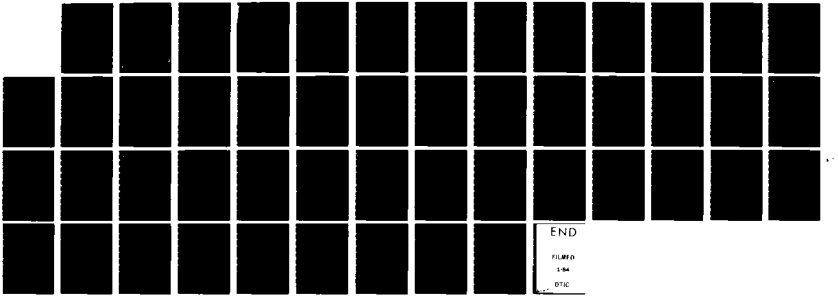
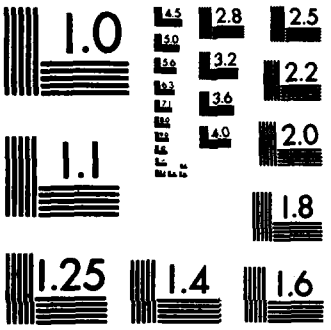
AD-A136 112 REDUCED SOLUBILITY OF POLYMER-ORIENTED WATER FOR SODIUM SALTS SUGARS AMIN..(U) PENNSYLVANIA HOSPITAL PHILADELPHIA DEPT OF MOLECULAR BIOLOGY.. 1/1

UNCLASSIFIED G N LING ET AL. 1983 N00014-79-C-0126 F/G 6/3 NL





(2)

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

AD-A136 112

by

Gilbert N. Ling, Cheryl Walton and

T. Janine Bersinger

Running Title: Solvency of Polymer-Oriented Water

1117

Accession For	
NTIS GRA&I	X
DTIC TAB	☐
Unannounced	☐
Justification	per ltr
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

N00014-79-C-0126

Department of Molecular Biology

Pennsylvania Hospital

8th and Spruce Streets

Philadelphia, Pennsylvania 19107

Currently there are two diametrically opposed concepts of the physico-chemical nature of the living cells. The membrane-pump theory places major emphasis on the property and activity of a submicroscopic plasma membrane which controls the distrubution of solutes in the cells by its permeability or impermeability and by the continuous operations of a battery of inward and outward pumps in the membrane (1,2,3). The cytoplasm is seen as having the property of a dilute salt solution. The alternative theories, exemplified by the association-induction hypothesis, see the living cells in a different light (4,5,6). Being alive in this theory signifies the maintenance of the assembly of cooperatively associated proteins-water-ions of the cells at a high energy state called the living state. The distribution pattern of a particular permeant free 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; dissolution in cell water which tends to keep the level of large solutes in the cell water at a level below that in the surrounding medium.

More specifically, the association-induction hypothesis proposes that certain proteins in the 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 constitute what is called an NP-NP-NP system (7,8). Water between the parallel chains is polarized in multi-

layers. For both entropic and enthalpic reasons, the solubility of a solute in this water may to varying degree be reduced when compared with

that in normal water (9,10). While such an NP-NP-NP system is the most effective in the long-range polarization of water, a matrix of chains containing properly spaced negative and vacant sites (an NO-NO-NO system) or positive sites and vacant sites (an OP-OP-OP system) (where O stands for a "vacant" site or a neutral site) may also function like an NP-NP-NP system in reducing the solvency of bulk phase water (11).

Although as part of the association-intuction hypothesis, the theory deals primarly with the living cells, the basic concepts should be more widely applicable to non-biological water containing systems as well. The present paper presents results of a study of the solvent properties of water associated with a broad selection of natural and synthetic polymers, which to varying degree, serve the useful role as models of the living cell.

Materials and Methods

ρ-value determination

The basic procedure used was to determine the apparent equilibrium distributive coefficient of a probe solute in the polymer-water systems. First a concentrated aqueous solution of a polymer was prepared often with aid of gentle heating. The polymer solution was then inserted into 1/4 inch dialysis tubing, the ends of the sac tied with Deknatel

silk threads, These loaded sacs were then placed in a suitable volume of an aqueous solution usually containing Na_2SO_4 or Na-citrate at various concentrations and one or more other probe solutes such as sucrose, glycine or Mg SO_4. One or more of these solutes may be radioactively labelled (e.g., Na^{22}, C^{14}- sucrose, H^3- glycine). The tubes containing the sacs were placed horizontally and shaken at about 60 excursions per minute, each excursion usually 3/4 inch in distance. After a length of time long enough to insure equilibrium distribution of the solute under study, the sacs were taken out of 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 whose distribution between the water in the sac and out of it was under study. The results were expressed as an apparent distribution coefficient, ρ (10) where

$$\rho = \frac{\text{concentration of the solute in the water in the sac}}{\text{concentration of the solute in the water bathing the sac}}$$

It is to be noted that 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). ρ-value would exceed the q-value, if part of the solute in the sac is adsorbed on the polymer. In this study no attempt is made to sort out the adsorbed and the dissolved fraction, hence the use of the ρ-value throughout.

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

At the end of incubation sac contents were as a rule dissolved in 1N HNO_3

Aliquots of ^{14}C or ^{3}H labelled extracts were then (either counted directly for assay of ^{22}Na or for assay of ^{3}H and for ^{14}C)

Time Course Studies

Two basic methods were used for determining the time course of isotope exchange: an influx method and an efflux method. In the influx method, properly filled sacs about 1 cm in length were placed in the bathing medium containing radioactive ^{22}Na and after different lengths of time, taken out, blotted dry carefully, and weighed before the entire sac was placed in the bottom of a well-type γ-Scintillation counter to assay its radioactivity. After this counting the sac was returned to the radioactive bathing solution for further incubation and again taken out after an interval. The radioactivity per unit weight of the sac at each time is then plotted against 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-labelled Na^+, ^{35}S labelled SO_4) and the concentrated solution then placed in the dialysis tubing. The filled sac was then tied at both ends. After weighing the sac it was washed in 10 ml portions of a non-labelled Na_2SO_4 or Na-citrate solution for a certain length of time before it was moved to another tube filled also with non-labelled solution and the process repeated many times until after the final washing, the sacs were weighed once again and its contents taken out, dissolved in 1N HNO_3. Aliquots of each of the washing solution as well as that 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 25°C in a constant temperature room maintained at the temperature ±1°C. In other experiments at lower or higher temperatures carefully sealed screw-capped tubes containing the sacs were placed horizontally in

a Aminco constant temperature shaker bath maintained to within $\pm 0.1^{\circ}C$. Often a stack of tubes were placed inside a plastic box and the entire box totally immersed in the shaker bath.

Materials

From ICN, Irvine, Calif. we obtained the following: D_2O (99.8 atom %) (Lot 20328), ^{22}Na (Lot 32,39 and 40), ^{14}C-sucrose (Lot 696571, 640171), ^{3}H-glycide (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. we obtained the following: ^{35}S-SO_4 (Lot 8751), ^{3}H-sucrose (Lot 581-128), ^{3}H-glycine (Lot 929-042), ^{3}H-tyrosine (Lot 433-249) ^{3}H-leucine (Lot 965-076), ^{3}H-isolencine (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-Reseach, Orangeburg, N.Y., we obtained ^{3}H-L-aspastic acid (Lot 650). From Nuclear Chicago, Chicato, Ill, we obtained ^{14}C- sorbitol (Bath 21).

From Sigma Chemical Co. (St. Louis, Mo.) the following were obtained: gum arabic (64C-0252; gum ghatti (42C-2380; gum guar (32C1930); gum Karaya (103C-0720); gum locust bean (42C-2900); gum tragacanth (74C-0207); and gum xantham (888-0200); corn starch (6813-0216); potato starch (65B-2060); pectin (107B-0090); alginic acid (766-818); also polyvinyl alcohol (127C-0196). Gelatin was obtained from Eastman and were made 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 gifts: Kelzan®, a bacterial polysacchaide from Kelco Co., Clark, N.J.; poly(vinyl-methyl ether (Gantrez M-154®)(Batch 185) from GAF Corp., N.Y.; Methocel F4M (Hydroxylpropol methylcellulose)(Lot QP-3218410-F), from Dow Chemical Co., Coral Gables, Fla.; poly(ethylene oxide), Polyox 205 from Union Carbide, New York.

Results

The Time to Reach ^{22}Na, $^{35}SO_4$ Distribution Equilibrium

Figure 1 shows the time course of ^{22}Na uptake by 4 different polymers in ¼ inch dialysis tubing: polyvinylpyrrolidone (PVP) (A), poly(vinyl methyl ether (PVME) (B) and gelatin (C). The initial concentration of each polymer were respectively 40%, 40%, and 50%. and the temperature was 0°C for all except gelatin which also has a time course at 25°C. All uptake curves seem to have a fast component reaching equilibrium before 20 hours and then a slower component which in the case of PEO, did not stop even 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 were essentially similiar at 25°C as at 0°C.

Two alternative interpretations for this separation into a fast and slow uptake are: that the polymer-water contains pockets of low ^{22}Na diffusibility or that rapid attainment of distribution equilibrium of ^{22}Na was accompanied by a continual change of the polymer-water systems in a direction toward a higher ρ-value for ^{22}Na.

Figure 2 and 3 provides an answer in favor of the second explanation. In each of these efflux studies the radioactive isotopes Na^{22} were introduced into the water before the polymer was dissolved. Had there been pockets of low diffusibility of the magnitude required, the time course of ^{22}Na efflux from gelatin slices and PVP filled sacs would have one or more large very slow components. In fact, in both cases these isotopes reached 99% exchange in a matter of 1 hour(gelatin slices) or 4 hours (PVP sacs). These experiments as well as those in a companion paper dealing with diffusibility through denatured protein filled sacs (11) set 48 hours as adequate duration for equilibrium distrubution studies. Actual incubation times, however, often lasted much longer.

Solute exclusions and accumulation in gelatin-water and PVP-water systems

Table 1 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 carrying no cationic or anionic side chains yet the amino acids, histidine, leucine, and isolucine are accumulated with a ρ-value considerably above unity. Even greater degree of accumulation in PVP is observed for adenine, and ouabain, suggesting the specific association of these solute with PVP.

On the other hand, the amino acids glycine, alanine, argine, aspartic acid and glutamic acid are significantly excluded as are all four sugars and sugar alcohol listed in Table 1.

What Polymers affect the solubility properties of water?

Table 2 lists the ρ-value of Na (as citrate or sulfate), Mg (as sulfate), sucrose, and glycine for polymers under 3 general headings: A. gums; B. polysacchaides; and C. synthetic polymers. In general, virtually all aqueous solutions of these polymers in high enough concentrations show reduced solubility for the probe materials. However, the effectiveness varies a great deal. Among all of these polymers, the most effective in reducing water solvency are, methylcellulose, polyvinylmethylether (PVME); polyethelene-oxide(PEO); polyvinylprrolidone (PVP), and gelatin.

The effect of the concentration of Na_2SO_4 and of Na-citrate in the external medium on the water contents and the ρ-values for various polymer-water systems.

Figure 4 plots against the concentration of Na_2SO_4 or Na-citrate, the water contents and the ρ-values for these Na salts of gelatin. In general the ρ-values and the water contents decreased with increasing salt concentration. Also mole for mole, Na citrate is more effective than Na_2SO_4. One reason is simply that each molecule of Na-citrate ionized into 4 ion particles while each molecule of Na_2SO_4 disassociates only into 3. Nevertheless, mole for mole, a citrate anion is

considerably more effective than a sulfate anion. Thus at a concentration of 0.46 M Na citrate, $\rho_{\text{Na-citrate}}$ is only 0.6 while $\rho_{Na_2SO_4}$ is 0.78, even though the water contents are equal at this point. In both Na_2SO_4 and Na citrate, the ρ-values steadily decreased with increasing external salt concentrations.

Figures 5 and 6 show similar concentration effect on the water contents and ρ-values in PEO (Polyox-205) and PVME (Gantrez M154). In PEO both water contents and $\rho_{\text{Na-citrate}}$ steadily decreases, 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 an upward trend at high citrate concentration (see Figures 8 and 9 below).

Figure 7 shows the ρ-value of Na-citrate in the water of a methyl-cellulose-water system. Notice that in this polymer-water system with increasing Na-citrate concentration the water content continues to decrease as in the case of gelatin PEO and PVME. However, the $\rho_{\text{Na-citrate}}$ first goes down and then goes up again in a very pronounced manner.

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

The Effect of the Initial Polymer Concentration on The Water Contents and ρ-Values

Figures 8 and 9 show the water contents of ρ-values for Na^+ and Mg^{++} in PVP respectively at the initial polymer concentrations at 20% and 40%. The data indicates syneresis; much lower ρ-values and H_2O contents were observed in the polymer solution with a higher initial concentration.

The Effects of Temperature On The Water Content and ρ-values

Figures 10 and 11 show respectively the H_2O content and ρ-value for Na citrate in PVP at 4 different temperatures. Between 0° and $20^\circ C$, there was relatively little change in water contents but an even rise of ρ-values throughout the entire concentration range of Na citrate. In this respect, the curves resemble the behavior patters of gelatin shown in Figure 4. From 20° to $40^\circ C$, there was a rather abrupt fall in both water content and ρ-value at Na citrate concentrations near 0.4 M. However, at Na-citrate concentrations near 0.9 M, there was a reversal of the trend toward a higher water content and ρ-value, a trend becoming must pronounced at $60^\circ C$.

In the case of gelatin (Figures 12 and 13), the overall pattern appears simpler: The general trend was a rise of <u>both</u> water content and ρ-value with increasing temperature. However a minor downward shift in both was seen between 0° and $20^\circ C$ at the higher salt concentrations.

Quite the opposite trend is seen in the case of PVME (Gantrez). There was a gentle fall of both H_2O content and ρ-value for Na-concentrations (Figure 14) as well as for sucrose (Figure 15). However, between 25° and $35^\circ C$, there was a precipitous fall in both water content and ρ-values.

The Effect of To-and Fro-Shaking on the Water Contents of ρ-value of NaCitrate in PVP.

Figure 16 to 19 show the water contents and ρ-value of Na Citrate in PVP at two different temperatures and either shaken or quiescent. Shaking, which tends to line up the linear polymer in a parallel manner causes a decrease of water contents but much more pronounced decrease of ρ-value. A more limited demonstration of this shaking effect was presented in an earlier paper and its significance discussed (11).

The ρ-value of various Na salts, sucrose and glycine.

