and z-average molecular weights is of particular relevance to the study of radiation degradation by ultracentrifugation, where $\bar{M}_w(D)$ and $\bar{M}_z(D)$ for an irradiated sample may be obtained from the same sedimentation equilibrium experiment. Although gel permeation chromatography is also used for that purpose, sedimentation equilibrium is the technique of choice for determining $\bar{M}_w(D)$ and $\bar{M}_z(D)$ because it avoids the errors inherent in chromatographically estimated molecular weight averages unless account is taken of differences in the elution volumes of linear and cross-linked polymer chains with the same molecular weight. An advantage of the proposed procedure is the sensitivity of $\bar{M}_z(D)$ to cross-linking, which enables $G(S)$ and $G(X)$ to be obtained from studies of samples subjected to low radiation doses. On the basis of analyses of simulated radiation degradation data, the present procedure of evaluating $G(S)$ and $G(X)$ from the ordinate intercepts of plots of $([\bar{M}_i(0)/\bar{M}_i(D)] - 1)/D$ versus D (i = w, z) applies to a wide range of initial molecular weight distributions and $G(S)/G(X)$ ratios. Finally, an experimental verification of the theoretical treatment is provided by an examination of results for the γ-irradiation of polystyrene.

Acknowledgment. We thank the Australian Research Council and the Australian Institute of Nuclear Science and Engineering for financial support and Drs. D. J. T. Hill and P. J. Pomery for useful discussions of this research. C.L.W. is the recipient of a Commonwealth Postgraduate Research Award.

Registry No. Polystyrene, 9003-53-6.

References and Notes

(1) Charlesby, A. *Atomic Radiation and Polymers*; Pergamon Press: Oxford, 1960.
(2) Chapiro, A. *Radiation Chemistry of Polymeric Systems*; Interscience: New York, 1962.
(3) Charlesby, A.; Pinner, S. H. *Proc. R. Soc. London, Ser. A* **1959**, *249*, 367.
(4) Saito, A. In *The Radiation Chemistry of Macromolecules*; Dole, M., Ed.; Academic Press: New York, 1972; Vol. 1, Chapter 11.
(5) O'Donnell, J. H.; Rahman, N. P.; Smith, C. A.; Winzor, D. J. *Macromolecules* **1979**, *12*, 113.
(6) Inokuti, M.; Dole, M. *J. Chem. Phys.* **1963**, *38*, 3006.
(7) Schulz, G. V. *Z. Phys. Chem. Leipzig* **1939**, *B43*, 25.
(8) Zimm, B. H. *J. Chem. Phys.* **1948**, *16*, 1099.
(9) O'Donnell, J. H.; Smith, C. A.; Winzor, D. J. *J. Polym. Sci., Polym. Phys. Ed.* **1978**, *16*, 1515.
(10) Chervenka, C. H. *Anal. Biochem.* **1970**, *34*, 24.
(11) Yphantis, D. A. *Biochemistry* **1964**, *3*, 297.
(12) Budd, P. M. *J. Polym. Sci., Part B: Polym. Phys.* **1988**, *26*, 1143.
(13) Budd, P. M. *Br. Polym. J.* **1988**, *20*, 33.
(14) Nichol, J. M.; O'Donnell, J. H.; Rahman, N. P.; Winzor, D. J. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 2919.

Volume Effects of Amylose-Water Interactions[†]

U. P. Strauss,* R. J. Porcja, and S. Y. Chen

Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903. *Received January 9, 1989; Revised Manuscript Received May 11, 1989*

ABSTRACT: Volume changes resulting from the sorption of water by amylose have been determined by a newly developed dilatometric procedure involving the dissolution in potassium hydroxide of samples previously equilibrated to a range of desired water contents. The data are represented by an empirical equation containing two exponentials. A theoretical expression including parameters obtainable from sorption isotherms was developed by which the volume changes could be resolved into contributions arising from contact adsorption and from adsorption in more distant layers. The contribution from the latter was very small amounting to a volume decrease of less than 0.04 mL/g of sorbed water. The contact adsorption, in contrast, produced very large volume decreases ranging from 0.47 mL in nearly dry samples to 0.32 mL/g of water for samples containing 0.14-0.29 g of water/g of dry amylose. The change in these values suggests more than one type of sorption site. Their large magnitude points to a mutual filling of molecular cavities by the participating species.

