

5-1952

The effects of pH and salts on the viscosity of gelatin sol (the Hofmeister series)

Frank Z. Pollara

Union College - Schenectady, NY

Follow this and additional works at: <https://digitalworks.union.edu/theses>



Part of the [Chemistry Commons](#)

Recommended Citation

Pollara, Frank Z., "The effects of pH and salts on the viscosity of gelatin sol (the Hofmeister series)" (1952). *Honors Theses*. 2237.
<https://digitalworks.union.edu/theses/2237>

This Open Access is brought to you for free and open access by the Student Work at Union | Digital Works. It has been accepted for inclusion in Honors Theses by an authorized administrator of Union | Digital Works. For more information, please contact digitalworks@union.edu.

The Effects of pH and Salts
on the
Viscosity of Gelatin Sol

UC

(The Hofmeister Series)

A thesis presented to the Department of Chemistry of
Union College in partial fulfillment of the requirements
for the Degree of Master of Science in Chemistry.

By Frank J. Pollara

Approved By Howard E. Luffer

UNION COLLEGE
LIBRARY

May 12, 1952

8
UO2
P771e
1952
c.2

Contents

	<u>Page</u>
Acknowledgment	1
Introduction	2
Historical	3-6
Nature of Gelatin	7-10
Viscosity	11-18
Experimental (Viscosity)	
Chemicals and Apparatus	19-20
Outline of Procedure	21
Details and Comments on Procedure	22-29
Results	30-32
Supplementary Experimental Work (Acid combining Capacity of gelatin)	
Procedure	33
Results	34-35
Discussion	36-50
Summary	51
References	52
Appendix	53-54

Acknowledgment

It is without hesitation that I express my sincerest gratitude to those individuals who have given me aid and encouragement in this work.

In particular, it would be most gratifying to me if this thesis be worthy of the patient guidance and consideration given to me and this problem by my research director and advisor, Dr. H. E. Sheffer.

I am also very much obliged to my other professors, Dr. C. B. Hurd, Dr. G. W. Ewing, Dr. J. F. Flagg, Mr. H. K. Holt, and their colleague, Dr. E. K. Bacon for the role they have played in making this work possible.

For my part, I hope that this unpretentious thesis will contribute even in the smallest possible fashion to the great pioneering work of Loeb, Michaelis and others.

Lastly, I do not think it out of place here to thank my good wife for the cheerful manner in which she typed these pages.

Introduction

The purpose of this investigation was to accurately determine the effect of monovalent sodium salts on the viscosity of gelatin sol as a function of pH and to establish the Hofmeister series by this method.

The gelatin investigated was derived from pigskin and the salts used were NaCl, NaNO₃, and NaI, of various concentrations.

In order to justify and make reasonable the conclusions reached in this investigation, these conclusions must be viewed in the light of the work previously done by other investigators of this field. With this in mind, a short historical survey of the origin and development of the so-called Hofmeister series and further, a brief sketch of the nature of gelatin will first be given.

Historical

The work of Hofmeister₁ (1888), namely, the coagulation albumin sol by various salts produced results such that one would conclude that these salts had differing coagulating power; coagulating power meaning the concentration of salt necessary to cause precipitation of the protein.

For negative albumin sol the following series can be formulated from Hofmeister's data;

for anions;

citrate, tartrate, SO_4 , acetate, Cl, NO_3 , ClO_3 , I, CNS

for cations;

Li, K, Na, NH_4 , Mg

The aforementioned series has henceforth become known as the Hofmeister or Lyotropic series.

Later, Pauli₂ (1903) modified the series for anions as follows:

F, SO_4 , PO_4 , citrate, tartrate, Ac, Cl, NO_3 , ClO_3 , Br, I, CNS

In addition to coagulation this series has been found to manifest itself in other physical measurements₃, i.e. viscosity, osmotic pressure, conductivity, swelling, membrane potential etc., as has been shown by many investigators.

However, Loeb₄ has contested the validity of the results (the Hofmeister series) of these researchers on the basis that they neglected to take into consideration the acidity of the sols investigated. This view was brought about by his experiments from which he concluded that for

a sol kept at constant pH the Hofmeister series does not manifest itself except in accordance with the valency rule, that is, the effect is the function of the charge and not the species.

Reviewing the work of Loeb, Michaelis₅ showed that Loeb's conclusion was correct in so far as his experiments carried him. However, he also showed through his and his co-workers' investigations, the existence of the Hofmeister series by an extension of the salt concentration range covered by Loeb. A review of some of these experiments at this point will make this last statement clear and will elucidate what is to follow.

The following table taken from Hofmeister's work shows the concentration of the various electrolytes needed to coagulate negative albumin sol:

<u>Sodium Salt</u>	<u>Moles/Liter</u>
Citrate	0.56
Tartrate	0.78
Sulphate	0.80
Acetate	1.69
Chloride	3.62
Nitrate	5.42
Chlorate	5.52
Iodide	Sat.
Thiocyanate	No precipitate

Here it may be noted that these values reflect the minimum amount of salt required to bring about precipitation. There can be no doubt from this data that a series is indicated.

Bearing the above experiment in mind it is advisable to turn to Loeb's work on the swelling of gelatin in which the pH was controlled. For exactness the following simplified curve (see figure 1) and the statement made by Loeb, with regard to the effect of seven monovalent sodium salts on the swelling of gelatin is presented.

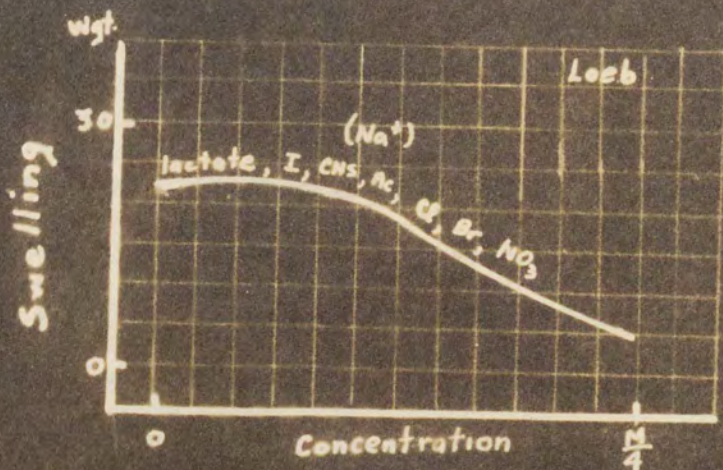
"It is obvious that the value of the effect of these seven salts lie on one curve, and that variations are essentially the chance variations due to limits of experimental accuracy. This is proved by the fact that the same variations occur when the concentration of the salt is zero, that is, when no salt is added. There is not the slightest indication of the Hofmeister Anion series. Slight influences of the salt on the cohesion of the gelatin may exist, but they are too small to play much of a role." The above needs no further comment.

The experiments of Dokan exhibit the effect of electrolytes on the swelling of agar-agar (Figure 2). Here Michaelis shows from the work of Dokan that at lower concentrations the results are in agreement with Loeb; that is, no Hofmeister series is apparent but that at higher concentration the Hofmeister series manifests itself.

Surely, then a Hofmeister series does exist but its sensitivity depends upon the concentration of salt that is employed. This point shall be dealt with more fully in the final discussion.

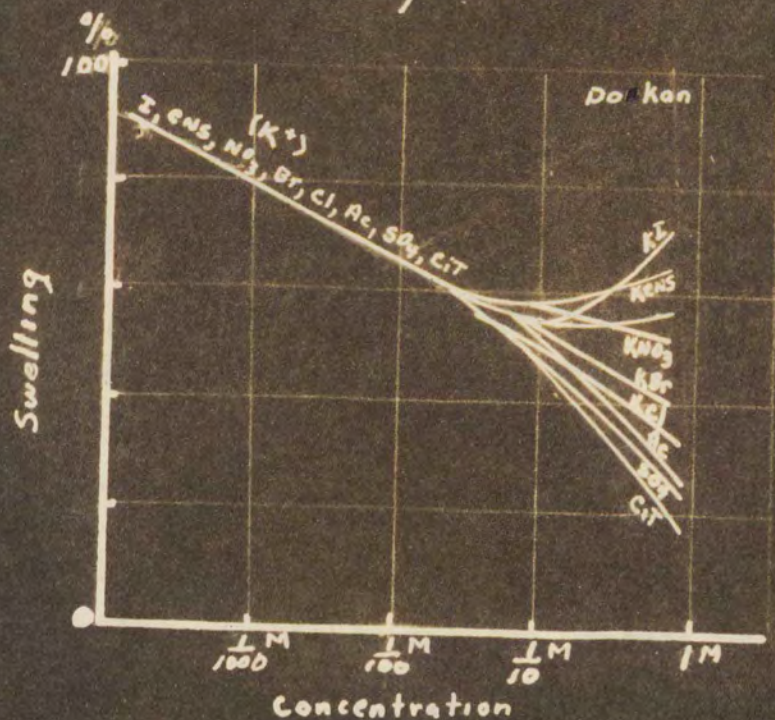
(Fig. 1.)

THE EFFECT of SALTS
on the
SWELLING of GELATIN



(Fig. 2.)

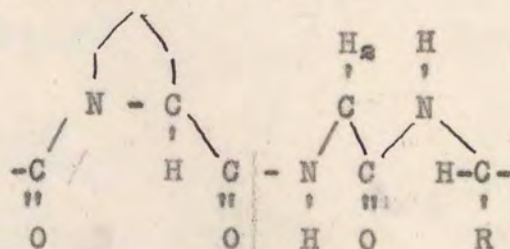
THE EFFECT of SALTS
on the
SWELLING of AGAR-AGAR



The Nature of Gelatin

Gelatin is perhaps one of the most worked with substance of which comparatively little is known with regard to its structure. Because of its complexity the interpretation of any physical measurement based on chemical interaction becomes a difficult task. However, from the preponderance of studies made on this substance a more or less justifiable interpretation can be given to many of the physical measurements made and, even at this, we find in the literature much conflict of opinion.

Gelatin is the product of boiling collagen in water. The structure of collagen based largely on X-ray analysis₆ has been suggested to be:



In the conversion of collagen to gelatin, Cherbuliez and Meyer₇ state that this process is carried out in two steps; namely, (a) the breaking down of a network of crystalline collagen into an amorphous substance which is of a loose tridimensional structure (b) the breaking of reticular bond of the tridimensional structure by further hydrolytic sission. Gies and Emmett₈ claim that in this conversion gelatin is not the product of hydrolysis, but a rearrangement of the collagen structure. Sokolou and Fel'daman₉ have shown that gelatin is the result of grad-

ual destruction of cross bonds which hold together the polypeptide chains; the initial destruction being accomplished by the alkali used in the treatment of hides, and the boiling in water being the continuation of the process through thermal action. This is essentially the result of the previous work mentioned. However, these authors have shown that collagen treated for different lengths of time yield gelatin with differing isoelectric points, the result being the liberation of (different numbers) polar groups.

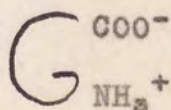
As an indication of the great number of polar groups characteristic of proteins, the following amino acids derived from gelatin are listed:

<u>Amino Acid</u>	<u>% of Total</u>
Glycine	25.5
Alanine	8.7
Valine	0.0
Leucine and isoleucine	7.1
Phenylalanine	1.4
Tyrosine	0.0
Tryptophan	0.0
Threonine	-
Glutamic acid	5.8
Hydroxyglutamic acid	0.0
Aspartic acid	3.4
Proline	9.5
Hydroxyproline	14.1
Serine	0.4
Cystine	0.2
Methionine	-
Arginine	8.2
Histidine	1.0
Lysine	5.9
Ammonia	0.4

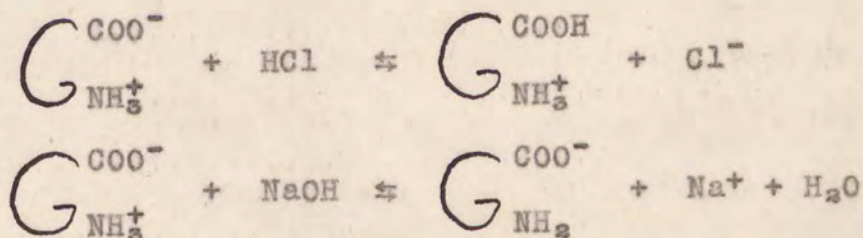
In addition to the aforementioned some work has been done with regard to X-ray analysis of gelatin₁₀. Here it was found that gelatin contains some crystalloidal aggregates and that upon stretching, gelatin yields a complete fibrous pattern much the same as an intermediate between rubber and cellulose.