Figure 20 compares the ρ value of the 3 Na salts: NaCl, Na_2SO_4, and Na_3-citrate and glycine and sucrose in PVP. The ρ-value for the 3 Na salts are in the order NaCl>Na_2SO_4>Na_3-citrate. A similar comparison is made for the polymer, polyvinyl alcohol (PVA) which is different from the other synthetic polymers cited above in that PVA contains only H-donating groups (OH) rather than only H-accepting sites groups. The results are qualitatively similar (Fig. 21).

Even though NaCl is the least excluded, it nonetheless exercises similar thc much less effective onthe polymer water contents and on ρ-values. This is shown the case of methyl cellulose in Fig. 22.

The relation of ρ-value of a solute and its molecular size and complexity

Figure 22 shows that the ρ-value in PVME for the smallest hydroxylic compound in the list, methanol, is close to unity; it is intermediate for the sugars, xylose and sucrose; it becomes lower for labelled inulin (M.W. 3000 to 4000) but it is much lower for labelled polyethylene glycol (M.W. 4000).

Is water the only solvent whose solubility properties can be altered by polymers?

Figure 23 shows that PVP dissolved in a variety of 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 Na sulfate. Similarly PVP dissolved in DMSO and ethanol and in 50% DMSO and 50% H_2O have similar ρ-value to glycine. The only exception is PVP dissolved in a 50-50 mixture of water and ethanol. In the latter case ρ-glycine becomes nearly unity.

Discussion

I. Polar groups of a linear polymer, a basic requirement for the long-range effect on water solvency

A. Polarity: In the polarized multilayer theory of cell water, a matrix of more-or-less parallel array of extended polypeptide chains polarize the bulk of cell water. It is argued that the negatively charged C=O groups are the primary seats of the polarization of water but the positively charged NH groups offer significant polarizing effect on its own, augmenting the total polarizing effect. However, theoretically speaking, a matrix of chains carrying alternating negative and vacant sites (NO-NO-NOsystem) or positive and vacant sites (PO-PO-PO- system) should also be effective if the distance between the neighboring sites are correct. The fact that 3 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 $(CH_2\text{-}\underset{H}{N}\text{-}CH_2)_n$. In its place, however, we have $(CH_2\text{-}\underset{OH}{CH})_n$ i.e., polyvinyl alcohol (PVA). These proton-donating OH groups are also effective in polarizing water but less strongly as shown in the below unity p-values for Na-citrate and SO_4.

Thus, a regular array of properly spaced proton-accepting groups or properly spaced proton-donating groups can both have an 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 C=O group and proton accepting NH groups will have a synergistic effect and will offer under the proper conditions, more effective action on water solvency.

B. 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 alternatingly 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 orientations and hence cohesion due to the cooperative interaction of each water with all of its surrounding neighboring water molecules (11).

While detailed comparison of the distances separating polar groups of all the effective polymers, must await future study, it is interesting to note that three of the most effective polymers share the basic vinyl group (PVP, PVA and PVME) and at least 2 carbon atoms separate each neighboring oxygen atom in PEO and one carbon and nitrogen separates 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 poly(ethylene oxide) is highly soluble in water, closely related polymers are insoluble in water. The related, water insoluble species include the polymers of formaldehyde and acetaldehyde ..., trimethylene oxide, ... tetramethylene oxide ..." Thus a reduction of one or addition of one (or more) methylene groups between the oxygen atoms of PEO, have both the same effect, i.e., the elimination of its solubility in water altogether. Obviously a polymer with no solubility in water can have no effect on water solvency.

C. <u>The unmasked state of the polar groups</u>. In a preceding paper we have discussed at length both the theory and experimental evidence demonstrating that the effect of polymer like protein on water solvency can be completely annuled if its oppositely charged polar groups C = 0 and NH form intra- or intermolecular H-bonds among themselves. We have also shown that when these masked groups are unmasked, for exampke, by urea denaturation, the effect on water solvency is restored.

While special efforts must be spent to disassociate H-bonds between the NH and CO groups, in order to reveal the water solvency effect of proteins, no such effort is necessary for 4 of the most effective artificial polymers: gelatin, PEO, PVP, and PVME, the first because its large proline and hydroxyproline content prevents the formation of α-helical conformation, the other 3 simply have no positively charged

proton-donating groups and are unable to form inter- or intrachain H-bonds.

D. The dominant role of the C=0 group of a polypeptide chain in solvency-reducing action on water. A compariso. of the ρ-value for, say, Na-citrate in PVA-water system with PVP, PEO, and PVME-water systems under similar conditions leaves little doubt of the much greater effect of a carbonyl or ether oxygen than the hydroxyl groups 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 example, that the vapor pressure of aqueous acetamide is little effected by substitution of methyl groups for proton on nitrogen. He argued the "interaction of water with carbonyl groups (rather than with the N-H protons) is mainly responsible for the hydrophobic character of the acetamide". This finding has great importance for another concept introduced in the AI hypothesis: the H-bonding strength of the polypeptide chains with the electron-donating or withdrawing effect of the side chains (5).

II. The alignment in a matrix of extended polypeptide chains and models.

That to and from agitation of a PVP-water system produces an enhancement of the ability of the system to exclude Na-citrate supports the expectation that the maximum effect on water solvency if the proper chains are arrayed in parallel as we have pointed out in an earlier paper (15). In the same light perhaps one can explain the much more profound solvency effect of methylcellulose than the large majority of natural gums and polysacchaides. It is well known that cellulose molecules represent straight chains. The interposition of methyl or other groups separates these chains, thereby presenting a regular arrays of water polarizing chains. By comparing with PEO, PVME, and PVP, one is inclined to think that in methyl cellulose the ether oxygen on the glucose ring must play a major role in polarizing water.

III. Molecular size and complexity and their q-value.

The AI hypothesis offers two mechanisms for solute exclusion from water polarized

in ultilayers: an enthalpic mechanism and an entropic mechanism (10). In both the predicted q-value decreases with increasing size and complexity of the probe molecules in question as seen in the ρ-value for NaCl, Na_2SO_4, and Na_3-citrate where the major determing factor appears to be the anion. Similarly the data shown in Fig. 22 also support this theory. It is also significant to point out roughly the same sequential order of preference for this series of hydroxylic compound is seen in living cells (15).

IV. Structure-breaking and structure-altering effects of high concentrations of Na_2SO_4 or Na_3-citrate.

Figure 5 shows that as the concentration of Na-citrate increases, the ρ-value for Na-citrate in methyl-cellulose water system decreases and then increases. A simple interpretation for the secondary rise is the structure breaking action of the Na-citrate at high concentration. However, a closer look suggests that the situation may be more complex. Thus after the secondary rise, the ρ-value does not continue to rise until it reaches complete destruction of water structure, with ρ = 1.0. Actually the ρ-value levels off at a ρ-value considerably below 1.0 and sometimes bends down again.

A similar sign of complex effect of high citrate concentration is seen in the study of the ρ-values for 4 different probe molecules in PVME (Fig. 6). Here, the ρ-value for Mg steadily increases from its lowest point at 0.2 M citrate. A comparison with the ρ-values of other probe molecules in the same samples shows, however, the point of minimum as well as maximum ρ-value varies from one probe molecule to another, arguing again against a general breakdown of water structure, but suggests the assumption of a somewhat altered water structure.

Summary

We studied the properties of polymer-oriented water which excludes Na^+, sucrose and free amino acids and other solutes found in low levels in many living cells. Altogether 23 polymers were investigated, of which 15 are natural gums and polysaccharides and are synthetic. More intensive studies were made on one natural (gelatin) and 4

synthetic polymers (methyl cellulose, polyvinylpyrrolidone, poly(vinyl methyl ether) and poly(ethelene oxide). With equilibrium dialysis methods, we determined the apparent equilibrium distribution coefficients or ρ-values of Na^+ (as sulfate and citrate), sucrose and glycine, etc. between the water in the polymer-water filled dialysis sacs and water in the external bathing solutions. We studied the effects on the water contents in the polymer-water system of the ρ-values for Na^+, etc., of the following variables: 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 overall observations are in general accord with the polarized multilayer theory of bulk-phase cell water, a part of the association-induction hypothesis.

Acknowledgements

We thank Kelco Co., Clark, N.J.; GAF Corporation, New York; Dow Chemical Co., Coral Gables, Fla.; and Union Carbide, New York 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-R01-CA16301-03-04.

References

1. Pfeffer, W. "Osmotische Untersuchungen," (2nd ed.), Engelmann, Leipzig, 1921.

2. Dean, R. Biol. Symp. 3:331, 1941.

3. Glynn, I. M. and Karlish, S. J. D. Ann. Rev. Physiol. 37:13, 1975.

4. Ling, G. N. "A Physical Theory of the Living State: The Association-Induction Hypothesis", Blaisdell, New York, 1962.

5. Ling, G. N. Inter. Rev. Cytology 26:1, 1969.

6. Ling, G. N. Molec. & Cell Biochem 15:159. 1977.

7. Ling. G. N. in "Water and Aqueous Solutions", ed. R. A. Horne, Wiley-Interscience, New York, 1972.

8. Ling, G. N. in"Water Studies at Their Water-Polymer Interface", ed. H. H. Jellinek, Plenum Press, New York, 1972.

9. Ling, G. N. Ann. N.Y. Acad. Sci. 125:401, 1965.

10. Ling, G. N. and Sobel, A. M. Phsiol. Chem. Phys. 7:415, 1975.

11. Ling, G. N., Ochsenfeld, M. M., Walton, C.L., and Bersinger, T. J. (Submitted to Science).

12. Ling, G. N. Fed. Proc. Symp. 24: S103, 1965.

13. Stone, F. W. and Stratta, J. J. Encyc. Polym. Sci. & Techn. 6: 103, 1967.

14. Wolfenden, R. Biochem. 17:201, 1978.

15. Ling, G. N., Miller, C., and Ochsenfeld, M. M. Ann. N.Y. Acad. Sci. 204:6, 1973.

Legends

Figure 1 - Time course of ^{22}Na uptake by several polymers:

A. Polyvinylpyrrolidone (PVP)(A)

B. Poly(vinyl methyl ether) (B)

C. Gelatin (C)

The initial polymer concentrations in the sacs were 40%, 50%, and 40%.

Figure 2 - Time course of labelled Na efflux from gelatin slices (25°C). Dry gelatin was dissolved in 1.5 volume of 400 mM ^{22}Na-labelled Na Citrate washed in 1.0 M Na Citrate.

Figure 3 - Time course of labelled 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.

Figure 4 - ρ-values of Na and H_2O contents of gelatin in various concentrations of Na_2SO_4 and Na Citrate (0°C). Dissolved gelatin (30%) were in dialysis tubing sacs.

Figure 5 - ρ-values of Na and H_2O contents in poly(ethylene oxide) (Polyox 205) in various concentrations of Na Citrate (25°C). Initial concentration of polymer was 40%.

Figure 6 - The ρ-values of Na, Mg, glycine, and sucrose and H_2O contents of poly(vinyl methyl ether) in various concentrations of Na_2SO_4 (25°C). Initial polymer concentration was 50%.

Figure 7 - The ρ-values of Na and H_2O contents of methyl cellulose (DOW A4M) in varying concentrations of Na citrate. Initial methyl cellulose concentration was 10%.

Figure 8 - The ρ-values of Na and Mg water contents of polyvinylpurrolidone in varying concentrations of Na_2SO_4 (25°C). Initial polymer concentration was 20%.

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

Figure 10 - The water contents of polyvinylpyrrolidone in varying concentrations of Na Citrate ard 4 different temperatures. Initial polymer concentration was 20%.

Figure 11 - The ρ-value of Na of Polyvinylpurrolidone in varying concentrations of Na Citrate and 4 different temperatures. Same experiment as that shown in Figure 10.

Figure 12 - The water contents of gelatin in varying concentrations of Na Citrate and 4 different temperatures. Initial gelatin concentration was 30%.

Figure 13 - The ρ-value of Na of gelatin in varying concentrations of Na Citrate and 4 different temperatures. Same experiment as that shown in Figure 12.

Figure 14 - The ρ-values of Na and H_2O contents of poly(vinyl methyl 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.

Figure 15 - The ρ-value of sucrose and H_2O contents of poly(vinyl methyl ether) Gantrez at different temperatures. Initial polymer concentration was 50%. The incubation solution contained 100 mM Na Citrate and 100 mM sucrose. Incubation lasted 23 days.

Figure 16 - The H_2O contents of quiescent and shaken sacs of polyvinylpyrrolidone solutions in varying concentrations of Na Citrate ($0^{\circ}C$). The initial polymer concentration was 20%.

Figure 17 - The ρ-values of Na in quiescent and shaken sacs of polyvinylpyrrolidone solutions in varying concentrations of Na Citrate ($0^{\circ}C$). Same experiment as that shown in Figure 16.

Figure 18 - The H_2O contents of quiescent and shaken sacs of polyvinylpyrrolidone solution in varying concentrations of Na Citrate ($20^{\circ}C$). The initial polymer concentration was 20%.

Figure 19 - The ρ-values of Na in quiescent and shaken sacs of polyvinylpyrrolidone solutions in varying concentrations of Na Citrate ($25^{\circ}C$). Experiment in the same as shown in Figure 18.

Figure 20 - The H_2O contents and ρ-values of Na Citrate, Na_2SO_4, NaCl, sucrose and glycine in solutions of poly(vinyl-methyl ether) (25°C). The incubation solutions contained respectively 100 mM of each of the following Na Citrate, Na_2SO_4, NaCl, sucrose, and glycine. The widths of horizontal lines at the end of each bar represent 2 X standard error.

Figure 21 - The H_2O contents and ρ-values of Na Citrate, Na_2SO_4, NaCl, sucrose and glycine in solutions of polyvinyl alcohol. The incubation solutions were the same as described in the legend for Figure 20. Distance between horizontal bars represent 2 X standard error.

Figure 22 - The water contents and ρ-values of alcohol, sugars and other compounds in poly(vinyl-methyl ether) solutions (25°C). Initial polymer concentration was 50%. All incubation contained 100 mM Na_2SO_4 plus 10 mM of each the compounds presented except inulin and polyethylene glycol in which cases only tracer amount of labelled materials were added. Incubation lasted 4 days.

Figure 23 - The ρ-value of Na and H_2O contents in methyl cellulose-H_2O system (25°C) in varying concentration of NaCl. Initial methylcellulose concentration was 10%. Incubation lasted 4 days.

Figure 24 - The effect of variations of the solvent upon the ρ-value of Na and that of glycine in polyvinylpyrrolidone solution (25°C). Initial polymer concentration was 20%. Distances between the two horizontal bars at the end of each bar represents 2 X S.E. Incubation lasted 3 days.

