# Acid Dissociation under Hydrostatic Pressure: Structural Implications for Volumetric Parameters

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Abstract

Here, we evaluate the pressure dependencies of the acid dissociation constants ($pK_a$) of pH indicator dyes and amino acids by spectroscopic methods, including chemometric-assisted direct and indirect absorption spectrometry. These data can be useful for considering molecular properties under extreme conditions, such as in deep sea. We confirmed a positive correlation between the molar volume difference ($\Delta V^\circ$) and the molar compressibility difference ($\Delta\kappa$) data for the deprotonation of molecules examined here and in literature. This relationship is discussed based on structural changes in the molecules upon deprotonation and associated hydration changes. Deprotonation from a carboxyl or phenolic hydroxyl group results in a negative $\Delta V^\circ$, whereas a molecule has positive $\Delta V^\circ$ when it loses a positive charge by deprotonation. This can be interpreted as a deprotonation-induced change in the size of the hydration sphere around the molecule. The contraction of the hydration sphere by deprotonation leads to negative $\Delta V^\circ$ and $\Delta\kappa$, particularly for carboxylic acids and phenols. In contrast, when deprotonation causes the hydration sphere to expand, both $\Delta V^\circ$ and $\Delta\kappa$ are positive. Thus, a positive correlation between $\Delta V^\circ$ and $\Delta\kappa$ data is interpreted based on the hydration structural change upon deprotonation.

Keywords: hydrostatic pressure; molar volume difference; molar compressibility difference; acid dissociation; hydration.

## Introduction

Pressure is a fundamental intensive thermodynamic property that governs various reactions, equilibria, and kinetic rates in various reactions [1-6]. Abe et al. discussed the activity enhancement of polygalacturonases, which are produced by a deep-sea yeast, at high pressures and low temperatures based on the activation volume of the enzyme complexes with a substrate [7]. They considered that the volume expansion of the enzyme-substrate complexes in the transition state causes a decrease in the activation energy for hydrolysis under deep-sea conditions. Ivanović-Burmazović et al. studied the spin crossover and kinetics of heme binding under deep-sea conditions [8]. The applied pressure reduced the binding of a small molecule to vacant coordination site of the heme Fe center and stabilized the high-spin state of the Fe center. It was speculated that this behavior reduces metabolic activity and enzymatic side reactions at high pressures. Ringo and Evans reported the effects of pressure on the enantiomeric complexation of β-cyclodextrin [9], as a model for hard-site binding interactions in enzymes and proteins [10, 11]. The pressure caused a small but notable difference in the partial molar volume between the solvated complexes of warfarin enantiomers. Thus, understanding the effects of pressure on chemical reactions is important for a diverse range of fields, not only in chemistry but also in life and environmental science.

The acid dissociation constant ($K_a$) is an important thermodynamic parameter that represents the proton transfer ability of a molecule in a solution. Because proton transfer is essential in chemical and biological systems, the pressure dependence of $K_a$ is of fundamental and practical importance [12-14]. The effects of pressure are related to the volumetric properties of the reaction, that is, the differences in molar volume ($\Delta V°$) and compressibility ($\Delta\kappa$). Because high pressure affects various reactions, not only proton transfer reactions but also supramolecular complexation and photoreactions [15-17], the quantitative interpretation of pressure effects based on these volumetric parameters is of fundamental importance. The $\Delta V$ values due to the proton transfer of acids and bases in aqueous solutions are determined by density [18], conductance [19, 20], and potentiometric measurements [21, 22].

Sue et al. determined the dissociation constants of hexanoic, heptanoic, and benzoic acids at pressures up to 30 MPa by potentiometry [23]. However, because the liquid junction potential was unstable under such conditions, potentiometric measurements were severely restricted [24]. Spectrometry is an obvious alternative technique for such measurements. Raghuraman et al. examined the pressure dependence of the p$K_a$ of phenol red and showed that p$K_a$ decreases as pressure increases up to 65.5 MPa [24]. Because widely used buffer components have mostly no effective chromophores, indirect methods

have also been used, where the pressure-induced pH changes of a buffer are detected by the changed color of a trace pH indicator. Kumar et al. reported the pressure dependence of the p$K_a$ values of acetic, benzoic, mandelic, and succinic acids using bromocresol green as a pH indicator [25]. Nueman et al. also determined $\Delta V^\circ$ and $\Delta\kappa$ for cacodylic acid, $H_2PO_4^-$, and $trisH^+$ using 2,5-dinitrophenol (2,5-DNP) and *p*-nitrophenol (*p*-NP) as pH indicators [26]. However, only a limited number of p$K_a$ values at high pressures are available despite the importance of this property. In this study, we determine the effects of pressure on the p$K_a$ of several pH indicator dyes, which cover a wide pH range of 1.5–8. Knowledge of the pressure dependencies of the p$K_a$ for pH indicators will allow us to evaluate those for acids and bases that contain no chromophores. To demonstrate the usefulness of this approach, the pressure dependencies of the first acid dissociation constants of some amino acids (p$K_a \approx 2$) were determined. The determined volumetric properties, $\Delta V^\circ$ and $\Delta\kappa$, are discussed in terms of the hydration nature of related chemical species to reveal general trends in these properties under conditions of acid-base equilibria in water.

