

Definition of Bound Water by Water Activity Depression

Kenneth D. Ross

Eastern Regional Research Center*,
600 East Mermaid Lane
Philadelphia, 19118 (U.S.A.)

(Received September 11, 1978; Accepted March 31, 1979; lwt 573)

Water binding is frequently cited as the cause of water activity (a_w) depression in aqueous solutions and food systems. Thermodynamic arguments indicate, however, that a_w is lowered by solutes primarily because of the entropy of mixing. The depressions of a_w in ideal solutions comes about because of the dilution of the solvent by the solute. Deviations from Raoult's Law occur because attractive or repulsive forces allow formation or disruption of molecular clusters between solvent molecules or between solvent and solute molecules, a type of binding in the general sense. This type of water binding can be quantified from the magnitude of a_w depression and the extent of deviation from Raoult's Law. No universal correlation between a_w and bound water may be obtained, however, since each solute has its own characteristic water binding.

Introduction

The search for a meaningful definition of a bound water has persisted because of the notion that at low values of a_w not all water is «available» for processes such as microbial growth, chemical reactions, or mass transfer. The portion «unavailable» for various types of processes has been labeled bound water. Since these processes differ markedly, it is not surprising that the various determinations of bound water do not consistently agree. The word bound connotes only restraint, a concept which is sufficiently vague to encompass not only chemical bonding but all manner of interactions including physical entrapment. These various states of water in foods have been discussed in several recent review articles (15, 16, 18).

Intuitively or operationally defined subsets of water need not be selected as bound water. Greater clarity is afforded by defining bound water in terms of average properties of all water within a system. Specifically, the observed water activity, a_w , best reflects the average thermodynamic state of water. This paper discusses the ways in which water binding may affect the observed a_w . This logically leads to a definition of bound water based on a_w , an easily measured thermodynamic property. Furthermore, arguments based on thermodynamics are presented to demonstrate that water binding is not a strict requirement for a_w depression.

Results and Discussion

The cause of depression of water vapor pressure in solutions,

i.e., the depression of a_w below 1, may be determined by considering either the thermodynamic consequences of the condition of equilibrium or the statistics of mixing two or more components.

For a multicomponent system at equilibrium the Gibbs-Duhem relation applies:

$$\sum x_i d\mu_i = 0 \quad \text{Equ. [1]}$$

where x_i = moles of component i and μ_i = chemical potential of i .

Specifically, by expressing μ_i in terms of activity, a_i , in Eqn [1], a two-component system at equilibrium must obey the following:

$$n_1 d(\ln a_1) = -n_2 d(\ln a_2) \quad \text{Eqn. [2]}$$

Integration of Eqn. [2] may be used to compute the activity of one component in a binary solution from values of the activity of the other over a range of mole fraction (11). The solute activity in a solution of mole fraction 0 is also 0, and the solute activity would have to remain equal to 0 at all concentrations in order for a_w to remain 1. If the solute activity is to assume a nonzero value and increase with mole fraction, then a_w must decrease. The depression of a_w can be argued strictly from thermodynamic considerations without having to postulate mechanisms.

The statistical derivation of the free energy of mixing of an ideal solution has been outlined by WALL (27) and HILL (14). An ideal solution will have, by definition, no binding, and $\Delta H_{\text{mix}} = 0$ and $\Delta V_{\text{mix}} = 0$. By calculating the number of ways of distributing two classes of distinguishable but equivalent molecules over a lattice of possible locations, one finds that the entropy of mixing is:

* Federal Research, Science and Education Administration, U.S. Department of Agriculture.

$$\Delta S_{\text{mix}} = -R \sum_i \ln_i(N_i) \quad \text{Eqn. [3]}$$

with N_i = mole fraction of component i. Since $\Delta H_{\text{mix}} = 0$,

$$\Delta G_{\text{mix}} = RT \sum_i n_i(N_i) \quad \text{Eqn. [4]}$$

but

$$\Delta G_{\text{mix}} = \sum_i n_i[\mu_i - \mu_i^0] \quad \text{Eqn. [5]}$$

Thus,

$$\ln(N_i) = \ln(a_i) \quad \text{Eqn. [6]}$$

and

$$N_i = a_i \quad \text{Eqn. [7]}$$

which is a statement of Raoult's Law.

