

Ionization Enthalpies of Some Common Zwitterionic Hydrogen-Ion Buffers (HEPES, PIPES, HEPPS and BES) for Biological Research

Teresa Roig,[†] Per Bäckman* and Gerd Olofsson

Division of Thermochemistry, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

Roig, T., Bäckman, P. and Olofsson, G., 1993. Ionization Enthalpies of Some Common Zwitterionic Hydrogen-Ion Buffers (HEPES, PIPES, HEPPS and BES) for Biological Research. – Acta Chem. Scand. 47: 899–901.

The ionization enthalpy $\Delta_i H$ has been determined for *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES), *N*-(2-hydroxyethyl)piperazine-*N'*-(3-propanesulfonic acid) (HEPPS) and *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) at three temperatures (288.15, 298.15 and 310.15 K). For three of the buffers (HEPES, PIPES and BES), standard ionization enthalpies at infinite dilution were derived from the variation of $\Delta_i H$ with ionic strength at 298.15 K.

As many biological processes are accompanied by uptake or release of hydrogen ions, the reaction media used in *in vitro* experiments frequently contain a hydrogen-ion buffer to stabilize the pH. With the increased use of calorimetric methods in a multitude of biological research areas,¹ the availability of thermodynamically well characterized buffer systems suitable for biological studies has become a serious problem. It has long been realized that traditional buffers such as phosphate, borate and tris(hydroxymethyl)aminomethane (Tris) may have considerable chemical reactivity and other undesirable properties. However, the use of biologically more suitable buffers such as the 'Good' buffers,^{2–4} has been hampered by the lack of reliable thermodynamic data. We have therefore determined the ionization enthalpy $\Delta_i H$ for the following 'Good' buffers: *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES); piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES); *N*-(2-hydroxyethyl)piperazine-*N'*-(3-propanesulfonic acid) (HEPPS); and *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES). These buffers are all zwitterionic acids with pK_a values between 6.8 (PIPES) and 8.1 (HEPPS), and are well suited to biological work.

Experimental

Buffer substances (HEPES, PIPES, HEPPS and BES) of the highest quality were purchased from Sigma Chemical

Company (St. Louis, MO) and used without further purification. Dilute hydrochloric acid was prepared from concentrated titration standards (Titrisol[®], Merck, Darmstadt, Germany). All solutions used in this study were prepared and handled under an N₂ atmosphere in order to avoid contamination by CO₂. Reagent-grade water produced with a Milli-Q[®] filtration system was used throughout this study.

Enthalpies of protonation ($\Delta_p H = -\Delta_i H$) were determined using a four-channel isothermal heat-conduction microcalorimeter⁵ ('Thermal Activity Monitor', Thermo-Metric AB, Järfälla, Sweden) equipped with a stirred stainless steel titration vessel,⁶ capacity 1 ml. The vessel was charged with 0.9 ml buffer (20 mmol l⁻¹)[‡] in 0.15 M NaCl, (if not otherwise stated) at a pH adjusted to $pK_a + 0.30$ using 0.2 M NaOH (i.e., a third of the buffer being protonated at the start of the experiment). The titrant, 0.2 M hydrochloric acid, was injected into the vessel using a motor driven 100 μ l gas-tight Hamilton syringe equipped with a 24 carat gold capillary tube. The injection volume was 3 μ l and each experiment consisted of 10 consecutive injections at 5 min intervals. About a third of the buffer was protonated during a titration series. The output signal of the calorimeter was corrected for the thermal inertia of the vessel using the Tian equation with a time constant τ equal to 190 s, as described earlier.⁷ The time constant and the calibration constant were determined using an electrical heater inserted into the vessel.⁸

* To whom correspondence should be addressed.

[†] Permanent address: Unitat de Biofísica, Facultat d'Odontologia, Universitat de Barcelona. c/Feixa Llarga s/n, E-089 07 L'Hospitalet, Spain.

[‡] In the following, M is used to denote mol l⁻¹.

Table 1. Enthalpy and heat capacity changes for the ionization of some zwitterionic buffers.^a

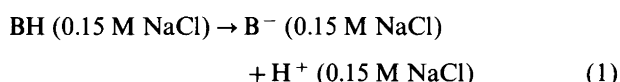
Buffer	$\Delta_i H_m / \text{kJ mol}^{-1b}$			$\Delta C_{p,m} / \text{J K}^{-1} \text{mol}^{-1}$
	288.15 K	298.15 K	310.15 K	
HEPES	21.08 ± 0.14	21.68 ± 0.08	22.52 ± 0.08	65.8 ± 6.2
PIPES	11.70 ± 0.06	12.11 ± 0.08	12.43 ± 0.04	33.0 ± 4.2
HEPPS	21.66 ± 0.09	22.45 ± 0.06	23.40 ± 0.12	79.2 ± 5.6
BES	25.67 ± 0.03	25.78 ± 0.08	25.76 ± 0.04	4.2 ± 5.3

^aAll values refer to the deprotonation of 20 mM buffer in 0.15 M NaCl. ^bValues are given as the mean of six independent series of experiments, with twice the standard error of the mean (SEM).

