In Situ Measurement of pH Under High Pressure

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In situ measurement of pH under high pressure is complicated by the need to remain functional under extreme pressures, electrically noisy environments, and the difficulty in inferring chemical equilibrium conditions under pressure. We describe a pH sensor wherein the current flowing at a given input voltage is directly related to hydrogen ion concentration and can be operated within extreme environments. The sensor was tested at 25 °C for in situ pH measurement of several buffer solutions under high pressure up to 784.6 MPa. We found generally an increase in the acidity of the buffer solutions with increasing pressure. In all cases, pH changes were found to be less than predicted using inferred reaction volume data from the literature. Sensor response and pH changes were found to be completely reversible upon depressurization at the same temperature.

Introduction

The measurement of pH is universal, especially in biological and food systems. Conventionally, pH is measured electrochemically using a pH-sensitive glass electrode and a reference electrode. However, there are many other alternative methods and devices for pH measurement based on electrochemical and nonelectrochemical principles. All the available methods and devices, so far, are either of limited or no use for in situ pH measurement under extreme pressures (>150 MPa).

Scientific investigations under high pressure are of interest in food, geological, and marine sciences. In the food industry, a commercial processing technology involves subjecting food to high hydrostatic pressure to inactivate harmful microorganisms and to obtain products with extended shelf life and characteristics similar to those of fresh products. It has been known that pressure affects ionization equilibria, particularly the dissociation of weak acids and bases, causing pH changes under pressure.[1] During high pressure processing, the pH value affects many biochemical reactions, such as protein denaturation, enzymatic activity, and the growth and mortality of microorganisms.[2,3] Measurement of pH is therefore a key research need and assists in product and process development. Furthermore, the knowledge of pH at great ocean depth is of particular interest in the field of oceanography.

The measurement of pH under pressure has been investigated over the past several decades. Disteche[4] made a glass pH electrode capable of withstanding pressure up to 1500 kg/cm² (147 MPa). The electrode assembly consisted of two Ag/AgCl electrodes and was designed to measure pH at great ocean depth. Crolet and Bonis[5,6] also used a glass pH electrode with a Ag/AgCl reference electrode to measure pH under pressure from 0.1 up to 100 MPa. However, the fragile nature of the glass pH electrode limits its use under pressures above 150 MPa, which are typical in high pressure processing. It is also reported that the electrode potential of the Ag/AgCl reference electrode is pressure-dependent,[7] which complicates pH measurement under pressure. Furthermore, the weak electrical signals (millivolt-level readings) produced by these electrodes are prone to external interference. In situ pH measurements (up to 450 MPa) have also been performed using optical methods.[8–10] These methods involve indicator dyes and are not suitable for opaque samples. The pH of buffer solutions has also been calculated using electrical conductivities measured under high pressure.[11] This is, however, not applicable for complex samples. A common calculation procedure involves the use of reaction volume data determined under atmospheric pressure to calculate pH under high pressure using El'yanov and Hamann's equation.[12–14] Stippl et al.[1] state that this equation is based upon a crude model, which assumes that the properties of water, such as the dielectric permittivity, are not influenced by the electric field of the ion.

The objectives of this work were to develop a pH sensor,[15] test it for in situ pH measurement over a wide range of pressure, and to determine pH of selected buffer solutions under pressure.

Experimental Section

Measurement Principle. The electrical conductivity of an electrolyte solution is the sum of the contributions from its individual ions. If it were possible to block the movement of all but hydrogen ions, the conductivity or (for a given geometry and voltage) the current would be due to the flux of hydrogen ions alone. Under such circumstances, it would be possible to relate the current to pH. We used this principle in the development of our pH sensor. The selective movement of hydrogen ions was achieved by means of a Nafion membrane in combination with two thin film reverse osmosis (RO) membranes (GE Sepa CF TF), as described below. The passage of hydrogen ions creates a charge separation across this membrane filter. However, the net charge of the electrolyte/test solution remains unchanged, obeying the principal of electroneutrality.

Fabrication of the pH Sensor. The Nafion membrane was sandwiched between the two RO membranes and then placed between the electrodes in the test solution (Figure 1). With an applied voltage, the first (from either side) RO membrane rejects the passage of salt ions and suspended materials in the test solution, since they are larger than the membrane pores. Since

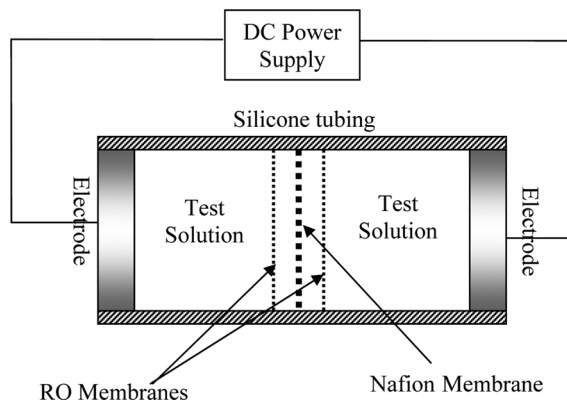* Corresponding author. Phone: (614) 292-3508. Fax: (614) 292-9448. E-mail: sastry.2@osu.edu.

