Biochem. J. (1981) **195**, 259–262
Printed in Great Britain

A common source of error in pH measurements

John A. ILLINGWORTH
Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.

(*Received 27 October 1980/Accepted 17 December 1980*)

Glass-electrode assemblies in which the reference half-cell contains a porous ceramic type of liquid junction are likely to produce misleading pH measurements under normal service conditions. The error arises from substantial liquid-junction potentials, associated with the porous ceramic plug, which vary with the ionic composition of the solution under test. The error is not revealed by conventional two-point calibration procedures, since the majority of standard buffer solutions have a similar total ionic strength, but will nevertheless be present when the unknown solution differs in ionic strength from the standardizing buffers. The size of the error is proportional to the ratio between the salt concentration in the standard buffers and the concentration present in the unknown solution, and varies from one electrode specimen to another. The fault was present in 24 out of 30 electrodes in normal use selected at random from seven laboratories, and the mean error was 0.2pH unit per 10-fold salt-concentration difference between standard and test solutions. It is estimated that errors of this order must be widespread in the recent literature. Older pH determinations are likely to be more reliable, since the original reference electrode design with a free-flowing liquid junction is apparently free from the artefact.

The combined type of pH-measuring electrode has now almost completely displaced the older design involving separate glass and reference electrodes for all but the most exacting applications. Although it is widely acknowledged by electrode manufacturers (e.g. Westcott, 1976) that difficulties may occasionally arise with the porous ceramic type of liquid junction, which is an almost universal feature of combined-electrode designs, the majority of investigators seem to regard these problems as obscure trivial effects that only affect a small minority of workers. I was abruptly displaced from this complacent position while attempting to measure rates of metabolic CO_2 production by a perfused heart preparation, and it is the purpose of the present paper to report that liquid-junction artefacts are apparently a widespread source of substantial errors in the majority of laboratory pH determinations.

The nature of the error ensures that it will not be detected during conventional electrode-calibration procedures. The size and frequency of the effect must make it a prime suspect when difficulties are encountered in reproducing results in different laboratories.

Materials and methods

All reagents were AnalaR grade and supplied by BDH Ltd., Poole, Dorset, U.K. The buffer systems used are listed in Table 1. Buffers 1, 5 and 9 are American National Bureau of Standards formulations for which detailed pH information is widely available (Dawson *et al.*, 1969; Bates, 1973; Robinson, 1974; Durst, 1975). Published data on the remaining buffers, together with the effects of dilution, are taken from Bates (1973).

In order to provide a wholly independent test of buffer accuracy, I measured the electronmotive force (e.m.f.) of a hydrogen electrode in contact with buffers 2–9, by using a saturated-KCl calomel (Hg_2Cl_2) reference electrode at 25°C, and calculated the pH by using a value of 0.2434 V for the calomel-half-cell potential. I also measured the pH of the other solutions relative to buffer 5 (which served as overall primary standard) by using a new glass electrode (Russell type SWL) and an Ag/AgCl reference electrode that did not contain a ceramic/liquid junction. This reference electrode, which was designed to produce the lowest attainable liquid-junction potentials, consisted of a silver wire (chloridized by brief anodic oxidation in 10mM-HCl) suspended in 3M-KCl saturated with AgCl. Contact between this electrode and the buffer under test was achieved via a nylon capillary (30cm × 0.5mm), and the efflux of KCl was restricted to a suitably low value by controlling the admission of air to the electrode reservoir. The saturated KCl/calomel reference electrode differed in potential from

0306-3275/81/040259-04$01.50/1 © 1981 The Biochemical Society

FORMYCON-1105

Table 1. *Buffer compositions and measured properties*

E.m.f. data were obtained with a hydrogen electrode and saturated KCl/calomel reference half-cell. The buffer pH calculated in column 2 is based on a value of 0.2434 V for the calomel-electrode potential. Published pH data are taken from Bates (1973), and an asterisk (*) indicates interpolation between tabulated values. Measured pH values are based on the standard value of pH 6.865 for buffer 5, and were obtained with a new glass electrode and an Ag/AgCl reference half-cell with a flowing-liquid junction. The observed error values in the final column refer to a defective 'used' electrode combination that was selected for further tests. All measurements were made at 25°C.

