and l exceed their random coil values (that is, above the polymer chain "Θ" point).

Since this work deals only with the first term for $P(\mu)$, in the limit of $x \rightarrow \infty$, the exact expressions for A and B and for the coefficient of $x^{-(3+l)/2}$ are of secondary importance. It suffices to state that these coefficients can be readily expressed in closed form, for a wide variety of the shape functions $F(z)$. However, the fact that the two-term (for $l = 0$) or the three-term (for $l > 0$) asymptotic equation for the scattering function depends both on the shape of the distribution function and the polydispersity of the polymer makes it more imperative to restrict analytical considerations to polymers of very high molecular weight.

References and Notes

(1) (a) National Bureau of Standards; (b) University of Akron.
(2) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).
(3) See, for example, P. Kratochvil, "Light Scattering from Polymer Solutions", M. B. Huglin, Ed., Academic Press, New York, N.Y., 1972.
(4) E. L. Slagowski, Ph.D. Thesis, University of Akron, 1972.
(5) A. J. Hyde, *Trans. Faraday Soc.*, **56**, 591 (1960).
(6) (a) J. Mazur, *J. Res. Natl. Bur. Stand., Sect. A*, **69**, 355 (1965); (b) C. Domb, J. Gillis, and G. Wilmers, *Proc. Phys. Soc., London*, **85**, 625 (1965).
(7) P. H. Verdier, *J. Chem. Phys.*, **45**, 2122 (1966).
(8) O. B. Ptitsyn, *Zh. Fiz. Khim.*, **31**, 1091 (1957).
(9) C. Loucheux, G. Weill, and H. Benoit, *J. Chim. Phys. Phys.-Chim. Biol.*, **55**, 540 (1958).
(10) A. Peterlin, Proceedings of the Interdisciplinary Conference on Electromagnetic Scattering, Potsdam, 1962, Macmillan, New York, N.Y., 1963, p 357.
(11) T. E. Smith and D. K. Carpenter, *Macromolecules*, **1**, 204 (1968).
(12) D. Carpenter, *J. Polym. Sci., Part A-2*, **4**, 833 (1966).
(13) P. Flory and R. L. Jerrigan, *J. Am. Chem. Soc.*, **90**, 3128 (1968).
(14) D. McIntyre, J. Mazur, and A. Wims, *J. Chem. Phys.*, **49**, 2887 (1968).
(15) B. H. Zimm, *J. Chem. Phys.*, **16**, 1093 (1948); **16** 1099 (1948); G. V. Schulz, *Z. Phys. Chem., Abt. B*, **43**, 251 (1939).
(16) S. Edwards, *Proc. Phys. Soc., London*, **85**, 613 (1965).
(17) J. Mazur, D. McIntyre, and A. Wims, *J. Chem. Phys.*, **49**, 2896 (1968).
(18) C. Domb and F. T. Hioe, *J. Chem. Phys.*, **51**, 1920 (1969).
(19) F. T. Wall and J. J. Erpenbeck, *J. Chem. Phys.*, **30**, 634 (1959).
(20) F. McCrackin, J. Mazur, and C. M. Guttman, *Macromolecules*, **6**, 859 (1973).
(21) D. McIntyre, L. J. Fetters, and E. Slagowski, *Science*, **170**, 1041 (1972).
(22) E. Slagowski, L. J. Fetters, and D. McIntyre, *Macromolecules*, **7**, 394 (1974).
(23) J. Mazur and F. McCrackin, *J. Chem. Phys.*, **49**, 648 (1968).
(24) W. H. Stockmayer, *Makromol. Chem.*, **35**, 541 (1960).
(25) S. F. Edwards, *Natl. Bur. Stand. (U.S), Misc. Publ.*, **No. 273** (1966).
(26) C. Domb, paper presented at the van der Waals Centenary Conference, Amsterdam, 1973.
(27) P. G. deGennes, *Phys. Lett. A*, **38**, 339 (1972).
(28) R. B. Griffiths, *Phys. Rev. Lett.*, **24**, 1479 (1970).
(29) C. Domb, A. J. Barrett, and M. Lax, *J. Phys. A: Math. Nucl. Gen.*, **6**, 82 (1973).
(30) H. Utiyama, Y. Tsunashima, and M. Kurata, *J. Chem. Phys.*, **55**, 3133 (1971).

