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Enthalpy and Heat Capacity Changes for the Proton Dissociation of Various Buffer Components in 0.1 M Potassium Chloride

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ABSTRACT **Enthalpy and heat capacity changes for the deprotonation of 18 buffers were calorimetrically determined in 0.1 M potassium chloride at temperatures ranging from 5 to 45°C. The values of the dissociation constant were also determined by means of potentiometric titration. The enthalpy changes for the deprotonation of buffers, except for the phosphate and glycerol 2-phosphate buffers, were found to be characterized by a linear function of temperature. The enthalpy changes for the second dissociation of phosphate and glycerol 2-phosphate where divalent anion is formed on dissociation were fitted with the second order function of temperature rather than the first order. Temperature dependence of buffer pH calculated by using the enthalpy and heat capacity changes obtained was in good agreement with the temperature variation of the pH values actually measured in the temperature range between 0 and 50°C for all the buffers studied. On the basis of the results obtained, a numeric table showing the temperature dependence of p*K* values for the 18 buffers is presented. Proteins 33:159–166, 1998.** © 1998 Wiley-Liss, Inc.

Key words: calorimetry; proton dissociation; enthalpy; heat capacity; buffer

INTRODUCTION

Biochemical experiments are usually carried out in buffer solutions, because most biological and biochemical processes (including thermally and chemically induced conformational transitions of biopolymers) are accompanied by proton release or uptake. Ever since Good et al.[1] introduced a number of buffer substances compatible with most media for biochemical and physiological interests, they have been widely used in biochemical experiments, including cellular level phenomena, under the name "Good's buffers." Most of the calorimetric investigations conducted to determine basic thermodynamic quantities of biochemical systems are also performed in buffered solutions. It will be clear that in calorimetric measurements of a reaction system in which the proton release or uptake take place simultaneously, a sum of the enthalpy changes due to the reaction and to the change in the protonation state in the buffer components is measured. For example, protein-ligand interactions are generally expressed by the following scheme:

$$\mathrm{P} + \mathrm{L} + n\mathrm{H}^+ \rightleftharpoons \mathrm{PL}(n\mathrm{H}^+);\ \Delta H \qquad (1)$$

where P and L denote protein and ligand, respectively, and n represents the number of protons taken up during the process of complex formation. If the reaction is conducted in a buffered solution, the protons to be taken up are released from the buffer component as expressed by the following scheme:

$$\mathrm{BH}^+ \rightleftharpoons \mathrm{B} + \mathrm{H}^+;\ \Delta H_\mathrm{i} \qquad (2)$$

where B denotes the buffer component.

If we define the net enthalpy change of complex formation to be ΔH and the enthalpy change associated with the proton release from the buffer to be ΔH_i, then in an actual calorimetric measurement the observed enthalpy change of reaction ΔH_obs is given as the sum of the above two contributions as expressed by the following equation:[2,3]

$$\Delta H_\mathrm{obs} = \Delta H + n\,\Delta H_\mathrm{i}\,. \qquad (3)$$

This means that to correctly determine the net enthalpy change ΔH, contribution from the buffer side on the observed heat effects must be subtracted

Abbreviations: ACES, *N*-(2-acetamido)-2-aminoethanesulfonic acid; BES, *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine; CAPS, 3-cyclohexylamino-1-propanesulfonic acid; EPPS, *N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); TAPS, *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

Dedicated to Professor Julian M. Sturtevant of Yale Univeristy, New Haven for his 90th birthday.

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from the observed enthalpy change. To attain the appropriate correction for the heat effect from the buffer component, both the values of n and ΔH_i must be known. In practice, the value of n is often determined by a pH-stat method and ΔH_i is calorimetrically determined under a separate measurement.[4] A more convenient method is to perform the calorimetric measurement with two or more buffer solutions at a definite pH.[2,3,5] In this case, the observed enthalpy changes ΔH_{obs} are plotted against the buffer deprotonation heats ΔH_i, which are determined by separate calorimetric measurements, using the relation given by equation 3. From the plot the values of n and the net enthalpy change ΔH are given by the slope and the intercept on ΔH_{obs} axis. It has been shown that the latter method is more advantageous than others (such as a pH-stat method) in some cases.[5]