## Experimental

### Materials

Phenol red (PR), thymol blue (TB), L-tryptophan (Trp, >98.5%), L-leucine (Leu, >99.0%), and L-phenylalanine (Phe, >98.0%) were purchased from Fujifilm Wako Chemicals Co., Ltd. (Osaka, Japan). Ethyl orange (EO), Congo red (CR, >98.0%), methyl red (MR, >98.0%), and 2,4-dinitrophenol (2,4-DNP, >98.0%) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The chemicals were used as received. The structures of the protonated species of the indicators are shown in Figure 1.

### Spectroscopic measurements

UV-vis spectra of pH indicators at various pH values under ambient pressure were measured with a spectrometer (Shimadzu, UV-3100PC). A UV-vis spectrometer (JASCO, V-560) was used to measure the spectra under hydrostatic pressure. Hydrostatic pressures were controlled using a custom-built high-pressure apparatus (Teramecs Co., Kyoto, Japan). A quartz cell (inner dimensions: 3 mm × 2 mm, height: 7 mm) connected to a short Teflon tube was used for spectrometric measurements. The cell was filled with a sample solution and sealed with a cap. The sample solution was pressurized to 320 MPa. The buffer concentration (phosphate and acetate) was 10 mM for spectrometric

measurements at ambient pressure. A buffer was not used for high-pressure measurements. The concentrations of pH indicators for spectroscopic measurements were 100 μM for TB, 50 μM for CR, 150 μM for MR, 200 μM PR, 150 μM for DNP, and 300 μM for EO.

Alternating Least Squares

The alternating least squares (ALS) method was used to separate the absorption spectra for acidic and basic species of the pH indicators. The matrices for collection of absorption spectra, **A**, are given by a component spectral matrix **K** and concentration matrix **C:**

$$\mathbf{A} = \mathbf{CK}.$$

The above equation can be rewritten as follows in terms of the unknown **K**, where **C** is substituted with random numbers.

$$\mathbf{K} = (\mathbf{C}^t\mathbf{C})^{-1}\mathbf{C}^t\mathbf{A}.$$

A negative value of **K** may be obtained as a result. Thus, after the negative numbers are replaced by zero, **C** is calculated as:

$$\mathbf{C} = \mathbf{A}\mathbf{K}^t(\mathbf{K}\mathbf{K}^t)^{-1}.$$

We again replaced the negative numbers in **C** by zero and recalculated **K**. This procedure was repeated until **K** and **C** converged.

## Results and Discussion

### Determination of acid dissociation constants of pH indicators at an ambient pressure.

The molar extinction coefficients ($\varepsilon$) of the acidic and basic forms of the pH indicators were determined to evaluate their acid dissociation constants. Figure 2 shows the pH-dependent UV-vis spectra of 50 μM TB at $P$ = 0.1 MPa; the ionic strength ($I$) was adjusted using NaCl at $I$ = 0.1 M. The absorbance at 544 nm (arising from the acidic form of TB) decreased, whereas that at 437 nm (arising from the basic form of TB) increased with increasing pH; the isosbestic point was observed at 490 nm. The p$K_a$ of TB is so low that the absorption spectrum of the pure acidic form of TB could not be recorded experimentally. Therefore, the absorption spectra of TB were decomposed by ALS to obtain the spectra of the pure acidic form of TB as well as that of the basic form. Figure S1 shows the component absorption spectra decomposed from Figure 2. We determined the molar extinction coefficient for the acidic and basic forms of TB at 544 nm ($\lambda_1$) and 437 nm ($\lambda_2$) as follows: $\varepsilon_{\lambda 1}^{\mathrm{a}}$ = 6.00×10$^4$, $\varepsilon_{\lambda 2}^{\mathrm{a}}$ = 2.59×10$^3$ L mol$^{-1}$ cm$^{-1}$, $\varepsilon_{\lambda 1}^{\mathrm{b}}$ = 606 and $\varepsilon_{\lambda 2}^{\mathrm{b}}$ = 1.73×10$^4$ L mol$^{-1}$ cm$^{-1}$. The concentrations of the acidic ([A]) and basic species ([B]) were determined using these values to determine $K_a$. The details are described in the SI.

Figure 3 shows the relationship between log([B]/[A]) and pH for TB ($I$ = 0.1 M).

The pH can be calculated from the following equation:

$$\mathrm{pH} = \mathrm{p}K^{0}_{\mathrm{a(ind)}} + \log\frac{[\mathrm{B}]}{[\mathrm{A}]} \ , \qquad (1)$$

where $\mathrm{p}K^{0}_{\mathrm{a(ind)}}$ is the acid dissociation constant of the pH indicator at ambient pressure. From the pH giving log ([B]/[A]) = 0, $\mathrm{p}K^{0}_{\mathrm{a(ind)}}$ = 1.66 for TB ($I$ = 0.1 M). Similarly, the $\mathrm{p}K^{0}_{\mathrm{a(ind)}}$ value of TB without added NaCl was determined to be 1.32, which agrees with values presented in literature [27].

The $\varepsilon^{a}$ and $\varepsilon^{b}$ values for other indicators were determined under sufficiently acidic or basic conditions, where the major species formed more than 99.9% of the total indicator concentration. Thus, $\mathrm{p}K^{0}_{\mathrm{a(ind)}}$ values for EO, 2,4-DNP, CR, MR, and PR were 3.00, 3.31, 4.10, 5.38, and 7.60, respectively, which are in close agreement with values reported in literature [28-35]. The experimental and reported $\mathrm{p}K^{0}_{\mathrm{a(ind)}}$ values are summarized in Table 1.