Buffer no.	Composition	E.m.f. (V)	pH value: Calculated	pH value: Published	pH value: Measured	Observed error
1	50 mM-Potassium hydrogen phthalate			4.008	4.020	+0.030
2	100 mM-Acetic acid/100 mM-sodium acetate	0.5195	4.680	4.652	4.660	+0.040
3	10 mM-Acetic acid/10 mM-sodium acetate	0.5235	4.747	4.718	4.730	−0.220
4	250 mM-KH_2PO_4/250 mM-Na_2HPO_4	0.6280	6.519		6.520	+0.355
5	25 mM-KH_2PO_4/25 mM-Na_2HPO_4	0.6480	6.858	6.865	6.865	
6	2.5 mM-KH_2PO_4/2.5 mM-Na_2HPO_4	0.6610	7.078	7.075*	7.065	−0.470
7	100 mM-Tris base/100 mM-Tris chloride	0.7265	8.188	8.201*	8.155	−0.095
8	10 mM-Tris base/10 mM-Tris chloride	0.7225	8.120	8.125*	8.130	−0.435
9	10 mM-Disodium tetraborate	0.7855	9.188	9.180	9.175	−0.305

the 3 M-KCl/AgCl electrode by 35 mV when both were immersed in either saturated KCl or buffer 6 at 25°C, which is in close agreement with theoretical expectation (37 mV) after making due allowance for the differing KCl concentrations. It therefore seems most unlikely that either of these two 'standard' reference electrodes was seriously defective.

Glass-electrode assemblies and pH-meters were kindly loaned by colleagues in my department, and in six neighbouring University of Leeds Life Sciences departments. Of the electrodes, thirty-eight were combination type, three had separate reference half-cells and all employed a ceramic/liquid junction. Five different manufacturers were represented in the sample (Beckman, Corning, Pye–Ingold, Radiometer and Russell). Eleven of the electrodes were new, whereas the remaining thirty had been in service for periods ranging from a few weeks to several years, and as far as practicable represent an unbiased sample of the generality of laboratory pH-electrodes in current use at the University of Leeds.

All the electrodes were tested by using the following protocol at ambient temperature (20–25°C). The electrode was suspended in National Bureau of Standards phosphate (buffer 5) until the reading stabilized, and the meter zero was then adjusted so as to achieve an accurate result. Buffers 1, 4, 6 and 9 were then tested in random order, the electrode being rinsed with distilled water between each measurement, and checks being made frequently for zero shifts by returning to buffer 5. Several electrodes exhibited a prolonged slow drift in some of the buffers, and in these cases I accepted the reading after several minutes when the drift had slowed to an almost imperceptible rate, at the point where I considered that other reasonable investigators would have behaved similarly. If drifting was a problem, the electrode was invariably re-equilibrated in buffer 5 and the meter zero re-adjusted if necessary before proceeding further. The temperature compensator on the pH-meter was always set to the actual ambient temperature and the electrode slope adjustment (where provided) to 58 mV/pH unit at 20°C throughout these experiments.

The entire procedure was then repeated for 18 'used' electrodes, chosen at random, with the integral reference electrode and associated porous plug disconnected from the measuring circuit, and replaced by the substitute Ag/AgCl half-cell (described above) that lacked a ceramic junction.

Several electrode assemblies that showed large errors were then selected for further investigation. The liquid-junction potentials produced by the reference half-cells were measured relative to the substitute half-cell with no ceramic junction in equimolar KH_2PO_4/Na_2HPO_4 over a 500-fold range of phosphate concentrations. The errors produced by these electrodes were also compared in all nine buffer systems in order to ascertain whether one particular ion was responsible for the problem.

Results

My findings are summarized in Table 2. It is apparent that removal of the ceramic/liquid junction resulted in a highly significant improvement in accuracy over the entire range tested, but that the effect with the 5 mM- and 500 mM-phosphate buffers was particularly marked. This strongly suggests that the nature of the error is in some way dependent on the ratio between the salt concentration in the standard buffer and the concentration in the solution under test, particularly since the error changes sign on passing from 5 mM- to 500 mM-buffer.

