Reconciling Measurement and Prediction of Free and Solvated Water in Solution

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ABSTRACT: In 2019, Wexler showed that, by applying Raoult’s law rigorously to aqueous solutions and properly accounting for hydration of solutes, equations can be derived that accurately describe solute and solvent activity over the full range of concentrations from infinitely dilute to pure solutes. One feature of this theory is that it also predicts the amount of water that is free and the amount that is bound to the solute. In 2004 and 2005, Choi and co-workers used an electrodynamic balance to measure (i) the concentration of a range of organic and electrolyte solutes as a function of water activity and (ii) the amount of free and bound water in these solutions using the fluorescence of pyranine as a probe. In the current work, we compare the predictions of Wexler’s theory to the measurements of Choi. After the adjustments to the amount of free water obtained by these measurements, the predictions of Wexler’s theory match the measurements of Choi. The potential reasons for the adjustments are discussed.

1. INTRODUCTION

Aqueous solutions are highly complex due to a number of factors including the strong dipole moment of water molecules and their ability to hydrogen-bond to each other and to solutes. In a recent work, Wexler1 showed that this complexity can be captured relatively simply by assuming that Raoult’s law2 applies to all constituents in solution and that the solutes may hydrate as molecules bound to the solute and is given by

\[ m = \frac{1}{M_w} \frac{1}{1 - a_w} + H_w(a_w) \]

where \( a_w \) is the water activity, \( M_w = 0.0182 \) kg/mol is the molar mass of water, and \( H_w(a_w) \) is the average number of water molecules bound to the solute and is given by

\[ H_w(a_w) = \frac{\sum_i j a_i^w \prod_{q=1}^i K_{iqw}}{1 + \sum_i a_i^w \prod_{q=1}^i K_{iqw}} \]

The \( K_{iqw} \) are the equilibrium constants between solute \( i \) with \( q \) bound waters, whose activity is \( a_{iqw} \) and solute \( i \) with \( q-1 \) bound waters, with activity \( a_{i(q-1)w} \)

\[ K_{iqw} = \frac{a_{iqw}}{a_ia_{i(q-1)w}} \]

The corresponding equation for electrolytes is

\[ m = \frac{1}{M_w} \frac{1}{1 - a_w} + \frac{1}{2} \{ \frac{\sum_i j a_i^w K_{iqw}}{1 - a_i^w} + H_i(K_{iqw}^DH_w) \} + \frac{1}{2} \{ \frac{\sum_i j a_i^w K_{iqw}}{1 - a_i^w} + H_i(K_{iqw}^DH_w) \} \]

where \( \nu_\alpha \) and \( \nu_\beta \) are the stoichiometric coefficients for the electrolyte, and \( K_{iqw}^DH \) is Pitzer’s form of the Debye–Huckel term

\[ K_{iqw}^DH = \exp \left( -\frac{2A_i^{3/2}}{1 + \rho I^{1/2}} \right) \]

where \( I \) is the ionic strength, \( A_i = 2.917 \) at 298 K relates the electric permittivity of the solvent to the change in water activity due to solute ions, and \( \rho \) is a fit parameter related to the ion size.

The resulting equations have two sets of free variables that are fit to data. The first set comprises the equilibrium constants that govern the equilibrium between each water molecule free in solution and bound to the solute. The second set comprises the \( \rho \)'s in Pitzer’s form of the Debye–Huckel term that describes the long-range electrostatic interaction for ions in solution. The derivation of these equations can be found in Wexler1.

