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# The Effects of pH and Salts on the Viscosity of Protein Solutions

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The Effects of pH and Salts  
on the  
Viscosity of Protein Solutions

The Effects of pH and Salts  
on the  
Viscosity of Protein Solutions

by

John H. Smith

A thesis presented to the Department of Chemistry of Union  
College in partial fulfillment of the requirements for the  
Degree of Bachelor of Science with a Major in Chemistry.

By \_\_\_\_\_

Approved by \_\_\_\_\_

*HE Shiff*

May 30, 1963



## TABLE OF CONTENTS

Introduction . . . . .	1
Historical . . . . .	2
Experimental . . . . .	3
Discussion . . . . .	6
Appendix . . . . .	24
Bibliography . . . . .	26



## INTRODUCTION

The purpose of this work was to determine, as a function of pH, the viscosity of gelatin solutions containing various monovalent anions. The sequence of these anions in their ability to depress viscosity, that is, the Hofmeister or Lyotropic series of anions, will be validated for the following salts: Sodium bromide, sodium iodate, sodium formate, sodium chloride and sodium nitrate.



## HISTORICAL

In 1888, Hofmeister first reported the observation that various salts had certain characteristic effects on the viscosity of albumin sol. He further stated that these salts could therefore be listed in a series according to their relative power in altering the original viscosity of the sol. Hofmeister's series was later modified by Pauli in 1903, to read (in the order of decreasing ability to depress viscosity):

$\text{F}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ , citrate $^{3-}$ , tartrate $^{2-}$ , acetate $^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{ClO}_3^-$ , Br $^-$ , I $^-$ , CNS $^-$

However, in 1922 Loeb,<sup>5</sup> experimenting with acidic gelatin sols, could only show that the pH, and not the specific identity of the cation, was the primary factor in determining viscosity. He therefore contested Hofmeister's work and the validity of the series.

Other subsequent experiments<sup>2,3,9</sup> have proven that a series is prevalent only at concentrations of salt greater than 0.01 M. Thus, Loeb and Hofmeister were justified in their disagreement.

In a more recent work on this subject,<sup>8</sup> it was found that the salts sodium iodide, sodium nitrate and sodium chloride appeared to enhance the viscosity of acidic gelatin in the order given. This order is, however, just the reversal of the accepted Hofmeister order and, because of this, further investigation of the viscosity of gelatin solutions was undertaken.



## EXPERIMENTAL

All viscosity measurements were performed on Eastman Purified Pigskin Gelatin (Isoelectric Point pH 8.6, Lot No. 171, February 1, 1951) and with Chemically Pure salts and acids. An Ubbelohde Viscometer and a stopwatch were utilized to obtain relative viscosity measurements, i.e., time of outflow of gelatin solution divided by time of outflow of distilled water. It was previously found by Pollara that at a temperature of 30°C there was very little change in the viscosity of gelatin with time. All data were therefore taken at this optimum temperature, by use of a constant temperature water bath set at 30°C  $\pm$  .01°C. Ph was recorded on a Beckman battery operated pH meter.

The gelatin solutions were prepared by heating 2.00g of solid gelatin and water at a temperature of 60°C for at least 11 hours. This lengthy heating time was necessitated by the fact that the solution of gelatin in water occurred very slowly and even after eleven hours, lumps of undissolved gelatin were still apparent. These lumps could not be dissolved, but it was found that the total undissolved gelatin was only about 0.001g. This was negligible compared to the original weight (2g) of gelatin added. After heating overnight, therefore, the gelatin solution was filtered into a 200 ml volumetric flask giving a total solution of 10g gelatin per liter of solution. The solution had to be filtered through a cloth towel, rather than filter paper, because the pores of the latter were blocked by the undissolved lumps, thus making filtration too long and tedious a process. The appropriate



amount of salt was then added to the 200 ml of gelatin solution and the volumetric flask was placed in the 30°C water bath. The salts, sodium bromide, sodium iodate and sodium formate were investigated at 0.01 M gelatin concentration and the salts, sodium bromide, sodium iodate, sodium formate, sodium chloride and sodium nitrate were investigated at 0.05 M.

The 200 ml solution was partitioned into four equal portions of 50 ml each, corresponding to the four pH ranges 2-4, 4-6.4, 6.4-9 and 9-11. Starting, then, at a pH of 6.4 (the approximate pH of the gelatin solution) weak solutions of acid or base could be added to obtain small changes of pH in the "sensitive range" (4 pH - 9 pH), while in the "less sensitive range" (2 pH - 4 pH or 9 pH - 11 pH) strong solutions of acid or base could be used. If the solution was divided into only 2 parts (corresponding to the acidic and basic side of 6.4) the added volume of weak acid or base needed to produce even a small pH change at the larger pH's would alter the protein concentration of the solution. Using our method, however, when the volume change resulting from weak acid or base becomes appreciable, the solution can be discarded and the less voluminous strong acid or base can be added to a new solution.

Viscosity measurements were taken at various pH readings using different salts, by depositing 10 ml of the specified solution in the viscometer, waiting 2 minutes for the solution to come to temperature, and finally noting the time of descent with the aid of a stopwatch.



The viscometer was rinsed out with a new solution before another viscosity determination was performed.

Curves were obtained for the gelatin solution, the gelatin solution plus the various salts, and the gelatin solution using various acids to traverse the pH range. In all cases, it was necessary to use that salt of the acid which was being used to alter the pH, or else the anion of the acid would be partly responsible for the viscosity change (which was supposed to be attributed wholly to the added electrolyte). The experimental results are plotted on graphs 1, 2, and 3.