Introduction

It has been well established that the physical and chemical properties of starch are critically affected by its interactions with water.[1-4] These interactions have been studied by a variety of experimental methods.[5] Perhaps the most exhaustively applied of these methods has been the determination of vapor pressure-sorption isotherms which, in addition to their practical applications, give useful thermodynamic information.[3,6,7] An originally empirical expression, known as the GAB (Guggenheim, Anderson, De Boer) equation,[8-11] has been found to give a satisfactory representation of such isotherms over nearly the whole range of water activities.[6,12] The equation may be derived by means of a multilayer adsorption model in which the sorbed water molecules are divided into two classes: one consisting of strongly adsorbed molecules in contact with the surface and the other of weakly adsorbed molecules in the remaining layers.[10]

It is important to realize that the same model also allows the calculation of the distribution of sorbed water between the two classes. Knowledge of this distribution makes it possible to test the two-class model by means of other experimental methods.

We present here the results of dilatometric measurements aimed at obtaining the volume changes accompanying the interactions of water with amylose, one of the two major components of starch. Since on the molecu-

---

[†] Paper No. D10535-22-88 of the NJAES Journal Series.

lar level both liquid water and dry amylose are known to be rather loosely packed,[13,14] significant volume decreases would be expected from mixing the two. The few reported density studies indicate that such volume changes do indeed occur[14-17] but lack the precision and detail necessary for obtaining the desired quantitative correlation between data and model, i.e. for determining whether the volume changes can be resolved into the contributions from two constant components corresponding to the hypothesized two classes of sorbed water.

The measurements were performed with Carlsberg dilatometers described by Rasper and Kauzmann,[18] based on the original design by Linderstrom-Lang and Lanz.[19] However, these dilatometers were previously used only to measure volume changes arising from the mixing of two liquids.[18-20] They are not suitable for directly mixing amylose powders with small amounts of water where equilibration of the adsorption may take several weeks. Therefore, the samples must be equilibrated to known water contents before starting the dilatometry experiment. Each sample is then mixed in the dilatometer with a suitable aqueous solvent to bring it into solution. If the final solution state is the same in each experiment, the differences between the volume changes obtained with the various samples lead to the desired information concerning the volume changes resulting from the adsorption.

Experimental Section

Amylose isolated by Avebe from potato starch was obtained from National Starch and Chemical Corp. The sample was washed with water and centrifuged several times to remove residual magnesium sulfate which had remained from the original isolation process. The weight and number-average molecular weights determined by HPLC with the use of dextran standards[21] were 500 000 and 130 000, respectively. Two stock samples were freeze-dried to water contents close to saturation. Other samples were prepared from these stock samples by water desorption under vacuum or by addition of water. The moisture contents of the samples were determined either directly by weight loss upon drying or indirectly by weight change attending water gain or loss of other samples with previously measured water contents. Samples were assumed to be dry when exposure to vacuum of better than 0.01 Torr at 23 °C produced no further loss of weight. Usually about 2 weeks were found to be sufficient for this purpose.

In a typical experiment a sample of known weight and water content was placed in one leg of the dilatometer vessel, an inverted "Y" tube. The other leg contained a KOH solution whose concentration was adjusted to bring its concentration after mixing with the sample to 2 *m*. The filling of the vessel was completed with *n*-heptane which served as the inert manometric fluid. In a few instances isooctane was used instead, with identical results. Degassing was carried out under a partial vacuum of about 250 Torr for 15–30 min in order to avert the formation of gas bubbles on the subsequent dissolution of the sample and to prevent the base-catalyzed oxidation of amylose. The vessel was then provided with a measuring capillary tube. The completed dilatometer assembly was mounted in a 30 °C water bath controlled to within 0.001 °C with a Tronac Model PTC-41 thermoregulator.