Table 1 - The H_2O contents and ρ-values of 9 amino acids, 4 sugars and 4 other physiologically interesting compounds in the water of a polyvinylpyrrolidone solution (25°C). Initial concentration of the polymer was 20%. Bathing solution contained 1.5 M Na-Citrate and 10 mM of each of the compounds whose distribution were studied.

Table 2 - The H_2O contents and ρ-values of Na, Mg, sucrose, and glycine in gums, polysacchaides, and synthetic polymers.

	Conc. Na-citr (M)	Conc. Na_2SO_4 (M)	Na-citr	Na_2SO_4	$MgSO_4$	sucrose	glycine
Agarose		0.1		$0.800^{+}_{-}.042$ (76.4%)	$0.702^{+}_{-}.071$ (76.4%)	$0.608^{+}_{-}.084$ (76.4%)	$0.722^{+}_{-}.017$ (76.4%)
Alginic Acid	1.5		$1.06^{+}_{-}.011$ (63.0%)				
	0.5		$1.20^{+}_{-}.005$ (82.4%)				
		0.1		$2.023^{+}_{-}.034$ (75.8%)	$2.59^{+}_{-}.076$ (75.8%)	$0.809^{+}_{-}.015$ (75.8%)	$1.343^{+}_{-}.028$ (75.8%)
Carragenan	1.5		$0.921^{+}_{-}.004$ (71.6%)				
	0.5		$0.832^{+}_{-}.020$ (85.9%)				
		0.1		$1.107^{+}_{-}.004$ (96.6%)	$1.326^{+}_{-}.070$ (96.6%)	$0.829^{+}_{-}.019$ (96.6%)	$0.872^{+}_{-}.039$ (96.6%)
Glycogen		0.1		$0.755^{+}_{-}.075$ (65.2%)	$0.560^{+}_{-}.051$ (65.2%)	$0.709^{+}_{-}.064$ (65.2%)	$0.725^{+}_{-}.069$ (65.2%)
Pectin	1.5		$0.928^{+}_{-}.008$ (65.3%)				
	0.5		$1.01^{+}_{-}.025$ (81.6%)				
		0.1		$1.432^{+}_{-}.074$ (84.2%)	$1.718^{+}_{-}.077$ (84.2%)	$0.849^{+}_{-}.068$ (84.2%)	$0.856^{+}_{-}0.100$ (84.2%)
Starch (Corn)	1.5		$0.836^{+}_{-}.006$ (49.5%)				
	0.5		$0.924^{+}_{-}0.004$ (72.5%)				
		0.1		$0.918^{+}_{-}.003$ (79.6%)	$0.901^{+}_{-}.013$ (79.6%)	$0.794^{+}_{-}.027$ (78.6%)	$0.754^{+}_{-}.009$ (78.6%)
Starch (Potato)	1.5		$0.852^{+}_{-}0.006$ (44.3%)				
	0.5		$0.905^{+}_{-}0.003$ (70.8%)				
		0.1		$0.926^{+}_{-}.010$ (79.2%)	$0.768^{+}_{-}.020$ (79.2%)	$0.848^{+}_{-}.003$ (78.3%)	$0.947^{+}_{-}.04$ (78.3%)

	Conc. Na-citr (M)	Conc. Na_2SO_4 (M)	$\rho_{Na\text{-}citr}$	$\rho_{Na_2SO_4}$	ρ_{MgSO_4}	$\rho_{sucrose}$	$\rho_{glycine}$
Gum arabic	1.5		0.850±.003 (64.1%)				
	0.5		0.906±.004 (78.2%)				
		0.1		1.324±.084 (83.6%)	1.493±.114 (83.6%)	0.824±.039 (83.6%)	0.857±.036 (83.6%)
Gum ghatti	1.5		0.855±.003 (66.3%)				
	0.5		0.936±.004 (83.1%)				
		0.1		1.206±.064 (84.7%)	1.531±.072 (84.7%)	0.749±.055 (85.8%)	0.915±.030 (85.8%)
Gum guar	1.5		0.919±.003 (64.9%)				
	0.5		0.950±.003 (81.3%)				
		0.1		0.959±.002 (83.7%)	0.953±0.107 (83.7%)	0.864±.027 (83.7%)	0.877±.047 (83.7%)
Gum karaya	1.5		0.834±.007 (67.9%)				
	0.5		0.960±.004 (84.9%)				
		0.1		1.590±.023 (86.7%)	2.335±.092 (86.7%)	0.650±.075 (85.5%)	0.845±.053 (85.5%)
Gum locust bean	1.5		1.06±.008 (61.3%)				
	0.5		0.960±.004 (83.0%)				
		0.1		0.945±.004 (83.5%)	0.957±.050 (83.5%)	0.823±.034 (86.0%)	0.914±.027 (86.0%)
Gum tragacanth	1.5		0.887±.003 (69.7%)				
	0.5		0.940±.003 (85.4%)				
		0.1				0.855±0.012 (87.4%)	0.909±.003 (87.4%)
Gum xantham	1.5		0.900±.003 (69.2%)				
	0.5		0.961±.004				

	Conc. Na-citr. (M)	Conc. Na_2SO_4	$\rho_{Na\text{-}citr}$	$\rho_{Na_2SO_4}$	ρ_{MgSO_4}	$\rho_{sucrose}$	$\rho_{glycine}$
Carbopole 940		0.1		$1.249 \pm .029$ (76.3%)	$1.057 \pm .031$ (76.3%)	$0.773 \pm .011$ (76.3%)	$0.999 \pm .164$ (76.3%)
Dextran 170	1.5		$0.889 \pm .032$ (62.9%)				
	0.5		$0.929 \pm .018$ (77.4%)				
		0.1		$0.882 \pm .007$ (79.5%)	$0.976 \pm .105$ (79.5%)	$0.791 \pm .02$ (81.8%)	$0.841 \pm .017$ (81.8%)
Gantrez M154		0.1		$0.294 \pm .024$ (74.4%)	$0.204 \pm .021$ (74.4%)	$0.524 \pm .033$ (74.4%)	$0.463 \pm .027$ (74.4%)
Kelzan		0.1	$1.602 \pm .02$ (84.4%)		$2.134 \pm .105$ (84.4%)	$0.846 \pm .022$ (84.4%)	$0.925 \pm .020$ (84.4%)
Methyl cellulose	1.5		$0.402 \pm .032$ (31.5%)				
	0.5		$0.452 \pm .012$ (66.0%)				
		0.1		$0.689 \pm .008$ (83.4%)	0.763 ± 0.120 (83.4%)	$0.769 \pm .014$ (83.4%)	$0.653 \pm .028$ (83.4%)
Polyox 205		0.1		$0.584 \pm .005$ (94.3%)	$0.172 \pm .046$ (94.3%)	$0.762 \pm .028$ (94.3%)	$0.730 \pm .017$ (94.3%)
Polyvinyl alcohol		0.1		$0.762 \pm .009$ (78.9%)	$0.772 \pm .027$ (78.9%)	$0.952 \pm .023$ (78.9%)	$0.625 \pm .011$ (78.9%)
Polyvinylpyrrolidone		0.1		$0.644 \pm .007$ (81.3%)	$0.555 \pm .088$ (81.3%)	$0.914 \pm .010$ (81.3%)	$0.792 \pm .017$ (81.3%)

TABLE 2C

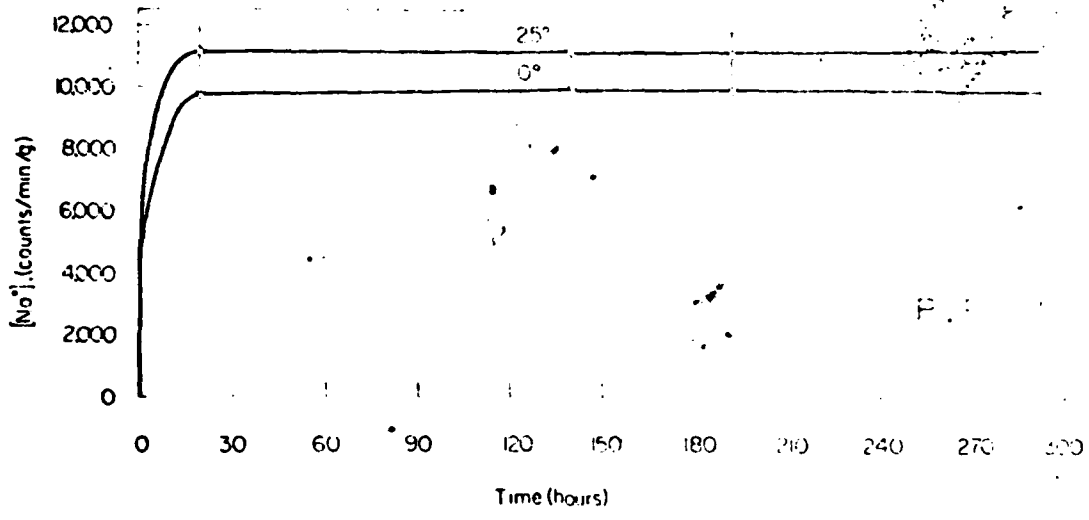

12,000
10,000
8,000
6,000
4,000
2,000
0
25°
0°
[Na+], (counts/min/g)
0
30
60
90
120
150
180
210
240
270
300
Time (hours)

