The Effect of Pressure on the Dissociation of Weak Acids in Aqueous Buffers

Motoyuki Tsuda, Ichimin Shirotani,* Shigeru Minomura,* and Yoshio Terayama
Department of Physics, Sapporo Medical College, Sapporo 060
**Institute for Solid State Physics, The University of Tokyo, Roppongi, Tokyo 106*

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The effect of pressure on the dissociation of boric acid, imidazole HCl, and $H_2PO_4^-$ in aqueous buffers has been investigated up to 6 kbars, using as optical indicators *p*-nitrophenol and Cresol Red. The limiting volume change on ionization at atmospheric pressure ($\Delta\bar{V}^\circ$) and the compressibility change ($\Delta\bar{\kappa}^\circ$) for these weak acids were obtained. The values of $\Delta\bar{V}^\circ$ ($cm^3 mol^{-1}$) and $\Delta\bar{\kappa}^\circ$ ($cm^3 mol^{-1} kbar^{-1}$) are -10.1 and -0.4 for Cresol Red, -30.2 and -4.3 for boric acid, and -2.4 and -0.6 for imidazole H^+.

A large number of measurements have been made of the dissociation constants of weak electrolytes under pressure using the method of the concentration cell e.m.f., and density and conductivity measurements.[1,2] They have shown that the dissociation of a weak acid HA in water, to form H^+ and A^- ions, is increased by pressure. Pressure lowers the free energy of solvation of the ions, because it is accompanied by a contraction, or electrostriction, of the surrounding solvent; water is much more densely packed around the ions than around the corresponding undissociated molecules. One important consequence of this is that the pH of aqueous buffer solutions varies with the pressure.[3] Biochemical systems are best studied under buffered conditions, and the conformation of biomolecules is sensitive to the pH. For the study of the pressure dependence of conformational change in biomolecules,[4-8] a detailed knowledge of pressure-induced changes in the buffer pH is required. The results obtained in this study have been extensively used in an investigation of pressure effects on the spectral changes in heme proteins and invertebrate rhodopsin.[9]

Gibson and Loeffer[10] attempted to measure the effects of pressure on the pH by the use of an optical indicator; however, no quantitative results were reported. Recently Newman *et al.*[3] extensively studied the pressure effects of weak acid ionization in aqueous buffers using as optical pH indicators 2,5-dinitrophenol and *p*-nitrophenol; they gave the values of the limiting volume change ($\Delta\bar{V}^\circ$) and the compressibility change ($\Delta\bar{\kappa}^\circ$) upon ionization for acetate, cacodylate, phosphate, and tris buffer. Here we wish to report on the effect of pressure on the acid dissociation of dihydrogen phosphate ($H_2PO_4^-$), boric acid, and imidazole HCl, using as optical pH indicators *p*-nitrophenol and Cresol Red up to 6 kbars. No data have been available for Cresol Red and imidazole HCl. However, the values of $\Delta\bar{V}^\circ$ at atmospheric pressure for the ionization of boric acid have been reported on the basis of limited pressure studies utilizing a glass electrode;[11] they will serve as reference data for the optical experiments reported here.

Experimental

The *p*-nitrophenol was recrystallized from ethanol–water and given long needles were filtered and dried *in vacuo*. The Cresol Red was titrated with 0.1 M NaOH to pH 11 and filtered. The filtrate was then neutralized with 0.1 M HCl. The suspension was filtered, and the residue was washed with water. This procedure was repeated. The residue was then dried at 60 °C overnight. The imidazole, recrystallized from ethanol, was neutralized with hydrochloric acid. The other reagents, sodium borate, boric acid, sodium phosphate, and potassium dihydrogenphosphate, were reagent-grade chemicals, and were used without further purification. A stock solution of *p*-nitrophenol (0.01 M) was prepared by weighing it and by dissolving it in distilled deionized water. Solutions of this indicator in the various buffers were prepared just prior to use by diluting 1.2 ml of the stock to 10 ml with the appropriate buffer. A stock solution of Cresol Red (0.001 M) was prepared in water containing an equivalent amount of sodium hydroxide. Solutions of this indicator in various buffers were prepared just prior to use in the way described above.

