Assignment of Standard pH Values (pH*(S)) to Buffers in 20 and 30% (w/w) Dimethyl Sulfoxide/Water Mixtures at Normal and Subzero Temperatures

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Standard pH values have been assigned to buffers in water (pH(S)) and dimethyl sulfoxide-water mixtures (pH*(S)) at temperatures between +25 and −12 °C by using the cell Pt;H_2(g, 1 atm), reference solution,$KCl_{(m)}$,AgCl;Ag. The reference solutions were (1) phosphates (mixtures of potassium dihydrogen phosphate and disodium hydrogen phosphate), (2) TES/TESNa (a solution of *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid and its sodium salt), and (3) potassium hydrogen phthalate.

Introduction

The need for reliable pH measurements at low temperatures in mixed solvents such as dimethyl sulfoxide (Me_2SO) and water has become increasingly apparent in recent studies of the preservation of biological cells, tissues, and organs (*10*, *15*, *17*, *18*, *21*). However, investigations into the importance of pH changes for the integrity of biological systems during exposure to low temperatures have been restricted by the limited validity of pH measurements made with conventional electrodes at room temperature (*10*, *21*) or with modified electrodes at subzero temperatures (*22*). In both cases the measurements were subject to serious errors due to the indeterminable contributions made to the cell potential by the asymmetry potential of the glass electrode and the liquid-junction potentials within the cell. The magnitudes of all these potentials are affected not only by the temperature but also the solvent composition.

The conventional electrometric technique for pH measurement is dependent upon an operational definition of pH (*4a*, *6*), which is expressed in the following way:

$$\mathrm{pH(X)} = \mathrm{pH(S)} + \frac{(E_x - E_s)F}{RT \ln 10} \tag{1}$$

where X denotes the solution of unknown pH and S the standard reference solution of known or assigned pH, while E is the electromotive force of a suitable galvanic cell consisting of an electrode reversible to hydrogen ions (usually a glass electrode) coupled with a suitable reference electrode (commonly calomel or silver-silver chloride electrode). The symbols R, T, and F represent the gas constant, the absolute temperature, and the Faraday, respectively. In recent times, with increasing interest in the measurement of pH values in solvents other than pure water, similar definitions and conventions have been adopted for mixed solvent systems (*3*). In this case standardization is made with reference to the standard state in the mixed solvent, S, and an operational scale in each medium is defined as

$$\mathrm{pH^*(X)} = \mathrm{pH^*(S)} + \frac{(E_x - E_s)F}{RT \ln 10} \tag{2}$$

where the asterisk denotes a quantity referred to the standard state in a solvent other than pure water (*4b*) and the operational unit pH* is analogous to the operational quantity pH for aqueous solutions. The procedure depends upon some convention for deriving the numerical value of the activity coefficient of the chloride ion, ${}_s\gamma_{Cl}$, in the mixed solvent, and a similar expression to that used in aqueous solutions has been recommended for mixed solvents, taking into account the necessary modifications due to changes in the dielectric constant of the media.

The determination of standard molal emf's for the hydrogen, silver-silver chloride electrochemical cell containing mixtures of dimethyl sulfoxide (Me_2SO) and water at temperatures between +25 and −12 °C has been described (*20*) and these now permit the assignment of standard pH values to reference solutions in Me_2SO-H_2O mixtures by using accepted procedures and conventions.

Procedure for Assigning Standard pH Values

Standard pH values of reference buffer solutions, denoted by pH(S) in water or pH*(S) in any solvent other than pure water, were assigned by using the procedure and conventions adopted by the American National Bureau of Standards (*4c*). This procedure involves three steps: (i) The first step is the measurement of the emf of the cell:

$$\mathrm{Pt;H_2(g),reference\ solution,KCl_{(\mathit{m})},AgCl;Ag} \tag{3}$$

when the reference solution contains a soluble chloride (potassium chloride) at one of three or four low and accurately known molalities; 0.020, 0.015, 0.010, and 0.005 m were used. The emf, E, of this cell is directly related to the standard potential, $E°$, the activities of the hydrogen and chloride ions in solution, and natural constants so that a quantity p_sH, known as the acidity function, can be evaluated from the expression

$$p_sH = \frac{(E - E°)F}{2.3026RT} + \log m_{Cl} \tag{4}$$

in which F is the Faraday, R is the gas constant, and T is the absolute temperature. The acidity function, p_sH, in solvent S (or p_wH in water) is equivalent to $-\log(m_H\gamma_H\gamma_{Cl})$.