FIGURE 1A

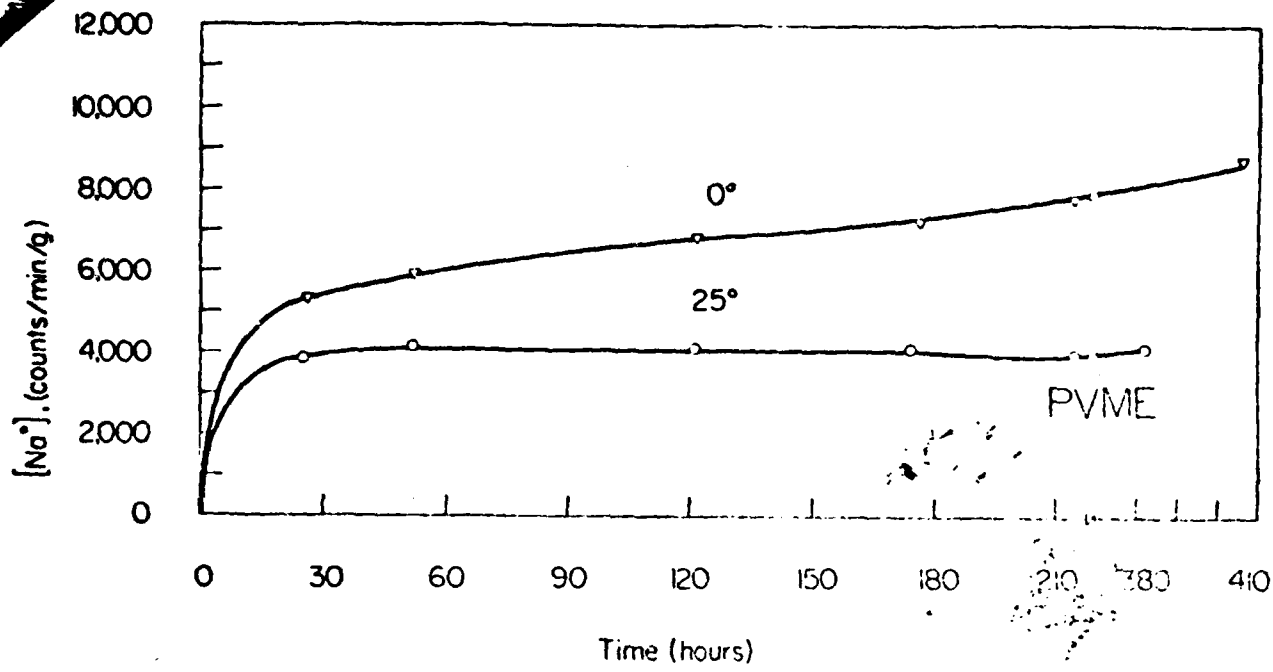


FIGURE 1B

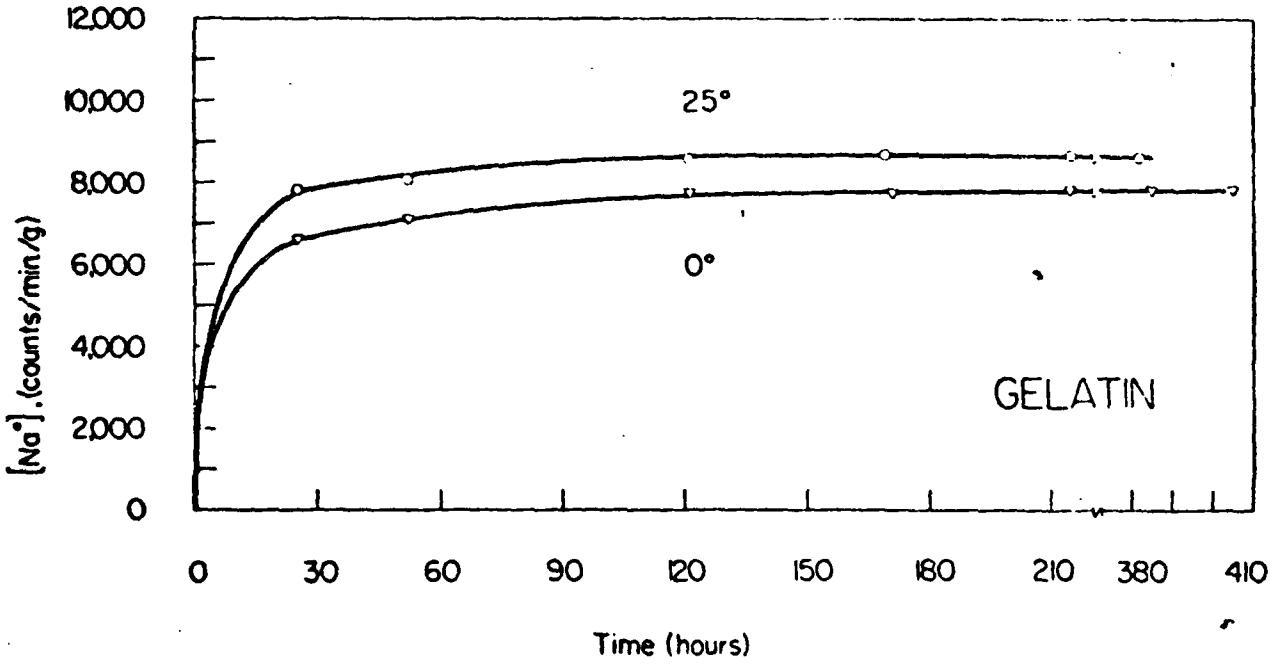


FIGURE 1C

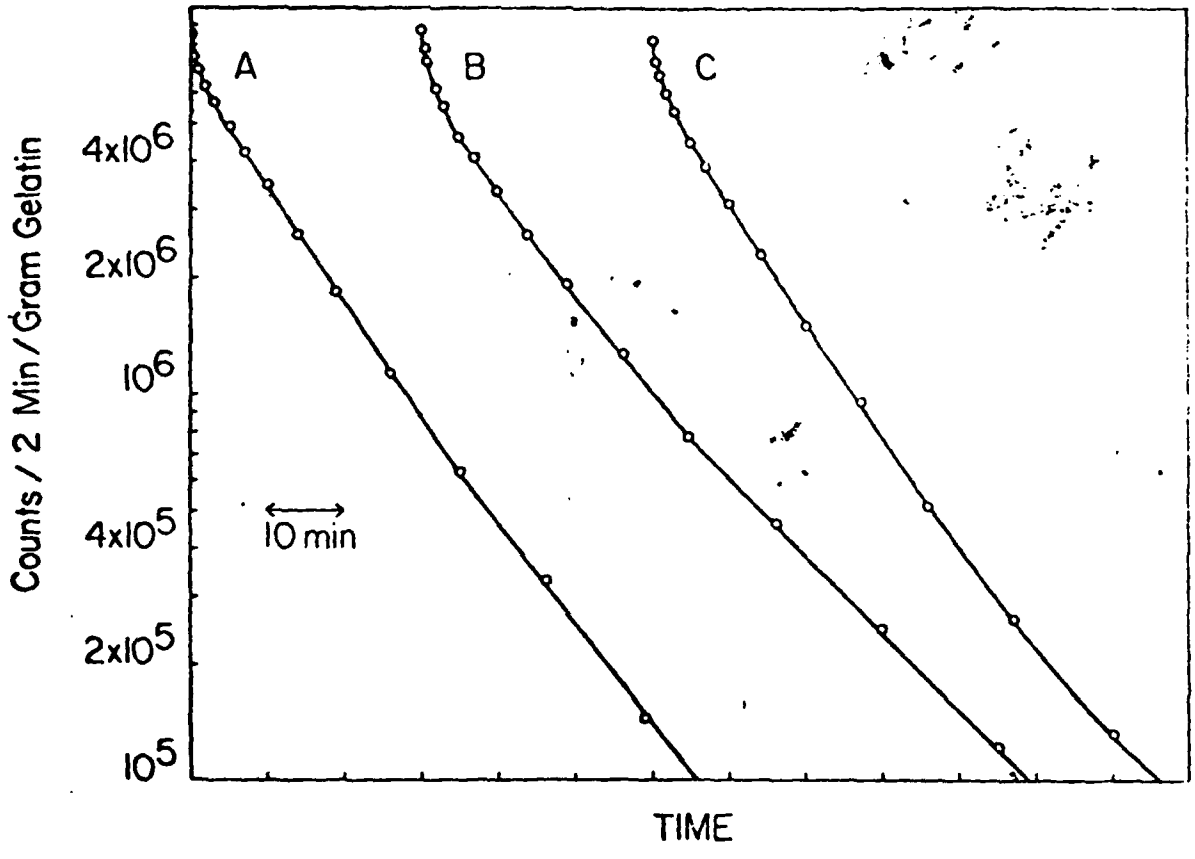

A
B
C
4x10^6
2x10^6
10^6
4x10^5
2x10^5
10^5
Counts / 2 Min / Gram Gelatin
10 min
TIME

FIGURE 2

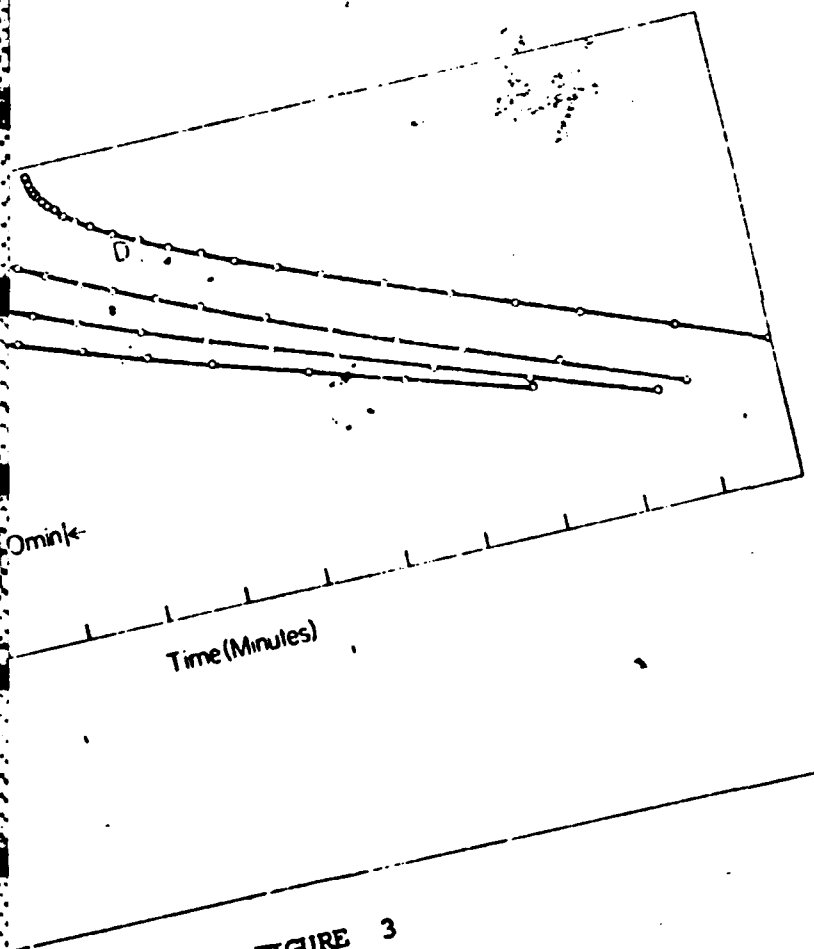


FIGURE 3

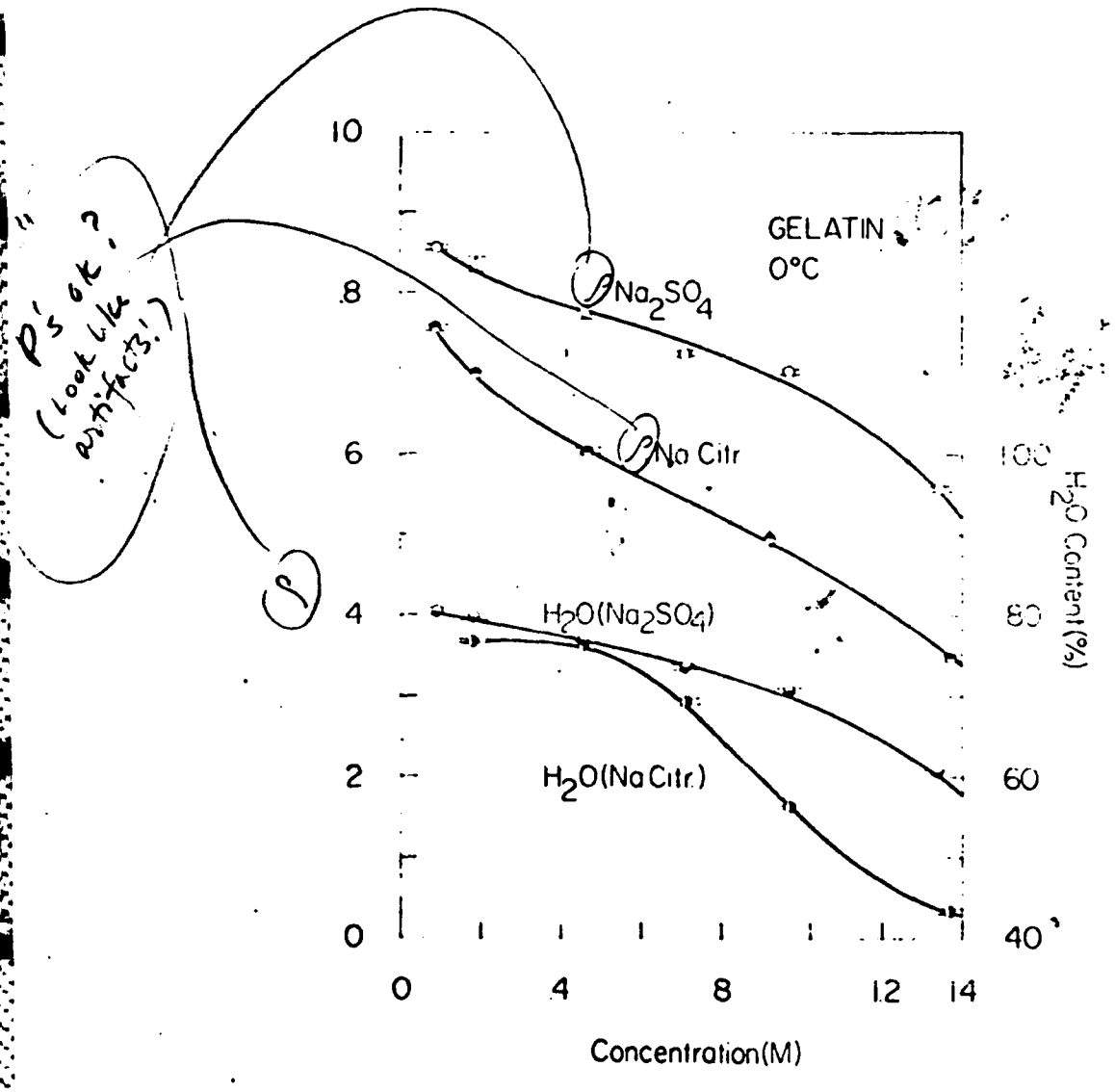

GELATIN
0°C
Na_2SO_4
Na Citr
$H_2O(Na_2SO_4)$
H_2O(Na Citr.)
H_2O Content(%)
Concentration(M)
0
2
4
6
8
10
40
60
80
100
0
4
8
12
14

FIGURE 4

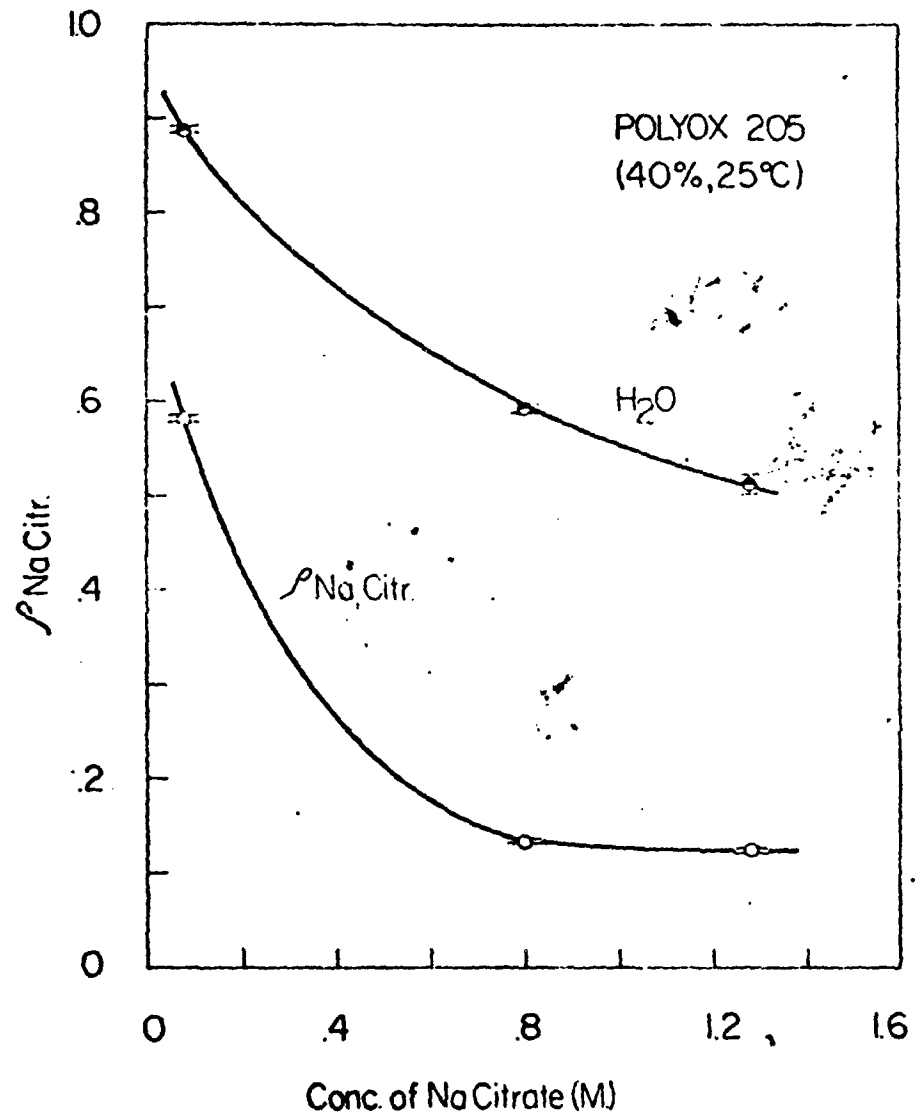

1.0
.8
.6
.4
.2
0
POLYOX 205
(40%, 25°C)
H2O
ρ Na Citr.
ρ Na Citr.
0
.4
.8
1.2
1.6
Conc. of Na Citrate (M)