## DISCUSSION

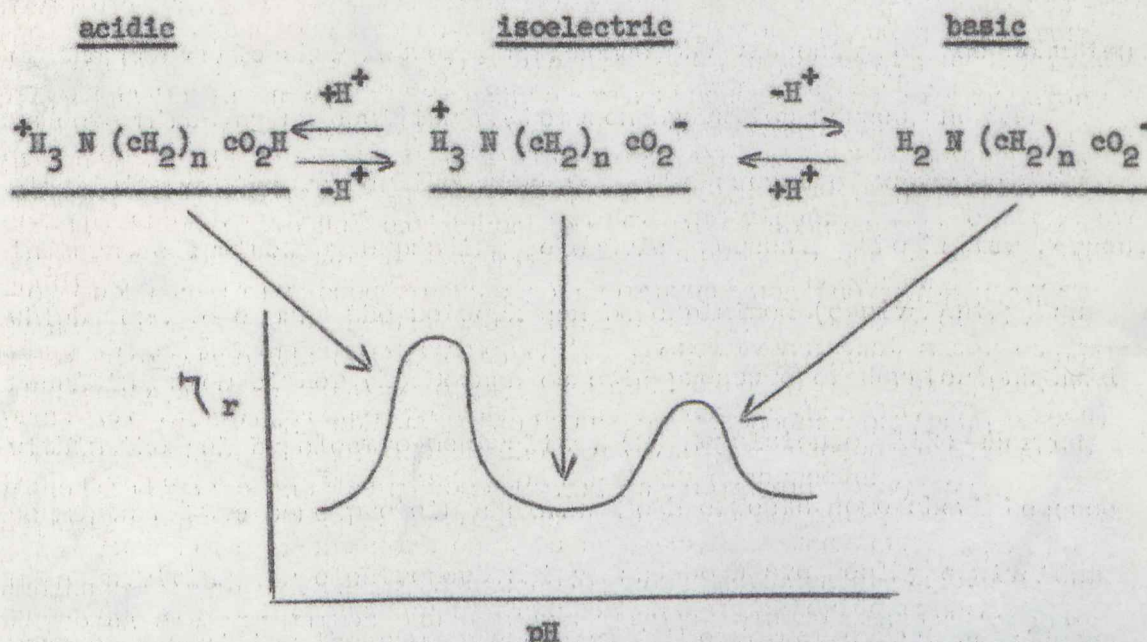
Gelatin is an amphoteric linear polyelectrolyte that forms a poly-molecular solution. The macromolecule of gelatin in solution is a randomly kinked coil which is cross-linked to other macromolecules by means of chemical bonding and simple interlocking of coils. Dry gelatin, when dissolved in water, takes up a definite amount of hydration water. Some of this water (about one molecule thick) is firmly bound to the colloidal chain by strong hydration forces. Most of the water, however, is "free" water which is only present between the expanded network of strands as loosely held occlusion water. The overall size of the macromolecular network, and hence the viscosity of the solution is determined by an equilibrium between hydration forces and the elasticity of the rubberlike network. The amount of water held is in turn determined by expanding "electric" forces. These latter forces, which tend to expand the network can be explained (equivalently)<sup>7</sup> as resulting from either (1) Donnan osmotic pressure of counterions held within the gel by electro-neutrality, (2) Repulsion of like-charged segments of the same protein chain. Both effects come about because of a "striving for electroneutrality" and both are really equivalent ways of explaining the same phenomena. The former effect considers the  $B^+$  ion (the ion resulting from the reaction of  $H^+$  with the protein) on the "inside" of the network, thus giving rise to an osmotic pressure "push" against the membrane walls; the latter considers the  $B^+$  ion on



the "outside," thus giving rise to an electrostatic "pull" on the walls. Both do not occur simultaneously, but each taken separately results in a swelling of the network and a consequent increase in viscosity. Both of these concepts will be applied in explaining the shape of the curves in graphs No. 1 and No. 2.

### The Donnan Equilibrium

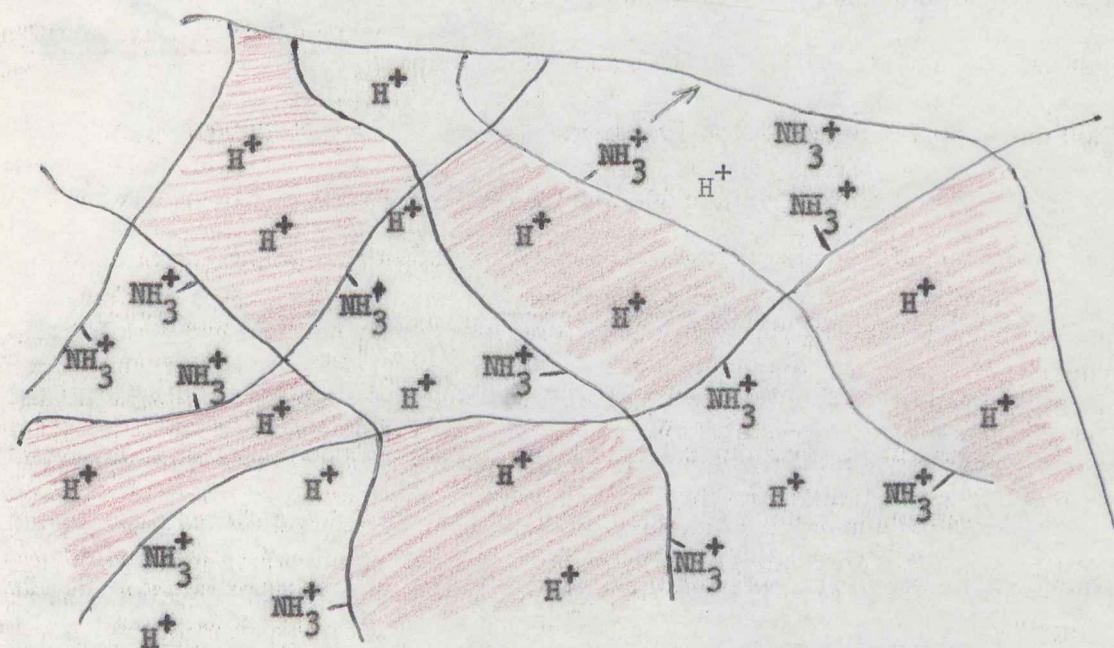
Gelatin, being amphoteric, reacts with either acid or base as follows:



The forms prevalent at the maximums and minimum on the experimental viscosity vs. pH curve is indicated at the appropriate pH.