Another important aspect of the thermodynamic properties of the buffer ionization state is that they affect the temperature variation of the buffer pH. For example, it may be easily shown by a simple pH measurement that the pH of Tris buffer adjusted at 25°C varies with approximateley −0.7 units when it is heated up to 50°C.[6–8] This effect is obviously due to the large enthalpy change of deprotonation in Tris buffer. This fact indicates that when one studies the thermal denaturation of a protein in a buffered pH to quantitatively discuss its thermodynamic stability, care must be taken how much the pH of the sample solution changes with increasing temperature. It would be unnecessary to mention that such a change in buffer pH with temperature is quantitatively described by a basic principle of physical chemistry using the values of enthalpy and heat capacity changes of the protonation/deprotonation process of the buffer components.

In the present study, we determined the enthalpy and heat capacity changes for the proton dissociation of 18 buffers that are widely used in biochemistry research experiments, i.e., Good's buffers (PIPES, HEPES, EPPS, MES, MOPS, ACES, BES, TES, Tricine, Bicine, TAPS, and CAPS), imidazole, triethanolamine, acetic acid, cacodylic acid, glycerol 2-phosphate, and phosphate. As for the phosphate and glycerol 2-phosphate buffers, because of their common use only the second dissociation—the pK values of which are around 7 and 6, respectively, at room temperature—was studied in this work.

MATERIALS AND METHODS

Materials

Disodium glycerol 2-phosphate was purchased from Merck, Darmstadt, Germany. Good's buffers studied were PIPES, HEPES, EPPS, MES, MOPS, ACES, BES, TES, Tricine, Bicine, TAPS, and CAPS. They were the products of Dojindo Laboratories, Kumamoto, Japan. Other chemicals were from Wako Pure Chemicals, Osaka, Japan. All reagents were of reagent grade and used without further purification. Certified 1 N or 0.1 N hydrochloric acid and potassium hydroxide solutions (also purchased from Wako Pure Chemicals) were diluted immediately before measurements. Doubly distilled and deionized water was used for the preparation of the solutions.

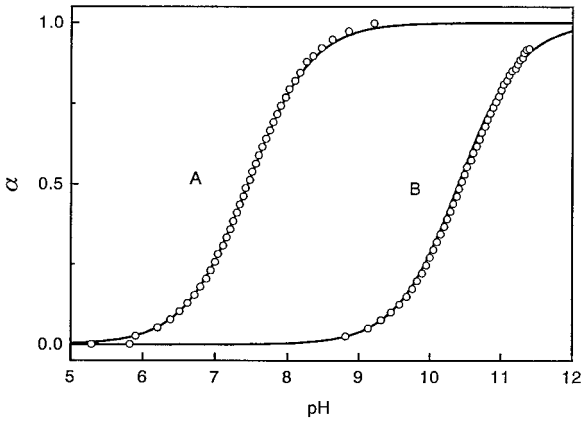

Fig. 1. Fractional deprotonation α as a function of pH obtained by the pH titration for the 0.01 M HEPES (**A**) and CAPS (**B**) buffers containing 0.1 M KCl at 25°C. The open circles are the data points actually observed, and the solid lines are the calculated curves. pK = 7.45 for HEPES, and pK = 10.39 for CAPS.

Calorimetry

Heats of protonation were determined by using a flow calorimeter with gold-tubing cell[4] at various temperatures ranging from 5 to 50°C. Although in principle the measurement is also possible with the deprotonation process, it was not used in the present study because a correction for a large heat effect due to the water formation would be required. Such a procedure introduces relatively large errors. The buffer solutions prepared at a concentration of 0.02 M and the 0.006 M HCl solution, both containing 0.1 M potassium chloride (KCl), were sent to the calorimetric cell through teflon-tubings for 1 or 2 min at a flow rate of 0.137 ml min^{-1} and were mixed in the gold-made mixing joint in the calorimetric unit; thus, the buffer concentration after mixing was diluted to 0.01 M. The buffer solutions for acidic substances were prepared with KOH solution so that the final concentration with respect to KOH was 0.013 M, while those for basic substances were prepared with HCl solution so that the final concentration with respect to HCl was 0.006 M. With these procedures, the pH of the prepared solutions was higher than the pK of each buffer by approximately +0.25 units, irrespective of the type of buffers. When the buffers prepared were mixed with an equal volume of 0.006 M HCl, their pH was lowered by about 0.50 in pH unit, the final pH thus being approximately equal with pK −0.25.

