Re-evaluation of pH and Tonicity of Pharmaceutical Buffers at 37°

ANTHONY J. CUTIE and BARTLEY J. SCIARRONE

Abstract □ Commonly employed isotonic buffer mixtures of pharmaceutical interest were studied at body temperature with emphasis placed on the tonicity and pH of these preparations. Tonicity data interpreted from vapor pressure osmometer data are presented for the official phosphate buffers, boric acid buffer, and Palitzsch buffers. Human serum was also checked at this temperature to validate the accepted base line of 0.90% as being isoosmotic with body fluids. Interesting discrepancies between freezing point depression data and vapor pressure osmometer data are discussed. Reformulated isotonic Palitzsch and boric acid buffers at 37° are presented. A critical assessment of accepting tonicity values at 0° and extrapolating them to body temperature is attempted.

Keyphrases □ Buffers, pharmaceutical—pH, tonicity □ Isotonicity determinations—37° □ Serum, human—NaCl solution osmotic equivalency □ Freezing point depression, tonicity values compared—37° experimental values □ Vapor pressure osmometer—analysis

Pharmaceutical solutions which are intended for parenteral injection or are to be applied to delicate membranes of the body are routinely adjusted to be approximately isotonic with body fluids to avoid discomfort and possible injury. More often than not the methods used to make solutions isotonic (really isoosmotic) with body fluids employ sodium chloride equivalent values which have been determined cryoscopically (1, 2). The objective of this investigation was to make a serious attempt at refining tonicity determinations by investigating the colligative properties elicited by body fluids and solutions that come in contact with body fluids at 37°. Performing this study at body temperature allowed the collection of tonicity data at simulated body conditions and eliminated the need for temperature extrapolations, something intrinsically disadvantageous to the freezing point depression tonicity data (FPD).

It was felt that the most immediate attention be devoted to isotonic buffered diluting solutions because of their routine use in pharmaceutical compounding and because these solutions represent the greatest portion of ophthalmic and parenteral preparations. Specifically the isotonic buffers re-examined and re-evaluated, when necessary, for both pH and tonicity in this study were the boric acid buffer, the Sorensen buffer, and the Palitzsch buffer. However, prior to this re-evaluation, human serum was checked at 37° to verify the commonly accepted reference value of 0.90% sodium chloride as being the osmotic equivalent of blood (3).

Table I—Vapor Pressure Osmometer Data on Human Blood Serum at 37°

Subject	Tonicity
1	0.88
2	0.87
3	0.88
4	0.87
5	0.88

EXPERIMENTAL

Human Serum Samples—The human serum used in this study was obtained from unadulterated whole blood taken from five male volunteers. The whole blood was placed into absolutely dry test tubes from syringes that had the needles removed to avoid hemolysis on transfer. The blood was allowed to clot undisturbed at room temperature and the serum was removed.

Preparation of Buffer Mixtures and Determination of pH—All chemicals used were reagent grade quality and de-ionized water was used throughout the investigation. The buffer mixtures, usually 100-ml. samples, were prepared analytically from prescribed formulas (4) and divided into two unequal portions—one part, approximately 30 ml., for pH determinations, and a larger part for osmometric measurements. The pH was checked at 25 and 37° with a pH meter (Corning model 12) operated on expanded scale. The electrode system was a calomel reference electrode (Corning) and indicating glass electrode. A water bath (Sargent) was used to maintain constant temperature within ±0.5° during the pH determinations.

The isotonic buffers were prepared by simply weighing out a quantity of sodium chloride suggested by the literature (4) into 50-ml. volumetric flasks and making up to volume with the remaining portion of the buffer mixture. This procedure was repeated in reformulating the isotonic buffer mixtures at 37° with the only difference being the quantities of sodium chloride added.

Determination of Osmotic Concentration of Buffer Mixtures and Human Serum—A vapor pressure osmometer (VPO, Mechrolab model 301A) was used for determining the tonicity of buffer mixtures and human serum because unlike most classical colligative property measuring devices, it can be used at various temperatures (5). The instrument was thermostatically controlled at 37.0 ± 0.2° and a matched pair of aqueous thermistors (Hewlett Packard) were used as the detection probes (6890 ohms). The VPO was used in the following manner for determining the tonicity of human serum, buffer mixtures and isotonic buffer mixtures. Four to five standard solutions of sodium chloride ranging from 0.30 to 1.50% were prepared and tested daily with the VPO. From this data a plot of instrumental reading ΔR (ohms) *versus* percent sodium chloride was constructed and employed as a calibration curve. Once the calibration curve was constructed, the colligative effect elicited by subse-