One feature of Wexler’s activity model is that the free water and bound water are separate terms, so the model not only predicts the molality–activity relationship but also the amount
of free and bound water per solute as a function of water activity. In eq 1, \(a_s/(1 - a_s)\) is the free water per solute, whereas in eq 4, \(K_{DH}^w a_s(1 - K_{DH}^w a_s)\) is the free water per ion. Similarly, the bound water per solute in eq 1 is given by \(H_s(a_s)\), whereas the bound water per ion in eq 4 is given by \(H_i(K_{DH}^w a_s)\). Thus, by fitting the \(K_{qw}\)'s and \(\rho\)'s to molality–water activity data, the model also predicts the partitioning of the water between the free and bound pools.
In a paper published in 1992, Chakraborty and Berglund used pyranine to probe the amount of free water in solution. Because they were performing these measurements in bulk solution, they were limited in concentration range to the solute’s solubility. In papers published in 2004 and 2005, Choi and co-workers used the pyranine fluorescence techniques pioneered by Chakraborty and Berglund with a single-droplet electrodynamic balance to measure the molality−water activity relationship and the free water in sub- and supersaturated solutions of sucrose, glucose, sodium chloride, sodium sulfate, ammonium sulfate, magnesium nitrate, and magnesium sulfate. The goal of the present work is to examine the ability of Wexler’s model to predict the partitioning of water between the free and bound pools by comparing the model predictions to the measurements of Choi and colleagues.

2. RESULTS

2.1. Fitting Model Parameters. Figure 1 shows the model fit for sucrose using data from Scatchard et al., Tokuoka and Ishitani, Robinson et al., Ruegg and Blanc, and Stokes and Robinson. The vertical axis shows the osmotic coefficient defined as \( \phi = -\ln \left( \frac{a_w}{\nu M_w m} \right) \) because it is very sensitive to differences between the model and data. When the model fits the osmotic coefficient data, the solute activity will also be accurate. The horizontal axis is the square root of the mole fraction. The square root is taken to expand the more dilute portion of the curve, which better shows the behavior of the model and data in this region. Sucrose contains eight OH moieties that could hydrogen-bond to water. Instead of fitting eight or more \( K_{iqw} \) equilibrium constants, we assumed that all the equilibrium constant values fit a logistic function given by

\[
K_{iqw} = \frac{K_{iw}^x}{1 + \exp((q - q_0)/\Delta q)}
\]  

Equation 6 has two plateaus: \( K_{iqw} = K_{iw}^x \) for \( q \ll q_0 \) and \( K_{iqw} = 0 \) for \( q \gg q_0 \). For small values of \( \Delta q \), the transition between these two plateaus is sharp, whereas for large values of \( \Delta q \), this transition is more gradual. For small values of \( \Delta q, q_0 \) essentially represents the maximum number of sites where water molecules can bind to the solute. So, instead of eight parameters to fit, we only fit two parameters. We used the solver in Microsoft Excel.
to minimize the difference between the measurements and the model, finding $K^{w}_{vw} = 1.0$ and $q_0 = 11.1$, which gives a hydration number of 5.3.

Figure 4. Fit of the model to sodium sulfate data.

Figure 5. Fit of the model to ammonium sulfate data.

Figure 2 shows the fit for glucose. Equations 1 and 6 are not able to fit the data. Glucose is known to polymerize in solution, forming glycogen, a storehouse for sugar in metabolism.
We assumed that glucose exists in monomer form in dilute solutions and, at increasing concentrations, forms a dimer. Appendix contains the derivation for the molality as a function of water activity for dimerizing compounds. We used eqs A11 and 6 to model the glucose solution. Table 1 contains the parameter fit values. The high value of \( K_{AA} \), the equilibrium constant between the monomer and the dimer, means that the dimer forms even in quite dilute solutions.

Figure 3 shows the fit for sodium chloride wherein the data were obtained from Robinson and Stokes\textsuperscript{14} for sub-saturated conditions and Chan et al.\textsuperscript{15} for sub- and supersaturated conditions. As with all the electrolytes, the solver within Microsoft Excel was used to minimize the difference between the

![Figure 6](image6.png)

**Figure 6.** Fit of the model to magnesium nitrate data.

![Figure 7](image7.png)

**Figure 7.** Fit of the model to magnesium sulfate data.
measurements and the model by adjusting $\rho$ in the Debye–Huckel term and the equilibrium constants for each ion. In the solver, we constrained the fit so that the equilibrium constants were positive and so that they were not increasing in value. That is, we assumed that the first water binds to the ion at least as strongly as the second water. Likewise, for subsequent bound waters. For sodium chloride, and often other electrolytes, this resulted in equilibrium constants with the same values, not unlike the distribution represented in eq 6, but sometimes with two plateaus, which we interpret as an inner layer of bound waters and an outer layer. The fit values are given in Table 2.