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Figure 1. Schematic diagram of the pH sensor.

the Nafion membrane is permeable to water and cations and is impermeable to anions, hydrogen ions (H_3O^+) become the only ionic species passing through the membranes, resulting in the passage of current through the sensor. The second RO membrane permits the application of the electric field from the other side of the test solution. It also filters the very small fraction of cations that might have passed through the first RO and the Nafion membranes. The pH sensor, consisting of this membrane filter, was made with flexible walls to permit the environmental pressure to be transmitted to the test solution.

In use, the sensor was filled with the test solution and was then sealed with luer fittings. The lead wires (Chromel wires), running through the luer fittings, also served as electrodes. The high-pressure electrical lead-throughs and all electrical connections were insulated with Teflon shrink tube and insulating epoxy (Devcon, 1233, Glenview, IL). The sensor was then immersed in a pressure vessel of the hydrostatic pressure generating system described below.

Pressure Generating System. A hydrostatic pressure-generating system (Harwood Engineering, 26190, Walpole, MA), rated to 1000 MPa and 150 °C, was used for these experiments. A 50:50 mixture of propylene glycol (Houghton Chemical, Safe-T-Therm, Allston, MA) and distilled deionized water (Reagent Laboratory Store, The Ohio State University, Columbus, OH) was used as the pressurizing fluid. The pressure-generating system consisted of four cylindrical jacketed pressure vessels, each of diameter 2.54 cm and depth 15.3 cm, respectively. The top closure of each vessel housed wire feed-throughs that allowed electrical and temperature measurements in each vessel. A thermocouple attached to the feed-throughs was placed adjacent to the pH sensor. Temperature in the pressurizing vessel was controlled by circulating a heat exchange fluid, usually propylene glycol, through the jacket around the vessel.

Sensor Response to pH under Atmospheric Pressure (0.1 MPa). The sensor was connected to a constant voltage DC power supply (Hewlett-Packard, 6236B, Palo Alto, CA) via the electrical feed-throughs from the outside of the pressure vessel. Temperature inside the pressure vessel was stabilized at 25 ($\pm$1) °C for 2 min under atmospheric pressure, then the sensor was energized with a voltage of 10 V (DC), and current and voltage were collected and recorded every second for 5 s using a data logger (Agilent, 34970, 22 Bit Board Resolution, Palo Alto, CA) connected to a computer. The current and voltage data were averaged for the calculation of pH. The above experimental measurements were made for several test solutions of known pH. These test solutions were prepared by adding HCl to the 0.05 m biphthalate (($pH)_0 = 4.01$) and 0.025 m phosphate (($pH)_0 = 6.86$) buffer solutions described below.

According to the Nernst−Einstein equation,[16] the electrical conductivity due to the movement of hydrogen ions (i.e., proton conductivity) can be written as

$$\sigma_{H+} = C_{H+}D_{H+}F^2/RT = \text{cell constant} \times (I_{H+}/V) \quad (1)$$

Thus, the pH can be related to the sensor response as follows:

$$\log(V/I_{H+}) = pH + \log \text{constant} \quad (2)$$

We tested the above relationship for the acidified buffer solutions.

Operation for in Situ pH Measurement under High Pressure. The current and voltage were collected and recorded in the same manner as described above under hydrostatic pressure for the buffer solutions described below. The measurements were carried out at 98.1, 392.3, 588.4, and 784.6 MPa by successive pressurization from the atmospheric pressure (0.1 MPa) after stabilizing the temperature inside the pressure vessel at 25 ($\pm$1) °C for 2 min at each pressure.

pH Determination under Pressure. pH is defined in terms of hydrogen ion activity a_{H+}[17] (eq 3).

$$pH = -\log a_{H+} \quad (3)$$

The activity a_{H+} is an "effective hydrogen ion concentration" and is traditionally calculated by multiplying hydrogen ion concentration (mol/L) by an activity coefficient (γ_{H+}).[17] Since the activity a_{H+} represents the effective concentration, pH can be alternately written as:

$$pH = -\log C_{\text{eff(H+)}} \quad (4)$$

where $C_{\text{eff(H+)}}$ is the effective hydrogen ion concentration (mol/L) in a solution.

In the use of our pH sensor, the effective hydrogen ions in the test solution react with the Nafion membrane and pass through the membrane filter, resulting in the passage of current through the sensor. Therefore, from eq 4,

$$pH = -\log(n_{H+}/v) \quad (5)$$

where n_{H+} and v are the number of moles of effective hydrogen ions and the volume (in L) of the test solution in the sensor, respectively.

$$pH = \log v - \log n_{H+} \quad (6)$$

Under atmospheric pressure (0.1 MPa),

$$(pH)_0 = \log(v)_0 - \log(n_{H+})_0 \quad (7)$$

Under high pressure,

$$(pH)_p = \log(v)_p - \log(n_{H+})_p \quad (8)$$

$(7 - 8)$ $(pH)_0 - (pH)_p = \log(v)_0/(v)_p - \log(n_{H^+})_0/(n_{H^+})_p$

$$(9)$$

The $(v)_0/(v)_p$ ratio can be determined by density, since the mass of the test solution in the sensor is constant at each pressure during pressurization (eq 10).

$$(v)_0/(v)_p = (\rho)_p/(\rho)_0 \qquad (10)$$

where $(\rho)_0$ and $(\rho)_p$ are the densities of the test solution under atmospheric and high pressure, respectively. For dilute aqueous solutions, the density data for water available from The National Institute of Standards and Technology (NIST)[18] can be used.