TABLE I. Entalpy and Heat Capacity Changes for the Dissociation of Protonated Buffer Substances in 0.1 M KCl at 25° C

Buffer substances	p*K**	ΔH (kJ mol^{-1})	ΔC_p (J K^{-1} mol^{-1})	$\partial \Delta C_p / \partial T$ (10^{-3} J K^{-2} mol^{-1})
Acetate	4.62	0.49 ± 0.02	−128 ± 2	—
MES	6.07	15.53 ± 0.03	16 ± 2	—
Cacodylate	6.14	−1.96 ± 0.02	−78 ± 2	—
Glycerol 2-phosphate	6.26	−0.72 ± 0.02	−179 ± 2	0.79 ± 0.39
PIPES	6.71	11.45 ± 0.04	19 ± 4	—
ACES	6.75	31.41 ± 0.05	−27 ± 4	—
Phosphate	6.81	5.12 ± 0.03	−187 ± 3	2.01 ± 0.22
BES	7.06	25.17 ± 0.07	2 ± 5	—
MOPS	7.09	21.82 ± 0.03	39 ± 3	—
Imidazole	7.09	36.59 ± 0.06	−16 ± 5	—
TES	7.42	32.74 ± 0.03	−33 ± 3	—
HEPES	7.45	21.01 ± 0.07	49 ± 5	—
EPPS	7.87	21.55 ± 0.05	56 ± 4	—
Triethaolamine	7.88	33.59 ± 0.04	48 ± 3	—
Tricine	8.00	31.97 ± 0.05	−45 ± 4	—
Bicine	8.22	27.05 ± 0.05	2 ± 4	—
TAPS	8.38	41.49 ± 0.06	23 ± 5	—
CAPS	10.39	48.54 ± 0.07	29 ± 6	—

*Standard error is within ±0.01.

Corrections were made for the dilution heats of buffer and of HCl and for viscous heating in a manner described previously.[4] These amounts of heat were measured separately for each run of the calorimetric measurements. The dilution heats were usually very small under the studied conditions, being less than 1% of the total observed heats. Viscous heating was also found to be almost zero at the flow rate used in the measurements. The calorimeter was calibrated at each temperature of the measurements by using the neutralization heat of HCl with NaOH reported by Grenthe et al.[9] Details of the calorimeter structure and its operation have been described elsewhere.[4] In practical calorimetric measurements, the pHs of the buffers after mixing with 0.1 M KCl alone and with 0.006 M HCl solution containing 0.1 M KCl were checked at a given temperature to determine the difference in the buffer protonation states before and after reaction with HCl. Under the conditions used, the majority of the protons added in the form of HCl was found to be taken up by the buffer components (the process of protonation). Some protons also reacted with hydroxyl ions to form water or remained unreacted as free ions. However, the fractions of these two were found to be negligibly small, being less than 0.1% of the total added protons, at neutral pH region. For example, the pH lowering of 0.01 M HEPES buffer was only 0.52 (from pH 7.72 to 7.20) at 25°C when it was mixed with an equal volume of 0.006 M HCl. In this case, it was estimated that 99.99% of the protons added were bound to HEPES, and the calculated heat effect due to the water formation amounted to only 0.034% of the observed heat of reaction. The situation was slightly different in the alkaline region; the observed heat had to be corrected for the secondary heat effect for some buffers, usable in the pH range above 9. For example, the pH of CAPS buffer changed from 10.51 to 10.02 when mixed with the HCl solution in the calorimeter. In this case, the fraction of protons bound to the buffer component was estimated to be 92.80% of the total added protons, the corresponding heat of water formation amounting to 8.3% of the total observed heat. Thus, the heat effect due to water formation with CAPS was larger than that with any of the other buffers. Despite this situation, the enthalpy change of protonation was determined on the basis of the bound protons after correction for the heat effect due to water formation[9] for all buffers studied.

