Physiol. Chem. & Physics 12 (1980)

REDUCED SOLUBILITY OF POLYMER-ORIENTED WATER FOR SODIUM SALTS, SUGARS, AMINO ACIDS, AND OTHER SOLUTES NORMALLY MAINTAINED AT LOW LEVELS IN LIVING CELLS

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• ***Water oriented by 14 natural and 8 synthetic polymers, and by 1 polypeptide, was studied for solubility effectson Na^+, sucrose, free amino acids, and other solutes commonly present in living cells. Additional intensive investigation was made of 1 natural polymer (gelatin) and 4 synthetic polymers (methylcellulose, polyvinylpyrrolidone, polyvinylmethly ether, and polyethylene oxide). Equilibrium dialysis was used to determine distribution coefficients (ρ-values) between solutes in polymer-oriented water and in external bathing solutions. Effects on oriented water and on ρ-values for each solute were measured for external salt concentrations, initial polymer concentrations, temperature, agitation, molecular size, and kind of solvent. Results supported the polarized multilayer phase of cell water, a corollary of the association-induction hypothesis.***

INTRODUCTION

Currently prevalent are two diametrically opposed concepts of the physicochemical nature of living cells. One concept, the membrane-pump theory, places major emphasis on the existence and activity of a submicroscopic plasma membrane that controls the distribution of solutes in the cells by its permeability or impermeability and by the continuous operation of a battery of inward and outward pumps within the membrane.[1-3] The cytoplasm is seen as having the properties of a dilute salt solution. According to the alternative concept, the association-induction hypothesis, being alive means maintaining the assembly of cooperatively associated proteins, water, and ions of the cell in a high energy state.[4-6] The distribution pattern of a particular permeant solute in the cell is determined by two mechanisms with opposing effects: specific adsorption, which tends to raise the level of a solute above that in the surrounding medium, and dissolution in cell water, which tends to keep the level of solutes in the cell below that in the surrounding medium.

More specifically, the association-induction hypothesis proposes that certain proteins in living cells exist in a fully extended conformation, with their positively charged NH and negatively charged CO groups directly exposed to the bulk-phase cell water. A matrix of a more or less regular, parallel array of these protein chains with alternating, properly spaced negative (N) and positive (P) sites constitutes what is called an NP-NP-NP system.[7,8] Water between the parallel chams is polarized in multilayers. For both entropic and enthalpic reasons, the solubility of a solute in this bulk-phase water may be reduced by varying degrees as compared to its solubility in normal water.[9,10] Such an NP-NP-NP system is the most effective for long-range polarization of water. But a matrix of chains containing properly spaced negative sites and vacant sites (an NO-NO-NO system, O signifying a vacant, or neutral site) or positive sites and vacant sites (a PO-PO-PO system), may also function like an NP-NP-NP system in reducing the solvency of bulk-phase water.[11]

Although the association-induction hypothesis deals primarily with living cells, its

basic principles should be more widely applicable to nonbiological water-containing systems as well. This paper presents the results of a study concerning the solvent properties of water within a broad selection of natural and synthetic polymers which in varying degree serve as models of the living cell.

METHODS AND MATERIALS

Determination of *ρ-value*. In order to determine the apparent equilibrium distribution coefficient of a probe solute in the polymer-water systems, a concentrated aqueous solution of a polymer was prepared, in most cases with the aid of gentle heating. The polymer solution was then inserted into $-inch dialysis tubing, and the ends of the sacs tied with Deknatel silk threads. These loaded sacs were then placed in a suitable volume of an aqueous solution usually containing sodium sulfate or sodium citrate at various concentrations as well as one or more additional probe solutes such as sucrose, glycine, or $MgSO_4$. One or more of these solutes may have been radioactively labeled (e.g., ^{22}Na, ^{14}C-sucrose, ^{3}H-glycine). The test tubes containing the sacs were positioned horizontally and shaken at about 60 excursions per min, with each excursion usually ¾ inch in distance.

Once the equilibrium distribution of the solute under study was assured, the sacs were removed from the solution and cut open. Part of the gel or solution from the sac was then weighed and dried at 100°C in preweighed aluminum pans to yield the water content of the sample. Other portions of the sample were assayed for one or more of the solutes in order to study the distribution between the water both within and outside the sac. The results were expressed as an apparent distribution coefficient, ρ,[10] where

$$\rho = \frac{\text{solute concentration in water within sac}}{\text{solute concentration in water bathing sac}},$$

the concentration being expressed as moles/liter water.

It should be noted that the ρ-value may equal the q-value, or true equilibrium coefficient, which refers only to solute dissolved in the water of the polymer-water system.[12] The p-value would exceed the *q*-value if part of the solute in the sac had been adsorbed to the polymer. Since in this study no attempt was made to sort out the adsorbed and the dissolved fractions, the ρ-value has been used throughout.

For most of the data reported here, four probe solutes were used: ^{22}Na-labeled Na^+, ^{14}C- or ^{3}H-labeled sucrose, ^{3}H- or ^{14}C-labeled glycine, and Mg^{2+} (not labeled). ^{22}Na was assayed on a Nuclear Chicago automatic γ-scintillation counter, while ^{14}C and ^{3}H were assayed on a Packard TriCarb β-scintillation counter. Mg^{2+} was assayed with a Perkin-Elmer atomic absorption spectrometer.

At the end of incubation, the contents of the sacs were, as a rule, dissolved in 1 N HNO_3. Aliquots of ^{14}C- or ^{3}H-labeled extracts were then counted directly either for assay of ^{22}Na or for assay of ^{3}H and ^{14}C.

Time course studies. Both an influx method and an efflux method were used to determine the time course of isotope exchange.

In the influx method, properly filled sacs about 1 cm in length were placed in the bathing medium containing radioactive ^{22}Na. After differing periods of time, these were taken out, carefully blotted dry, and weighed before the entire sac was placed in the bottom of a well-type γ-scintillation counter to assay radioactivity. After this count, the sac was returned to the radioactive bathing solution for further incubation and after an

interval was again taken out. Each time, radioactivity per unit of weight of the sac was measured and plotted against the time of incubation.

In the efflux method, usually the dry polymer powder was dissolved in water containing the radioactive-label materials (e.g., ^{22}Na-labeled Na^+, ^{35}S-labeled SO_4), and the concentrated solution was then placed in the dialysis tubing. The filled sac was tied at both ends. After the sac was weighed, it was washed in 10-ml portions of a solution of nonlabeled sodium sulfate or sodium citrate for a certain length of time before it was moved to another tube also filled with nonlabeled solution. The process was repeated many times until the final washing, at which point the sacs were weighed once again and their contents removed and dissolved in 1 N HNO_3. Aliquots of each of the washing solutions as well as of the HNO_3 extract were assayed for radioactivity. The data provide the basis for a semilogarithmic plot of the remaining radioactivity in the sac after t minutes of washing against the time of washing, *t*.

Temperature. Most experiments were carried out at a constant temperature of 25° ± 1°C. In the few experiments at lower or higher temperatures, carefully sealed screw-capped tubes containing the sacs were placed horizontally in an Aminco constant-temperature shaker bath maintained to within ± 0.1°C. Often a stack of tubes was placed inside a plastic box and the entire box immersed in the shaker bath.

Materials. From ICN (Irvine, Calif.): D_2O (99.8 atom %) (Lot 20328), ^{22}Na (Lots 32, 39, 40), ^{14}C-sucrose (Lots 696-571, 640171), ^{3}H-glycine (Lot 760776), D-^{3}H-L-glutamic acid (Lot 461-081), ^{3}H-L-arginine (Lot 465-086), ^{14}C-trehalose (Lot 5144-37), ^{14}C-D-arabinose (Lot 578065).

From New England Nuclear (Boston, Mass.): ^{35}S-SO_4 (Lot 8751), ^{3}H-sucrose (Lot 581-128), ^{3}H-glycine (Lot 929-042), ^{3}H-tyrosine (Lot 433-249), 3H-leucine (Lot 965-076), ^{3}H-isoleucine (Lot 443-132), ^{3}H-1-D-mannitol (Lot 292-47), ^{3}H-5-D-arabinose (Lot 61-9265), ^{14}C-urea (Lot 952-178), ^{14}C-1,2-polyethylene glycol (Lot 729-136).

From Schwarz Bio-Research (Orangeburg, N.Y.): ^{3}H-L-aspartic acid (Lot 6501).

From Nuclear Chicago (Chicago, Ill.): ^{14}C-sorbitol (Batch 21).

From Sigma Chemical Co. (St. Louis, Mo.): gum arabic (64C-0252), gum ghatti (42C-2380), gum guar (32C1930), gum karaya (103C-0720), gum locust bean (42C-2900), gum tragacenth (74C-0207), gum xantham (888-0200), corn starch (6813-0216), potato starch (65B-2060), pectin (107B-0090), alginic acid (766-818), polyvinyl alcohol (127C-0196), and polyvinylpyrrolidone (mol. wt. 360,000) (94C-0049, 103C-2380). Gelatin, obtained from Eastman, was from pig skin (Batch 70-2727, 1EP 9.3; Lot 176, 1EP 8.7) and from calf skin (Lot B4B, 1EP 4.7).

The following polymers were donated: Kelzan,® a bacterial polysaccharide from Kelco Co., Clark, N.J.; polyvinylmethyl ether (Gantrez M-154,® Batch 185) from GAF Corp., N.Y.; Methocel A4M (hydroxylpropyl methyl-cellulose, Lot QP-32184-10-F) from Dow Chemical Co., Coral Gables, Fla.; polyethylene oxide (Polyox-205) from Union Carbide, N.Y.; and Carbopol 940 (carboxy polymethylene polymer, mol. wt. 4,000,000) from B. F. Goodrich Co., Cleveland, Ohio.

RESULTS

Time needed for ^{22}Na to reach distribution equilibrium. Figure 1 shows the time course of ^{22}Na uptake by three different polymers in ¼-inch dialysis tubing: polyvinylpyrrolidone (PVP), polyvinylmethyl ether

