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ISOELECTRIC FOCUSING AS A METHOD FOR THE CHARACTERIZATION OF AMPHOLYTES

II. pH MEASUREMENTS IN SOLVENT MIXTURES USED IN DENSITY-GRADIENT ISOELECTRIC FOCUSING

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SUMMARY

When pH values are measured in mixed aqueous-organic solvents by means of the usual electrometric procedure, a correction (δ) must be applied in order to obtain physically meaningful pH values (*i.e.*, values that can be interpreted as the negative logarithm of the hydrogen ion activity). δ values have been determined in sucrose-water, glycerol-water and ethylene glycol-water mixtures at 25° and 4°, for use in density-gradient isoelectric focusing.

INTRODUCTION

In a previous paper¹, we drew attention to the shortcomings of isoelectric focusing as a method for the characterization of ampholytes. It was shown that only those variants of the technique in which the solvent composition of the focused zones is standardized (*e.g.*, zone convection, gel and thin-layer isoelectric focusing) are useful as characterization methods. In these variants, the standard deviation of a measured pI value is governed by the temperature difference between the final focusing temperature and the temperature of the pH measurement. Its magnitude was estimated to be about 0.005 pH unit per degree of temperature difference if the carrier ampholytes employed are matched to the investigated ampholytes with respect to their protolytic groups.

For density-gradient isoelectric focusing, we showed that, in addition, systematic and random errors are introduced as a result of the influence of the solvent composition on the isoelectric point of an ampholyte and upon the difference between the measured pH and $pa_{H^+}^*$. Definite conclusions about the importance of these errors

* An asterisk is used to denote that the quantity under consideration (here the activity of H⁺ ions) is referred to an infinitely dilute solution in the same solvent. When the asterisk is omitted, the quantity under consideration is referred to an infinitely dilute solution in water (or is considered in a general way).

were precluded by the lack of data pertinent to the most utilized solvents in isoelectric focusing, *viz.*, sucrose-water, glycerol-water and ethylene glycol-water mixtures, although it was argued that their importance may be far greater than that due to temperature differences.

In this paper, we present electrometric measurements of $\delta = \text{pH} - \text{p}a_{\text{H}}^*$ in these solvents, with the aid of which the measured pH value of a focused zone in the solvents of interest can be converted into the corresponding $\text{p}a_{\text{H}}^*$ value, if the solvent composition in the zone is known. This enables one to make a reliable comparison of measured isoelectric points of the same ampholyte in different solvent mixtures.

THEORETICAL

It is well recognized that pH values of solutions in non-aqueous or partly aqueous solvents, obtained by using the operational pH definition and aqueous standard buffer solutions for the calibration of the pH meter, cannot be identified with $\text{p}a_{\text{H}}^*$, but differ from it by a quantity δ . This quantity δ accounts for the influence of the solvent on both the liquid junction potential, E_j , at the tip of the calomel electrode and the standard potential, E_{glass}° , of the glass electrode:

$$\delta \equiv \text{pH} - \text{p}a_{\text{H}}^* = \frac{(E_j^* - E_j) - (E_{\text{glass}}^{\circ*} - E_{\text{glass}}^\circ)}{2.303 RT/F} \quad (1)$$

where R represents the molar gas constant, T the absolute temperature and F the Faraday constant.

δ values can be easily measured, but they can be used universally for the calculation of $\text{p}a_{\text{H}}^*$ values from measured pH values only if three conditions are fulfilled:

(1) $E_j^* - E_j$ should not depend on the nature of the (buffering) solutes in the standard and sample solutions. In methanol-water and ethanol-water mixtures, this condition has been proved²⁻⁴ to be generally valid for (buffer) solutions with pH (in water) ranging from 2.5 to 10 and for contents of the organic component up to about 70% of methanol and 100% of ethanol.

(2) $E_j^* - E_j$ should not depend upon the type of the device forming the liquid junction at the tip of the calomel electrode. This condition has also been proved⁵ to be generally valid in methanol-water and ethanol-water mixtures.