Work on dissolved molecules of gelatin revealed that they are folded and coiled up to approximately 1/5 their extended length and as such are still rod like in shape₁₁.

Up to this point, it could be concluded that gelatin is a long fibrous like molecule coiled up in rod shape with many of the polar groups of amino acids extending between the folds and from the surface of the particle. This picture has been represented by some authors as



where the zwitter-ion theory is used. Accordingly, the work of Loeb, Sørensen, Pauli and Michaelis indicate that such a structure goes into chemical combination in accordance with the classical laws of chemistry.



In connection with the above, the work of Friedman, Klem and Thompson₁₂ on the acid and base combining capacity of gelatin is of importance. They conclude that this combination is apparently more complex than just the combination of H^+ or OH^- with gelatin. However, in the process of coming to this conclusion, they summarized the stoichiometric relation between HCl and gelatin obtained by various methods. As will be seen, the combining capacity of gelatin for acid₁₃ will indirectly prove fruitful in the interpretation of the results obtained from this investigation.

Viscosity

Since viscosity measurements is the modus operandi of this investigation, it is appropriate that a discussion of viscosity measurements with regard to large solute particles now be given. Further, it will be seen that these considerations have a direct bearing on the choice of procedure used.

In the derivation of Poiseuille's equation₁₄ for viscosity, the liquid is assumed to move in streamline flow through a capillary and that the liquid adjacent to the wall of the tube remains fixed. As a consequence of these assumptions, it is found that the velocity at any radius from the axis of the tube is given by:

$$v = - \frac{r^2 P}{4\eta l} + C$$

where

P= pressure due to head of liquid

l= length of tube

η = viscosity coefficient

C= constant of integration

From the above equation it can be seen that the velocity is a parabolic function of the radius. When the liquid contains a solute of large particle size it follows that such particles are subject to an inhomogenous velocity gradient. This problem has been considered by Einstein₁₅ who, through hydrodynamic considerations, produced an

equation for the relative viscosity for such cases. The expanded form of this equation is:

$$\frac{\eta}{\eta_0} = 1 + 2.5\phi$$

where

ϕ = aggregate volume of solute in unit volume
of suspension

η_0 = viscosity of the solvent

This equation holds for dilute solutions in which the particles are spherical. However, there have been several modifications of Einstein's formula which have been shown to agree better with the actual observation of proteins. Among these modifications is the Arrhenius equation:

$$\ln \eta - \ln \eta_0 = \theta \phi$$

where

θ = a constant

This equation is useful when dealing with higher viscosities.

Although no attempt will be made to use these equations directly, the functional dependence of η on ϕ will be needed and used in the final conclusions.

In addition to the above concentration effect (ϕ) Smoluchowski₁₆ states that particles which are charged should show an increase in viscosity over and above that due to the Einstein effect on the basis that such charged particles in motion set up an opposing electromotive force. On this assumption, he has produced the following viscosity equation:

$$\frac{\eta}{\eta_0} = 1 + 2.5\phi \left[1 + \frac{1}{\lambda \eta_0 r^2} \left(\frac{D \zeta}{2 \pi} \right)^2 \right]$$

where

λ = the specific conductivity

r = radius of particle

D = dielectric constant

ζ = electrokinetic potential

n = viscosity of solvent

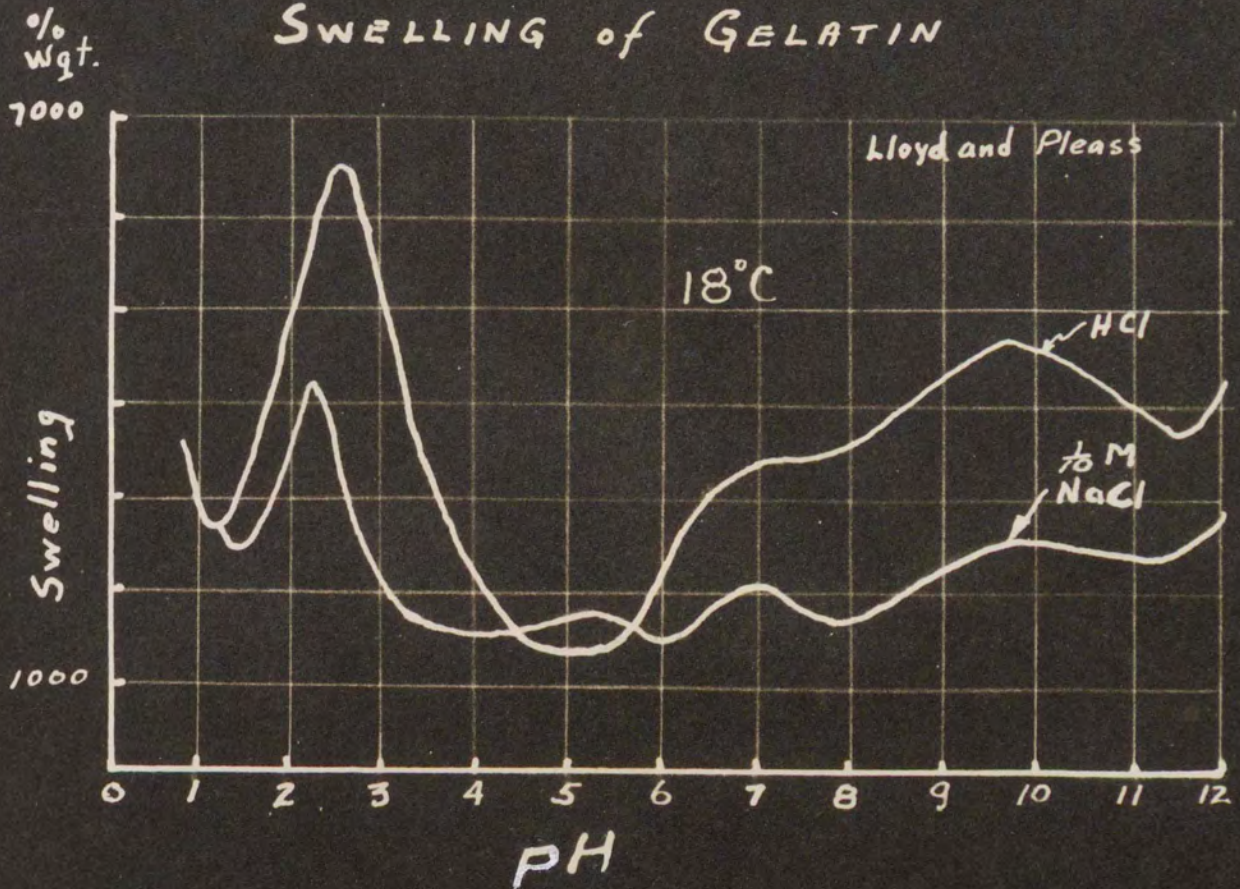
Although the factors of volume fraction and charge are predominant in viscosity, temperature, degree of solvation, thermal and mechanical treatment, presence of reacting and non reacting electrolytes add to the complexity of the interpretation of viscosity measurements.

With regard to reacting electrolytes, Lloyd and Pleass¹⁷, Freundlich¹⁸, Loeb⁴, and others have shown that the viscosity and swelling of gelatin is a function of pH. This is shown in Figures 3 and 4. In both the curves of Freundlich, and Lloyd and Pleass, it should be noted that at a pH of ca. 4.7 a minimum is evident. This pH at which the minimum occurs is called the isoelectric point. That is, the point of minimum charge where the protein is predominantly in the $\text{C}_{\text{NH}_3^+}^{\text{COO}^-}$ form.

In contrast to this isoelectric point of pH of 4.7 Davis and Oaks¹⁹ work on viscosity revealed an isoelectric

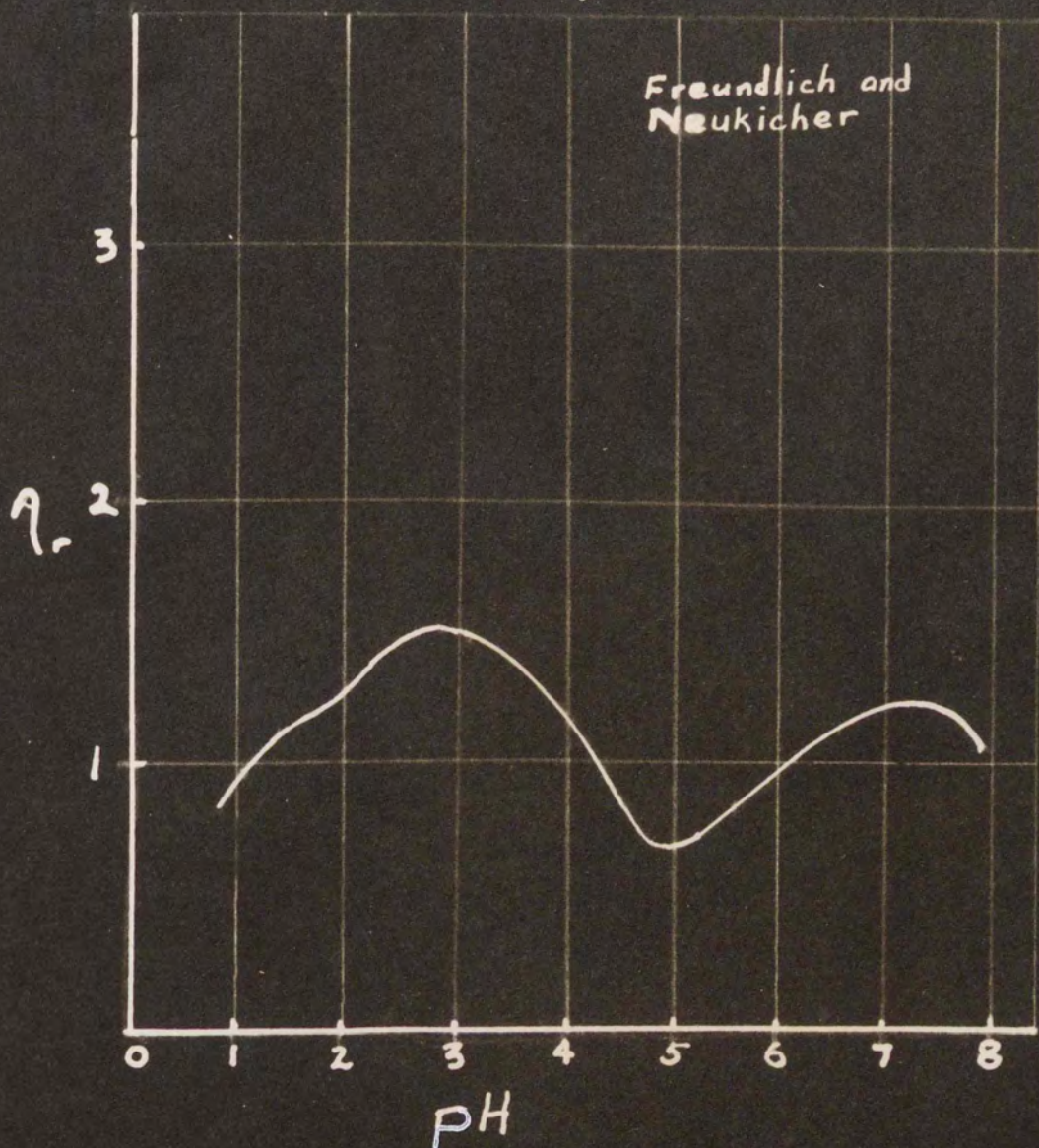
(Fig. 3)

THE EFFECT of pH and SALT
on the
SWELLING of GELATIN



(Fig. 4)

THE EFFECT of pH
on the
VISCOSITY of GELATIN



point of pH 8 and in the present work one of pH 8.6 (see Figure 13). This variation can be accounted for by the work of Wilson and Kern¹⁹ who showed that gelatin exists in two equilibrium forms; these being known as a sol form that will not gel and a gel form which will gel, both being stable at 35° C and 15° C respectively. The work of Loeb, Freundlich and Lloyd being carried out at ca. 20° C yielded the lower isoelectric point and that of Davis and Oaks and the present investigation being carried out at 40° and 30° C respectively yielded the higher isoelectric point. That the two isoelectric points can exist at one temperature is shown in Figure 5. The above will also explain the change in slope at the region of pH 8 shown on the swelling curves of Lloyd and Pleass (see Figure 3).