**Pressure effects on $\mathrm{p}K_{\mathrm{a(ind)}}$**

The $\mathrm{p}K_{\mathrm{a(ind)}}$ values at high pressures ($P \leq 320$ MPa) were determined by the same method applied to the ambient pressure results. Neumann and Pollmann studied the pressure effects on the absorbance of CR solutions up to 240 MPa and showed that the difference in the molar extinction coefficients between 0.1 and 240 MPa was not more

than 1% [36]. Therefore, pressure effects on the molar extinction coefficients were ignored, and those determined at ambient pressure were used to determine $K_{\mathrm{a(ind)}}$ at high pressures. Although the solute concentrations increase because of contraction of the solution volume, this effect is canceled out because $K_{\mathrm{a(ind)}}$ is derived from the ratio of the concentrations of the deprotonated and protonated species. Figures 4A and S2–S7 show the pressure dependencies of $\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(ind)}}$ for TB ($I$ = 0.1 M), TB, CR, MR, PR, 2,4-DNP, and EO, respectively. The $\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(ind)}}$ values for TB and 2,4-DNP decreased with increasing $P$, whereas those for CR, MR, PR, and EO increased. Byrne et al. reviewed the influence of pressure on chemical equilibria in aqueous systems.[1] The effect of pressure on an equilibrium constant is formulated as follows [37]:

$$RT\ln\left(\frac{K^{\mathrm{p}}_{\mathrm{a}}}{K^{0}_{\mathrm{a}}}\right) = -\Delta V_0(P - P^0) + 0.5\Delta\kappa(P - P^0)^2 \quad (2)$$

where $R$ is the gas constant, $T$ is the temperature, and $\Delta V_0$ and $\Delta\kappa$ are the changes in the molar volume and compressibility upon deprotonation of a molecule, respectively. We can discuss the solvation and structural changes from these parameters. Figure 4B shows the relationship between $RT\ln\left(\frac{K^{\mathrm{p}}_{\mathrm{a}}}{K^{0}_{\mathrm{a}}}\right)$ and $(P - P^0)$ obtained for TB ($I$ = 0.1 M). The solid curve represents the result of regression with Eq. (2) with $\Delta V^\circ$ and $\Delta\kappa$ as fitting parameters; $\Delta V^\circ$ and $\Delta\kappa$ were determined to be $-0.228\times10^{-6}$ m$^3$ mol$^{-1}$ and $1.75\times10^{-15}$ m$^3$ mol$^{-1}$ Pa$^{-1}$, respectively.

Table 1 summarizes $\Delta V°$ and $\Delta\kappa$ values for indicators determined in this way. The $\Delta V°$ value of 2,4-DNP has been reported to be $-1.1\times10^{-5}$ $m^3$ $mol^{-1}$ [38], which almost agrees with the corresponding value determined here. Raghuraman et al. reported $\Delta V° = -1.096\times10^{-5}$ $m^3$ $mol^{-1}$ for PR [24], their estimation involved some errors because the effects of pressure on the dissociation of phosphates were not considered. In this study, spectra at high pressures were recorded in unbuffered solutions to avoid this complexity. The use of a buffer solution for $pK_a$ determination at high pressures may lead to incorrect results. Thus, $\Delta V° = 6.45\times10^{-6}$ $m^3$ $mol^{-1}$ for PR reported in Table 1 differs from the reference value [24]. Other $\Delta V$ and $\Delta\kappa$ values have not been reported, to the best of our knowledge.

**Indirect spectrometric determination of $pK_a$ for amino acids under hydrostatic pressures**

The pressure dependence of $pK_{a(ind)}$ allows us to determine the $pK_a$ of solutes, which have no effective absorption bands in the UV-vis range. The indicators studied in this study cover a wide range of $pK_a$ values from 1 to 7. The pressure effects on the first acid dissociation constants for Trp, Lue, and Phe were evaluated by an indirect spectrometric method using TB as a pH indicator. The $pK_a$ values of Trp, Lue, and Phe at

$P$ = 0.1 MPa ($\mathrm{p}K^{0}_{\mathrm{a(amn)}}$) have been reported to be 2.38, 2.33, and 2.16, respectively [39]. In general, it is difficult to determine such low p$K_\mathrm{a}$ values; however, the use of TB allows the determination of pH changes arising from the dissociation of amino acids even under hydrostatic pressures.