It will be remembered that Gelatin in solution is a chaotically built meshwork of long strands of molecules which are joined together by various types of bonds. A cross-section of such a network of intertwining strands may look as follows in acid solution (see Organic Colloids, Jirgenson's, Fig. 93, p. 262, Elsevier Press 1958):



It is seen that certain compartments (actually 3-dimensional compartments) were drawn containing the non-diffusible, attached,  $\text{NH}_3^+$  ion and that other compartments (red) have no such ions. This is, of course, a simplification of the actual situation where one compartment has more  $\text{NH}_3^+$  ions than another, etc. Also, there are present (not shown above) in each compartment enough  $\text{Cl}^-$  ions (from the added acid,  $\text{HCl}$ ) to make



each compartment electrically neutral. Now, of one red and one plain compartment are singled out, are arbitrarily designated as "inside" and "outside" (according to where the  $\text{NH}_3^+$  group is relative to the other compartment), and finally, are inspected at equilibrium, the following distribution will have occurred:<sup>6</sup>

<u>"inside"</u> (red)			<u>"outside"</u> (plain)	
$\text{NH}_3^+$	$\text{H}^+$	$\text{Cl}^-$	$\text{H}^+$	$\text{Cl}^-$
Z	y	y + Z	X	X

It is noted here that the designations "inside" and "outside" do not refer to the relative availabilities of the  $\text{H}^+$  ion but are used to distinguish between the compartments which contain the  $-\text{NH}_3^+$  ion and those which do not. That is to say, there is no  $\text{H}^+$  ion "inside" anything; and hence no  $\text{H}^+$  ion (either "inside" or "out") that cannot be recorded on a pH meter. The  $\text{H}^+$ 's in either compartment are equally mobile, for any strongly bound water present (which might retard movement) is only present at the walls of each compartment and is only one molecule thick. Now, an electrode of a pH meter, when inserted into such a solution, will be in contact with both types of compartment simultaneously and it is assumed an average pH will be recorded, i.e.,

$$\text{pH} = -\log \left( \frac{X + Y}{2} \right)$$



The total osmotic pressure,  $\pi_T$ , may be written

$$\pi_T = \pi_p + \pi_i$$

where  $\pi_p$  is the partial osmotic pressure due to the protein molecules themselves, and  $\pi_i$  is the pressure resulting from the unequal distribution of diffusible ions across a membrane. It is this  $\pi_i$ , then, that must be considered here. It can be shown,<sup>1</sup> that  $\pi_i$  is defined as follows (at infinite dilution, a simplifying approximation):

$$\pi_i = RT (\sum c_i - \sum c_o)$$

where  $\sum c_i$  equals the sum of the concentrations of the diffusible ions on the inside and  $\sum c_o$  their sum on the outside. Thus, from the previous final distribution,

$$\pi_i = RT (y + y + Z - (x + x))$$

or

$$\pi_i = RT (2y + Z - 2x) \quad (1)$$

Now, since at equilibrium the activity of the acid must be the same on both sides of the membrane, the product of their concentrations on the inside equals their product on the outside:

$$(H_1^+) (Cl_1^-) = (H_0^+) (Cl_0^-)$$

$$y (y + z) = x^2$$

$$z = \frac{x^2}{y} - y \quad (2)$$

Therefore, substituting (2) into (1),

$$\pi_1 = RT \left( y + \frac{x^2}{y} - 2x \right)$$

or

$$\pi_1 = RT \left( \frac{y^2 + x^2 - 2xy}{y} \right)$$

and

$$\pi_1 = RT \frac{(x - y)^2}{y} \quad (3)$$

Thus, this equation determines the osmotic pressure changes on the inside as more acid is added to the isoelectric gelatin. This equation should therefore also predict the observed maximum in viscosity at a pH of 3, for an increase in osmotic pressure increases the size of the aggregate and thus is proportional to viscosity.

The equations relating the variables are as follows:

$$x^2 = y (y + z)$$

and

$$pH = -\log \left( \frac{x + y}{2} \right) \text{ or } x + y = 2 \times 10^{-pH}$$



Thus,

$$x = 2 \times 10^{-pH} - y \quad (4)$$

and

$$y = \frac{4 \times 10^{-2pH}}{Z + 4 \times 10^{-pH}} \quad (5)$$

Substituting equations (4) and (5) in (3),

$$\pi_1 = \frac{RT \left[ 2 \times 10^{-pH} - \frac{8 \times 10^{-2pH}}{Z + 4 \times 10^{-pH}} \right]}{\frac{4 \times 10^{-2pH}}{Z + 4 \times 10^{-pH}}}$$

Since at a pH of 3 a maximum in viscosity was observed, this equation should have a maximum value at a pH = 3. If this is the case, the above equation's derivative at pH = 3, when set to 0, will give the value of Z where the maximum occurs, that is,

$$\frac{d}{dZ} \pi_1 (Z_m) = 0,$$

where  $Z_m$  is that value of Z which gives a maximum value of  $\pi_1$  (and  $\eta_r$ ). When this is done (see Appendix)  $Z_m$  equals  $8 \times 10^{-3}$ . From this value of Z, other relative values of Z can be assigned and from these, corresponding values of x and y can be calculated at each pH (from equations (4) and (5)), in order to get a more quantitative (but still empirical) picture of the occurrences.