The effect of the presence of indifferent electrolytes has been investigated by many workers. Of interest in this connection is the work of Ostwald and Stuart²⁰ who showed that the viscosity of 1.4% gelatin sol at the isoelectric point is a function of the activity coefficient given by

$$\eta - 1 = k \gamma$$

where

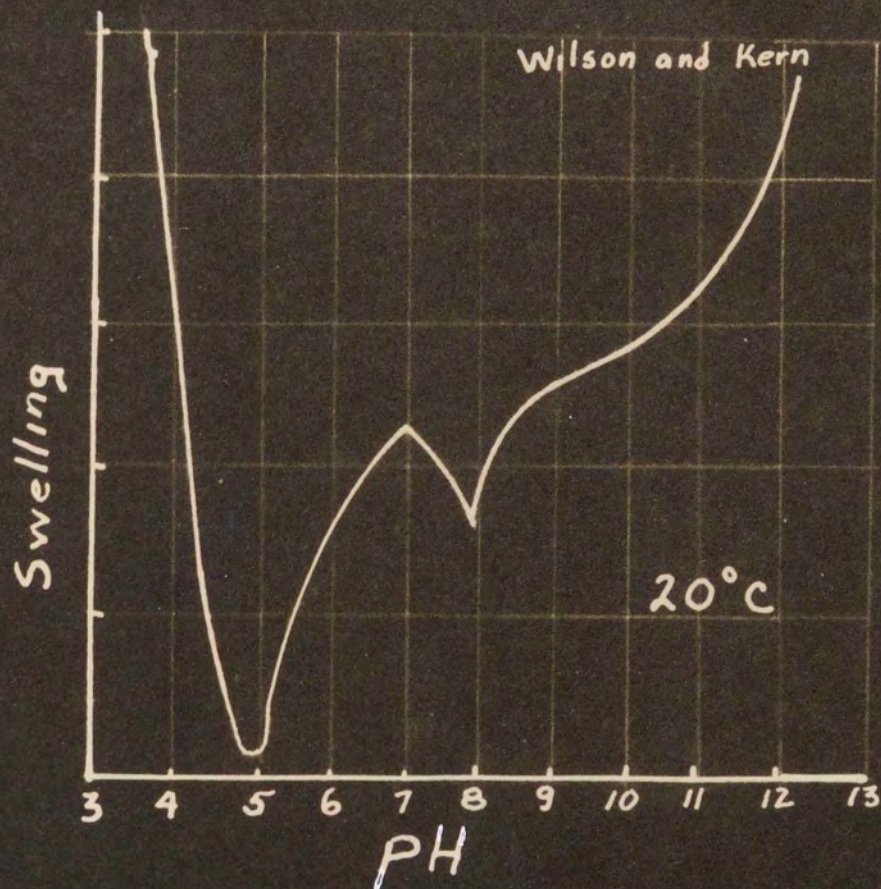
k = a constant

γ = the activity coefficient

The results of this relation were given for NaCl, Na₂SO₄ and Na₄Fe(CN)₆. Unfortunately, the data was not obtained

(Fig. 5)

THE TWO ISOELECTRIC
POINTS of GELATIN



for I, nor the NO_3 .

Loeb_{21,4} attributes the high viscosity of gelatin to the amount of occluded H_2O as regulated by Donnan Equilibrium. A more detailed explanation of this point will be held over for later discussion.

Dhar₂₂ claims that the gelatin absorbs more electrolytes of opposite charge than an ion having the same charge which results in a net decrease of charge. This is supposed to increase hydration and manifest itself as an increase in viscosity. He also points out that peptization, due to the addition of acid or base, is associated with increase hydration and hence an increase in the viscosity.

The factors of temperature on ageing of gelatin and pH will be discussed under the experimental part of this thesis to eliminate duplication.

It is believed that the above survey should give a sufficient background for the introduction to the present problem.

Experimental

The experimental portion of this thesis has been divided into the following four parts:

I Chemicals

II Apparatus

III Outline of Procedure

IV Details and Comments on Procedure

A few remarks with regard to parts III and IV will be useful. As will be noted, part III is given in extended step by step form. This has been done for recording the most expedient and systematic procedure found by this experimenter with regard to this investigation. Further, in the practice of carrying out the procedure given in part III, it was found that the working substance (gelatin) has properties foreign to the more common solutions and as such, many variations of ordinary techniques had to be employed in the handling of this material. Part IV covers these variations in detail as guides for subsequent investigators.

In part III each major operation has been numbered. This number was carried over to part IV; thus associating the practice and precautions followed in each step.

I Chemicals

1. Eastman Purified Pigskin Gelatin

Isoelectric Point pH - 8.6

pH on 1% solution 5.69

(continued on next page)

I Chemicals (continued)

Ash on moisture free

basis 0.02%

Albumin on free basis 0.02%

Lot# 171-Feb. 1, 1951

Made by Eastman Kodak

Co., Rochester, N.Y.

Research Laboratory

2. Salts - NaCl , NaNO_3 , NaI

3. Acids - HCl , HNO_3 , HI

4. Buffers for pH meter

II Apparatus and Miscellaneous Materials:

1. 2 - Ostwald Viscometers (Will)

Time of H_2O (outflow)

#1 - 111.0 sec.

#2 - 115.0 sec.

2. 2 - Stopwatches

0.2 sec.

3. 1 - Constant temperature bath equipped as follows:

1- Thermometer 100°C

2- Heating elements (knife type)

1- Thermo regulator, Seargent 5 - 81835

0.01°C

1- Motor stirrer and speed control

1- Power control unit complete

4. 1 - Beckman pH meter equipped as follows:

1- glass electrode (outside use)

1- calomel electrode (outside use)

1- thermometer 100°C

III Outline of Procedure

- 1 - Adjust constant temperature bath to 30° C
- 2 - Weigh gelatin
- 3 - Add necessary amount of solvent
- 4 - Let stand
- 5 - Dissolve gelatin
- 6 - Filter gelatin
- 7 - Divide gelatin into two portions (Bulk I and II)
- 8 - Place I and II in constant temperature bath
- 9 - Zero in pH meter
- 10 - Clean two viscometers
- 11 - Rinse viscometers with solution I
- 12 - Pipette 5 ml. of solution I into viscometers
- 13 - Make time run for both viscometers
- 14 - During step 13 measure pH of a portion of bulk I
- 15 - After 14, return solution to bulk I and slightly acidify bulk with proper acid
- 16 - Record findings of step 13 and 14
- 17 - Leaving one viscometer undisturbed, repeat steps 10 - 14 with the acidified solution
- 18 - Make rerun of undisturbed solution
- 19 - Continue as outlined down pH scale
- 20 - Repeat steps 10 to 19 for bulk II using NaOH for pH adjustment

IV Details and Comments on Procedure

(1) The gelatin which was obtained in sheet form (approximately $2 \frac{3}{4}$ grams per sheet) was weighed accurately and enough solvent (water or salt solution) added to give a solution whose composition would contain 1 gram of gelatin as supplied to 100 grams of H_2O (or 0.902 grams of moisture free gelatin per 100.1 grams of water) irrespective of whether the solvent was water or one of the salt solutions used. (2) This was accomplished by calculating the weight of salt solution required to yield the desired grams of water necessary. The total weight of solvent, gelatin, and a 1000 ml beaker used to contain the mixture, was determined.

The gelatin and beaker were then placed on a platform balance and enough solvent added to make up the total weight. The specific solvents used in this investigation are tabulated below:

<u>SALT</u>	<u>MOLARITY</u>			
	<u>0</u>	<u>1/100</u>	<u>1/10</u>	<u>1</u>
NaCl	x	x	x	x
NaI	x	x	x	x
NaNO ₃	x	x	x	x

(3) Of course, the molarity of the various solvents was altered due to the addition of the gelatin. This change was determined by measuring the volume of a dry sheet of gelatin which amounted to about 200. Hence, in 250 ml of solution, (the approximate amount made from each sheet), the molarity would be lowered approximately 8 parts per

1000, which would not lead to any severe error if the solutions were considered to be of the original molarity.

(4) One of the mixtures now made up was covered and allowed to stand for 24 hours at room temperature, allowing it to imbibe water, hence facilitating the disintegration of the gelatin. It was noted at this point that after imbibition, the gelatin was many times its original size and still intact.

(5) The mixture was then placed over a steam boiler (1000 ml beaker) and the contents heated to 60° C while being continually beaten to break up the gelatin. In each case, it was noted that complete disintegration to a homogeneous solution could not be accomplished. There always remained some macroscopic particles which were about 1/8 of an inch long and 1/64 of an inch in diameter; only rod shaped ones being observed. After maximum disintegration, the complete system was again weighed and enough water added to make up that lost by evaporation.

(6) The solution was then filtered to eliminate the visible particles which would be detrimental in the course of viscosity measurements. To determine the loss in weight of the gelatin due to filtration, the filter papers were weighed before and after (dry). This showed approximately 3.5 mg of gelatin lost per 2.75 grams of gelatin, approximately a loss of 1 part per 1000. Hence, the composition is assumed to be the same as before filtration.

(7) The filtered solution was now divided into two parts (one for the addition of acid, the other for the addition of base) and placed in a water bath held at 30°C.

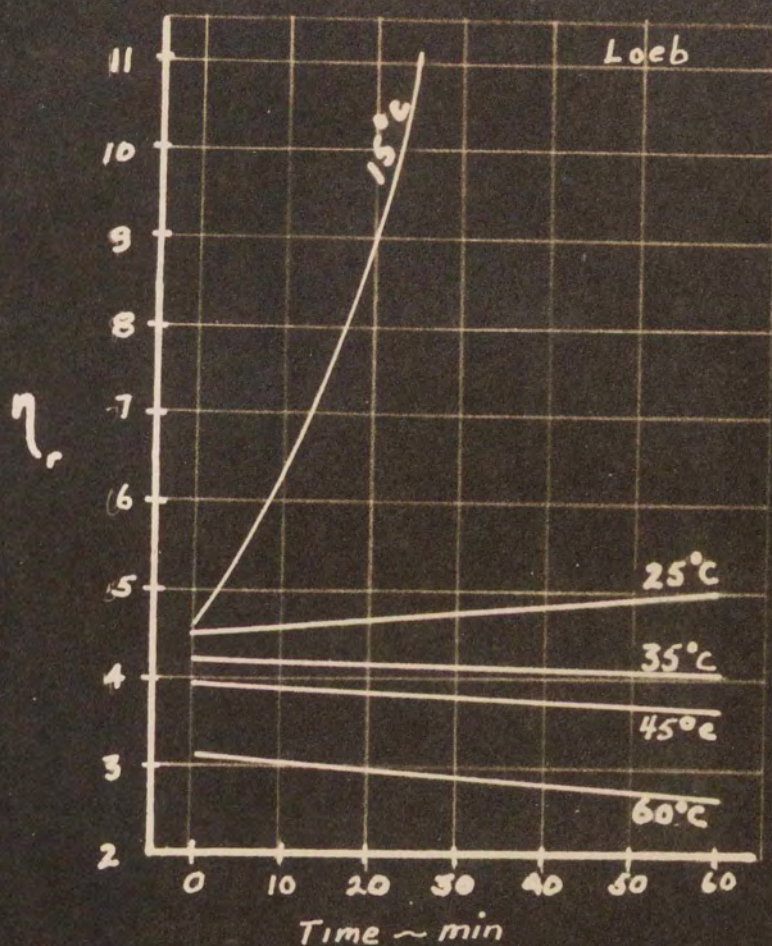
(8) This temperature was chosen for the following reasons:

1. Lower temperature would be impossible to control due to room temperature conditions exceeding that of bath.
2. Loeb₄ has shown that with gelatin above ca. 30° C, the viscosity drops off with age and below ca. 30° C the viscosity increases rapidly with age (see figure 6). Hence, to eliminate the variable of viscosity as a function of ageing, the gelatin was used at this temperature.