HCl was used to adjust the pH of the initial solution to ~$\mathrm{p}K^{0}_{\mathrm{a(amn)}}$; thus, the solution was buffered by the acid-base equilibrium of the amino acid to be studied. The solution pH at high pressures can be determined from spectrometric changes of TB based on the following equation:

$$\mathrm{pH^{p}} = \mathrm{p}K^{\mathrm{p}}_{\mathrm{a(ind)}} + \log\frac{[\mathrm{B_{ind}}]^{\mathrm{p}}}{[\mathrm{A_{ind}}]^{\mathrm{p}}}, \tag{3}$$

where $\mathrm{A_{ind}}$ and $\mathrm{B_{ind}}$ are the acidic and basic forms of TB, respectively. For an amino acid, we can write the following equations analogous to Eq. (3).

$$\mathrm{pH^{0}} = \mathrm{p}K^{0}_{\mathrm{a(amn)}} + \log\frac{[\mathrm{B_{amn}}]^{0}}{[\mathrm{A_{amn}}]^{0}}\ , \tag{4}$$

$$\mathrm{pH^{p}} = \mathrm{p}K^{\mathrm{p}}_{\mathrm{a(amn)}} + \log\frac{[\mathrm{B_{amn}}]^{\mathrm{p}}}{[\mathrm{A_{amn}}]^{\mathrm{p}}}\ . \tag{5}$$

From Eqs. (4) and (5), the following equation is obtained:

$$\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(amn)}} = \mathrm{pH^{p}} - \mathrm{pH^{0}} + \mathrm{p}K^{0}_{\mathrm{a(amn)}} - \log\frac{[\mathrm{B_{amn}}]^{\mathrm{p}}[\mathrm{A_{amn}}]^{0}}{[\mathrm{A_{amn}}]^{\mathrm{p}}[\mathrm{B_{amn}}]^{0}}. \tag{6}$$

We can determine $\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(amn)}}$ by substituting the charge and mass balance equations into Eq. (6).

Figure 5 shows the pressure dependence of $\mathrm{p}K_{\mathrm{a(amn)}}$ for Trp (A), Lue (B), and

Phe (C). Obviously, the $pK^{p}_{a(amn)}$ values decrease with increasing pressure. Using Eq. (2), $\Delta V^{\circ}$ and $\Delta\kappa$ of the proton dissociation of the amino acids were also calculated, as summarized in Table 1. All of the amino acids studied here have negative values of $\Delta V^{\circ}$ and $\Delta\kappa$. Taulier and Chalikian studied these volumetric parameters for some α, ω-aminocarboxylic acids [40]. This paper shows that $\Delta V^{\circ} = -6\times10^{-6}$ m$^3$ mol$^{-1}$ and $\Delta\kappa = -7\times10^{-15}$ m$^3$ mol$^{-1}$ for glycine; similar values were obtained for β-alanine. The accuracy of the volumetric parameters determined in this study is discussed later.

**Correlation of $\Delta V$ and $\Delta\kappa$ values**

Figure 6 shows the correlation between the $\Delta V^{\circ}$ and $\Delta\kappa$ values, including those determined in this study and those reported in literature [25, 26, 40]. First, they are classified in terms of the signs of $\Delta V^{\circ}$. MR, PR, EO, and CR have positive $\Delta V^{\circ}$ values, whereas the others have negative values. Nobel et al. examined $\Delta V^{\circ}$ values in various chemical processes and showed that the deprotonation of a carboxyl group generally results in a negative $\Delta V^{\circ}$ [14]. Here, the amino acids, mandelic acid, and succinic acid had $\Delta V^{\circ}$ values ranging from –11 to $-14\times10^{-6}$ m$^3$ mol$^{-1}$, which are in agreement with the reported values and also with the considerations of Nobel et al. [25]. The $\Delta V^{\circ}$ values for phenols are also in this range, suggesting that deprotonation from a phenolic hydroxyl

group results in a similar change in the molar volume. In fact, a similar negative $\Delta V^\circ$ value (= $-18.4\times10^{-6}$ $m^3$ $mol^{-1}$) has been reported for the dissociation of phenol [38].

The negative $\Delta V^\circ$ for these molecules can be discussed in terms of the change in size of the hydration sphere upon deprotonation. Fedotova et al. studied the interaction of acetic acid and water molecules through the use of the one-dimensional RISM (reference interaction site model) approach [41]. This study indicated that the number of hydrogen bonds increases, particularly around carboxylate group when acetic acid dissociates. In addition, the distance between the carboxylate group and neighboring water molecules becomes shorter. Thus, deprotonation of acetic acid causes the hydration sphere to shrink, resulting in a negative $\Delta V^\circ$. The deprotonation of carboxyl and phenolic hydroxyl groups causes similar changes in the hydration structures around these functional groups.

Shimada et al. revealed the pH-dependent structures of TB using UV-vis spectrometry and chemometric analysis [42]. According to their study, TB is zwitterionic at low pH and becomes a monovalent anion by deprotonation (Figure S8). Because PR has almost the same molecular skeleton as that of TB, the structural changes for PR can be discussed in a similar manner. In addition, the cationic charges of the acidic forms of CR, MR, and EO are neutralized by deprotonation. Thus, these dyes lose a positive charge by deprotonation. Hamann et al. showed that the deprotonation of substituted anilinium

ions results in a positive $\Delta V^{\circ}$ [43]. Variation of $\Delta V$ for pH indicators in the range of 0–$6\times10^{-6}$ $m^3$ $mol^{-1}$ may arise from functional groups. Hamann et al. and Kumar et al. investigated $\Delta V^{\circ}$ of substituted phenol, aniline, and benzoic acid [25, 37, 43]. Methyl, sulfonic, and carboxylic groups had negative $\Delta V^{\circ}$ values, whereas nitro, amino, and hydroxyl groups had positive $\Delta V^{\circ}$ values. Although TB has the same molecular skeleton as that of PR, except for the ethyl and methyl groups, $\Delta V^{\circ}$ of TB is smaller than that of PR because of the effect of alkyl groups. Thus, the main influence on $\Delta V^{\circ}$ originates from deprotonation and other substitute groups.