Some Aspects of Water Clusters in Polymers

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ABSTRACT: Cluster theory has been applied to gain new insights into the relationships of water molecules adsorbed in polymers to each other and to the polymer. In many polymers at low relative humidities, it appears that two or more water molecules must interact to make a substantial contribution to the partial pressure. In polymethyl methacrylate, nylon, and cellulose beyond a certain point, newly adsorbed water forms bridges between previously uncorrelated water molecules to reduce the number and increase the size of the clusters. In serum albumin at higher relative humidities, water is added to previously absorbed water molecules to increase the average cluster size from one to two without changing the number of clusters.

Sorption isotherms for water in polymers frequently have a characteristic sigmoidal shape. At low relative humidities, the curve is concave toward the humidity axis showing that polymer–water contacts are favored. At high humidities, the curve is convex toward the humidity axis. In this range, there is a tendency for water molecules to be in contact with each other. Among the theoretical isotherms which have been presented to describe these phenomena are those of Brunauer, Emmett, and Teller[1] and White and Eyring.[2]

An approach to determining the degree of clustering or nonrandom mixing in a two-component system was developed by Zimm[3] following the work of Kirkwood and Buff[4] and applied to mixtures of polymers and solvents by Zimm and Lundberg.[5] This treatment is especially attractive, because it does not depend on any preconceived model. Its application to the sorption of water in a number of polymers has been discussed in an earlier note.[6]

The mean number of water molecules in the neighborhood of a given water molecule in excess of the mean concentration of water is given by

$$c_1G_{11} = (1-\phi_1)\left(\frac{\partial \ln \phi_1}{\partial \ln a_1}\right)_{p,T} - 1 \qquad (1)$$

where G_{11} is the cluster integral, and c_1, ϕ_1, and a_1 are the molar concentration, volume fraction, and activity of water. From this expression, it follows that the average number of water molecules in a cluster may be taken to be $c_1G_{11} + 1$, at least at high relative humidities. At low humidities this quantity may be less than unity, because the tendency to form polymer–water contacts may cause the water molecules to be more isolated from each other than they would be in a random mixture.

The effect of the surface/volume ratio is very small. Assuming that at most three molecular layers, say 10 Å, of water are absorbed on the surface of a fiber 0.2 mils (5 μ) in diameter or a film 1–10 mils thick, the maximum volume fraction of surface water would be 10^{-5}–10^{-3}.

Crystallinity would have a serious effect on the calculation only if the crystalline or water inaccessible fraction changed reversibly with relative humidity, and this is not known to happen. The effect of irreversible changes can be minimized by using the desorption isotherm. The choice of the entire polymer or the amorphous fraction has surprisingly little effect on the value of the clustering function. The sorption isotherm and the clustering function which is derived from it do not depend on anisotropy or orientation, per se.

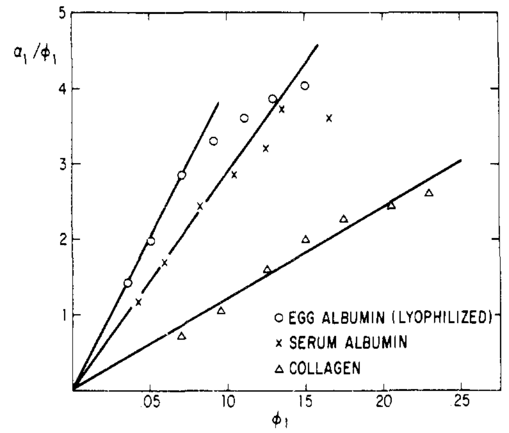

Figure 1. Three proteins at low humidities.

