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Buffers for the Physiological pH Range: Acidic Dissociation Constants of Zwitterionic Compounds (ACES and CHES) in Water from 5 to 55 °C

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The precise electromotive force (emf) measurements of the cells without liquid junction, Pt|H_2|ACES (m_1) + NaACESate (m_2) + NaCl (m_3)|AgCl, Ag and Pt|H_2|CHES (m_1) + NaCHESate (m_2) + NaCl (m_3)|AgCl, Ag, have been made to collect emf data from (5 to 55) °C, with molalities $m_1 = m_2 = m_3$ in the range 0.01 to 0.1 mol·kg^{-1}. The hydrogen and silver + silver chloride electrodes were used. The second thermodynamic dissociation constants (pK_2) of *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) and 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES), along with the thermodynamic quantities associated with the ionization of ACES and CHES, have been calculated. The values of pK_2 for ACES are expressed by the equation pK_2 = 2548.60/(T/K) − 20.03159 + 3.2172 ln(T/K), and for CHES, by pK_2 = 1917.66/(T/K) + 5.7940 − 0.4971 ln(T/K) with standard deviations of ±0.000 58 and ±0.000 97, respectively.

Introduction

The zwitterionic buffers recommended by Good and coworkers (*8*, *9*) are suitable as standard buffers for the standardization of glass-electrode pH meter assembly. Harned and Ehlers (*11*) demonstrated how cells without liquid junction could be used for the determination of equilibrium constants of weak acid−base systems including zwitterionic amino acids (*7*, *15*, *16*, *20*).

Biological buffers are of utmost importance for research in biomedicine. They are vital in experimentation ranging from stabilizing biological molecules to fertilizing ova in vitro. The future of biomedical research depends on reliable biological buffer systems, as they provide pH stabilizers in the optimum pH range for specific biological molecules, such as nucleic acids and proteins. In the present study, the ampholyte ACES is useful as a biological buffer in the pH range 6.5−7.5. Chappell (*6*) showed that ACES improved phenotyping of α_1-antitrypsin by isoelectric focusing on agarose gel. The buffer substance CHES is also important as a physiological buffer in the pH range 8.5−9.5. In order to obtain pH values for these buffer compounds with greater accuracy as well as to recommend a suitable pH buffer standard at an ionic strength of 0.16 mol·kg^{-1} for biochemical applications, it is essential to determine very accurately the thermodynamic dissociation constants (pK_2) of ACES and CHES as a function of temperature. To our knowledge, very limited and unreliable data are available in the literature (*10*) for ACES and CHES, which are the derivatives of the simplest amino acid, taurine. For the determination of pK_2 values from (5 to 55) °C, the most precise emf measurements of the following cells without liquid junctions have been carried out:

Pt; H_2 (g, 1 atm)|ACES (m_1), NaACESate (m_2), NaCl (m_3)|AgCl, Ag (A)

Pt; H_2 (g, 1 atm)|CHES (m_1), NaCHESate (m_2), NaCl (m_3)|AgCl, Ag (B)

where m_1, m_2, and m_3 indicates the molalities of undissociated molecules of ACES (or CHES), the sodium salt of the zwitterionic substance, and the neutral salt NaCl, respectively.

Theory and Equations

ACES (or CHES) is usually present in the form of a zwitterion with a large dipole moment. The second dissociation constant involves the dissociation of a proton from the protonated amino group of a dipolar molecule, $z^{\pm}$. The structural formulas of ACES and CHES in the zwitterionic forms are given below:

$H_2N{-}COCH_2{-}N^+H_2CH_2CH_2SO_3^-$ (ACES)

$C_6H_{11}{-}N^+H_2{-}CH_2{-}CH_2SO_3^-$ (CHES)

The dissociation process is indicated by

$$z^{\pm} = z^- + H^+ \qquad (1)$$

where $z^{\pm}$ is ACES or CHES and z^- is NaACESate or NaCHESate.