Table II—Sodium Chloride Equivalents[a] of Buffer Components at 37°

Buffer Component	Stock Solution, g./l.	NaCl Equiv. at Concentration Used
Boric acid	17.700	0.51 (0.47)
Boric acid	12.404	0.51 (0.49)
Sodium tetraborate	19.108	0.45 (0.35)
Sodium acid phosphate (monohydrate)	9.208	0.43 (0.40)
Sodium phosphate (anhydrous)	9.470	0.54 (0.53)
Sodium carbonate (monohydrate)	24.801	0.63 (0.58)
Potassium chloride	2.857	0.77 (0.76)

[a] Values in parentheses are E values determined at 0° (see *References* 1 and 2).

Table III—Re-evaluated pH and Tonicity for Modified Palitzsch Buffer Mixtures[a]

0.2 *M* Boric Acid Solution, ml.	0.05 *M* Sodium Tetraborate Solution, ml.	pH at 25° (Lit.[b])	Exptl. pH 25°	Exptl. pH 37°	Tonicity of Buffer Mixtures Lit.[c]	Tonicity of Buffer Mixtures Exptl. (37°)
97	3	6.77	6.93	6.92	0.68	0.63
94	6	7.09	7.27	7.25	0.68	0.63
90	10	7.36	7.51	7.49	0.68	0.64
85	15	7.60	7.74	7.71	0.67	0.64
80	20	7.78	7.90	7.87	0.66	0.64
75	25	7.94	8.06	8.02	0.66	0.65
70	30	8.08	8.18	8.14	0.65	0.66
65	35	8.20	8.28	8.24	0.65	0.67
55	45	8.41	8.48	8.42	0.64	0.69
45	55	8.60	8.64	8.57	0.63	0.71
40	60	8.69	8.71	8.64	0.63	0.73
30	70	8.84	8.84	8.76	0.62	0.76
20	80	8.98	8.96	8.88	0.61	0.79
10	90	9.11	9.10	8.99	0.60	0.83

[a] Formula: Boric acid solution(0.2 *M*): boric acid, 12.404 g.; de-ionized water up to 1000 ml. (pH 4.96 at 25°). Sodium tetraborate solution(0.05 *M*): $Na_2B_4O_7 \cdot 10H_2O$(borax), 19.108 g.; de-ionized water up to 1000 ml. (pH 9.22 at 25°). [b] Original values reported in *Reference* 6. The original formulation contained 0.2 *M* sodium chloride in the boric acid solution. [c] The tonicity was calculated by Goyan and Hind (4).

quent solutions of unknown tonicity was readily interpretable in terms of percent sodium chloride. The sodium chloride equivalents of the buffer components were also checked individually at different concentrations in a similar manner.

RESULTS AND DISCUSSION

Human Serum Studies—In this study it was found that human serum has an average osmotic concentration equivalent to a 0.88% sodium chloride solution at 37°. For a breakdown of individual values and the extent of individual variation, Table I should be reviewed. The average value obtained corresponded quite well to the officially accepted work of Krough *et al.* (3) and thus for practical compounding purposes the commonly accepted reference value of 0.90% sodium chloride was employed throughout this study as the base line for preparing isotonic solutions.

Palitzsch Borate Buffer—The components of the modified Palitzsch Buffer (0.2 *M* boric acid and 0.05 *M* borax) were studied individually at their stock solution concentration at 37° to note the colligative effect elicited by these components at this temperature. The sodium chloride equivalent value (*E* values) determined from VPO data and reported from FPD (1, 2) for these two components can be found in Table II. Table II shows that the *E* value of boric acid determined in this study at 37° is only slightly higher (approximately 6% higher) than that determined at 0°. However, the *E* value determined for sodium tetraborate using the VPO differs quite substantially with the values reported from cryoscopic data. The *E* value at 37° is approximately 0.45 at the stock solution's concentration while the *E* value at 0° assigned to sodium tetraborate at the same concentration is 0.35. This indicates that there are approximately 20 to 25% more particles in solution at 37° than at 0°. It therefore follows that a solution of sodium tetraborate that is prepared isotonic from FPD data will in effect exhibit considerable hypertonic activity when used ophthalmically. Because sodium tetraborate in solution exists as different aggregates depending on concentration (*E* values at 0° vary from 0.48 at 0.5% and 0.37 at 2.6%) and temperature, this behavior is expected since the extent of solute aggregation as a general rule is less at elevated temperatures.