Both of the preceding analyses of the nature of a_w depression in solution speak for the view that a_w depression is primarily the result of dilution, rather than binding, of water by solute. This same conclusion was expressed by ANDREWS (1). Two consequences of this view are immediately apparent: (A) The word "humectant" is an unfortunate choice for describing solutes in food systems. Regardless of whether water binding occurs, solutes depress the a_w of the water within a food. Therefore, they permit a higher equilibrium moisture content for the entire food system *at a specified a_w* , than if they were not present. (B) Since a_w is depressed by solutes regardless of their water binding properties, a universal one-to-one correlation of bound water and a_w is impossible. That is, a measurement of bound water in some unknown sample cannot be used to calculate the a_w of that sample without additional information.

Definition of bound water in terms of deviation from ideal behavior

One might imagine that water binding by solutes should affect a_w in some manner, even though it is not the primary cause of a_w depression. Numerous experimental techniques have measured hydration of solutes (5, 21, 23) and the results indicate that solutes have coordinated, or complexed, water molecules. Real solutions generally show deviations from Raoult's Law, indicating preferential interactions between species in the mixture as well as differences in molecular dimensions (13). These deviations from Raoult's Law provide the basis for a definition of bound water which is not purely operational (*e.g.*, unfreezable water) but subject to unambiguous measurement.

The definition of bound water proposed here is based on the properties of the entire system and refers to the average interaction of all water molecules with solute molecules. Calculation of the magnitude of this average interaction may be performed by assigning the deviation from ideality to a change in the quantity of solvent water, as though some water were a structural part of the solute species. Raoult's Law may be written in the usual manner, except for the use of an effective molal concentration of solute, m' , instead of the true molality, m . The change from m to m' results from the hypothetical change in solvent water content, or "binding"

$$a_w = \frac{55.51}{55.51 + m'} \quad \text{Eqn. [8]}$$

$$m' = \frac{1000 m}{X} \quad \text{Eqn. [9]}$$

where X = g free (solvent) $\text{H}_2\text{O}/\text{kg}$ total H_2O

$$B = 1000 - X = \text{g bound } \text{H}_2\text{O}/\text{kg total } \text{H}_2\text{O} \quad \text{Eqn. [10]}$$

or

$$B_m = \frac{(1000 - X)}{(18 m)} \\ = \text{mol bound water/mol solute} \quad \text{Eqn. [11]}$$

or

$$B_g - (1000 - X)/M = \text{g bound water/g solute} \quad \text{Eqn. [12]}$$

where M = molecular weight of solute

There have been other attempts to treat nonideal aqueous systems with a modified form of Raoult's Law. For example, "semi-ideal" behavior was proposed by SCATCHARD (24) and again by STOKES and ROBINSON (26) to calculate average hydration numbers for sugars and polyols. In these two cases the best average hydration numbers were fit to data at all a_w values. The present formalism does not presuppose one value for bound water, or hydration, but emphasizes the total bound water level at any given a_w . Raoult's Law was also proposed as part of a mathematical description of the sorption isotherms of polymers (12). The water-polymer system was divided into free water, free polymer, and various polymer hydrate species (only the monohydrate was used in practical application). In order to account for non-ideality, the experimental sorption data was fit by calculating an "effective molecular weight" and two other constant terms.

The interpretation of non-ideality proposed here, and those other interpretations mentioned above, do not rely on the use of activity coefficients. One may account for deviations from ideality by means of the activity coefficient of water, γ_w , which, when multiplied by the ideal Raoult's Law expression, gives the true a_w of a non-ideal system. Although the use of γ_w is conceptually attractive for dealing with non-ideality, and a clear relationship exists between γ_w and B_m , the use of B_m allows for quantitating a certain amount of water which may be called "bound." Bound water in this formalism may thus be compared with that derived from alternative formalisms or from experiment.

Bound water in solutions

Tab. 1 lists the results of calculations for common solutes. The a_w data at 25°C for sucrose and NaCl were obtained from ROBINSON and STOKES (21), the data for glucose from STOKES and ROBINSON (26). The calculated values of B_m do vary with concentration, but to a much greater extent with the electrolyte than with the two sugars. Similar behavior was observed in calculations for other non-electrolytes including polyols and sugars. Comparison of the B_m values for non-electrolytes with experimental hydration data indicated fairly good correlation. For example, STEINBERG and LEUNG (25), using wide-line NMR, found 6.2 mol H_2O bound per mol sucrose and a much smaller, unspecified amount bound to glucose. Experimental values for hydration of NaCl have ranged from about 0.5 mol/mol to 72 mol/mol (23), which result from the strong concentration dependence indicated in **Tab. 1**.