The "apparent" thermodynamic dissociation constant is expressed as

$$pK_2' = pK_2 - \log_{10}\{\gamma_z \pm \gamma_{Cl^-}\}/\gamma_{z^-} \qquad (2)$$

$$= (E - E°)F/(R(T/K)\ln 10) + \log_{10}(m_1 m_3/m_2) \qquad (3)$$

where E is the corrected emf (at a hydrogen pressure of 1 atm or 101 325 Pa) listed in Tables 1 and 2 for ACES and CHES, respectively; γ indicates the activity coefficients of the respective species, and m refers to the molalities. The activity coefficient of a dipolar molecule is assumed to behave like an uncharged molecule. The values of $E°$, the standard electrode potential of the silver + silver chloride electrodes were determined by making emf measurements of 0.01 mol·kg^{-1} HCl solution, assuming a value of γ_{HCl} = 0.904 (*1*, *2*) at 25 °C in this concentration. In eq 3, for the calculation of pK_2, the values of $E°$ used were (0.234 31, 0.231 62, 0.228 78, 0.225 77, 0.222 59, 0.219 28, 0.215 87,

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Table 1. Electromotive Force of the Cell Pt; H_2 (g, 1 atm)|ACES (m_1), NaACESate (m_2), NaCl (m_3)|AgCl, Ag from (5 to 55) °C

mol·kg^{-1}			*E*/V					
m_1	m_2	m_3	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
0.01	0.01	0.01	0.744 81	0.745 48	0.745 97	0.746 37	0.746 67	0.746 87
0.01	0.01	0.01	0.744 63	0.745 30	0.745 80	0.746 19	0.746 49	0.746 69
0.02	0.02	0.02	0.728 78	0.729 16	0.729 35	0.729 44	0.729 44	0.729 35
0.02	0.02	0.02	0.728 61	0.728 99	0.729 18	0.729 28	0.729 28	0.729 17
0.02	0.02	0.03	0.719 35	0.719 56	0.719 58	0.719 50	0.719 32	0.719 05
0.02	0.02	0.03	0.719 18	0.719 39	0.719 40	0.719 33	0.719 15	0.718 87
0.04	0.04	0.03	0.719 94	0.720 15	0.720 16	0.720 08	0.719 90	0.719 63
0.04	0.04	0.03	0.719 78	0.719 99	0.720 01	0.719 93	0.719 75	0.719 46
0.05	0.05	0.05	0.708 58	0.708 58	0.708 37	0.708 07	0.707 67	0.707 17
0.05	0.05	0.05	0.708 42	0.708 41	0.708 20	0.707 91	0.707 50	0.707 00
0.08	0.08	0.03	0.721 12	0.721 34	0.721 35	0.721 27	0.721 09	0.720 81
0.08	0.08	0.03	0.720 95	0.721 17	0.721 18	0.721 10	0.720 92	0.720 63
0.08	0.08	0.04	0.714 52	0.714 62	0.714 50	0.714 30	0.713 99	0.713 59
0.08	0.08	0.04	0.714 36	0.714 46	0.714 34	0.714 14	0.713 83	0.713 42
0.08	0.08	0.08	0.699 09	0.698 90	0.698 48	0.697 98	0.697 37	0.696 67
0.08	0.08	0.08	0.698 93	0.698 73	0.698 31	0.697 81	0.697 20	0.696 49