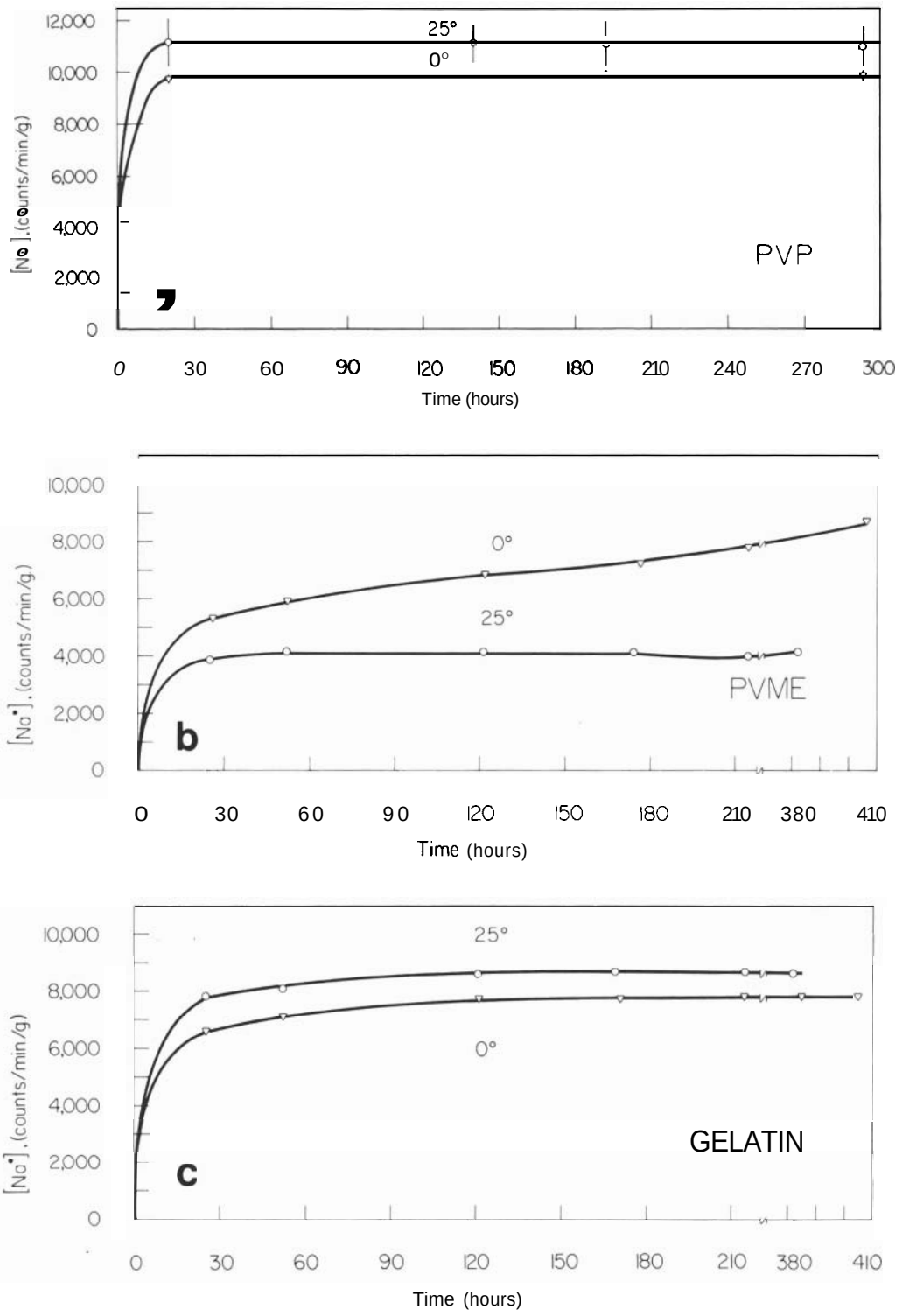

FIGURE 1. Time course of ^{22}Na uptake by several polymers: (A) polyvinylpyrrolidone (PVP), (B) polyvinylmethyl ether (PVME), (C) gelatin. The initial polymer concentrations in the sacs were 40%, 50%, and 40% respectively.

TABLE I. Water Contents and p-Values of Nine Amino Acids, Four Sugars, and Four Other Physiologically Interesting Compounds in the Water of a Polyvinylpyrrolidone (PVP) Solution *(25°C)*. (Initial concentration of the polymer was 20%. Bathing solution contained 1.5 M sodium citrate and 10 mM of each compound studied.)

		H_2O Content %	p-Value (mean ± S. E.)
Amino Acids	alanine	51.7	0.785±0.002
	arginine	51.9	0.461±0.007
	aspartic acid	51.2	0.436±0.007
	glutamic acid	51.5	0.529±0.012
	glycine	50.7	0.443±0.022
	histidine	53.2	1.337±0.017
	isoleucine	51.8	1.183±0.012
	leucine	51.2	1.300±0.0024
	tyrosine	52.6	1.040±0.030
Sugars	fructose	52.6	0.582±0.007
	glucose	52.6	0.595±0.010
	trehalose	50.7	0.633±0.024
	sorbitol	51.8	0.604±0.012
Other Compounds	acetic acid	52.6	1.234±0.003
	adenine	51.9	6.03±0.063
	ouabain	52.0	3.89±0.338
	urea	53.2	1.516±0.014

(PVME), and gelatin. The initial concentration of each polymer was 40%, 50%, and 40%, respectively, and the temperature was either 0°C or 25°C. All uptake curves seemed to have a fast component that reached equilibrium before 20 hours, but PVME at 0°C has a slower component that did not reach equilibrium after 12 days. Except for the fact that at 25°C more ^{22}Na was taken up, the time course of ^{22}Na uptake by gelatin was essentially the same at 25°C as at O°C.

Alternative interpretations for this separation into fast and slow uptake by PVME are (1) that the polymer-water contains pockets of low ^{22}Na diffusibility or (2) that rapid attainment of distribution equilibrium of ^{22}Na was accompanied by a continual change of the polymer-water system toward' a higher ρ-value for ^{22}Na. Figures 2 and **3** provide information in support of the second explanation. In each of these efflux studies the radioactive isotopes of ^{22}Na were introduced into the water before the polymer was dissolved. Had there been pockets of low diffusibility of the magnitude required,

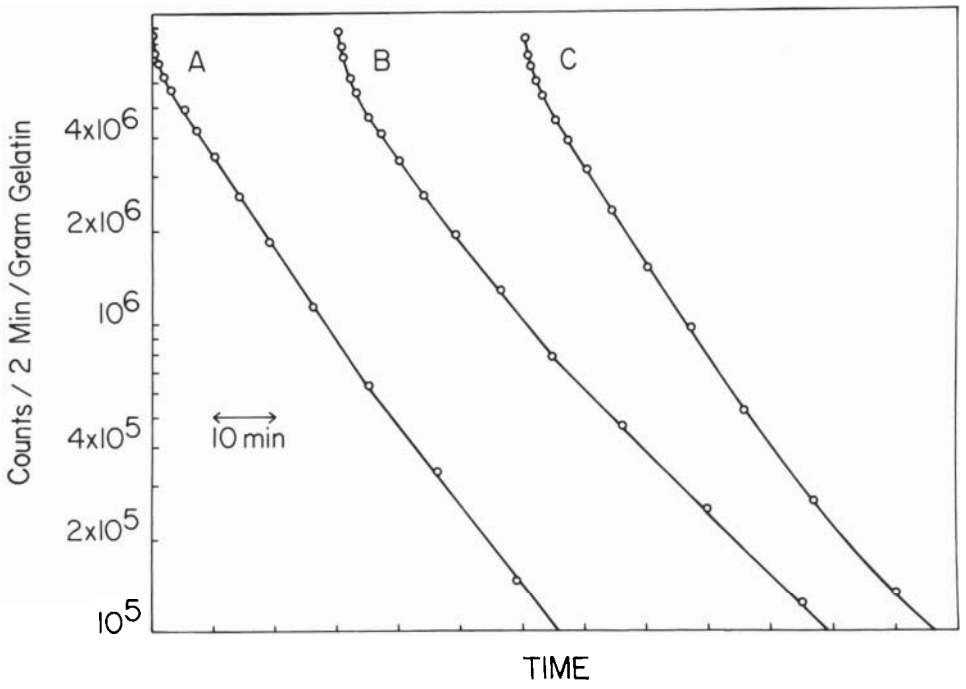

FIGURE 2. Time course of labeled Na efflux from gelatin slices **(25°C).** Dry gelatin was dissolved in 1.5 vol of **400 mM** ^{22}Na-labeled sodium citrate washed in **1.0** M sodium citrate.

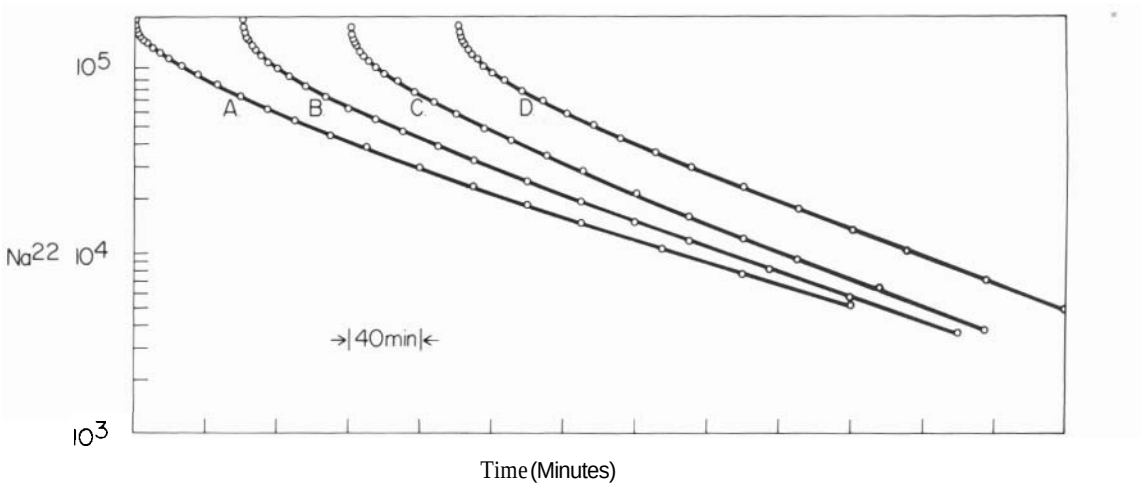

FIGURE 3. Time course of labeled Na efflux from sacs filled with ^{22}Na-loaded polyvinylpyrrolidone solution **(25°C** initial temperature). Na_2SO_4 concentration in the sacs was **0.5** M. Washing solution contained 1.0 M Na_2SO_4.

the time course of ^{22}Na efflux from gelatin slices and PVP-filled sacs would have one or more large, extremely slow components. In fact, in both cases these isotopes reached 99% exchange in a matter of one hour (gelatin slices) or four hours (PVP sacs). These experiments as well as those described in another report that deals with diffusibility through denatured protein-filled sacs[11] established that 48 hours is an adequate time

period for equilibrium distribution studies. Actual incubation times, however, often lasted much longer.

Solute exclusions and accumulation in PVP-water systems. Table I presents some examples of the ρ-values of free amino acids, sugars, and a few other organic compounds of interest to cell physiologists. PVP is a neutral polymer that carries no cationic or anionic side chains, yet the amino acids histidine, leucine, and isoleucine are accumulated with a ρ-value considerably above unity. An even greater degree of accumulation in PVP is observed for adenine and ouabain, suggesting a specific association between these solutes and PVP.

On the other hand, the amino acids glycine, alanine, arginine, aspartic acid, and glutamic acid are significantly excluded as are all four sugars and sugar alcohol listed in Table I.

What polymers affect the solubility properties of water? Table II lists the ρ-value of Na (as citrate or sulfate), Mg (as sulfate), sucrose, and glycine for polymers under three general headings: gums, polysaccharides, and synthetic polymers. Virtually all aqueous solutions of these polymers in high enough concentrations show reduced solubility for some probe materials. However, in these polymers that carry net charges the effectiveness varies a great deal. Among all these polymers, the most effective in reducing water solvency are methylcellulose, PVME, polyethylene oxide (PEO), PVP, and gelatin.

