A Simple Method for Measuring the pH of Acid Solutions Under High Pressure

Murielle Hayert, Jean-Marie Perrier-Cornet, and Patrick Gervais*

Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, 1, esplanade Erasme, F-21000 Dijon, France

Received: July 28, 1998; In Final Form: December 7, 1998

Measurements of pH with fluorescein fluorescent intensity have been undertaken in order to measure the pH evolution of acidic solutions under pressure up to 250 MPa. This technique is quite rapid and allows to monitor changes of pH in real time. When the pressure increases, pH is shown to change even for buffers. Indeed, a pressure of 200 MPa increases the dissociation of orthophosphoric acid (ΔpH = −0.92) and acetic acid (ΔpH = −0.40). It also increases the dissociation of water (ΔpH = −0.31) and potassic buffers (ΔpH = −0.16 for potassium hydrophtalate and ΔpH = −0.28 for potassium dihydrogenophosphate). Nevertheless, the same pressure leads to a decrease in the dissociation of morpholineethanesulfonic acid buffer (ΔpH = + 0.50). Owing to these dissociation data against pressure, we have obtained the standard partial volume changed on ionization for the different acidic solutions used. The similarity in these values with the previous literature data is gratifying and supports the validity of the procedure used.

Introduction

Over the past 60 years, investigations were made in order to determine pH under pressure and also to develop high pressure techniques. Because the conformation of molecules depends on pH and so as pH varies under pressure, a detailed knowledge has to be realized to understand chemical or biochemical evolutions of various systems under high pressure treatments and so numerous experiments were made on the dissociation of electrolytes under high pressure during the 60−70 year period.

During high pressure food processing, pH controls lots of phenomena such as proteins properties (gelification, enzymatic activities, etc.),[1−3] growth and mortality of microorganisms or spores,[4,5] and chemical reactions kinetics.[6,7]

Some hypothesis have been proposed in order to explain the cumulative effect of pH stress with pressurization, the most used one was the pressure induced dissociation of weak acids. But some conflicting data have been stated about the pH variations under high pressure. Indeed, neutral pH water at atmospheric pressure and ambient temperature was reported to undergo an important decrease of −0.73 pH unit at 100 MPa[8,9] whereas Kunugi[10] and Marshall and Franck[11] related a decrease of only −0.39 pH unit for the same pressure increase. This discordance could be explained by the non linear relation between pH and pressure. Indeed the apparent volume change for ionization of an acid is equal to $\overline{\Delta V_a} = -RT\,(\partial \ln K_a)/(\partial P)$, with K_a, the molal acidic ionization equilibrium. As the pressure dependence of ln K_a is not linear, values of $\overline{\Delta V_a}$ change with pressure. Thus, direct pressure experiments are necessary to obtain accurate measurements of pH changes, and previous discrepancies could be attributed to the different indirect methods of pH measurements under pressure: by emf measuring of glass electrode,[12] by density and conductivity measuring,[13−15] or by measuring the optical density of indicators (2,5-dinitrophenol, *p*-nitrophenol, or Cresol red).[16,17]

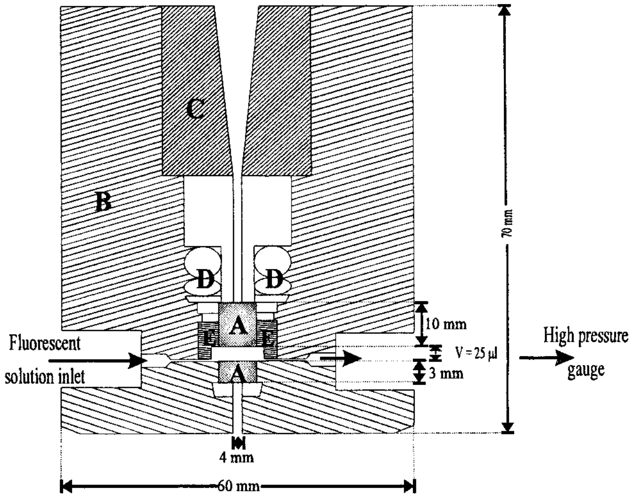


Figure 1. Cross section of the high pressure optical bomb: (A) sapphire windows, (B) high pressure rig, (C) screw cap, (D) seal rings, (E) cell chamber delimiter.