The 14 modified Palitzsch buffers generated from these two stock solutions were re-evaluated for pH and tonicity at 37°. Table III is a compilation of the pH and tonicity values reported in the literature and determined experimentally for the specified Palitzsch buffer mixtures that were modified by Goyan and Hind (4). A comparison of the literature values cited by Goyan and Hind with the experimental values elucidates two apparent discrepancies. The discrepancy that exists between pH values cited in the literature and those determined experimentally can be attributed to the fact that the values reported were not experimentally determined but rather transcribed from the original formulation (6). In their transcription the authors (4) retained the original pH values but diminished the concentration of sodium chloride found in the acid stock solution from 0.2 *M* to a concentration calculated for isotonicity. Diminution of sodium chloride naturally increased pH values as demonstrated experimentally because of the decreased ionic strength. The Debye-Huckel theory predicts such a change. Table III also indicates that the pH at 25 and 37° differs slightly with the lower pH mixtures which have little or no temperature effect while higher pH values have a slightly negative coefficient due to increased concentration of borax.

In Table III it can be seen that the tonicity values of the buffer mixtures calculated by Goyan and Hind (4), using freezing point depression data, disagree with experimental data collected at 37°. In fact, the two authors predicted a decrease in tonicity when a portion of boric acid was replaced with borax. However, as can be seen, experimental evidence indicates just the opposite. Experimental tonicity data show that a mixture, predominantly boric acid (97 to 3 ratio) had a lower tonicity value than that calculated and, as already indicated, increased rather than decreased tonicity with the replacement of boric acid with borax. It should be pointed out that an increase in tonicity upon the replacement of a 0.2 *M* boric acid solution by a 0.05 *M* borax solution requires that borax must dissociate to such an extent that it generates at least four times the number of species than that to which boric acid dissociates. Since both the literature values and experimental tonicity values change in magnitude for each buffer mixture but opposite in direction, there is a point where the literature and experimental values approxi-

Table IV—Test of Reported Tonicities of Modified Palitzsch Buffers[a]

0.2 *M* Boric Acid Solution, ml.	0.05 *M* Sodium Tetraborate Solution, ml.	Tonicity of Buffer Components Lit.[b]	Tonicity of Buffer Components Exptl.	NaCl to Render Isotonic(Lit.)[c] (mg./100 ml.)	Tonicity of Resulting Solution Lit.[c]	Tonicity of Resulting Solution Exptl. (37°)
94	6	0.68	0.63	220	0.9	0.86
85	15	0.67	0.64	230	0.9	0.87
70	30	0.65	0.66	250	0.9	0.91
40	60	0.63	0.73	270	0.9	0.99
10	90	0.60	0.82	300	0.9	1.11

[a] Formula: Boric acid solution (0.2 *M*): boric acid, 12.404 g.; de-ionized water up to 1000 ml. (pH 4.96 at 25°). Sodium tetraborate solution(0.05 *M*): $Na_2B_4O_7 \cdot 10H_2O$(borax), 19.108 g.; de-ionized water up to 1000 ml. (pH 9.22 at 25°). [b] The reported tonicity was calculated by Goyan and Hind (4) using a method devised by Wells (7). [c] The amount of sodium chloride added to make the mixture isotonic was determined by Goyan and Hind (4) by merely subtracting the calculated buffer mixtures' tonicities from 0.9% sodium chloride, but was not experimentally validated.

Table V—pH and Tonicity Values for Palitzsch Buffer Mixtures Corrected to Be Isotonic[a]

0.2 *M* Boric Acid Solution, ml.	0.05 *M* Sodium Tetraborate Solution, ml.	Revised NaCl Added, mg./100 ml.	Tonicity of Revised Buffer at 37°	Exptl. pH	
				25°	37°
97	3	270	0.91	6.82	6.80
94	6	270	0.90	7.16	7.13
90	10	270	0.91	7.44	7.41
85	15	260	0.90	7.67	7.64
80	20	260	0.90	7.85	7.81
75	25	250	0.90	7.98	7.95
70	30	240	0.89	8.12	8.11
65	35	230	0.90	8.23	8.20
55	45	210	0.89	8.43	8.39
45	55	190	0.90	8.60	8.54
40	60	180	0.90	8.67	8.62
30	70	140	0.90	8.81	8.76
20	80	110	0.90	8.95	8.88
10	90	70	0.90	9.08	8.99

[a] Formula: Boric acid solution (0.2 *M*): boric acid, 12.404 g.; de-ionized water up to 1000 ml. (pH 4.96 at 25°). Sodium tetraborate solution (0.05 *M*): $Na_2B_4O_7 \cdot 10H_2O$(borax), 19.108 g.; de-ionized water up to 1000 ml. (pH 9.22 at 25°).

mate each other. This occurs at a 75–25 mixture of boric acid–sodium tetraborate. This unforseen reversed trend of tonicity values for the buffer mixtures indicates clearly that experimental data is essential in evaluating complex borate systems such as Palitzsch buffer mixtures.