(3) $E_{\text{glass}}^{\circ*} - E_{\text{glass}}^\circ$ should not depend upon the type of glass electrode. In an investigation⁶ of the validity of this condition we found, in methanol-water and ethanol-water mixtures, a slight dependence of $E_{\text{glass}}^{\circ*} - E_{\text{glass}}^\circ$ on the method of fabrication and pre-conditioning in the laboratory (rather than on the composition of the glass). This means that the use of average δ values, holding for all glass electrodes, results in a rather large error (about 0.04 pH unit).

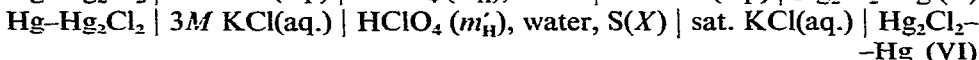
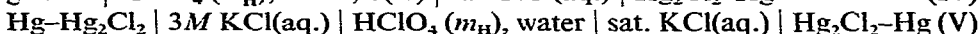
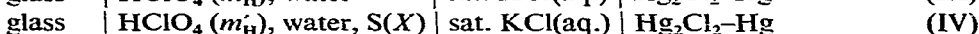
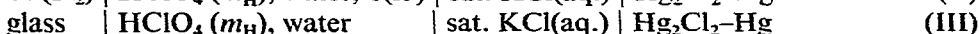
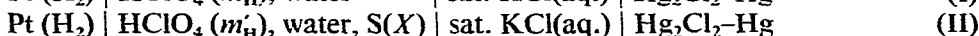
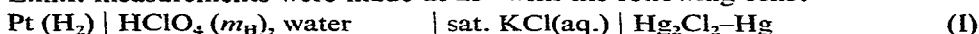
Douhéret^{7,8} measured δ values in solutions of hydrochloric acid of various concentrations in a series of partly aqueous solvents, using a glass electrode of a manufacture not included in the above-mentioned investigation⁶. In methanol-water, ethanol-water, ethylene glycol-water and 2-ethoxyethanol-water mixtures δ appeared to be a constant, characteristic of the solvent composition and independent of the acid concentration at $\text{p}a_{\text{H}}^* > 3$. Moreover, the differences between his δ values in

methanol-water and ethanol-water mixtures and those measured by Geisema and co-workers^{5,6} ranged from 0 to 0.06 pH unit (depending on the solvent composition), which is in reasonable accordance with the variability of $E_{\text{glass}}^{c*} - E_{\text{glass}}^{\circ}$ values mentioned above.

All of this evidence points to the feasibility of obtaining meaningful pH values (*i.e.*, $p\alpha_{\text{H}}^*$ values) by simple correction of measured pH values with appropriate δ values also in the solvents used in isoelectric focusing. Therefore, we measured δ in sucrose-water, glycerol-water and ethylene glycol-water mixtures, for saturated and 3 M potassium chloride-calomel electrodes. We also measured the values of $\delta' \equiv (E_j^* - E_j)/2.303 (RT/F)$ and of $(E_{\text{glass}}^{c*} - E_{\text{glass}}^{\circ})/2.303 (RT/F)$ separately. These measurements were made at 25°, the standard temperature for pH and pK determinations, but, as many investigators perform pH measurements in isoelectric focusing experiments at low temperatures, some δ values were also measured at 4°.

EXPERIMENTAL

E.m.f. measurements were made at 25° with the following cells:



where $m_{\text{H}} \approx m'_{\text{H}} \approx 0.001 \text{ mole} \cdot \text{kg}^{-1}$, S represents sucrose, glycerol and ethylene glycol, respectively, and X, representing the weight percentage of component S, has the values 15, 30, 45 and 60% for sucrose and 20, 40, 60 and 80% for glycerol and ethylene glycol. The following chemicals were used: perchloric acid (Merck, Darmstadt, G.F.R., p.a. grade), sucrose (J. T. Baker, Phillipsburgh, N.J., U.S.A., analyzed grade), glycerol (Merck, p.a. grade) and ethylene glycol (Merck, p.a. grade). The design of cells I and III was given by De Ligny and Rehbach⁹.

