persistence dipole moment is 36 whereas for CC it is 21, suggesting a more rigid conformation of AC which is in qualitative agreement with the molecular parameters deduced from light-scattering measurements.[2] The monomer dipole moment in the AC chain is higher than the corresponding value $\mu_0 = 0.9$ D found for CC, which is a further proof of the different conformations of the two polysaccharides.

Conclusion

On the basis of dipole moment determinations, the rigidity as well as the monomer dipole moment in the chain direction are higher for AC than for CC, which implies that their conformations are different. As in the case of CC, a thermally induced conformational transition was found for AC in both dioxane and ethyl acetate. This transition shifts with molecular weight for both molecules. The occurrence of a high-frequency absorption whose magnitude is temperature dependent is a noteworthy consequence of this transitional change.

References and Notes

(1) A. K. Gupta, E. Marchal, and W. Burchard, *Macromolecules*, 8, 843 (1975).
(2) W. Burchard, *Br. Polym. J.*, **3**, 214 (1971).
(3) H. Janeschitz-Kriegl and W. Burchard, *J. Polym. Sci., Part A-2*, **6**, 1953 (1968).
(4) E. Marchal, Thesis, University of Strasbourg, 1964.
(5) B. Pfannemüller and W. Burchard, *Makromol. Chem.*, **121**, 1 (1969).
(6) B. Pfannemüller, H. Mayerhöfer, and R. C. Schulz, *Biopolymers*, **10**, 243 (1971).
(7) O. Kratky and G. Porod, *Recl. Trav. Chim. Pays-Bas*, **68**, 1106 (1949).
(8) H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).
(9) C. Dufour and E. Marchal, *Biopolymers*, **11**, 1021 (1972).
(10) J. E. Hearst, *J. Chem. Phys.*, **38**, 1062 (1963).
(11) J. G. Kirkwood, *Recl. Trav. Chim. Pays-Bas*, **68**, 649 (1949); *J. Polym. Sci.*, **12**, 1 (1954).
(12) J. E. Hearst and W. H. Stockmayer, *J. Chem. Phys.*, **37**, 1425 (1962).
(13) H. Yamakawa and M. Fuji, *Macromolecules*, **6**, 407 (1973).

Water Binding on Collagen by Inverse Phase Gas Chromatography: Thermodynamic Considerations

U. Coelho, J. Miltz, and S. G. Gilbert*

Department of Food Science, Cook College, Rutgers, The State University, New Brunswick, New Jersey 08903.

Received September 20, 1978

ABSTRACT: In a previous publication, the details of using inverse phase gas chromatography (IPGC) for determining the moisture sorption isotherms of proteins were outlined. In the present article, the moisture sorption isotherms of collagen at five temperatures in the range of 40–60 °C are described using this method. The thermodynamics of the sorption process are considered by application of the BET equation, the Clausius–Clapeyron equation, and the Zimm–Lundberg cluster theory. Almost the entire region of the isotherms studied was found to be in or below the monomolecular layer as defined by the BET equation. The calculated values of the Zimm–Lundberg cluster function were negative throughout, indicating the absence of water clustering. The isosteric heat of adsorption was found to be highest at the lowest moisture contents studied and then dropped slightly before leveling off at a constant value of 6.8 kcal/mol characteristic of hydrogen bonding.

The theories of gas chromatography (GC) which relate peak shape to sorption isotherms also include retention volume as part of the definition of equilibrium conditions as derived from plate theory. The following equations described by Kiselev and Yashin[1] illustrate the application of these theories to actual conditions:

$$u = m_pS_p/ms \tag{1}$$

$$p = m_pqhRT/sw \tag{2}$$

where u = uptake (in our specific case water on collagen), g/g; m_p = mass of probe (water), g; S_p = total area under curve, cm^2; m = mass of stationary phase (collagen), g; s = peak area, cm^2; p = partial pressure of probe in carrier gas, atm; q = chart speed, cm/min; h = peak height, cm; T = temperature, K; w = corrected carrier gas flow rate, cm^3/min; and R = universal gas constant, (cm^3 atm)/(mol K).