$\pi_1$	$\frac{(x-y)^2}{y}$	pH	Z B <sup>+</sup>	y H <sup>+</sup>	y+Z Cl <sup>-</sup>	x H <sup>+</sup>	x Cl <sup>-</sup>
0	0	8 <sup>(iso-electric)</sup>	0	$1 \times 10^{-8}$	$1 \times 10^{-8}$	$1 \times 10^{-8}$	
$5.9 \times 10^{-9}$	$2.37 \times 10^{-10}$	7	$1 \times 10^{-8}$	$9.76 \times 10^{-8}$	$10.8 \times 10^{-8}$	$1.024 \times 10^{-7}$	
$0.00174 \times 10^{-1}$	$7.02 \times 10^{-6}$	6	$1 \times 10^{-5}$	$2.86 \times 10^{-7}$	$1.0 \times 10^{-5}$	$1.71 \times 10^{-6}$	
$0.0174 \times 10^{-1}$	$7.02 \times 10^{-5}$	5	$1 \times 10^{-4}$	$2.86 \times 10^{-6}$	$1.0 \times 10^{-4}$	$1.71 \times 10^{-5}$	
$0.174 \times 10^{-1}$	$7.02 \times 10^{-4}$	4	$1 \times 10^{-3}$	$2.86 \times 10^{-5}$	$1.0 \times 10^{-3}$	$1.71 \times 10^{-4}$	
$1.32 \times 10^{-1}$	$5.31 \times 10^{-3}$	3	$8 \times 10^{-3}$	$3.34 \times 10^{-4}$	$8.33 \times 10^{-3}$	$16.6 \times 10^{-4}$	
$0.496 \times 10^{-1}$	$2.0 \times 10^{-3}$	2	$1 \times 10^{-2}$	$8.0 \times 10^{-3}$	$1.8 \times 10^{-2}$	$1.2 \times 10^{-2}$	
$0.092 \times 10^{-1}$	$3.71 \times 10^{-4}$	1	$1.3 \times 10^{-2}$	$9.7 \times 10^{-2}$	$6.81 \times 10^{-2}$	$1.03 \times 10^{-1}$	

In assigning values of Z (these are the only variables arbitrarily assigned, all others are calculated from Z), the value of  $8 \times 10^{-3}$  was kept in mind and also the fact that the amount of  $\text{-NH}_3^+$  will increase rapidly at first, with small addition of  $\text{H}^+$ , and then level off after nearly all gelatin has reacted. If the values of  $\pi_1$  are multiplied by a proportionality factor of 25.0 to connect osmotic pressure to viscosity, and then a value of 1.70 is added to account for  $\pi_p$ , the following values result:

pH	$\eta_r$
1	1.93
2	2.94
3	5.00
4	2.14
5	1.74
6	1.70
7	1.70
8	1.70



These are plotted on graph No. 3 for comparison to experimental observations. A similar curve would result if the basic side was considered. (The value of  $Z = 8 \times 10^{-3}$  obtained from the integration could perhaps be used in the equation,<sup>6</sup>

$$Z = \frac{C_o y}{y + \frac{K_w}{K_b}}, \quad C_o = \text{original conc. of gelatin.}$$

as a method for determining either the molecular weight of  $K_2$  of gelatin, if one of these constants were known.)

The Donnan Equilibrium can also account for the depressing effects salts have on viscosity, although the prediction of a Hofmeister series is not possible with this concept. Considering, however, the addition of Na Cl to the previous mixture of gelatin and acid, one can appraise the following final distribution of ions as was done above:

<u>inside</u>				<u>outside</u>		
Z	y	w	y + Z + w	x	V	x + V
$-\text{NH}_3^+$	$\text{H}^+$	$\text{Na}^+$	$\text{Cl}^-$	$\text{H}^+$	$\text{Na}^+$	$\text{Cl}^-$

Here the osmotic pressure is

$$\pi_1 = RT (2y + 2w + Z - 2x - 2V)$$

and the equations to be considered are:



$$a) \quad y(y + Z + w) = x(x + V)$$

$$b) \quad xw = Vy$$

$$c) \quad pH = -\log\left(\frac{x + y}{2}\right)$$

$$d) \quad V + w = 0.05$$

where b) was obtained by dividing  $w(y + Z + w) = V(x + V)$  by a)

and d) was obtained by considering a 0.05 m concentration of NaCl.

Now, obtaining x, v, and w in terms of y:

$$x = 2 \times 10^{-pH} - y$$

$$v = 0.05 - \frac{0.05}{2 \times 10^{-pH}} y$$

$$w = \frac{0.05}{2 \times 10^{-pH}} y$$

and substituting into a),

$$y(Z + 2 \times 10^{-pH} + 0.1) - 10^{-2pH} - 0.05 \times 10^{-pH} = 0$$

then, at a pH of 3, using the same value of Z as before (since the amount of  $B^+$  probably would not be altered by the addition of a salt)

i.e.,  $Z = 8 \times 10^{-3}$ , the following values for y, x, V, and w result:

$$y = 9.286 \times 10^{-4}$$

$$x = 1.0714 \times 10^{-3}$$



$$v = 2.678 \times 10^{-2}$$

$$w = 2.322 \times 10^{-2}$$

$$\text{Thus, } \pi_1 = RT (0.00186 + 0.04644 + 0.0080 - 0.00214 - 0.05356)$$

$$\pi_1 = RT (6 \times 10^{-4}) = 0.0149$$

Multiplying by 25.0 and adding 1.70 as before,

$$\gamma_r = 2.07 \text{ at salt conc.} = 0.05 \text{ M. at pH} = 3$$

from previously

$$\gamma_r = 5 \text{ at salt conc.} = 0 \text{ at pH} = 3$$

Thus it is seen that the addition of 0.05 moles of Na Cl per l of gelatin solution depresses the viscosity of the latter to almost one half.

As stated above, however, the Donnan Equilibrium concept does not predict any specific ionic sequence of depressing power. Partly because of this fact, Loeb, who regarded Donnan effects as the essence of colloid behavior, could see no reason to accept the work of Hofmeister and the existence of a Lyotropic Series.