FIGURE 5

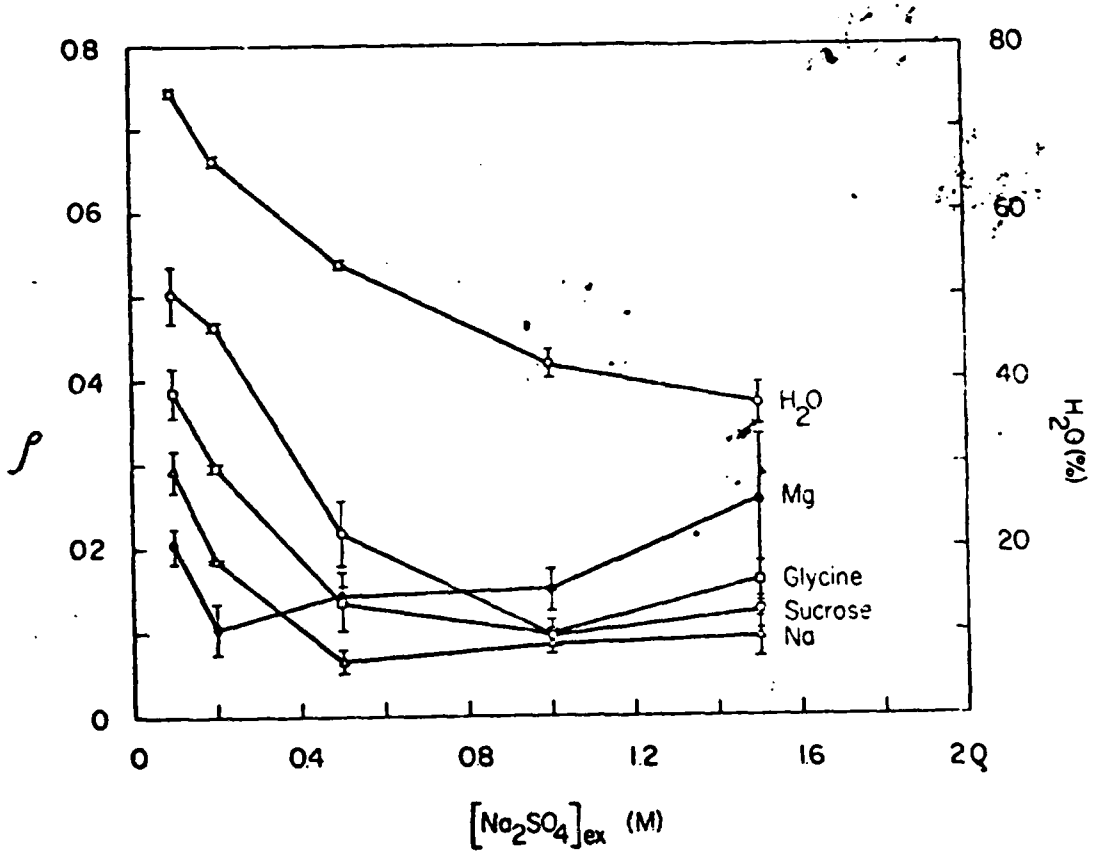

08
06
04
02
0
80
60
40
20
ρ
H2O (%)
H2O
Mg
Glycine
Sucrose
Na
0
0.4
0.8
1.2
1.6
2.0
[Na2SO4]ex (M)

FIGURE 6

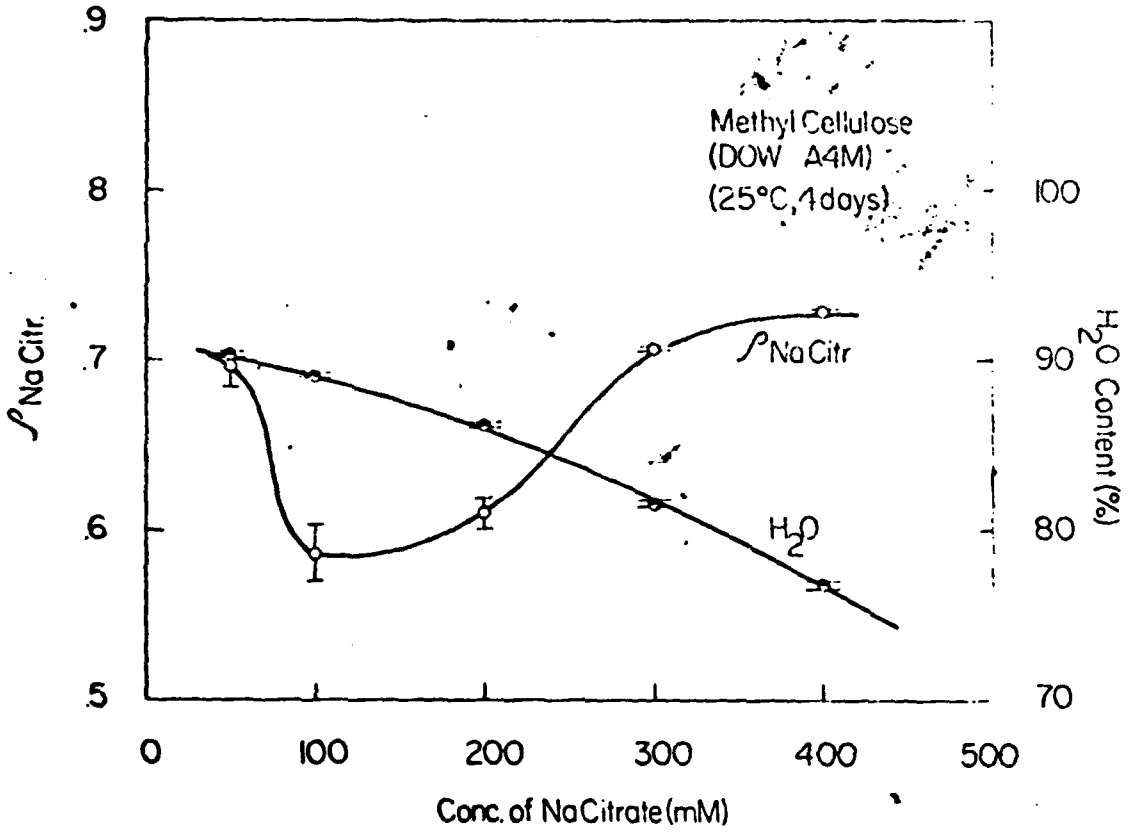

Methyl Cellulose
(DOW A4M)
(25°C, 1 days)
ρ Na Citr.
ρ Na Citr
H2O
H2O Content (%)
.9
.8
.7
.6
.5
100
90
80
70
0
100
200
300
400
500
Conc. of Na Citrate (mM)

FIGURE 7

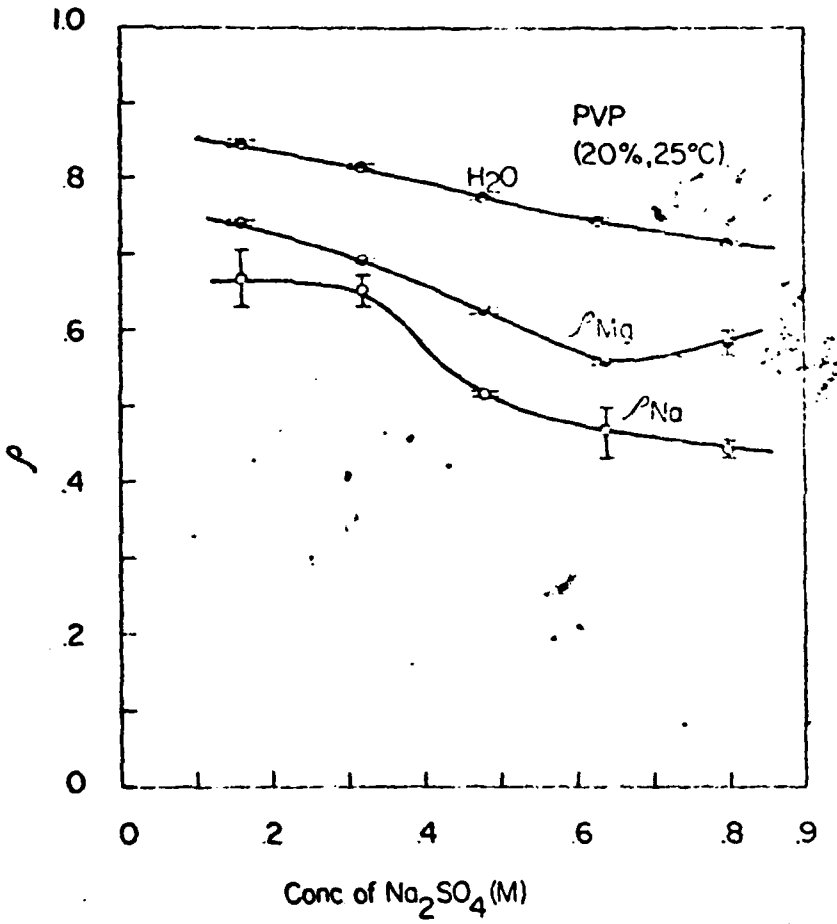


FIGURE 8

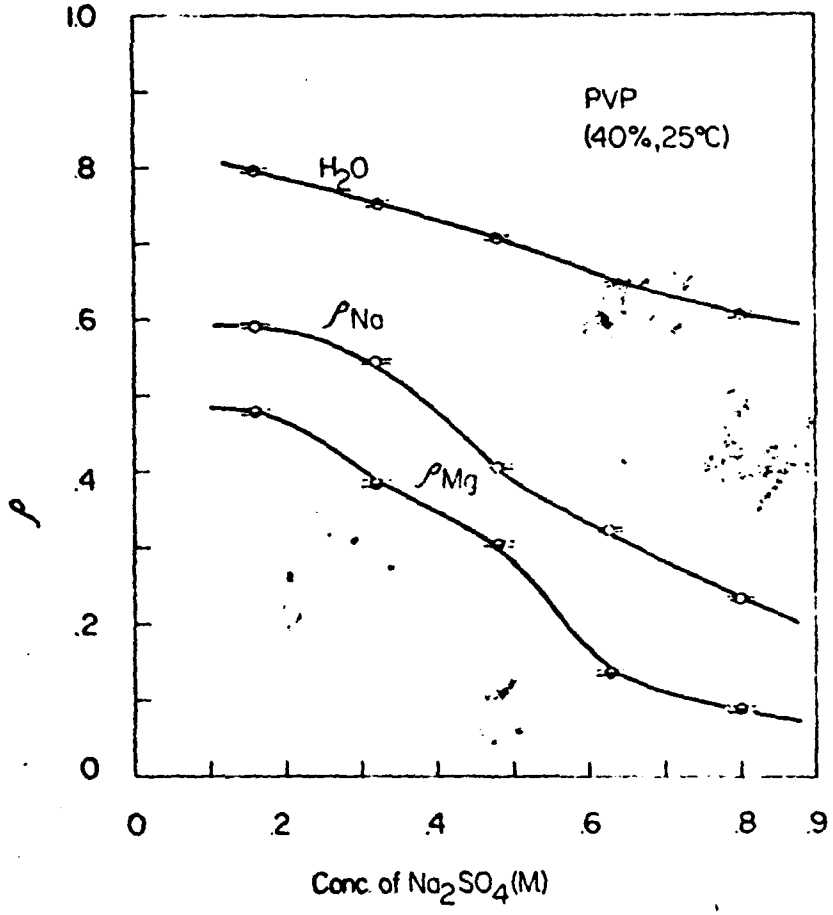

1.0
.8
.6
.4
.2
0
PVP
(40%,25°C)
H_2O
ρ_{Na}
ρ_{Mg}
ρ
0
.2
.4
.6
.8
.9
Conc. of Na_2SO_4(M)

FIGURE 9

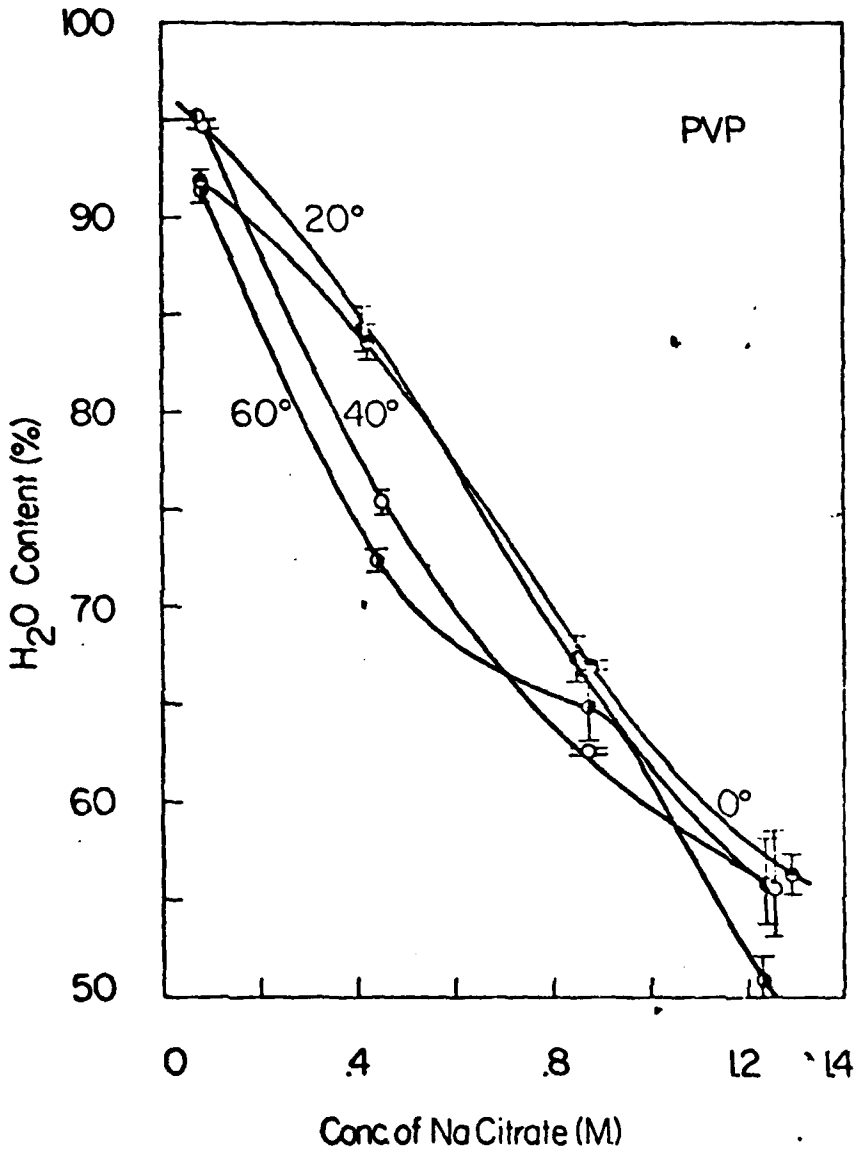

PVP
20°
40°
60°
0°
100
90
80
70
60
50
H_2O Content (%)
0
.4
.8
1.2
1.4
Conc of Na Citrate (M)