Usually about ten dilatometers were followed in one run, two or three of which contained no amylose to serve as blanks for monitoring temperature stability. After the manometer readings became constant, each dilatometer was gently tilted inside the bath to allow the KOH solution to flow into the leg containing the amylose powder. In order to expedite quick dissolution, a magnetic stirring bar inside the tube was agitated from the outside with a magnetic stirring bar retriever. After dissolution was complete and temperature equilibrium had been reestablished, the manometer readings were found to be constant over several hours, indicating that there were no effects of possible slow reactions on the solution volume.

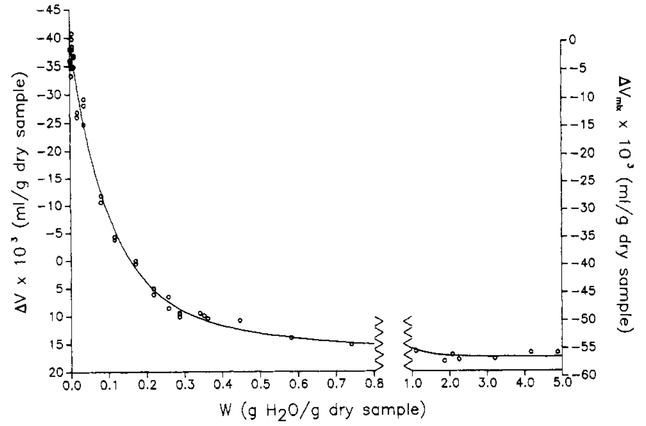

Figure 1. Volume change resulting from dissolution in KOH (left ordinate) and volume change resulting from mixing 1 g of amylose with *W* g of water (right ordinate) as a function of *W*. See text.

Results

The results obtained in the dilatometry experiments are given in Figure 1, where ΔV, in mL/g of dry amylose, is presented by the left ordinate scale as a function of W, expressed in g of water/g of dry amylose. The data are corrected for the volume change resulting from the dilutions of the KOH solution by water contained in the amylose sample. The corrections that are quite substantial for the samples with high water content were determined in separate dilatometry experiments. It was found convenient for subsequent calculations to represent the data by an analytical expression of the form

$$\Delta V = \Delta V_\infty - a_1 \exp(-k_1 W) - a_2 \exp(-k_2 W) \qquad (1)$$

The NLIN procedure of SAS Version 5.16 of the Rutgers University mainframe computer was employed for this purpose. We made use of the Marquardt method to obtain the following least-squares estimates of the parameters for the above nonlinear equation: $\Delta V_\infty = 17.48 \times 10^{-3}$ mL g^{-1}, $a_1 = 9.97 \times 10^{-3}$ mL g^{-1}, $a_2 = 46.53 \times 10^{-3}$ mL g^{-1}, $k_1 = 1.85$, and $k_2 = 9.74$. The quality of the fit between the data and the equation is indicated by the root-mean-square residual of 2.17×10^{-3} mL, a value close to the experimental uncertainty of the data.

The volume change on mixing 1 g of amylose with W grams of water, ΔV_{mix}, is equal to $[\Delta V(W = 0) - \Delta V]$ and is given by the same curve as ΔV, but with the ordinate scale on the right-hand side of Figure 1. The empirical equation for ΔV_{mix} derived from eq 1 may be represented by the simple form

$$\Delta V_{\text{mix}} = -a_1[1 - \exp(-k_1 W)] - a_2[1 - \exp(-k_2 W)] \qquad (2)$$