#### Repulsion of Like-Charged Groups

This second method of explaining graphs No. 1 and No. 2 can best be understood by drawing a simplified picture of bridgeformation between two protein chains:



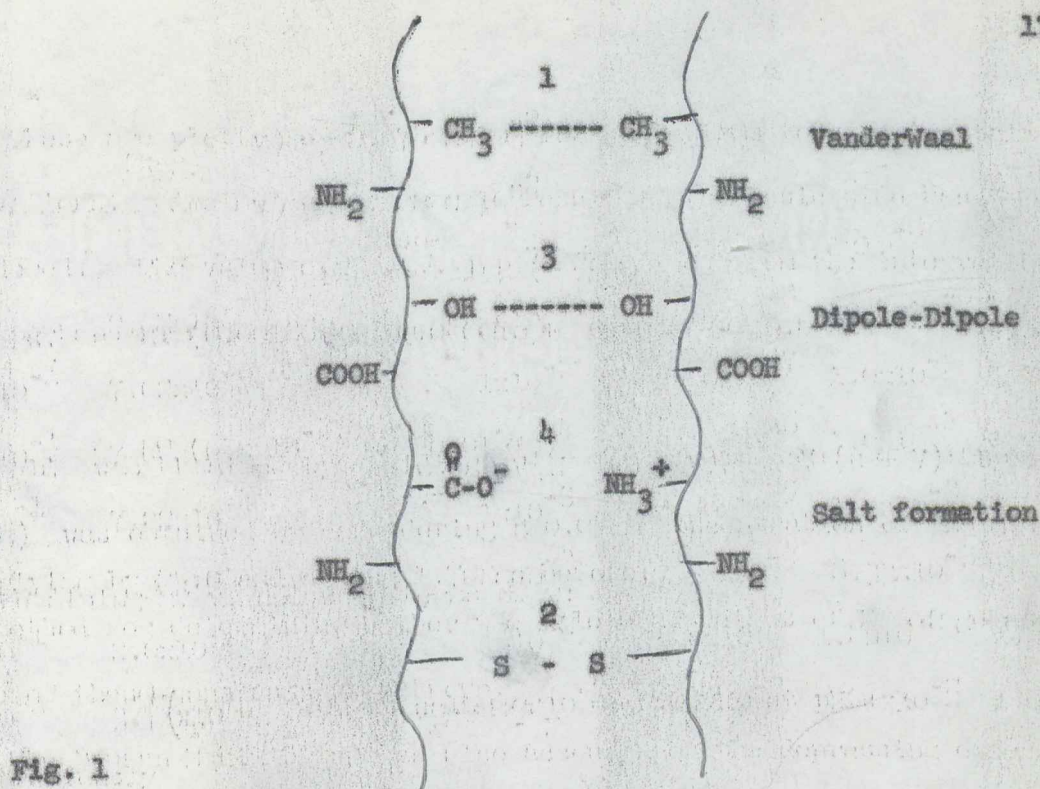


Fig. 1

Bonds No. 1 and 2 cannot be broken by solvent while bonds 3 and 4 are easily broken. Thus, when gelatin is dissolved in water, and acid is slowly added, two things occur. First, the  $\text{H}^+$  reacts with the  $\text{NH}_2$  forming  $\text{NH}_3^+$  groups, and second, bonds 3 and 4 are loosened by the solvent. Also, since like charges repel, the mutual repulsion of the  $\text{NH}_3^+$  groups tend to make the network expand as follows:

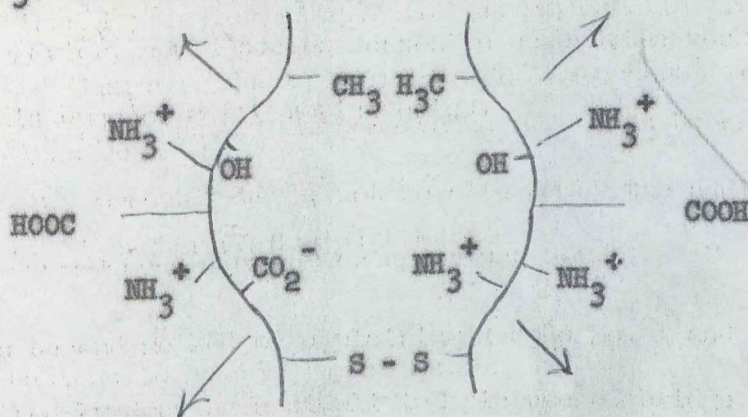


Fig. 2



Therefore, it is seen that with the addition of small amounts of acid, the viscosity should increase, as a result of the increased size of the aggregate. This increase in viscosity will occur as long as there exists still unreacted  $\text{-NH}_2$  and a low concentration of the acid's counterion (say  $\text{Br}^-$ ). However, when the amount of  $\text{NH}_3^+$  groups start to become small relative to the amount of  $\text{Br}^-$  (due to the exhaustion of unreacted  $\text{NH}_2$  and the increasing quantity of acid being added), a "screening" effect will occur. That is to say, the  $\text{Br}^-$  ions will tend to cluster around the repelling  $\text{NH}_3^+$  groups and neutralize their repelling force:

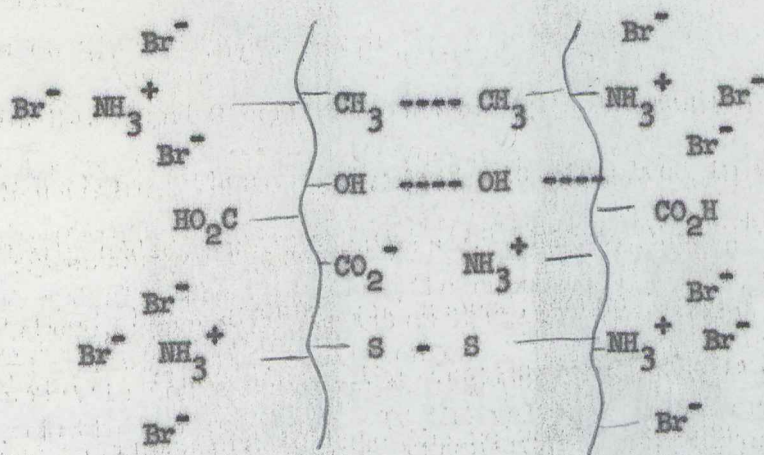


Fig. 3

Consequently the aggregate will shrink to its "normal" size with a resulting decrease in viscosity. A similar explanation can be given for the maximum observed on the basic side of the pH range.