pH Measurements

A Hitachi-Horiba M-7 pH meter (Horiba, Kyoto, Japan) equipped with a Metrohm glass electrode (Herisan, Switzerland) was used for pH measurements. Half-protonated buffer solutions containing 0.1 M KCl were prepared and placed in the thermostated bath for the pH measurements at various temperatures from 0 to 50°C. The pH meter was calibrated at each temperature of measurements using the standard buffers.

Potentiometric Titration

To determine the dissociation constant p*K*, potentiometric titration was conducted with the above pH meter with a Metrohm electrode. Twenty milliliters of 0.01 M buffer component containing 0.1 M KCl was titrated with certified 0.2 M KOH or HCl by means of a microburet at 25°C under a nitrogen atmospheric condition. The titrations were per-

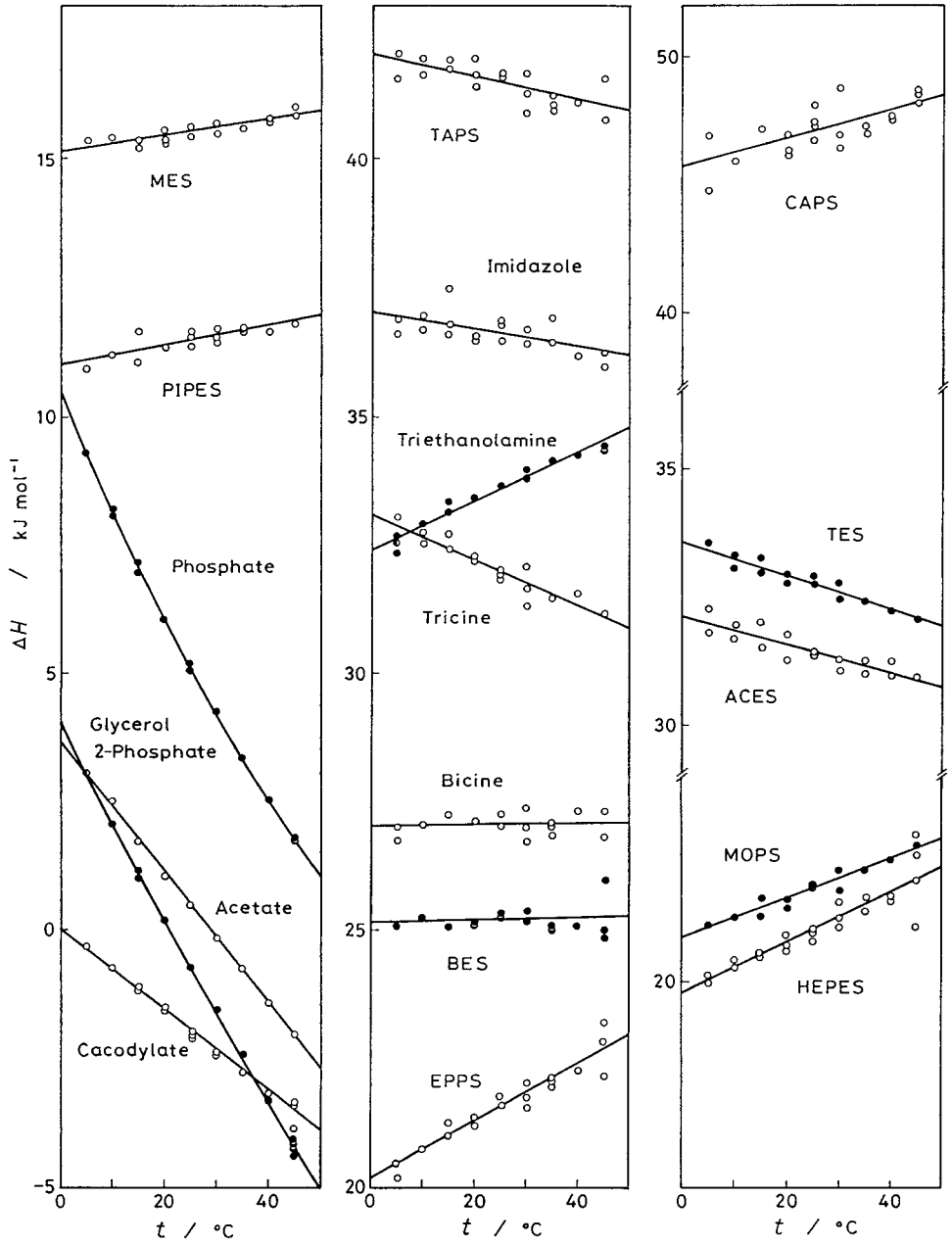

Fig. 2. The enthalpy change for the deprotonation of various buffers as a function of temperature in 0.1 M KCl. The solid lines were drawn by the least-squares method.

formed at least three times to obtain the average values of p*K* for all the buffers studied.

RESULTS AND DISCUSSION

An example of the result of potentiometric titration is shown in Figure 1, in which the fractional deprotonation of buffer α (the fraction of species B in equation 2) is plotted against the pH of the buffer titrated. The open circles are the experimentally determined data points and the solid line is the calculated curve obtained by using the determined dissociation constant p*K*. The values of p*K* obtained for the 18 buffers are summarized in the second column of Table I.