This hypothesis was confirmed directly by measuring the liquid-junction potential associated

Table 2. *New- and used-electrode performances*

The pH readings obtained at ambient temperature were corrected, where necessary, to a constant temperature of 25°C with the aid of National Bureau of Standards temperature coefficients of phthalate, phosphate and borate buffers. The data have been expressed ± S.D. to demonstrate the improved accuracy and reproducibility of measurements taken with the substitute half-cell lacking a ceramic plug. The 'best' pH estimates represent my opinion in the light of the data shown in Table 1.

	pH reading				
Buffer no. (see Table 1) ...	1	4	5	6	9
Composition ...	50 mM-Phthalate	500 mM-Phosphate	50 mM-Phosphate	5 mM-Phosphate	10 mM-Borate
Electrode sample					
'Used' electrodes In normal service ($n = 30$)	4.045 ± 0.083	6.711 ± 0.113	6.865	6.860 ± 0.133	9.049 ± 0.113
With substitute reference half-cell ($n = 18$)	4.013 ± 0.028	6.518 ± 0.014	6.865	7.061 ± 0.013	9.175 ± 0.051
New electrodes with integral reference half-cell ($n = 11$)	4.062 ± 0.063	6.594 ± 0.088	6.865	7.001 ± 0.057	9.079 ± 0.139
'Best' pH estimate	4.008	6.520	6.865	7.070	9.180

with the porous ceramic plug. Typical results are shown in Fig. 1. It is apparent that a near-linear relationship existed between liquid-junction potential and log (salt concentration), with a slope in the example shown of 34 mV per decade, which is in reasonable agreement with the error of 0.52 unit in the pH measurements. Strictly speaking, a small part of the e.m.f. measured in Fig. 1 might have arisen from electrolyte concentration effects (if the 'defective' electrode had been filled with a solution other than 3 M-KCl) or from a liquid-junction potential associated with the 'standard' reference electrode, rather than the 'defective' electrode under test. It is unlikely that these additional contributions would exceed 10 mV (Bates, 1973) and the bulk of the measured potential can therefore be ascribed to the porous ceramic plug on the 'defective' electrode. It should be noted that all of the 'defective' electrodes tested were of the Ag/AgCl type, so that the possibility of a difference in standard electrode potentials does not arise.

Several electrodes were tested by using all nine buffer systems, and typical results appear in the last column of Table 1. It was apparent that errors were not confined to one particular buffer system, and that the largest artefacts were produced when the salt concentration in the test solution differed considerably from the salt present in the standard buffer no. 5.

Results from the new electrodes were obviously better than those from used electrodes, although several electrodes were defective when unpacked. I have no detailed time-course information for electrodes in laboratory service, but it is clear that the artefacts described here can appear relatively quickly, certainly within a matter of weeks.

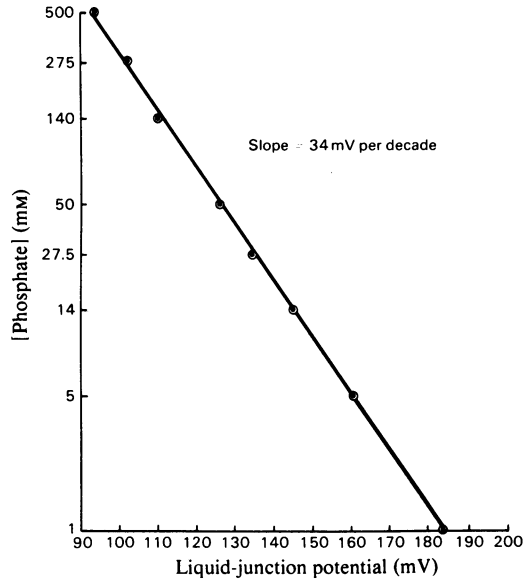

Fig. 1. *Liquid-junction potential produced by a defective reference electrode*

The measurements were made at 25°C by using the substitute Ag/AgCl electrode with the flowing liquid junction to complete the circuit. The two electrodes were immersed in a series of dilutions prepared from buffer 4.

Two other improvements were also noted after eliminating the porous plugs, in that electrode drift or hysteresis was almost abolished, and the results became much less dependent on stirring speed. It is my experience that these two most irritating effects are diagnostic of serious reference-electrode malfunction.