(ii) The second step is the determination of $p_sH°$, the limit approached by p_sH for each buffer solution as the molality of added chloride is reduced. This was achieved by extrapolating the chloride molality to zero ($m_{Cl} = 0$).

(iii) The final step is to calculate standard pH values by using the expression

$$\mathrm{pH(S)} = p_sH° + \log \gamma_{Cl} \tag{5}$$

in which the activity coefficient of the chloride ion, γ_{Cl}, is obtained by using a conventional definition (*3*, *6*) consistent with the Debye-Hückel equation; i.e.

$$-\log \gamma_{Cl} = \frac{AI^{1/2}}{1 + 1.5I^{1/2}} \tag{6}$$

where A is a constant dependent upon the dielectric constant of the solvent and the temperature and I is the ionic strength.

Experimental Procedures

Emf measurements of cell 3 containing the reference buffer solutions with added potassium chloride were made with a Vibron

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electrometer in conjunction with a dc millivolt calibrator as described in a previous communication (*20*). Platinized hydrogen electrodes and silver-silver chloride electrodes of the thermal electrolytic type were prepared in the same way as previously described (*20*). Aliquots of 15 cm^3 of cell solution were equilibrated with purified hydrogen and constancy of emf readings to 0.1 mV for at least 30 min was taken to be the criterion for equilibration.

Standard molal buffer solutions were prepared by weight by using preboiled, deionized water with a conductivity of less than $1.0 \times 10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$. The concentrations of phosphate buffers and of potassium hydrogen phthalate used as standards in this work were taken as those recommended as International pH Standards (*2*). However, because of the limited solubility of disodium hydrogen phosphate in mixtures of Me_2SO and water at temperatures in the region of 0 °C and below, an alternative reference solution was sought for standardization in the physiologically important pH range of 7–8. The compound *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), one of the zwitterionic buffers introduced by Good et al. in 1966 (*11*), was chosen because its pK_a is 7.5 in water at 20 °C (that is, the compound offers maximum pH buffering in the region of physiological pH) and also because it was soluble to the extent of 2.6 M at 0 °C (*11*). A 100-m*m* solution of TES in 30% Me_2SO-H_2O was titrated at 20 °C against standard sodium hydroxide to determine the ratio of TES to its sodium salt necessary to achieve a solution with a pH of 7.4.

The titration showed that a three-tenths neutralized TES solution produced a solution having a pH value in the desired range. A solution containing 70 mmol of TES and 30 mmol of its sodium salt, TESNa, was therefore chosen as the reference buffer solution for the pH range 7–8.

The compositions of buffer solutions prepared for standardization were as follows: (A) phosphate buffer I, 0.025 *m* potassium dihydrogen phosphate plus 0.025 *m* disodium hydrogen phosphate; (B) phosphate buffer II, 0.008 695 *m* potassium dihydrogen phosphate plus 0.030 43 *m* disodium hydrogen phosphate; (C) TES + TESNa, 0.070 *m* *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and 0.030 *m* sodium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate (TESNa); (D) phthalate buffer, 0.05 *m* solution of potassium hydrogen phthalate (KH phthalate).

Standard pH values were assigned to these buffers which were dissolved in either pure water or water containing 20% (w/w) or 30% (w/w) Me_2SO at temperatures between 25 and −12 °C.

Results and Calculations

Values of the acidity function $p(a_H\gamma_{Cl})$ were derived, for each set of conditions of solvent composition and temperature, from the emf (E), the molality of chloride (m_{Cl}), and the standard emf ($E°$) by using eq 4. The values of $E°$ and $2.3026RT/F$ for each set of conditions are given in Table I.

When the acidity functions for each buffer solution were plotted as a function of m_{Cl}, straight lines of small slope were obtained and the intercepts, namely, $p_sH°$, at $m_{Cl} = 0$ were obtained by extrapolating the linear regression lines to zero concentration of chloride by the method of least squares. Typical regression lines are shown in Figure 1 for the TES + TESNa buffer in the various solvents.

Finally, pa_H for the chloride-free buffer solutions was calculated by using the convention for the activity coefficient of the chloride ion expressed by eq 6. Values for the Debye–Hückel slope, A, are given in Table I.