The measurements were performed as follows. A stream of carbon dioxide and oxygen-free hydrogen was bubbled through the cell. When a stable e.m.f. value (E_I, E_{II}) had been reached (within 1 h), one of the two platinum electrodes was replaced with a glass electrode and the e.m.f. ($E_{\text{III}}, E_{\text{IV}}$) was measured. The glass electrode was kept in an aqueous buffer solution between the measurements. Details of the platinization of platinum electrodes and the purification of hydrogen were given elsewhere¹⁰. A Radiometer 4 pH meter and an Ingold glass electrode*, Type 10271/3005, were used. All e.m.f. measurements were measured in duplicate at least (see Tables I and II).

δ' values for the saturated calomel electrode were calculated by means of the equation

$$\delta'_{\text{sat}} \equiv \frac{E_j^* - E_j}{2.303 RT/F} = \frac{E_{\text{II}} - E_{\text{I}}}{2.303 RT/F} + \log \frac{m'_{\text{H}}}{m_{\text{H}}} + \log \frac{\gamma_{\text{H}}^*}{\gamma_{\text{H}}} \quad (2)$$

* The glass electrode had been given the pre-treatment prescribed by the manufacturer.

where γ_{H} and γ_{H}^* represent the activity coefficients of the hydrogen ion in water and in the partially aqueous solvent, respectively. These data were calculated by the equation

$$-\log \gamma_{\text{H}} (\gamma_{\text{H}}^*) = \frac{1.8144 \cdot 10^6 (DT)^{-3/2} (m_{\text{H}} d_0)^{\pm}}{1 + 201.21 (DT)^{-\pm} (m_{\text{H}} d_0)^{\pm}} \quad (3)$$

where D represents the dielectric constant of the solvent and d_0 its density. Values of D and d_0 were taken from the literature¹¹⁻¹⁴.

δ values for the saturated calomel electrode were calculated by means of the equation

$$\delta_{\text{sat}} \equiv \frac{(E_j^* - E_j) - (E_{\text{glass}}^* - E_{\text{glass}}^{\circ})}{2.303 RT/F} = \frac{E_{\text{IV}} - E_{\text{III}}}{2.303 RT/F} + \log \frac{m_{\text{H}}'}{m_{\text{H}}} + \log \frac{\gamma_{\text{H}}^*}{\gamma_{\text{H}}} \quad (4)$$

Values of $E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ}$ can then easily be found:

$$E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ} = 2.303 RT/F (\delta' - \delta) \quad (5)$$

E.m.f. measurements were also performed on the cells III and IV at 4°, at $m_{\text{H}} \approx m_{\text{H}}' 0.001 \approx \text{mole} \cdot \text{kg}^{-1}$ and with X representing 60% of sucrose, 80% of glycerol and 80% of ethylene glycol, respectively. The resulting δ values were calculated by using eqns. 3 and 4.

E.m.f. measurements on cells V and VI (E_{V} , E_{VI}) were made in quadruplicate. The difference of the δ values, holding for saturated and 3 M calomel electrodes, can be calculated as follows:

$$\delta_{\text{sat}} - \delta_{3\text{M}} = \frac{E_{\text{VI}} - E_{\text{V}}}{2.303 RT/F} \quad (6)$$

TABLE I

VALUES OF δ' , δ AND $(E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ})/2.303 (RT/F)$ AT 25° IN SUCROSE-WATER, GLYCEROL-WATER AND ETHYLENE GLYCOL-WATER MIXTURES