The highpressure optical bomb is shown in Fig. 1(a). This bomb was constructed from hardened-steel and had three sapphire windows and a electrode for thermocouples. A

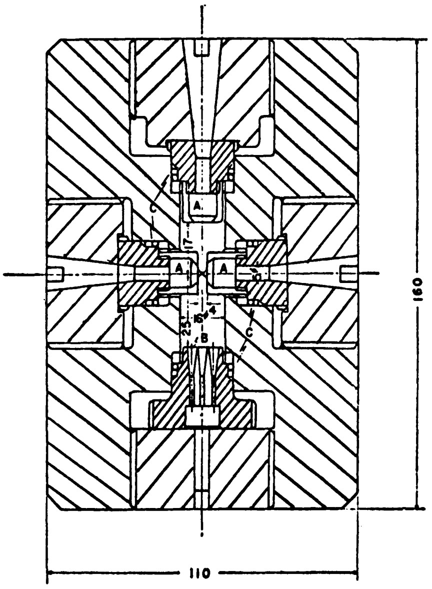

Fig. 1(a). The high pressure optical bomb.
A: sapphire window, B: electrode, C: O-ring (mm).

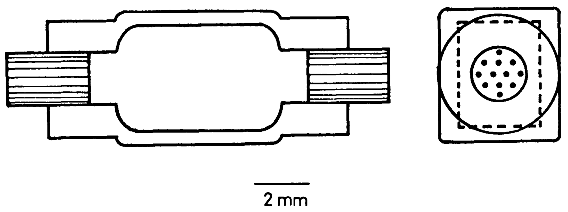

Fig. 1(b). The micro optical cell of quartz with Teflon piston.

hydrostatic pressure up to 6 kbars was applied by means of the pressuring fluid (silicone oil) through a pressure intensifier.

The pressure was determined by a Harwood manganine resistance gauge and a Heise Bourdon tube gauge. The indicator solutions were contained in an internal micro optical cell of quartz (0.3 cm path length and 1.0 cm overall length of the cell). The travelling Teflon piston in the separator served as a physical barrier to the mixing of the indicator solution with the pressuring fluid. The micro optical cell is exhibited in Fig. 1(b).

A thermostatting jacket was constructed for the high-pressure vessel. This was made of brass and was machined to fit snugly along the entire length of the highpressure vessel. The vessel, with its thermostatting jacket, was securely fastened into a metal supporting frame which was itself positioned directly into the sample compartment of a Shimadzu SV-50 spectrophotometer. The frame was designed so that the optical aperture of the cell automatically coincided with the light beam when the frame was in place.

The method of operating was to equilibrate the sample at a low pressure. After the data were obtained, the pressure was raised slightly and the system was allowed to reequilibrate. After the highest pressure had been attained, the system was returned to the initial low pressure to check for reversibility. The pH values of the buffers and the other solutions studied were measured using a Radiometer pH meter and Radiometer electrodes.

The absorbance of any solution in the optical cell of a fixed length will increase with an increase in the pressure due to compression. Before two-state equilibrium constants can be determined, the experimentally determined absorbances must be corrected for this effect. This correction** can be achieved by multiplying the experimental values by $\mathrm{OD_{I.P.}}$ (1 atm)/ $\mathrm{OD_{I.P.}}$ (P) for the isosbestic point of the absorption spectra of the indicator holds in its acid-base equilibrium. $\mathrm{OD_{I.P.}}$-(P) implies the absorbance values at the wavelength at the isosbestic point under the pressure, P.

Methods

The molar equilibrium constant (K_a) for the ionization of weak acids is pressure- or volume-dependent. When the buffer concentration is sufficiently high, as it is these studies (0.05—0.10 M), pressure-induced changes in the pH of buffer solutions do not significantly alter the concentration ratio of the acid, HA, and its conjugate base A^-.

$$\frac{\partial \mathrm{pH}}{\partial P} = \frac{\partial \mathrm{p}K_a}{\partial P} + \frac{\partial}{\partial P}(\log([\mathrm{A}^-]/[\mathrm{HA}]) \simeq \frac{\partial \mathrm{p}K_a}{\partial P} \qquad (1)$$

