pH Corrections in Chemical Denaturant Solutions

Orlando Acevedo,* Mercedes Guzman-Casado,† Maria M. Garcia-Mira,‡ Beatriz Ibarra-Molero,‡ and Jose M. Sanchez-Ruiz†

*Departamento de Física, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia; and †Facultad de Ciencias, Departamento de Química Física, Universidad de Granada, 18071 Granada, Spain.

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The need to apply corrections to obtain pH values from glass-electrode pH-meter readings in mixed solvents has been recognized in the literature for many years (1–6). Mixed solvents (water/urea and water/guanidinium chloride) are routinely used in experimental studies on protein folding kinetics and energetics and the effect of concentrated denaturants on pH-meter readings for protein solutions was already considered in early protein folding studies (see, for instance, Refs. 7 and 8). However, to the best of our knowledge, values for the pH-correction terms in chemical denaturant solutions have not been available until very recently (9). Thus, we have recently determined the pH-correction terms in water/guanidinium chloride (9) and found them to be significant for concentrated guanidinium chloride solutions (about 0.8 pH units for 6 M guanidinium chloride). This implies that guanidinium-chloride-induced denaturation experiments addressed at determining protein thermodynamic stability have been carried out so far at a constant value of the pH-meter reading. It is uncertain, however, whether the experimental procedure for chemical denaturation should be modified so that a constant value of true pH is obtained; thus, as we noted (9), it could be argued that pH should be changed with denaturant concentration in such a way that the protonation state of the ionizable groups relevant for stability does not change (although this kind of experiment may be difficult to carry out in practice). On the other hand, it is clear that denaturation Gibbs energy values reported in the literature for the same “pH” (actually, pH-meter reading) and derived from different kinds of denaturation experiments (urea-induced, guanidinium-chloride-induced, thermal) may correspond to different values of “true” pH, which may contribute to the discrepancies often found. In connection with this, we pointed out (9) the interest of determining the pH-correction terms for water/urea; these are reported in the present paper and compared with the values we obtained previously in water/guanidinium chloride (9).

Urea used in this work was from Sigma and hydrochloric acid was analytical grade from Panreac. Deionized water was used throughout. Water/urea mixtures were passed through an ion-exchange resin (AG501-X8(D) from Bio-Rad) and used immediately. Urea concentrations were determined from refraction index measurements (10) using an Atago R500 refractometer. pH measurements were performed at 25°C with a Crison 52-09 glass electrode connected to a Crison Digit-501 pH-meter that can detect 0.01 units of pH. The scale of the pH-meter was adjusted with aqueous standard buffers from Crison. Dielectric constants for water/urea mixtures at 25°C were determined from capacity measurements (11) using a dekameter DK 300 from WTW (Weilheim, Germany).

The pH of a solution is the minus logarithm of hydrogen ion activity,

\[ \text{pH} = -\log_{10}[H^+] \]


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The continuous line is the best fit of a second-order polynomial to the dielectric constant values ($\epsilon = 78.41 + 1.669 \times 10^{-2} + 8.686 \times 10^{-4} \times C^2$). (Middle) Logarithm of the activity coefficient of the hydrogen ion in mixtures water/urea at 0.1 M ionic strength as calculated using the Debye-Hückel limiting law (Eq. [3]) and the Debye-Hückel equation that includes an ion-size parameter (Eq. [4]). Note that the difference between the two values of $\log_{10} \gamma_H^*$ is only about 0.01 units. (Bottom) pH-correction terms in water/urea. ○, average of four experiments with CIH concentrations 2.1 × 10^{-3} M, 4.1 × 10^{-3} M, 9.8 × 10^{-3} M, and 1.35 × 10^{-2} M; ●, average of four experiments with CIH concentrations 2.6 × 10^{-3} M, 4.3 × 10^{-3} M, 5.6 × 10^{-3} M, and 6.3 × 10^{-3} M; ■, average of four experiments with CIH concentrations 1.96 × 10^{-3} M, 3.79 × 10^{-3} M, 9.62 × 10^{-3} M, and 1.35 × 10^{-2} M. The standard errors associated with these averages were always in the range 0.01–0.02, values which are smaller than the size of the symbols. The continuous line for water/urea is the fit of fourth-degree polynomial to the urea-concentration dependence of the $\delta \log_{10} \gamma_H^*$ values: $\delta \log_{10} \gamma_H^* = -0.394 \times C + 6.015 \times 10^{-2} \times C^2 - 7.157 \times 10^{-1} \times C^3 + 3.382 \times 10^{-2} \times C^4$.

where the correction term ($\delta \log_{10} \gamma_H^*$) contains contributions from the transfer Gibbs energy of the proton from aqueous solution to the mixed solvent and from the liquid junction potential. Again, see Ref. (9) for a more detailed discussion.