The pH determination was realized under pressure owing to fluorescent intensity measurements of fluorescein and compared to the previous literature data.

Experimental Apparatus and Procedure

Pressure Equipment. In order to measure the pH under pressure, a high pressure rig (Figure 1), previously described by Perrier-Cornet et al.,[18] was adapted for spectrofluorometry (Figure 2). The high pressure bomb included a reactor of 25 μL delimited by two translucent sapphire windows. The pressure cycle was controlled by a manual operated piston screw pump (Novaswiss, Switzerland), and the pressure was measured with a high pressure gauge (G515/700 Sedeme Kistler, France). The high pressure bomb was placed on an inverted light microscope (Fluovert, Leitz-Weitzlar, Germany). The fluorescent light source of the microscope was filtered and allowed to excite the solution in the pressure bomb at a given wavelength. The emitted

---

* Corresponding author. Tel.: 33.(0)3.80.39.66.54. Fax: 33.(0)3.80.39.66.11. E-mail: gervais@u-bourgogne.fr.

10.1021/jp983204z CCC: $18.00

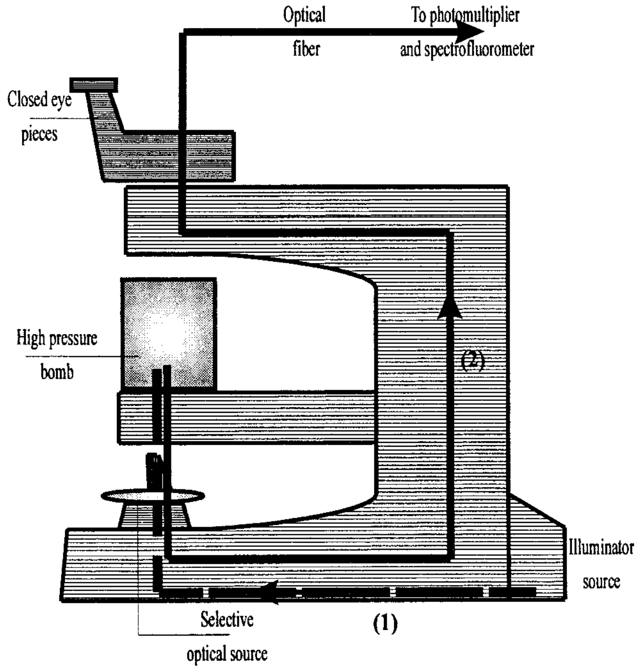


Figure 2. Schematic diagram of the microscope set-up used to adapt the high pressure bomb for the spectrofluorometer: (1) filtered fluorescent excited light from microscope ($\lambda_{500nm}/\lambda_{435nm}$), (2) emitted light read at λ_{540nm}.

light spectrum was seized through an optical fiber by a photomultiplier connected to a spectrofluorometer (Oriel, Stratford, MA) where the output signal was digitized and all the data were analyzed by a computer which was interfaced to the digitizer.

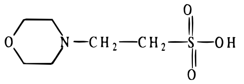
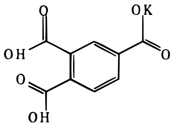
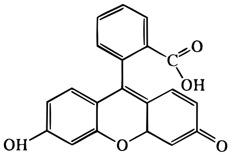
In order to limit interference with other light sources, this equipment had to be put in a black box.

Acid Solutions. Different reagent-grade chemicals or buffers were used in order to compare the pH measured with fluorescence method to the literature data. They consisted of (i) aqueous solution of 5.5 mM 2-[*N*-morpholino]ethanesulfonic acid (MES), the pH of which was regulated by the addition of NaOH at pH 5.0 and pH 6.0; (ii) potassic buffers (PB) of pH 4.0, 5.0, and 7.0 consisting of aqueous solution of 0.5 M potassium hydrogenophtalate for solutions at pH 5.0 and 4.0, and the pH 7.0 potassic buffer was prepared with potassium dihydrogenophosphate. All these pH were obtained by addition of Na_2HPO_4; and (iii) orthophosphoric (OPA) and acetic (AA) acid aqueous solutions were respectively at pH 7.2 and pH 4.1. These solutions (the characteristics of which are summarized in Table 1) were prepared in distilled water (DW) at pH 5.8 which was considered as the reference.