Effect of sodium sulfate and sodium citrate concentration in external medium on water contents and *ρ-values* for various *polymer*-water systems. In Fig. 4 the concentration of sodium sulfate and sodium citrate is plotted against water contents and ρ-values

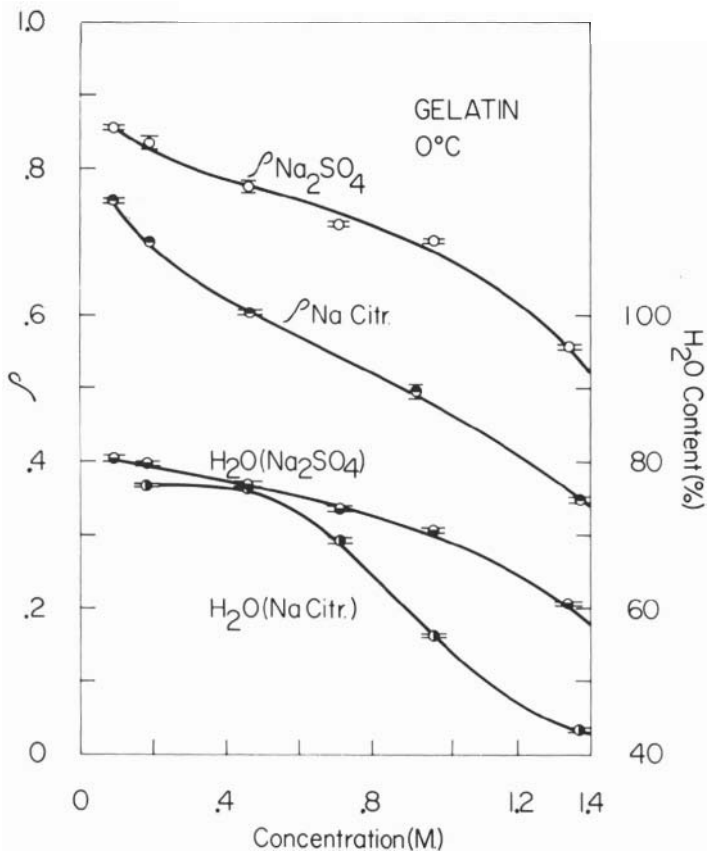

FIGURE 4. ρ-values of Na and water contents of gelatin in various concentrations of Na_2SO_4 and sodium citrate (0°C). Dissolved gelatin (30%) was in dialysis sacs.

TABLE II. Water Contents and ρ-Values of Na, Mg, Sucrose, and Glycine in Gums, Polysaccharides, and Synthetic Polymers (Values in parenthesis represent percentage of water in materials studied.)

	Concentration (M)						
	Na-citr	Na_2SO_4	$\rho_{Na\text{-}citr}$	$\rho_{Na_2SO_4}$	ρ_{MgSO_4}	$\rho_{sucrose}$	$\rho_{glycine}$
Gum arabic		0.1		1.324±0.084 (83.6%)	1.493±014 (83.6%	0.824±0.039 (83.6%)	0.857±0.036 (83.6%)
	1.5		0.850±0.003 (64.1%)				
	0.5		0.906±0.004 (78.2%)				
Gum ghatti		0.1		1.206±0.064 (84.7%)	1.531±0.072 (84.7%)	0.749±0.055 (85.8%)	0.915±0.030 (85.8%)
	1.5		0.855±0.003 (66.3%)				
	0.5		0.936±0.004 (83.1%)				
Gum guar		0.1		0.959±0.002 (83.7%)	0.953±0.107 (83.7%)	0.864±0.027 (83.7%)	0.877±0.047 (83.7%)
	1.5		0.919±0.003 (64.9%)				
	0.5		0.950±0.003 (81.3%)				
Gum karaya		0.1		1.590±0.023 (86.7%)	2.335±0.092 (86.7%)	0.650±0.075 (85.5%)	0.845±0.053 (85.5%)
	1.5		0.834±0.007 (67.9%)				
	0.5		0.960±0.004 (84.9%)				

Gum locust bean		0.1		0.945±0.004 (83.5%)	0.957±0.050 (83.5%)	0.823±0.034 (86.0%)	0.914±0.027 (86.0%)
	1.5		1.06±0.008 (61.3%)				
	0.5		0.960±0.004 (83.0%)				
Gum tragacanth		0.1				0.855±0.012 (87.4%)	0.909±0.003 (87.5%)
	1.5		0.887±0.003 (69.7%)				
	0.5		0.940±0.003 (85.4%)				
Gum xantham	1.5		0.900±0.003 (69.2%)				
	0.5		0.961±0.004 (86.5%)				
Agarose		0.1		0.800±0.042 (76.4%)	0.702±0.071 (76.4%)	0.608±0.084 (76.4%)	0.722±0.017 (76.4%)
Alginic acid		0.1		2.023±0.034 (75.8%)	2.59±0.076 (75.8%)	0.809±0.015 (75.8%)	1.343±0.028 (75.8%)
	1.5		1.06±0.011 (63.0%)				
	0.5		1.20±0.005 (82.4%)				
Carregenin		0.1		1.107±0.004 (96.6%)	1.326±0.070 (96.6%)	0.829±0.019 (96.6%)	0.872±0.039 (96.6%)
	1.5		0.921±0.004 (71.6%)				
	0.5		0.832±0.020 (85.9%)				
Glycogen		0.1		0.755±0.075 (65.2%)	0.560±0.051 (65.2%)	0.709±0.064 (65.2%)	0.725±0.069 (65.2%)

TABLE II. *(Continued)*

	Concentration (M)						
	Na-citr	Na_2SO_4	$\rho_{Na\text{-}citr}$	$\rho_{Na_2SO_4}$	ρ_{MgSO_4}	$\rho_{sucrose}$	$\rho_{glycine}$
Pectin		0.1		1.432±0.074 (84.2%)	1.718±0.077 (84.2%)	0.849±0.068 (84.2%)	0.856±0.100 (84.2%)
	1.5		0.928±0.008 (65.3%)				
	0.5		1.01±0.025 (81.6%)				
Starch (corn)		0.1		0.918±0.003 (79.6%)	0.901±0.013 (79.6%)	0.794±0.027 (78.6%)	0.754±0.009 (78.6%)
	1.5		0.836±0.006 (49.5%)				
	0.5		0.924±0.004 (72.5%)				
Starch (potato)		0.1		0.926±0.010 (79.2%)	0.768±0.020 (79.2%)	0.848±0.003 (78.3%)	0.947±0.04 (78.3%)
	1.5		0.852±0.006 (44.3%)				
	0.5		0.905±0.003 (70.8%)				
Carbopole 940		0.1		1.249±0.029 (76.3%)	1.057±0.031 (76.3%)	0.773±0.011 (76.3%)	0.999±0.164 (76.3%)
Dextran 170		0.1		0.882±0.007 (79.5%)	0.976±0.105 (79.5%)	0.791±0.02 (81.8%)	0.841±0.017 (81.8%)
	1.5		0.889±0.032 (62.9%)				
	0.5		0.929±0.018 (77.4%)				

Gantrez M-154		0.1		0.294±0.024 (74.4%)	0.204±0.021 (74.4%)	0.524±0.033 (74.4%)	0.463±0.027 (74.4%)
Kelzan		0.1	1.602±0.02 (84.4%)		2.134±0.105 (84.4%)	0.846±0.022 (84.4%)	0.925±0.020 (84.4%)
Methylcellulose		0.1		0.689±0.008 (83.4%)	0.763±0.120 (83.4%)	0.769±0.014 (83.4%)	0.653±0.028 (83.4%)
	1.5		0.402±0.032 (31.5%)				
	0.5		0.452±0.012 (66.0%)				
Polyox-205		0.1		0.584±0.005 (94.3%)	0.172±0.046 (94.3%)	0.762±0.028 (94.3%)	0.730±0.017 (94.3%)
Polyvinyl alcohol		0.1		0.762±0.009 (78.9%)	0.772±0.027 (78.9%)	0.952±0.023 (78.9%)	0.625±0.011 (78.9%)
Polyvinylpyrrolidone		0.1		0.644±0.007 (81.3%)	0.555±0.088 (81.3%)	0.914±0.010 (81.3%)	0.792±0.017

for these Na salts of gelatin. In general the ρ-values and water contents decrease with increasing salt concentration. Also, mole for mole, sodium citrate is more effective than sodium sulfate. One reason is simply that each molecule of sodium citrate is ionized into four ion particles whereas each molecule of sodium sulfate disassociates into only three. Nevertheless, mole for mole, a citrate anion is considerably more effective than a sulfate anion. Thus at a concentration of 0.46 M sodium citrate, $\rho_{Na\text{-}citrate}$ is only 0.60 while $\rho_{Na\text{-}sulfate}$ is 0.78, even though the water contents are equal at this point. In both sodium sulfate and sodium citrate, the ρ-values steadily decrease with increasing external salt concentrations.

Figures 5 and 6 show a similar concentrating effect on the water contents and ρ-values in PEO (Polyox-205) and PVME (Gantrez M-154). In PEO, both water contents and $\rho_{Na\text{-}citrate}$ steadily decrease the ρ-value to a low of 0.14. In PVME, the water contents again fall steadily with increasing Na concentration. However, the four ρ-values for Na, Mg, glycine, and sucrose all show varying degrees of upward trend at high citrate concentration (see Figs. 8, 9).

Figure 7 shows the ρ-value of sodium

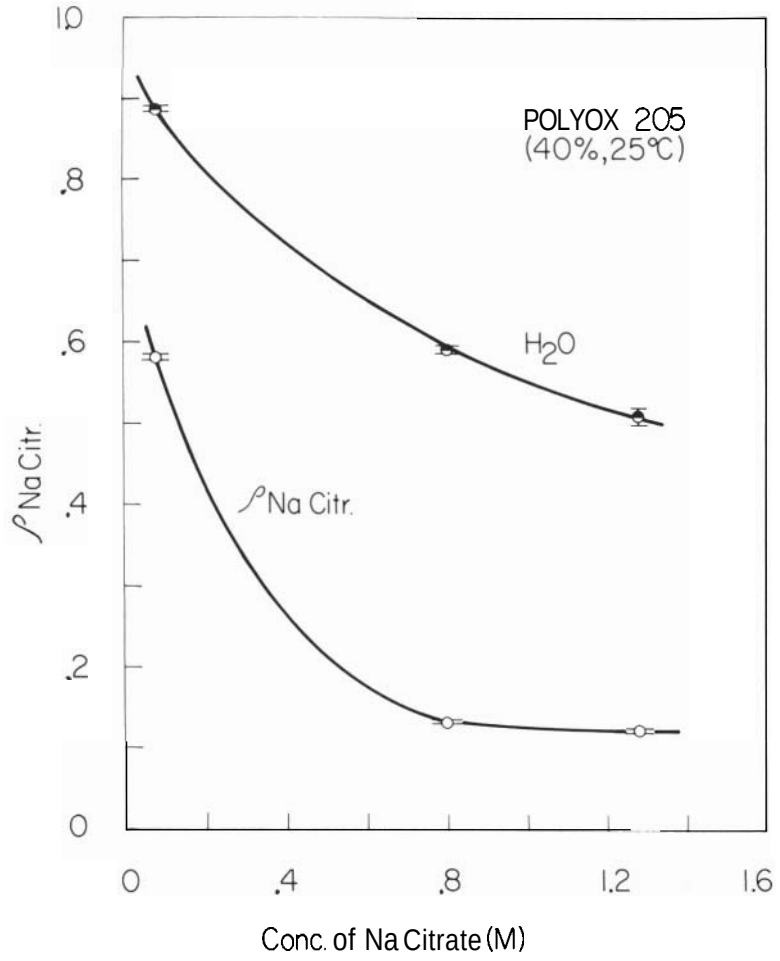

FIGURE 5. ρ-values of Na and water contents in polyethylene oxide (Polyox-205) in various concentrations of sodium citrate (25°C). Initial concentration of polymer was 40%.