The parameters of eq 1 and 2 lead to several quantities of physical significance. Thus $-(a_1 + a_2)$ represents ΔV_{mix} of 1 g of amylose with excess water. Hence

$$-(a_1 + a_2) = \bar{V}_{\text{sp,Am}}(W = \infty) - \bar{V}_{\text{sp,Am}}(W = 0) \qquad (3)$$

where $\bar{V}_{\text{sp,Am}}$ represents the partial specific volume of amylose. Similarly, by differentiation of ΔV_{mix} with respect to W, we find

$$a_1 k_1 + a_2 k_2 = V^\bullet_{\text{sp,W}} - \bar{V}_{\text{sp,W}}(W = 0) \qquad (4)$$

where $V^\bullet_{\text{sp,W}}$ and $\bar{V}_{\text{sp,W}}(W = 0)$ represent the specific volume of pure water and the partial specific volume of water in pure amylose, respectively. The uncertainties in the values of these quantities can be obtained with the SAS program by appropriate changes of variables.

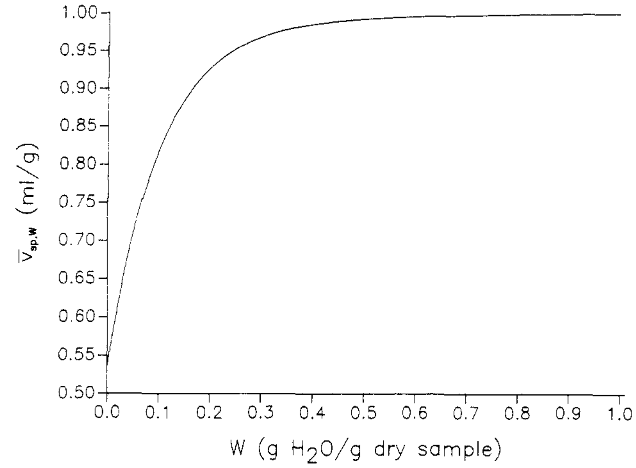

Figure 2. Partial specific volume of water in amylose as a function of water content.

Thus, we find that $(a_1 + a_2) = 0.0565 \pm 0.0012$ mL g^{-1} and $(a_1k_1 + a_2k_2) = 0.47 \pm 0.04$ mL g^{-1}.

The quantity ΔV_∞ in eq 1 represents the volume change of transferring 1 g of amylose from an environment of excess water to an environment of excess 2 *m* KOH. The positive value of $(17.48 \pm 1.1) \times 10^{-3}$ mL arises from the transfer of protons from polysaccharide to hydroxide ions and indicates that hydroxide ions are more effective in electrostricting water than are the oxyanions resulting from the deprotonation of the alcohol groups of amylose.

The partial specific volume of water is presented in Figure 2 as a function of water content. The values of $\bar{V}_{\mathrm{sp,W}}$ are obtained from the equation

$$\bar{V}_{\mathrm{sp,W}} = V^{\bullet}_{\mathrm{sp,W}} + \frac{\partial \Delta V_{mix}}{\partial W} \tag{5}$$

with the value of $V^{\bullet}_{\mathrm{sp,W}} = 1.00$ mL g^{-1} at 30 °C. The small values of $\bar{V}_{\mathrm{sp,W}}$ at low W indicate very strong interactions between water and amylose, very likely resulting from a mutual filling of molecular cavities in both components.

Discussion

We now turn to our attempt to apportion contributions to ΔV_{mix} from sorption in the contact layer and in more distant layers according to the equation

$$\Delta V_{\mathrm{mix}} = xW\Delta V_{\mathrm{b}} + (1-x)W\Delta V_{\mathrm{r}} \tag{6}$$

where ΔV_{b} and ΔV_{r} are the volume changes resulting from the sorption of 1 g of water in the contact layer and from the sorption of 1 g of water in all other layers, respectively, and x is the fraction of water in contact.