The screening effect is also significant in showing why the addition of salts depress the viscosity of gelatin solutions, but does



not as yet explain why these salts should follow a series in their depressing power (Hofmeister series). Consider the above two chains before any salt (Na Br) was added; the  $\text{CH}_3$  ----  $\text{CH}_3$  and the S - S bonds are still strong, as they are impervious to water, but bonds No. 2 and 3 have become weakened by polar water molecules squeezing between the two bond-making groups and are thus easily ruptured. As a result, the two chains, appear as follows in acid solution, with no salt added:

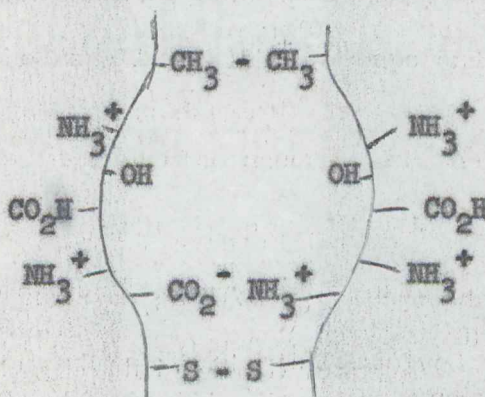


Fig. 4

The hourglass shape is a result of the mutual repulsion between the like-charged  $\text{NH}_3^+$  groups (or  $\text{COO}^-$  groups in basic salt). Now, when salts are added, as shown in Fig. 3, the screening effects begin to occur, i.e., the ions of the salts attach onto or surround the ionized group of the protein chain. As a result, the repulsion between like-charged  $\text{NH}_3^+$  groups is diminished, due to their cancelation by oppositely charged ions of the salt, and the two chains relax to parallel lines with the reformation of the weak  $\text{OH} - \text{OH}$  and  $\text{O}=\text{C} - \text{O} \text{NH}_3^+$  bonds.



Thus, it is seen that the gelatin cage-like structures become swelled when no salts are present, resulting in a high viscosity acidic or basic solution, and become condensed when salts are present, resulting in low viscosity acidic or basic solution. However, when the protein is in neither acid or base solution, that is, when it is in its isoelectric state, with equal numbers of  $\text{NH}_3^+$  and  $\text{COO}^-$  groups on the same chain, the addition of salt has just the opposite effect. Now, without salt, the gelatin chain is cramped together because of the attraction between the unlike charges on the same chain.

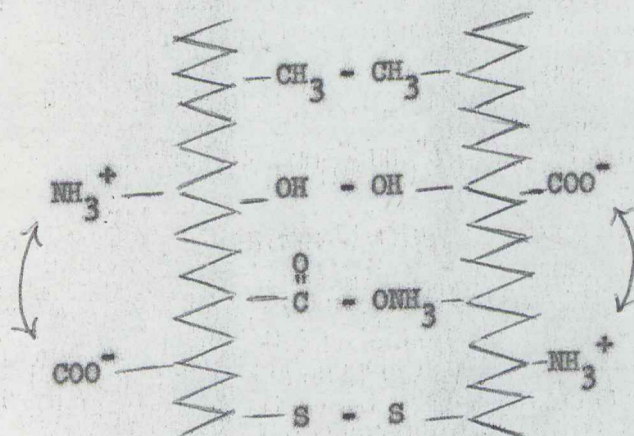


Fig. 5

When salts are added the ions again surround or attach onto the  $\text{COO}^-$  and  $\text{NH}_3^+$  groups and the charges of these groups are again canceled. As a result, the two chains assume the form of the first diagram, as less electrostatic attraction exists; but with this increase in size, there is a corresponding increase in viscosity. Thus, it is seen that if salt is added to isoelectric gelatin, an increase in viscosity occurs,



while if the same salt is added to acidic or basic gelatin, a decrease in viscosity occurs, although the latter effect is more pronounced than the former.

The two remaining bonds, 3 and 4, must now be considered. As stated previously, these bonds can be loosened or broken by polar groups such as water molecules. If these bonds are broken, the two chains will expand, somewhat like in diagram No. 2, to the most probable state, that is the state of maximum entropy which is of larger size than the form of Fig. 1 but not as large as in Fig. 2 (because no electrostatic forces are being considered as in Fig. 2). The chains would spread apart indefinitely if the opposing elastic forces, anchored by the still intact bonds No. 1 and No. 2, did not restrain their movements. Consider the process whereby some water molecules are inserted between the OH-OH bond, thus loosening the bond. If the salt is now added, the ions tend to pull the above tightly bound water molecules from between the bond with a strength proportional to the salts integral heat of dilution. That is to say, salts retard the breaking of bonds No. 3 and No. 4, and thus retard both the expansion of the chains and an increase in viscosity with an intensity dependent on the salts heat of dilution. Let the partial thermodynamic quantities referring to the bonds and to the salt solution be denoted by subscripts 1 and 2 and consider again the process of squeezing water molecules between the bonds. Then the relations for the partial free energies for this process are:



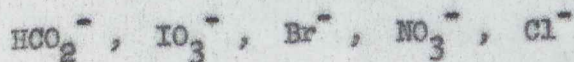
$$\Delta F_1 = \Delta H_1 - T\Delta S_1 \quad \text{and} \quad \Delta F_2 = \Delta H_2 - T\Delta S_2$$

the total relation is

$$\Delta F_T = \Delta F_1 - \Delta F_2 = \Delta H_1 - \Delta H_2 + T\Delta S_1 - T\Delta S_2.$$

The process will take place if  $\Delta F_{\text{TOTAL}}$  is  $-$ . As seen, the larger  $-\Delta H_2$  is for a certain salt, the more spontaneously the process will occur (the value of  $T\Delta S_2$  remains constant here). In other words, the salts with the largest negative heat of dilution will encourage the breakage of the bonds to the greatest extent and will therefore simultaneously depress the normal, no salt, viscosity to the least extent. Thus, for example, the heat of dilution of  $\text{NH}_4\text{NO}_3$  is  $-1442$  joules<sup>(4)</sup> (i.e., if to 1 gm. mole of  $\text{KCl}$  and 25 gm. moles of  $\text{H}_2\text{O}$  at  $30^\circ\text{C}$  is added 25 gm. moles of  $\text{H}_2\text{O}$ ,  $1442$  joules of heat must be supplied to keep the temperature at  $30^\circ\text{C}$ ) while that for  $\text{NH}_4\text{Cl}$  is  $+25$  j.<sup>(4)</sup> under the same conditions. The  $\text{NO}_3^-$  should therefore decrease the viscosity less than  $\text{Cl}^-$ . As seen on graph No. 1 this is borne out by experiment.

