Fig. 5. Acetate buffer in 0.1, 0.01, and 0.001M KCl. 1, 2, 3: $(E_1 - E_p) = 2.3\ RT/F \log k_p'/k_1'$ as a function of the total acetate and acetic acid concentration. 1', 2', 3': extrapolation of $\Delta_p K' = -\log k_1' + \sqrt{\mu_1'} \log k_p' - \sqrt{\mu_p'}$ (Eq. [2] and [4]) to zero buffer concentration.
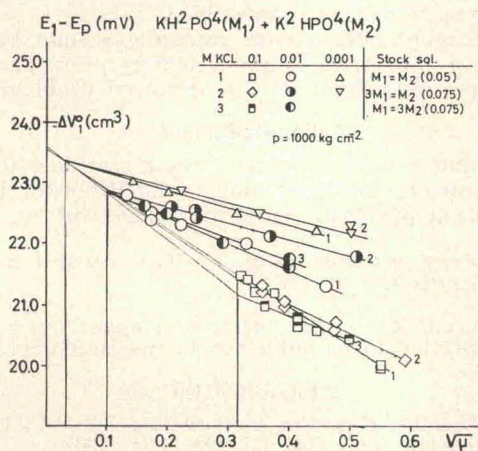

Fig. 6. Phosphate buffer $K_{(2)}$ in 0.1, 0.01, and 0.001M KCl. Extrapolation of $(E_1 - E_p) = 2.3\ RT/F \log k_p'/k_1'$ to zero KCl and buffer concentration at three different buffer ratios; $-\Delta V_1° = 24.0\ cm^3\ mole^{-1}$.

we take the necessary data to extrapolate $-\Delta V_1$ to [KCl] $= 0$ on the $\sqrt{\mu}$ scale as shown in Fig. 2. $-\Delta V_1° = 9.2\ cm^3\ mole^{-1}$ in fairly good agreement with the conductivity determinations (Table I) [8.8 cm^3 $mole^{-1}$, Hamann (10)]. Data for formate buffer, treated in the same way as the acetate buffer, yield $-\Delta V_1 = 7.4$ $cm^3\ mole^{-1}$ (Fig. 2).

It is seen on this same figure that the curves for acetic acid and formic acid, for acetate buffer and for formate buffer, run nearly parallel with each other.

Phosphate buffer (2nd step).—In Fig. 6, the results obtained for three different buffer ratios are plotted on the $\sqrt{\mu}$ scale and the double extrapolation leads without any difficulty to $-\Delta V_1° = 24.0\ cm^3\ mole^{-1}$, which is to be preferred to a preliminary estimate, 23.4 cm^3 $mole^{-1}$, published in 1962.

Density measurements predict $-\Delta V_1° = 28.1\ cm^3$ $mole^{-1}$ (14) but dilatometric experiments by Linderstrøm-Lang and Jacobsen [see ref. (11)] give 24.1 cm^3 $mole^{-1}$.

Adenosine triphosphate and phosphorylcreatine.— Figure 7 indicates that $-\Delta V_1°$ (24.0 $cm^3\ mole^{-1}$) is the same in phosphate buffer and in ATP solutions at $pH = 7.0$, the double extrapolation being done on the $\sqrt{m}$ and $\sqrt{\mu}$ scales. A slightly smaller value is obtained if both extrapolations are made on the $\sqrt{\mu}$ scale (dotted line).

Figure 8 shows the corresponding results for phosphorylcreatine. $-\Delta V_1° = 21.2\ cm^3\ mole^{-1}$ and is considerably smaller than the volume change observed for phosphate buffer $(K_{(2)})$ and ATP.
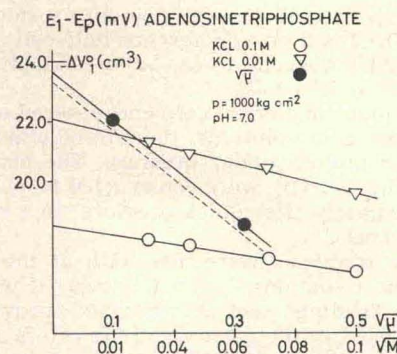