BET Isotherm. The isotherm model in most general use is that of Brunauer et al.[2] This general equation is known as the BET isotherm and can be derived kinetically, from statistical mechanics and from thermodynamic considerations, as described by Adamson:[3]

$$\frac{V}{V_m} = \frac{Ca}{(1-a)[1+(C-1)a]} \tag{3}$$

where V = amount sorbed, V_m = monolayer value, a = water activity, and $C = Ke^{(Q_s/RT)}$ [K = (accomodation coefficient)/(frequency factor) $\simeq$ 1, Q_s = heat of adsorption, R = universal gas constant, and T = temperature (K)]. Generally, eq 3 is rearranged to the form:

$$\frac{a}{(1-a)V} = \frac{1}{V_mC} + \frac{a(C-1)}{V_mC} \tag{4}$$

A plot of $a/(1-a)V$ vs. a should give a straight line. From the slope and intercept of this line, the monolayer coverage value, V_m, can be calculated.

Zimm–Lundberg Cluster Theory. Zimm and Lundberg[4] proposed a "cluster theory" in order to explain the sorption of vapors by high polymers. The theory defines a function that measures the tendency of the absorbed molecules to cluster.

Zimm[5] derived the following relationship between the activity coefficient ($\gamma_1 = a_1/\phi_1$) and the cluster integral G_{11}

$$G_{11}/V_1 = -\phi_2[\partial(a_1/\phi_1)/\partial a_1]_{p,T^{-1}} \tag{5}$$

where ϕ_2 is the volume fraction of the polymer and is equal to $(1-\phi_1)$, while V_1 is the partial molar volume.

The clustering function G_{11}/V_1 is the mean number of water molecules in the neighborhood of a given water

0024-9297/79/2212-0284$01.00/0 © 1979 American Chemical Society

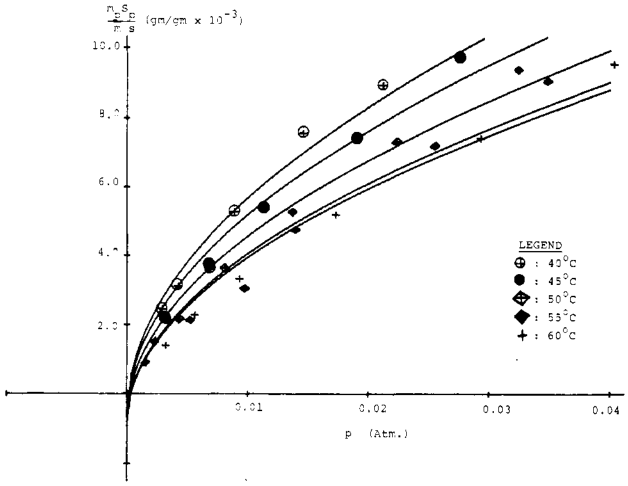

Figure 1. Sorption isotherms.

molecule in excess of the mean concentration of water. C_1G_{11} in which C is the molar concentration of component 1 is a simple way of examining the clustering phenomenon. If the clustering function is more negative than −1, the solvent molecules are widely dispersed and are attached to specific separate sites on the polymer. If the clustering function is positive, for instance $C_1G_{11} = 3$, the interpretation is that a cluster of four molecules is present, namely three additional molecules in excess of the original one are forming a cluster.

Isosteric Heat of Adsorption. The isosteric heat of adsorption can be calculated from the chromatographic data using the Clausis–Clapeyron equation:

$$\left[\frac{\partial \ln p}{\partial(1/T)}\right]_u = \left[\frac{\Delta H(\mathrm{ads})}{R}\right]_u \tag{6}$$

where p is the partial pressure of the probe in the gas phase. The subscript u indicates quantities measured at a given value of u, the amount of probe sorbed on the adsorbent. Gray and Guillet[6] investigated the sorption of n-hexanol on poly(methyl methacrylate), while Neumann[7] used alumina to study the sorption of benzene, cyclohexane, and n-hexane. These researchers found that eq 6 gave good results when retention volumes depend on sample size, as is the case with the collagen/water system under consideration.