The $(n_{H^+})_0/(n_{H^+})_p$ ratio can be related to the proton conductivity (σ_{H^+}), which is essentially the specific proton current and is independent of the sensor dimensions and the voltage across the sensor.

$$\sigma_{H^+} \ (A \ m^{-1} \ V^{-1}) = \text{cell constant} \times (I_{H^+}/V) \qquad (11)$$

Since the electrical charge of one mole of hydrogen ions is $9.6487 \times 10^4 \ C \ mol^{-1}$ (F),

$$n_{H^+} = \sigma_{H^+}/F \quad (\text{in mol } m^{-1} \ V^{-1} \ s^{-1}) \qquad (12)$$

Therefore,

$$(n_{H^+})_0/(n_{H^+})_p = (\sigma_{H^+})_0/(\sigma_{H^+})_p \qquad (13)$$

From eq 9,

$$(pH)_0 - (pH)_p = \log(\rho)_p/(\rho)_0 + \log(\sigma_{H^+})_p/(\sigma_{H^+})_0 \qquad (14)$$

Thus, the pH under pressure can be determined by the proton conductivity ratio, $(\sigma_{H^+})_p/(\sigma_{H^+})_0$.

Since the sensor is designed to deform under pressure, the cell constant may change at different pressures. Furthermore, the pressure may affect the transport of mass through the membranes.[19] Therefore, a correction procedure is necessary when calculating the proton conductivities. We used the following procedure to correct the $(\sigma_{H^+})_p/(\sigma_{H^+})_0$ ratio for the pressure effects.

Correction for the Pressure Effects on the Sensor. HCl is a strong acid and is considered fully dissociated in water (eq 15) under atmospheric pressure.

$$HCl \Rightarrow H^+ + Cl^- \qquad (15)$$

If the dissociation of water and other background reactions (e.g., see eqs 28−30) do not generate hydrogen ions during pressurization, the number of moles of hydrogen ions in a given mass of an HCl solution is constant at all the pressures. The pH change of the HCl solution is therefore due to only the change in the volume and the activity coefficient of hydrogen ion. Our correction procedure relies on this concept, and a correction factor (CF) was developed using the HCl data as follows.

We prepared a dilute HCl solution (0.100 m, $(pH)_0 = 1.35$) using distilled deionized water. The HCl concentration was arbitrarily chosen to represent the concentrations of all the buffer solutions described below. The current and voltage measurements were carried out for the HCl solution as well as for the distilled deionized water at each pressure, as in the pH measurements described above.

Since, in reality, the dissociation of water and other background reactions (eqs 28−30) do generate hydrogen ions during pressurization, we subtracted the contribution from the background to determine the proton conductivity of HCl only, $(\sigma_{H^+})_{HCl}$ (eq 16).

$$(\sigma_{H^+})_{HCl} = (\sigma_{H^+})_{HCl+H_2O} - (\sigma_{H^+})_{H_2O} \qquad (16)$$

where $(\sigma_{H^+})_{HCl+H_2O}$ and $(\sigma_{H^+})_{H_2O}$ are the proton conductivities of the HCl solution and the distilled deionized water, respectively.

Using the experimental measurements of current and voltage, eq 16 can be written as

$$(\sigma_{H^+})_{HCl} = (L/A) \times [(I_{H^+}/V)_{HCl+H_2O} - (I_{H^+}/V)_{H_2O}] \qquad (17)$$

where L/A is the cell constant of the sensor.

Under atmospheric pressure (0.1 MPa),

$$[(\sigma_{H^+})_{HCl}]_0 = (L/A)_0 \times [(I_{H^+}/V)_{HCl+H_2O} - (I_{H^+}/V)_{H_2O}]_0 \qquad (18)$$

Under high pressure,

$$[(\sigma_{H^+})_{HCl}]_p = (L/A)_p \times [(I_{H^+}/V)_{HCl+H_2O} - (I_{H^+}/V)_{H_2O}]_p \qquad (19)$$

$(19/18)$ $[(\sigma_{H^+})_{HCl}]_p/[(\sigma_{H^+})_{HCl}]_0 = (L/A)_p/(L/A)_0 \times$

$[(I_{H^+}/V)_{HCl+H_2O} - (I_{H^+}/V)_{H_2O}]_p/[(I_{H^+}/V)_{HCl+H_2O} - (I_{H^+}/V)_{H_2O}]_0$ (20)

Thus, eq 20 gives the experimental value of the proton conductivity ratio of the HCl solution in the absence of the reactions generating hydrogen ions during pressurization, $\{[(\sigma_{H^+})_{HCl}]_p/[(\sigma_{H^+})_{HCl}]_0\}_{experimental}$.