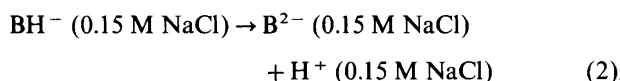
Enthalpies of dilution of 0.2 M hydrochloric acid were determined in separate experiments and corrected for when significant.

Results and discussion

Ionization enthalpies $\Delta_i H_m$ of the four buffers HEPES, PIPES, HEPPS and BES were determined from microcalorimetric titration measurements of hydrochloric acid additions to buffer solutions at three different temperatures, 288.15, 298.15 and 310.15 K. The results are summarized in Table 1. For HEPES, HEPPS and BES the given values refer to the process shown in eqn. (1),



where BH and B⁻ are the protonated and unprotonated forms, respectively, of the buffer at a total concentration of 0.020 M. For PIPES, which has two sulfonic acid groups, the corresponding process is shown in eqn. (2).



In all cases, the change in $\Delta_i H_m$ with temperature could be described with sufficient accuracy by a constant molar heat capacity change $\Delta C_{p,m}$, eqn. (3).

$$\Delta_i H_m(T_2) = \Delta_i H_m(T_1) + \Delta C_{p,m} \Delta T \quad (3)$$

As can be seen from Table 1, the observed values of $\Delta C_{p,m}$ are fairly small. It should be noted that eqn. (3)

Table 2. Variation in pK_a with temperature for some zwitterionic buffers.

Buffer	$pK_{a,293}^a$	$(dpK_a/dT)/K^{-1b}$
HEPES	7.55	-0.0127 (-0.014)
PIPES	6.8	-0.0071 (-0.0085)
HEPPS	8.1	-0.0132 (-0.015)
BES	7.15	-0.0151 (-0.016)

^a pK_a values are taken from Refs. 2 and 3. ^bThe variation in pK_a with temperature calculated as described in the text; errors (± 2 SEM) are in all cases $\leq 0.0001 \text{ K}^{-1}$. The figures in parentheses represent values published in the literature.^{2,3}

provides a means of deriving $\Delta_i H_m$ values valid for any temperature within the measured range. As the ionization enthalpies of these buffers are relatively temperature independent, reliable estimates of the variation of pK_a with temperature can be obtained using the van 't Hoff equation, eqn. (4).

$$dpK_a/dT = -\Delta_i H_m / (\ln 10 RT^2) \quad (4)$$

Values of dpK_a/dT calculated using the $\Delta_i H_m$ values at 298.15 K (Table 1) are listed in Table 2 together with values of pK_a and dpK_a/dT taken from the literature.^{2,3} As can be seen in Table 2, our slopes are consistently somewhat lower than previously reported values. The differences are probably not significant and may be due to differences in experimental conditions.

Although the enthalpy values listed in Table 1 are directly applicable to most biological and biochemical systems, they are not to be regarded as standard enthalpies of ionization. In general, molar enthalpy changes for reactions involving electrolytes can be expected to deviate significantly from the values at infinite dilution even at moderate concentrations owing to long-range electrostatic interactions. Therefore, the ionization enthalpies of HEPES, PIPES and BES were measured at 298.15 K in salt solutions of varying concentration (0, 0.10 and 0.15 M NaCl). As shown in Fig. 1, the measured $\Delta_i H_m$ values increased linearly with the square root of the

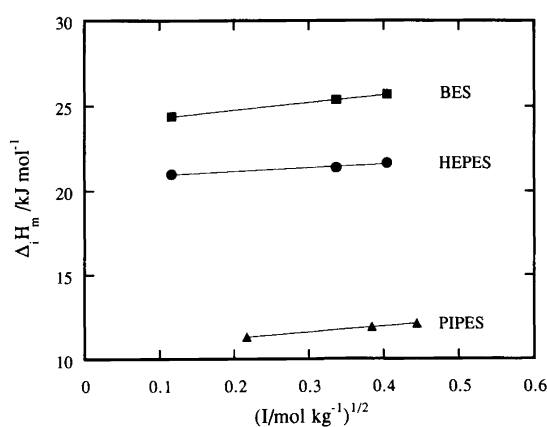


Fig. 1. The ionization enthalpies, $\Delta_i H$, of HEPES, PIPES AND BES as a function of $I^{1/2}$. Errors (± 2 SEM) are, in all cases, smaller than the size of the symbols.

Table 3. Changes in the ionization enthalpy with ionic strength for some zwitterionic buffers.