The data for NaCl also show "negative hydration" at low concentrations. In this formalism, "negative hydration" simply means a positive deviation from Raoult's Law. Nevertheless, "negative hydration" has been discussed by SAMOILOV (23) and others (*e.g.*, ref. (19) on the basis of experimental evidence for the disruption of water structure by certain ions. Ions which are "structure breakers" in the nomenclature of FRANK and WEN (10) tend to show

“negative hydration” experimentally. Both Na⁺ and Cl⁻ are on the borderline between structure making and structure breaking; the change in sign of B_m with concentration may reflect a concentration-dependent change in their relative contributions to water structure.

B_m values for other salts containing ions with “negative hydration” are listed in Table 2. Again, the negative values of B_m are highly concentration-dependent. Experimentally (*i.e.*, by studies of self-diffusion of water and by the temperature-dependence of ion mobilities), water molecules in solutions of these salts are more mobile and have faster exchange rates than molecules of pure water (23). Quantitation of this effect has been reported in terms of interaction energy, rather than hydration number, but the results of Samoilov correlate well with the B_m values shown in **Tab.2**. The experimental observation of “negative hydration” results from measurement of bulk properties, just as the calculation

Tab.1 Bound water in solution of common solutes^a

Molal concentration	B _m (mol H ₂ O/mol solute)		
	Sucrose ^b	NaCl ^b	Glucose ^c
0.5	4.84	-8.58	-
1.0	4.99	-2.85	1.54
2.0	4.91	0.51	1.79
3.0	4.63	1.78	1.84
4.0	4.28	2.41	1.80

^a Calculated from sorption isotherms by means of Eqn. [8]–[11].

^ba_w Data from ROBINSON and STOKES (ref. 21)

^ca_w Data from STOKES and ROBINSON (ref. 26)

Tab.2 Bound water in solutions of salts with “negative hydration”

Molal concentration	B _m (mol H ₂ O/mol solute) ^a			
	KCl	CaCl	CsI	CaNO ₃
0.5	-8.95	-16.03	-16.87	-26.87
1.0	-3.47	-8.29	-11.17	-19.14
1.2	-2.51	-6.85	-7.77	-17.55
1.4	-1.74	-5.75	-6.67	-16.51
2.0	-0.28	-3.42	-4.62	-
3.0	+0.78	-1.55	-3.03	-

^aa_w Data from ROBINSON and STOKES (ref. 21), calculation according to Eqn. [8]–[11]a

Tab.3 Bound water calculated from protein isotherms^a

a _w	Serum albumin		β-Lactoglobulin		Salmine	
	W ^b	B _g ^c	W ^b	B _g ^c	W ^b	b _g
0.1	4.35	4.35	3.54	3.54	5.35	5.33
0.3	8.10	8.09	6.96	6.94	8.16	8.06
0.5	10.80	10.77	10.90	10.86	15.40	15.18
0.7	16.24	16.18	15.47	15.35	27.60	27.07
0.9	28.70	28.46	28.25	27.79	66.70	64.67
0.95	35.70	35.19	39.20	38.24	113.00	108.72

^a Isotherms reported by BULL (ref. 6) calculations according to Eqn. [8]–[12].

^bW = total moisture, g H₂O/100 g protein

^cB_g = bound water, g H₂O/100 g protein.

of B_m refers to the entire solutions, just as the calculation of B_m refers to the entire solution and not a subset of water molecules.

Negative values of hydration have no physical significance on a molecular level. Pure water is highly structured because of extensive hydrogen bonding, which produces clusters of water molecules. This structure accounts for many unusual properties of water in comparison to homologous compounds (*e.g.*, low vapor pressure, high viscosity). In such an environment of highly polar solvent, all charged species must have an inner hydration shell of tightly bound water molecules. However, the region just beyond the inner hydration shell must be disoriented in comparison to bulk water (10). Since the value of B_m (and Samoilov’s interaction energy) refers to an overall mean value for water molecules throughout the system, a negative B_m implies a dominant role for the outer, disorganized hydration shell.

One may conclude from the foregoing calculations that by taking the extent of deviation from Raoult’s Law as a measure of bound water, one arrives at calculated values for B_m which are reasonable when viewed as an indication of the average structure of water in a mixture compared to the structure of pure water. That is, bound water is related to the *magnitude* of a_w depression, not the *fact*. A second point, considered in greater detail below is that for each solute considered (including many others not reported here) the calculated values of B are dependent on concentration, or a_w.