The pH of buffers and prepared acid solutions were measured using a glass electrode (Mettler Toledo, Switzerland) connected to a pHmeter (Inceltech, Toulouse, France) at atmospheric pressure and ambient temperature before used. All the chemicals were from Sigma (St. Quentin, France).

Measurement of the Variation of pH under Pressure. 0.001% (w/v) of fluorescein was added to aqueous acid solutions. The pH was measured again at atmospheric pressure and ambient temperature in order to control the initial pH of the solution with fluorescein, and then the acid solutions were admitted into the high pressure bomb. Fluorescence spectra were recorded at emission wavelengths between 300 and 660 nm, and the ratio of the intensities at two excitation wavelengths (500 nm over 435 nm) was taken at optimal emission fluorescent (around 540 nm).

TABLE 1: Characterization of the Chemical Reagents Used

Chemical reagents	Structure	Characteristics
Acetic acid	$CH_3—COOH$	pHused = 4.1 / pKa = 4.75
Orthophosphoric acid	H_3PO_4	pHused = 7.2 / pKa$_1$ = 7.2
MES buffer (+ NaOH)		pHused = 6.0 pHused = 5.0
Potassic buffers (+ Na_2HPO_4) ·		
* Potassium hydrogenophosphate	KH_2PO_4	pHused = 5.0 pHused = 4.0
* Potassium dihydrogenophtalate		pHused = 7.0
Fluorescein		pK$_a$ = 6.1

The molecular structure of fluorescein and its characteristics are represented in Table 1. This fluorescent compound exhibits acid and base forms which fluoresce at different colors. Indeed, the intensity at 435 nm represents acid solution and the one at 500 nm basic solution.[19] The absorbance of any solution in the optical cell increases with an increase in the pressure due to compression, also an indirect correction, was made by calculating the ratio of the two different intensities (QCI). It ensured that the measurement was not related to any signal errors caused by variations in concentration and pathlength.

The measured intensities were corrected with the background and also with the basis line. Usually, the decimal logarithmic of the intensities quotient was reported to be well correlated to the pH, so the evolution of the pH under pressure was measured through the modification of the decimal logarithmic quotient. More than three measures were made for each solution at atmospheric pressure up to 250 MPa, and the error estimation was evaluated through confidence interval at 0.05% level. The logarithmic fluorescence quotient values (log (QCI)), obtained for each aqueous solution at different pH and at atmospheric pressure, allowed for the establishment of the relation between pH and the mean logarithmic quotients of corrected fluorescence intensities. The method of operating was to equilibrate the sample at a low pressure. After the data were obtained, the pressure were raised slightly and the systems was allowed to reequilibrate. After the highest pressure had been reached, the system was returned to the initial low pressure to check reversibility.

Results and Discussion

The literature data could be distributed in two categories: the one corresponds to theoretical values or cited values without any reference, the other is constituted by the experimental ones. Even for distilled water, previous data on pH variations at 100 MPa are very different: −0.16,[20] −0.39,[11,21] and −0.73[8,9] pH unit. Moreover, the pressure dependence profile was rarely indicated. So it seems obviously interesting to give a theoretical approach of the pressure dependence evolution of pH.

Theoretical Aspects of pH Evolution under Pressure. In aqueous solutions, the influence of the pressure P on activity coefficient of a molecule i (a_i) is expressed as

$$\frac{\partial \ln a_i}{\partial P} = \frac{1}{RT}\left[\frac{\partial \mu_i}{\partial P} - \frac{\partial \mu_i^0}{\partial P}\right] \tag{1}$$

where μ_i is the chemical potential of i (J mol^{-1}) and the sign ^{0} represents the standard state, T the constant standard temperature of 293 K, and R the molar gas constant equal to 8.31 J K^{-1} mol^{-1}.