mol·kg^{-1}			*E*/V					
m_1	m_2	m_3	35 °C	37 °C	40 °C	45 °C	50 °C	55 °C
0.01	0.01	0.01	0.747 01	0.747 07	0.747 01	0.746 85	0.746 78	0.746 46
0.01	0.01	0.01	0.746 81	0.746 86	0.746 79	0.746 64	0.746 56	0.746 24
0.02	0.02	0.02	0.729 18	0.729 11	0.728 85	0.728 38	0.727 98	0.727 33
0.02	0.02	0.02	0.728 99	0.728 91	0.728 64	0.728 16	0.727 76	0.727 11
0.02	0.02	0.02	0.718 70	0.718 55	0.718 17	0.717 52	0.716 94	0.716 10
0.02	0.02	0.02	0.718 51	0.718 36	0.717 97	0.717 30	0.716 73	0.715 88
0.04	0.04	0.04	0.719 28	0.719 12	0.718 72	0.718 04	0.717 44	0.716 55
0.04	0.04	0.04	0.719 10	0.718 93	0.718 52	0.717 85	0.717 24	0.716 35
0.05	0.05	0.05	0.706 59	0.706 32	0.705 72	0.704 84	0.703 98	0.702 82
0.05	0.05	0.05	0.706 40	0.706 13	0.7055 6	0.704 62	0.703 77	0.702 60
0.08	0.08	0.08	0.720 44	0.720 26	0.719 81	0.719 10	0.718 46	0.717 50
0.08	0.08	0.08	0.720 25	0.720 07	0.719 61	0.718 88	0.718 24	0.717 28
0.08	0.08	0.08	0.713 10	0.712 86	0.712 33	0.711 48	0.710 70	0.709 60
0.08	0.08	0.08	0.712 92	0.712 68	0.712 13	0.711 27	0.710 50	0.709 39
0.08	0.08	0.08	0.695 86	0.695 48	0.694 72	0.693 53	0.692 42	0.690 94
0.08	0.08	0.08	0.695 67	0.695 29	0.694 52	0.693 31	0.692 20	0.690 72

Table 2. Electromotive Force of the Cell Pt; H_2 (g, 1 atm)|CHES (m_1), NaCHESate (m_2), NaCl (m_3)| AgCl, Ag from (5 to 55) °C

$m_1 = m_2 = m_3$/mol·kg^{-1}	*E*/V											
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	37 °C	40 °C	45 °C	50 °C	55 °C
0.01	0.890 99	0.892 81	0.894 46	0.895 94	0.897 18	0.898 24	0.899 24	0.899 60	0.900 03	0.900 65	0.901 26	0.901 61
0.01	0.890 84	0.892 64	0.894 27	0.895 75	0.896 98	0.898 05	0.899 04	0.899 44	0.899 83	0.900 43	0.901 26	0.901 44
0.02	0.874 78	0.876 31	0.877 62	0.878 80	0.879 78	0.880 55	0.881 21	0.881 46	0.881 72	0.882 04	0.882 33	0.882 45
0.02	0.874 66	0.876 13	0.877 46	0.878 63	0.879 58	0.880 38	0.881 02	0.881 34	0.881 52	0.881 85	0.882 13	0.882 24
0.03	0.865 49	0.866 84	0.867 97	0.868 97	0.869 77	0.870 40	0.870 84	0.871 03	0.871 19	0.871 33	0.871 44	0.871 37
0.03	0.865 33	0.866 67	0.867 79	0.868 79	0.869 59	0.870 21	0.870 65	0.870 91	0.871 00	0.871 15	0.871 24	0.871 16
0.04	0.859 03	0.860 25	0.861 24	0.862 09	0.862 81	0.863 31	0.863 60	0.863 76	0.863 85	0.863 86	0.863 83	0.863 63
0.04	0.858 88	0.860 07	0.861 05	0.861 92	0.862 64	0.863 14	0.863 41	0.863 64	0.863 65	0.863 68	0.863 63	0.863 42
0.06	0.850 10	0.851 15	0.851 97	0.852 66	0.863 23	0.853 59	0.853 62	0.853 75	0.853 73	0.853 57	0.853 33	0.852 94
0.06	0.850 04	0.851 00	0.851 80	0.852 40	0.853 05	0.853 41	0.853 44	0.853 62	0.853 54	0.853 39	0.853 13	0.852 73
0.08	0.844 10	0.845 03	0.845 66	0.846 20	0.846 67	0.846 94	0.846 77	0.846 89	0.846 80	0.846 51	0.846 11	0.845 59
0.08	0.843 91	0.844 87	0.845 50	0.846 01	0.846 49	0.846 76	0.846 57	0.846 76	0.846 60	0.846 32	0.845 93	0.845 38
0.09	0.841 70	0.842 47	0.843 14	0.843 61	0.844 06	0.844 30	0.844 03	0.844 15	0.844 03	0.843 68	0.843 23	0.842 65
0.09	0.841 54	0.842 41	0.842 98	0.843 44	0.843 88	0.844 11	0.863 84	0.844 02	0.843 84	0.843 50	0.843 03	0.842 43
0.10	0.839 45	0.840 44	0.840 93	0.841 36	0.841 78	0.841 99	0.841 64	0.871 74	0.841 61	0.841 21	0.840 69	0.840 06
0.10	0.839 60	0.840 27	0.840 75	0.741 17	0.841 59	0.841 80	0.841 44	0.841 62	0.841 41	0.841 03	0.840 49	0.839 84