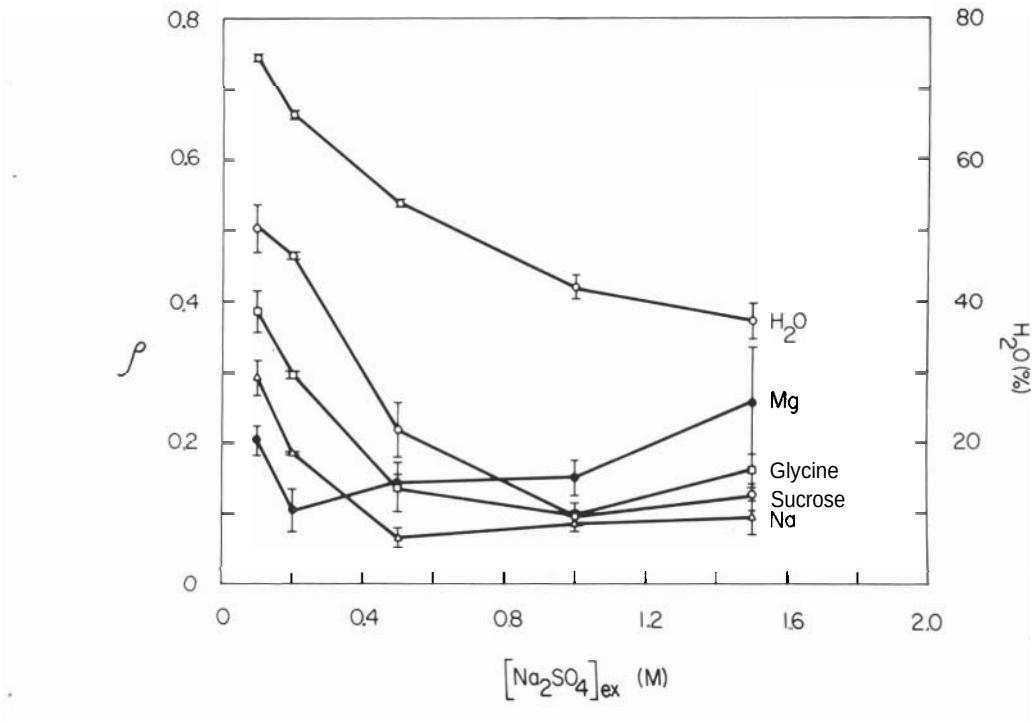

FIGURE 6. ρ-values of Na, Mg, glycine, and sucrose and water contents of polyvinylmethyl ether in various concentrations of Na_2SO_4 (25°C). Initial concentration of polymer was 50%.

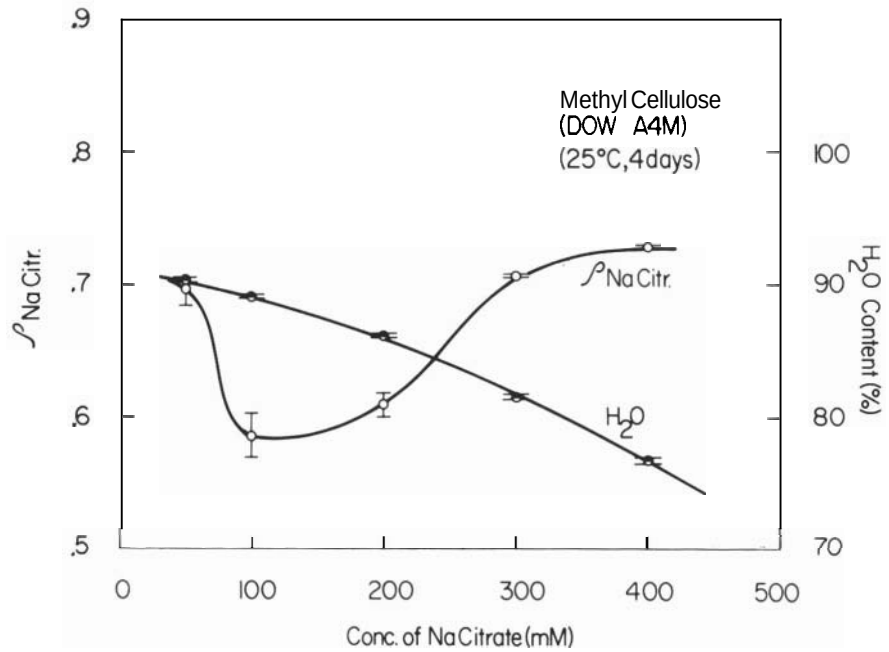

FIGURE 7. ρ-values of Na and water contents of methylcellulose (DOW F4M) in varying concentrations of Na citrate. Initial concentration of methylcellulose was 10%.

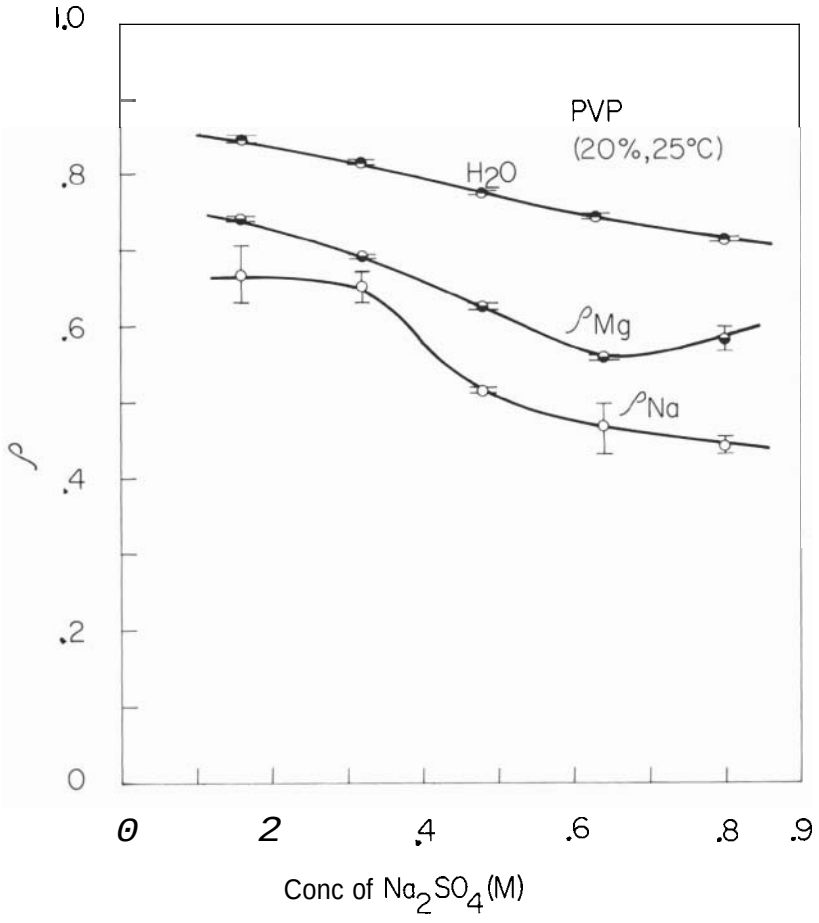

FIGURE 8. ρ-value **of Na and Mg water contents of polyvinylpyrrolidone in varying concentrations of Na_2SO_4 (25°C). Initial concentration of polymer was 20%.**

citrate in the water of a methylcellulose-water system. Note that in this polymer-water system, with increasing sodium citrate concentration the water content continues to decrease as in the case of gelatin, PEO, or PVME. However, the $\rho_{\text{Na-citrate}}$ first decreases and then increases again in a very pronounced manner.

It should also be mentioned that the extremely low ρ-values shown in Figs. 5 and 6 were achieved, in part at least, with a tight-sac technique in which imbibition of water of the originally concentrated polymer-water system (40% PEO, 50% PVME) was limited. If looser sacs had been used, more water would have been taken up and as a rule the ρ-value would have been higher.

Effect of initial polymer concentration on water contents and ρ-*values.* Figures 8 and 9 show the water contents and ρ-values in PVP for Na+ and Mg^{2+} at the initial polymer concentrations of 20% and 40%. The data indicate syneresis; much lower ρ-values and water contents were observed in the polymer solution with a higher initial concentration.

Effects of temperature on water content and ρ-*values.* Figures 10 and 11 show respectively the water content and ρ-value for sodium citrate in PVP at four different

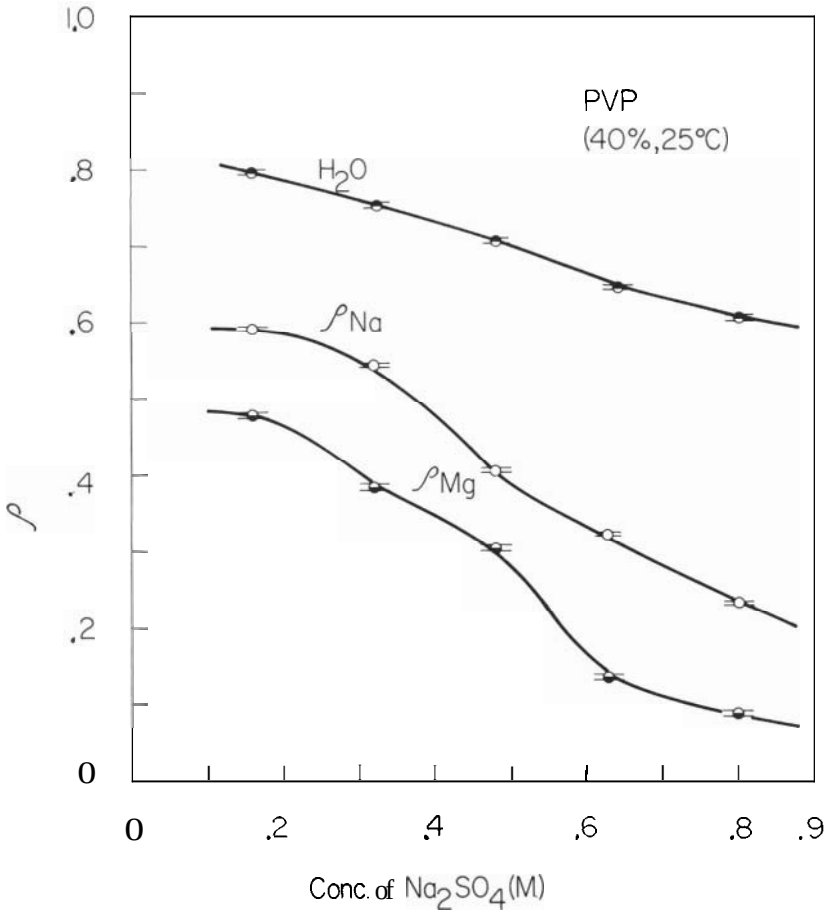

FIGURE 9. p-values of Na and Mg and water contents of polyvinylpyrrolidone in varying concentrations of Na_2SO_4 (25°C). Initial concentration of polymer was 40%.

temperatures. Between 0" and 20°C there is relatively little change in water contents but an even rise of ρ-values occurs throughout the entire concentration range. In this respect, the curves resemble the behavior pattern of gelatin shown in Fig. 4. From 20" to 40°C, there is a rather abrupt fall in both water content and ρ-value at sodium citrate concentrations near 0.40 M. However, at sodium citrate concentrations near 0.90 M, there is a reversal of the trend toward a higher water content and ρ-value that becomes most pronounced at 60°C.

In the case of gelatin (Figs. 12, 13), the overall pattern appears simpler: the general trend is a rise of both water content and ρ-value with increasing temperature. However, a minor downward shift in both is seen between 0" and 20°C at the higher salt concentrations.

Quite the opposite trend is seen in the case of PVME (Gantrez). There is a gentle fall of both water content and ρ-value for sodium concentrations (Fig. 14) as well as for sucrose (Fig. 15). However, a precipitous fall in both water content and ρ-value occurs between 25" and 35°C.