Because generally the ideality could not be assumed, the partial molal volume of i, $\partial \mu_i / \partial P = \overline{V_i}$, has to be taken into account and then eq 1 becomes

$$\frac{\partial \ln a_i}{\partial P} = \frac{\overline{V_i} - \overline{V_i^0}}{RT} = \frac{\overline{\Delta V_i}}{RT} \tag{2}$$

As pH = $-\log a_{H^+}$, the pH variation under pressure is expressed by $\Delta pH = (\Delta V_{H^+})/(\ln 10\ RT)$ where ΔV_{H^+} is the volume variation of one mole of H^+ ions infinitely diluted from atmospheric pressure to a pressure P in defined state. Below 100 MPa, values of ΔV_{H^+} were found to range between -20.4[15] and -22[8,9,21,22] mL mol^{-1}.

In the case of water, $K_w = a_{H^+} \cdot a_{OH^-} / a_{H_2O}$ with $a_{H_2O} = 1$ and ions activities can be expressed by their molar concentrations.[23] As $[H^+] = [OH^-]$ in pure water,

$$(\partial pH/\partial P) = -(1/2 \ln 10\, [H^+]^2)(\partial K_w/\partial P) \tag{3}$$

Holzapfel[20] and Parson[24] gave empirical equation relating acidity coefficient and pressure. According to these equations, pH variations at 200 MPa were respectively -0.33 and -0.49. In the case of other weak acids, relations between pH and pressure were less known and developed. Nevertheless, for an infinitely diluted monoacid, the pH variation can be related to the pK$_a$ variation in relation to P by

$$\frac{\partial pH}{\partial P} = \frac{\partial pK_a}{\partial P} + \frac{\partial}{\partial P}\left(\log\frac{[A^-]}{[HA]}\right) \tag{4}$$

$$\frac{\partial pH}{\partial P} = \frac{\partial pK_a}{\partial P} - \frac{1}{2 - \dfrac{[H^+]}{C_{AH}}}\left[\frac{\partial pK_a}{\partial P} - \frac{\chi T}{\ln 10}\right] \tag{5}$$

Many authors used the approximation of the constancy of the ratio $[A^-]/[AH]$ and thus $\Delta pH = \Delta pK_a$. In eq 5, χ_T is the isothermal compressibility of the acid solution and C_{AH} the initial undissociated acid concentration.

Lown et al.[25] have developed a simple equation to describe the relation between acid ionization and pressure (up to 200 MPa):

$$\ln 10\, RT \log\frac{K_P}{K_0} = -\overline{\Delta V_a^0} P + 0.5 \Delta\chi^0 P^2 \tag{6}$$

with $\overline{\Delta V_a^0}$, the limiting volume change for ionization of weak acid at atmospheric pressure, and $\Delta\chi^0$, the compressibility term which accounts for the pressure dependence.

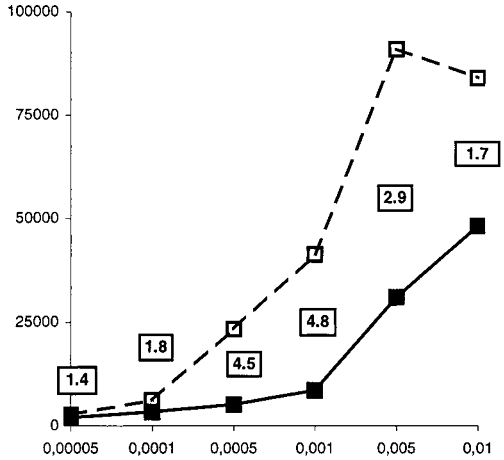


Figure 3. Fluorescence intensities of different concentrations of fluorescein at excitation wavelengths of 500 nm (□) and 435 nm (■) and fluorescence intensities ratio (in rectangle).

Because chemists studying steam generators and biochemists studying hyperbaric life needed general descriptions not only of the ionization behavior of water but also of low acid media, a large number of measurements of the dissociation constants of weak electrolytes has been made previously under pressure using the method of the concentration cell emf,[12] density and conductivity[12,14,15] measurements, direct pH measurement with a glass electrode,[26] and also O.D. measurements.[16,17] In this work, the pH determination was carried out owing to fluorescent intensity measurements and compared to the mentioned data.

Fluorescent Method to Determine pH Evolution under Pressure. The measurement of the fluorescence intensities of fluorescein which is a well-known molecule in the pH determination, more particularly intracellular pH,[27−29] has allowed for the obtaining of the pH variation of five solutions (i.e., distilled water as reference, potassic and MES buffers, orthophosphoric and acetic acids) in different initial acidic conditions up to 250 MPa.