The pHs of solutions can be determined by an examination of the absorption spectra of pH indicators, using the following equation:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{I} - \log\left(\frac{\Delta \mathrm{OD}}{\mathrm{OD}}\right), \ \Delta\mathrm{OD} = \mathrm{OD_I} - \mathrm{OD} \qquad (2)$$

where $\mathrm{p}K_\mathrm{I}$ is the dissociation constant of the acid form of the indicator, $\mathrm{OD_I}$ is the optical density at $\lambda_{\max}$ corresponding to the complete conversion of the indicator to its conjugate base, and OD is the observed optical density at the same wavelength due to the conjugate base of the indicator acid. The relation of the pressure dependence of $\mathrm{p}K_a$ and $\mathrm{p}K_\mathrm{I}$ can thus be determined using Eq. 3, which arises from a combination of Eqs. 1 and 2.

$$\frac{\partial(\mathrm{p}K_a)}{\partial P} = \frac{\partial(\mathrm{p}K_\mathrm{I})}{\partial P} - \frac{\partial \log(\Delta\mathrm{OD}/\mathrm{OD})}{\partial P} \qquad (3)$$

The effect of the pressure on $\mathrm{p}K_\mathrm{I}$ can be determined if the pressure dependence of $\mathrm{p}K_a$ is known. Equation 1 shows that the pressure-induced changes in $\mathrm{p}K_a$ can be attributed directly to changes in the pH. Thus, changes in the pH of the buffer with the pressure can be determined from the pressure dependence of the OD of the indicator.

The pressure dependencies of $\mathrm{p}K_a$ for boric acid and imidazole HCl were determined using as optical indicators *p*-nitrophenol ($\mathrm{p}K_\mathrm{I}=7.05$) and Cresol Red ($\mathrm{p}K_\mathrm{I}=8.3$). The pressure dependence of $\mathrm{p}K_\mathrm{I}$ for *p*-nitrophenol was determined but that of $\mathrm{p}K_\mathrm{I}$ for Cresol Red is not known. Accordingly the pressure dependence for Cresol Red was calculated from data available for $\mathrm{H_2PO_4^-}$.[3]

The limiting volume change for the weak acid at atmospheric pressure ($\Delta\bar{V}^\circ$) and the compressibility change ($\Delta\bar{\kappa}^\circ$) during ionization may be readily obtained by means of thermodynamics. The change in the partial molar volume can be determined from the pressure derivative of the free energy, *i.e.*:

$$\left(\frac{\Delta\partial F^\circ}{\partial P}\right) = -\left(\frac{\partial RT\ln K}{\partial P}\right)_T$$
$$= \bar{V}^\circ(\mathrm{H^+ + A^-}) - \bar{V}^\circ(\mathrm{HA}) = \Delta\bar{V}^\circ \qquad (4)$$

where $\bar{V}^\circ$ (HA) and $\bar{V}^\circ$ ($\mathrm{H^+ + A^-}$) are the partial molar volumes of acid and ionized forms of the weak acid. On the other hand, V varies with the pressure, the derivative

$$\left(\frac{\partial \Delta V^\circ}{\partial P}\right)_T = -\Delta\bar{\kappa}^\circ \qquad (5)$$

being termed the standard partial molar isothermal compressibility on ionization. It follows that

$$\frac{RT\ln(K_p/K_o)}{P} = -\Delta\bar{V}^\circ + \frac{1}{2}\Delta\bar{\kappa}^\circ P, \qquad (6)$$

where the $\Delta\bar{\kappa}^\circ$ term accounts for the pressure dependence of $\Delta\bar{V}^\circ$. Using the experimental data, the values of $\Delta\bar{V}^\circ$ and $\Delta\bar{\kappa}^\circ$ were obtained from the slope and intercept of $\log(K_p/K_o)/P$ *vs.* P (Eq. 6).

Results and Discussion

The absorption spectra of *p*-nitrophenol and Cresol Red in phosphate, borate and imidazole (Im) buffer were recorded at various pressures from 1 bar to 6 kbars. The absorption band of the alkali form of the indicators ($\lambda_{\max}$; 570 nm for Cresol Red and 400 nm for *p*-nitrophenol) in phosphate and borate buffers increased in intensity and shifted slightly to a higher wavelength, but that of the indicator in the imidazole buffer decreased in intensity when it also shifted slightly to a higher wavelength under pressure. These changes were completely reversible upon the release of the pressure. An example is given in Fig. 2, which shows the absorption spectra of Cresol Red in the borate buffer (pH=8.4) recorded as a function of the pressure up to 6 kbars. The spectra are not corrected for contraction. The absorption band of

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** As the pressure-induced shift of absorption maximum ($\lambda_{\max}$) is small, this treatment may be correct for the present experimental accuracy.