Theoretically, the same proton conductivity ratio of the HCl solution in the absence of the reactions generating hydrogen ions during pressurization can be determined by the following method.

From eq 12,

$$(\sigma_{H^+})_{HCl} = n_{H^+} \times F \qquad (21)$$

The number of moles of effective hydrogen ions (n_{H^+}) in the sensor can be written as

$$n_{H^+} = \gamma_{H^+} \times \text{moles of hydrogen ions in the sensor} \qquad (22)$$

where γ_{H^+} is the activity coefficient of hydrogen ion.

Since the mass of the HCl solution in the sensor is constant, the number of moles of hydrogen ions is the same at each

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TABLE 1: Some Data Used in the Correction Procedure for the Pressure Effects on the Sensor

pressure (MPa)	ρ^a (kg/dm^3)	ε^b	$\gamma_{H^+}{}^c$	CF × [$(L/A)_p/(L/A)_0$]
0.1	0.99705	77.92	0.688	1.000
98.1	1.0372	80.80	0.696	0.662
392.3	1.1247	89.45	0.724	0.554
588.4	1.1678	95.22	0.741	0.548
784.6	1.2041	100.98	0.757	0.524

[a] Pressure−density data (25 °C) for water.[18] [b] Pressure−dielectric constant data (300 K) for water.[20] Values of ε given up to 500 MPa were fitted into a straight line with linear regression. The regression equation, $\varepsilon = 0.0294 \, (P) + 77.917$, $R^2 = 0.9968$, was then employed to determine ε at each pressure, assuming the existence of the same equation, even beyond 500 MPa. [c] γ_{H^+} (∼25 °C) of 0.100 m HCl determined by the Debye−Huckel law (eq 24).

pressure. Therefore, the theoretical value of the proton conductivity ratio of HCl can be written as

$$\{[(\sigma_{H^+})_{HCl}]_p/[(\sigma_{H^+})_{HCl}]_0\}_{theoretical} = (\gamma_{H^+})_p/(\gamma_{H^+})_0 \tag{23}$$

The activity coefficient of hydrogen ion at 25 °C can be determined by the Debye−Huckel law according to the following equation[14] (eq 24):

$$\log \gamma_{H^+} = -354.3(\rho I/\varepsilon^3)^{0.5} \tag{24}$$

where ρ, ε, and I are the density, the dielectric constant, and the ionic strength of the HCl solution, respectively.

Since the HCl solution is dilute, the pressure−density data[18] and the pressure−dielectric constant data[20] for water were assumed. The ionic strength ($I = 0.1$) is constant at all the pressures because HCl is a fully dissociated acid. Table 1 shows the values of ρ, ε, and γ_{H^+} determined by eq 24 at different pressures.

We then define the CF as a ratio between the theoretical and the experimental values of proton conductivity ratios of HCl (eq 25).

$$CF = \{[(\sigma_{H^+})_{HCl}]_p/[(\sigma_{H^+})_{HCl}]_0\}_{theoretical}/$$
$$\{[(\sigma_{H^+})_{HCl}]_p/[(\sigma_{H^+})_{HCl}]_0\}_{experimental} \tag{25}$$

The values of CF × [$(L/A)_p/(L/A)_0$] are shown in Table 1. The proton conductivity ratio of the test solution (in eq 14) multiplied by the CF corrects for the pressure effects on the sensor and eliminates the need to find the cell constants under pressure (eq 26).

$$\{(\sigma_{H^+})_p/(\sigma_{H^+})_0\}_{corrected} = (L/A)_p/(L/A)_0 \times (I_{H^+}/V)_p/$$
$$(I_{H^+}/V)_0 \times CF \tag{26}$$

The above correction procedure was also performed without the use of the Debye−Huckel law. In that case, the activity coefficient of the hydrogen ion was assumed to be one ($\gamma_{H^+} = 1$) at all pressures. We found that not using the Debye−Huckel law resulted in a change of less than 0.04 pH units at the highest pressure; thus, the effect is small.