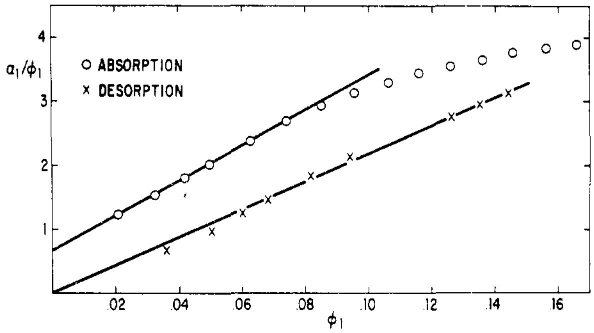

Figure 2. Data for keratin at low humidities.

The Low Humidity Region

It has previously been reported[6] that log–log plots of ϕ_1 vs. a_1 frequently have slopes of 0.5 in the region of low relative humidities. In the past[1,2] this portion of the sorption isotherm has usually been interpreted in terms of modifications of the Langmuir isotherm which treats sorption on a finite number of specific sites. The exponential relationship suggests a somewhat different approach. Since

$$(\partial \ln \phi_1/\partial \ln a_1)_{p,T} \simeq 0.5 \qquad (2)$$

the activity or partial pressure of water is at least approximately proportional to the square of its volume fraction. This indicates that there is a significant contribution to the partial pressure only when two absorbed water molecules find themselves by chance in the same neighborhood.

In principle, isolated water molecules must make some contribution to the partial pressure, and the relationship should be linear at sufficiently low concentrations. The isotherm for this region may be written in one of the following forms.

$$a_1 = K_1\phi_1 + K_2\phi_1^2 \qquad (3)$$

$$a_1/\phi_1 = K_1 + K_2\phi_1 \qquad (4)$$

Thus, the constants, K_1 and K_2, are the intercept and slope for a plot of a_1/ϕ_1 vs. ϕ_1. The data of Bull[7] for egg albumin, serum albumin, and collagen are plotted in Figure 1. Within experimental uncertainty, K_1 is negligible for these materials.

The sorption isotherms for many polymers exhibit significant degrees of hysteresis. Examples are found in the studies of Morrison and his coworkers on keratin[8] and cellulose.[9] These data are plotted in Figures 2 and 3, respectively. Perhaps, the tightest bonding of water to polymer takes time to develop. This could be the reason why K_1 has a measurable value for absorption but not for desorption in keratin and cellulose. The values for K_1 and K_2 as defined in eq 3 and 4 are listed in Table I.

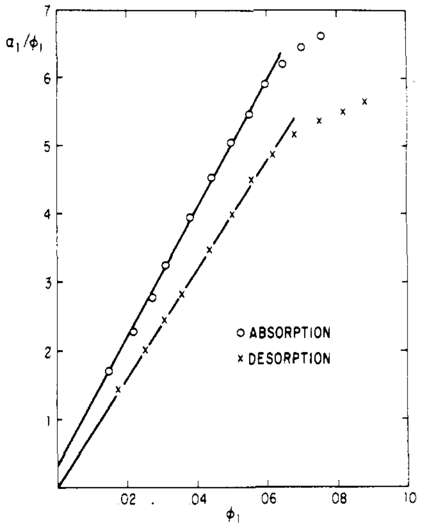

Figure 3. Data for cellulose at low humidities.

Table I
Quadratic Parameters for the Sorption of Water at Low Humidities

Polymer	K_1	K_2	Approximate limits of applicability ϕ_1	 a_1
Egg albumin (lyophilized)	Nil	40	0.07	0.20
Serum albumin	Nil	29	0.10	0.30
Collagen	Nil	12	0.20	0.50
Keratin				
Absorption	0.67	27	0.08	0.25
Desorption	Nil	22	0.14	0.45
Cellulose				
Absorption	0.32	94	0.06	0.35
Desorption	Nil	80	0.06	0.30

It appears that these equations are applicable up to 6 to 20% water by volume depending on the polymer. This supports the interpretation that isolated molecules of water are tightly bound to the polymer and that there is an appreciable tendency to evaporate only when two water molecules come in contact. In some cases, larger numbers of water molecules may have to interact to allow evaporation. The data of Bull[7] indicate that up to $a_1 = 0.2$, a_1 is proportional to ϕ_1^3 for gelatin and proportional to ϕ_1^4 for salmin. The principal characteristic of clusters in the low humidity region is their instability and the way in which they govern the partial pressure of the absorbed water.