0.214 57, 0.212 29, 0.208 51, 0.204 74, and 0.200 59) V for (5, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50, and 55) °C, respectively. These values of $E°$ at other temperatures were obtained from the temperature variations of $E°$ from the literature (*4*). The oxygen gas was always removed from HCl cell solutions containing 0.01 mol·kg^{-1} and 0.1 mol·kg^{-1}. Appropriate activity coefficients of HCl(aq) were used for the determination of $E°$ of the silver−silver chloride electrode at 25 °C. These results are in excellent agreement with earlier communications (*17*).

The last term in eq 3 containing the activity coefficients of the participating species is small because of the charge type, and should be directly proportional to the ionic strength I, if $\gamma_{z^\pm}$ is assumed to be unity. The more convenient form of eq 4 given below was used to calculated the values of pK_2 by linear regression methods (plot of pK_2' versus I). The intercept at $I = 0$ yields pK_2. Equation 3 reduces to

$$\mathrm{p}K_2' = \mathrm{p}K_2 + \beta I \qquad (4)$$

where β is the slope parameter and $I = m_2 + m_3$. The values of pK_2 together with standard deviations for ACES and CHES are entered in Tables 3 and 4, respectively. It is important to indicate that the hydrolysis correction to m_1/m_2 for the dissociation process of ACES is unnecessary since the dissociation of z^- is small ($\approx 10^{-7}$ mol kg^{-1}), and $m_{z^\pm} = m_1 + m_{OH^-} = m_1$, and $m_{z^-} = m_2 - m_{OH^-} = m_2$. For

Table 3. Second Dissociation Constants of ACES from (5 to 55) °C, Slope Parameters β, and the Standard Deviations of the Intercept $\sigma(pK_2)$ and Slope Parameters $\sigma(\beta)$

t/°C	pK_2	$\sigma(pK_2)^a$	β/kg·mol^{-1}	$\sigma(\beta)^b$/kg·mol^{-1}
5	7.2375	0.0007	0.546	0.007
10	7.1343	0.0008	0.529	0.008
15	7.0341	0.0009	0.518	0.010
20	6.9385	0.0009	0.509	0.009
25	6.8475	0.0008	0.499	0.008
30	6.7599	0.0007	0.489	0.007
35	6.6758	0.0008	0.476	0.008
37	6.6420	0.0009	0.464	0.010
40	6.5953	0.0007	0.441	0.007
45	6.5180	0.0008	0.418	0.009
50	6.4441	0.0009	0.396	0.010
55	6.3749	0.0008	0.361	0.008

[a] Standard deviation of the intercept. [b] Standard deviation of the slope parameters.

Table 4. Second Dissociation Constants of CHES from (5 to 55) °C, Slope Parameters β, and the Standard Deviations of the Intercept $\sigma(pK_2)$ and Slope Parameters $\sigma(\beta)$

t/°C	pK_2	$\sigma(pK_2)^a$	β/kg·mol^{-1}	$\sigma(\beta)^b$/kg·mol^{-1}
5	9.8897	0.0007	0.382	0.005
10	9.7597	0.0007	0.375	0.006
15	9.6342	0.0008	0.354	0.006
20	9.5132	0.0008	0.343	0.006
25	9.3944	0.0008	0.357	0.006
30	9.2790	0.0008	0.361	0.006
35	9.1687	0.0008	0.322	0.006
37	9.1236	0.0005	0.335	0.004
40	9.0603	0.0008	0.333	0.006
45	8.9562	0.0008	0.326	0.006
50	8.8551	0.0008	0.309	0.006
55	8.7596	0.0008	0.301	0.006

[a] Standard deviation of the intercept. [b] Standard deviation of the slope parameters.