According to Eq. [2], the pH correction for a given water–cosolvent mixture can be calculated (as $\log_{10} \gamma_H^*$) from the pH-meter reading for a CIH solution in the mixture, provided that a value of $\log_{10} \gamma_H^*$ for that solution can be calculated as $-\log_{10} \gamma_H^* = \log_{10} (\text{activity coefficient})$ (see Eq. [1]). The hydrogen ion concentration required for this $\log_{10} \gamma_H^*$ calculation can be easily obtained from a pH measurement in a matching solution in water with the same CIH concentration as that in the water–cosolvent mixture, as we have described (9). The activity coefficient, on the other hand, must be estimated theoretically; in our previous study on water/guanidinium chloride (9), we showed that $\gamma_H^*$ could be taken as unity for guanidinium chloride concentrations of about 1 M or higher, due to the screening of the ion–ion interactions caused by guanidinium chloride. We cannot assume, however, $\gamma_H^* = 1$ in concentrated urea solutions, since

\[
\text{pH}^* = \log_{10} \gamma_H^* = -\log_{10} \chi_H^*.
\]
urea is not a salt; we have resorted, then, to the Debye-Hückel limiting law,
\[
\log_{10} \gamma^*_H = -A \cdot \sqrt{T},
\]
where \(I\) is the ionic strength and \(A\) is the Debye-Hückel constant which depends on temperature and the dielectric constant of the medium (see Chap. 3 in Ref. 12). The dielectric constants for water/urea mixtures determined in this work (see Fig. 1, top) were used to obtain the urea-concentration dependence of \(A\) in Eq. [3]. The calculated activity coefficients for a representative ionic strength are shown in Fig. 1 (middle). As we have previously discussed (9), the estimation of the activity coefficient is not a critical step in the calculation of the pH corrections. Thus, for instance, the \(\gamma^*_H\) coefficient can also be evaluated using an "extended" form of the Debye-Hückel law that includes an ion-size parameter (see page 10 in Ref. 4),
\[
\log_{10} \gamma^*_H = -A \cdot \sqrt{T} / \left(1 + B a_0 \gamma^*_H\right),
\]
where \(B\) is a constant that varies with temperature and dielectric constant (see Chap. 3 in Ref. 12) and \(a_0\) is the ion-size parameter. However, Eq. [4] with \(a_0 = 9\) Å (from Table 3.3, page 49, in Ref. 4) gives values for \(\log_{10} \gamma^*_H\) which differ about 0.01 or less from those obtained using Eq. [3] (see Fig. 1, middle); thus we could use either equation to estimate the activity coefficient and obtain the same (to all practical purposes) pH-correction values. Actually, even assuming ideal behavior (that is, setting \(\gamma^*_H = 1\)) would not lead to grossly different values for the correction terms (differences of about 0.05 or less from the values obtained using Eq. [3] or [4]).

We show in the lower panel of Fig. 1 values of \(\delta pH^*\) calculated as \(pH^* = pH_0\), where \(pH_0\) is the pH-meter reading for a CIH solution in water/urea and \(pH^*\) is the true pH value of the solution calculated using Eq. [1] with \([H^+]\) determined from the pH measurement in a matching CIH solution in water (as explained in Ref. 9) and \(\gamma^*_H\) given by Eq. [3]. Note that there is an excellent agreement between the \(\delta pH^*\) values determined using different CIH concentrations (within the 0.002–0.14 M range).

For comparison, we also include in Fig. 1 the correction terms determined previously for water/guanidinium chloride mixtures (9). The \(\delta pH^*\) values in both water/urea and water/guanidinium chloride are large in absolute value, but they have opposite signs! This means that the pH-meter reading for a concentrated guanidinium chloride solution is lower than the true pH value (\(pH^*\)), while the pH-meter for a concentrated urea solution is higher than \(pH^*\). Since, as we have discussed above, the effect of the \(\gamma^*_H\) coefficient is rather minor, it also means that the \(pH^*\) of, for instance, a 0.01 M CIH solution (in water, water/urea, or water/guanidinium chloride), is close to 2, while the pH-meter reading of a 0.01 M CIH solution in concentrated guanidinium chloride is significantly lower than 2 and the pH-meter reading of a 0.01 M CIH solution in concentrated urea is significantly higher than 2, facts that the interested reader may easily verify by him/herself.

Of course, the linear extrapolation method (LEM)\(^3\) commonly used to calculate denaturation Gibbs energies from equilibrium chemical denaturation data is expected to automatically correct for a significant part of the difference between pH-meter reading and true pH. That is, for a chemical denaturation experiment carried out at a constant value of the pH-meter reading, both the denaturation Gibbs energy and the \(pH^*\) value can be taken to change linearly with denaturant concentration within the narrow transition region and it appears reasonable to conceive the LEM as a simultaneous extrapolation of these two dependencies down to zero denaturant concentration, resulting in an extrapolated pH* value perhaps not too different from the pH-meter reading. We note, however, that the LEM will not correct completely for the difference between pH-meter reading and \(pH^*\), since the denaturant-concentration dependence of the pH-correction term is not linear in a wide concentration range (Fig. 1). In any case, the fact that the pH-correction terms in water/urea and water/guanidinium chloride are now available (Fig. 1) should make it possible for the parameters obtained from chemical denaturation experiments to be assigned to specific \(pH^*\) values in an unambiguous manner.

REFERENCES


\(^3\) Abbreviation used: LEM, linear extrapolation method.