The results of graphs No. 1 and No. 2 definitely seem to validate the Hofmeister series at both 0.05 and 0.01 Molar concentrations of salt. The series is as follows (in the order of increasing flocculating power):





There is also present, however, an anomalous maximum at about  $\text{pH} = 7$ .

One explanation was given by Wilson and Kern (J. Am. Chem. Soc. 44, 2633-6 (1922)) who found gelatin to consist of 2 equilibrium forms. One form can be made to gel while the other form cannot. At  $20^{\circ}\text{C}$  these researchers found two isoelectric points, one at  $\text{pH} = 5$  and one at  $\text{pH} = 8$ . According to graph No. 3, at  $30^{\circ}\text{C}$  our pigskin gelatin has an isoelectric point at  $\text{pH} = 6.7$  and  $\text{pH} = 8$ . This difference (and indeed the entire trouble of reproducibility in gelatin research) is probably caused by varying degrees of gelatin purity.

Another explanation could be merely that  $\text{H}^{+}$  and  $\text{OH}^{-}$  ions dehydrate the gelatin network to a small extent before the Donnan Effects become appreciable, and thus decrease the viscosity on either side of  $\text{pH} = 7$ . This is consistent with the fact that  $\text{H}^{+}$  is a good dehydrator, and also the fact that the gelatin molecule at a  $\text{pH}$  of 7 is in a swelled condition due to the discharging effect of the various salts on the zwitter ion. It might therefore be relatively easy for the  $\text{H}^{+}$  (or  $\text{OH}^{-}$ ) ion to remove some of this loosely bound water of the zwitter ion and thus decrease the viscosity of the gelatin solution to the normal (no salt) viscosity at that particular  $\text{pH}$ .



APPENDIX

$$\pi_1 = \frac{RT}{\frac{10^{-6}}{|Z| + 4 \times 10^{-3}}} \left[ \frac{10^{-3} - \frac{4 \times 10^{-6}}{|Z| + 4 \times 10^{-3}}}{10^{-6}} \right]^2$$

The absolute value of Z is used here because Z cannot be negative.

$$\frac{d(\pi_1)}{dZ} = 0 = \frac{\left[ \frac{2 \times 10^{-6}}{|Z| + 4 \times 10^{-3}} \right] \left[ \frac{10^{-3} - \frac{4 \times 10^{-6}}{|Z| + 4 \times 10^{-3}}}{10^{-6}} \right] \left[ \frac{4 \times 10^{-6}}{(|Z| + 4 \times 10^{-3})^2} \right] + \frac{10^{-12}}{(|Z| + 4 \times 10^{-3})^2} + \left[ \frac{10^{-3} - \frac{4 \times 10^{-6}}{|Z| + 4 \times 10^{-3}}}{10^{-6}} \right]^2 \frac{10^{-6}}{(|Z| + 4 \times 10^{-3})^2} = 0$$

$$= \frac{8 \times 10^{-3}}{|Z| + 4 \times 10^{-3}} - \frac{32 \times 10^{-6}}{(|Z| + 4 \times 10^{-3})^2} + \frac{10^{-12} - \frac{8 \times 10^{-15}}{|Z| + 4 \times 10^{-3}} + \frac{16 \times 10^{-18}}{(|Z| + 4 \times 10^{-3})^2}}{10^{-12}}$$