Effect of to-and-fro shaking on water content and ρ-value of sodium citrate in PVP. Figures 16 to 19 show the water con-

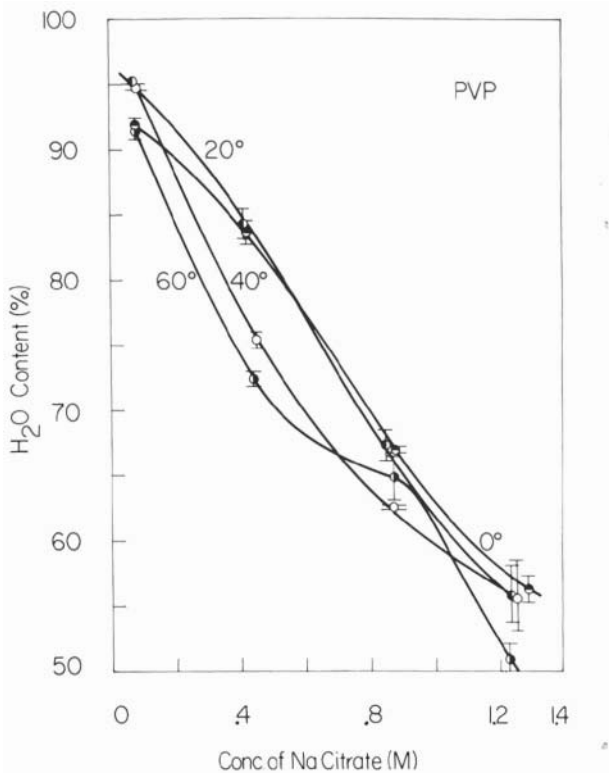

FIGURE 10. Water contents of polyvinylpyrrolidone in varying concentrations of Na citrate and at four different temperatures. Initial concentration of polymer was 20%.

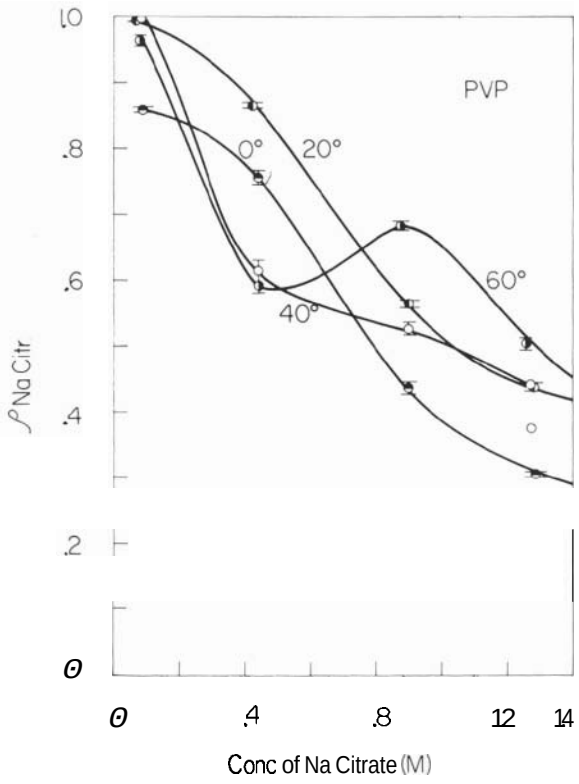

FIGURE 11. p-value of Na of polyvinylpyrrolidone in varying concentrations of Na citrate and at four different temperatures. Same experiment as that shown in Fig. 10.

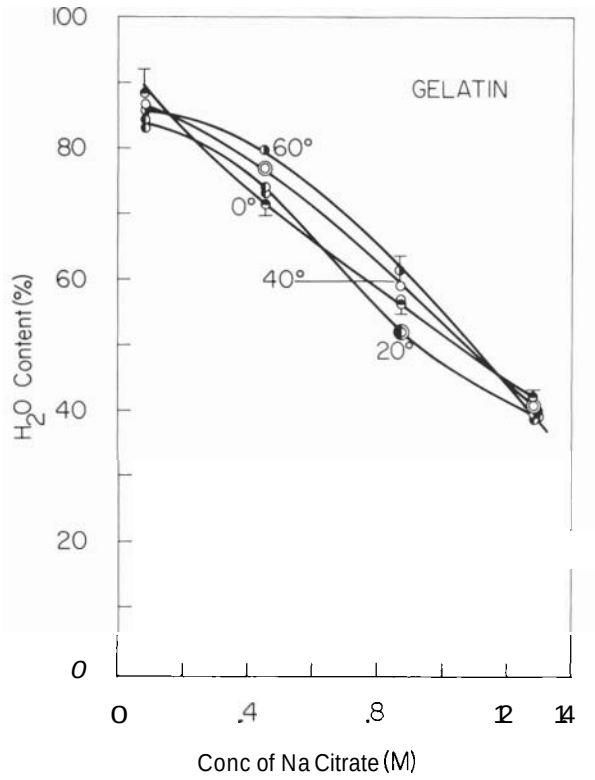

FIGURE 12. Water contents of gelatin in varying concentrations of Na citrate and at four different temperatures. Initial concentration of gelatin was **30%.**

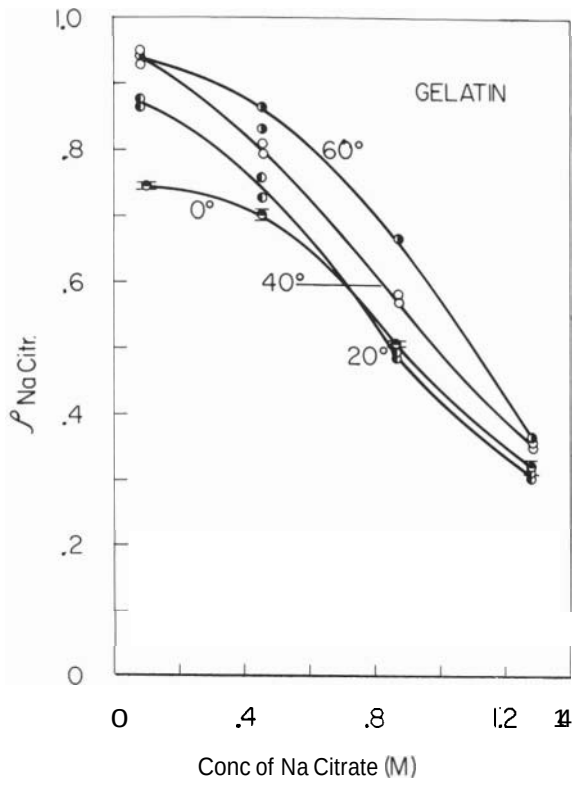

FIGURE 13. ρ-value of Na of gelatin in varying concentrations of Na citrate and at four different temperatures. Same experiment as that shown in Fig. 12.

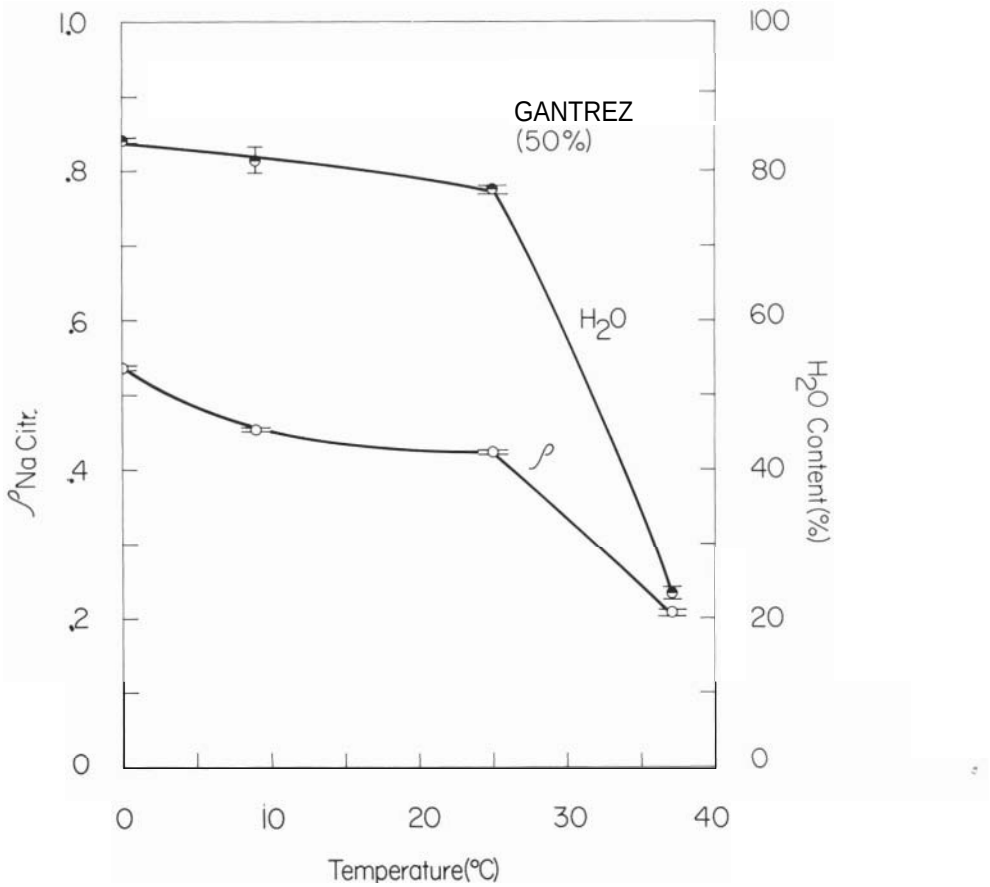

FIGURE 14. p-values of Na and water contents of polyvinylmethyl ether (Gantrez) at different temperatures. Initial concentration of polymer was 50%. The incubation solution contained 100 mM Na citrate and 100 mM sucrose. Incubation lasted 21 days.

tent and p-value of sodium citrate in PVP at two different temperatures and either shaken or quiescent. Shaking, which tends to cause the linear polymer to line up in a parallel manner, results in a decrease of water content but a much more pronounced decrease of p-value. A more limited demonstration of this shaking effect was presented and its significance discussed in an earlier paper.[11]

p-value of various sodium salts, sucrose, and glycine. Figure 20 compares the ρ-values of the three sodium salts (NaCl, Na_2SO_4, and Na_3-citrate), glycine, and sucrose in PVME. The p-values for the sodium salts are in the order NaCl > Na_2SO_4 > Na_3-citrate. A similar comparison is made for the polymer polyvinyl alcohol (PVA), which differs from the other synthetic polymers given above in that PVA contains only H-donating groups (OH) rather than only H-accepting groups. The results show a much more uniform p-value for the same probes (Fig. 21).

Even though NaCl is the least excluded, it nonetheless exercises a similar though much weaker effect on the polymer water contents and ρ-values. This is shown in the case of methylcellulose (Fig. 22).