Experimental Section

A Beckman GC-5 equipped with a thermal conductivity detector was used in this study. The analysis was run at five different column temperatures: 40, 45, 50, 55, and 60 °C. The other operating conditions were: detector temperature, 160 °C; carrier gas, helium; and carrier gas flow rate, 50 cm^3/min. After each change in the column temperature, a minimum of 24 h was allowed for the column to attain thermal equilibrium.

Quantities of water varying from 1.0 to 9.0 μL were pulsed through the column at regular intervals (75 min) and the chromatogram recorded. Duplicate analyses were run at each column temperature setting. The peak areas of eq 1 and 2 were obtained by photocopying the chromatograms, carefully cutting out the required areas and weighing on an analytical balance. The values of areas used in the calculations are an average of triplicate determinations. The use of an integrator would yield more accurate values of the required areas.

Results and Discussion

The moisture sorption isotherms of collagen are shown in Figure 1 and the mean values of sorption at 50 °C are listed in Table I. The experimental isotherms may be directly related to the physical processes of sorption–desorption and other thermodynamic parameters which have practical significance. The sorption data at different temperatures indicate that the higher the temperature the less moisture is adsorbed at any given vapor pressure. The change in moisture content with the increase in temperature is greater at higher vapor pressures than at lower ones. The isotherms show greater differences in the range of 40–50 °C as compared to the one of 50–60 °C. This is consistent with the greater kinetic energy of the system at the higher temperatures.

According to the BET isotherm, eq 4, a plot of $a/(1 - a)V$ vs. a should yield a straight line. From the slope and intercept of this line, the monolayer uptake value for the system can be calculated. A linear regression analysis on

Table I
Sorption Data at 50 °C

m_p, g × 10^{-3}	u,[a] g/g × 10^{-3}	p,[a] atm	% moisture, (g water)/(g of collagen)
1	1.51	0.0023	0.15
2	2.15	0.0043	0.22
3	3.63	0.0081	0.36
5	5.26	0.0137	0.53
7	7.31	0.0223	0.73
9	9.33	0.0324	0.93

[a] Represents an average of two runs.

Table II
Bet Monolayer Values

temp, °C	monolayer value, [(g of water)/(g of collagen)] × 10^{-3}
40	8.19
50	8.70
55	9.30
60	9.66

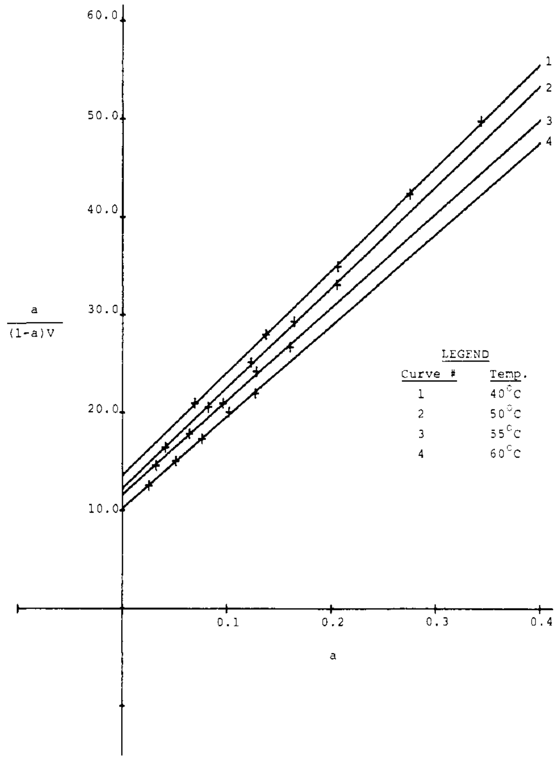

Figure 2. BET isotherms.