$$= - \frac{16 \times 10^{-6}}{(|Z| + 4 \times 10^{-3})^2} + 1 = 0$$

$$+ 16 \times 10^{-6} = Z^2 + 8|Z| \times 10^{-3} + 16 \times 10^{-6}$$



$$|z| (|z| + 8 \times 10^{-3}) = 0$$

$$|z| = -8 \times 10^{-3}$$

$$z^2 = 64 \times 10^{-6}$$

$$|z| = \pm \sqrt{64 \times 10^{-6}} = \pm 8 \times 10^{-3}$$

$$|z| = + 8 \times 10^{-3}$$



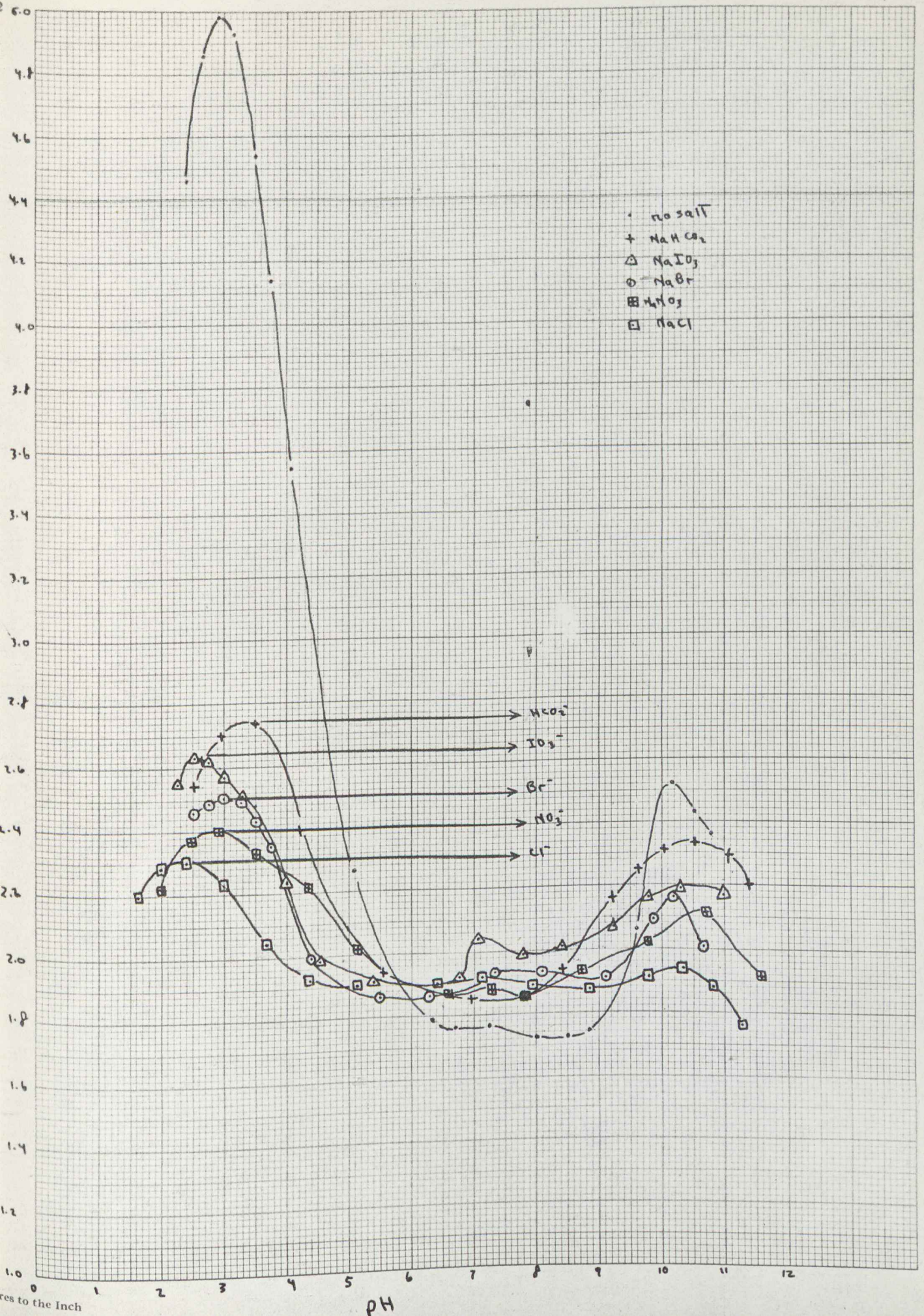
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Graph #1

.05 Molar

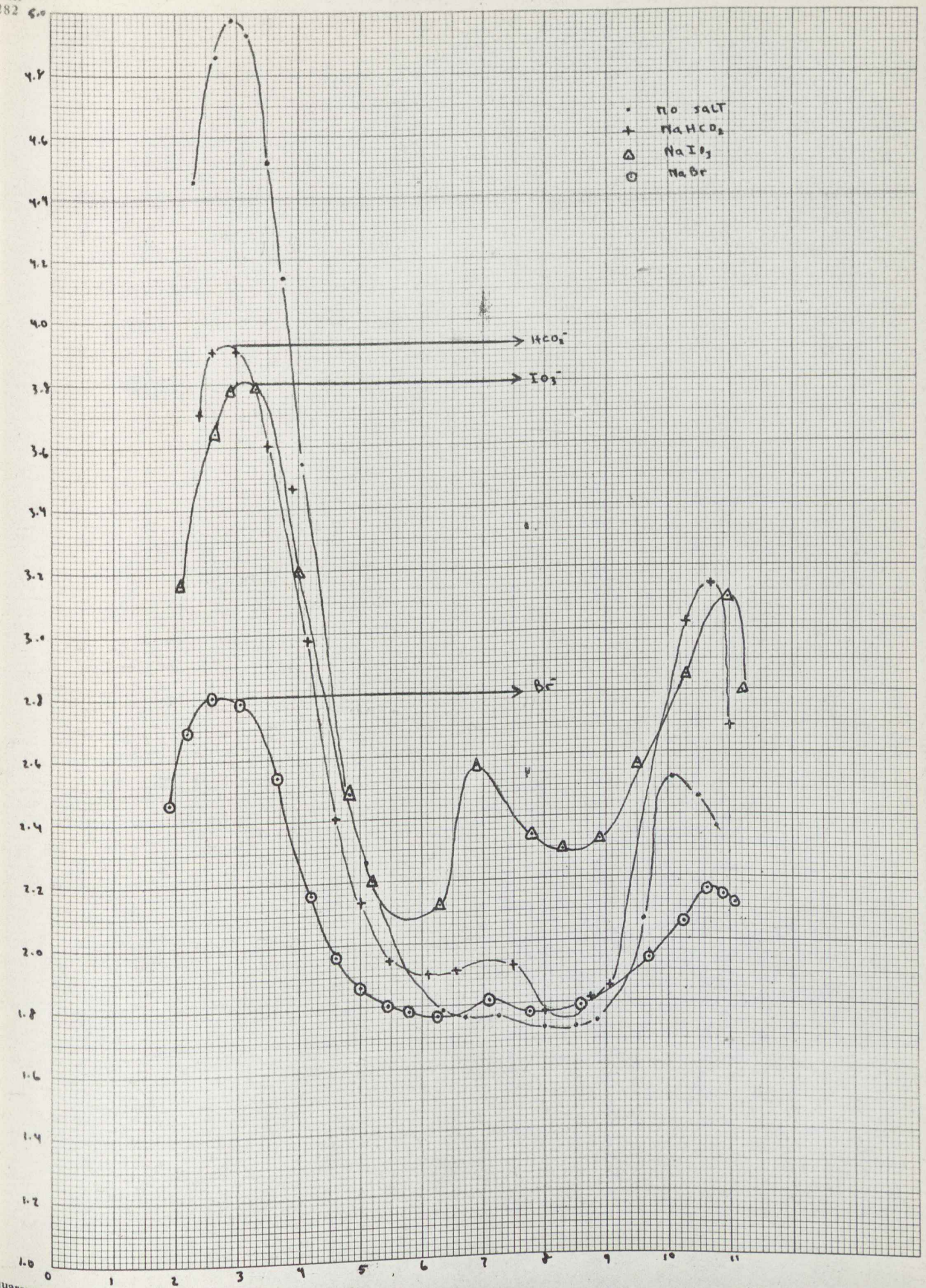
relative viscosity  
vs. pH



Graph # 2

0.1 Molar

relative viscosity vs. pH





Graph #3

relative viscosity  $\eta_r$   
pH

