p*H, between 2 and 7, was parallel within 0.2 mv. to that of a hydrogen electrode. Both electrodes were supported in a water-jacketed vessel similar to that used in the previous work.[4] The temperature of the cell was kept within 0.05° of 25 or 38° by water which flowed through the jacket from a regulated bath. Electromotive force was measured to 0.1 mv. by a Type K potentiometer, Type R galvanometer and thermionic amplifier (Leeds and Northrup). The galvanometer deflection was 4 mm. for 0.1 mv. Electrical stability was obtained by placing the potentiometer and amplifier on a grounded copper sheet, enclosing the electrode vessel in a grounded metal box, grounding the water in the glass bath, and putting a condenser and resistor in series across the terminals of the thermoregulator.[10]

Procedure.—At the beginning and end of each set of measurements, the cell was filled with a standard solution, 0.01 *N* hydrochloric acid in 0.15 *N* sodium chloride. The electromotive force was measured at five-minute intervals until it was constant to 0.1 mv., the cell was then refilled, and the measurements were repeated. Before filling the cell with a new solution it was rinsed at least three times. The measurements were reproducible to about 0.3 mv.

Results

The results are reported as the difference between the electromotive forces obtained when the same electrodes were bathed by the unknown and standard solutions. This difference, *E* volts, is the electromotive force of a concentration cell without transference:

Glass electrode, buffer + NaCl, AgCl, Ag—
Ag, AgCl, HCl (0.01 *N*) + NaCl (0.15 *N*), glass electrode

Values of C_H were calculated by means of the equation

$$E = k \log (0.0016/C_H C_{Cl}) \qquad (1)$$

in which *k* was 0.05915 for 25° and 0.06173 for 38°. The value of C_{Cl} was taken as the total chloride concentration in the saline buffer solution. As a test of this method of measuring C_H, the standard solution was compared at 25° with a few other solutions of hydrochloric acid and sodium chloride of the same ionic strength. The results are shown in Table I; the greatest divergence between the observed and calculated values is 0.2 mv., or 0.003 in log C_H.

TABLE I

MEASUREMENT OF C_H IN HYDROCHLORIC ACID–SODIUM CHLORIDE SOLUTIONS AT 25°

C_{HCl}	C_{NaCl}	*E*, volts Obs.	*E*, volts Calcd.
0.002	0.158	0.0412	0.0413
.005	.155	.0179	.0178
.020	.140	− .0176	− .0178

The results obtained with the buffer solutions are given in Tables II and III. The values of $-\log C_H$ were obtained from those of *E* and C_{Cl} by means of equation (1). Values of K_c were calcu-

TABLE II

IONIZATION IN SALINE ACETATE BUFFERS

Total acetate = 0.0250 = C_1; total acid = C_2; total chloride = 0.1350 + C_2 = C_{Cl}.

10^3C_2	*E*	$-\log C_H$	$-\log K_c$
	Part I, 25°		
2.50	0.2092	5.471	4.516
5.00	.1877	5.116	4.513
7.50	.1733	4.879	4.510
12.50	.1508	4.515	4.513
17.50	.1285	4.152	4.514
20.00	.1148	3.928	4.517
22.50	.0957	3.611	4.520
			Av. 4.515
	Part II, 38°		
5.00	0.1969	5.133	4.530
12.50	.1584	4.531	4.529
20.00	.1206	3.939	4.529
			Av. 4.529

TABLE III

IONIZATION IN SALINE PHOSPHATE BUFFERS

Total phosphate = 0.0250 = C_1; total acid = C_2; total chloride = 0.0850 + 2 C_2

10^3C_2	*E*	$-\log C_H$	$-\log K_c$
	Part I, 25°		
2.51	0.3450	7.583	6.631
5.02	.3232	7.238	6.638
7.53	.3083	7.008	6.642
12.55	.2839	6.638	6.641
17.57	.2593	6.259	6.633
20.08	.2444	6.025	6.636
22.59	.2217	5.658	6.630
			Av. 6.636
	Part II, 38°		
5.00	0.3368	7.230	6.628
12.50	.2954	6.622	6.622
20.00	.2546	6.017	6.619
			Av. 6.623

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(9) Brown, THIS JOURNAL, **56**, 646 (1934).