The $\Delta\kappa$ value represents a change in the rigidity of a solvation sphere upon deprotonation. Obviously, there is a positive correlation between $\Delta V^{\circ}$ and $\Delta\kappa$, as shown in Figure 6. For carboxylic and phenolic compounds, the deprotonated species are more strongly hydrated and form a more rigid hydration sphere than the corresponding protonated one, leading to a negative $\Delta\kappa$. In contrast, because dye molecules with dissociative cationic groups have more rigid hydration spheres than the corresponding deprotonated species, their $\Delta\kappa$ values are basically positive.

Although most compounds follow the same tendency and fall on a single $\Delta V^{\circ}-\Delta\kappa$ line, MR and succinic acid deviate from this relationship. Khalid et al. suggested that deprotonation occurs from neutral or zwitterionic MR species to anionic species (Figure

S8) [44]. In the neutral species, the intramolecular interactions between the carboxyl and azo groups prevent the formation of a rigid hydration sphere. The hydration sphere becomes rigid after deprotonation because the intramolecular binding is dissociated and formation of a hydration sphere is facilitated. Because succinic acid has two carboxyl groups, which have similar p$K_a$ values of 4.21 and 5.64 [39], both dissociations may be involved in the reported $\Delta V^\circ$ and $\Delta\kappa$ values. Thus, the behaviors of MR and succinic acid are different from those of other compounds. Hence, the correlation between $\Delta V^\circ$ and $\Delta\kappa$ holds for most of the deprotonation equilibria and is well interpreted by the change in the strength of hydrogen bonding around the molecule upon deprotonation.

## Conclusion

The positive correlation between $\Delta V^\circ$ and $\Delta\kappa$ is interpreted based on changes in the strength of hydrogen bonding with surrounding water molecules and in the size of the hydration sphere upon deprotonation. The dissociation of a carboxyl and phenolic hydroxyl group results in stronger hydrogen bonding with water molecules, leading to a contraction of the hydration sphere and in turn, negative $\Delta V^\circ$ and $\Delta\kappa$ values. In contrast, pH indicators, which lose their positive charge upon deprotonation, have positive $\Delta V^\circ$ and $\Delta\kappa$ values, except MR, for which intramolecular interactions play a critical role in the

determination of its hydration.

The findings of this study facilitate the prediction of the pressure shift of p$K_a$ from structural changes to a molecule during deprotonation. In addition, if we know one of the volumetric parameters of a given deprotonation reaction, the other is precisely estimated from the relationship in Figure 6. We expect that a comprehensive compilation of the volumetric parameters for various reactions will facilitate the prediction of unknown parameters. This will give an understanding of molecular behaviors under extreme conditions, such as in deep sea and extrasolar systems.

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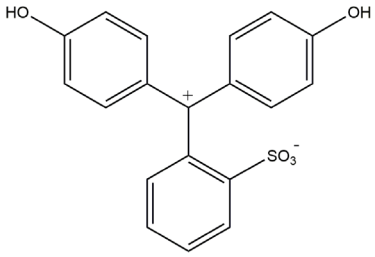
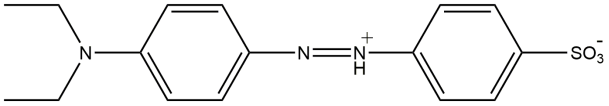
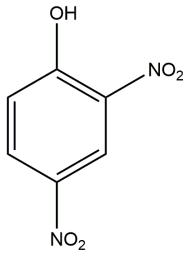


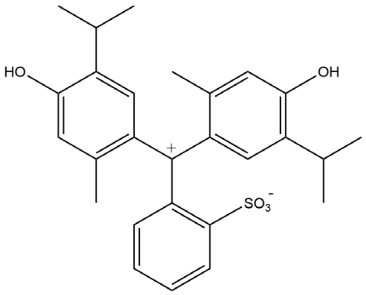

Phenol red



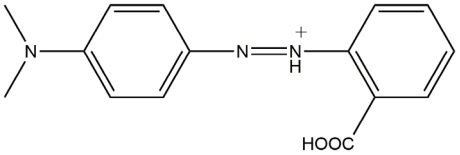

Ethyl orange



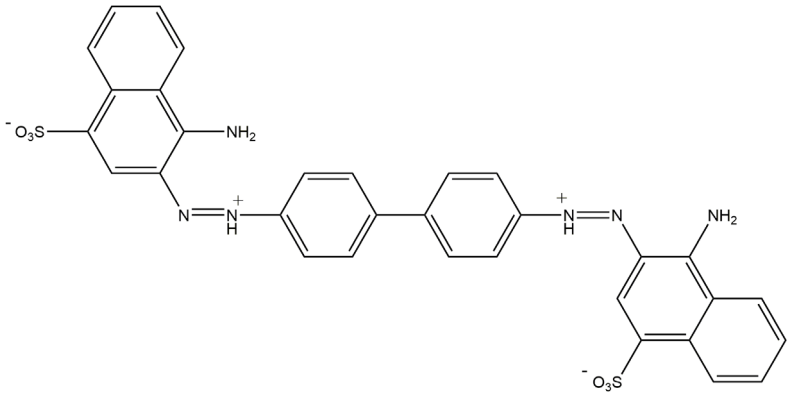

2, 4-Dinitrophenol




Thymol blue




Methyl red




Congo red

Figure 1. Structures of pH indicators used in this study. All indicators represent protonated species.