The ionic strength of both phosphate solutions was taken as 0.1. The ionic strength of the TES/TESNa buffer solutions was taken to be the same as the molality of the sodium salt since it was assumed that the zwitterionic form of TES made no

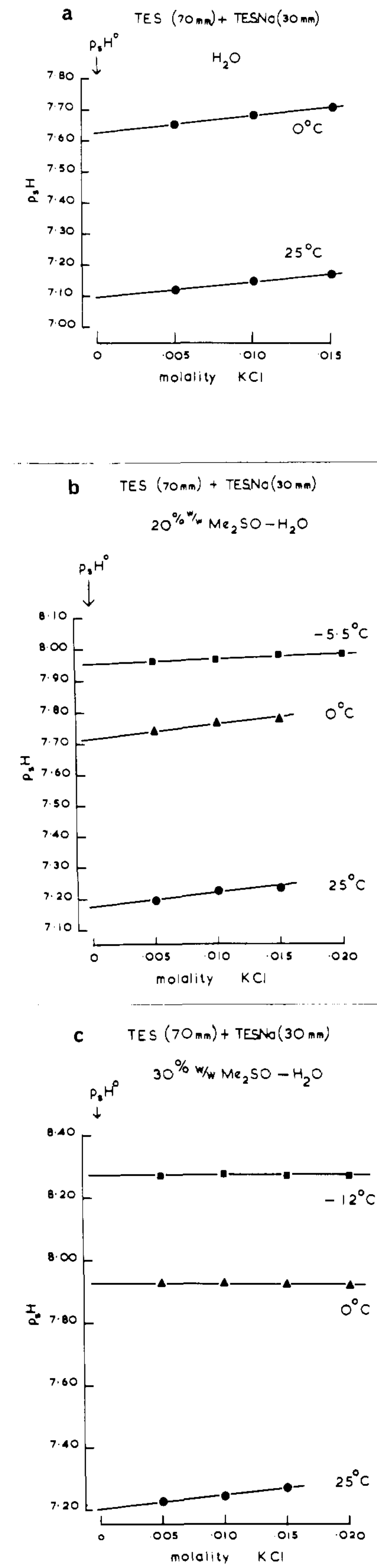

Figure 1. Graphs to show the variation of acidity function, p_sH, with the molal concentration of chloride added to various buffers in mixed solvents at temperatures between 25 and −12 °C. The lines drawn in each case are the least-squares regression lines which were extrapolated to zero concentration of chloride to obtain the limiting values, $p_sH°$, of the acidity functions. These were used to calculate standard pH values for the buffer solutions: (a) TES/TESNa in H_2O; (b) TES/TESNa in 20% (w/w) Me_2SO-H_2O; (c) TES/TESNa in 30% (w/w) Me_2SO-H_2O.

Table I. Data Used in the Calculation of Acidity Functions, p_sH, from Equation 4 for Different Solvents at Temperatures between +25 and −12 °C

solvent	temp, °C (K)	$E°$ [a]	$2.3026RT/F$	A
water	25 (298.16)	0.222 27	0.059 15	0.5116
	0 (273.16)	0.236 74	0.054 19	0.4918
20% (w/w) Me_2SO-H_2O	25 (298.16)	0.219 38	0.059 15	0.5159
	0 (273.16)	0.234 82	0.054 19	0.4956
	−5.5 (267.16)	0.236 71	0.053 00	0.4938
30% (w/w) Me_2SO-H_2O	25 (298.16)	0.218 39	0.059 15	0.5205
	0 (273.16)	0.232 99	0.054 19	0.4994
	−12 (261.16)	0.238 88	0.051 81	0.4921

[a] From Taylor 1978 (*20*). A is the Debye-Hückel constant; values were derived from the expressions given by Robinson and Stokes (*16*); i.e., $A = (1.825 \times 10^6)/\epsilon T^{3/2}$ $mol^{-1/2}$ $L^{1/2}$ $k^{3/2}$, R = 8.31 433 J K^{-1} mol^{-1}, and $F = 9.648\ 70 \times 10^4$ C mol^{-1}.

contribution to the ionic strength. (See ref 7.) The data of Hamer, Pinching, and Acree (*13*) for the calculation of the ionic strength of 0.05 *m* potassium hydrogen phthalate solutions in the temperature range 0–60 °C showed that the ionic strength changed by only 0.35% for a temperature change of 35 °C. These data were therefore extrapolated to lower temperatures and a value of 0.053 was used for the ionic strength of the potassium hydrogen phthalate standard. Table II summarizes the data derived for the assignment of standard pH values to the four buffer solutions, giving the acidity function at each level of added chloride, the limit of p_sH as the concentration of chloride approaches zero, and finally the assigned values of pH(S) or pH*(S).