Discussion

The question of an acceptable error in pH determinations plainly depends on the application in view. Third-decimal accuracy is feasible with care, and the standards are defined to this level, although for the majority of numerical calculations, second-decimal accuracy would be sufficient. Many laboratory operations are relatively insensitive to pH, and for the bulk of preparative and analytical work an error of ± 0.1 pH unit would be entirely acceptable. Only six out of thirty 'used' electrodes tested achieved even this modest degree of accuracy. The mean error after a 10-fold change in buffer concentration was 0.2 pH unit, and many electrodes performed far worse than this. Particularly serious problems are to be expected during isoelectric-focusing experiments, where pH measurements are necessarily made at very low ionic strength, and in ion-exchange chromatography and electrophoretic separations, where the sample is commonly applied in very dilute buffer, at an 'accurately' known pH. Difficulties will also arise in tissue-culture and organ-perfusion experiments, since the salt concentration in physiological media is three times higher than that in the standard buffers used to calibrate the electrode. It is fortunate that the majority of physiological media were devised with an older type of reference electrode that was capable of giving the correct answer at physiological salt concentrations. It should be noted that modern blood-gas analysers normally incorporate special reference electrode designs that eliminate the error described here.

A particularly insidious aspect of the present artefact arises from the near-constant ionic strength of the most widely used standard buffers, a situation that leads to a fortuitous cancellation of liquid-junction errors when two or more standard buffers are compared. This has plainly lulled many investigators (including myself) into a false sense of security for many years. The enormous errors evident in the last column of Table 1 were produced by an electrode assembly that gave an entirely adequate response in both phthalate and phosphate standard buffers, and that was only discarded as a result of the present survey. Previous studies have suggested that artefacts were more likely with Tris buffers (Ryan, 1969), but it is clear from Table 1 that Tris is in no way unique, and that it is the salt concentration that is the crucial factor.

It is the variation from one electrode to the next that is likely to cause the greatest difficulty in practice, and in this connection it is noteworthy that the new electrodes were better than the 'used' specimens, probably because the new porous ceramic plugs were thoroughly impregnated with 3 M-KCl, so that the liquid junction actually formed at the surface of the plug rather than deep within it. Support for this interpetation came from two old electrodes that actually gave quite satisfactory results, but were excluded from the survey because they were not in routine use at the time of the tests. Both had been discarded by their owners as unserviceable, and allowed to dry out on the bench with the result that solid KCl had crystallized around the ceramic plug and on the pH-sensitive membrane. This treatment clearly resulted in a considerable improvement, though it is doubtful if it can be recommended as a routine procedure!

All the glass-electrode half-cells tested gave good results with the substitute reference electrode that had no ceramic plug, despite the presence of numerous abrasions resulting from long periods of heavy use. It would appear that modern glass half-cells can be relied upon to give accurate results for many years, until the membrane is finally smashed by an accidental blow. Most electrodes were tested with their usual amplifier, but six were re-tested using my own pH-meter with essentially the same results. There was never any occasion to suspect the amplifiers, and the fact that every electrode tested gave an adequate response with its usual amplifier and the substitute reference electrode indicates that amplifier malfunction is relatively uncommon. It is hoped that the present paper will stimulate manufacturers to improve their reference-cell designs, for until this is achieved it is apparent that many current pH measurements as well as those published over the last 10–15 years should be regarded with a certain degree of suspicion. In the meantime it is relatively easy to test an electrode for the problem described here by diluting National Bureau of Standards equimolar phosphate (buffer 5) (1 vol.) with distilled water (9 vol.) and checking the apparent pH. The meter should read pH 7.065 ± 0.01, at 25°C in the diluted buffer, and 6.865 in the full-strength solution.

References

Bates, R. G. (1973) *Determination of pH*, 2nd edn., Wiley–Interscience, New York

Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research*, 2nd edn., pp. 475–508, Oxford University Press, London

Durst, R. A. (1975) *Standardisation of pH measurements* (*National Bureau of Standards Special Publication no. 260–53*), U.S. Government Printing Office, Washington, D.C.

Robinson, R. A. (1974) in *Handbook of Chemistry and Physics*, 55th edn. (Weast, R. C., ed.), pp. D112–D119, CRC Press, Cleveland, OH

Ryan, M. F. (1969) *Science* **165**, 851

Westcott, C. C. (1976) *Beckman Technical Information Bulletin no. 987-EC-76-7T*, Beckman Scientific Instruments Division, Irvine, CA