Fluorescein concentration was set at 0.001% (w/v) to read maximum intensities ratio. Figure 3 represents the emission peaks dependence at the two excitation wavelengths (500 and 435 nm) and their ratio upon concentration of fluorescein in water. Fluorescent properties were controlled not to change with pressure. An example is given in Figure 4 which shows the spectra of fluorescein in water obtained at the two excitation wavelengths at atmospheric pressure, 100 MPa and 200 MPa. Fluorescent intensity changed with pressure only, without any modification of wavelength and shape. These changes were completely reversible upon the release of the pressure.

The logarithmic ratio of fluorescence intensities (log QCI) is represented in Figure 5 as a function of pressure for each chosen acid and for the reference (distilled water). This measurement decreased with pressure except for MES buffer. In order to express pH out of logarithmic quotient of fluorescence intensities, the fluorescent measurements at atmospheric pressure were used. They induced a good correlation ($r = 0.96$) between pH and the logarithmic quotient of fluorescence intensities, as shown in Figure 6. Owing to this logarithmic correlation, ΔpH had been calculated for all the chosen aqueous acid solutions and for each level of pressure. The results are summed up in Figure 7 and, for 100 and 200 MPa, in Table 2. Furthermore, using these calculated values of pH and the relation $\Delta pH = (\Delta V_{H^+})/\ln 10\, RT)$, values of ΔV_{H^+} were obtained by calculating (Table 2).

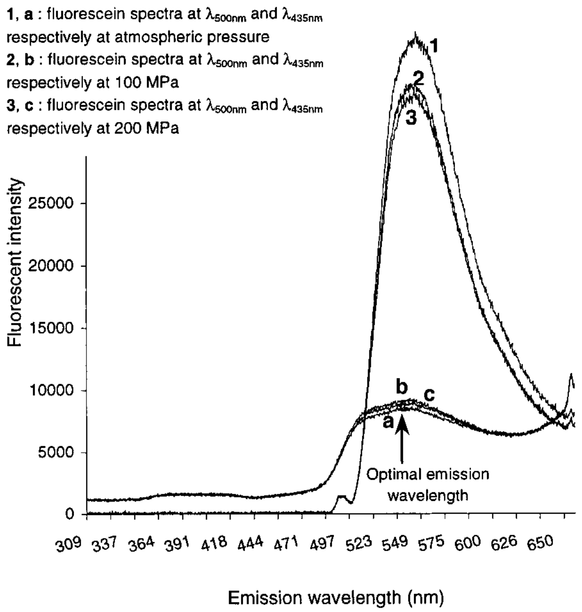


Figure 4. Spectra of fluorescein in water obtained at excitation wavelengths (500 nm and 435 nm) at atmospheric pressure, 100 MPa and 200 MPa.

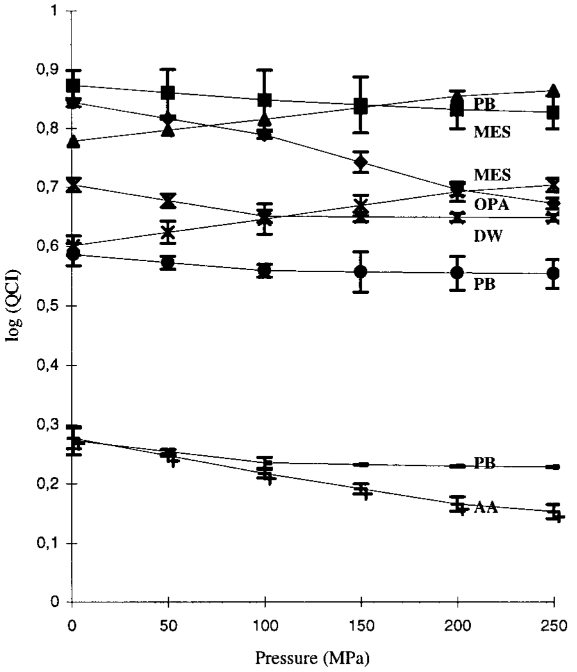


Figure 5. Pressure dependence of log (QCI) for the 5 acidic solutions at chosen pH$_i$ (initial pH). Error bars represented confidence intervals at level 0.05 of more than 3 experiments: (+) acetic acid (pH$_i$ = 4.1), (×) distilled water (pH$_i$ = 5.8), (−) potassic buffer (pH$_i$ = 4.0), (▲) MES buffer (pH$_i$ = 6.0), (●) potassic acid (pH$_i$ = 5.0), (◆) orthophosphoric acid (pH$_i$ = 7.2), (*) MES buffer (pH$_i$ = 4.0), (■) potassic buffer (pH$_i$ = 7.0).