In order to relate x to a sorption isotherm, consider the simple multilayer adsorption scheme where the layer occupancy is governed by the equilibria

$$\frac{\theta_i}{\theta_{i-1}} = K_i a \qquad i = 1, 2, 3, ..., \infty \tag{7}$$

where θ_i is the fraction of sites containing i water molecules, K_i represents the equilibrium constant, and a is the activity of water. These equations lead to the expressions

$$\theta_i = \theta_0 K_1 K_2 K_3 ... K_i a^i \tag{8}$$

and

$$\sum_{i=0}^{\infty} \theta_i = 1 = \theta_0 S \tag{9}$$

where

$$S = 1 + K_1 a + K_1 K_2 a^2 + ... \tag{10}$$

The average number of water molecules per site, N_{w}, is given by

$$N_{\mathrm{w}} = \sum_{i=1}^{\infty} i\theta_i \tag{11}$$

which, together with eq 8–10, leads to the equation

$$N_{\mathrm{w}} = \frac{a}{S}\frac{\partial S}{\partial a} \tag{12}$$

Assuming $K_2 = K_3 = K_4 = ... \equiv K$ and denoting K_1 by CK, eq 10 can be simplified to

$$S = \frac{1 + (C-1)Ka}{1 - Ka} \tag{13}$$

The simplification involves an infinite geometric series expression for which Ka must be smaller than unity. Since, as will be shown below, $K < 1$ for amylose, this condition is satisfied here.

Applying eq 12 to eq 13 and replacing N_{w} by W/W_{m}, where W_{m} is the maximum weight of water that can be adsorbed in a monolayer per gram of dry amylose, we obtain

$$\frac{W}{W_m} = \frac{CKa}{(1-Ka)[1+(C-1)Ka]} \tag{14}$$

the well-known GAB equation which has been shown to represent sorption equilibria of amylose up to values of $a = 0.9$.[22]

The weight of water adsorbed in the contact layer, xW, is given by the expression

$$xW = (1-\theta_0)W_{\mathrm{m}} \tag{15}$$

which, after application of eq 9 gives

$$xW = \left(\frac{S-1}{S}\right)W_{\mathrm{m}} \tag{16}$$

After combination with eq 13 and 14, we find

$$x = 1 - Ka \tag{17}$$

Equation 6 now becomes

$$\Delta V_{\mathrm{mix}} = W(1-Ka)\Delta V_{\mathrm{b}} + WKa\Delta V_{\mathrm{r}} \tag{18}$$

Thus, if ΔV_{b} and ΔV_{r} are constants, a plot of $\Delta V_{\mathrm{mix}}/W(1-Ka)$ against $Ka/(1-Ka)$ should result in a straight line with intercept ΔV_{b} and slope ΔV_{r}. Such a plot is shown in Figure 3. The parameters needed for the application of eq 14 were $C = 16.279$, $K = 0.565$, and $W_{\mathrm{m}} = 0.140$, determined with the SAS program from the desorption isotherm for amylose given on p 136 in ref 3. The values of ΔV_{mix} were obtained from eq 2. The plot is seen to be linear over a substantial interval of the abscissa, ranging from 0.25 to 1.15. The corresponding ranges of a and W are 0.35–0.95 and 0.14–0.29, respectively. From the intercept and slope of the (extrapolated) linear portion of the curve we find that $\Delta V_{\mathrm{b}} = -0.319$ mL g^{-1} and $\Delta V_{\mathrm{r}} = -0.036$ mL g^{-1}. The last of these values indicates that the sorption of water in the second and higher layers produces only a very small volume change. The value of ΔV_{b} indicates a very large volume decrease for the sorption of water in the contact layer. It is noteworthy that at the point where the curve becomes linear, i.e. at $Ka/(1-Ka) = 0.25$, the fraction of occupied contact sites, $(S-1)/S$, is already 0.80, so that most of the contact sorption occurs in the first, nonlinear region of the curve.