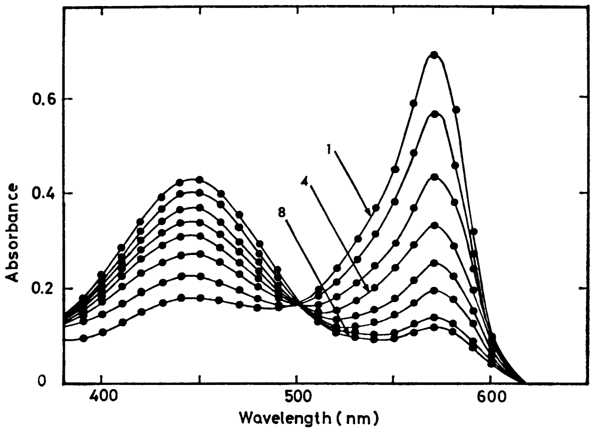

Fig. 2. The effect of pressure on the absorption spectrum of Cresol Red in the borate buffer (0.05 M, pH 8.4) and at appropriate pressures at 25 °C.
1: 1 bar, 2: 706 bar, 3: 1570 bar, 4: 2420 bar, 5: 3340 bar, 6: 4180 bar, 7: 5000 bar, 8: 5480 bar.

the alkali form of Cresol Red decreased, while that of the acid form increased, in intensity and shifted slightly to higher wavelength under pressure. The isosbestic point of the spectra shifted. This is due to the contraction of the sample under pressure. After the correction of the values of OD, the values of log (ΔOD/OD) were calculated for each buffer-indicator system; a summary of these data is presented in Fig. 3. These values of Δlog (ΔOD/OD) are equal to $\Delta pK_I - \Delta pK_a$, where the Δ symbol indicates the difference between the quantity at 1 bar and at a high pressure, *P*.

The values of Δlog(ΔOD/OD) for *p*-nitrophenol in the borate buffer and the imidazole buffer in conjunction with previously available data for the pressure dependence of pK_I for *p*-nitrophenol permitted the determination of the pressure dependence of pK_a for boric acid and imidazole HCl. Further, the pressure dependence of the pK_a of boric acid and imidazole HCl was examined using Cresol Red as the indicator. The pressure dependence of the pK_I for the Cresol Red was calculated from the data available for $H_2PO_4^-$.[3] These pK_I values in conjunction with the data for Cresol Red in the borate and imidazole buffers, gave an alternate determination of ΔpK_a values for boric acid and imidazole HCl. The results are given in Fig. 4. With the exception of imidazole HCl, the dissociation of the acids is favored by pressure. The increases in the ΔpK_a value for Cresol Red are very similar to those for *p*-nitrophenol. The changes in the pK_a for boric acid are much greater. The results show that the pH of boric acid changes by more than two units up to the pressure of 6 kbars. On the other hand, the pH of imidazole HCl is almost pressure-insensitive up to 6 kbars.

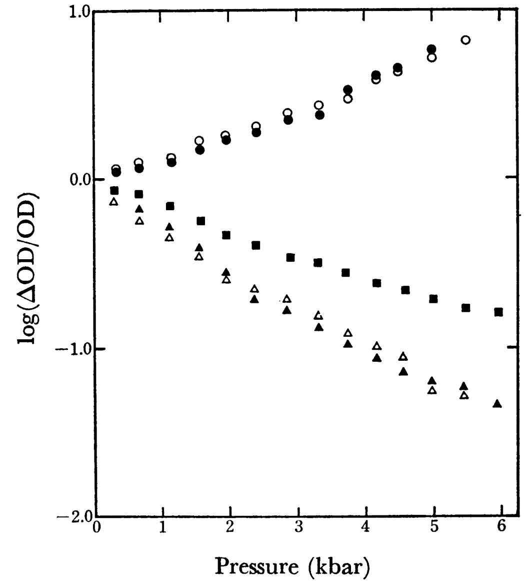

Fig. 3. Pressure dependence of log (ΔOD/OD) for the optical indicator Cresol Red in borate (●), phosphate (■) and imidazole (▲) buffers and for *p*-nitrophenol in borate (○), and imidazole (△) buffers.