Water bound to macromolecules

The bound water formalism can be extended to larger molecules, specifically proteins. As KUNTZ and KAUFMANN (17) have explained, there are no apparent discontinuities in the properties of protein-water mixtures over the entire range of moisture content. Thus, even at very low moistures, such systems may be viewed as highly concentrated solutions. As such, they are amenable to calculations of B_g as long as the molecular weight is known. It may be shown, however, that in heterogeneous proteins the number average molecular weight is required.

Tab.3 lists the data of BULL (6) for three representative proteins. Total moisture content is labeled W, and B_g is bound water. Molecular weights were: Salmine 8000 daltons (20), bovine β-lactoglobulin 36800 (28, and serum albumin 69000 (28). The higher molecular weight species have no appreciable “free moisture” below a_w 0.9–0.95, the point on the sorption isotherm where moisture content begins its steepest rise. The limit of detection of freezable water by DSC or DTA is also frequently found to be in that same range of a_w (9.22). Thus, calculation of B_g by the Raoult’s Law formalism again yields results which are reasonable in light of experimental measurements.

Calculation of sorption isotherms for proteins solely on the basis of selecting an appropriate value for bound water and using Equ. (8)–(12) should now be possible. With the hypothetical isotherm labeled B_g = 0 on **Fig.1**, in which the molecular weight has been chosen to be 20000, up to a_w 0.90 there is virtually no moisture content at equilibrium. A higher molecular weight would shift the onset of moisture sorption to even higher a_w. In the limit of infinite molecular weight, no moisture would be sorbed at all until a_w 1.0, at which point the moisture content in all systems is undefined.

If a value for B_g of 25 g/100 g solids were selected and the isotherm were computed for the 20000 dalton protein, the value of Q at a_w 0 would be 25 g/100 g. The isotherm would approach 25 g/100 g asymptotically, in sharp contrast to the normal shape of protein isotherms. From previous calculations for low molecular weight solutes, one may hypothesize that B_g varies with a_w, perhaps linearly. In other words, B_g = B^o a_w. The remaining isotherms in Figure 1 were com-

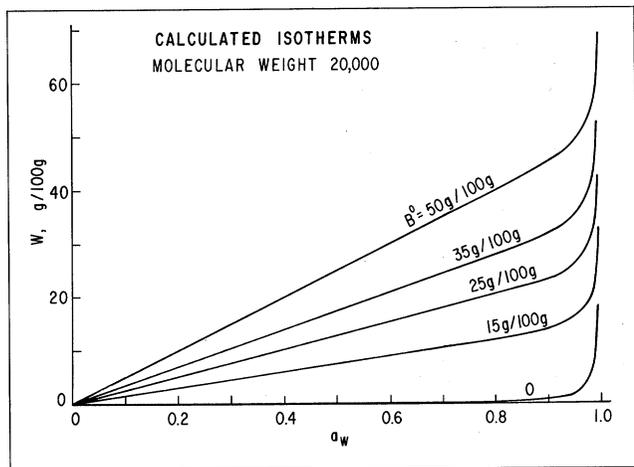


Fig. 1 Isotherms calculated by Raoult's Law, Eqn. [8]–[12], for a hypothetical protein of 20 000 daltons. Bound water was assumed to depend on a_w : $B_g = B^0 a_w$.

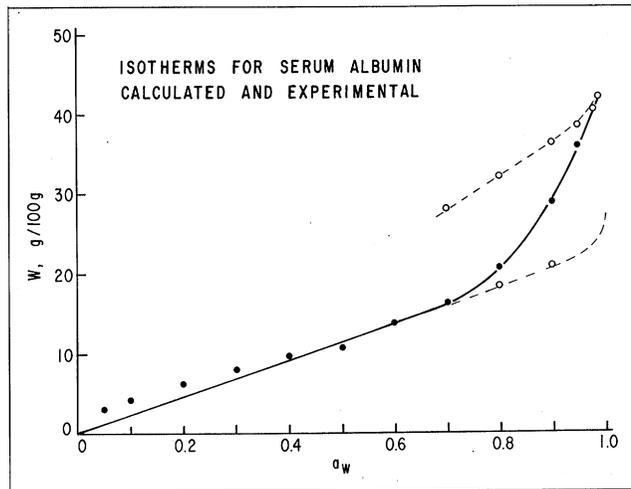


Fig. 2 Calculated and experimental isotherms for β -lactoglobulin. Solid circles are experimental data points of BULL (ref. 6). Open circles are two $B^0 = 55\text{g}/100\text{g}$. The solid line is a composite calculated isotherm in which B^0 varies linearly with a_w from 22 to 55/100g between a_w 0.75 and 1.0.