When the solution cooled to 30°C, 5 ml rinse portions were pipetted into two Ostwald viscometers (time of H₂O discharge 111.5 and 115.0 sec. respectively). (11) These portions were removed from the viscometers by means of an aspirator until no drainage showed in the U tube of the viscometer. This use of a wet viscometer is contrary to normal practice, but since it was known that changes of pH effects the viscosity so readily, it was decided that two rinses of the solution to be measured would eliminate variations in pH from other sources such as H₂O washing and drying solvents and that further justification could be found in the design of the Ostwald viscometer to minimize any error in head due to such a variation of procedure.

(Fig. 6)

THE AGING of GELATIN
as a
FUNCTION of TEMPERATURE



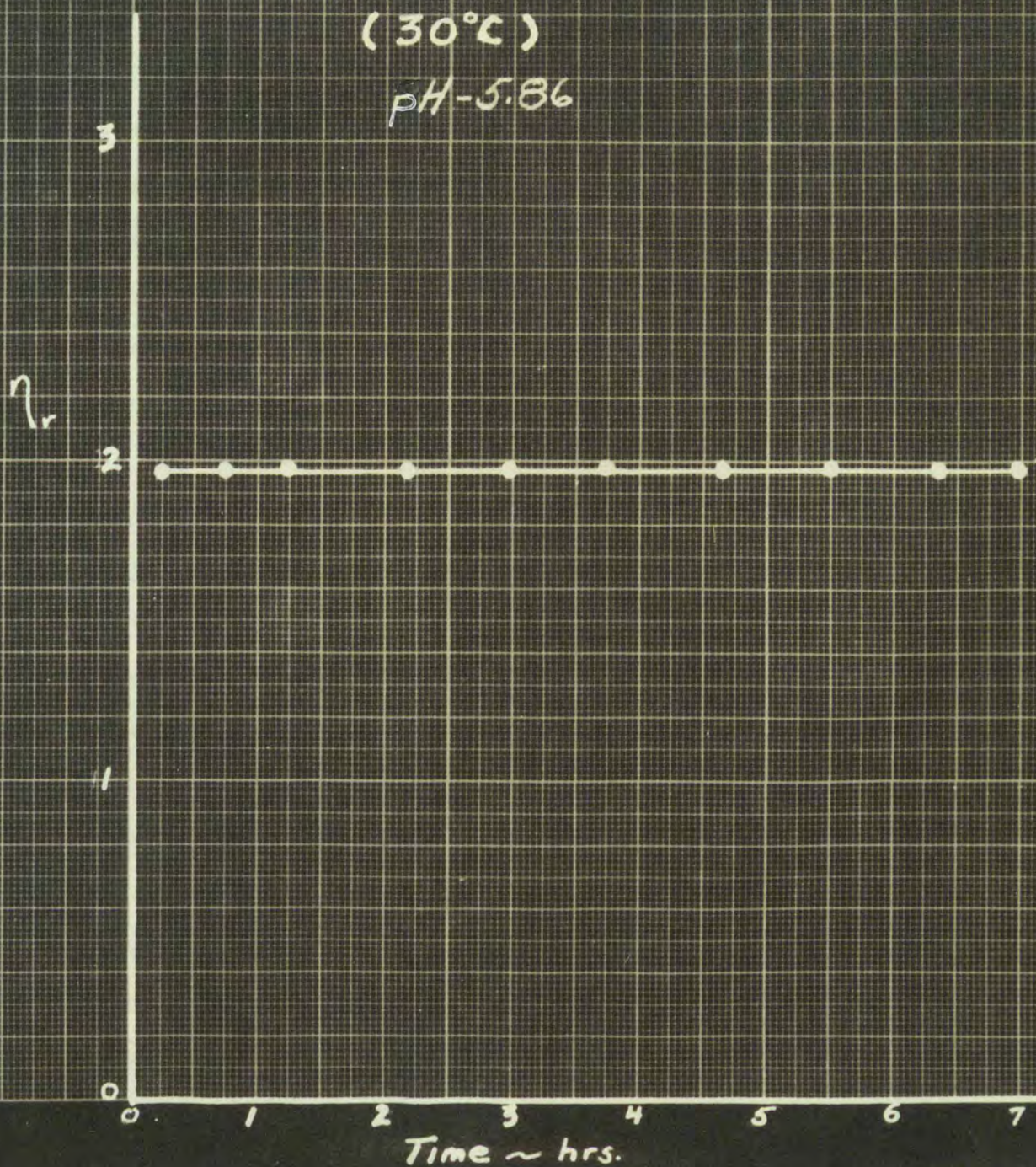
(12) Upon pipetting the third 5 ml portion into the viscometers care was exercised to make sure the solution did not splash. This is mentioned not with regard to lost droplets but with regard to a foam that is produced upon agitation of the gelatin solution. This foam in the large tube makes the head uncertain and also leads to a train of many minute air bubbles which travel up the capillary tube, counter-current to the downward flow of liquid and hence cannot be tolerated. Further, as the solution becomes more viscous, chance of escape of these bubbles before the liquid proceeds down the tube is not enhanced.

(17) It should be stated at this point why only two viscometers were used. It has been shown by Hatschek₁₄ that the viscosity of elongated molecules varies with the velocity gradient. This being the case, only one viscometer can give comparable results unless more than one can be found with the same radius (same time of outflow). Hence, to eliminate this problem completely only one viscometer was used for the viscosity measurements made as a function of pH. (18) The purpose of the second viscometer was to determine if any change in viscosity took place due to ageing as pointed out under the discussion of temperature control. As was found, no change took place at this temperature. (see Figure 7)

(15) The pH of the solutions was adjusted by the addition of minute amounts of concentrated acid or base of the corresponding salt shown in above table. Concentrated

(Fig. 7)

THE VISCOSITY of GELATIN SOL
as a
FUNCTION of TIME
(30°C)
pH-5.86



acid and base were used to minimize any change in volume. The corresponding acid was employed in order that only one anion species would be present.

Measurement of pH was accomplished by the use of a Beckman pH meter equipped with outside glass and calomel electrodes. These electrodes were always washed and then rinsed with the solution to be studied, rather than washed and wiped clean for the same reason given in (11). It should also be pointed out that in the actual measurement of pH, enough time was to be allowed and swirling of the solution was to be employed in order to obtain equilibrium between the electrodes and the solution. These two last precautions were found necessary in order to obtain a steady reading on the meter in as short a time as possible.

As was pointed out in the outline under step 19, it was necessary to traverse the pH scale, as prescribed in step 15, by the addition of concentrated acid or base. Here it should be pointed out that this process is a one way procedure. Once acid has been added to the solution, it is not correct to add base if the pH desired is overshoot; for such a practice would result in the production of salt which would invalidate all subsequent measurements made on the remaining solution.

Observing and adhering to all that has been said, the time of outflow and pH of the various solutions were measured. The results obtained are shown in graphical form in Figures 8, 9, 10, plotted as relative viscosity verses pH.

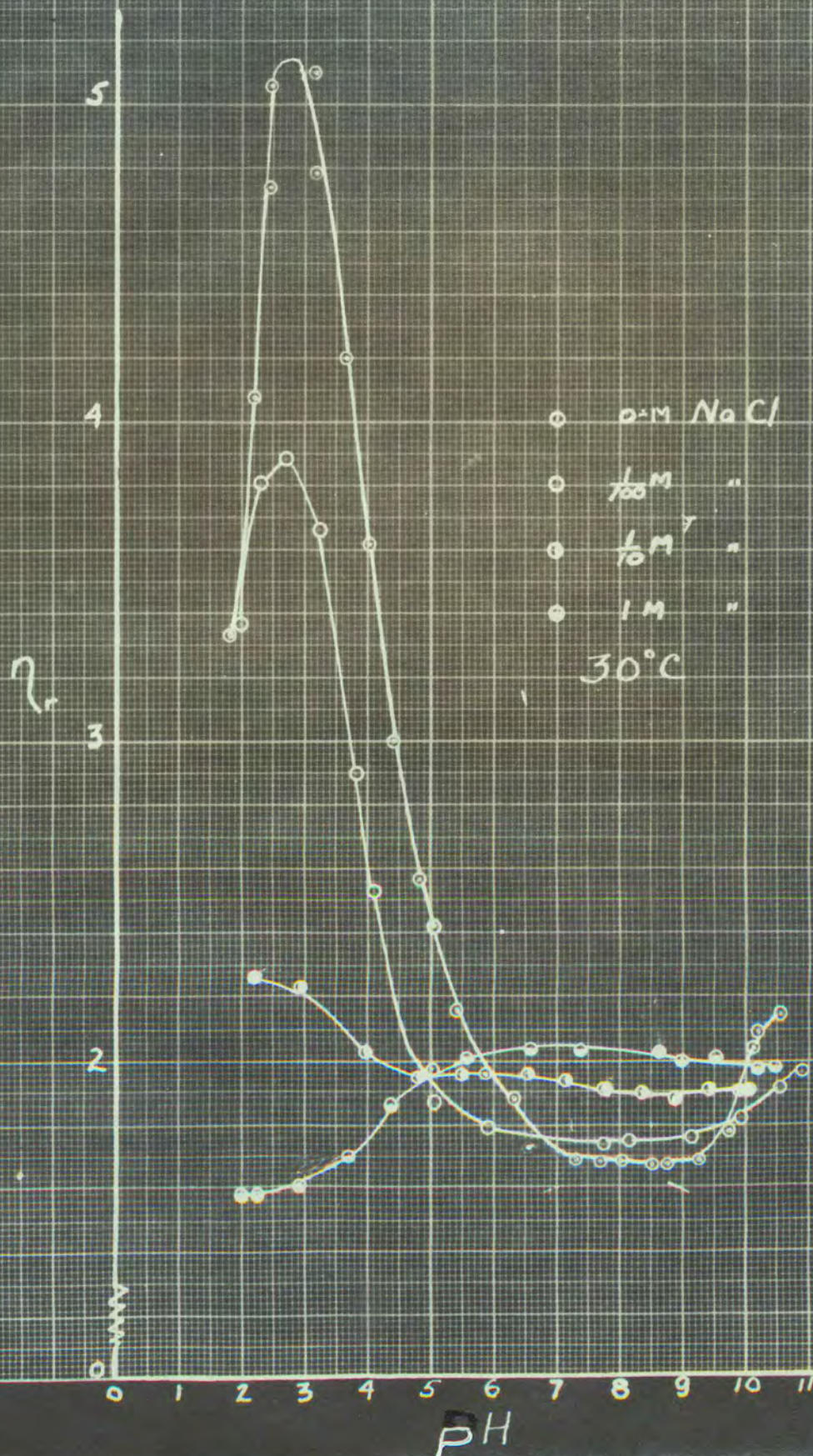
$$\text{Relative viscosity} = \frac{(\text{Time of outflow of sol})(\text{density of sol})}{(\text{Time of outflow of H}_2\text{O})(\text{density of H}_2\text{O})}$$

Since the ratio of the two densities in this equation are ca. 1, the equation may be modified to:

$$\text{Relative viscosity} = \frac{\text{Time of outflow of sol}}{\text{Time of outflow of H}_2\text{O}}$$