FIGURE 10

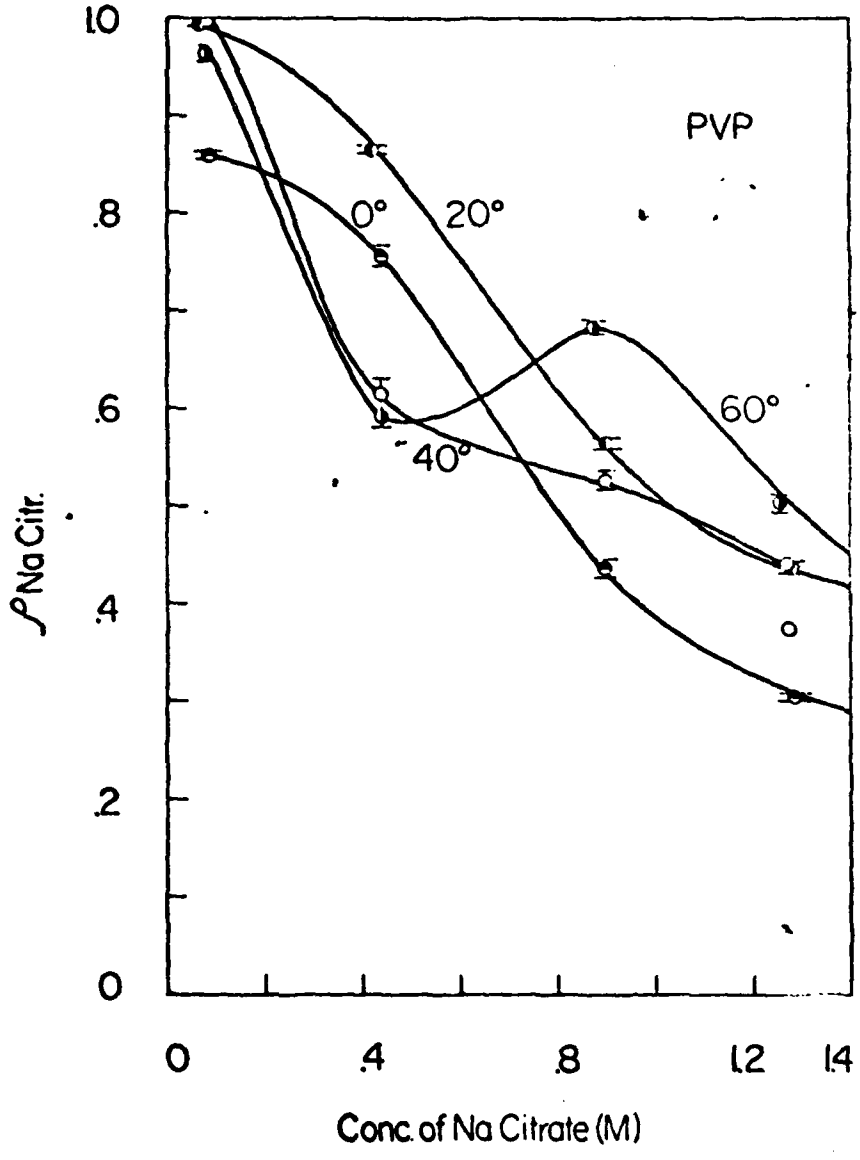


FIGURE 11

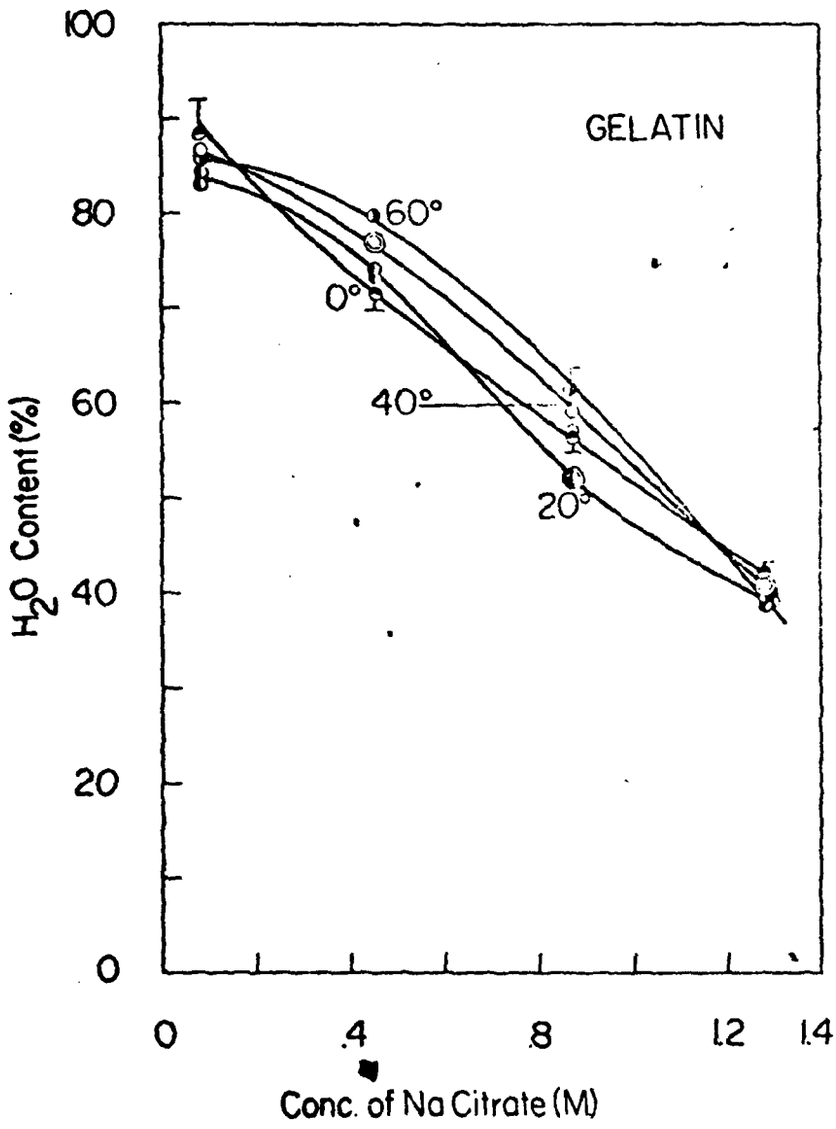

GELATIN
60°
0°
40°
20°
100
80
60
40
20
0
H_2O Content (%)
0
.4
.8
1.2
1.4
Conc. of Na Citrate (M)

FIGURE 12

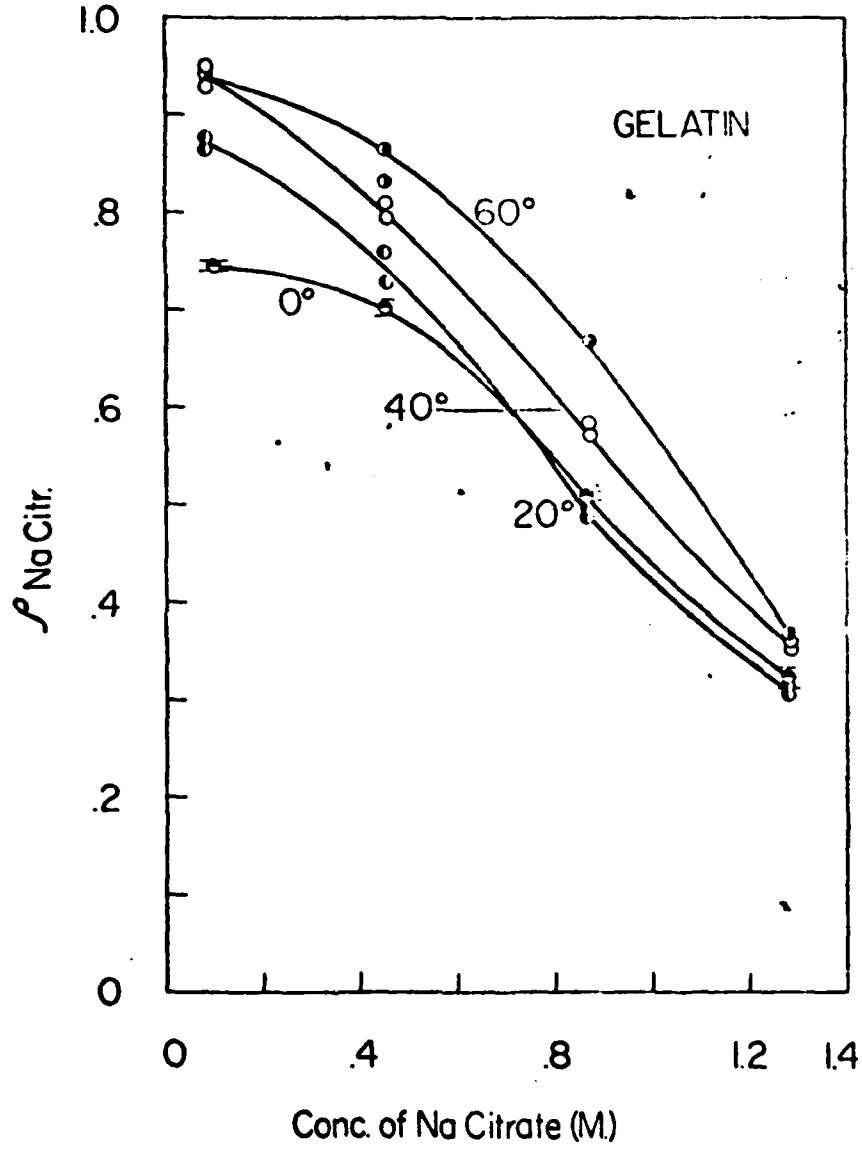

GELATIN
60°
0°
40°
20°
1.0
.8
.6
.4
.2
0
0
.4
.8
1.2
1.4
ρ Na Citr.
Conc. of Na Citrate (M.)

FIGURE 13

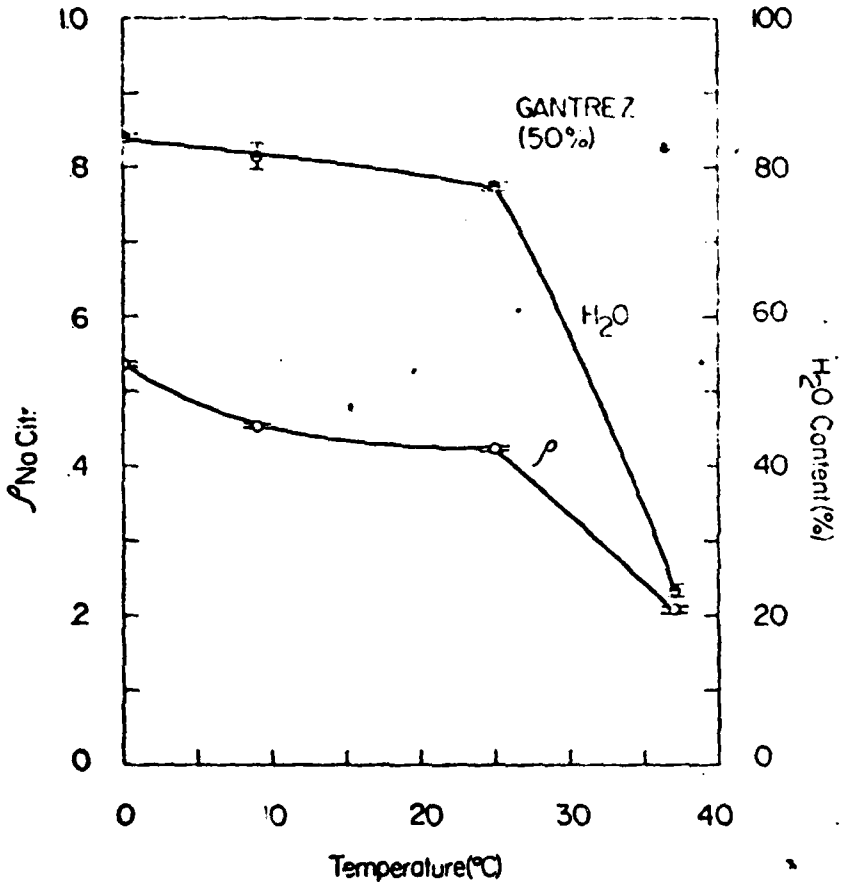

GANTREZ
(50%)
H_2O
ρ
ρ No Cit
H_2O Content (%)
Temperature(°C)
0
2
4
6
8
10
0
20
40
60
80
100
0
10
20
30
40

FIGURE 14

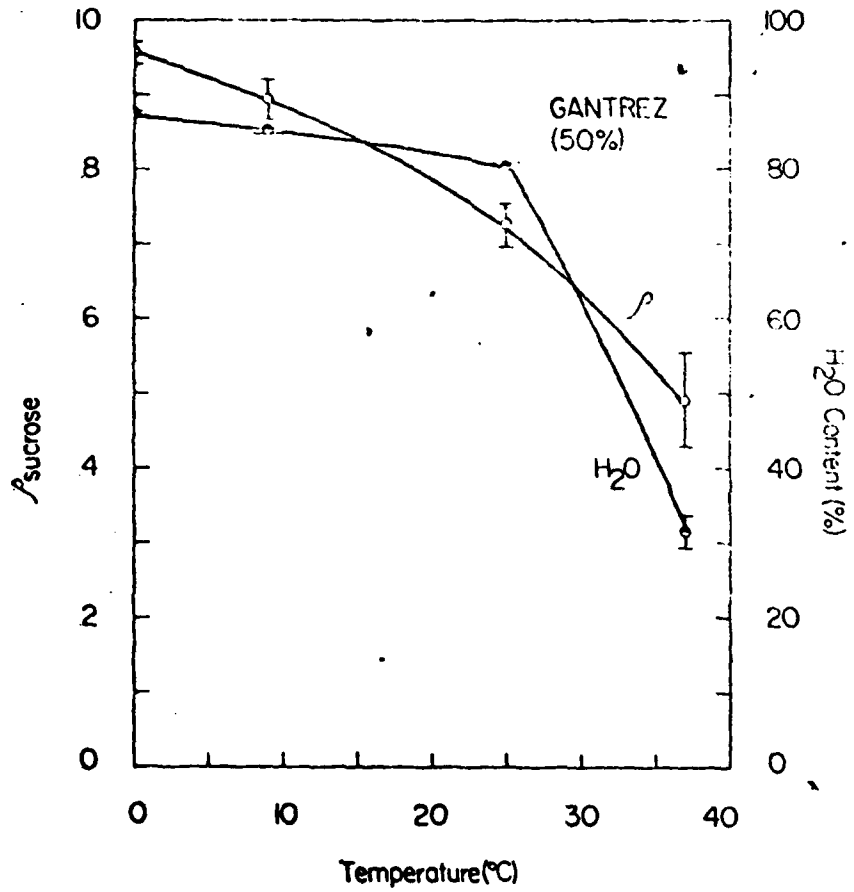

GANTREZ
(50%)
ρ
H2O
ρsucrose
H2O Content (%)
Temperature(°C)
0
2
4
6
8
10
0
20
40
60
80
100
0
10
20
30
40