Buffer Solutions. Acetate (0.01 m, $(pH)_0 = 4.72$), biphthalate (0.05 m, $(pH)_0 = 4.01$), phosphate (0.025 m, $(pH)_0 = 6.86$), and sulfanilate (0.01 m, $(pH)_0 = 3.23$) buffer solutions described
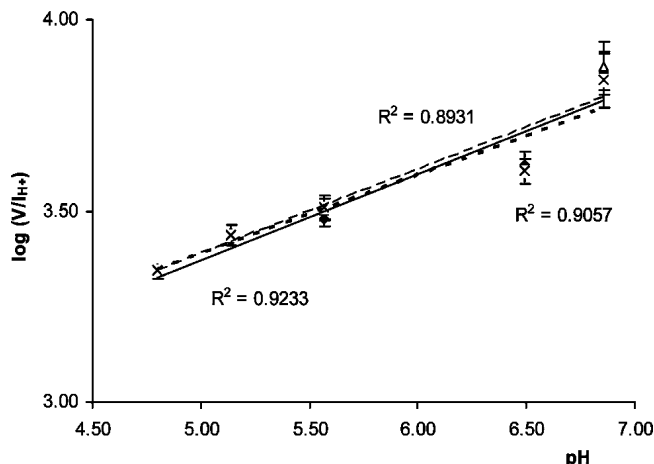
Figure 2. Sensor response to pH under atmospheric pressure (25 °C) for the acidified 0.025 m Phosphate buffer.
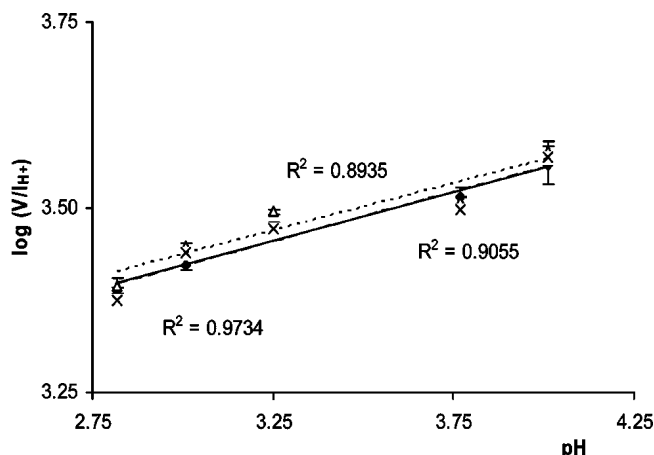
Figure 3. Sensor response to pH under atmospheric pressure (25 °C) for the acidified 0.05 m biphthalate buffer.

by El'yanov;[12] and ACES (0.09 M, $(pH)_0 = 7.0$), citrate (0.09 M, $(pH)_0 = 4.0, 5.0$), HEPES (0.09 M, $(pH)_0 = 5.0, 7.0$), MES (0.09 M, $(pH)_0 = 4.0, 5.0$), and TRIS (0.09 M, $(pH)_0 = 8.1$) buffers well-known in biological studies were used as test solutions. All the test solutions were prepared using distilled deionized water ($(pH)_0 = 5.5$).

Results and Discussion

The pH Sensor. Figures 2 and 3 show that the sensor is, indeed, responsive to pH under atmospheric pressure and follows the relationship in eq 2. Thus, upon calibration, the sensor can be employed to determine the pH of unknowns. However, there are no standard calibrating buffers available for sensor calibration under high pressure. The reported pH values[2,9,12] for different buffers under high pressure have not been verified and are not considered as standards. We, therefore, used the above alternative approach (eqs 3−26) for pH determination with this particular sensor.

The sensor yielded an increase in current for all the test solutions with increasing pressure (Figure 4). The voltage across the sensor was virtually unchanged at each pressure. Furthermore, these sensor measurements were always reversible upon depressurization at the same temperature (25 °C). This reversibility of sensor measurements indicates the reversible pH changes of the test solutions under pressure. The reversibility further indicates that the pressure does not cause any irreversible effect on the membranes.
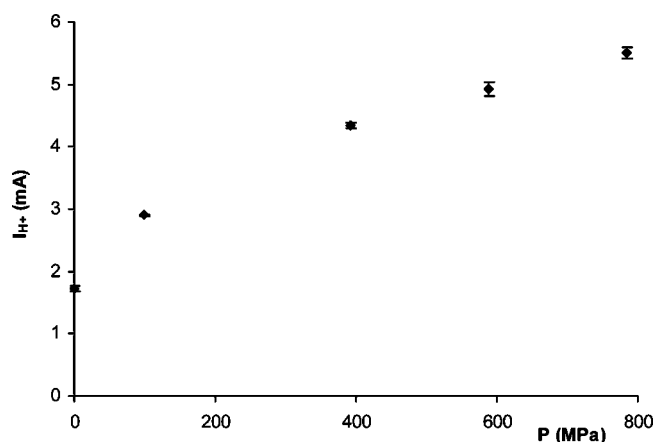
Figure 4. Typical current vs pressure (25 °C) for the 0.05 m biphthalate buffer at the energized voltage of 10 V (DC).
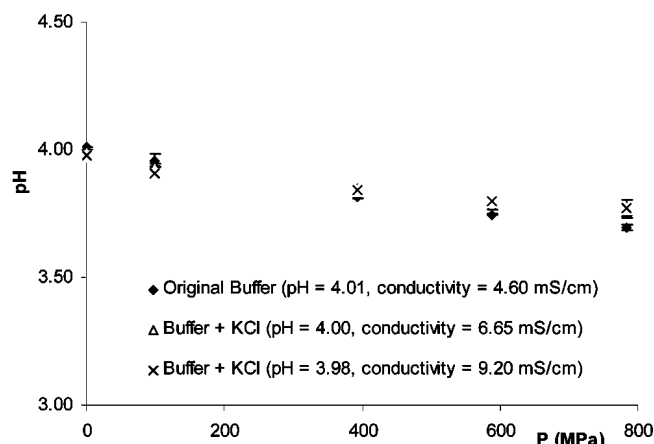

Figure 5. pH vs pressure (25 °C) for the 0.05 m biphthalate buffer at different initial electrical conductivities. The electrical conductivities were varied by adding KCl.