Figure 4 shows the fit for sodium sulfate. The measurements up to saturation are from Robinson and Stokes and Rard and Miller. Supersaturated measurements are from Tang and Munkelwitz. See the sodium chloride section for how the model was fit for this and subsequent electrolytes. We were able to find a parameter set that fits the peak in the osmotic coefficient data (shown) or a set that fits the trough in the data, but not both. The fit parameters are shown in Table 2.

Figure 5 shows the fit for ammonium sulfate. Subsaturated data are from Wishaw and Stokes. Data that extend into the supersaturated regime are from Zhang and Chan and Tang and Munkelwitz. The two supersaturated data sets disagree somewhat so the fit averaged between them.

Figure 6 shows the fit for magnesium nitrate. Subsaturated data are from Robinson and Stokes. Supersaturated data are two data sets from Ha and Chan.

Figure 7 shows the fit for magnesium sulfate. Subsaturated data are from Robinson and Stokes and Rard and Miller. Supersaturated data are two data sets from Ha and Chan.

2.2. Comparison of Model Predictions of Bound and Free Water to Measurements. Choi and co-workers following the work of Chakraborty and Berglund used pyranine as a probe to measure free water in solution. Chakraborty and Berglund performed their measurements in bulk solutions by limiting the concentration to the solubility of the solute. Choi and co-workers performed their measurements in bulk solutions but also in an electrodynamic balance so that the free/bound water partitioning could be measured deep into the supersaturated range. The free water-to-bound water ratio is measured by fluorescence of the pyranine. The total water is measured by the voltage on the electrodynamic balance, which gives the mass of the droplet. The water activity is controlled by the relative humidity in the balance.

The solution thermodynamic model presented by Wexler predicts both the free and bound water associated with the solute. The number of moles of free water per mole of solute is given by $\nu K_w a_w (1 - K_w a_w)$, where $\nu$ is the number of moles produced by the solute per mole of solute in solution. For sucrose, $\nu = 1$. For sodium chloride, $\nu = 2$. For ammonium and sodium sulfate and magnesium nitrate, $\nu = 3$. Since we assumed that glucose dimerizes in solution, $\nu = 0.5$ for sufficiently concentrated solutions such that effectively all the glucose has formed dimers. The important
point here is that, for sucrose, there are no free parameters governing the free water content, while for the electrolytes, the only free parameter is $\rho$, which primarily influences the free water content in the dilute solution range. The equilibrium constant fit parameters all relate to the bound water.

Figures 8–14 compare the free and bound water predictions to the measurements of Choi and co-workers. The open square markers are the raw free water measurements, while the open triangular markers are the raw bound water measurements. The points with a cross through them correspond to measurements of the bulk solution. The other points were taken in the electrodynamic balance (EDB).

Sodium sulfate is the only compound in the set where the predictions and measurements agree. For the others, we multiplied the measured free water by adjustment factors listed in Table 3. When the free water measurements were adjusted this way to match the predictions, (i) the shape of the free water measurement curve came close to that of the prediction and (ii) the bound water measurements also approached the bound water predictions.

Why was this adjustment necessary? One reason is measurement uncertainty. In all but Figure 14, there are bulk and EDB measurements interspersed. They rarely agree and the extent of disagreement is similar to the disagreement with the predictions. Chakraborty and Berglund,5 and others cited in their work, used pyranine as a fluorescent probe for the free water content in solution. Fluorescence is excited at 342 nm. The peaks in emission occur at 440 and 511 nm, corresponding to protonated (PyOH*) and deprotonated (PyO−*) pyranine in the excited state, respectively. The ratio of these peaks is used as a marker for the free water content. There are a number of reasons why pyranine may not be an accurate probe for the free water content of solutions leading to the adjustments listed in Table 3:

- Fundamentally, the literature does not seem to contain any validation of pyranine as a quantitative indicator of the free and bound water contents. That is, there is no independent measurement of the free and bound water contents of solutions that can be used to validate pyranine fluorescence as a probe.
- A number of assumptions are employed to convert fluorescence emissions into bound and free water contents:
  1. Fluorescence of PyOH* at $\sim$440 nm is due to the presence of bound water.
  2. Fluorescence of PyO−* at $\sim$510 nm is due to the presence of free water.
  3. Only one free water can participate in the proton exchange for the reaction $\text{PyOH}^* \leftrightarrow \text{PyO}^- + \text{H}^+$
  4. Fluorescence quantum yields of PyOH* and PyO−* are the same.

Assumptions (1), (2), and (4) might not be valid. For instance, the $pK_a$ for PyOH* $\leftrightarrow$ PyO−* + H+ is below 0.4,21 and therefore, the fluorescence at 510 nm would disappear in a very acid environment.
regardless of the availability of free water. Even a small change in pH can bias the relative fluorescence of PyOH* and PyO*. Hence, pH likely plays a major role in the work of Choi and co-workers, especially at high concentrations. Even though the salts are neutral, evaporation or condensation of small quantities of volatile acids or bases such as ammonium or nitric acid may have a large influence on pH.

For the solutes considered here, the bound water is much less than the free water for most or all of the water activity range explored in the measurements. The bound water content is obtained by essentially subtracting the free water from the total water. Thus, the experimental errors in measurement of the free and total water are amplified in the relatively small amount of bound water. This may explain the large scatter in the bound water data and the negative values in some cases.

3. CONCLUSIONS

We compared measurements of free and bound water in aqueous solutions of seven solutes to those predicted by a new theory of solution thermodynamics recently posited by Wexler. Here, the theory was expanded to dimerizing solutes. The measurements were performed in an electrodynamic balance, permitting measurements to be made in supersaturated solutions. The measurements and predictions only agree for sodium sulfate. For the other six solutes, an adjustment in the free water by a factor ranging from 0.8 to 1.5 was needed to bring the predicted and measured free water into agreement. But with that factor, both the free and bound water measurements are generally in agreement with the predictions. The free and bound water measurements used fluorescence of pyranine as a probe. We discuss a number of reasons why pyranine may not be a quantitative indicator of free and bound water in solution. After the adjustment, the shape of the predicted free water and the shape of the measured free water are in general agreement, supporting both the theory and the fact that pyranine may be useful as a qualitative probe of the free water.

APPENDIX A. DIMERIZING SOLUTES

Here, we follow the derivation presented by Wexler but altered to account for dimerization. The definitions of the variables are the same.

Some solutes, such as glucose, urea, and acetic acid, react with each other in aqueous solutions to form dimers. We term the monomer A and dimer AA satisfying the equilibrium

\[ a_{AA} = K_{AA} \alpha_a^2 \]  

Here, the total amount of water in the solution is

\[
\begin{align*}
n_{w_{Tot}} &= n_w + n_{wT} + n_{AAwT} \\
&= \left( \frac{a_w}{1 - a_w} + H_A \right) n_{AT} + \left( \frac{a_w}{1 - a_w} + H_{AA} \right) n_{AAT}
\end{align*}
\]  

(A2)

Dividing both sides by \( n_{w_{Tot}} \) yields

\[
\frac{a_{AA}}{a_A^2} = K_{AA}
\]  

(A1)
The total solute mole fraction is
\[ M_m = \frac{n_{AA}}{n_{wTot}} = \frac{n_{AT}}{n_{wTot}} + 2 \frac{n_{AAT}}{n_{wTot}} \]  
(A4)

Using this in eq A3 to eliminate \( n_{AT}/n_{wTot} \) gives
\[ 1 = \left( \frac{a_w}{1 - a_w} + H_A \right) M_{aw} + \left( H_{AA} - 2H_A - \frac{a_w}{1 - a_w} \right) \frac{n_{AAT}}{n_{wTot}} \]  
(A5)