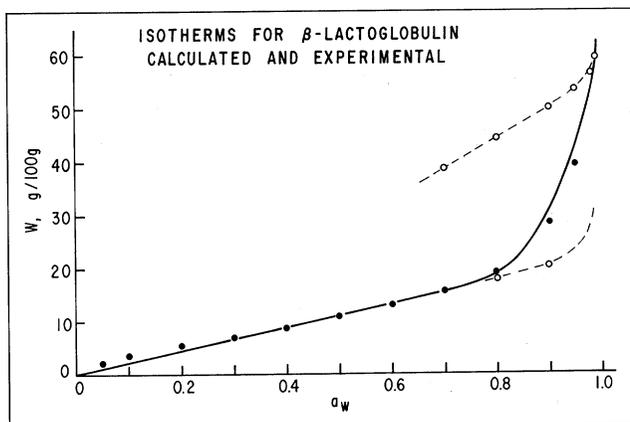


Fig. 3 Calculated and experimental isotherms for serum albumin. The solid line was computed by letting B^0 vary linearly with a_w from 23 to 40g/100g between a_w 0.70 and 1.0

puted in this manner, for various selections of B^0 . These isotherms are more nearly representative of the usual shape for isotherms of proteins and many other food components. DOLE (8) addressed the problem of moisture sorption by polymers with a statistical mechanical treatment involving binding constants (c_i) for the various layers of sorbed water. A linear relationship between B_g and a_w is equivalent to a harmonic decrease in c_i (i.e., $c_i = \frac{1}{i} c_1$) in Dole's notation. One conclusion from this treatment of sorption isotherms is that an experimental measurement of bound water can give B^0 only at a_w 1.0, which will never be obtained in solution. Therefore, measurements of bound water should be reported for a specified a_w or osmotic pressure. The variability of protein hydration determinations may in part reflect the lack of uniformity of a_w of the protein sample from one study to the next.

The isotherms of most proteins are approximately linear in the low and intermediate range of a_w , and B_g is also linear with a_w in that range **Tab. 3**. The slope of the plot of B_g vs a_w is B^0 , which may then be used to compute the entire isotherm. **Fig. 2** shows the results of a calculation for bovine β -lactoglobulin, with $B^0 = 22\text{ g}/100\text{ g}$. The experimental data (6) fit with the calculated points reasonably well up to a_w 0.75, but deviate markedly thereafter. On the other hand, if $B^0 = 55\text{ g}/100\text{ g}$, as obtained calorimetrically by BERLIN *et al.* (2), only the very high range of a_w may be fit. The solid curve between a_w 0.75 and 1.0 was computed by letting B^0 increase linearly from 22 g/100 g to 55 g/100 g over that a_w range. By this procedure, the calculated isotherm fits fairly well with the experimental isotherm.

A similar calculation for serum albumin is presented in **Fig. 3**. B^0 at low a_w is 23 g/100 g; B^0 at high a_w is 40 g/100 g (17). The solid curve represents a linear increase in B^0 from 23 to 40 g/100 g over the a_w range 0.7 to 1.0

The apparent increase in B^0 may reflect swelling at higher moisture content or some other conformational change that allows greater contact between protein and water. In addition, the appearance of at least two conformational states, or two limiting values for bound water, implies the possibility of hysteresis: the higher value of B^0 may be retained on desorption if the expanded conformation does not immediately collapse.

Independent evidence for at least a two-state model for binding is limited and inconclusive. In studies of heat capacity versus moisture content, the partial specific heats of sorbed water and of protein were significantly different at higher moisture contents than at low moisture (3, 7). Hysteresis between adsorption and desorption has also been observed by partial specific heat measurement of whey solids (4). Similarly, a significant change in ΔH of vaporization at 18 g/100 g for β -lactoglobulin and several other proteins has been reported (2). The authors attributed the change in ΔH to swelling.