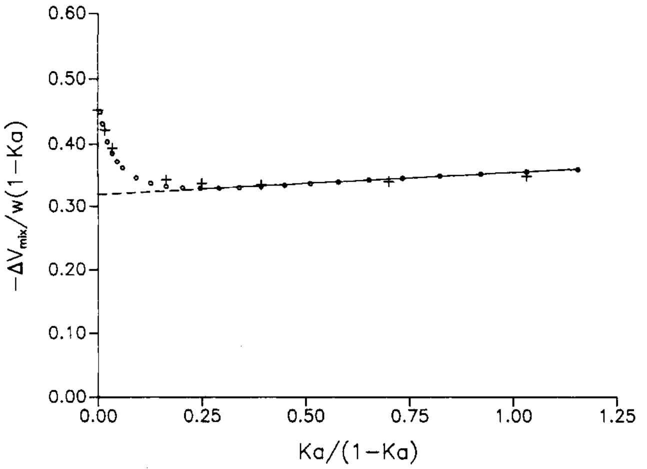

Figure 3. Test plot of eq 18 and 20. Circles correspond to the experimental data as represented by eq 2. Crosses calculated by eq 20. See text.

If we assume that ΔV_r remains constant, the second term on the right-hand side of eq 18 becomes negligibly small compared to the first term in this nonlinear region, so that the ordinate in Figure 3 represents the value of ΔV_b.

It is possible to account for the large change in ΔV_b in the initial region by assuming two types of sites, with different affinities for water molecules, which produce different volume changes on contact adsorption. These types of sites could belong to the crystalline and amorphous regions of amylose, both of which are known to sorb water.[14,23] It will be shown elsewhere that with this assumption eq 14 and 18 may be extended to the forms

$$W = \frac{Ka}{1-Ka}(g_1+g_2) \qquad (19)$$

$$\frac{\Delta V_{mix}}{W(1-Ka)} = \left(\frac{g_1}{g_1+g_2}\right)\Delta V_{b1} + \left(\frac{g_2}{g_1+g_2}\right)\Delta V_{b2} + \left(\frac{Ka}{1-Ka}\right)\Delta V_r \qquad (20)$$

where

$$g_i = \frac{C_i W_{mi}}{1+(C_i-1)Ka} \qquad i = 1, 2 \qquad (21)$$

with the subscripts 1 and 2 referring to the crystalline and amorphous sites, respectively. In the derivation of these equations it has also been assumed that only the contact adsorption is affected, so that single parameters K and ΔV_r which refer to the adsorption in the more remote layers can be used for both types of sites. We have found close fits to both eq 19 and 20 with the following values of the parameters: $K = 0.565$, $W_{m1} = 0.0336$, $W_{m2} = 0.1064$, $C_1 = 41.0$, $C_2 = 13.0$, $\Delta V_{b1} = -0.80$ mL g^{-1}, $\Delta V_{b2} = -0.15$ mL g^{-1}, and $\Delta V_r = -0.036$ mL g^{-1}. It should be noted that K and ΔV_r have been left intact and that W_{m1} and W_{m2} have been chosen to correspond to 24% and 76%, respectively, of the previously found value of W_m, in line with possible percentages of crystalline and amorphous regions of amylose. The fit of these parameters with the experimental curve is shown in Figure 3 where several points calculated by means of eq 20 have been indicated by crosses. In view of the relatively large number of parameters at one's disposal, no great significance should be attached to the values just given. Other combinations with different percentages of crystallinity also provide good fits with eq 19 and 20. The primary reason for presenting the applicability for eq 19 and 20 is to show that the assumption of two different contact sites is quantitatively compatible with the experimental data.