CHES, because of somewhat high pK_2, the hydroxyl ion concentration, $m_{OH}-$, obtained from the hydrolysis of $z^{\pm}$ and z^{-} was attempted. Since $m_{OH}-$ is small in comparison with $m_1 + m_{OH}-$, and $m_2 - m_{OH}-$, the value of $m_{OH}-$ can be evaluated with reasonable accuracy by the following equation

$$\log m_{OH^-} = \log K_w + pw_H \qquad (5)$$

where $pw_H = (E - E°)/k + \log m_{Cl},$; $k = (R(T/K) \ln 10)/F$, and pK_w is the ionization constant of water. For example at 25 °C, the contribution of $m_{OH}-$ to the value of pK_2 for CHES is within ±0.0004 pK unit, which is well within experimental uncertainty. Hence, also for CHES, the hydrolysis correction was ignored.

Experimental Section

The emf measurements with a precision to ±0.02 mV, originally introduced by Harned and Ehlers (*11*), were performed using a Keithley Model 191 digital voltmeter. For a particular run, the emf readings of the initial and final measurements (at the end of the temperature run) at 25 °C agreed on the average to 0.03 mV. All emf data in the temperature range (5−55) °C were corrected to a hydrogen partial pressure of 1 atm (101 325 Pa). The preparation of the hydrogen electrode, AgCl|Ag electrodes (*3*, *4*), and buffer solutions, the design of glass cells (*11*), and other experimental details have been published elsewhere (*5*, *15*, *17*). The bias potential of Ag|AgCl electrodes was always within ±0.03 mV. The temperature of the bath was regulated to ±0.005 K with a digital thermometer (Guideline Model 9540).

ACES and CHES were obtained from Research Organics Inc. (Cleveland, OH) and were used as received. Preliminary measurements with recrystallized sample (with 70 mass % ethanol) and untreated buffer substance yielded emf readings within ±0.05 mV, which is well within experimental error. The purified sample for ACES and CHES assayed 99.95% and 99.94% by titrations with standard NaOH solution. The buffer materials were always dried and stored over Drierite. The ACS-certified reagent grade NaCl was recrystallized twice from deionized doubly distilled water and dried at 115 °C. The cell solutions were prepared by mass from ACES (or CHES), NaCl, and standard solutions of NaOH, doubly distilled water in appropriate amounts so that the molalities of ACES (or CHES), NaACES (or NaCHES), and NaCl were equal in most cases.

Results and Discussion

The values of $E°$(AgCl|Ag) obtained in the present study are in excellent agreement with those previously published (*17*). For example at 25 °C, $E°$(AgCl|Ag) value of 0.222 59 V is the same as reported before (*17*). The variation of the experimental values of pK_2 (from Tables 3 and 4) with thermodynamic temperature T was found to be given by the following expressions:

For ACES,

$$pK_2 = 2548.60/(T/K) - 20.03159 + 3.2172 \ln(T/K) \quad (6)$$

and for CHES,

$$pK_2 = 1917.66/(T/K) + 5.7940 - 0.4971 \ln(T/K) \quad (7)$$

where T lies between (278.15 and 328.15) K. The standard deviation for regression was 0.000 58, and 0.000 97, respectively.

The standard changes of Gibbs energy ($\Delta G°$), enthalpy ($\Delta H°$), entropy ($\Delta S°$), and heat capacity ($\Delta C_p°$) for the dissociation processes of ACES and CHES can easily be derived from the constants of eqs 6 and 7 by simple thermodynamic relationships. Equations 6 and 7 are of the form (*12*)

Table 5. Thermodynamic Quantities with Uncertainties for the Dissociation of ACES and CHES at (5, 25, 37, and 55) °C