Relation of p-value of a solute and its molecular size and complexity. Figure 23 shows that the p-value in PVME for the

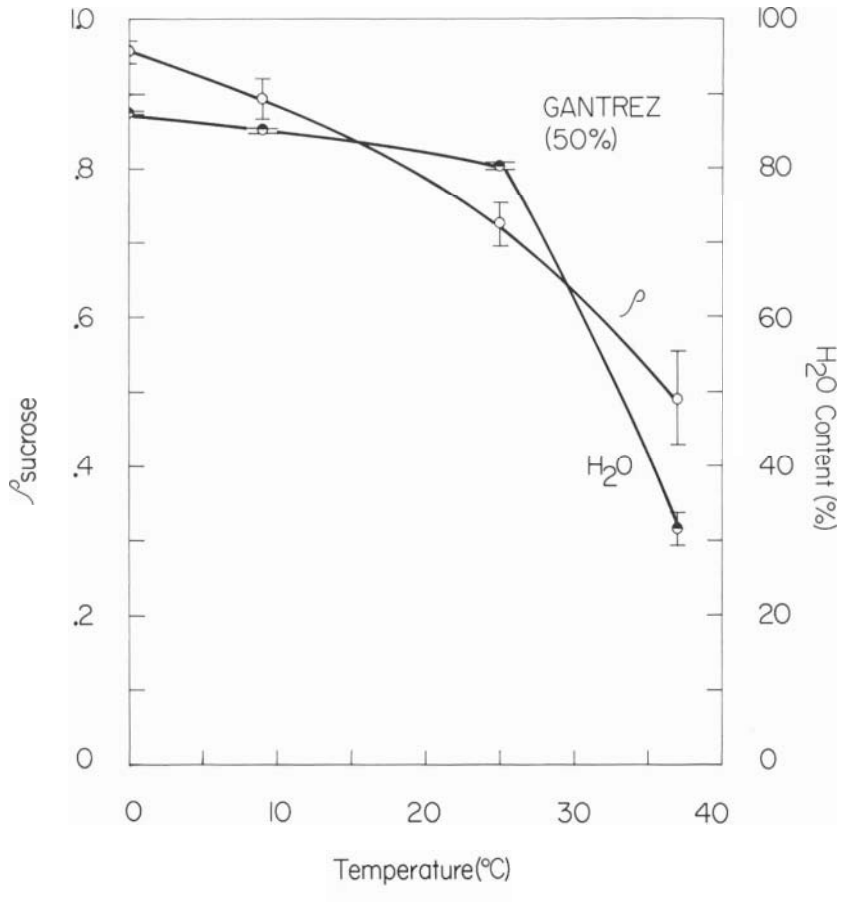

FIGURE 15. ρ-value **of sucrose and water contents of polyvinylmethyl ether (Gantrez) at different temperatures. Initial concentration of polymer was 50%. Incubation solution contained 100 mM Na citrate and 100 mM sucrose. Incubation lasted 23 days.**

smallest hydroxylic compound in the list, methanol, is close to unity; it is intermediate for the sugars, xylose, and sucrose; it becomes lower for labeled inulin (mol. wt. 3000 to 4000), but it is much lower for labeled polyethylene glycol (mol. wt. 4000).

Is water the only solvent with solubility properties that can be altered by polymers? Figure 24 shows that **PVP** dissolved in other pure polar solvents (D_2O and formamide) as well as solvents mixed with water in approximately 50-50 proportion ($H_2O + D_2O$, formamide, dioxane [9.5 M urea], and ethanol), show reduced ρ-value for sodium sulfate. Similarly, **PVP** dissolved in DMSO and ethanol or in 50% DMSO and 50% H_2O has a ρ-value similar to that of glycine in water. The only exception is **PVP** dissolved in a 50-50 mixture of water and ethanol, in which case ρ-glycine almost equals unity.

DISCUSSION

Polar Groups of a Linear Polymer—A Basic Requirement for the Long-Range Effecton Water Solvency

Polarity. In the polarized multilayer theory of cell water, a matrix of a more or less parallel array of extended polypeptide chains polarizes the bulk of cell water. It is argued that the negatively charged CO

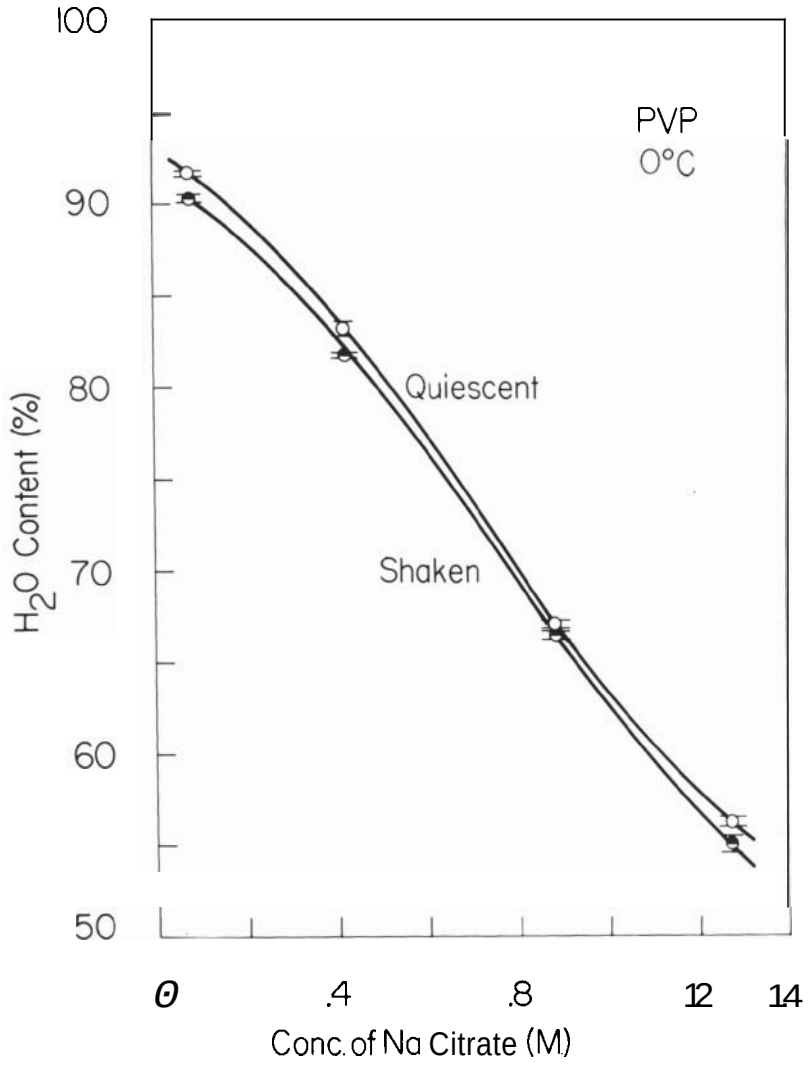

FIGURE 16. Water contents of shaken and quiescent sacs of polyvinylpyrrolidone solutions in varying concentrations of Na citrate *(20°C)*. Initial concentration of polymer was 20%.

groups are the primary seats of the polarization of water but that the positively charged NH groups augment the total polarizing effect. However, theoretically a matrix of chains carrying alternating negative and ***vacant*** sites (NO-NO-NO system) or positive and ***vacant*** sites (PO-PO-PO system) should also be effective if the distance between the neighboring sites is correct. The fact that three of the effective artificial polymers in water polarization—PVME, PEO, and PVP—are in fact NO-NO-NO systems confirms this view. We have not been able to obtain a polymer of the kind $(CH_2—N—CH_2)_n$, with H on the N, In its place, however, we have $(CH_2—CH)_n$, with OH on the CH, i.e., polyvinyl alcohol (PVA). These protein-donating OH groups are also effective in polarizing water but less strongly,

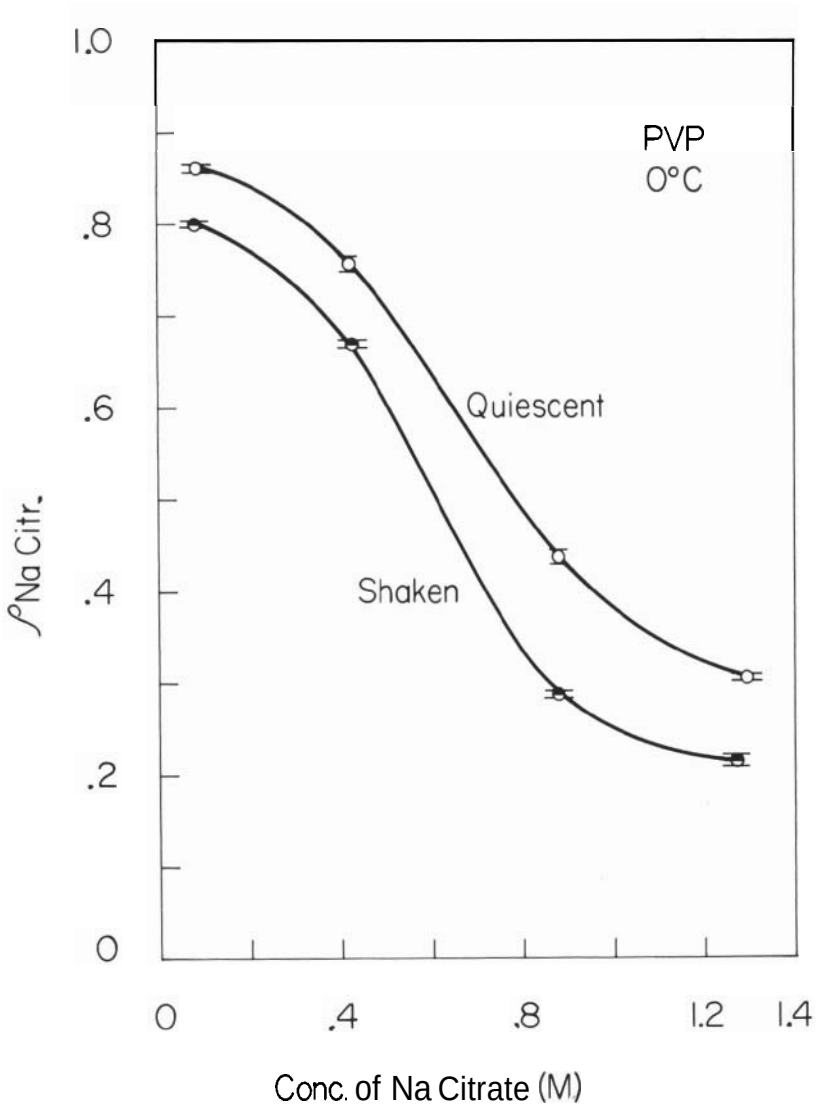

FIGURE 17. ρ-values of Na in shaken and quiescent sacs of polyvinylpyrrolidone solutions in varying concentrations of Na citrate ***(0°C)***. Same experiment as that shown in Fig. 16.

as shown in the ρ-values for sodium citrate and sodium sulfate.

Thus in a regular array of properly spaced proton-accepting groups and properly spaced proton-donating groups, both can have the effect of reducing the solubility of water. Together they support the concept that an extended protein chain endowed with a sequential array of both proton-accepting CO groups and proton-donating NH groups will have a synergistic effect and under the proper conditions will act more effectively on water solvency.