Conclusions

1. Water activity depression in solution is not caused solely by hydration of solute molecules, and therefore no universal correlation between bound water and a_w is possible.
2. In systems where hydration does occur, water binding may be defined in terms of deviations from Raoult's Law. In this manner, the correlation between water binding and a_w requires the additional parameter of solute concentration.
3. Sorption isotherms for proteins may be approximated over the entire range of a_w by means of Raoult's Law and a value for bound water which varies linearly with a_w at low a_w and parabolically at high a_w .

References

- 1 ANDREWS, F.C., *Science* 194, 567, (1976)
- 2 BERLIN, E., KLIMAN, P.G. and PALLANSCH, M.J., *J. Colloid Interface Sci.*, 34, 488 (1970)
- 3 BERLIN, E., KLIMAN, P.G. and PALLANSCH, M.J., *Thermochimica Acta*, 4, 11 (1972)
- 4 BERLIN, E. and KLIMAN, P.G., in *Analytical Calorimetry*, 3, 497, Ed. Porter, R.S. and Johnson, J.F. Plenum Press, New York, 1974
- 5 BOCKRIS, J.O'M., *Quart. Rev.*, 3, 173 (1949)
- 6 BULL, H., *J. Amer. Chem. Soc.* 66, 1499 (1944)
- 7 BULL, H. and BREESE, K., *Arch. Biochem. Biophys.*, 128, 497 (1968)
- 8 DOLE, M., *Ann. N.Y. Acad. Sci.* 51, 705 (1949)
- 9 DUCKWORTH, R.B., *J. Food Technol.*, 6, 317 (1971)
- 10 FRANK, H.S. and WEN, W.Y., *Disc. Faraday Soc.*, 24, 133 (1957)
- 11 GLASSTONE, S., *Chemical Thermodynamics*, 2nd ed. D. van Nostrand Co., Inc., New York, pp. 687-9, 1946
- 12 HAILWOOD, A.J. and HORROBIN, S., *Trans. Faraday Soc.*, 42B, 84 (1946)
- 13 HILDEBRAND, J.H. and SCOTT, R.L., *The solubility of nonelectrolytes*. 3rd Ed. Reinhold Publ. Co., New York, ACS Monograph Series No. 17, 1950, p.29
- 14 HILL, T.L., *An introduction to statistical thermodynamics*. Addison-Wesley Publ. Co., Inc., Reading, MA, 1960, p.371
- 15 KAREL, M., *CRC Crit. Rev., Food Technol.*, 3, 329 (1973)
- 16 KAREL, M., *Physico-chemical modification of the state of water in foods - A speculative survey*. In *Water Relations of Foods*, Ed. R.B. Duckworth, Academic Press, London, 1975, p.639
- 17 KUNTZ, I.D. and KAUZMANN, W., *Adv. Protein Chem.*, 28, 239, (1974)
- 18 LABUZA, T.P., Interpretation of sorption data in relation to the state of constituent water. In *Water Relations of Foods*, Ed. R.B. Duckworth, Academic Press, London, 1975, p.155
- 19 MREVLISHVILI, G.M. and PRIVALOV, P.L., *Zh. Strukt. Khim.*, 9, 8 (1968)
- 20 NEURATH, H. and BAILEY, K., *The Proteins*, 1st ed., Vol. 1 Part A, Academic Press, Inc., New York, 1953, p.211
- 21 ROBINSON, R.A. and STOKES, R.H., *Electrolyte Solutions*, 2nd ed. (Revised). Butterworths Publ. Ltd., London, 1965
- 22 RUEGG, M., MOOR, U. and BLANC, B., *Biochem. Biophys. Acta*, 400, 334 (1975)
- 23 SAMOILOV, O.YA., *Disc. Faraday Soc.*, 24, 141, (1957)
- 24 SCATCHARD, G., *J. Amer. Chem. Soc.*, 43, 2406 (1921)
- 25 STEINBERG, M.P. and LEUNG, H., Some applications of wide-line and pulsed nuclear magnetic resonance investigations of water in foods. In *Water Relations of Foods*, Ed. R.B. Duckworth, Academic Press, London, 1975, p.233
- 26 STOKES, R.H. and ROBINSON, R.A., *J. Phys. Chem.*, 70, 2126 (1966)
- 27 WALL, F.T., *Chemical Thermodynamics*, 2nd ed., W.H. Freeman Co., San Francisco, 1965, p.349-50
- 28 WEBB, B.H., JOHNSON, A.H., and ALFORD, J.A. (Eds.), *Fundamentals of Dairy Chemistry*, 2nd ed., Avi Publ. Co., Westport, CT., 1974, p.90