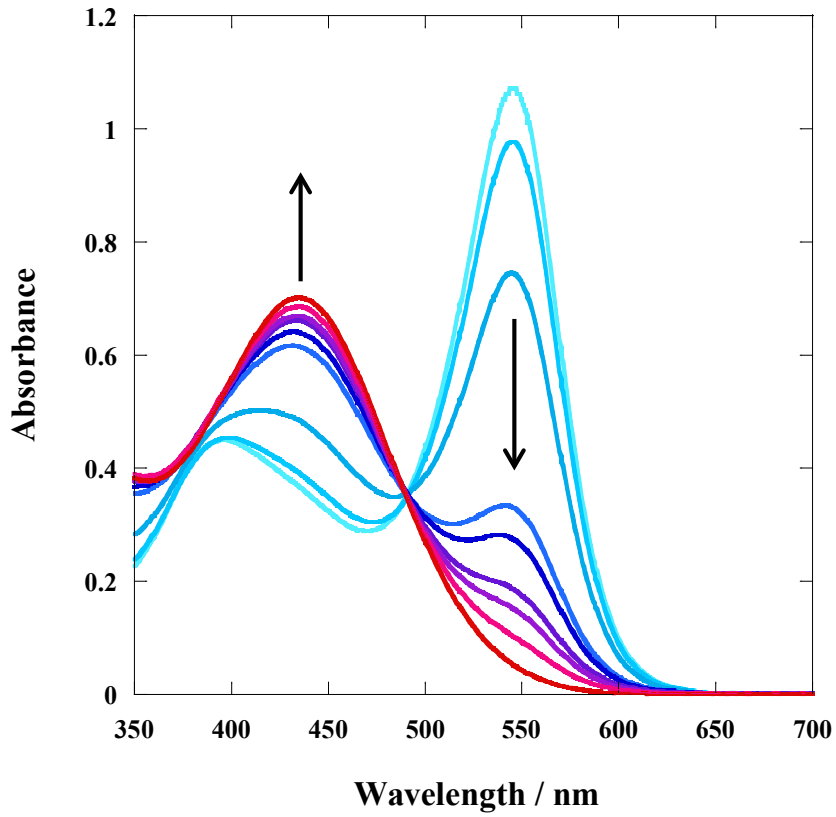



Figure 2. pH dependence of UV-vis spectra of 50 μM TB in 10 mM acetate buffer with various pH ($I_{NaCl}$ = 0.1 M). Arrows indicate a pH increase in the order of 1.00, 1.10, 1.40, 1.52, 1.68, 2.05, 2.28, 2.68, and 4.05.

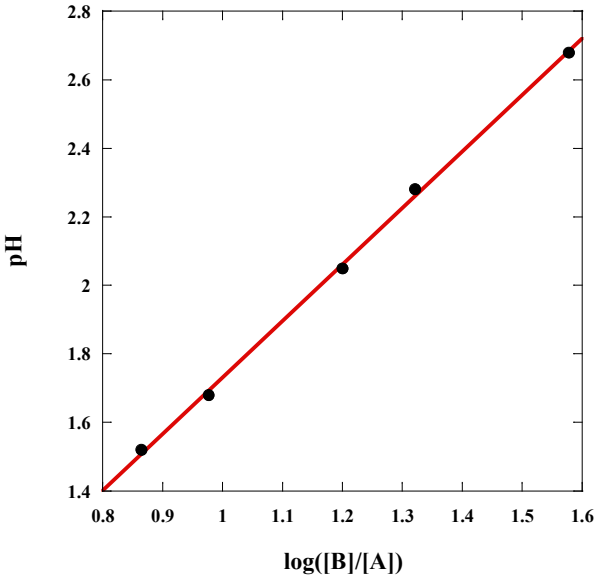



Figure 3. Relationship between log ([B]/[A]) and pH for TB ($I_{NaCl}$ = 0.1 M). [TB] = 50 μM. [A] and [B] represent the protonated and deprotonated species of TB, respectively.

Table 1 $pK_a^0$, $\Delta V°$, and $\Delta\kappa$ of pH indicator, amino acids, and weak acids.