Table III
Zimm-Lundberg Cluster Analysis

	40 °C		45 °C		50 °C		55 °C		60 °C	
a_1	G_{11}/V_1	C_1G_{11}	G_{11}/V_1	C_1G_{11}	G_{11}/V_1	C_1G_{11}	G_{11}/V_1	C_1G_{11}	G_{11}/V_1	C_1G_{11}
0.025							−2.6254	−0.2935	−2.5626	−0.3003
0.050	−2.5180	−0.4062	−2.5404	−0.4136	−2.5871	−0.4486	−2.5006	−0.4501	−2.4300	−0.4668
0.075									−2.3321	−0.5770
0.100	−2.3716	−0.5744	−2.3921	−0.5882	−2.4264	−0.6238	−2.3352	−0.6314	−2.2592	−0.6520
0.150							−2.2314	−0.7299		
0.200	−2.1988	−0.7425	−2.2210	−0.7471	−2.3263	−0.7193				
0.250	−2.1403	−0.7919	−2.1632	−0.7956	−2.2538	−0.7821				

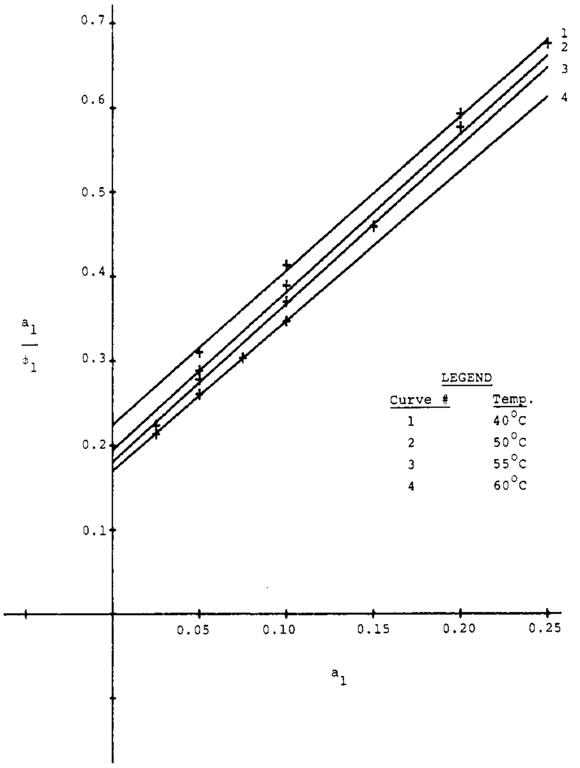

Figure 3. Zimm-Lundberg cluster function.

the data yielded the plots represented graphically in Figure 2. The monolayer coverage values obtained from these plots are listed in Table II. As determined by the BET equation, it can be seen from the sorption isotherms (Figure 1) that nearly all of the range of concentrations investigated in this sorption study lie below the monomolecular layer.

The Zimm-Lundberg cluster theory defines a clustering function for studying the interactions between the sorbed molecules themselves. This clustering function, defined as C_1G_{11}/V_1 in eq 5, is calculated from the slopes of plots of the experimental values a_1/ϕ_1 vs. a_1. Such plots are shown in Figure 3 and values of the clustering functions tabulated in Figure 3. The calculated values of the Zimm-Lundberg cluster function are negative throughout the range of moisture contents studied. This result agrees with our findings from the BET analysis, which indicated coverages below the monomolecular layer.

Lieberman et al.[8] studied the water/collagen system, while Zimm and Lundberg,[4] Starkweather,[9] and Merten[10] calculated and compared the clustering functions of water for proteins such as collagen, keratin, serum albumin, and egg albumin. All these investigators observed that at low relative humidities the cluster function was comparatively strongly negative. These negative values indicate the absence of clustering of water molecules (water-water interactions) and the presence of water-polymer interactions at dispersed and separate sites.