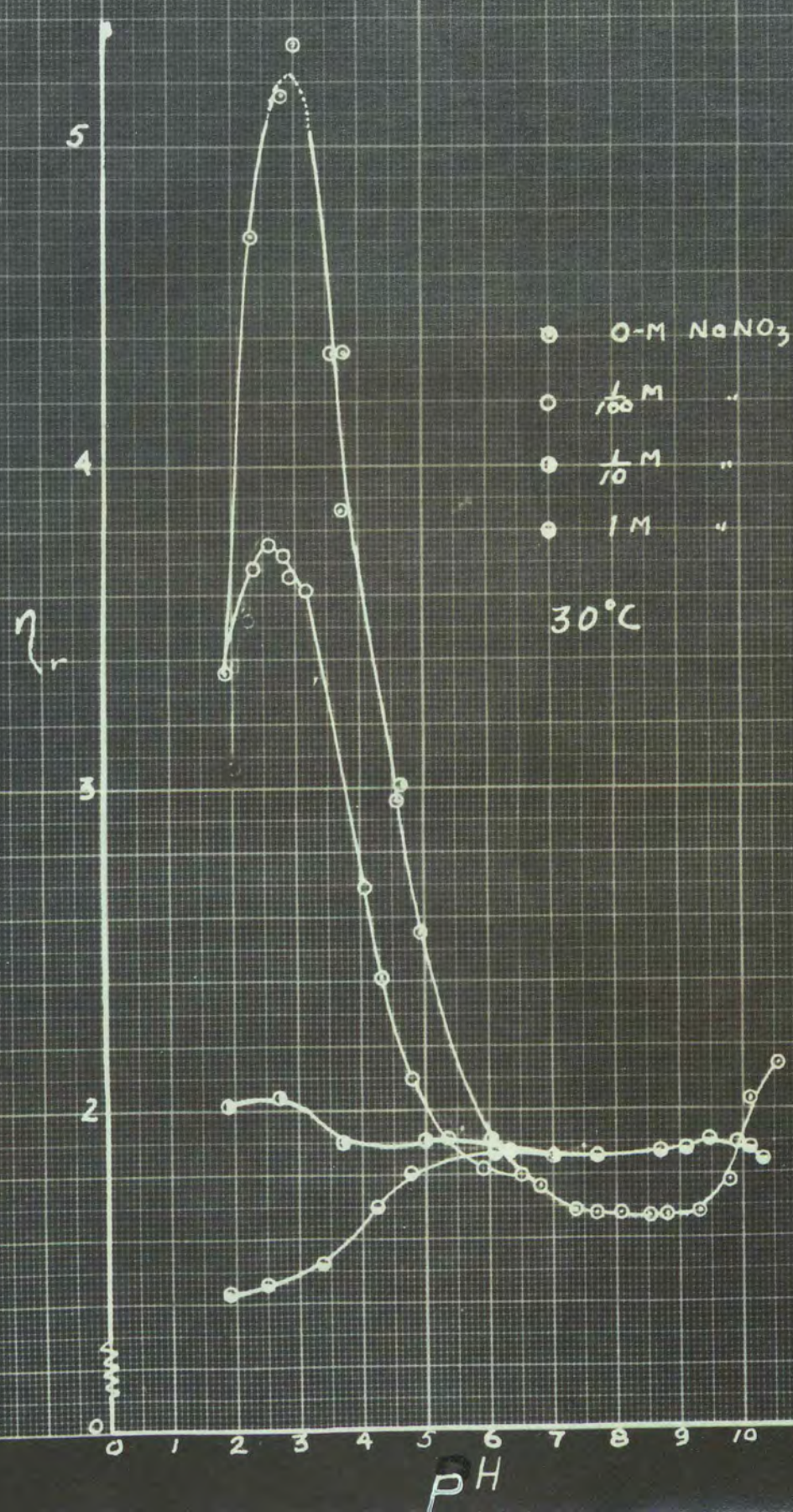
(Fig. 8)

THE VISCOSITY of GELATIN-NaCl Sol.
as a
FUNCTION of pH and CONCENTRATION



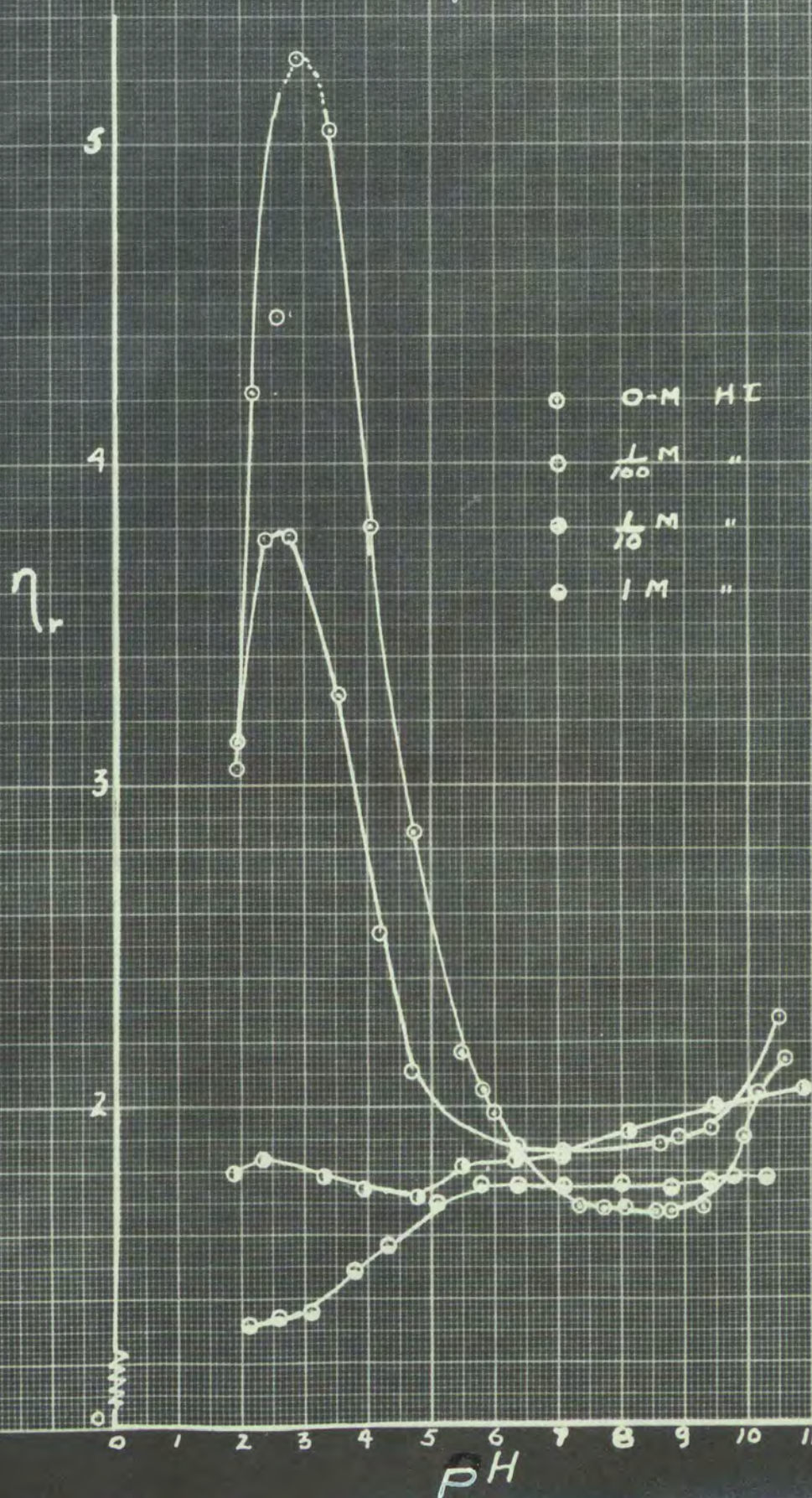
(Fig. 9)

THE VISCOSITY of GELATIN - NaNO_3 SOL
as a
FUNCTION of pH and CONCENTRATION



(Fig. 10)

THE VISCOSITY OF GELATIN-NaI SOL
as a
FUNCTION of pH and CONCENTRATION.



Supplementary Experimental Work

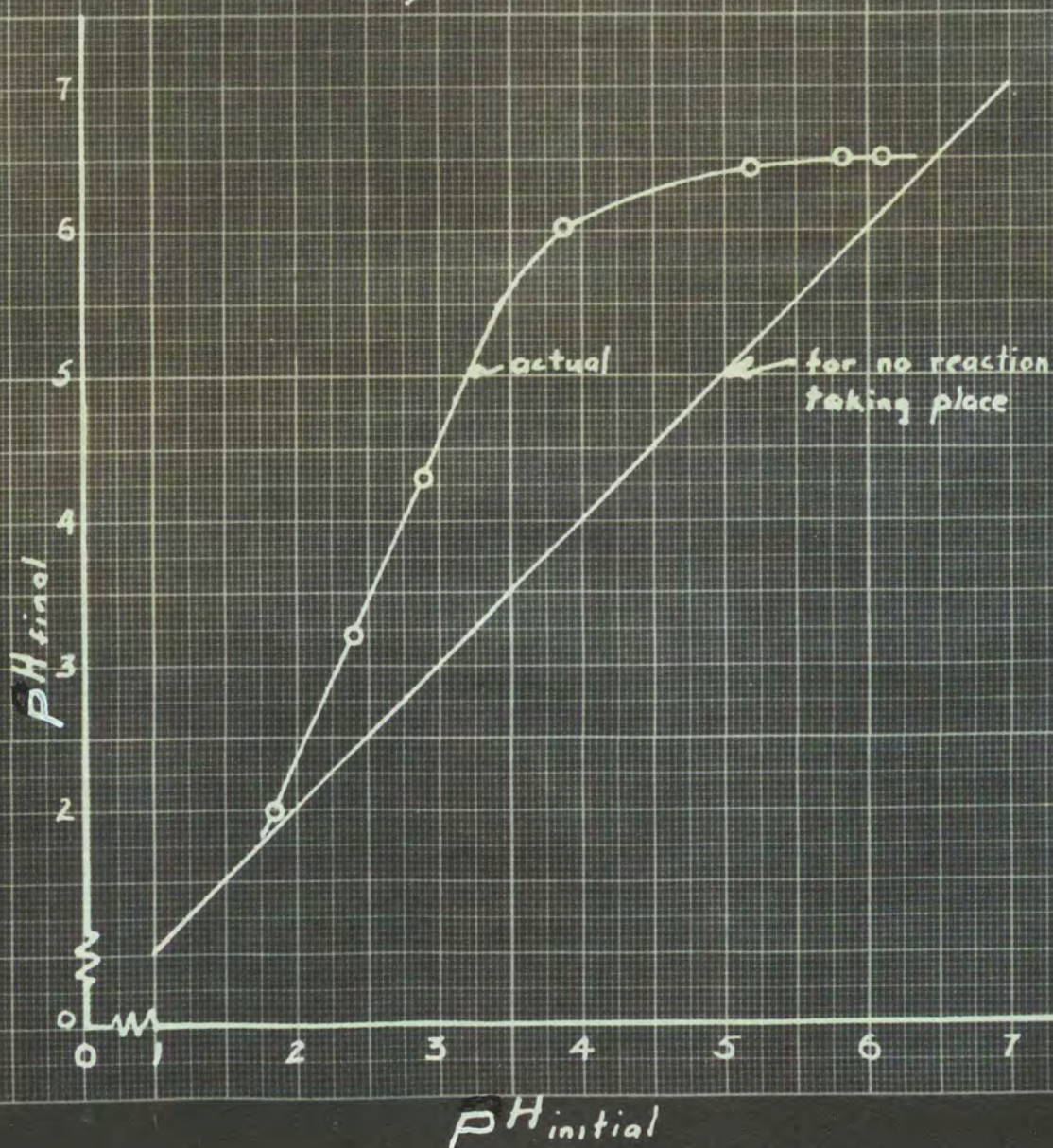
In conjunction to the aforementioned, it was found that additional information was needed for the interpretation of these curves. Hence, the following experimental work was performed. This work covers the amount of acid combined with the gelatin as a function of pH.