Fig. 7. Adenosinetriphosphate in 0.1 and 0.01M KCl. Extrapolation of $(E_1 - E_p) = 2.3\ RT/F \log k_p'/k_1'$ to zero ATP concentration ($\sqrt{m}$ scale) and to zero KCl concentration ($\sqrt{\mu}$ scale); $-\Delta V_1° = 24.0\ cm^3\ mole^{-1}$. Dotted lines, both extrapolations are made on the $\sqrt{\mu}$ scale.
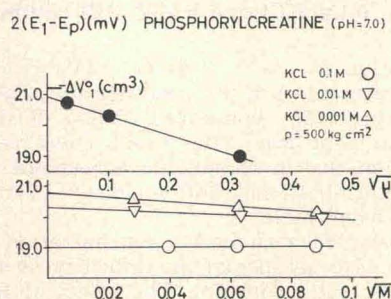

Fig. 8. Phosphorylcreatine in 0.1, 0.01, and 0.001M KCl. Extrapolation of $2\ (E_1 - E_p) = 2 \times 2.3\ RT/F \log k_p'/k_1'$ to zero phosphorylcreatine concentration (lower curves) and to zero KCl concentration (upper curve); $-\Delta V_1° = 21.2\ cm^3\ mole^{-1}$.
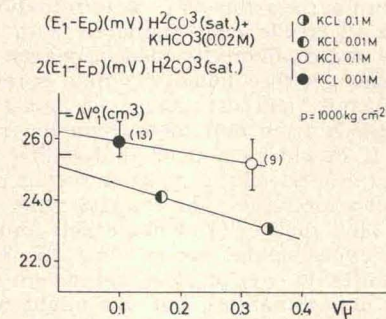

Fig. 9. Extrapolation of $2\ (E_1 - E_p) = 2.3\ RT/F = \log k_p'/k_1'$ for carbonic acid (saturated solution) and $(E_1 - E_p) = 2.3\ RT/F \log k_p'/k_1'$ for bicarbonate buffer (H_2CO_3 sat. + 0.02M $KHCO_3$) to zero ionic strength; $-\Delta V_1° = 26.6\ cm^3\ mole^{-1}$ and 25.5 $cm^3\ mole^{-1}$. The circles on the upper curve represent average values derived from 13 and 9 determinations in 0.01M KCl and 0.1M KCl respectively; the horizontal lines indicate the highest and lowest values.

The ΔV values are calculated from readings at 500 kg cm^{-2} because of the rather marked curvature of the $(E_1 - E_p) = f(p)$ curve.

Control analysis before and after a pressure run failed to indicate any hydrolysis.

It is well known (12) that the $pK_{(2)}$ of phosphoric acid in phosphorylcreatine is strongly affected by the methylguanidinium group, its value being 4.6 instead of 7.2. The difference observed for $\Delta V_1°$ is related to this phenomenon insofar as it is related to the ionic solvation energy difference between H_3PO_4 and its ester.

Carbonic acid and bicarbonate buffer.—Our previous determinations of ΔV_1 for carbonic acid in 0.1M KCl (1) gave values as high as 31.0 and 32.3 $cm^3\ mole^{-1}$, but only 22.8–23.9 $cm^3\ mole^{-1}$ for the bicarbonate buf-

fer (values corrected for the asymmetry due to KCl when 0.1M HCl is used as reference half-cell). Density data (6) predict 29.0 cm³ mole⁻¹ and conductivity data give 26.5 cm³ mole⁻¹ (13).

Experimental difficulties were encountered especially with carbonic acid solutions, the pH of which shows long-term instability under pressure. The curves giving $(E_1 - E_p) = f(p)$ were constructed from readings taken immediately after quick pressure steps separated by long intervals.

The same problems were met with at the start of the new determinations. Later, however, it was found that stable readings can be obtained provided the silicone oil used in the glass electrode cell is saturated with CO_2 as well as the aqueous solutions and the cell filled from pipettes where the gas phase is pure CO_2. These precautions limit the possibility of CO_2 exchanges between both liquid phases and the escape of CO_2 in the pressure bomb or elsewhere.

The circles on the upper curve of Fig. 9 correspond to the average value of, respectively, 9 and 13 determinations in 0.1M KCl and 0.01M KCl saturated with CO_2.

Extrapolation at $\sqrt{\mu} = 0$ gives $-\Delta V_1° = 26.6$ cm³ cm⁻³ in agreement with the conductivity experiments.

Bicarbonate buffer containing 0.02M KHCO₃ was tested in the same way. The results show very much less dispersion, are in reasonable agreement with our previous determinations, and yield on extrapolation $-\Delta V_1° = 25.5$ cm³ mole⁻¹.