The High Humidity Region

At higher levels of humidity, the tendency of water molecules to cluster increases steadily.[6] It is of interest to understand something of the details of this clustering process because of its impact on the properties of polymers containing absorbed moisture. From eq 1, it follows that the average number of water molecules in a cluster is

$$c_1G_{11} + 1 = (1 - \phi_1)(\partial \ln \phi_1/\partial \ln a_1)_{p,T} \qquad (5)$$

If w is the amount of absorbed water expressed as grams

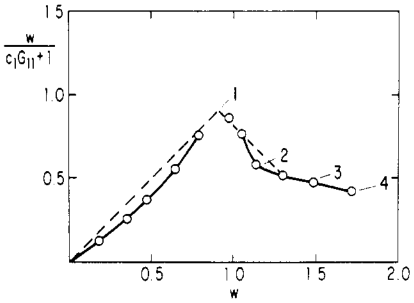

Figure 4. Clustering of water in poly(methyl methacrylate). Numbers refer to average cluster size.

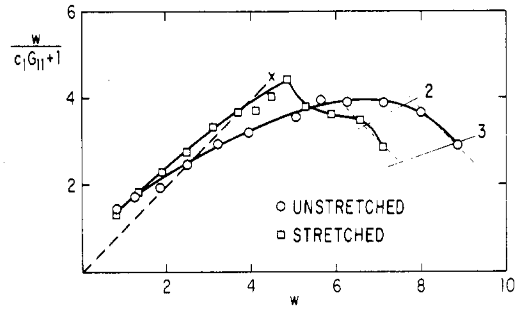

Figure 5. Clustering of water in fibers of nylon 66.

per 100 g of dry polymer, the number of water clusters in a given amount of polymer is proportional to

$$w/(c_1G_{11} + 1) \tag{6}$$

Plotting this quantity against w can give insights into the mechanisms of cluster building. If water molecules are absorbed at random, there will be a straight line with a slope of one because $c_1G_{11} = 0$. In a region where all newly absorbed molecules attach themselves to existing clusters, the slope will be zero, because the number of clusters is unchanged. If each added molecule forms a bridge between two previously uncorrelated water molecules, the slope will be -1, because the addition of a water molecule reduces the number of clusters by one. Other situations will have intermediate slopes. For example, if two newly absorbed molecules combine with two uncorrelated molecules to form a cluster of four, the slope will be -0.5.

A plot derived from the data of Brauer and Sweeney[10] for the sorption of water in poly(methyl methacrylate) is shown in Figure 4. Up to about 0.9% water and 75% relative humidity, the data fall just below the dashed line with a slope of one. This indicates almost random sorption of water molecules with possibly a slight tendency toward clustering. The next region which extends to 1.3% water is close to the dashed line having a slope of -1. The final portion of the curve has a much smaller negative slope. It appears that first one and then a second water molecule form a bridge between two initially uncorrelated water molecules to give an average cluster size of four near saturation. The final number of clusters is about half the maximum number. In these plots, the numbers at diagonal lines indicate the cluster size which is the reciprocal of the slope of the line.

Figure 5 is derived from the data of Bull[7] for stretched and unstretched fibers of nylon 66. At low moisture levels, the data are close to random sorption. Points to the left of the line indicate that water–polymer contacts are favored. The maximum number of clusters is close to the condition for one water molecule per two amide groups in the amorphous or water-accessible regions (marked X). Beyond this point, water is absorbed on previously absorbed water. Near saturation, the slope is about -1 (dashed lines) indicating bridging by newly absorbed water. In this region, the average size of a cluster is increasing from 2 to 3.

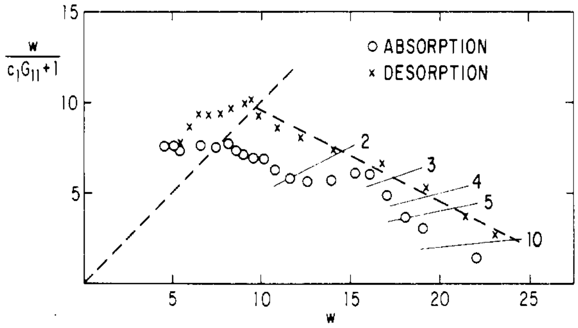

Figure 6. Clustering of water in cellulose.