Using the Beckman pH meter, an HCl solution of about pH-1 was made. Ten ml of this solution were pipetted into a 125 ml beaker and set aside. The balance of the solution was diluted to a higher pH, approximately 2, and this was pipetted into a beaker and also set aside. This procedure was continued until a series of acid solutions were prepared ranging from ca. pH-1 to pH 6. The exact pH values were recorded in each case. To each of these solutions, 10 ml of a 1 percent gelatin solution was then added after which the pH was again measured and recorded.

The results of this supplementary experiment are shown in figure 11, with an adjustment made in pH for the dilution of the original acid solutions by the gelatin added.

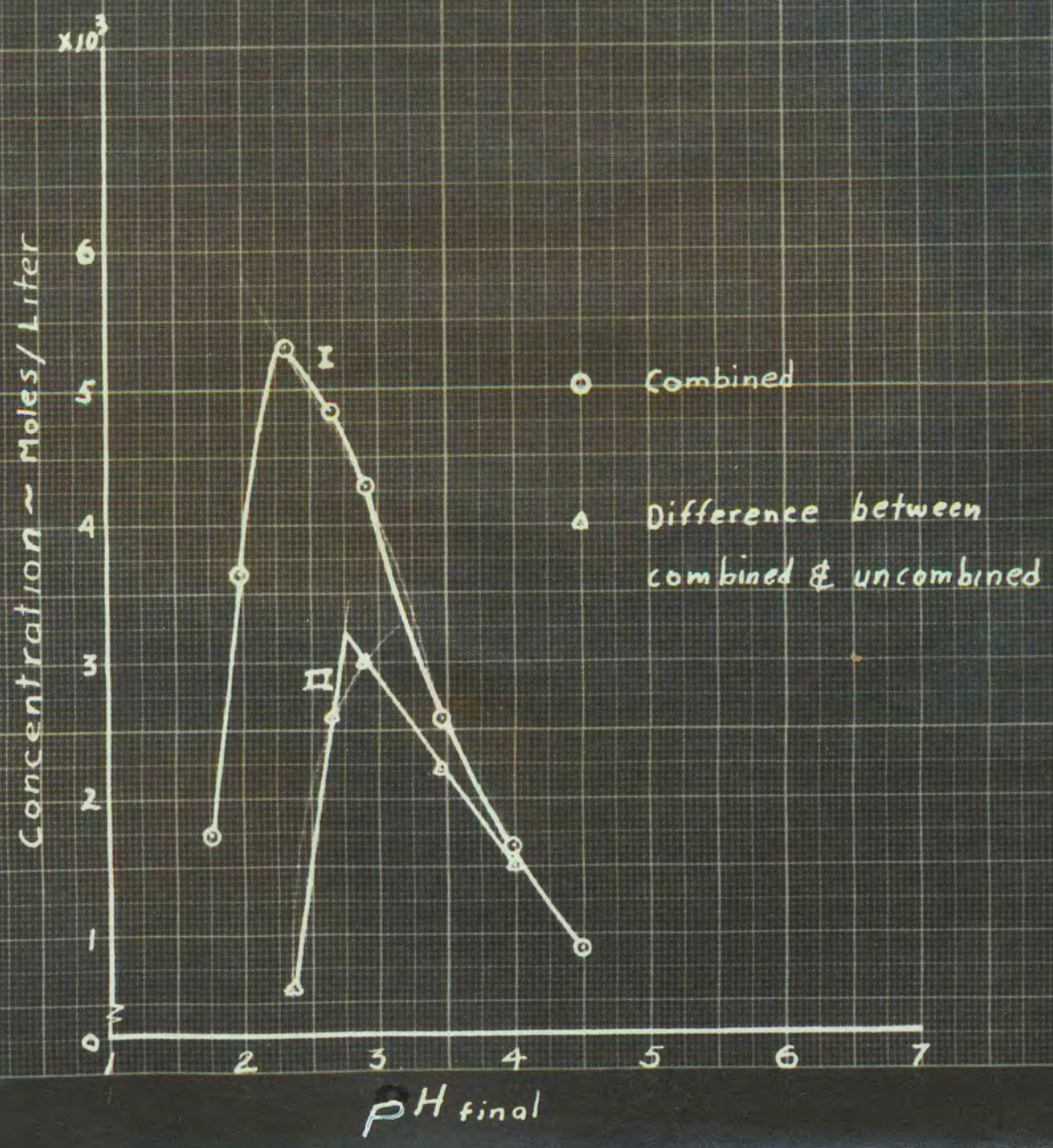
(Fig. 11)

THE COMBINING CAPACITY
of
GELATIN for HCl



(Fig. 12)

THE COMBINING CAPACITY of GELATIN for HCl



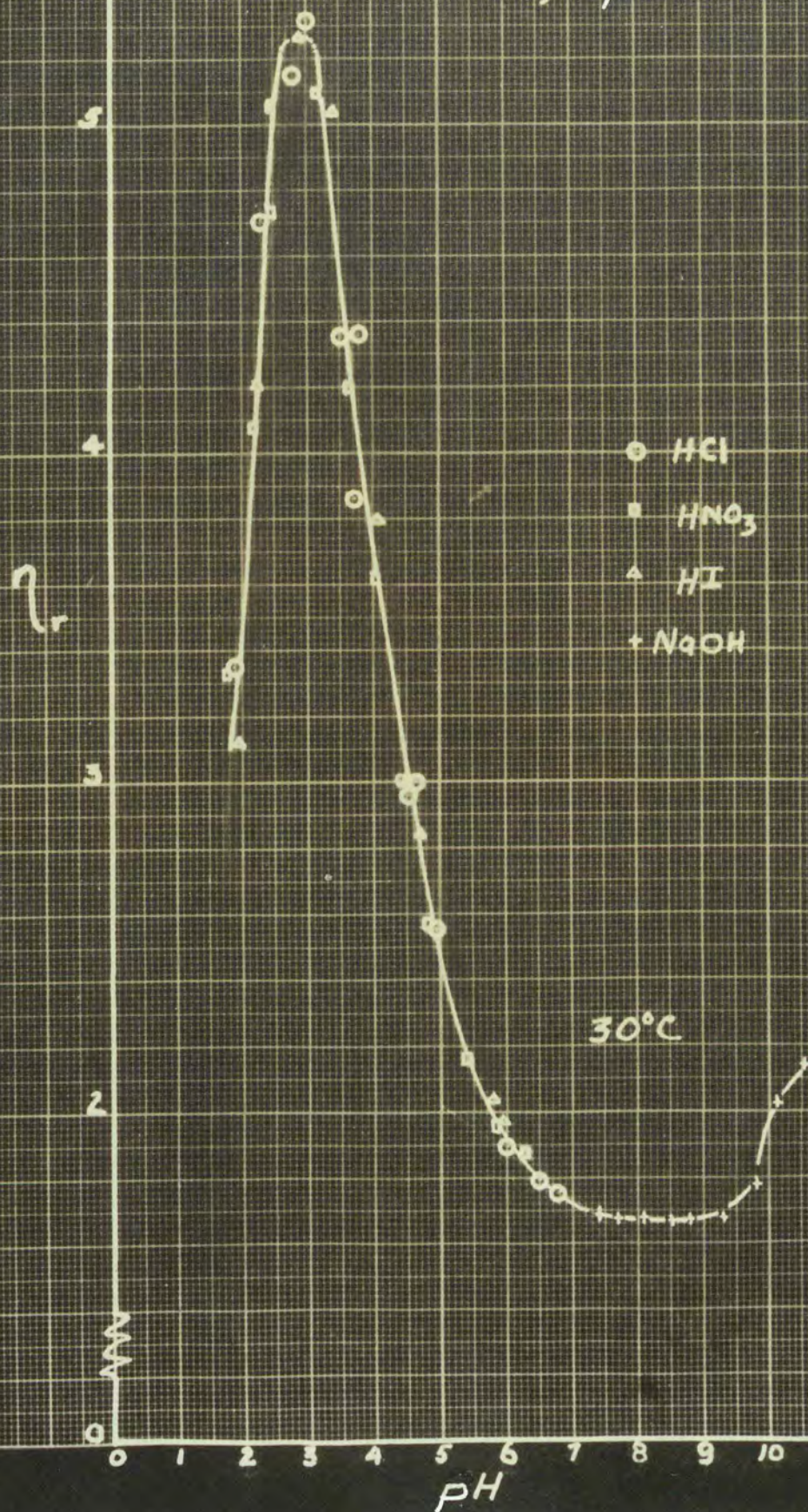
Discussion

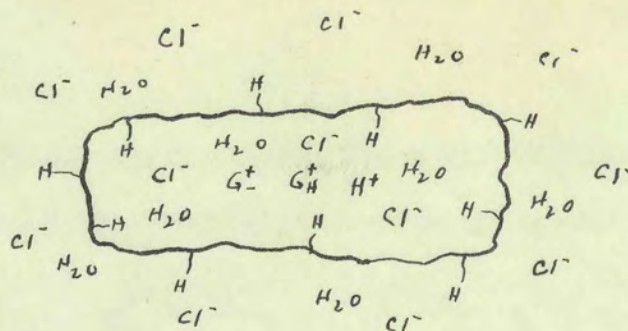
The effect of acid on viscosity will be selected as the first point of discussion. This effect is shown graphically by the uppermost curves of Figures 8, 9, and 10. For convenience, these curves have been summarized for the three acids in Figure 13. The first point to be noted is that the curve is in agreement to those of Lloyd and Pleass (see figure 3) and that of Freundlich (see figure 4) with the exception that the minimum comes at pH 8.6, whereas the others come at pH 4.7. However, this is consistent with isoelectric points shown in Figure 5 for reasons already explained.

Further, it is to be noted that as the pH is lowered from the isoelectric point there is a sharp rise in viscosity followed by a sharp drop; that is, the curve goes through a maximum. To explain this, the conclusion drawn in the description of the nature of gelatin, namely, that gelatin is a rod shaped particle with many amino acid groups extending between the folds and from the surface of the particles, will be used. When acid is added to the solution, some of it combines with the gelatin micelle in more than one fashion. These possibilities may be chemical combination, adsorption and occlusion. The degree of each of these possibilities depends on the concentration of acid used. The following model will make this acid - gelatin relation clearer:

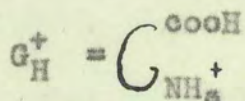
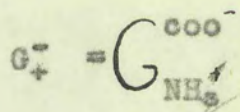
(Fig. 13)

THE VISCOSITY of GELATIN-ACID SOL
as a
FUNCTION of pH





where



Cl^- is in diffused double layer

When one considers that the reaction



it becomes evident that a Donnan equilibrium system is set up due to the production of a non diffusable ion. Since G_H^+ must be matched by Cl^- , there must be more of the latter held between the folds of the micelle than are present on the outside, as will become apparent from the following illustrations making use of the Donnan equilibrium equation:

$$[H^+]_{in} [Cl^-]_{in} = [H^+]_{out} [Cl^-]_{out}$$

as applied to the condition



Representing the product terms of the Donnan expression as areas of two rectangles with lengths

$$H_1^+ \boxed{\begin{matrix} Cl_1^- \\ \text{in} \end{matrix}} = \boxed{\begin{matrix} Cl_o^- \\ \text{out} \end{matrix}} H_o^+$$

H_1^+ , Cl_1^- , and H_o^+ , Cl_o^- respectively, the subsequent relations result:

- (a) The areas must be equal because of Donnan's expression.
- (b) The area of $(H_o^+)(Cl_o^-)$ can be represented as a square since H_o^+ and Cl_o^- are equal.
- (c) The area of $(H_1^+)(Cl_1^-)$ can be expressed by a rectangle because the Cl_1^- is in excess of the H_1^+ , since some of the Cl_1^- match the G_H^+ as well as the H_1^+ .
- (d) From the fact that the perimeters of a rectangle and a square containing the same area are unequal, it follows that the concentration of the ionic species on the inside will exceed those of the outside; that is:

$$[H^+]_1 + [Cl]_1 > [H^+]_o + [Cl^-]_o$$

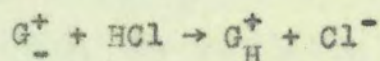
If this be the case, water will then enter between the folds due to osmotic pressure differences; thus swelling the micelle. This swelling will continue as long as G_-^+ is being converted to G_H^+ . When this reaction has gone to a maximum, additional acid will then decrease the difference in concentration between the in and out side of the micelle membrane and as a consequence, the particle will shrink.