| | $pK_a^0$ | $\Delta V°$ / $10^{-6}$ $m^3$ $mol^{-1}$ | $\Delta\kappa$ / $10^{-14}$ $m^3$ $mol^{-1}$ $Pa^{-1}$ |
|---|---|---|---|
| TB | 1.32 (1.5)[a] | −0.685 (0.047) | 0.0618 (0.036) |
| TB ($I$ = 0.1 M) | 1.66 (1.5)[a] | −0.225 (0.048) | 0.173 (0.037) |
| EO | 3.00 (4.1)[b] | 0.034 (0.0029) | −0.0215 (0.00023) |
| 2,4-DNP | 3.31 (4.09)[c] | −9.60 (0.13) | −0.976 (0.10) |
| CR | 4.10 (3.7)[d] | 5.63 (0.093) | 0.321 (0.71) |
| MR | 5.38 (4.82)[e] | 4.66 (0.68) | −1.05 (0.52) |
| PR | 7.60 (7.34)[f] | 6.45 (0.22) | 2.02 (0.17) |
| Trp ($I$= 0.1 M) | 2.38[g] | −2.72 (0.77) | 0.335 (0.17) |
| Leu ($I$= 0.1 M) | 2.33[g] | −3.15 (0.24) | −0.116 (0.18) |
| Phe ($I$= 0.1 M) | 2.16[g] | −4.94 (0.21) | −0.671 (0.16) |
| $CH_3COOH$[h] | | −11.1 | −1.7 |
| Benzoic acid[h] | | −11.3 | −2.0 |
| 2,5-DNP[i] | | −11.3 | −1.28 |
| *p*-NP[i] | | −11.3 | −1.94 |
| Cacodylic acid[i] | | −13.2 | −1.94 |

Values from references: [a]: ref. 27, [b]: ref. 35, [c]: ref. 30, [d]: ref. 29, [e]: ref. 28, [f]: ref. 31, [g]: ref. 38, [h]: ref. 25, [i]: ref. 26.

Standard deviations in parentheses.

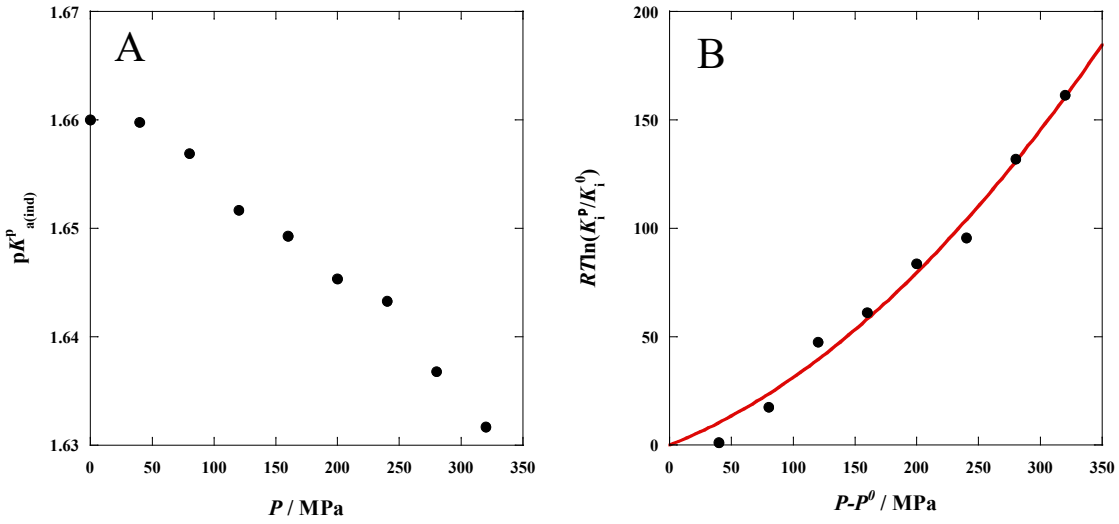



Figure 4. (A) Pressure dependence of $\mathrm{p}K_{\mathrm{a(ind)}}$ ($\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(ind)}}$) of TB ($I_{\mathrm{NaCl}}$ = 0.1 M). (B) Relationship between $RT\ln\left(\frac{K_{\mathrm{a}}^{p}}{K_{\mathrm{a}}^{0}}\right)$ and $(P - P^{0})$ of TB ($I_{\mathrm{NaCl}}$ = 0.1 M). Solid curve was calculated from Eq. (2) with the use of $\Delta V$ and $\Delta\kappa$ as fitting parameters.

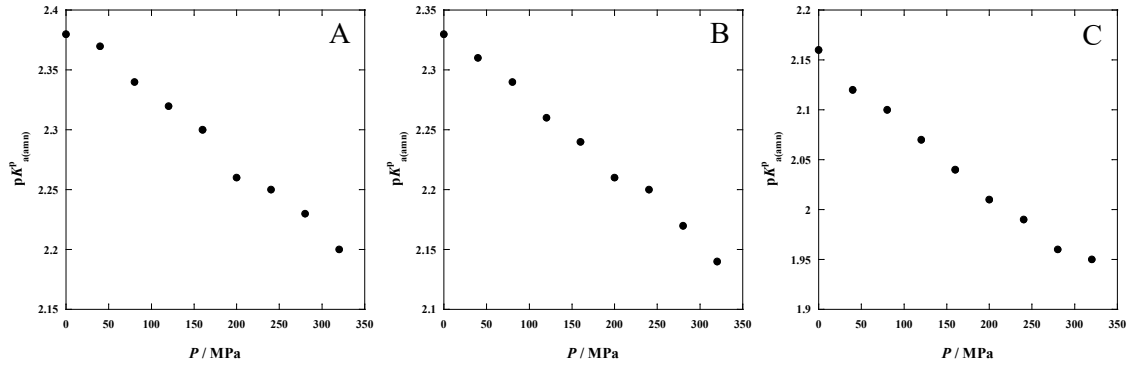



Figure 5. Pressure dependence of $\mathrm{p}K^{\mathrm{p}}_{\mathrm{a(amn)}}$ of Trp (A), Lue (B), and Phe (C) at $I_{\mathrm{NaCl}}$ = 0.1 M.