Since test solutions can have different electrical conductivities at the same pH, we examined the effect of initial electrical conductivity on the pH measurement with our sensor. Figure 5 shows the pH of the 0.05 m biphthalate buffer $((pH)_0 = 4.01)$ under pressure at different initial electrical conductivities. It can be seen that the buffer solutions with different electrical conductivities follow the same trend as the original buffer. Even though the initial electrical conductivity was varied by 100%, the alteration of pH was only ≤ 0.05 pH units at each pressure, except at 784.6 MPa, where it was 0.07 pH units.

pH Measurement of Water. Figure 6 shows the pH of commonly used water in our laboratory, distilled water $((pH)_0 = 6.7)$, and distilled deionized water $((pH)_0 = 5.5)$, under high pressure measured with our pH sensor. In the literature, the pH change of water has been reported up to 200 MPa by various authors. The reported values, however, vary quite widely in the range of -0.16 to -0.73 pH units at 100 MPa.[9] With our pH sensor, we observed pH changes of -0.16 (± 0.07) and -0.12 (± 0.08) pH units for the distilled water and the distilled deionized water, respectively, at 98.1 MPa. The difference in our results could be due to the difference in the original pH, $(pH)_0$, of the water samples.

It is important to note that the studies reported in the literature[9,13,14] always considered pure water and the reaction-volume for the self-ionization of pure water in determining the pH of water. However, in reality, water is not in the pure form and contains dissolved CO_2 and possibly many other substances that contribute to pH. For instance, the original pH of the
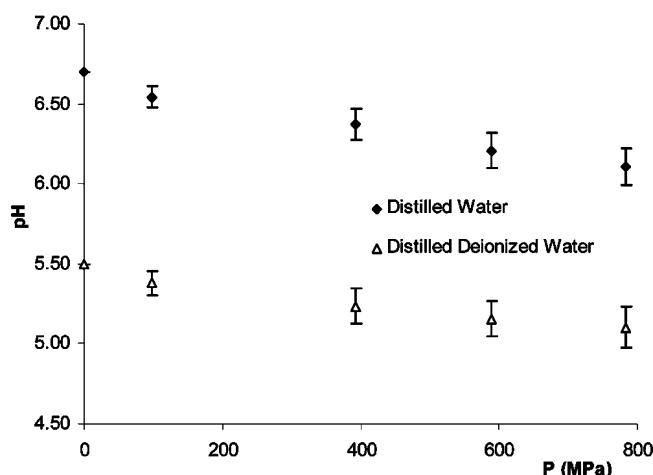

Figure 6. pH (25 °C) of distilled water and distilled deionized water under high pressure measured with the pH sensor.

distilled water $((pH)_0 = 6.7)$ is quite different from that of the distilled deionized water $((pH)_0 = 5.5)$, indicating different levels of dissolved substances present in the water samples. Upon pressurization, the dissolved substances can modify the theoretically expected pH for pure water. Reaction volumes for ionization reactions, including the self-ionization of pure water, so far, have been measured only under atmospheric pressure.[12−14] The atmospheric pressure reaction volume data were then used in calculating reaction volumes (and pH) as a function of pressure by several semiempirical relationships reported in the literature. Stippl et al.[1] have already reported the lack of accuracy of these relationships and highlighted the importance of a comprehensive theory, which is yet to be developed, to describe the pressure behavior of ionization reactions. Furthermore, these authors[1] and others[21] described structural changes of liquid water due to pressure. In particular, the existence of localized structural heterogeneities of liquid water[21] could have an influence on the movement of protons (and thereby pH) under high pressure. However, all the aforementioned semiempirical relationships treat water as a homogeneous medium, and none of them consider the structural changes of water directly.[1] Therefore, the differences in results between our experimental pH measurements and the reaction-volume-based studies reported in the literature are not surprising.

Another approach to reaction volume determination is the use of conductivity measurements under pressure to determine equilibrium constants, followed by use of Kohlrausch's law and the Debye−Huckel limiting law to determine the degree of ionization and thereby calculate reaction volumes.[11,22] This approach is again indirect, extrapolated to the dilute solution limit, and presumes that the chemistry under high pressures is identical to that under atmospheric pressure. The use of such data to infer pH changes in realistic systems under pressure is questionable.

pH Measurement of the Buffer Solutions. Tables 2 and 3 show that, in general, the buffer solutions become acidic with increasing pressure. The increased acidity induced by pressure can be explained by the following ionic equilibria.