The above process can be summarized by an explanation of the following equation:

$$\frac{Cl_i^-}{Cl_o^-} = \sqrt{1 + \frac{G_H^+}{H_i^+}} \quad (\text{see appendix})$$

where the symbols have the same meaning as given in the last treatment. The ratio of the chloride ions is used in this expression due to the fact that they reflect the apparent concentration of all ions on either side of the membrane, since in this system they are the only negative ions involved.

Upon small additions of acid, the G_H^+ and H_i^+ terms increase simultaneously, although G_H^+ increases more rapidly than H_i^+ because H^+ ions are readily bound up as:



From the above it is seen that the G_H^+/H_i^+ ratio increases, and as such, reflects itself as an increase in the Cl_i^-/Cl_o^- term of the above expression. The increase progresses until the G_H^+ concentration reaches its maximum limit. Further addition of acid then increases the H_i^+ concentration without changing the G_H^+ concentration. This results in a reduction of the G_H^+/H_i^+ ratio. This, of course, manifests itself in a reduction of the Cl ion ratio and brings about a more equal distribution of the ionic species.

Since, as already stated, the Cl^- governs the concentration of both sides of the membrane, the variations in

concentration of Cl^- between the inside and outside of the micelle, through osmotic pressure, result in a change of particle size. Therefore, a picture is arrived at where the particle changes its volume and goes through a maximum as a function of pH.

Reviewing this in the light of Einstein's or Arrhenius' equations:

$$\eta_r = 1 + 2.5\phi$$

$$\ln \eta - \ln \eta_0 = \phi$$

it is apparent that such changes in volume would reflect a change in ϕ and thus manifest itself in the viscosity.

To support the above point of view reference will now be made to the additional experiment performed in this investigation; namely, the combining capacity of acid and gelatin. A conversion of the experimental data shown in figure 11, that is, pH to moles per liter, gives the concentration of acid combined with gelatin (see figure 12 - curve I).

This curve reveals that the gelatin shows a maximum in its acid combining power. This maximum appears at a pH of ca. 2.25. However, when the difference in acid concentration between the combined and uncombined with gelatin is calculated (see figure 12, curve II) a maximum at a pH of 2.8 is found. This pH corresponds to the pH of maximum viscosity (see figure 13). The above, it is believed, is consistent with Loeb's suggested mechanism of swelling.

Reviewing next, the hydration of gelatin, there can be no doubt that gelatin with its numerous polar groups exhibits dipole-dipole and ion-dipole interaction with H_2O molecules. Referring back to our micelle model, it is seen that the net charge contained in the micelle is zero. However, the particle has a charge due to the exposed acid combining groups existing and extending from the surface of the micelle. This may account for any water shell which may come about by ion-dipole attraction. Further, from the fact that hydrogen bonds₂₃ exist only between the most electronegative atoms (O, N, in this case) it seems apparent that hydrogen bonding is responsible for a great deal of the water associated with proteins in addition to that already accounted for. This is substantiated by the work of Sponsler₂₄ who gives the number of water molecules coordinated by hydrophilic groups.

<u>Group</u>	<u>No. of Waters</u>	
	<u>Theoretical</u>	<u>Experimental</u>
H_2O	4	4
-OH	3	3
-COOH	4-5	4
=O	2	2
-NH ₂	3	3
-NH	2	-
=N-	1	-

From the above discussion of hydration, it seems inevitable that the increase in volume due to hydration (which should be more or less constant at various pHs) would

following sketch, applicable to the conditions under consideration, is presented:



At equilibrium, the ratio of the Cl^- ions will be indicative of the ratio of the concentrations of all the ions on either side of the membrane since they are again the only negative ions present. Thus, the following expression is offered in terms of Cl^- ion:

$$\frac{Cl_O^-}{Cl_1^-} = \sqrt{1 - \frac{G_H^+}{Cl_1^-}} \quad (\text{see appendix})$$

Since the G_H^+/Cl_1^- term governs the chloride ion ratio, it should be apparent that with constant G_H^+ , an increase in the Cl_1^- which comes about by the addition of salt, causes the G_H^+/Cl_1^- term of the equation to decrease. This results in an increase in the Cl_O^-/Cl_1^- ratio which finally approaches 1 in the limit, as more and more salt is added, meaning that the inside and outside concentrations become more equalized. Hence the difference in osmotic pressure on both sides of the membranes approach zero and as a consequence the volume approaches that of isoelectric

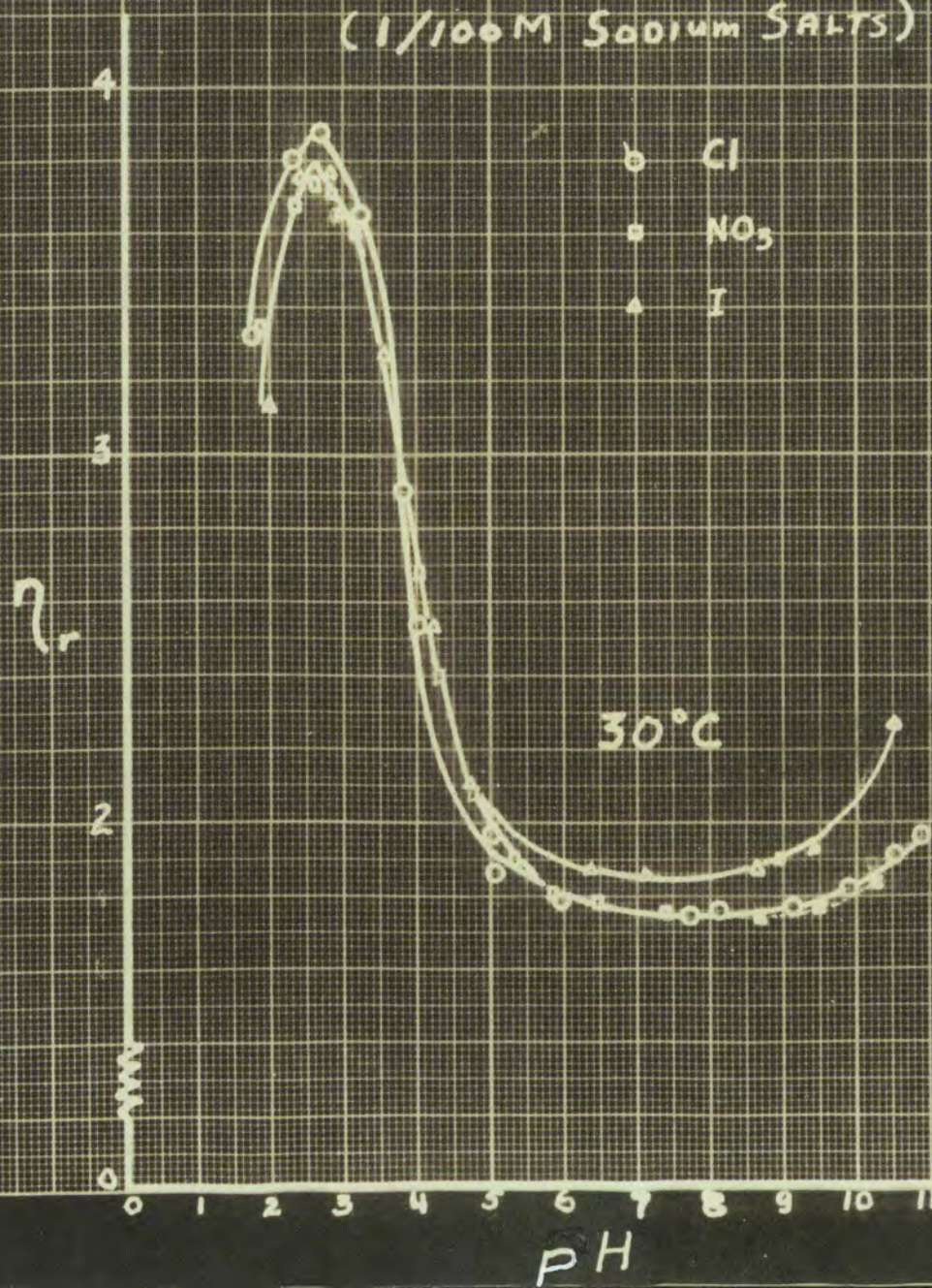
gelatin; that is, a minimum in volume is attained. This, of course, reflects a decrease and eventually a minimum in viscosity, depending on the salt concentration being used.

Turning now to figures 14, 15, 16, it is to be noted that not only is there a depressing effect in viscosity by the salts, but that the depressing effect manifests itself in a series I, NO_3 , Cl (Hofmeister series), the series becoming more predominant at higher concentrations. This is in accord with the work of Michaelis on the swelling of agar as pointed out at the outset of this thesis, and also with the work of Ando₂₅ who has confirmed the Hofmeister series for hemoglobin and egg albumin by viscosity measurements at controlled pH. As already noted, the results achieved from this investigation also show that a Hofmeister series can be obtained for gelatin through viscosity measurements (see figure 17). Why such a series should exist with these salts may perhaps be accounted for in the following manner.

From the micelle model already presented it can be seen that salt could effect both occluded H_2O and that of the shell as already pointed out. Since the water shell is not governed by Donnan equilibrium but mainly by the surface charge, it seems plausible to assume that the various dehydrating power of the various salts removes water from the shell in accordance with its ability to do so; thus changing the volume contribution made to the micelle by the H_2O shell.

(Fig. 14)

VISCOSITY of GELATIN-SALT SOL
as a
FUNCTION of pH ADJUSTED by
CORRESPONDING ACID
(1/100M Sodium SALTS)



(Fig. 15)

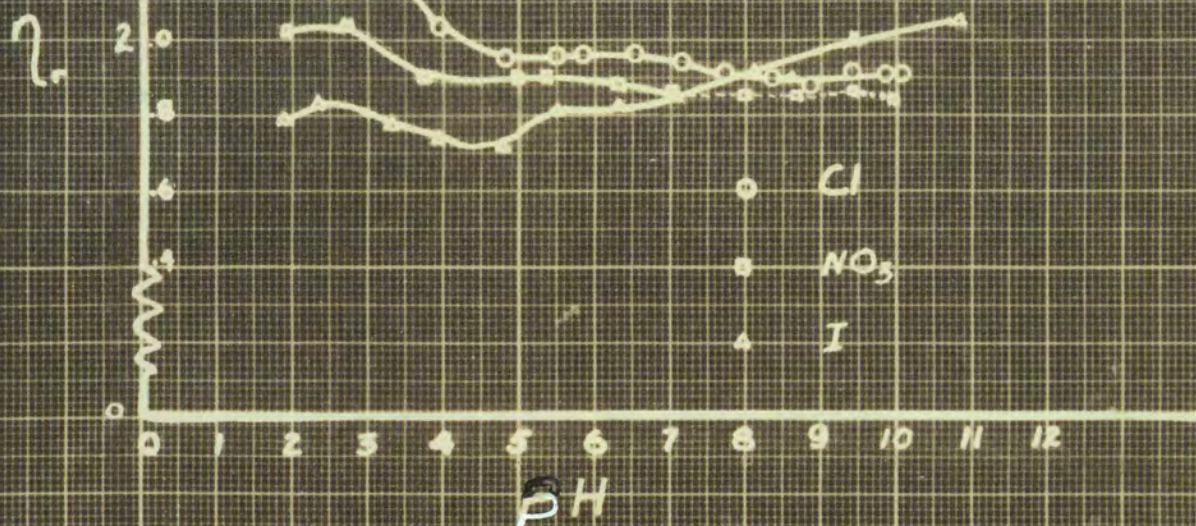
VISCOSITY of GELATIN ~ SALT SOL

as a

FUNCTION of pH ADJUSTED by
CORRESPONDING ACID

(1/10 M SODIUM SALTS)

30°C



(Fig. 16)