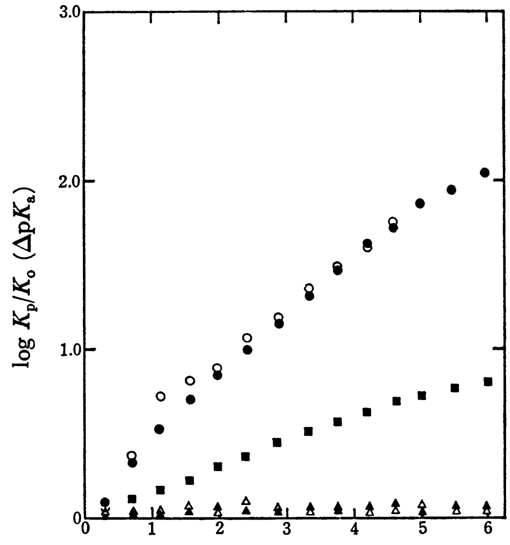

Fig. 4. Pressure dependence of calculated values of K_p/K_o for boric acid (○,●), Cresol Red (■), and imidazole HCl (△,▲).

The values of $\Delta\bar{V}^\circ$ and $\Delta\bar{k}^\circ$ were calculated, using Eq. 6, from the experimental values of $\log(K_p/K_o)$ for *p*-nitrophenol, boric acid, and imidazole HCl. They are given in Table 1, along with the approximate values of $\Delta\bar{V}^\circ$ for the imidazole HCl obtained. The values of $\Delta\bar{V}^\circ$ for Cresol Red is close to the values previously

Table 1.

Weak acid	$\Delta\bar{V}^\circ$(cm^3/mol)	$\Delta\bar{k}^\circ$(cm^{3}mol/kbar)	Coupled acid	pH
Cresol Red (CR)	−10.1	−0.4	$H_2PO_4^-$	7.60
Imidazole H$^+$	−2.4	−0.6	CR	7.84
	−2.0	−0.4	PNP	7.25
Boric acid	−30.2	−4.3	CR	8.26
	−30.9 (−31.4)[a]	−4.6	PNP	7.52
p-Nitrophenol (PNP)	(−11.3)[b]	(−1.43)[b]		

a) Observed by means of electrodes by A. Distèche and S. Distèche.[11] b) Observed by R. C. Neuman *et al.*[3]

reported for other phenols, that is, $-11.3\ \mathrm{cm^3\ mol^{-1}}$[3] and $-10.3\ \mathrm{cm^3\ mol^{-1}}$[2] for *p*-nitrophenol and $-11.8\ \mathrm{cm^3\ mol^{-1}}$[13] and $-11.3\ \mathrm{cm^3\ mol^{-1}}$[3] for 2,5-dinitrophenol. This fact supports the validity of the procedures used.

The value of $\Delta\bar{V}^\circ$ for boric acid is considerably larger than those of the acids. The reason for the large $\Delta\bar{V}^\circ$ value for the amine bases is that the positive charges of the cations are localized on the small nitrogen atoms.[1] Distèche[2] has suggested that this is probably also the reason why $\Delta\bar{V}^\circ$ is very small for boric acid. We suggest that another contributive factor may be the existence of a preliminary hydration equilibrium for boric acid;

$$\mathrm{H_3BO_3 + H_2O \rightleftharpoons H_3BO_3\cdot H_2O}$$

$$K_1 = \frac{[\mathrm{H_3BO_3\cdot H_2O}]}{[\mathrm{H_3BO_3}]} \tag{7}$$

$$\mathrm{H_3BO_3\cdot H_2O \rightleftharpoons B(OH)_4^- + H^+}$$

$$K_2 = \frac{[\mathrm{H^+}][\mathrm{B(OH)_4^-}]}{[\mathrm{H_3BO_3\cdot H_2O}]} \tag{8}$$

The dissociation constant, K, is based on the total concentration of boric acid, both free and hydrated, so that

$$K = \frac{[\mathrm{B(OH)_4^-}]\cdot[\mathrm{H^+}]}{[\mathrm{H_3BO_3}]+[\mathrm{H_3BO_3\cdot H_2O}]} = \frac{K_1K_2}{1+K_1} \tag{9}$$