Table IV
Isosteric Heat of Adsorption (Clausius-Clapeyron Equation)

$u \times 10^3$, g/g	ΔH(ads), kcal/mol	$u \times 10^3$, g/g	ΔH(ads), kcal/mol
1	−7.92	5	−7.03
2	−7.27	7	−6.76
3	−6.85	9	−6.46
4	−6.77		

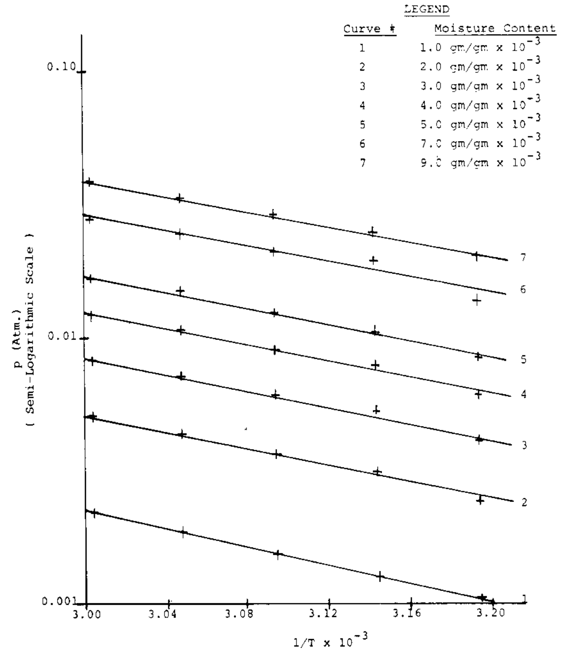

Figure 4. Isosteric heat of adsorption.

A more recent paper of Starkweather[11] suggests methods for examining the mechanism of water sorption by polymers. The data from this work were examined by the following equation:

$$a_1/\phi_1 = K_1 + K_2\phi_1 \qquad (7)$$

and by plotting $u/(C_1G_{11} + 1)$ against u. The plots for eq 7 were linear with the intercept $K_1 = 0$ and the slope $K_2 = 2$. This supports the interpretation that isolated water molecules are tightly bound to the polymer. The plot of $u/(C_1G_{11} + 1)$ vs. u gave a slope of 4 which is interpreted here as being indicative of a highly nonrandom distribution of energies of binding at active sites at these low humidities.

The sorption isosteres are illustrated in Figure 3 and were obtained by a linear regression analysis on the data obtained on application of the Clausius-Clapeyron equation. The calculated values of the isosteric heats of adsorption are listed in Table IV and the heat of adsorption dependence on the moisture content is shown in

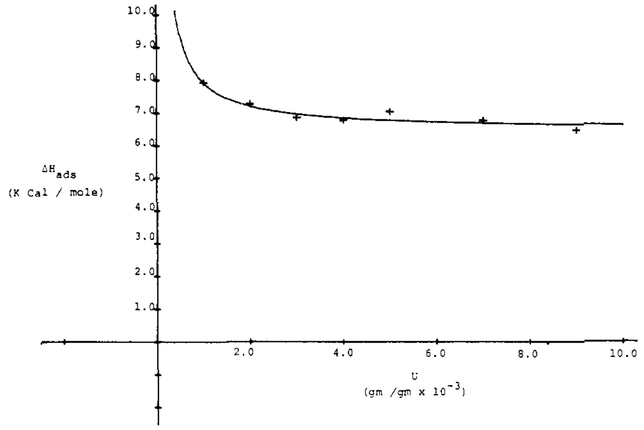

Figure 5. Dependence of isosteric heat of adsorption on moisture content.

Figure 4. The negative values of the heat of adsorption indicate an exothermic sorption process. Over the range of moisture contents studied, the isosteric heat of adsorption is highest at low moisture content and shows a slight decrease with an increase in the moisture content. Since the first molecules of water are taken up at the most active sites (highest activation energy), the evolution of heat corresponds to that of strong hydrogen bonding. The next molecules of water are adsorbed at weaker sites, as compared to the first sites occupied, and therefore lower activation energies are involved.