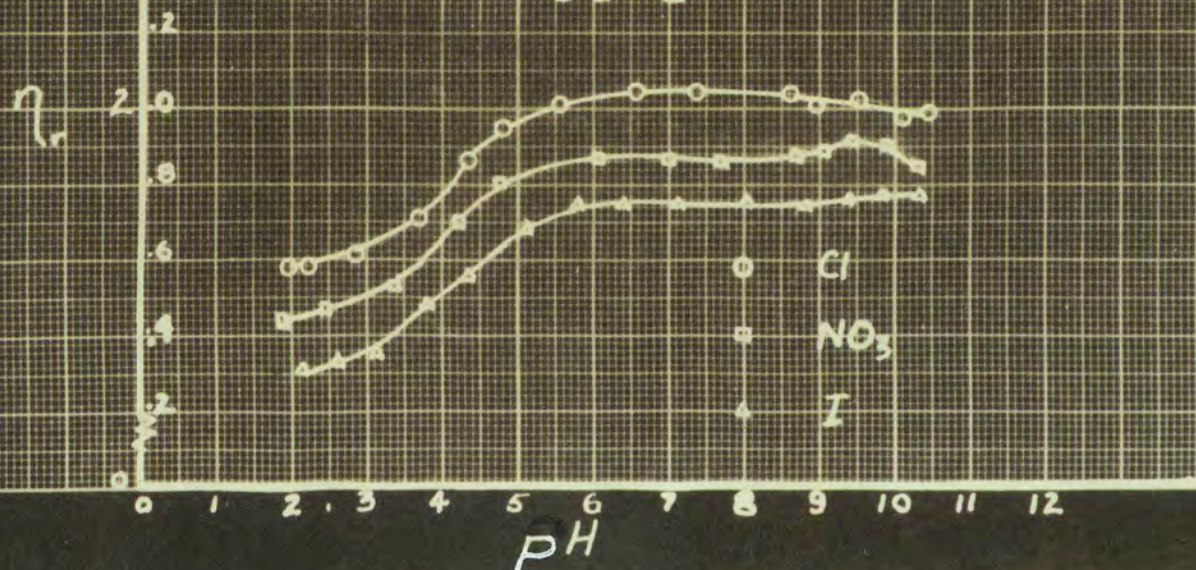
VISCOSITY of GELATIN ~ SALT SOL

as a

FUNCTION of pH ADJUSTED by
CORRESPONDING ACID

(1 M SODIUM SALTS)

30°C



Further, inasmuch as a double layer is assumed to exist at the surface of the micelle (explaining the electrophoretic properties of gelatin) it can be shown that the charge density and zeta potential (ζ) would be effected by the addition of electrolytes. This may be realized with the aid of the Debye-Hückel expression given as follows₂₆:

$$\zeta = \frac{Z e}{D a (1 + K a)}$$

where

Z = the valence of central ion

e = charge on electron

D = dielectric constant

a = radius of central ion

K = reciprocal of ionic radius

Since the ionic radius depends on the ionic strength,

$$K = b \sqrt{U}$$

where

U = ionic strength

b = a constant

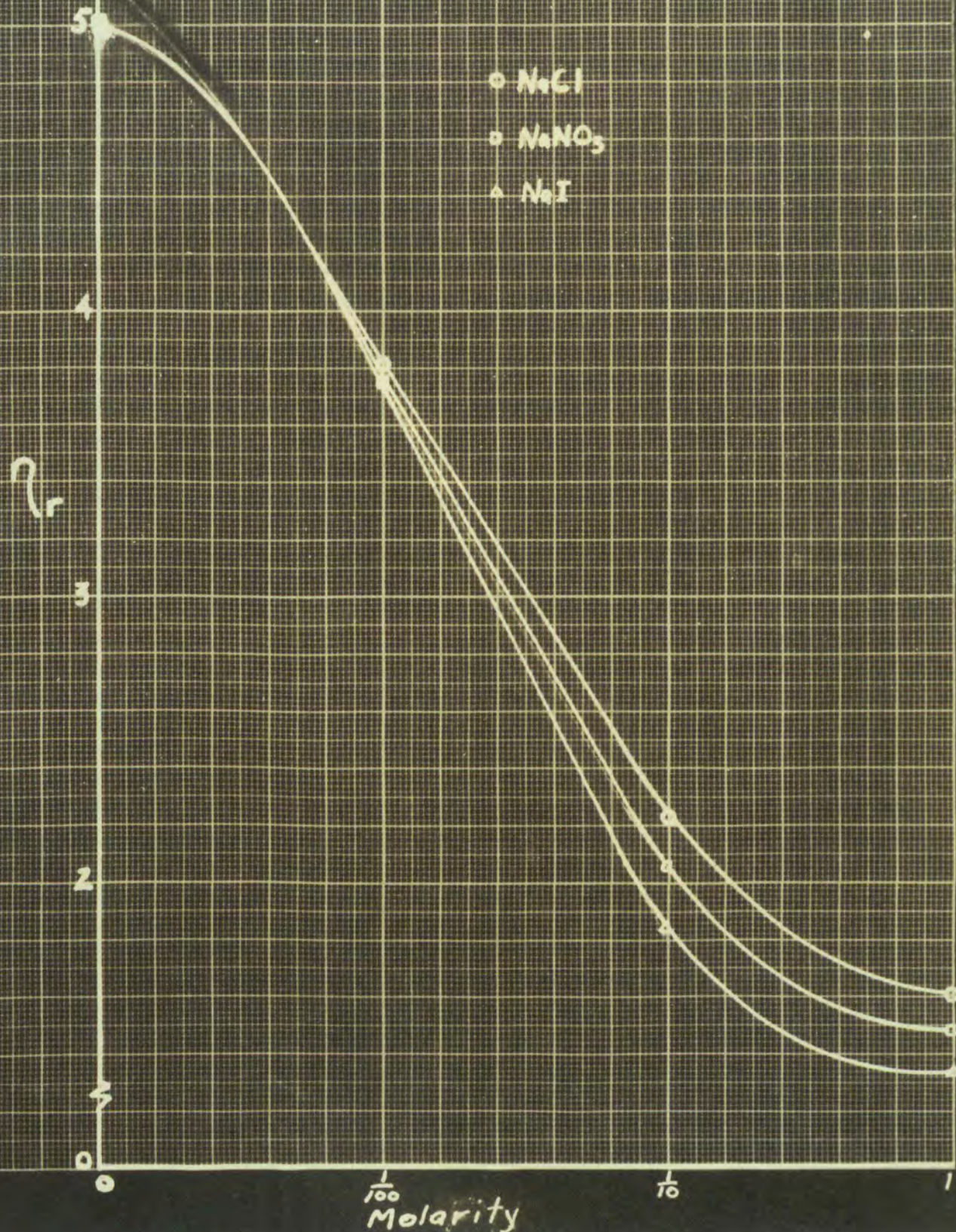
It is apparent that at high concentrations different salts must result in differing zeta potentials through their activity coefficients which are functionally related to the ionic strength. Hence, the last term in Smoluchowski's equation, $(\frac{D \zeta}{2 \eta})$, becomes a function of the nature of the salt species and as such is reflected as an electroviscous effect.

Thus, in the light of all the aforementioned, it seems that many factors contribute to viscosity variations as a

function salt concentration. At most one can conclude that the mechanism is quite complex and that it is governed by contributions from each of the points discussed.

(Fig. 17)

THE VISCOSITY OF GELATIN-SALT SOL
 AS A
 FUNCTION OF CONCENTRATION
 (PH \sim 2.5)



Summary

1. At low concentrations of salt the Hofmeister series is not apparent through viscosity measurements.
(Figure 17)
2. At high concentrations of salt the Hofmeister series manifests itself through viscosity measurements.
(Figure 17)
3. Viscosity variations as a function of pH are governed by Donnan equilibrium.
4. Viscosity variations as a function of salt concentration is governed by Donnan equilibrium and ionic strength of external solution.
5. Gelatin is a long fibrous like molecule coiled up in rod shape with many polar group of amino acids extending between the folds and from the surface of the particle.

References

- 1 - Arch. exptl. path. pharmacol; 24,247 (1888)
- 2 - Beitr. Chem. Physiol. Path., 3, 225 (1903)
- 3 - Colloidal Chem. - Weiser (1939)
- 4 - Proteins and Theory of Colloidal Behavior- Loeb,(2nd Ed.)(1924)
- 5 - The Effect of Ions in Colloidal Systems - Michaelis (1925)
- 6 - Textbook of Biochem. - Mitchell (2nd Ed.) (1950) Pg. 583
- 7 - Compt. rend. trav. Lab. Carlsberg, Ser' Chem.22, 118-24(1938)
- 8 - J. Biol. Chem. 3, xxxiii, (1907)
- 9 - Leghaya Prom. 3, No. 6, 19-22 (1943)
- 10- Colloidal Phenomena - Hauser (1939)
- 11- C.A. 35 - 3507
- 12- J. of Phy. Chem. 43, 1133-7(1939)
- 13- J. Phy. Chem. 33 (1929)
- 14- The Viscosity of Liquids - Hatschek (1928)
- 15- Ann. d. Physik (4) 19,289 (1906)
- 16- Ann. d. Physik (4) 18,194 (1916)
- 17- J. Biochem, 21, 1352 (1927)
- 18- Kolloid Z. 38 (181-1) (1926)
- 19- J. Am. Chem. Society 44, 2633-6 (1922)
- 20- Kolloid Z. 79 - 49-55 (1937)
- 21- J. Gen. Physiology 4,73-95 (1921)
- 22- J. Phy. Chem. 29, 1556-67 (1921)
- 23- Nature of the Chemical Bond - Pauling
- 24- J. Phy. Chem. 44,996 (1940)
- 25- J. Med. Science 2,110-23 (1927)
- 26- Colloidal Science - Alexander and Johnson (1949)

Appendix

I Derivation for the chloride ion ratio in the case where HCl and gelatin make up the system;

$$(H)_1(Cl)_1 = (H)_0(Cl)_0 \quad (1)$$

$$(Cl)_1 = (H)_1 + G_H^+ \quad (2a)$$

$$(Cl)_0 = (H)_0 \quad (2b)$$

substituting (2a,b) in (1)

$$(H)_1 (H)_1 + G_H^+ = (H)_0 (H)_0 \quad (3)$$

extending (3)

$$(H)_1^2 + (H)_1(G_H^+) = (H)_0^2 \quad (4)$$

dividing through by $(H)_1^2$

$$1 + G_H^+/H_1 = H_0^2/H_1^2 \quad (5a)$$

$$H_0/H_1 = \sqrt{1 + G_H^+/H_1} \quad (5b)$$

since from (1),

$$H_0/H_1 = Cl_1/Cl_0$$

therefore

$$Cl_1/Cl_0 = \sqrt{1 + G_H^+/H_1} \quad (6)$$

Appendix (continued)

II Derivation for the chloride ion ratio in the case where NaCl, HCl and gelatin make up the system:

$$(H)_1(Cl)_1 = (H)_0(Cl)_0 \quad (1)$$

$$(Na)_1(Cl)_1 = (Na)_0(Cl)_0 \quad (2)$$

$$(Na)_1 = (Cl)_1 - G_H^+ - H_1 \quad (3a)$$

$$(Na)_0 = (Cl)_0 - H_0 \quad (3b)$$

rearranging (1) and (2)

$$H_1/H_0 = Cl_0/Cl_1 = Na_1/Na_0 \quad (4)$$

substituting (3a,b) in (4)

$$H_1/H_0 = (Cl_1 - G_H^+ - H_1)/(Cl_0 - H_0) \quad (5)$$

cross multiplying and simplifying

$$(H)_1(Cl)_0 = (H)_0(Cl)_1 - H_0 G_H^+ \quad (6)$$

dividing through by $(H)_0(Cl)_1$,

$$(H)_1(Cl)_0/(H)_0(Cl)_1 = 1 - G_H^+/Cl_1 \quad (7)$$

substituting (4) into (7)

$$(Cl_0/Cl_1)^2 = 1 - G_H^+/Cl_1$$

$$(Cl_0/Cl_1) = \sqrt{1 - G_H^+/Cl_1} \quad (8)$$