The equilibrium for the protonic ionization of a buffering agent (HA) can be written as

$$HA \leftrightarrow H^+ + A^- \tag{27}$$

The equilibrium for the dissociation of water is

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TABLE 2: pH of El'yanov[12] Buffers under High Pressure at 25 °C

	pressure (MPa)				
	0.1	98.1	392.3	588.4	784.6
	0.01 m Acetate Buffer				
pH* (measured)	4.72[a]	4.69[a]	4.60[b]	4.54[c]	4.49[d]
	(0.00)	(0.01)	(0.01)	(0.01)	(0.03)
pH (El'yanov[12])	4.72	4.53	4.12	3.93	3.77
	0.05 m Biphthalate Buffer				
pH* (measured)	4.01[a]	3.96[a]	3.82[b]	3.75[c]	3.70[d]
	(0.00)	(0.03)	(0.01)	(0.00)	(0.01)
pH (El'yanov[12])	4.00	3.77	3.25	3.01	2.81
	0.025 m Phosphate Buffer				
pH* (measured)	6.86[a]	6.68[a]	6.60[a]	6.55[a]	6.54[a]
	(0.00)	(0.19)	(0.20)	(0.18)	(0.19)
pH (El'yanov[12])	6.90	6.49	5.57	5.14	4.80
	0.01 m Sulfanilate Buffer				
pH* (measured)	3.23[a,b]	3.26[a]	3.21[a,b]	3.18[b]	3.18[b]
	(0.00)	(0.02)	(0.02)	(0.03)	(0.03)
pH (El'yanov[12])	3.23	3.26	3.32	3.35	3.37

*Mean (standard deviation) of three replicate measurements.
[a-d] Means of each buffer with different superscript letters are significantly different ($P \leq 0.05$).

**TABLE 3: pH* of Some Biological Buffers (0.09 M) under High Pressure at 25 °C Measured with the pH Sensor**

pressure (MPa)				
0.1	98.1	392.3	588.4	784.6
ACES Buffer				
7.0[a] (0.0)	6.91[b] (0.02)	6.87[b] (0.03)	6.87[b] (0.03)	6.87[b] (0.04)
Citrate Buffer				
4.0[a] (0.0)	3.98[b] (0.01)	3.90[c] (0.01)	3.82[d] (0.01)	3.80[e] (0.00)
5.0[a] (0.0)	4.94[b] (0.01)	4.88[c] (0.00)	4.85[d] (0.01)	4.85[d] (0.01)
HEPES Buffer				
5.0[a] (0.0)	5.01[a] (0.01)	4.97[b] (0.01)	4.92[c] (0.01)	4.92[c] (0.01)
7.0[a] (0.0)	6.95[b] (0.01)	6.90[c] (0.02)	6.85[d] (0.03)	6.87[c,d] (0.03)
MES Buffer				
4.0[a] (0.0)	3.93[a] (0.14)	3.86[a] (0.12)	3.82[a] (0.11)	3.80[a] (0.12)
5.0[a] (0.0)	4.80[b] (0.01)	4.77[b,c] (0.01)	4.74[c] (0.02)	4.74[c] (0.04)
TRIS Buffer				
8.1[a] (0.0)	8.00[a] (0.08)	7.96[a] (0.08)	7.96[a] (0.07)	7.96[a] (0.08)

*Mean (standard deviation) of three replicate measurements.
[a-e] Means of each buffer with different superscript letters are significantly different ($P \leq 0.05$).

$$H_2O \leftrightarrow H^+ + OH^- \quad (28)$$

Dissolved CO_2 in distilled deionized water is at equilibrium, which can be written as

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \quad (29)$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (30)$$
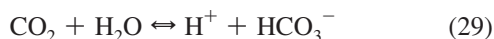
Furthermore, possibly many other dissolved substances (unknown), either present in distilled deionized water or accompanying the buffering agents as impurities, may have their own equilibrium reactions involving protons. A buffer solution is therefore a mixture of several ionic reactions that are at equilibrium.

By considering the ionic reactions 27−30, the overall equilibrium reaction can be written as follows (eq 31):

$$(27 + 28 + 29 + 30) \quad HA + CO_2 + 2H_2O \leftrightarrow 4H^+ + A^- + CO_3^{2-} + OH^- \quad (31)$$
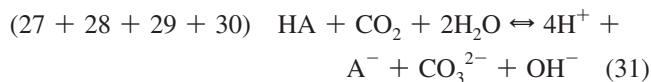
At any given pressure and temperature, the reaction volume for the above overall equilibrium (ΔV_{31}) is the sum of the reaction volumes of the individual reactions.

Thus,

$$\Delta V_{31} = \Delta V_{27} + \Delta V_{28} + \Delta V_{29} + \Delta V_{30} \quad (32)$$

where ΔV_{27}, ΔV_{28}, ΔV_{29}, and ΔV_{30} are the reaction volumes accompanying the reactions 27, 28, 29, and 30, respectively.