These observations are in general accord with the conclusions by Puffr and Sabenda.[11] They considered that the tightly bound water absorbed at low humidities forms bridges between pairs of carbonyl oxygens and is limited to one water molecule per two amide groups in the amorphous regions. This is also the water content at which the loss modulus for the β relaxation has its greatest value.[12] Puffr and Sabenda believe that at higher humidities loosely bound water is sorbed between CO and NH groups. Cluster theory can only state that the positions of these water molecules are correlated with those absorbed earlier.

The data of Morrison and Dzieciuch[9] for cotton cellulose were used in Figure 6. The points for the greatest number of clusters are close to the 45° line which represents a balance between water–water and water–polymer contacts. For desorption, this quantity is about three times the monolayer value[9] calculated from the BET equation[1] and is probably close to the number of hydroxyl groups in the regions accessible to water.[13] At higher water contents, the data for desorption lie close to the dashed line having a slope of $-\frac{1}{2}$. This corresponds to the situation in which a pair of water molecules forms a bridge between two clusters. For absorption, the pattern is more complex. At w = 8–11, the slope is about $-\frac{1}{2}$. This is followed by a region of near zero slope, corresponding to addition to clusters. At w = 16–19, the slope is about -1 which corresponds to one-molecule bridges.

In cellulose near saturation, the size of the clusters is very high as indicated by the numbers attached to the diagonal lines. At a given water content, w, the average cluster size is larger for absorption than for desorption. At saturation where $w \simeq 25$, extrapolation of the data for absorption indicates indefinitely large clusters. Here as in the low humidity region, it appears that the structural relationship between the polymer and the absorbed water may change over a period of time.

Another type of behavior is illustrated in Figure 7 which is based on the data of Bull[7] for serum albumin. In this case, the number of clusters is constant over most of the range. This means that newly absorbed water is located near water absorbed earlier and there is little, if any, bridging. Near saturation the average cluster size is two. The horizontal line corresponds to one cluster per peptide unit.

The tendency for absorbed water to form clusters in polymers seems to be related to a tension between hydrophilic and hydrophobic characteristics. Poly(vinyl pyrollidone) which dissolves in water exhibits very little clustering or departures from random mixing.[14] The polymers in which the number of clusters passes through a maximum

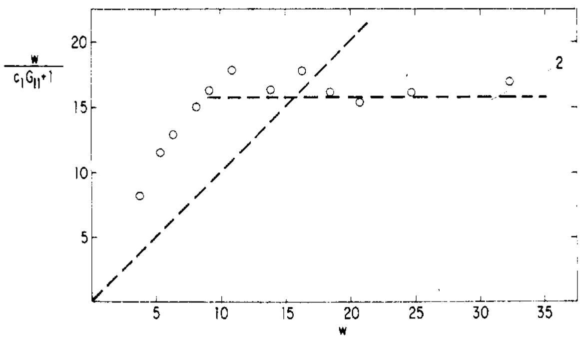

Figure 7. Clustering of water in serum albumin.

are those in which the hydrophilic–hydrophobic tension is most marked. It is in these cases that the water–polymer interaction is forced to assume its most highly structured character.

References and Notes

(1) S. Brunauer, P. H. Emmett, and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).
(2) H. J. White and H. Eyring, *Text. Res. J.*, **17**, 523 (1947).
(3) B. H. Zimm, *J. Chem. Phys*, **21**, 934 (1953).
(4) J. G. Kirkwood and F. P. Buff, *J. Chem. Phys.*, **19**, 774 (1951).
(5) B. H. Zimm and J. L. Lundberg, *J. Phys. Chem.*, **60**, 425 (1956).
(6) H. W. Starkweather, *Polym. Lett.*, **1**, 133 (1963).
(7) H. B. Bull, *J. Am. Chem. Soc.*, **66**, 1499 (1944).
(8) J. L. Morrison and J. F. Hanlan, *Proc. Int. Congr. Surf. Act., 2nd*, **2**, 322 (1957).
(9) J. L. Morrison and M. A. Dzieciuch, *Can. J. Chem.*, **37**, 1379 (1959).
(10) G. M. Brauer and W. T. Sweeney, *Mod. Plast.*, **32**, 138 (May 1955).
(11) R. Puffr and J. Sabenda, *J. Polym. Sci., Part C*, **16**, 79 (1967).
(12) J. Kolarik and J. Janacek, *J. Polym. Sci., Part C*, **16**, 441 (1967).
(13) J. L. Morrison, *Nature (London)*, **185**, 160 (1960).
(14) M. Dole and I. L. Faller, *J. Am. Chem. Soc.*, **72**, 414 (1950).

Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. IX. Glutamic Acid Parameters from Random Poly(hydroxybutylglutamine-*co*-L-glutamic acid)[1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-glutamic acid with N^5-(4-hydroxybutyl)-L-glutamine and the thermally induced helix–coil transitions of these copolymers in water and in 0.1 *N* KCl are described. The incorporation of L-glutamic acid was found to increase the helix content of the polymer at low pH and to decrease it at high pH even though the presence of 0.1 *N* KCl effectively eliminated the difference between the electrostatic free energies of the helix and the coil. The Zimm–Bragg parameters σ and *s* for the helix–coil transition in poly(L-glutamic acid) in water and in 0.1 *N* KCl were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The synthesis of *N*-acetyl-*N'*-methylglutamic acid amide and its titration, as well as that of the copolymers and poly(L-glutamic acid), in 0.1 *N* KCl are described.

There have been several previous investigations of the helix–coil transitions of poly(L-glutamic acid) in water and in salt solutions.[3-9] Both the homopolymer and copolymers in which glutamic acid is the principal component have been studied, and the effects of temperature and pH on the helix–coil transition have been observed. Using the Zimm–Rice theory,[10] the helix–coil stability constants for this amino acid have been evaluated.[3-10] These previous studies have, however, been hampered by the precipitation of poly(L-glutamic acid) at low pH and the difficulty of treating the electrostatic interactions at high pH. In the present paper, the use of random copolymers, in which glutamic acid is the minor component, eliminates these problems so that the Zimm–Bragg[11] helix–coil transition parameters for uncharged and charged glutamic acid can be calculated. For random copolymers in which the glutamic acid side chains are charged, 0.1 *N* KCl provides sufficient shielding of the charges to allow calculation of the Zimm–Bragg parameters using a near-neighbor theory, as in previous papers in this series.[12-19] The importance of short-range[20] and medium-range[21] interactions has been discussed elsewhere, and their effects on the conformation of glutamic acid residues in proteins and polypeptides will be examined in this paper and in the following paper.[22]

The synthesis of water-soluble random copolymers of L-glutamic acid with N^5-(4-hydroxybutyl)-L-glutamine (HBG) is described in section I. The experimental characterization of these copolymers and their melting and titration behavior are presented in section II along with the titration of poly(L-glutamic acid) and *N*-acetyl-*N'*-methylglutamic acid amide. Finally, in section III, the data are analyzed to determine the helix–coil stability parameters of L-glutamic acid in the neutral and charged forms in water and in 0.1 *N* KCl. These results are then compared with previous results for polypeptides and proteins.

I. Experimental Section

Preparation and Characterization of the Copolymers. The copolymers were prepared by first copolymerizing the *N*-carboxyanhydrides (NCA) of γ-*tert*-butyl L-glutamate and γ-benzyl L-glutamate in dioxane with triethylamine or sodium methoxide as an initiator. The γ-*tert*-butyl blocking group was removed using trifluoroacetic acid, and the benzyl blocking group was replaced by reaction with 4-amino-1-butanol.

A. Materials. Dioxane was purified shortly before use by refluxing and distilling over sodium. Hexane was dried over calcium sulfate and decanted just before use. Triethylamine (TEA) was refluxed and distilled with acetic anhydride, and then dried and distilled over KOH. Sodium methoxide was prepared by placing freshly cut sodium in anhydrous methanol and diluting with benzene. Ethyl acetate was dried over Linde Molecular Sieves (4A) and decanted just before use. Purified grade dichloroacetic acid