Buffer	$\Delta_i H_m / \text{kJ mol}^{-1}$ ^a			$\Delta_i H_m^\circ$ ^b kJ mol ⁻¹	$d\Delta_i H_m / dI^{1/2}$ ^c kJ mol ^{-3/2} kg ^{1/2}
	[NaCl]/M = 0	0.10	0.15		
HEPES	21.00 ± 0.08	21.41 ± 0.04	21.68 ± 0.08	20.7 ± 0.2	2.2 ± 0.6 (2.955)
PIPES	11.32 ± 0.05	11.91 ± 0.08	12.11 ± 0.08	10.6 ± 0.2	3.5 ± 0.6 (5.910)
BES	24.41 ± 0.13	25.39 ± 0.10	25.72 ± 0.08	23.9 ± 0.3	4.5 ± 0.8 (2.955)

^aValues refer to the deprotonation of 20 mM buffer in NaCl(aq) at 298.15 K and are given as the mean of six independent series of experiments with twice the standard error of the mean (SEM). ^bStandard ionization enthalpies calculated as described in the text. ^cThe variation of $\Delta_i H$ with $I^{1/2}$ as explained in the text; limiting law values are given in parentheses.

ionic strength [$I = 1/2 \sum (m_i z_i^2)$]. The values of $\Delta_i H_m$ obtained are listed in Table 3, together with the slopes $d(\Delta_i H_m)/dI^{1/2}$ evaluated using linear regression. The observed slopes provide a simple means of calculating enthalpies valid at varying ionic strengths. Included in this table are values for the standard molar enthalpies of ionization at infinite dilution $\Delta_i H_m^\circ$, derived by extrapolating the regression lines to zero ionic strength.

According to the Debye–Hückel model in its simplest form, the deviation of $\Delta_i H_m$ from the value at infinite dilution can be ascribed to electrostatic interactions according to eqn. (5) where ΔH_{el} is the enthalpy change

$$\Delta H_{el} = A_H / 2 \sum_i (v_i z_i^2) I^{1/2} \quad (5)$$

due to electrostatic interaction, A_H the Debye–Hückel limiting slope, v_i the stoichiometric number and z_i the ionic charge number. The theoretical value of A_H is 2955 J mol^{-3/2} kg^{1/2} at 298.15 K, as calculated by Clarke and Glew.¹⁰ The limiting slope of $\Delta_i H_m$ with $I^{1/2}$ is thus $2A_H$ for PIPES, where deprotonation results in the formation of a divalent ion, whereas it is equal to A_H for HEPES and BES [cf. eqns. (1) and (2)]. Owing to simplifying assumptions, relation (5) is valid only at ionic concentrations below about 10^{-3} M, and generally the behavior of electrolyte solutions differs significantly from the limiting law at higher concentrations. However, for the buffers studied, $\Delta_i H_m$ in fact varies linearly with $I^{1/2}$ and with a slope that is of the same order of magnitude as the limiting value (see Table 3).

To the best of our knowledge, calorimetrically determined values of $\Delta_i H_m$ have not been reported for BES

and HEPPS. Beres and Sturtevant¹¹ have determined, using flow calorimetry, the ionization enthalpies of the other two buffers at 298.15 K in 0.10 M NaCl. Their values of $\Delta_i H_m$ for HEPES and PIPES, 21.0 ± 1.1 and 11.5 ± 0.3 kJ mol⁻¹, are in good agreement with our values (Table 3).

Acknowledgments. This work was supported by grants from the Swedish National Board for Technical Development and *Fondo de Investigaciones Sanitarias* (FIS 92/552). T. R. was also the recipient of a Research Fellowship from *Ministerio de Asuntos Exteriores* of Spain.

References

- Lamprecht, L., Hemminger, W. and Höhne, G. W. H., Eds., *Calorimetry in the Biological Sciences*, *Thermochim. Acta* 193 (1991).
- Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S. and Singh, R. M. M. *Biochemistry* 5 (1966) 467.
- Good, N. E. and Izawa, S. *Methods Enzymol.* 24 (1972) 53.
- Ferguson, W. J. and Good, N. E. *Anal. Biochem.* 104 (1980) 300.
- Suurkuusk, J. and Wadsö, I. *Chem. Scr.* 20 (1982) 155.
- Görman, M., Laynez, J., Schön, A., Suurkuusk, J. and Wadsö, I. *J. Biochem. Biophys. Methods* 10 (1984) 187.
- Bastos, M., Hägg, S., Lönnbro, P. and Wadsö, I. *J. Biochem. Biophys. Methods* 23 (1991) 255.
- Briggner, L.-E. and Wadsö, I. *J. Biochem. Biophys. Methods* 22 (1991) 101.
- Pitzer, K. S. and Brewer, L. *Thermodynamics*, 2nd ed., McGraw-Hill, New York 1961, p. 339.
- Clarke, E. C. W. and Glew, D. N. *J. Chem. Soc., Faraday Trans. 1*, 76 (1980) 1911.
- Beres, L. and Sturtevant, J. M. *Biochemistry* 10 (1971) 2120.

Received December 18, 1992.