In Figure 2, the enthalpy changes for the deprotonation calorimetrically determined for the 18 buffer components in 0.1 M KCl are plotted against temperature of the measurements. Solid lines (except for those phosphate and glycero 2-phosphate buffers) are drawn on the basis of the assumption that the enthalpy change is simply given by a linear function of temperature, thereby the slope gives the heat

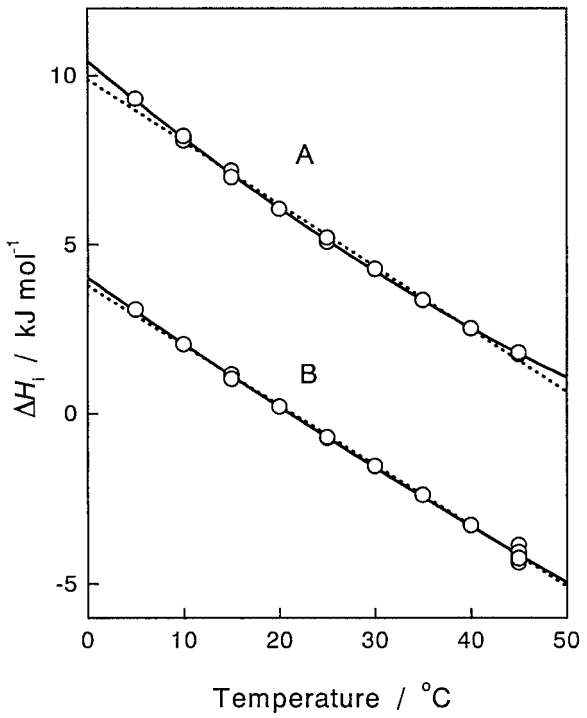

Fig. 3. Comparison of curves fitted by the linear (dotted lines) and second-order (solid lines) functions on the temperature dependence of the enthalpy change for the second dissociation of phosphate (**A**) and of glycerol 2-phosphate (**B**).

capacity change of the proton dissociation process ΔC_p. The enthalpy and heat capacity changes at 25°C obtained by the regression analysis are summarized, respectively, in the third and fourth columns of Table I. The temperature dependence of the enthalpy changes for the phosphate and glycerol 2-phosphate buffers were found to exhibit a slightly downward curvature rather than a straight line. Consequently, they were fitted with a second-order function, and the fitted curves are shown in Figure 3. The temperature coefficients of ΔC_p thus determined for the two buffers are shown in the last column of Table I, together with the values of ΔH and ΔC_p at 25°C determined by a procedure based on the fitting to the second-order function.