Solvent	X (wt.-%)	N	$-\delta'$ (pH units)	$-\delta$ (pH units)	$-(E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ})$
					$2.303 RT/F$ (pH units)
Sucrose-water	15	2	0.114 ± 0.004	0.104 ± 0.003	0.010 ± 0.005
	30	2	0.219 ± 0.001	0.210 ± 0.005	0.009 ± 0.005
	45	2	0.377 ± 0.004	0.333 ± 0.004	0.044 ± 0.006
	60	2	0.611 ± 0.017	0.505 ± 0.004	0.106 ± 0.017
Glycerol-water	20	3	0.134 ± 0.010	0.130 ± 0.010	0.004 ± 0.014
	40	3	0.278 ± 0.012	0.255 ± 0.010	0.023 ± 0.016
	60	3	0.466 ± 0.018	0.414 ± 0.017	0.052 ± 0.025
	80	3	0.780 ± 0.011	0.696 ± 0.007	0.084 ± 0.013
Ethylene glycol-water	20	2	0.113 ± 0.004	0.109 ± 0.003	0.004 ± 0.005
	40	2	0.211 ± 0.001	0.202 ± 0.002	0.009 ± 0.002
	60	2	0.318 ± 0.001	0.297 ± 0.003	0.021 ± 0.003
	80	2	0.667 ± 0.004	0.617 ± 0.016	0.050 ± 0.017

RESULTS

The values of $\delta_{\text{sat}} - \delta_{3M}$ did not differ significantly from zero. Values of δ , δ' and $(E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ})/2.303 (RT/F)$ at 25° are presented in Table I. The indicated errors are standard deviations calculated from replicate e.m.f. measurements; the number of replicates (N) is given in the table.

δ values are plotted as a function of the solvent composition in Fig. 1; the results obtained by Douh ret⁸ in ethylene glycol-water mixtures are included.

In Table II the δ values at 4° are given.

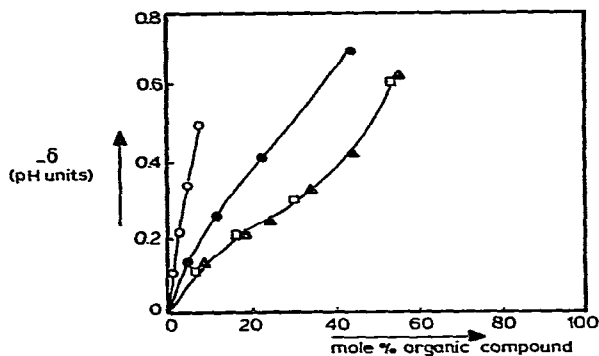


Fig. 1. Values of δ at 25° in sucrose-water (○), glycerol-water (●) and ethylene glycol-water (□) mixtures as a function of the mole fraction of the organic component. ▲, From ref. 8.

TABLE II

VALUES OF δ AT 4° IN 60% SUCROSE, 80% GLYCEROL AND 80% ETHYLENE GLYCOL

Solvent	X (wt.-%)	N	$-\delta$ (pH units)
Sucrose-water	60	3	0.475 ± 0.004
Glycerol-water	80	3	0.675 ± 0.006
Ethylene glycol-water	80	3	0.554 ± 0.004

DISCUSSION

It was demonstrated by Douh ret⁸ that δ values in mixtures of monovalent alcohols and ethylene glycol with water are related in a qualitative way to the Hammett acidity function¹⁵: low δ values correspond to a low basicity of the solvent mixture. As has been pointed out by Kalidas and Palit¹⁶, the relatively low basicity of ethylene glycol compared with monofunctional alcohols can be explained by the capacity of the molecules of ethylene glycol to form *inter*- and *intra*-molecular hydrogen bonds. As this tendency for intermolecular hydrogen bonding can be assumed to increase in the order ethylene glycol < glycerol < sucrose, δ values at equal mole fractions of the organic component would be expected to decrease in this order. Fig. 1 shows that the expected order obtains.

The values of $(E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ})/2.303 (RT/F)$ and their standard deviations, given in Table I, clearly demonstrate a significant influence of the solvent composition on the standard potential of the glass electrode in the three solvent systems studied. This result corroborates earlier findings in methanol-water and ethanol-water mixtures.