It follows that, when K_1 is very small, $K=K_1K_2$ and $\Delta\bar{V}^\circ=\Delta\bar{V}^\circ_1+\Delta\bar{V}^\circ_2$, where the subscripts 1 and 2 refer to the hydration and ionization reactions, 7 and 8 respectively. Ellis correctly pointed out that the abnormally large values of $\Delta\bar{V}^\circ$ for carbonic acid and sulforous acid can be ascribed to the contribution of $\Delta\bar{V}^\circ_1$ since, at the atmospheric pressure, $\mathrm{CO_2}$ and $\mathrm{SO_2}$ are only slightly hydrated in solution.[14]

In striking contrast to these results is the fact that the pH of an imidazole buffer solution is almost pressure-insensitive, below 6 kbars. This is understandable in terms of the ionization equilibrium. Substances that are stronger bases than water are able to extract protons from water molecules, whereupon the conjugate acid of the base, $\mathrm{BH^+}$, is formed, together with hydroxide ions:

$$\mathrm{Im + H_2O \rightleftharpoons ImH^+ + OH^-} \tag{10}$$

In an analogous way, acids such as $\mathrm{BH^+}$ are able to react with water molecules, which then function as a base;

$$\mathrm{ImH^+ + H_2O \rightleftharpoons Im + H_3O^+} \tag{11}$$

Reaction 10 may be taken as the basic dissociation of the base, Im, and the corresponding equilibrium constant is K_b. Reaction 11 is the acidic dissociation of acid $\mathrm{BH^+}$, and the corresponding equilibrium constant is K_bn. K_b and K_bn are related by means of the ion-product constant of water, K_w:

$$K_\mathrm{w} = K_\mathrm{b}K_\mathrm{bn}$$

Amines are usually characterized in terms of K_bn, because of the emphasis upon the hydrogen-ion concentration. The large volume changes for boric acid and amine bases with connected with Reaction 11 and with the effect of the pressure on K_b. The determinations of the pH yield $K_\mathrm{bn}=K_\mathrm{w}/K_\mathrm{b}$, so that the effect of the pressure on K_b is cancelled by the corresponding effect on K_w, $\Delta\bar{V}^\circ$ value for water being $-20.4\ \mathrm{cm^3\ mol^{-1}}$. This explains why, for the imidazole buffer, the observed $\Delta\bar{V}^\circ_\mathrm{bn}$ value is $-4.2\ \mathrm{cm^3\ mol^{-1}}$, which means that the $\Delta\bar{V}^\circ_\mathrm{b}$ value for Reaction 10 is $-16.2\ \mathrm{cm^3\ mol^{-1}}$.

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References

1) S. D. Hamann, "High Pressure Physics and Chemistry," R. S. Bradley, ed., Vol. 2, Academic Press, New York (1963).

2) A. Distèche, *Symp. Soc. Exp. Biol.*, **26**, 27 (1972).

3) R. C. Neuman, W. Kauzmann, and A. Zipp, *J. Phys. Chem.*, **77**, 2688 (1973).

4) K. Suzuki and Y. Taniguchi, *Symp. Soc. Exp. Biol.*, **26**, 103 (1972).

5) J. F. Brandts, R. J. Oliveira, and C. Westort, *Biochemistry.*, **9**, 1038 (1970).

6) A. Zipp and W. Kauzmann, *Biochemistry*, **12**, 4217 (1973).

7) A. A. Lamola, T. Yamane, and A. Zipp, *Biochemistry*, **13**, 738 (1974).

8) Q. H. Gibson and F. G. Carey, *Biochem. Biophys. Res. Commun.*, **67**, 747 (1975).

9) M. Tsuda *et al.*, unpublished data.

10) R. E. Gibson and O. H. Loeffler, *Trans. Am. Geophys. Un.*, **1941**, 503.

11) A. Distèche and S. Distèche, *J. Electrochem. Soc.*, **114**, 330 (1967).

12) D. A. Lown, H. R. Thirsk, and Lord Wynne-Jones, *Trans. Faraday Soc.*, **64**, 2073 (1968).

13) S. D. Hamann, *Division of Applied Chemistry*, *Technical Paper* No. 3, CSIRO, Australia (1972).

14) A. J. Ellis, *J. Chem. Soc.*, **1959**, 3689.