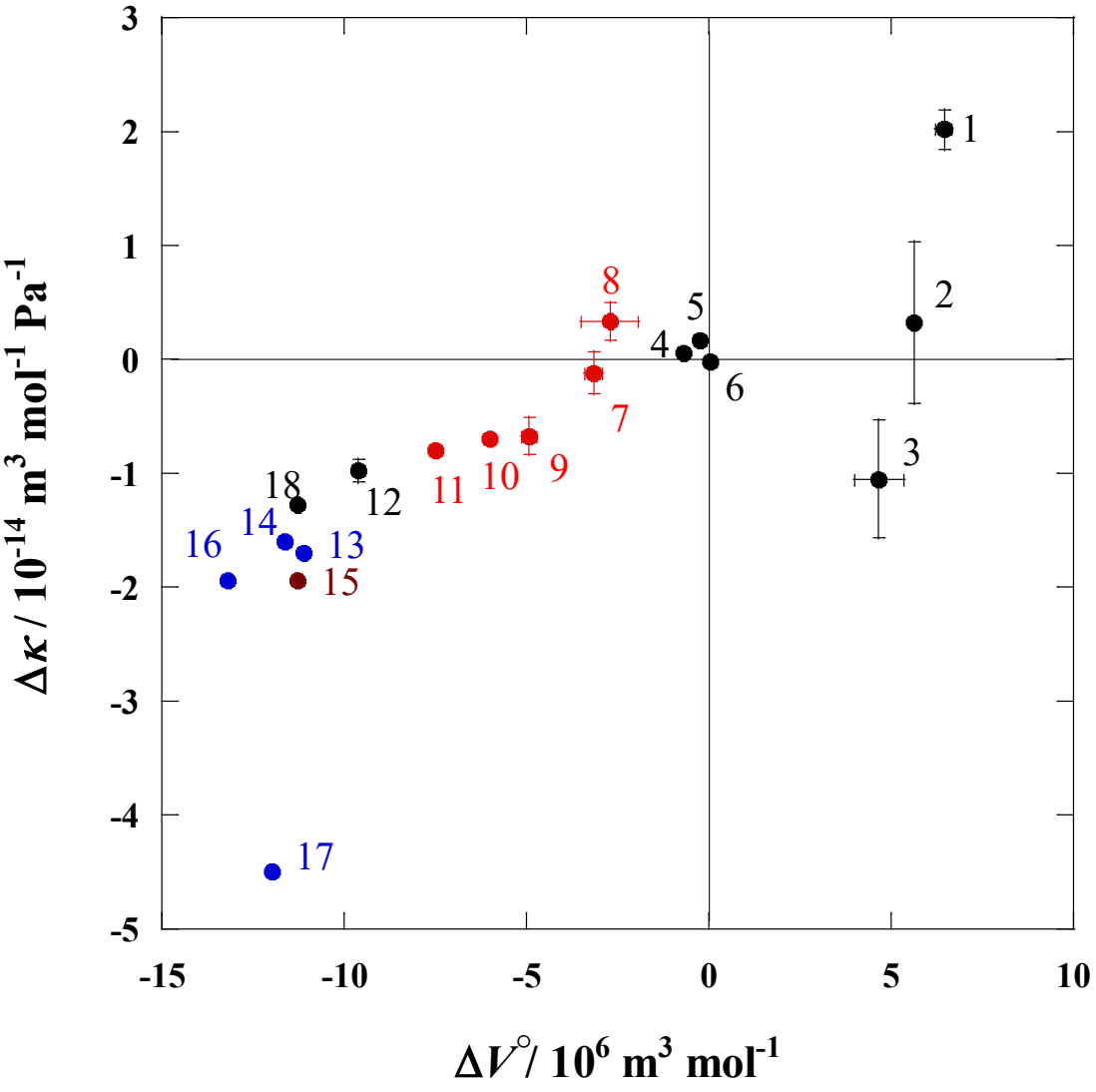
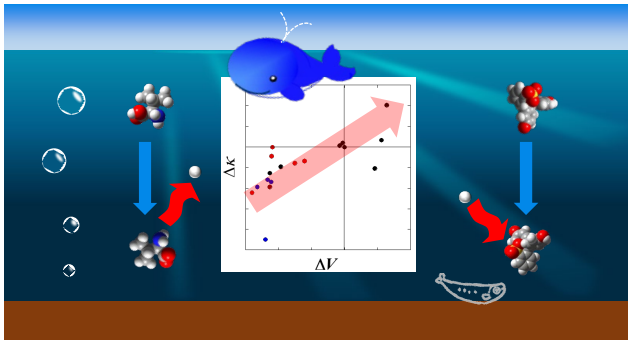



Figure 6. Relationship between $\Delta V^{\circ}$ and $\Delta\kappa$. 1: PR, 2: CR, 3: MR, 4: TB, 5: TB ($I$ = 0.1 M), 6: EO, 7: Lue, 8: Trp, 9: Phe, 10: glycine [40], 11: β-alanine [40], 12: 2,4-DNP, 13: acetic acid [25], 14: mandelic acid [25], 15: *p*-NP [26], 16: cacodyl acid [26], 17: succinic acid [25], and 18: 2,5-DNP [26].

Graphic abstract



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