However, from, at this point, a limited number of trials with different parameter sets involving reasonable percentages of crystallinity, we find that C_1 is considerably larger than C, that C_2 is slightly smaller than C, and that ΔV_{b1} represents a volume decrease of more than 0.6 mL g^{-1} while ΔV_{b2} lies in the neighborhood of −0.2 mL g^{-1}. The values of C_2 and ΔV_{b2} point to a rather strong contact interaction between the amorphous sites and water. However, the unusually large magnitudes of C_1 and ΔV_{b1} indicate that the strongest effects occur with the crystalline sites. More than half of the volume of water appears to get lost in this interaction. This finding is compatible with previous evidence indicating that the crystalline X-ray pattern of dry amylose becomes sharper on the adsorption of water[14,24] and that water forms an integral part of the crystal structure of amylose.[25-28]

Acknowledgment. This work was supported in part by N.J. Agricultural Experiment Station and the Center for Advanced Food Technology, a New Jersey Commission on Science and Technology Center.

Registry No. H_2O, 7732-18-5; K(OH), 1310-58-3; amylose, 9005-82-7.

References and Notes

(1) Banks, W.; Greenwood, C. T. *Starch and Its Components*, Edinburgh University Press: Edinburgh, 1975.
(2) *Starch. Chemistry and Technology*, 2nd ed., Whistler, R. L., Bemiller, J. N., Paschall, E. F., Eds.; Academic Press: Orlando, 1984.
(3) Van den Berg, C. *Vapour Sorption Equilibria and Other Water-Starch Interactions; A Physico-Chemical Approach*; Doctoral Dissertation, Agricultural University, Wageningen, 1981.
(4) Slade, L.; Levine, H. In *Recent Developments in Industrial Polysaccharides*; Stivala, E. S., Crescenci, V., Dea, I. C. M., Eds.; Gordon and Breach Science: New York, 1987; pp 387–430.
(5) References to these methods that include vapor pressure, calorimetry, density, refractive index, NMR, and dielectric absorption can be found in ref 1–4.
(6) Van den Berg, C.; Kasper, F. S.; Weldring, J. A. G.; Wolters, I. *J. Food Technol.* **1975**, *10*, 589.
(7) Reference 2, pp 215–219.
(8) Reference 3, p 106.
(9) Anderson, R. B. *J. Am. Chem. Soc.* **1946**, *68*, 686.
(10) Guggenheim, E. A. *Applications of Statistical Mechanics*; Clarendon Press: Oxford, 1966.
(11) De Boer, J. H. *The Dynamical Character of Adsorption*; Clarendon Press: Oxford, 1953.
(12) Reference 3, pp 147–148.
(13) Eisenberg, D.; Kauzmann, W. *The Structure and Properties of Water*; Oxford University Press, 1969, p. 150.
(14) Reference 3, pp 73–84.
(15) Nara, S. *Starch/Staerke* **1979**, *31*, 73.
(16) DeWilligen, A. H. A.; de Groot, P. W. *Starch/Staerke* **1967**, *19*, 368.
(17) Reference 2, pp 219–221.
(18) Rasper, J.; Kauzmann, W. *J. Am. Chem. Soc.* **1962**, *84*, 1771.
(19) Linderstrom-Lang, K.; Lanz, H. *Mikrochim. Acta* **1938**, *3*, 210.
(20) Begala, A. J.; Strauss, U. P. *J. Phys. Chem.* **1972**, *76*, 254.
(21) Kobayashi, S.; Schwartz, S. J.; Lineback, D. R. *J. Chromatog.* **1985**, *319*, 205.
(22) The derivation of the GAB equation given here is a simpler and more transparent version than one given previously[10] involving grand canonical ensembles.
(23) Hennig, H. J.; Lechert, H. *J. Colloid Interface Sci.* **1977**, *62*, 199.
(24) Kainuma, K.; French, D. *Biopolymers* **1972**, *11*, 2241.
(25) Cleven, R.; Van den Berg, C.; Van der Plas, L. *Starch/Staerke* **1978**, *30*, 223.
(26) Guilbot, A.; Mercier, C. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1985; Vol. 3, pp 254–256.
(27) Wu, H. C. M.; Sarko, A. *Carbohydr. Res.* **1978**, *61*, 7.
(28) Booy, F. P.; Chanzy, H.; Sarko, A. *Biopolymers* **1979**, *18*, 2261.