Experimental values of reaction volumes under atmospheric pressure (25 °C) at infinite dilution have been reported for protonic ionization of several buffering agents and the dissociation of water.[14] These data show that the dissociation of water accompanies a large negative reaction volume ($\Delta V_{28}^0 = -22.2$ cm^3/mol). For protonic ionization of buffering agents, the reaction volume (ΔV_{27}^0) is generally negative, except for the zwitterionic buffering agents (ACES, HEPES, MES, TRIS, and sulfanilate). However, the positive reaction volumes of these zwitterionic buffering agents are below $+10$ cm^3/mol. Although the reaction volume data for eqs 29 and 30 have not been reported, both ΔV_{29}^0 and ΔV_{30}^0 are expected to be negative, since no zwitterions are involved in these reactions. Therefore, the sum of the reaction volumes (ΔV_{31}^0) is negative, which indicates that the overall equilibrium (eq 31) shifts to the right to minimize the volume with increasing pressure according to the Le Chatelier principle.[1,23] As a result, the buffer solutions become acidic with increasing pressure.

We want to note that El'yanov[12] considered only the protonic ionization reaction (eq 27) and used its reaction volume ((ΔV_{27}^0) to calculate the pH of buffer solutions under high pressure. However, when a buffer solution is pressurized, the pressure affects the entire system (eq 31), not just one reaction. We also want to note that the in situ pH measurements by the optical methods[8-10] again considered only the protonic ionization reaction (eq 27), since the measured intensities of the buffer solutions were corrected for the solvent blank (water) and related to the pK_a values of the buffering agents.

Similarly, the pH calculations by electrical conductivity measurements[11] also completely relied on the protonic ionization reaction (eq 27). In addition to the unique consideration of the protonic ionization reaction (eq 27), all the above pH measurement methods were reaction-volume-based and involved semiempirical relationships we described in the preceding section. It has been already reported that these relationships neither fully describe the pressure behavior of ionization reactions nor directly consider the structural changes of liquid water under pressure.[1] Therefore, again, the differences in results between our experimental pH measurements and the reaction-volume-based studies reported in the literature are not surprising and reflect the methodological differences.

Our pH measurements (in Tables 2 and 3) show that the buffer solutions undergo ≤ 0.3 pH unit drop at the highest pressure (784.6 MPa). This means that the original pH of a buffer solution does not change much with increasing pressure. Thus, a buffer solution is, indeed, a buffer approximately at the same original pH, even under high pressure. Our results indicate a marked, in some cases, statistically significant ($P \leq 0.05$) pH drop up to 392.3 MPa, but a quite small and generally insignificant ($P > 0.05$) pH drop beyond 588.4 MPa for all the buffer solutions.

This can be explained by the more pronounced hydrogen bonding at lower pressures (<300 MPa) as compared with the disrupted hydrogen bonding at higher pressures (>300 MPa), hindering the mobility of hydrogen ions (H_3O^+).[7]

For the zwitterionic buffer solutions, in general, we observed a relatively smaller pH drop per 100 MPa in our pressure range. In particular, the sulfanilate, ACES, HEPES, and TRIS buffer solutions yielded less than 0.15 pH unit drop at the highest pressure (784.6 MPa). The zwitterionic buffering agents have positive reaction volumes,[12,14] which indicate that the pressure hinders the protonic ionization of these buffering agents (eq 27) according to the Le Chatelier principle.[1,23] Therefore, the observed pH drop is somewhat reduced for these buffer solutions. Interestingly, we observed an increase in pH for the sulfanilate and HEPES (($pH)_0 = 5.0$) buffer solutions at 98.1 MPa. However, these pH values are not statistically significant ($P > 0.05$).

Conclusions

A pH sensor has been developed on the basis of selective movement of hydrogen ions through a specially designed membrane filter in the presence of an electric field. Our pH sensor enables in situ pH measurement under high pressure by immersing the sensor in a pressurized fluid. The pH measurements have shown that, in general, pressure increases the acidity of the buffer solutions. In general, pH changes were lower than calculated using inferred reaction volumes.

The differences in results between our study and reaction-volume-based studies reported in the literature suggest that methodologies to measure pH in situ under pressure should continue to be developed until consensus for pH values under pressure exists.

Nomenclature

A	area of the current path of the sensor (m^2)
a_{H^+}	hydrogen ion activity
CF	correction factor
$C_{eff(H^+)}$	effective hydrogen ion concentration (mol/L)
C_{H^+}	concentration of hydrogen ions (mol/m^3)
DC	direct current (A)
D_{H^+}	mass diffusivity of hydrogen ions (m^2/s)
F	Faraday constant (9.6487×10^4 C mol^{-1})
I	ionic strength
I_{H^+}	proton current (A)
K_a	ionization constant of weak acid buffering agent
L	distance between the electrodes of the sensor (m)
n_{H^+}	number of moles of effective hydrogen ions
P	probability
R	universal gas constant (8.314 J/mol K)
RO	reverse osmosis
T	temperature (K)
V	voltage across the sensor (V)
υ	volume of the test solution in the sensor (L)
ΔV_i	reaction volume of reaction i (cm^3/mol)
ΔV_i^0	reaction volume of reaction i under atmospheric pressure at infinite dilution (cm^3/mol)

Greek Letters

ε	dielectric constant
ρ	density (kg/dm^3)
γ_{H^+}	activity coefficient of hydrogen ion
σ_{H^+}	proton conductivity (Sm^{-1})

Subscripts

0	atmospheric pressure
p	pressurized condition
m	in molal units (mol/kg)
M	in molar units (mol/L)

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