The quantity \( n_{AAT}/n_{wTot} \) can be evaluated in a number of ways, one of which is
\[ \frac{n_{AAT}}{n_{wTot}} = \frac{S_{AA} n_{AA}}{S_{wTot} n_{wTot}} = \frac{S_{AA} n_{AA}}{n_{AA}/n_t} = \frac{M_w m S_{AA} K_{AA a_A} a_A^2 + S_{AA} a_A}{2 + S_{AA} S_{AA} K_{AA a_A}} \]  
(A6)

where we have employed equations found by Wexler to carry through these equalities. Combining eqs A5 and A6 yields
\[ M_{aw} = \frac{1}{\frac{a_w}{1 - a_w} + H_A + \frac{S_{AA} K_{AA a_A} a_A^2}{2 + S_{AA} S_{AA} K_{AA a_A}}} \]  
(A7)

Equation A7 contains \( a_A \) in the denominator. Using the same procedure that Wexler did to derive the solute activity, we start with the total number of constituents in solution
\[ n_t = n_w + n_{AT} + n_{AAT} = n_w + S_A a_A + S_{AA} n_{AA} \]  
(A8)

Dividing both sides by \( n_t \) yields a quadratic in \( a_A \)
\[ S_{AA} K_{AA a_A} a_A^2 + S_A a_A - (1 - a_w) = 0 \]  
(A9)

which can be solved to find
\[ \frac{S_{AA} K_{AA a_A}}{S_A} = \frac{\sqrt{4S_{AA} K_{AA} (1 - a_w)/S_A^2 + 1} - 1}{2} \]  
(A10)

which also provides an expression for the solute activity. Equation A9 reduces to the equation for solute activity given by Wexler when the solute does not dimerize, that is, when \( K_{AA} = 0 \). Combining eqs A7 and A10 gives
\[ m = \frac{1}{M_w} \left[ \frac{a_w}{1 - a_w} + H_A + \frac{1}{2} \left( 1 - \frac{1}{\sqrt{1 + 4(1 - a_w) K_{AA a_A} S_A}} \right) \right] \right]^{-1} \]  
(A11)

As with prior formulas, the denominator (the square bracket in eq A11) is the total water per mole of solute. The single solute...
Figure 12. Comparison of predicted and measured free and bound water in \((\text{NH}_4)_2\text{SO}_4\) solutions. Not shown are the following high humidity bulk measurements with very high water content per solute.

Figure 13. Comparison of predicted and measured free and bound water in Mg(NO_3)_2 solutions.
term, \( a_w/(1 - a_w) + H_M \) is present, but as the dimer forms, the water associated with the monomer in free form and bound form decreases due to the terms \(-a_w/(1 - a_w) - 2H_A\). Equation A11 reduces to eq 1 for very dilute solutions \((a_w \approx 1)\) or when no dimer forms \((K_{AA} = 0)\). In the opposite limit, the quantity in the square root becomes very large so that the quantity in large parentheses becomes unity, in which case the solution is all dimer, the \( H_A \) terms cancel and the molality becomes \( m = 1/[2M_w(a_w/(1 - a_w) + H_{AA})]\), similar to eq 1 but with a factor of 2 in the denominator since the solutions now contain half as many solute molecules due to the dimerization.

### Table 3. Free Water Adjustment Factors

| solute         | adjustment factor |
|----------------|-------------------|
| sucrose        | 1.2               |
| glucose        | 1.5               |
| NaCl           | 0.8               |
| \( \text{Na}_2\text{SO}_4 \) | 1.0           |
| \((\text{NH}_4)_2\text{SO}_4\) | 1.3           |
| \( \text{Mg(NO}_3)_2 \) | 0.9           |
| \( \text{MgSO}_4 \) | 0.8           |

Figure 14. Comparison of predicted and measured free and bound water in MgSO4 solutions. Not shown are the following high humidity bulk measurements with very high water content per solute.

### ACKNOWLEDGMENTS

A.S.W. would like to thank Simon Clegg, University of East Anglia, for his valuable guidance. C.K.C. would like to acknowledge the Hong Kong Research Grants Council (grant #11302318) for their support.

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