Since the heat of adsorption remains almost constant over the rest of the region studied, it would indicate that the rest of the active sites have an almost constant bonding energy, primarily that of hydrogen bonding in the peptide group. The absence of a dramatic change in the heat of adsorption confirms the previous interpretation of the data, in that most of the range of concentrations studied lie below the monomolecular layer. It should be noted that these data contradict one of the basic assumptions of the BET equation, according to which the heat of sorption for the first layer is constant and equal to the total heat of vaporization plus a constant heat due to site interaction. Evidently, the heat due to site interaction is higher for the first few sites occupied, while the balance of the sites do have an almost constant heat due to site interaction.

References and Notes

(1) A. V. Kiselev and Y. I. Yashin, "Gas Adsorption Chromatography", Plenum Press, New York, 1969.
(2) S. Brunauer, P. H. Emmett, and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).
(3) A. W. Adamson, "Physical Chemistry of Surfaces", Interscience, New York, 1963.
(4) B. H. Zimm and J. L. Lundberg, *J. Phys. Chem.*, **60**, 425 (1956).
(5) B. H. Zimm, *J. Chem. Phys.*, **21**, 934 (1953).
(6) D. G. Gray and J. E. Guillet, *Macromolecules*, **3**, 316 (1972).
(7) M. G. Neumann, *J. Chem. Educ.*, **53**, 708 (1976).
(8) E. R. Lieberman, S. G. Gilbert, and V. Srinivasa, *Trans. N.Y. Acad. Sci.*, **34**, 694 (1972).
(9) H. W. Starkweather, *J. Polym. Sci. Part B*, 1 (3), 133 (1963).
(10) U. Merten, "Desalination by Reverse Osmosis", Massachusetts Institute of Technology Press, Cambridge, Mass., 1966, Chapter 2.
(11) H. W. Starkweather, *Macromolecules*, **8**, 476 (1975).

Kinetic Study on the Inter- and Intramolecular Acetalization Reactions of Polymeric Reactants

Hiroo Iwata and Yoshito Ikada*

Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan.
Received September 18, 1978

ABSTRACT: Inter- and intramolecular acetalizations of poly(vinyl alcohol) (PVA) carrying one or two terminal aldehyde groups were carried out in aqueous solutions as well as in dimethyl sulfoxide (Me_2SO) solutions over a wide range of the polymer concentration. The observed rate constant for intermolecular acetalization was in both the solutions independent of the polymer concentration and not significantly different from that for the acetalization of a conventional PVA with aldol, a homologous low molecular weight aldehyde. The results strongly suggest that the polymer coils interpenetrate rather freely with each other in concentrated solutions even of Me_2SO which is a good solvent of PVA (water is almost a Θ solvent of PVA). This finding is in agreement with the theory predicting that the excluded volume effect may not be large enough to be detectable under the experimental conditions of this study. In addition, the observed OH concentration effective for the intramolecular acetalization was compatible with that predicted by the statistics of polymer chains.

Intermolecular reactions of polymeric reactants have been frequently encountered, for instance, in the termination reaction of radical polymerization,[1] the gelation of polymers,[2] and syntheses of block and graft copolymers by coupling reaction.[3] Hydrolysis of a polymeric substrate with a polymer having catalytic sites such as enzymes or anionic or cationic synthetic polymers[4,5] also belongs to the polymer–polymer reactions. Kinetic studies of such polymer–polymer reactions are therefore of great significance. Although kinetics of diffusion-controlled intermolecular reactions have been studied in detail[1,6,7] especially on the termination reaction of propagating polymeric radicals in the radical polymerization, there are only a few experimental[5,8] and theoretical[9,10] studies on the chemically controlled intermolecular reactions, where the rate-determining step is not diffusion processes but chemical reactions. It is interesting to point out that Cho and Morawetz[5] found no excluded volume effect in such a polymer–polymer reaction, while Wetmur[10] found this effect to be very large.

It should be further emphasized that the kinetic studies of polymer–polymer reactions might provide valuable information with respect to the state of polymer chains in solution, since the reaction rate must be intimately related

0024-9297/79/2212-0287$01.00/0 © 1979 American Chemical Society