FIGURE 15

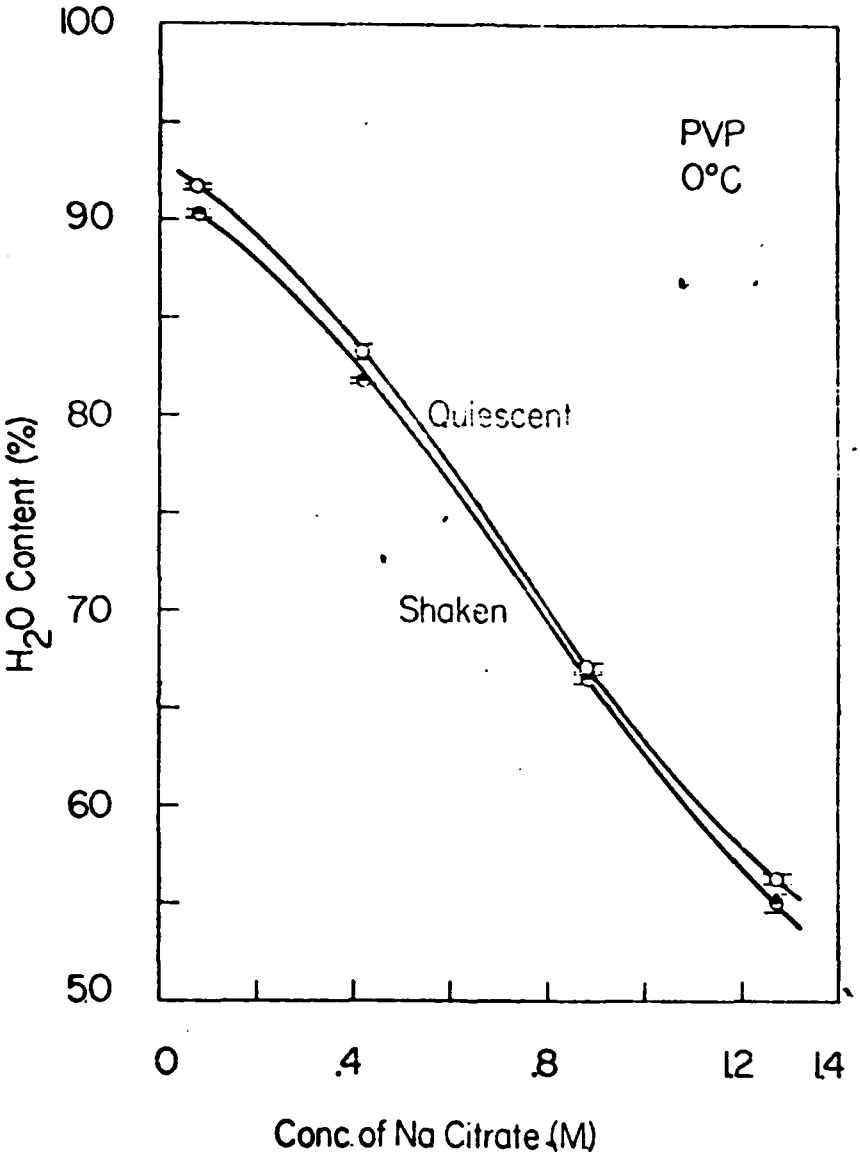

100
90
80
70
60
50
H_2O Content (%)
PVP
0°C
Quiescent
Shaken
0
.4
.8
1.2
1.4
Conc. of Na Citrate (M)

FIGURE 16

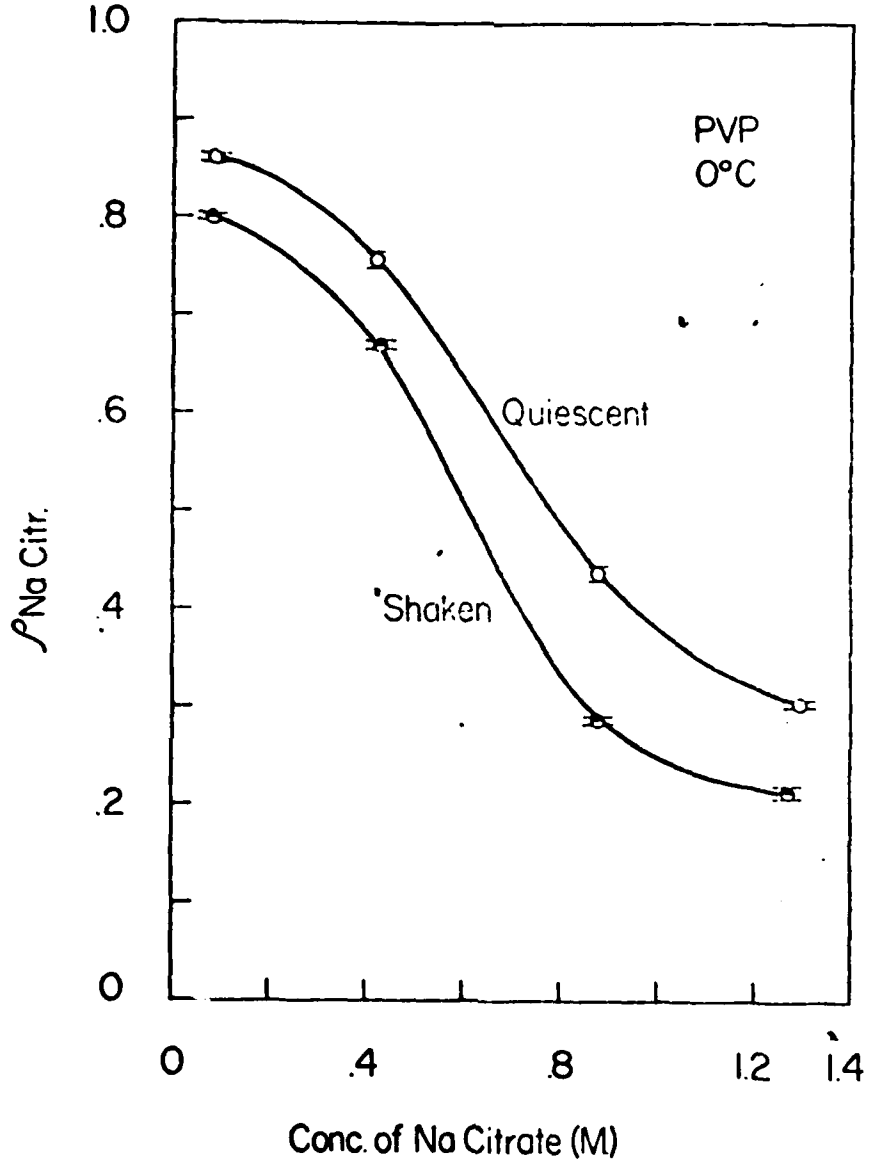

1.0
.8
.6
.4
.2
0
PVP
0°C
Quiescent
Shaken
ρNa Citr.
0
.4
.8
1.2
1.4
Conc. of Na Citrate (M)

FIGURE 17

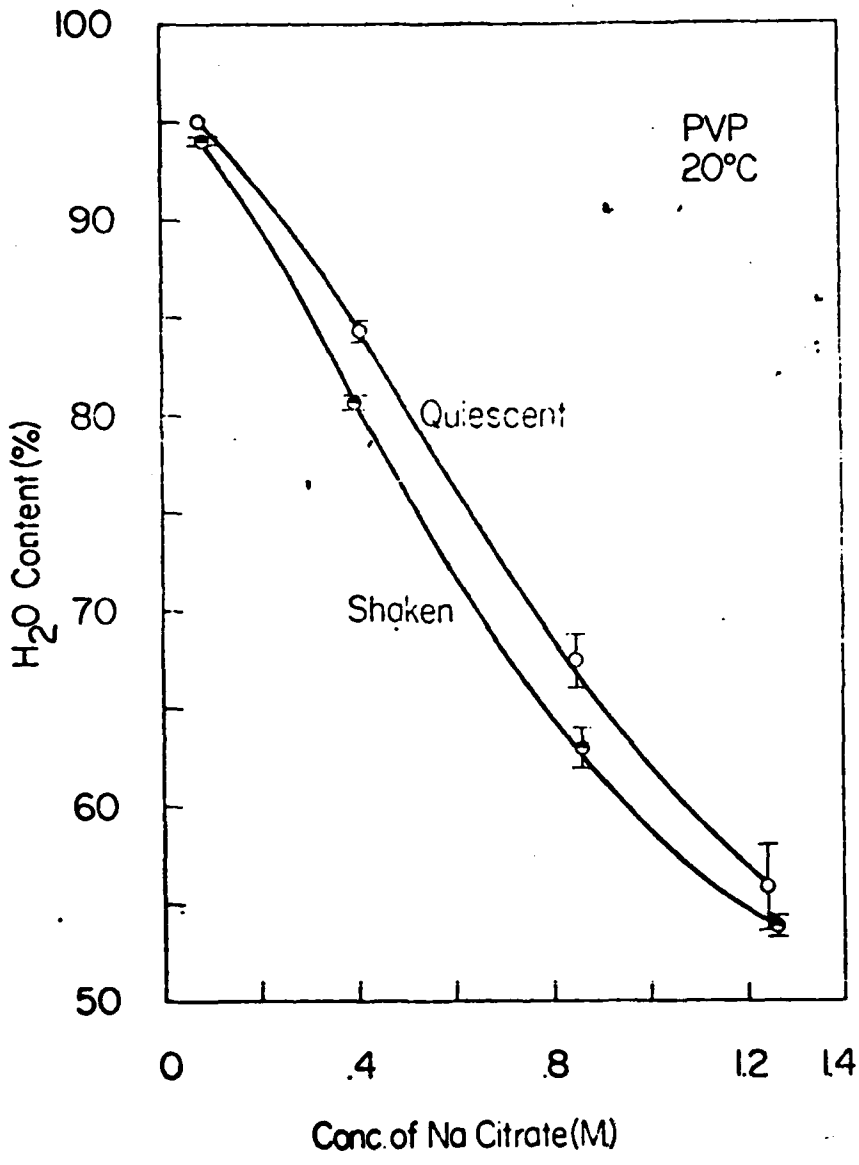

100
90
80
70
60
50
H_2O Content (%)
PVP
20°C
Quiescent
Shaken
0
.4
.8
1.2
1.4
Conc. of Na Citrate (M)

FIGURE 18

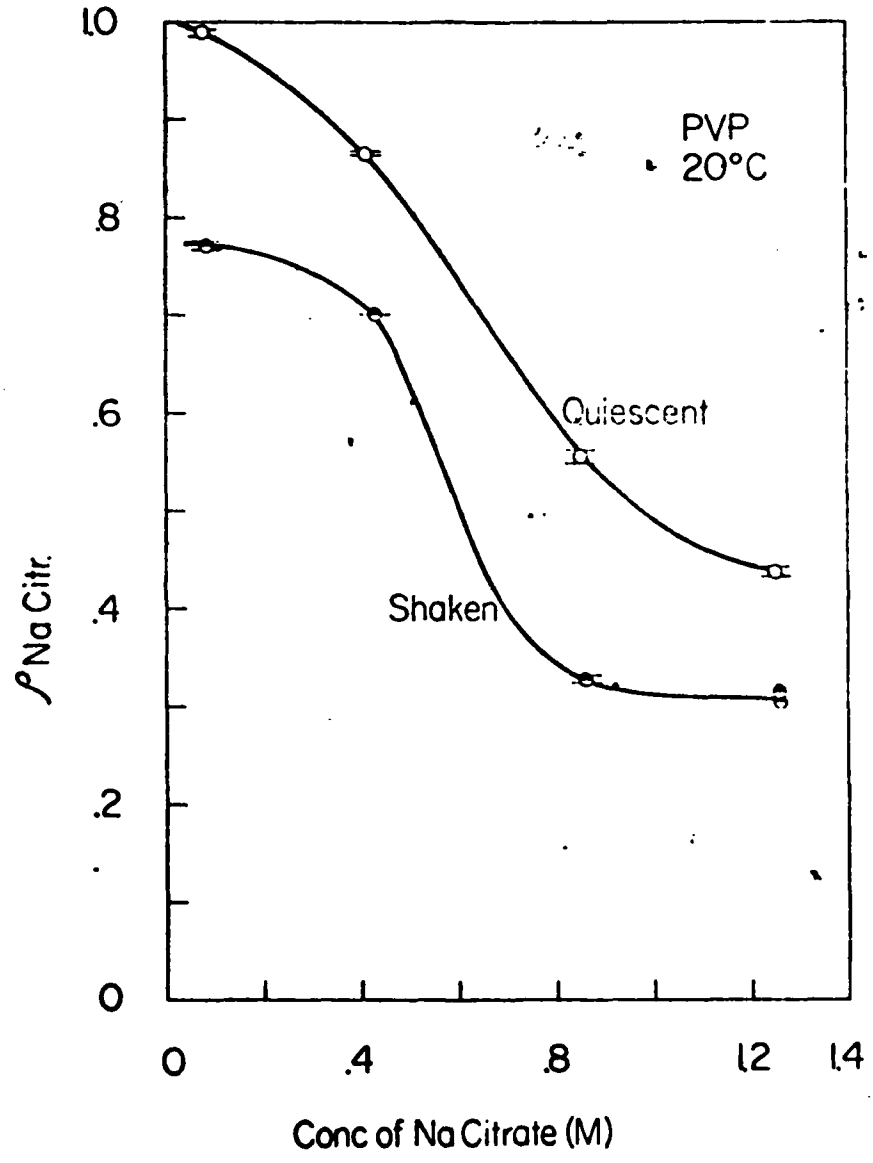

1.0
.8
.6
.4
.2
0
PVP
20°C
Quiescent
Shaken
ρ Na Citr.
0
.4
.8
1.2
1.4
Conc of Na Citrate (M)