Extrapolation at zero buffer concentration was not tried in this case because of the difficulty of maintaining the buffer ratio constant. The effect of H_2CO_3 on log K_p'/K_1', however, is small (5) compared to the effect of the ionic strength, so that it seems safe to extrapolate.

Discussion

Table I summarizes the $\Delta V_1°$ determinations made with a glass electrode cell compared with the data from density and conductivity measurements.

The agreement with conductivity data is fairly good for acetic, formic, and carbonic acid, but the value found for H_3PO_4 from emf measurements is about 2 cm³ higher. It should be noticed that H_3PO_4 is highly dissociated in diluted solutions which means relatively small pH shifts for large $\Delta pK'$ changes when pressure is applied. This makes ΔV calculations much more sensitive to experimental errors. The ΔV found for phosphate buffer $K_{(1)}$ is in close agreement with the conductivity determinations, but this might be fortuitous since we notice that systematically ΔV values for buffers, from pH measurements, are smaller than the values obtained for pure acid solutions.

The difference is much larger than possible experimental errors: 0.9, 1.8, 2.0, 1.1 cm³ for acetate, formate, phosphate $K_{(1)}$, and bicarbonate buffer, respectively.

The difference is identical with Na or K salts. This practically eliminates the possibility of an error due to asymmetry in cation composition between the cell compartments since the reference half-cell always contains KCl except for the experiments in 0.001M KCl where the effect of KCl is negligible. However, the cell cannot be made symmetrical with respect to anions. Though it seems rather unlikely, these could be adsorbed on the glass membrane and cause errors in the buffer measurements.

pK values are difficult to determine with precision when dissociation is appreciable and it is known that, for example, the data for phosphoric acid $K_{(1)}$ buffer at different buffer ratios extrapolate to different values of log K depending on the choice of the function to estimate the activity coefficients (17). It can however be shown that a change of the activity coefficients used to calculate m_H shifts both K_p and K_1 and has but little effect on log K_p/K_1 for phosphoric acid, phosphate buffer $K_{(1)}$, and formic acid. For acetic acid, acetate buffer and formate buffer m_H is small except at very high dilutions and can be completely neglected for carbonic acid and bicarbonate buffer.

The fact that the observed differences exist even at infinite dilution eliminates as explanation all the effects which affect the activity coefficients: ionic associations, uncomplete dissociations, salting in and out effects, etc., unless one admits that some of these effects only disappear at extreme dilutions where emf measurements become impossible.

More experiments on, for example, acetate buffer, covering a very wide range of buffer ratios, perhaps will throw more light on this unsolved problem.

Acknowledgment

REFERENCES

1. A. Distèche, *Rev. Sci. Instruments*, **30**, 474 (1959).
2. A. Distèche, *This Journal*, **109**, 1084 (1962).
3. A. Distèche and M. Dubuisson, *Bull. Inst. Océanographique Monaco*, **1174**, 1 (1960).
4. S. D. Hamann, *J. Phys. Chem.*, **67**, 2233 (1963).
5. H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2d ed., Reinhold Publishing Corp., New York (1958).
6. B. B. Owen and S. R. Brinkley, *Chem. Rev.*, **29**, 461 (1941).
7. H. S. Harned and B. B. Owen, *J. Am. Chem. Soc.*, **52**, 5079 (1930).
8. A. J. Ellis and D. W. Anderson, *J. Chem. Soc.*, **342**, 1765 (1961).
9. S. D. Hamann and W. Strauss, *Trans. Faraday Soc.*, **51**, 1684 (1955).
10. S. D. Hamann, "Physico-chemical Effects of Pressure," Butterworths Scientific Publications (1957).
11. F. K. Johnson, H. Eyring, and M. J. Polissar, "The Kinetic Basis of Molecular Biology," John Wiley & Sons, New York (1954).
12. W. D. Kumler and J. J. Eiler, *J. Am. Chem. Soc.*, **65**, 2355 (1943).
13. A. J. Ellis, *J. Chem. Soc.*, **750**, 3689 (1959).
14. O. Redlich and J. Bigeleisen, *Chem. Rev.*, **30**, 171 (1942); see ref. (5).
15. J. S. Smith, Dissertation Yale Univ. (1943); see ref. (5).
16. A. Bodanszky and W. Kauzmann, *J. Phys. Chem.*, **66**, 177 (1962).
17. R. Bates, *J. Res. Nat. Bureau Standards*, **47**, 127 (1951).