	5 °C	25 °C	37 °C	55 °C
		ACES		
$\Delta G°$/J·mol^{-1}	38 544 ± 5	39 082 ± 6	39 445 ± 6	40 043 ± 6
$\Delta H°$/J·mol^{-1}	31 660 ± 117	39 082 ± 40	39 445 ± 54	40 043 ± 130
$\Delta S°$/J·mol^{-1}·K^{-1}	−25.73 ± 0.41	−30.43 ± 0.14	−29.69 ± 0.14	−28.58 ± 0.41
$\Delta C_p°$/J·mol^{-1}·K^{-1}	−62 ± 4.4	−62 ± 4.7	62 ± 4.8	−62 ± 5.2
		CHES		
$\Delta G°$/J·mol^{-1}	52 668 ± 5	53 618 ± 6	54 1820 ± 6	55 020 ± 6
$\Delta H°$/J·mol^{-1}	39 360 ± 118	39 551 ± 40	39 665 ± 50	39 836 ± 131
$\Delta S°$/J·mol^{-1}·K^{-1}	−49.75 ± 0.41	−49.78 ± 0.14	−48.67 ± 0.14	−48.11 ± 0.41
$\Delta C_p°$/J·mol^{-1}·K^{-1}	−9.5 ± 4.3	9.5 ± 4.7	9.5 ± 4.9	9.5 ± 5.2

$$pK_2 = A/(T/K) + B + C \ln(T/K) \qquad (8)$$

After substitution of the thermodynamic formulas, one can write

$$\Delta G°/J{\cdot}mol^{-1} = 19.145(A + B(T/K) + C(T/K)\ln(T/K)) \qquad (9)$$

$$\Delta H°/J{\cdot}mol^{-1} = 19.145(A - C(T/K)) \qquad (10)$$

$$\Delta S°/J{\cdot}K^{-1}{\cdot}mol^{-1} = -19.145(B + C + C\ln(T/K)) \qquad (11)$$

$$\Delta C_p°/J{\cdot}K^{-1}{\cdot}mol^{-1} = -19.145C \qquad (12)$$

These equations were employed to calculate the thermodynamic quantities which are listed in Table 5 at (5, 25, 37, and 55). The estimates of the standard deviations determined by the method of Please (*14*) are also summarized in Table 5.

The pK_2 value for CHES at 25 °C is 9.394, whereas for taurine it is 9.061 (*13*). This decrease in acidic strength (increase in the value of pK_2) as well as small decrease in $\Delta H°$ (41.840 kJ·mol^{-1} for taurine, as compared with 39.551 kJ·mol^{-1} for CHES) is due to lengthening of the chain for hydrophobic cyclohexyl ($C_6H_{11}-$) substitution in the parent compound taurine. As apparent from Table 5, the negative value of $\Delta S°$ ($\Delta S° = -49.78$ J·K^{-1}·mol^{-1}), for CHES, as compared with taurine (*16*) ($\Delta S° = -33.05$ J·K^{-1}·mol^{-1}), might indicate that the stabilization of solvent structure causes an increased order in the proximities of molecules of uncharged base (*15*, *19*). In regard to the value of $\Delta C_p°$, for the second dissociation of CHES in H_2O it is about 9.5, whereas that of taurine is -33.5 J·K^{-1}·mol^{-1}. This indicates a change in the solvation pattern for the dissociation process.

According to Timimi and Everett (*19*), hydroxyethyl or hydroxymethyl substitution in taurine usually decreases the values of pK_2, $\Delta G°$, and $\Delta H°$. This is consistent with the trend found in our studies for ACES (Table 5), in which there is N-substitution in taurine by the group *N*-(2-acetamido). The change in $\Delta S°$ (from -33.05 for taurine to -30.43 J·K^{-1}·mol^{-1} for ACES at 25 °C) is very small, whereas the change in $\Delta C_p°$ from -33 J·K^{-1}·mol^{-1} for taurine to -62 J·K^{-1}·mol^{-1} for ACES may indicate a substantial change in the solvation pattern for the isoelectric dissociation process.

The results of pH for ACES/NaCl and CHES/NaCl buffers in an isotonic saline solution (0.16 mol·kg^{-1}) in the temperature range (5 to 55) °C including the body temperature (37 °C) will be reported in a separate communication (*18*).

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