FIGURE 19

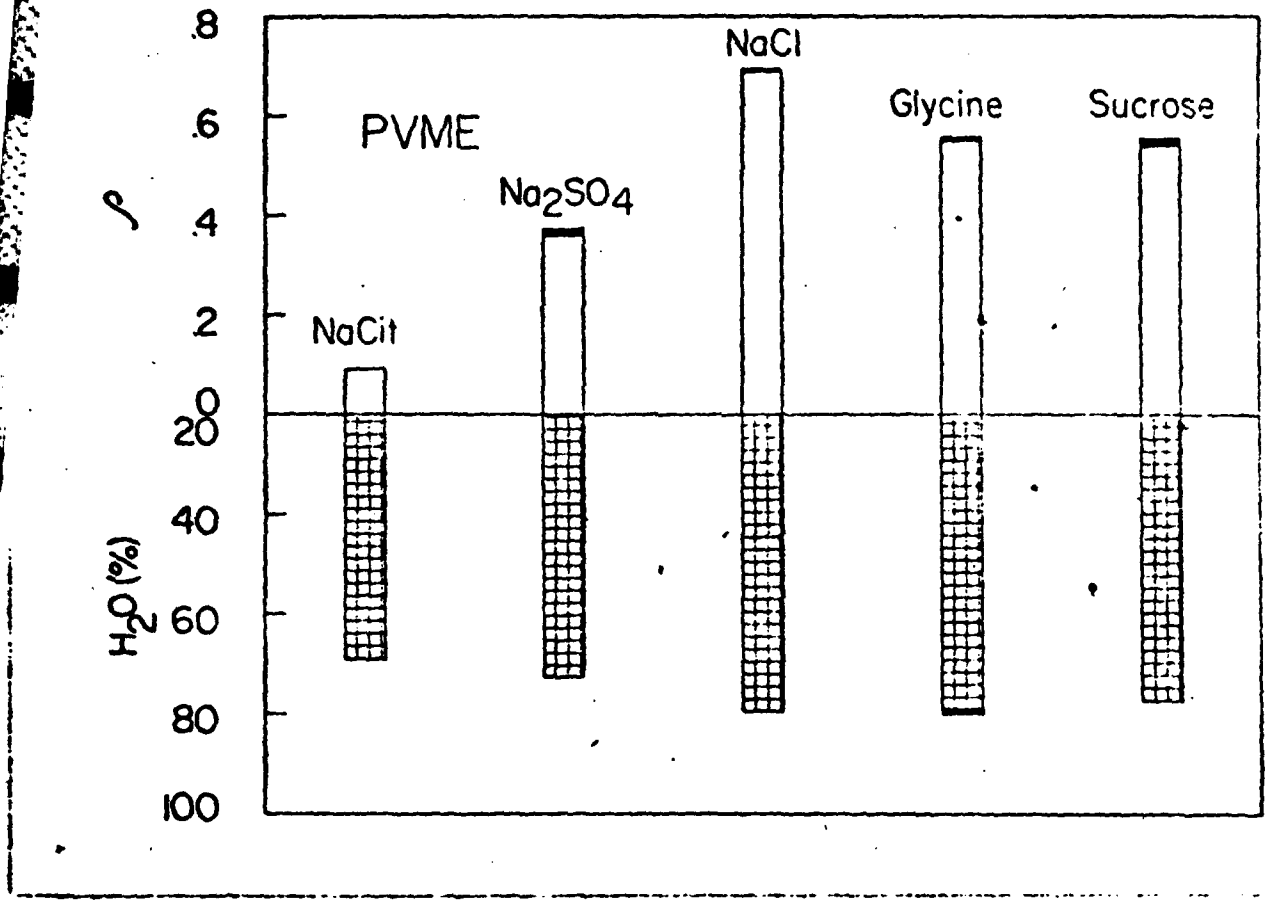

.8
.6
.4
.2
0
20
40
60
80
100
ρ
H2O (%)
PVME
NaCit
Na2SO4
NaCl
Glycine
Sucrose

FIGURE 20

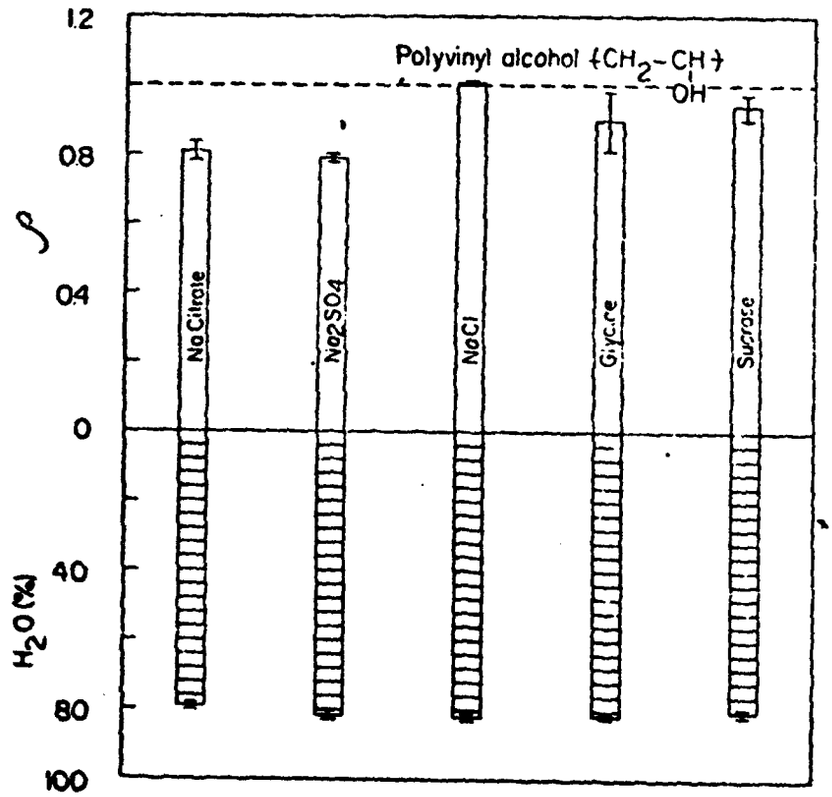

1.2
0.8
0.4
0
40
80
100
ρ
H2O(%)
Polyvinyl alcohol {CH2-CH}
OH
Na Citrate
Na2SO4
NaCl
Glycine
Sucrose

FIGURE 21

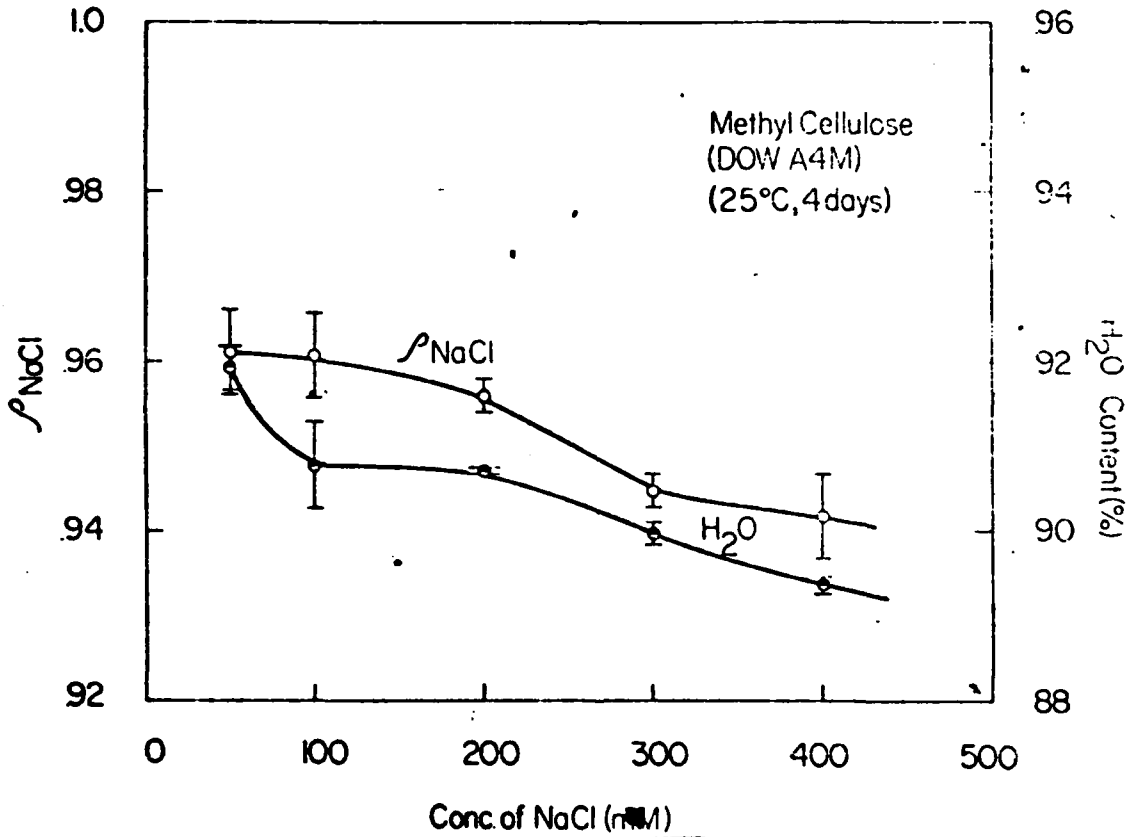

Methyl Cellulose
(DOW A4M)
(25°C, 4 days)
ρNaCl
H2O
ρNaCl
H2O Content(%)
Conc. of NaCl (mM)
1.0
.98
.96
.94
.92
96
94
92
90
88
0
100
200
300
400
500

FIGURE 13

22

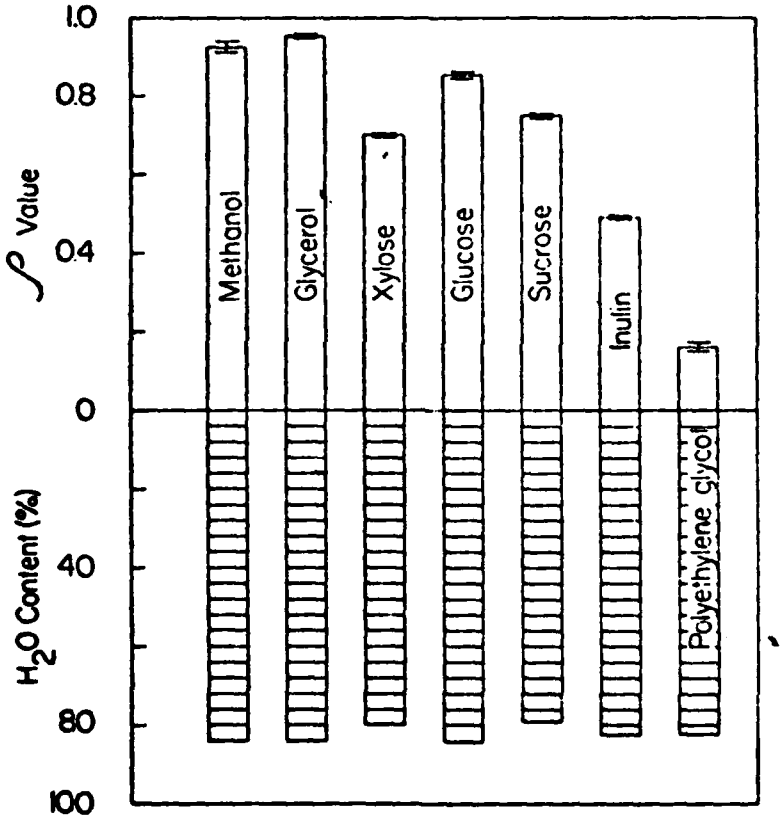

1.0
0.8
0.4
0
40
80
100
ρ Value
H2O Content (%)
Methanol
Glycerol
Xylose
Glucose
Sucrose
Inulin
Polyethylene glycol

FIGURE 12

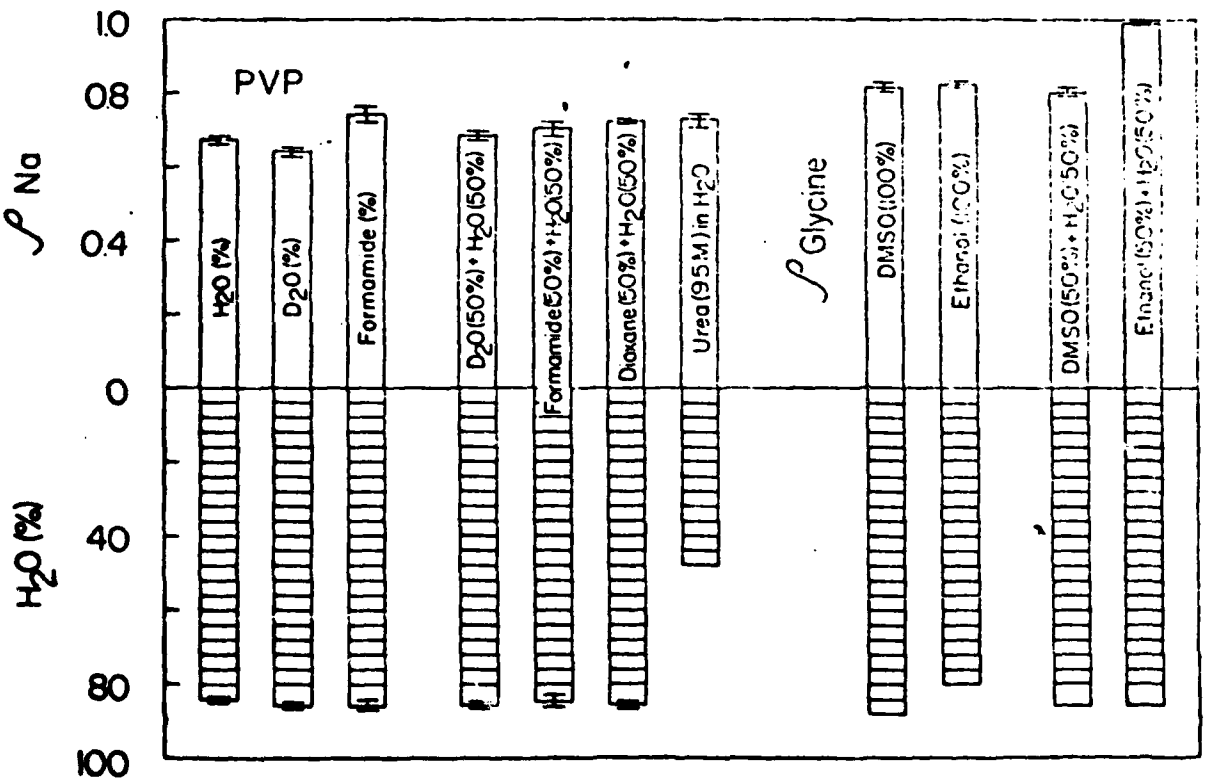

1.0
0.8
0.4
0
40
80
100
PVP
ρ Na
H2O (%) ρ H
H2O (%)
D2O (%)
Formamide (%)
D2O (50%) + H2O (50%)
Formamide (50%) + H2O (50%)
Dioxane (50%) + H2O (50%)
Urea (9.5 M) in H2O
ρ Glycine
DMSO (100%)
Ethanol (100%)
DMSO (50%) + H2O (50%)
Ethanol (50%) + H2O (50%)

FIGURE 24


FILM
1-8