As can be seen in Fig. 1, the differences between the δ values in ethylene glycol-water mixtures given in Table I, and those measured by Douhéret⁸ range from 0 to about 0.05 pH unit. These differences are reasonable, in view of the fact that different glass electrodes were used in the two studies and in view of the established variability of $E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ}$ values between different glass electrodes in methanol-water and ethanol-water mixtures (see Theoretical). The absolute values of $(E_{\text{glass}}^{\circ*} - E_{\text{glass}}^{\circ})/2.303 (RT/F)$, given in Table I, are of the same order of magnitude as those found earlier⁶ in methanol-water and ethanol-water mixtures (0–0.07 pH unit). Therefore, if the δ values in Table I are used to correct pH values measured with an arbitrary glass electrode, their standard deviation can be estimated to range from 0 to ± 0.04 pH unit as the solvent composition ranges from water-rich to water-poor, as found before⁶. Evidently, if pH measurements are performed with the same (Ingold) glass electrode as was used in the present investigation (to which the same pre-treatment has been given), the standard deviations of the δ values given in Table I apply.

At 4°, the δ values at the highest concentration of the organic constituent of the solvent mixtures are 0.02–0.06 pH unit less negative than those at 25° (see Table II). At lower concentrations of the organic component, values of δ at 4° can be estimated by assuming that the difference $\delta_{25^{\circ}} - \delta_{4^{\circ}}$ is proportional to the weight percentage X .

In Table III, δ values at both temperatures and at various values of X , obtained by graphical interpolation, are given.

TABLE III

VALUES OF δ (IN pH UNITS) AT 25° AND 4° IN SUCROSE-WATER, GLYCEROL-WATER AND ETHYLENE GLYCOL-WATER MIXTURES

X (wt.-%)	Sucrose-water		Glycerol-water		Ethylene glycol-water	
	$-\delta_{25^{\circ}}$	$-\delta_{4^{\circ}}$	$-\delta_{25^{\circ}}$	$-\delta_{4^{\circ}}$	$-\delta_{25^{\circ}}$	$-\delta_{4^{\circ}}$
5	0.03	0.03	0.03	0.03	0.03	0.02 ₅
10	0.06 ₅	0.06	0.06 ₅	0.06	0.05 ₅	0.04 ₅
15	0.10 ₅	0.09 ₅	0.10	0.09 ₅	0.08	0.07
20	0.14	0.13	0.13	0.12 ₅	0.11	0.09 ₅
25	0.17 ₅	0.16	0.16	0.15 ₅	0.14	0.12
30	0.21	0.19 ₅	0.19	0.18 ₅	0.16	0.13 ₅
35	0.24 ₅	0.23	0.22 ₅	0.21 ₅	0.18 ₅	0.15 ₅
40	0.29	0.27	0.25 ₅	0.24 ₅	0.20	0.17
45	0.33 ₅	0.31	0.29	0.28	0.22	0.18
50	0.38	0.35 ₅	0.32 ₅	0.31 ₅	0.24	0.20
55	0.43 ₅	0.41	0.36 ₅	0.35 ₅	0.26 ₅	0.22
60	0.50 ₅	0.47 ₅	0.41 ₅	0.40	0.29 ₅	0.25
65	—	—	0.47	0.45	0.34	0.28 ₅
70	—	—	0.53	0.51	0.40	0.34 ₅
75	—	—	0.60	0.58	0.49	0.43
80	—	—	0.69 ₅	0.67 ₅	0.62	0.55 ₅

CONCLUSIONS

Meaningful pH values (*i.e.*, values which can be interpreted as $-\log a_{\text{H}^+}^*$) can be obtained in sucrose-water, glycerol-water and ethylene glycol-water mixtures, which are used as solvents in density-gradient isoelectric focusing. This can be done by simply subtracting from the pH meter readings a quantity δ , which is characteristic of the solvent composition.

The resultant interpretation errors, $\text{pH} - \delta - \text{p}a_{\text{H}^+}^*$, range from zero in water to ± 0.04 pH unit in 60% sucrose, 80% glycerol and 80% ethylene glycol, if an arbitrary glass electrode is used. They are less than ± 0.02 pH unit if a glass electrode of the same manufacture as used in the present investigation is applied.

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