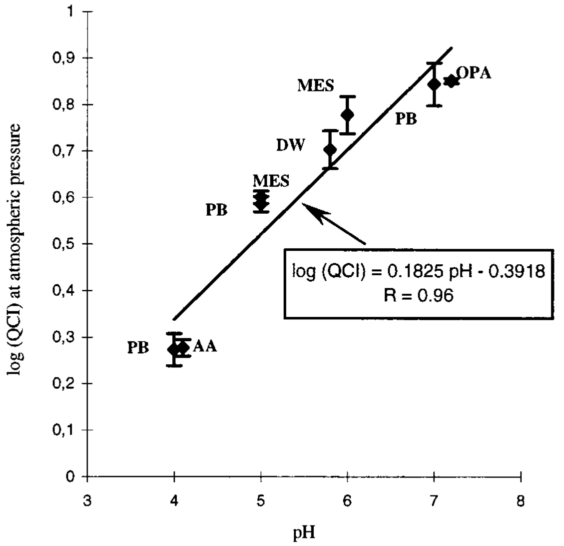


Figure 6. pH dependence of log QCI for the 5 acid solutions at atmospheric pressure. Error bars represented confidence intervals at level 0.05 of more than 3 experiments.

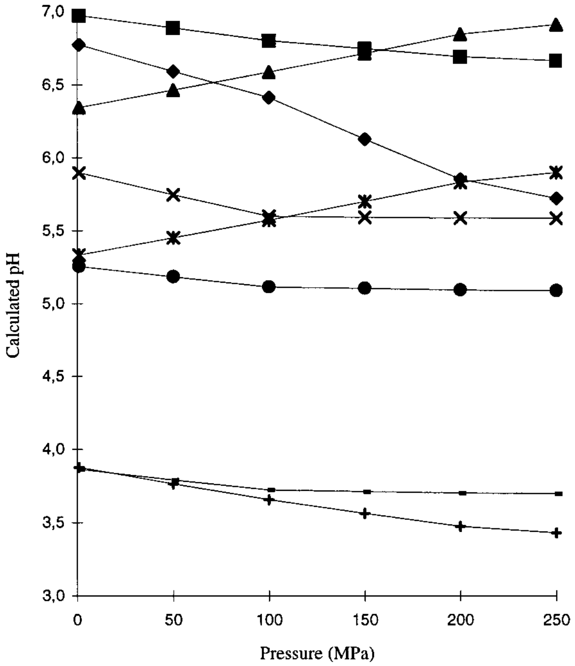


Figure 7. Evolution of calculated pH up to 250 MPa: (+) acetic acid (pH$_i$ = 4.1), (×) distilled water (pH$_i$ = 5.8), (−) potassic buffer (pH$_i$ = 4.0), (▲) MES buffer (pH$_i$ = 6.0), (●) potassic acid (pH$_i$ = 5.0), (◆) orthophosphoric acid (pH$_i$ = 7.2), (*) MES buffer (pH$_i$ = 4.0), (■) potassic buffer (pH$_i$ = 7.0).

Indeed, a pressure of 200 MPa increased the dissociation of orthophosphoric acid (ΔpH = −0.92) and acetic acid (ΔpH = −0.40). It also increased the dissociation of water (ΔpH = −0.31) and potassic buffers (ΔpH = −0.16 for potassium hydrophtalate and ΔpH = −0.28 for potassium dihydrogenophosphate). Nevertheless the same pressure led to a decrease in the dissociation of morpholineethanesulfonic acid buffer (ΔpH = + 0.50). Thus, beyond 200 MPa, the volume variation ΔV_{H^+} decreased when the pressure increased and was proved not to be constant with pressure except for MES.