Distance between neighboring groups of same polarity. According to the polarized multilayer theory of cell water, to achieve long-range ordering of water the linear arrays of alternating positive and negative sites, or alternating positive (or negative) and vacant sites, are essential. In these configurations, neighboring rows of water molecules polarized by the polymer chain have opposite

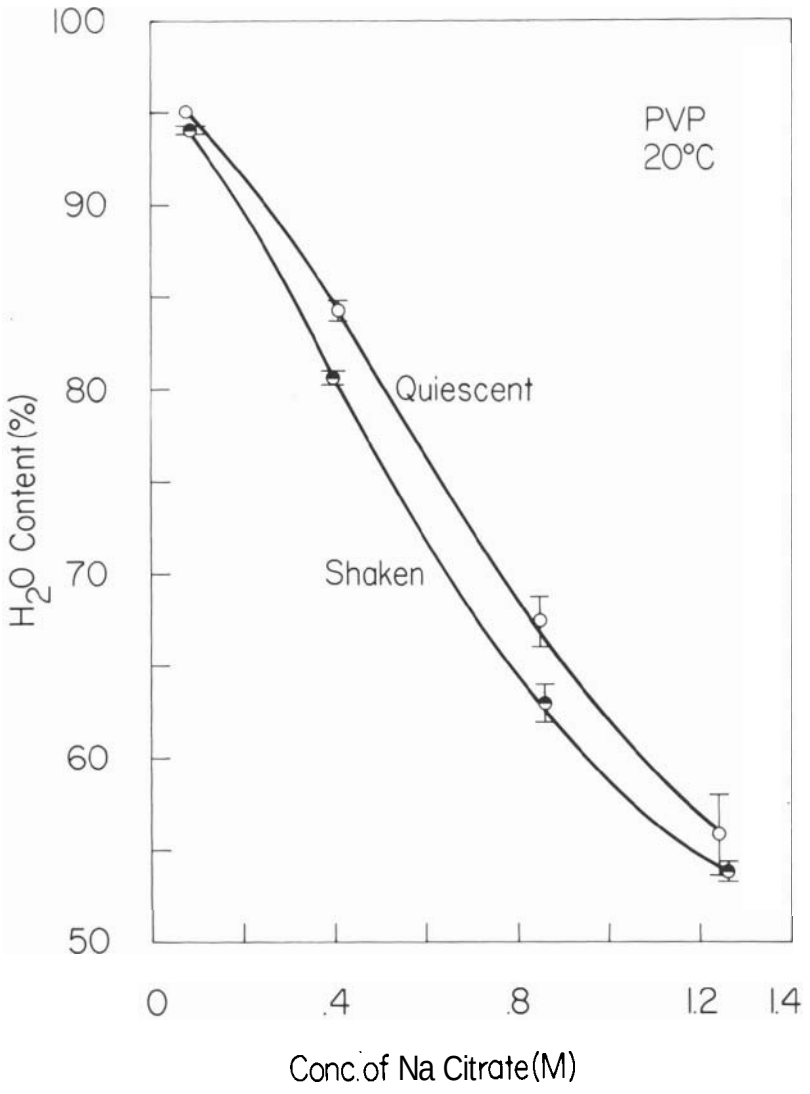

FIGURE 18. Water contents of shaken and quiescent sacs of polyvinylpyrrolidone solution in varying concentrations of Na citrate (20°C). Initial concentration of polymer was 20%.

orientation and hence cohesion owing to the cooperative interaction of each water molecule with all its neighboring water molecules.[11]

Detailed comparison of the distances separating polar groups of all the effective polymers must await future study. But it is interesting to note that three of the most effective polymers share the basic vinyl group (PVP, PVA, and PVME) and that at least two carbon atoms separate each neighboring oxygen atom in PEO and one carbon and one nitrogen atom separate the two carbonyl groups of extended protein chains.

Particularly interesting is the observation of Stone and Stratta[13] in their review on ethylene oxide polymers: "Although polyethylene oxide is highly soluble in water, closely related polymers are insoluble in water. The related water-insoluble species include the polymers of formaldehyde and

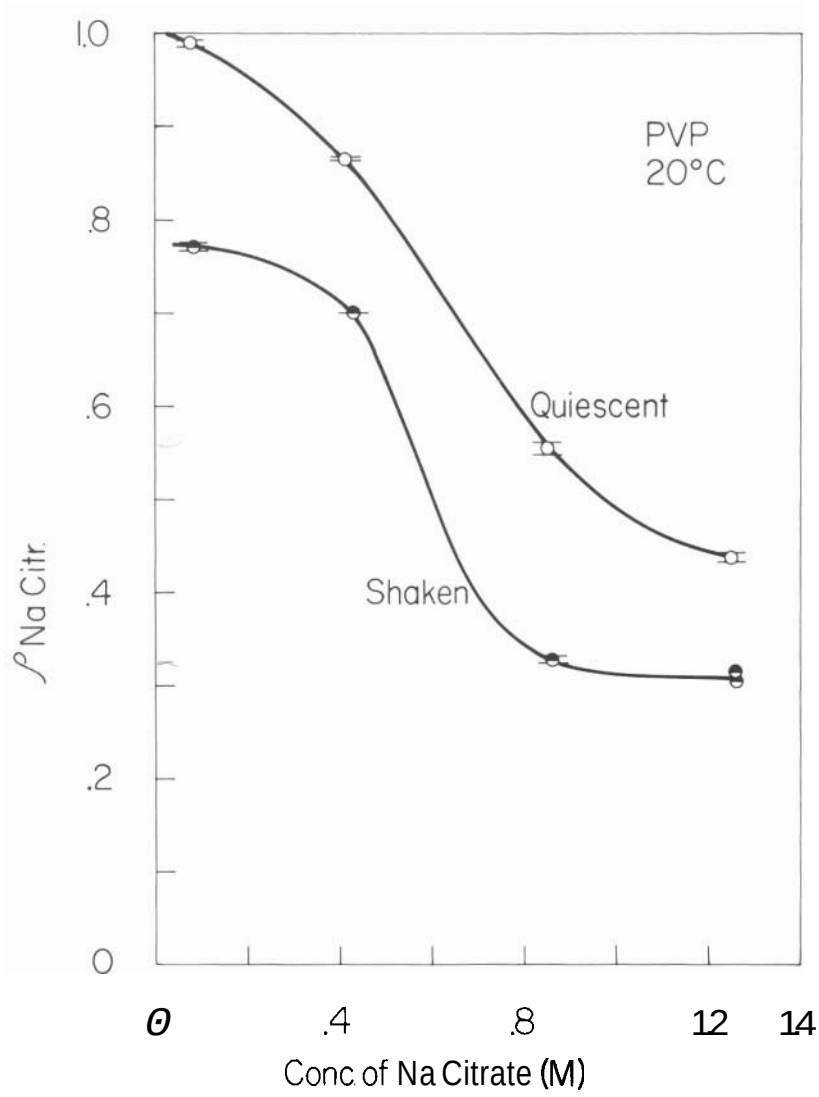

FIGURE *19.* **ρ-values of Na in shaken and quiescent sacs of polyvinylpyrrolidone solutions in varying concentrations of Na citrate (25°C). Same experiment as that shown in Fig. 18.**

acetaldehyde . . . , trimethylene oxide, . . . and tetramethylene oxide. . . ." Thus a reduction of one or addition of one (or more) methylene groups between the oxygen atoms of PEO has the same effect; i.e., it is no longer soluble in water. Obviously a polymer that is not soluble in water can have no effect on water solvency.

Unmasked state of the polar groups. In an earlier paper we discussed at length both the theory and the experimental evidence that the effect on water solvency of polymer-like protein can be completely annulled if its oppositely charged polar groups, CO and NH, form intra- or intermolecular H-bonds among themselves. We have also shown that when these masked groups are unmasked, as for example by urea denaturation, the effect on water solvency is restored."

While special effort must be made to disassociate H-bonds between the NH and

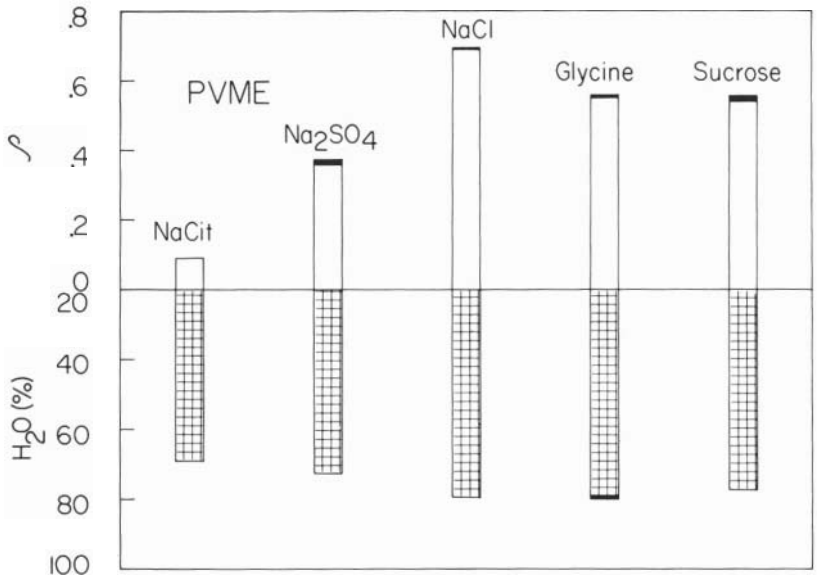

FIGURE 20. Water contents and p-values of Na citrate, Na_2SO_4, NaCI, sucrose, and glycine in solutions of polyvinylmethyl ether (25°C). Incubation solutions contained 100 mM of each of the following: Na citrate, Na_2SO_4, NaCI, glycine, and sucrose. Widths of horizontal lines at the end of each bar represent 2 × standard error.

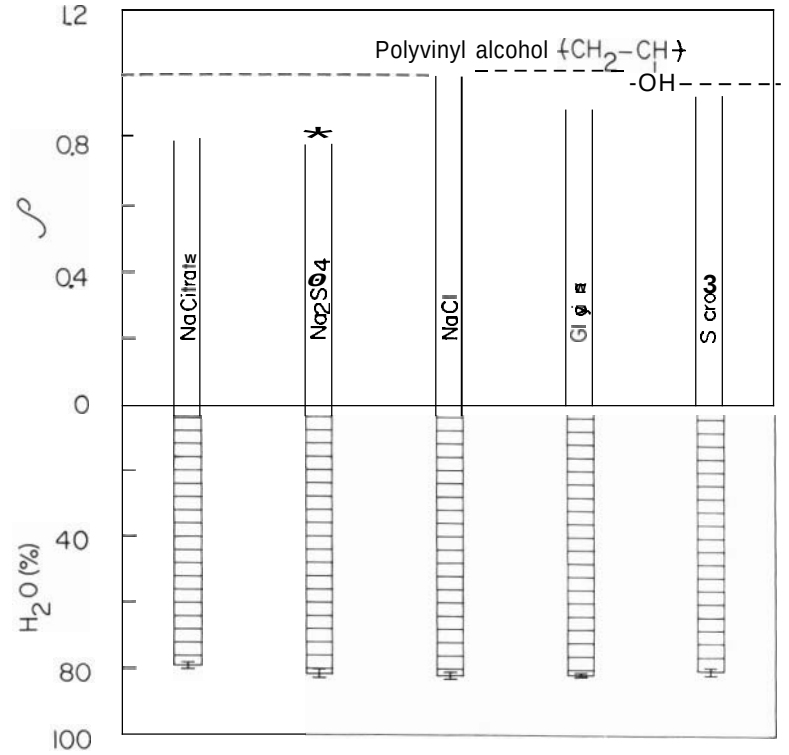

FIGURE 21. Water contents and p-values of Na citrate, Na_2SO_4, NaCI, glycine, and sucrose in solutions of polyvinyl alcohol. Incubation solutions were same as described in legend for Fig. 20. Distance between horizontal bars represents 2 × standard error.

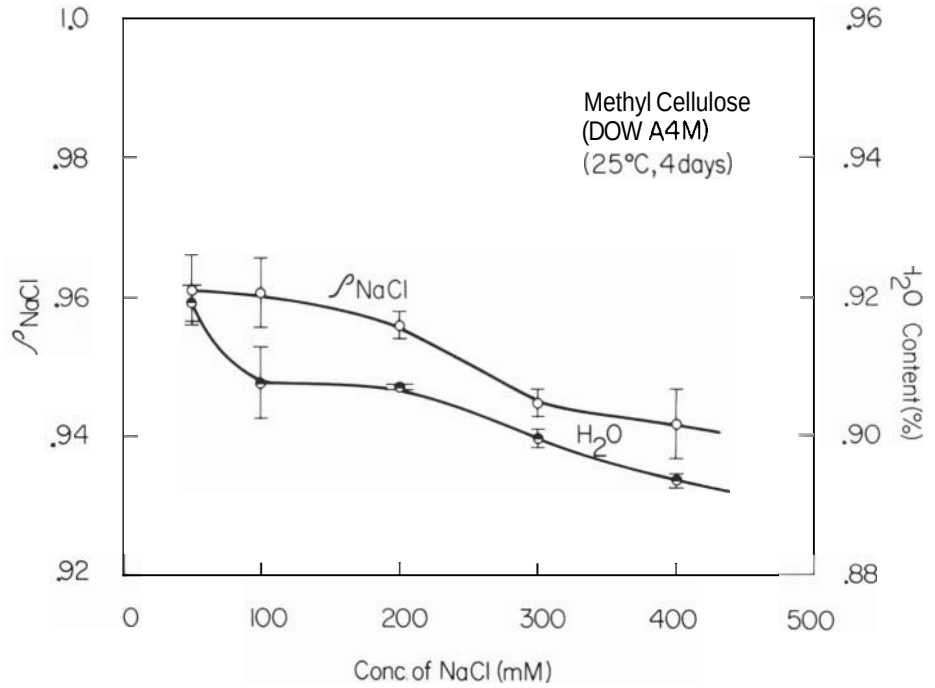

FIGURE 22. p-value of Na and water contents in methylcellulose-H_2O system (25°C) in varying concentrations of NaCl. Initial concentration of methylcellulose was 10%. Incubation lasted 4 days.