It can be known from Table I that relatively large heat capacity changes were observed with the buffer deprotonations where charge separation takes place. The enthalpy of deprotonation of some buffers had been determined calorimetrically at 25°C by Beres and Sturtevant,[10] ΔH/kJ mol^{-1} = 11.46 (PIPES) and 20.96 (HEPES), and by Roig et al.,[11] ΔH/kJ mol^{-1} and ΔC_p/J K^{-1} mol^{-1} = 12.11 and 33 (PIPES), 21.68 and 66 (HEPES), and 25.78 and 4 (BES), respectively. Imidazole deprotonation was reported to be 35.69 ± 0.59 kJ mol^{-1} from titration calorimetry by Marini et al.[12] Bates et al.[13–16] performed electromotive force (emf) measurements of Good's buffers at various temperatures to determine the thermodynamic quantities of proton dissociation. The same measurements have been applied to PIPES[17] and glycerol 2-phosphate.[18] The results obtained in the present study are in good agreement with these previously reported values.

The temperature dependence of the equilibrium constant is given by the van't Hoff equation,

$$\partial \ln K/\partial T = \Delta H/RT^2 \quad (4)$$

where R and T are the gas constant and the absolute temperature, respectively.

The enthalpy change varies with temperature according to the Kirchhoff equation,

$$\partial \Delta H/\partial T = \Delta C_p \quad (5)$$

When the heat capacity change ΔC_p is independent of temperature, the enthalpy change is given by a simple linear function of temperature as expressed in the form,

$$\Delta H = \Delta H(T_0) + \Delta C_p(T - T_0) \quad (6)$$

where $\Delta H(T_0)$ is ΔH at temperature T_0.

If ΔC_p varies with temperature and is assumed to have a constant temperature coefficient, i.e., $\partial \Delta C_p/\partial T$ is constant, then the heat capacity change at a given temperature T is

$$\Delta C_p = \Delta C_p(T_0) + (\partial \Delta C_p/\partial T)(T - T_0) \quad (7)$$

From equations 6 and 7, the enthalpy change is given by the equation,

$$\Delta H = \Delta H(T_0) + \Delta C_p(T_0)(T - T_0) + (\partial \Delta C_p/2\partial T)(T - T_0)^2 \quad (8)$$

where $\Delta C_p(T_0)$ is ΔC_p at T_0.

Using these relations, the dissociation constant pK is described as a function of temperature by the equation,

$$\mathrm{p}K = \mathrm{p}K(T_0) + [\{(\Delta H(T_0) - \Delta C_p(T_0)T_0 + (\partial \Delta C_p/2\partial T)T_0^2\}(1/T - 1/T_0) - \{\Delta C_p(T_0) - (\partial \Delta C_p/\partial T)T_0\} \cdot \ln(T/T_0) - (\partial \Delta C_p/\partial T)(T - T_0)]/2.303R \quad (9)$$

where p$K(T_0)$ is pK at T_0.

Of course, the temperature variation of the buffer is also expressed by the same relation. The pH measurements of buffers were made at various temperatures ranging from 0 to 50°C. The results obtained for 18 buffers are shown in Figure 4. The solid lines are the curves calculated on the basis of equation 9 with the values of $\Delta H(T_0)$, $\Delta C_p(T_0)$ and