Table IV represents selected modified Palitzsch buffer rendered isotonic using the calculated quantities of sodium chloride suggested by Goyan and Hind (4). It should be noted that the resulting mixtures, supposedly rendered isotonic, varied, from being hypotonic to hypertonic. Table V lists the modified Palitzsch buffer reformulated to be isotonic using VPO data. The pH values at 25 and 37° for the newly formulated buffers are also listed in Table V.

Boric Acid Buffer—The official buffer was found to be hypertonic at 37°, having a tonicity of 0.95% at the concentration suggested in the pharmaceutical compendiums (8, 9). To adjust the buffer to be isotonic with body fluids, an *E* value was determined at 37° from a series of solutions of different concentrations of boric acid. The *E* value at this temperature was unchanged over a concentration range of 0.5 to 3.0% and was equal to 0.51. On the other hand the reported *E* values determined from FPD (1, 2) vary with concentration (*E* value at 0.5% is 0.51 and at 1.9% is 0.47) and is equal to 0.47 at the concentration suggested to be isotonic at 0° by the official compendiums (8, 9). From the *E* value determined with the VPO, 17.7 g. instead of the officially recommended 19.0 g. should render 1000 ml. of water isotonic with body fluids. Experimentally it was found that a 1.77% boric acid solution is equivalent to 0.90% sodium chloride at 37°. The pH of this newly formulated isotonic boric acid buffer was found to be 4.96 at 25° and 4.85 at 37°.

Table VI—Sorensen Buffer Re-evaluated for pH and Tonicity[a]

Acid Stock Solution, ml.	Alkaline Stock Solution, ml.	pH at 25°			Exptl. pH at 37°	Tonicity	
		Lit.[b]	Calcd.	Exptl.		Exptl. at 37°	Lit.[c] at 0°
90	10	5.91	5.92	5.90	5.88	0.41	0.38
80	20	6.24	6.25	6.25	6.23	0.42	0.39
70	30	6.47	6.47	6.47	6.45	0.43	0.40
60	40	6.64	6.64	6.65	6.64	0.44	0.41
50	50	6.81	6.80	6.81	6.81	0.45	0.42
40	60	6.98	6.96	6.98	6.97	0.46	0.44
30	70	7.17	7.14	7.19	7.17	0.47	0.45
20	80	7.38	7.36	7.42	7.41	0.49	0.46
10	90	7.73	7.70	7.79	7.77	0.50	0.47
5	95	8.04	8.02	8.13	8.13	0.51	0.48

[a] Formula: Acid stock solution: sodium acid phosphate (monohydrate), 9.208 g.; de-ionized water up to 1000 ml. Alkaline stock solution: sodium phosphate (anhydrous), 9.470 g.; de-ionized water up to 1000 ml. [b] Values reported by Sorensen (10) in 1909. [c] Values determined cryoscopically by Goyan and Hind (4).

Table VII—Modified Sorensen Buffer Re-evaluated for pH and Tonicity[a]

Acid Stock Solution, ml.	Alkaline Stock Solution, ml.	NaCl Added to Render Solution Isotonic(Lit.)[b] (mg./100 ml.)	pH at 25°		pH at 37° Exptl.	Tonicity at 37°[c]
			Calcd.	Exptl.		
90	10	520	5.81	5.76	5.74	0.92
80	20	510	6.15	6.12	6.10	0.92
70	30	500	6.37	6.35	6.33	0.92
60	40	490	6.56	6.54	6.53	0.92
50	50	480	6.72	6.72	6.71	0.92
40	60	460	6.89	6.89	6.88	0.92
30	70	450	7.08	7.09	7.08	0.92
20	80	440	7.30	7.33	7.32	0.92
10	90	430	7.65	7.70	7.69	0.92
5	95	420	7.97	8.07	8.05	0.92

[a] Formula: Acid stock solution: sodium acid phosphate(monohydrate), 9.208 g.; de-ionized water up to 1000 ml. Alkaline stock solution: sodium phosphate (anhydrous), 9.470 g.; de-ionized water up to 1000 ml. [b] The amount of sodium chloride added to make the mixture isotonic was determined by Goyan and Hind (4) by merely subtracting the calculated buffer mixtures' tonicities from 0.9% sodium chloride, but was not experimentally validated. [c] Literature value assumed to be 0.90 by Goyan and Hind (4).