Discussion

Calculation of the acidity function, p_sH, from the emf of cell 3 involved a correction of the emf to a partial pressure of hydrogen of 1 atm. This was achieved in the same way as described previously for the determination of standard cell potentials under the same conditions of solvent composition and temperature (*20*).

It has been shown that the acidity function p_sH can be derived from eq 4 for decreasing concentrations of chloride in order that, in the limit, the pH of the chloride-free buffer solutions can be obtained by extrapolation. Several workers (*5*, *8*, *9*, *12*) have shown that values of p_sH for a given buffer solution, computed by using eq 4, vary linearly with low concentrations of added chloride. This has permitted the evaluation of values of $p_sH°$ at zero concentration of chloride by linear extrapolation by using the method of least squares.

The standard pH, that is pH(S), is formally defined as $-\log a_H = -\log \gamma_H m_H$ and is related to $p_sH°$ by eq 5.

The more reasonable and accurate the estimate of γ the closer the standard pH will approach the desired $-\log \gamma_H m_H$. It is necessary to evaluate the activity coefficient of a single ionic species in order to compute the activity; although this coefficient is not amenable to measurement, its uncertainty diminishes with decreasing ionic strength so that the pH(S) values obtained by the method described, by using the cell without liquid-junction potential, become increasingly accurate as the concentration of dissolved electrolyte decreases (*1*). The Debye–Hückel equation for the evaluation of γ with ionic strength, I, in the form that allows for the restriction of the attraction between ions due to their finite size (expression 7)

$$\log \gamma = -AZ^2 I^{1/2}/(1 + Ba° I^{1/2}) \qquad (7)$$

has been used to compute individual ionic activity coefficients, where A and B are constants dependent upon the dielectric constant of the solvent and temperature, Z is the ionic valence, and $a°$ is the ion size parameter. Although an equation of this form undoubtedly expresses the variation of γ_{Cl} with ionic strength at low concentrations, it is impossible to determine the correct value of the ion-size parameter, $a°$. Various reasonable methods of estimating this parameter lead to results differing by more than 0.01 unit in $\log \gamma_{Cl}$ and pH(S) when the ionic strength exceeds 0.1 (*1*). The best procedure, therefore, is to choose standard buffer solutions of sufficiently low concentration to be consistent with adequate buffering capacity. Under these conditions any reasonable value of $a°$ will yield pH(S) values with uncertainties less than 0.01 unit. In order to derive pa_H from $p(a_H\gamma_{Cl})$ by using the relationship

$$pa_H = -\log (m_H\gamma_H) = p(a_H\gamma_{Cl}) + \log \gamma_{Cl} \qquad (8)$$

Bates and Guggenheim (*6*) suggested that γ_{Cl} at ionic strengths not exceeding 0.1 be calculated by using the Debye–Hückel equation (7) with the term Ba^0 = 1.5 $kg^{1/2}$ $mol^{-1/2}$. This corresponds to a value of 4.56 Å for the ion size parameter of

Table II. Standard pH Values (pH(S) or pH*(S)) Derived (Equation 5) from the Acidity Functions in Water or Mixtures of Dimethyl Sulfoxide and Water at Temperatures between 25 and −12 °C

molal concn KCl		$p_sH \equiv p(a_H\gamma_{Cl})$						
solvent	temp, °C	0.020	0.015	0.010	0.005	$p_sH°$	$\log \gamma_{Cl}$	pH(S) or pH*(S)
			Phosphate I					
water	25		6.970	6.972	6.978	6.981	−0.1097	6.871
20% (w/w) Me_2SO-H_2O	25		7.494	7.502	7.509	7.517	−0.1105	7.407
30% (w/w) Me_2SO-H_2O	25		7.771	7.794	7.803	7.821	−0.1110	7.710
			Phosphate II					
20% (w/w) Me_2SO-H_2O	25		8.038	8.048	8.059	8.069	−0.1105	7.959
30% (w/w) Me_2SO-H_2O	25		8.342	8.344	8.368	8.377	−0.1110	8.266
			TES/TESNa					
water	25		7.173	7.153	7.120	7.096	−0.070	7.026
water	0		7.708	7.683	7.653	7.626	−0.068	7.558
20% (w/w) Me_2SO-H_2O	25		7.236	7.226	7.194	7.177	−0.071	7.106
20% (w/w) Me_2SO-H_2O	0		7.781	7.767	7.737	7.718	−0.069	7.649
20% (w/w) Me_2SO-H_2O	−5.5	7.982	7.982	7.968	7.964	7.957	−0.068	7.889
30% (w/w) Me_2SO-H_2O	25		7.274	7.243	7.226	7.200	−0.072	7.128
30% (w/w) Me_2SO-H_2O	0	7.923	7.924	7.928	7.926	7.929	−0.069	7.860
30% (w/w) Me_2SO-H_2O	−12	8.263	8.281	8.277	8.271	8.278	−0.068	8.210
			Potassium Hydrogen Phthalate					
water	25		4.099	4.098	4.096	4.095	−0.088	4.007
20% (w/w) Me_2SO-H_2O	25		4.551	4.552	4.557	4.559	−0.088	4.471
30% (w/w) Me_2SO-H_2O	25		4.789	4.809	4.830	4.850	−0.089	4.761
30% (w/w) Me_2SO-H_2O	−12	4.931	4.935	4.937	4.952	4.955	−0.084	4.870