The application of pressure improved dissociation of weak acids (acetic, potassic, orthophosphoric) but the induced pH decrease was inferior to −0.4 pH unit at 100 MPa and to −1.0 pH unit at 200 MPa (extreme pH variations were obtained for orthophosphoric acid as shown in Table 2). As water is much more densely packed around the ions than around the corresponding undissociated molecules, these observations are qualitatively explained by Le Chatelier's principle as follows: these acids ionize increasing the number of formal charges, so when charges are created, substantial volume contraction occurs due to solvation effects or electrostriction of water molecules around

TABLE 2: Measured pH Variations under 100 MPa and 200 MPa for Several Acid Solutions and Comparison with Some Literature Data

	experimental values					literature data		
		at 100 MPa		at 200 MPa		at 100 MPa		
aqueous acid solutions	pH_i	ΔpH	ΔV_{H^+} (mL mol^{-1})	ΔpH	ΔV_{H^+} (mL mol^{-1})	ΔpH	ΔV_{H^+} (mL mol^{-1})	
distilled water	5.8	−0.30	−16.9	−0.31	−8.7	−0.73	−22.1	8
						−0.35	−22.0	22
orthophosphoric acid	7.2	−0.36	−20.3	−0.92	−25.9	−0.45	−25.0	22
						−0.30	−16.0	22[a]
potassic buffer	7.0	−0.17	−9.6	−0.28	−7.9	−0.18	−11.2	25
	5.0/4.0	−0.14	−7.9	−0.16	−4.5			
MES buffer	6.0/5.0	+0.24	+13.5	+0.50	+14.1	+0.09	+5.0	10[b]
acetic acid	4.1	−0.22	−12.4	−0.40	−11.3	−0.20	−12.0	22
						−0.16	−9.2	30

[a] Values for the first equilibrium constant. [b] Without Na_2PO_4.

the ions. Thus, high pressure, which favors processes characterized by volume decrease, favors the ionized form. Nevertheless, MES buffer became more basic (Table 2). As pressure lowers the free energy of solvation of the ions because it is accompanied by a contraction of electrostriction of the surrounding solvent, and as this buffer contains NaOH, the pH increase could be explained by an increase in OH^- concentration with pressure and so, by a change in acid/base equilibrium.

Furthermore, the pH of water was not very significantly modified by an increase in pressure of 100 MPa. The distilled water pH decrease was experimentally determined to be approximately equal to −0.30 pH unit at 100 MPa and even 200 MPa, values comparable to the above-mentioned ones calculated by Holzapfel[28] and Parson,[24] but quite inferior to the ones given by Marquis[9] and Cheftel.[8]

Although the pH variations evaluated with fluorescein were close to former data,[10,11,22,25,30] some differences have been shown more particularly for the MES buffers (Table 2). These differences may be explained by the addition of basic reagents in order to control pH in MES and potassic buffers and also by the preparation of the aqueous solutions in distilled water at pH 5.8 instead of pure unaerated and decarboxylated water at neutral pH.

The pH variation values for 250 MPa (Figure 7) were not quite different from the 200 MPa values for all acid solutions. Indeed, the measured variations between 200 MPa and 250 MPa were inferior to 0.2 pH unit except for MES solutions, the pH of which still increased greatly with pressure. Therefore the pressure increase (inferior to 250 MPa) did not induce consequential modification on acid pH.

In conclusion, the proposed fluorescein method to measure the pH evolution under pressure has been correlated to the other previous methods and attests the low pH variation inferior to −0.4 pH unit at 100 MPa and −1.0 at 200 MPa of several acid solutions or buffers. With the exception of MES, dissociation of the acids is favored by pressure. The changes in pH are much greater for H_2PO_4 than for the others acids. These pressure experiments allow to obtain rapidly accurate measures of pH changes with pressure. The results obtained in this study are extensively used in an investigation of pressure effects on microorganisms, and it seems that the pH variation under pressure could not explain the increase in mortality of microorganisms pressurized at low pH.

References and Notes

(1) Mozhaev, V. V.; Heremans, K.; Frank, J.; Masson, P.; Balny, C. *Proteins: Struct., Funct., Genet.* **1996**, *24*, 81.