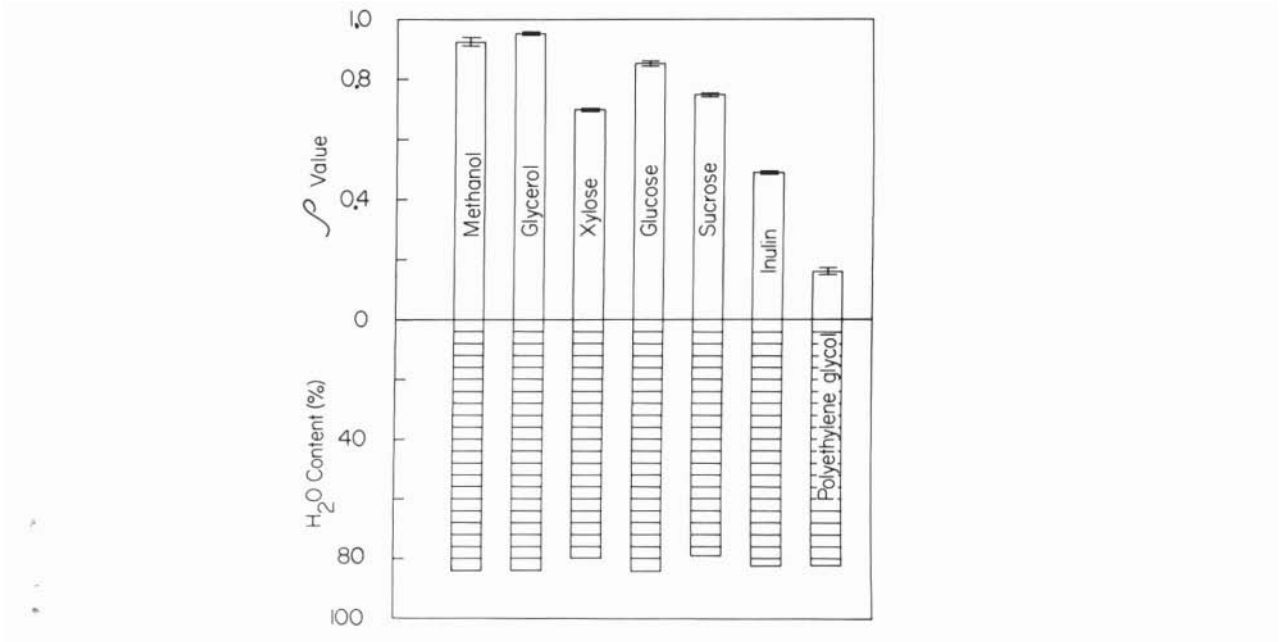

FIGURE 23. Water contents and p-values of alcohol, sugars, and other compounds in polyvinylmethyl ether solutions (25°C). Initial concentration of polymer was 50%. All incubation solutions contained 100 mM Na_2SO_4 plus 10 mM of each of the compounds presented except inulin and polyethylene glycol, in which cases only trace amounts of labeled materials were added. Incubation last 4 days.

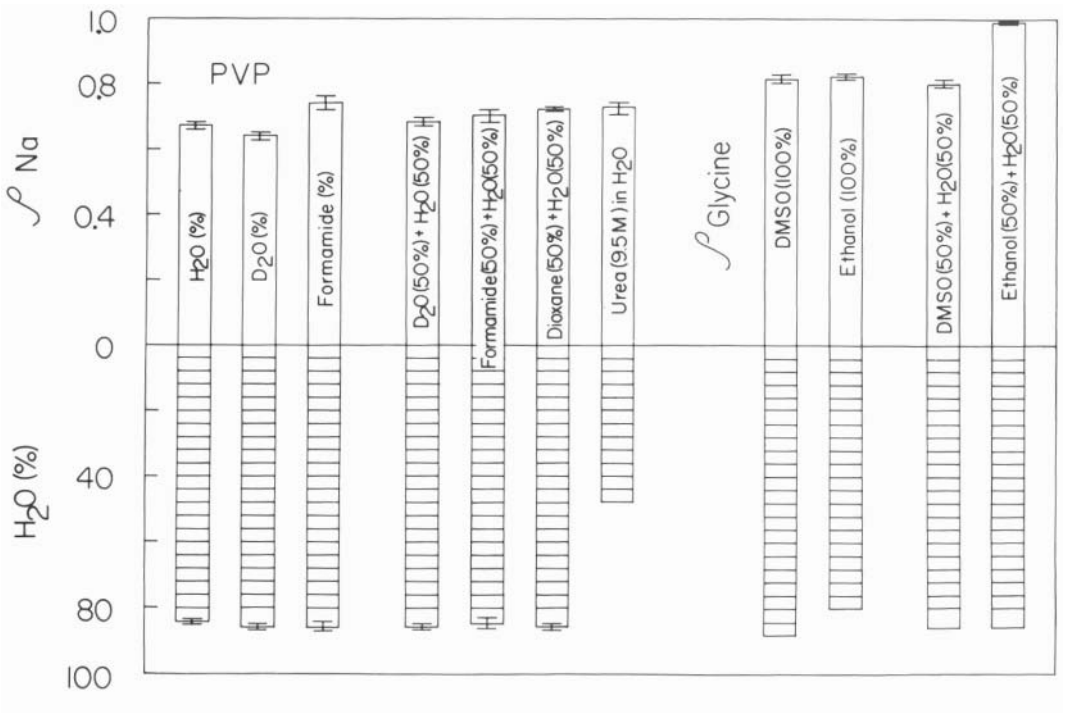

FIGURE 24. Effect of variations of solvent on ρ-values of Na and of glycine in polyvinylpyrrolidone solution **(25°C).** Initial concentration of polymer was 20%. Distances between the two horizontal bars at the end of each bar represent **2** × standard error. Incubation lasted **3** days.

CO groups in order to reveal the effect of proteins on water solvency, no such effort is necessary for four of the most effective artificial polymers: gelatin, PEO, PVP, and PVME. In the case of gelatin, its large proline and hydroxyproline contents prevents formation of the α-helix; the other three simply have no positively charged proton-donating groups and are unable to form inter- or intrachain H-bonds.

Dominant role of the CO group of a polypeptide chain in reducing water solvency. A comparison of the ρ-value for, say, sodium citrate in the PVA-water system with that in the PVP-, PEO-, and PVME-water systems under similar conditions leaves little doubt of the much greater effect of a carbonyl or ether oxygen than of a hydroxyl group in reducing the solvency of water.

Recently Wolfenden[14] reported his study of the interaction of the peptide bond with solvent water and noted, for instance, that the vapor pressure of aqueous acetamide is little affected by substitution of methyl groups for protons on nitrogen. He argued that the "interaction of water with carbonyl groups (rather than with the NH protons) is mainly responsible for the hydrophilic character in the acetamide." This finding has great importance for another concept introduced in the association-induction hypothesis: the relation of the H-bonding strength of the polypeptide chains to the electron-donating or withdrawing effect of the side chains."

Alignment in a Matrix of Extended Polypeptide Chains and Models

That to-and-fro agitation of a PVP-water system enhances its ability to exclude sodium citrate supports the expectation that

the maximum effect on water solvency if the proper chains are arrayed in parallel, as pointed out in an earlier paper.[15] In the same light, perhaps one can explain why the solvency effect of methyl cellulose is much more profound than that of the large majority of natural gums and polysaccharides. It is well known that cellulose molecules represent straight chains. The interposition of methyl or other groups separates these chains, thereby presenting a regular array of water-polarizing chains. One is inclined to think that in methyl cellulose, as compared to PEO, PVME, and PVP, the ether oxygen on the glucose ring must play a major role in polarizing water.

Effect of Molecular Size and Complexity on q-Value

The association-induction hypothesis offers two mechanisms for solute exclusion from water polarized in multilayers: an enthalpic mechanism and an entropic mechanism.[10] In both cases the predicted q-value decreases with increasing size and complexity of the probe molecules in question, as seen in the p-value for NaCl, Na_2SO_4, and Na_3-citrate, for which the major determining factor appears to be the anion. The data shown in Fig. 23 also support this theory. It is significant to point out that roughly the same sequential order of preference for this series of hydroxylic compounds is seen in living cells.[15]

Structure-Breaking and Structure-Altering *Effects of High* Concentrations of Sodium Sulfate or Sodium Citrate

Figure 7 shows that as the concentration of sodium citrate increases, the p-value for sodium citrate in the methyl cellulose water system decreases and then increases. A simple interpretation for the secondary rise is the structure-breaking action of the sodium citrate at high concentration. However, a closer look suggests that the situation may be more complex. Thus, after the secondary rise, the p-value does not continue to rise until the water structure is completely destroyed with p equal to 1.0. Actually, the slope of the p-value levels off at a p-value considerably below 1.0 and sometimes curves down again.

Similar evidence of the complex effect of high citrate concentration can be seen in the study of the ρ-values for four different probe molecules in PVME (Fig. 6). Here the p-value for Mg steadily increases from its lowest point at 0.2 M citrate. However, a comparison with the ρ-values of other probe molecules in the same samples shows that the point of minimum as well as maximum p-value varies from one probe molecule to another, again arguing against a general breakdown of water structure but suggesting a somewhat altered water structure.

SUMMARY

We studied the properties of polymer-oriented water, which excludes Na^+, sucrose, free amino acids, and other solutes found in low levels in many living cells. Altogether, twenty-three polymers were investigated of which fourteen were natural gums and polysaccharides, eight were synthetic, and one was a polypeptide. More intensive studies were done of one natural (gelatin) and four synthetic polymers (methyl cellulose, polyvinylpyrrolidone, polyvinylmethyl ether, and polyethylene oxide). With equilibrium dialysis methods, we determined the apparent equilibrium distribution coefficients, or ρ-values, of Na+ (as sulfate and citrate), sucrose, glycine, etc., between water in the dialysis sacs filled with polymer-water and water in the external bathing solutions. We studied the effects of the following variables on the water contents in the polymer-water

system and the ρ-values for each solute: external salt concentrations, initial polymer concentration in the sac, temperature, mechanical agitation, molecular size of the probe molecules, and the nature of the solvents. The observations made are generally in accord with the polarized multilayer phase of bulk-phase cell water, proposed under the association-induction hypothesis.

We thank Kelco Co., N.J.; GAF Corporation, N.Y.; Dow Chemical Co., Fla.; B. F. Goodrich Chemical Co., Ohio; and Union Carbide, N.Y. for their generous gifts of polymer samples; and Jean Brogan and Marilyn Hymowitz for much help. The above work was supported by NIH Grant 2-RO1-CA16301-03-04 and ONR Contract NOO14-71C-0178.

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(Received Septernber 10, 1979)