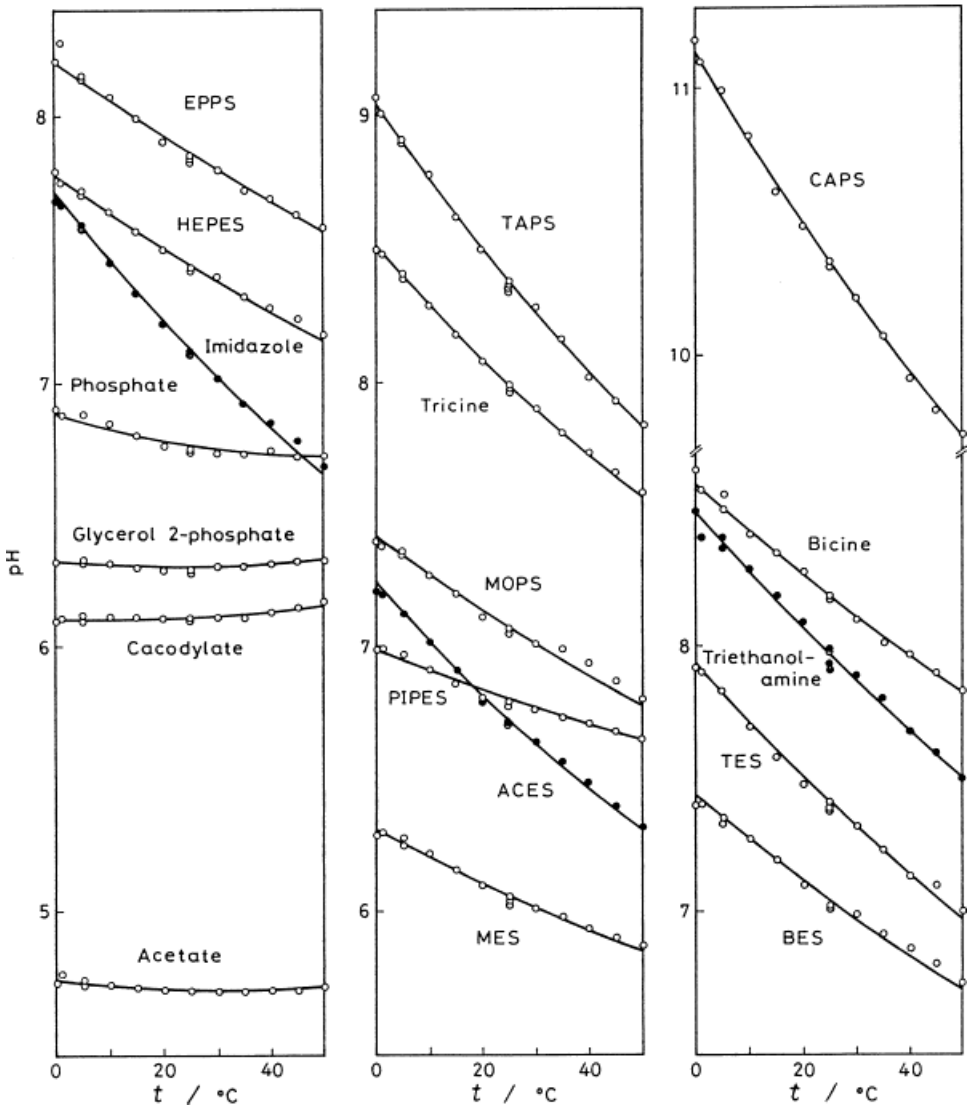

Fig. 4. Temperature dependence of buffer pHs in 0.1 M KCl. The filled and open circles are the experimental values, and the solid lines are the calculated curves according to van't Hoff equation (equation 9) using the thermodynamic parameters given in Table I.

$\partial\Delta C_p/\partial T$ given in Table I. With the exception of phosphate and glycerol 2-phosphate, the values of $\partial\Delta C_p/\partial T$ for all buffers were assumed to be zero as understood from the results given in Figure 2. From Figure 4 it is apparent that all the calculated curves are in good agreement with the experimental data determined by the direct pH measurements. These results obviously indicate that the thermodynamic quantities determined by calorimetric measurements in the present study are fully reliable and provide quantitative knowledge about the temperature dependence of the buffer pH. On the basis of the results obtained, numeric values showing the temperature variation in the pK values of the 18 buffers studied were calculated and are given in Table II.

It is evident from Table II that the buffers with the deprotonation enthalpy smaller than 10 kJ mol^{-1} show small pH changes with temperature, whereas in the case of buffers with ΔH as large as 30 kJ mol^{-1} their pH changes are about 1 unit in the temperature interval of ΔT = 50 K. This fact indicates that one should be very careful in choosing the appropriate buffer if the biochemical substances under study are very sensitive to the medium pH.

We believe that the numeric data given in Table II will be useful not only for studies based on isothermal and scanning calorimetry, but also for a wide area of biochemical research on cellular constituents that are sensitive to the hydrogen ion concentration in the medium.