the chloride ion. This convention has been adopted throughout this work, making necessary adjustments to the Debye–Hückel constant *A* which is dependent on the dielectric constant and temperature of the solvent. It is reasonable to use this value of $a°$ at other temperatures and for Me_2SO-H_2O solvents thus making the convention for ${}_s\gamma_{Cl}$ entirely consistent with the convention on which γ_{Cl} and standard pH values in aqueous solution are based. Paabo et al. (*14*) have used the same convention for methanol–water mixtures, and Bates (*3*) has recommended adoption of this convention for practical measurements of pH in mixed solvents.

Standard error estimates of the intercepts for regression analyses of plots of p_sH against the molal concentration of added chloride showed, in every case, that the errors were negligible (less than 0.001 units).

The limit of solubility of the mixed phosphate buffers in Me_2SO-H_2O solutions at 0 °C precluded their use as a standard for the region of the pH scale around neutrality. Although the optimum pH for biological integrity in mixed solvent media at low temperatures is not yet known, it was decided to maintain a pH value in the region of 7.4 as the reference point for a buffer used to replace the phosphates as a standard for the physiological range. The compound *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), a zwitterionic buffer used during cryopreservation studies on smooth muscle (*10*), is known to have a p*Ka* of 7.5 at 20 °C in water (*11*) and also to be soluble to the extent of 2.6 M concentration at 0 °C. Moreover, it can be seen from the work of Elford and Walter (*10*) that 196.3 mmol of TES remains soluble in solutions containing 60% (w/v) Me_2SO at temperatures down to −79 °C. The titration of TES against standard alkali in 30% (w/w) Me_2SO showed that it is necessary to convert 30% of the TES in a solution to its sodium salt in order to establish a buffer solution with a pH value of 7.4. These potentiometric titrations carried out in Me_2SO mixtures by using a measuring cell that was standardized with a purely aqueous buffer at 20 °C were subject to some error because of the indeterminate residual liquid-junction potential. Nevertheless, for the sole purpose of determining the relative concentrations of TES and its sodium salt to produce a buffer solution with a pH value in the region of 7.4, these approximate titrations were adequate since the exact hydrogen-ion activity of the solution was subsequently determined with the hydrogen, silver–silver chloride cell which is without liquid junction.

The pH(S) values obtained for phosphate and phthalate buffers in purely aqueous solution at 25 °C are in complete agreement with values in the literature (*2*). This served to demonstrate that the system used in this work for the assignment of pH standards conforms with those used to establish international pH scales. The pH*(S) values now assigned in some mixtures of dimethyl sulfoxide and water at temperatures between +25 and −12 °C permit the establishment of an operational definition of pH*, under these conditions, as described by expression 2. The new standards can now be used to calibrate conventional and modified electrodes for meaningful pH measurements in Me_2SO-H_2O mixtures at low temperatures, and the use of these standards for a preliminary investigation into the response of the glass/calomel pH cell at +25 and −12 °C in aqueous solutions containing dimethyl sulfoxide has already been published (*19*).

Glossary

Me_2SO	dimethyl sulfoxide
pH(S)	standard pH value assigned to a buffer solution in water
pH*(S)	standard pH value assigned to a buffer solution in a solvent other than pure water
p_sH	a quantity called the acidity function in solvent, S, and equivalent to $-\log (m_H\gamma_H\gamma_{Cl})$ (see eq 4)
TES	trivial name for *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
TESNa	sodium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate
γ_{Cl}	activity coefficient of the chloride ion

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