(10) We are glad to acknowledge the help of Dr. L. F. Nims, Mr. Delafield DuBois and Dr. R. A. Robinson in planning the electrical connections, and of Dr. B. B. Owen in checking the calibration of the standard cell.

lated from the concentrations by means of the equation

$$K_c = \frac{C_H(C_1 - C_2 + C_H)}{C_2 - C_H} \qquad (2)$$

Here C_1 is the total buffer concentration and C_2 is the total acid concentration. In the case of the phosphate mixtures, K_c was taken as $C_H(C_1 - C_2)/C_2$. The buffer ratio, $(C_1 - C_2)/C_2$, was varied from 9:1 to 1:9 in the 25° series and from 4:1 to 1:4 in the 38° series. Inspection of the last column in Tables II and III shows no regular trend of K_c with changing buffer ratio, except possibly for the phosphates at 38°. The greatest average deviation from the mean of log K_c is ± 0.004; this is within the estimated error in E.

Discussion

The validity of our values for C_H and K_c depends on the assumption that the mean activity coefficient of hydrochloric acid is the same in the standard and unknown solutions. Such an assumption was not made by Harned and Hickey[11] in their study of the ionization of unbuffered acetic acid in salt solutions. Their k_A is equivalent to our K_c at infinite dilution of the buffer in a neutral salt solution. From their data, as tabulated by Harned and Owen,[12] we find by interpolation that the negative logarithm of k_A for acetic acid at infinite dilution in 0.16 M sodium chloride solution at 25° is 4.524. The small difference between this value and 4.515, our figure for $-\log K_c$ in the saline acetate buffers, makes it probable that our figures for C_H and K_c are not in error by more than 2%.

Brönsted[6,13] showed that ionic equilibria can be accurately described by simple mass law equations in terms of concentrations, provided that the reaction in question is allowed to take place in solutions containing a constant and sufficiently great excess of other ions. This principle was applied to acid–base equilibria by Güntelberg and Schiödt,[14] Kilpatrick,[15] and others, but it has not been generally utilized for the preparation of buffer solutions. In a recent contribution, Brodersen[16] emphasized the value of the Brönsted principle in the selection of buffers. He found, by measurements with a ferro–ferricyanide electrode, that phosphate buffers of constant concentration gave a salt effect which varied in one direction with changes in the buffer ratio, while buffers of constant ionic strength (0.1) produced a similar trend in the opposite direction. These variations were abolished by a 1 to 10 dilution of the latter buffers with 0.1 M sodium chloride solution. Our results lend support to his recommendation that a diluted buffer in a relatively concentrated salt solution should be used for experiments in which the main variable is to be the hydrogen ion concentration.

Summary

Buffer solutions of 0.025 M sodium acetate or phosphate, with the acidity varied by the addition of 0.1 to 0.9 as much hydrochloric acid, were given a constant ionic strength of 0.16 by the addition of sodium chloride. Hydrogen ion concentrations at 25 and 38° were determined by means of a glass electrode in a cell without liquid junction. Dissociation constants, from which these concentrations can be exactly calculated without the use of activity coefficients, were obtained. The two sets of buffers covered the range of hydrogen ion concentrations between 2.5×10^{-4} and 2.5×10^{-8}.

NEW HAVEN, CONN. RECEIVED[17] JUNE 11, 1946

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(11) Harned and Hickey, THIS JOURNAL, **59**, 1284 (1937).

(12) Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Corp., New York, 1943, p. 523.

(13) Brönsted, *Trans. Faraday Soc.*, **23**, 416 (1927).

(14) Güntelberg and Schiödt, *Z. physik. Chem.*, **135**, 393 (1928).

(15) Kilpatrick, *Chem. Rev.*, **16**, 57 (1935).

(16) Brodersen, *Acta Physiol. Scand.*, **7**, 162 (1944).

(17) Original manuscript received April 21, 1942.

[CONTRIBUTION NO. 487 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

Heats of Dilution of Aqueous Solutions of α- and β-Alanine at 25°[1,2]

BY H. A. BENESI,[3] L. S. MASON AND A. L. ROBINSON

The properties of amino acids in solutions are in the process of extensive study. The monograph by Cohn and Edsall[4] contains a review of a considerable part of these investigations. Scatchard,[5] Kirkwood[6] and Fuoss[7] have examined the properties of dipolar molecules in solution theoretically.

The following results are presented in continuation[8] of a projected study of the heats of dilution of some of the simpler amino acids.

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[2] The financial assistance of the Buhl Foundation in support of this investigation is gratefully acknowledged.

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[4] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publishing Corp., New York, N. Y., 1943.

(5) G. Scatchard and J. G. Kirkwood, *Physik. Z.*, **33**, 297 (1932).

(6) J. G. Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

(7) R. M. Fuoss, THIS JOURNAL, **58**, 982 (1936).

(8) W. E. Wallace, W. L. Offutt and A. L. Robinson, *ibid.*, **65**, 347 (1943).