TABLE II. Temperature Dependence of pK/pH Values of Various Buffers

t /°C	Acetate	MES	Cacodylate	GPh	PIPES	ACES	Phosphate	BES	MOPS	Imidazole	TES	HEPES	EPPS	Tea	Tricine	Bicine	TAPS	CAPS
0	4.65	6.32	6.12	6.29	6.89	7.26	6.94	7.46	7.43	7.68	7.95	7.78	8.20	8.41	8.52	8.64	9.15	11.16
5	4.64	6.26	6.13	6.28	6.85	7.15	6.90	7.38	7.36	7.55	7.84	7.71	8.13	8.30	8.41	8.55	8.99	11.00
10	4.63	6.22	6.13	6.27	6.81	7.04	6.87	7.29	7.29	7.43	7.73	7.64	8.07	8.19	8.30	8.46	8.83	10.84
15	4.63	6.16	6.13	6.26	6.78	6.94	6.85	7.21	7.22	7.31	7.62	7.58	8.00	8.08	8.20	8.37	8.67	10.68
20	4.62	6.12	6.13	6.26	6.74	6.84	6.83	7.14	7.15	7.20	7.52	7.51	7.93	7.98	8.10	8.29	8.52	10.53
25	4.62	6.07	6.14	6.26	6.71	6.75	6.81	7.06	7.09	7.09	7.42	7.45	7.87	7.88	8.00	8.21	8.38	10.39
30	4.62	6.03	6.15	6.26	6.68	6.66	6.80	6.99	7.03	6.98	7.33	7.39	7.81	7.78	7.91	8.13	8.24	10.25
35	4.62	5.98	6.15	6.27	6.64	6.57	6.78	6.92	6.96	6.88	7.23	7.33	7.75	7.69	7.82	8.06	8.10	10.11
40	4.62	5.94	6.16	6.28	6.61	6.49	6.77	6.85	6.90	6.78	7.15	7.27	7.69	7.60	7.73	7.98	7.97	9.98
45	4.63	5.90	6.17	6.29	6.58	6.41	6.77	6.78	6.85	6.69	7.06	7.21	7.63	7.50	7.65	7.91	7.84	9.85
50	4.63	5.86	6.18	6.30	6.55	6.33	6.77	6.72	6.79	6.60	6.98	7.16	7.57	7.42	7.57	7.84	7.72	9.73
55	4.64	5.82	6.19	6.31	6.52	6.25	6.76	6.66	6.73	6.51	6.90	7.10	7.51	7.33	7.50	7.78	7.60	9.61
60	4.65	5.78	6.20	6.33	6.49	6.18	6.76	6.60	6.68	6.42	6.83	7.05	7.46	7.25	7.43	7.71	7.48	9.49
65	4.66	5.74	6.21	6.34	6.47	6.11	6.77	6.54	6.62	6.34	6.75	7.00	7.40	7.16	7.36	7.65	7.37	9.37
70	4.67	5.71	6.22	6.36	6.44	6.04	6.77	6.48	6.57	6.26	6.68	6.94	7.35	7.08	7.29	7.59	7.25	9.26
75	4.68	5.67	6.24	6.38	6.41	5.98	6.78	6.43	6.52	6.18	6.62	6.89	7.29	7.01	7.22	7.53	7.15	9.15
80	4.70	5.63	6.25	6.40	6.38	5.91	6.79	6.37	6.47	6.10	6.55	6.84	7.24	6.93	7.16	7.47	7.04	9.05
85	4.71	5.60	6.26	6.42	6.36	5.85	6.80	6.32	6.42	6.03	6.49	6.79	7.19	6.85	7.10	7.41	6.94	8.94
90	4.73	5.57	6.28	6.45	6.33	5.79	6.81	6.27	6.37	5.96	6.42	6.74	7.14	6.78	7.04	7.36	6.84	8.84
95	4.74	5.54	6.29	6.47	6.31	5.73	6.83	6.22	6.32	5.89	6.37	6.70	7.09	6.71	6.98	7.31	6.74	8.74
100	4.76	5.50	6.30	6.50	6.28	5.68	6.84	6.17	6.27	5.82	6.31	6.65	7.04	6.64	6.93	7.26	6.64	8.65

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