Sorensen Buffer—Table VI lists the literature and calculated values along with the experimentally determined values of pH for the 10 official phosphate buffer mixtures. It can be seen that the pH values reported by Sorensen (10) and cited by Goyan and Hind (4) in their formulation of isotonic phosphate buffer mixtures which have been transcribed into the USP (8) and NF (9) agree quite well with the experimental values obtained in this study at 25°. However, it should be pointed out that the pH values found in the USP (8) and NF (9) are somewhat ambiguous since they do not represent isotonic buffers' pH values as they might lead one to suspect; but instead represent the buffer mixtures' pH before the addition of the appropriate amounts of sodium chloride needed to render the solution isotonic. A rigorous approach involving all phosphate equilibria, mass balance, and charge balance was employed in calculating the pH. Because of the complexity of the relationship that results from such a rigorous approach, a computer (IBM-1130) was used to perform the required Newton-Raphson iteration. It can also be noted in Table VI that tonicity values calculated from freezing point depression data of the buffer mixtures are somewhat lower than those determined experimentally with the osmometer at 37°. This seems to indicate that at higher temperatures the ability of phosphate species to associate is slightly diminished. This was found to be true when the phosphate salts' E values were reviewed. Table II illustrates that the E values of both phosphate salts are 5 to 10% higher at 37° than at 0°. The pH values of the buffer mixtures at 37° are also included in Table VI.

The compilation of the data obtained in the re-evaluation of the official isotonic phosphate buffer found in Table VII demonstrates that the 10 buffer mixtures that were calculated to be isotonic at 0° are slightly hypertonic at 37°. The resulting solutions were hypertonic due to the low tonicity values assigned to the buffer mixtures which dictated the quantities of sodium chloride that would be necessary to render the buffers isotonic. The pH values for these buffer mixtures at 25 and 37° are listed in Table VII. The USP (8) and NF (9) do not list such values; however, it was thought more important to know the pH of the resulting mixtures than the pH of the buffer mixture itself.

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Determination of Micellar Weights for Di-Alkyl Sodium Sulfosuccinates in Anhydrous and Hydrous Hydrocarbon Solutions

SYLVAN G. FRANK* and GEORGE ZOGRAFI

Abstract □ Previous studies have demonstrated that di-(2-ethylhexyl) sodium sulfosuccinate, dissolved in various hydrocarbon solvents, is capable of solubilizing large amounts of water, whereas closely related compounds such as the di-*n*-octyl and di-*n*-hexyl derivatives exhibit negligible solubilizing capacity. In view of these observations the micellar size of these three compounds in *n*-octane was measured by light scattering. Large differences in micelle weight between di-(2-ethylhexyl) sodium sulfosuccinate in *n*-octane (13,000) and the other compounds (about 200,000) suggested a significant role for the 2-ethyl side chain. The active participation of water in the organization of these micelles is suggested by significant changes in micellar weight, well in excess of that accounted for by the added amount of water. The influence of different hydrocarbon solvents on micellar weight also was found to be quite significant.

Keyphrases □ Di-Alkyl Na sulfosuccinates—micellar weights □ Micellar weight determination—anhydrous, hydrous hydrocarbon systems □ Cohesive energy—micellar size □ Light scattering spectrometry—micellar weights □ Refractive index—micellar solutions

The present study is part of an overall effort to gain an understanding of the complex equilibria produced when relatively large amounts of water and lipids or oils are dispersed in the presence of surface-active micelle-forming molecules. Of particular concern, presently, is the ability of surface-active agents to solubilize water in nonpolar solvents. Pharmaceutical situations where this may be important include the formulation of clear oil-soluble liquids or gels to be applied to the skin, or the formulation of nonaqueous solutions for the depot injection of water-soluble allergenic extracts and vaccines. More basic still is the application of such knowledge toward an understanding of the properties of water at interfaces, particularly biological interfaces such as membranes.

Previous studies have demonstrated that di(2-ethylhexyl) sodium sulfosuccinate[1] dissolved in various hydrocarbon solvents is capable of solubilizing relatively large amounts of water (1–4), comparable to that solubilized by lecithin (5) and the more complex micro-

[1] Supplied commercially as Aerosol OT by the American Cyanamid Co.