(2) Morild, E. *Adv. Protein Chem.* **1981**, *34*, 93.

(3) Ashie, I. N. A.; Simpson, B. K.; Ramaswany, H. S. *J. Food Sci.* **1996**, *61* (2), 354.

(4) Mackey, B. M.; Forestiere, K.; Isaacs, N. *Food Biotechnol.* **1995**, *9* (1-2), 1.

(5) Sale, A. J. H.; Gould, G. W.; Hamilton, W. A. *J. Gen. Microbiol.* **1970**, *60*, 323.

(6) Frauenfelder, H.; Alberding, N. A.; Ansari, A.; Braunstein, D.; Cowen, B. R.; Hong, M. K.; Iben, I. E. T.; Johnson, J. B.; Luck, S.; Marden, M. C.; Mourant, J. R.; Ormos, P.; Reinisch, L.; Scholl, R.; Schulte, A.; Shyamsunder, E.; Sorensen, L. B.; Steinbach, P. J.; Xie, A.; Young, R. D.; Yue, K. T. *J. Phys. Chem.* **1990**, *94*, 1024.

(7) Cheftel, J. C.; Culioli, J. *Meat Sci.* **1997**, *46* (3), 211.

(8) Cheftel, J.-C. *Food Sci. Technol. Int.* **1995**, *1*, 75.

(9) Marquis, R. E. *Adv. Microb. Physiol.* **1976**, *14*, 159.

(10) Kunugi, S. *High Pressure Sciences for Food*; San Ei Publishing Company: Kyoto, 1991; pp 74−84.

(11) Marshall, W. L.; Franck, E. U. *J. Phys. Chem. Ref. Data* **1981**, *10* (2), 295.

(12) Distèche, A. *Rev. Sci. Instrum.* **1959**, *30*, 474.

(13) Gier, T. E.; Young, H. S. In *High Pressure Physics and Chemistry*; Bradley, R. S., Ed.; Academic Press: London, 1963; Vol. 1.

(14) Holzapfel, W. B.; Franck, E. U. *Ber. Bunsen-Ges. Physik. Chem.* **1966**, *70*, 1105.

(15) Hamann, S. D. *High Pressure Physics and Chemistry*, Academic Press: New York, 1963; Vol. 2, Chapter 7ii.

(16) Neumann, R. C., Jr.; Kauzmann, W.; Zipp, A. *J. Phys. Chem.* **1973**, *77*, 2687.

(17) Tsuda, M.; Shirotani, I.; Minomura, S.; Terayama, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49* (11), 2952.

(18) Perrier-Cornet, J. M.; Marechal, P. A.; Gervais, P. *J. Biotechnol*. **1995**, *41*, 49.

(19) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 5th ed.; Molecular Probes, Inc.: Eugene, 1992.

(20) Holzapfel, W. B. *J. Chem. Phys.* **1969**, *50*, 4424.

(21) Burnham, C. W.; Holloway, J. R.; Davis, N. F. *Thermodynamic Properties of Water to 100 °C and 10,000 Bars.* Special Paper No. 132; Geological Society of America: Boulder, CO, 1969.

(22) Distèche, A. *The Effects of Pressure on Organisms*; Cambridge University Press: Cambridge, 1972; pp 27−60.

(23) Emschwiller, G. *Chimie Physique: Equilibres en solutions - Phénomènes de surface*. Presses Universitaires de France: Paris, 1964; Tome II.

(24) Parson, R. *Handbook of Electrochemical Constants*; Butterworths: London, 1959.

(25) Lown, D. A.; Thirsk, H. R.; Wynne-Jones, L. *Trans. Faraday Soc.* **1968**, *63*, 2073.

(26) LeBris, N., and Birot, D. Anal. Chim. Acta, **1997**, *356*, 205−215.

(27) Slavik, J. *FEBS Lett.* **1982**, *140*, 22.

(28) Imai, T.; Nakajima, I.; Ohno, T. *J. Am. Soc. Bre. Chem.* **1994**, *1*, 5.

(29) Imai, T.; Ohno, T. *J. Biotechnol.* **1995**, *38*, 165.

(30) Owen, B. B.; Brinkley, S. R. *Chem. Rev.* **1941**, *29*, 461.