

6-1951

# The Effect of Electrolytes on The Viscosity of Protein Solutions

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THE EFFECT OF ELECTROLYTES ON  
THE VISCOSITY OF PROTEIN SOLUTIONS

THE EFFECT OF ELECTROLYTES ON THE  
VISCOSITY OF PROTEIN SOLUTIONS

by

Thomas Anthony White

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

By Thomas A. White

Approved by Howard E. Sheffer

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## Introduction

This project was undertaken to study the Hofmeister series and Loeb's contention that it is incorrect.

UH 9<sup>12</sup>  
W588e The protein chosen for study was gelatin.

1951  
C.2 The three salts used were: sodium chloride, sodium acetate and sodium nitrate.

## Historical

Oct. 9, 1951  
"In the development of the Hofmeister series a serious error has been committed, namely, the neglect of measuring hydrogen ion concentration of the protein solutions and protein gels." This was taken from Loeb's book and describes briefly and clearly why the project was undertaken and what was hoped to be decided.

Hofmeister claimed that the viscosity of protein solutions, along with several other of their physical properties; namely, osmotic pressure, solubility and swelling is directly affected by electrolytes. He claimed that each ion affects these properties differently and in a characteristic manner and definite order.

For the swelling of gelatin the Hofmeister series is:

$SO_4 < \text{tartrate} < \text{acetate} < Cl < Br < NO_3 < CNS$

where swelling is maximum in CNS solution.

\* Reference one

152828 ✓  
Left of author



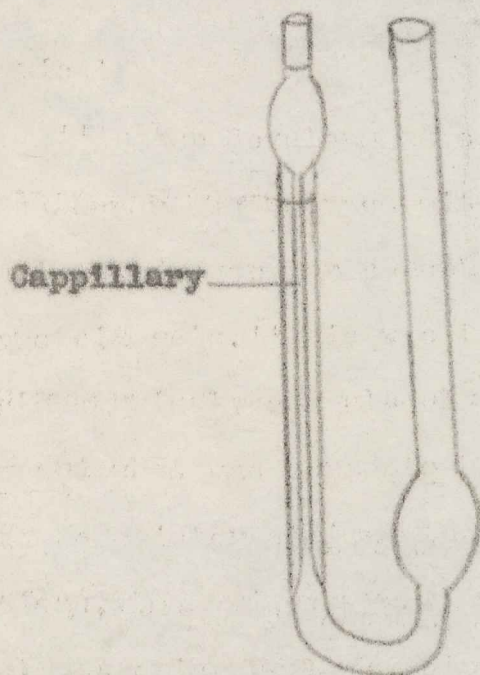
Loeb's work came out some twenty years later and from this he said that Hofmeister had neglected to take the pH of the solutions into consideration when he formulated his series and therefore the series were not valid. He claimed that the only important factor in the effect on proteins by electrolytes is the valence of the ion. All monovalent ions will depress the viscosity of a protein solution in the same way and to the same degree.

This is the problem as it was started in September, 1950. By taking pH into consideration and keeping all factors possible constant, varying only the concentration of salt used, an attempt was made to decide the question as to who is correct, Hofmeister or Loeb.

**Apparatus:**

Constant temperature bath, Ostwald pipette (viscometer), Beckman pH meter, purified gelatin, several chemically pure (C.P.) electrolytes and a stopwatch.

Ostwald Pipette



The time of flow of liquid between the two lines is taken with a stopwatch. The lower reservoir is filled with five millieliters.

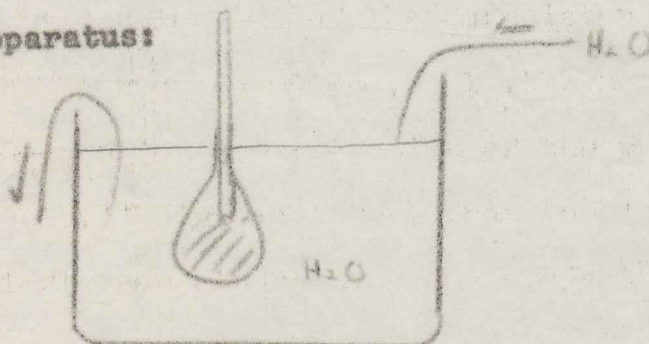


## Procedure

An attempt to purify the gelatin to be used by dialysis was made. This was done by preparing a one percent solution of gelatin in pure water.

The solution was placed in previously prepared colloidian membranes and dialysed in running distilled water. The viscosity was taken before the dialysis was started and during the dialysis.

The apparatus:



The process was abandoned because a noticeable change occurred in the solution within two days. Coagulation had occurred and a marked increase in the viscosity of the remaining solution was noted.

The gelatin used was obtained Eastman Kodak Company through the Will Corporation. It was from Lot 181 and the label read:

pH of 1% solution	5.5
Isoelectric point	8.5

The constant temperature bath was kept at  $30^{\circ} \pm 0.05^{\circ} \text{C}$ . Thirty degrees was chosen because it was above the normal room temperature, and at this temperature the viscosity of a gelatin solution does not change appreciably on standing. The time of measurement was kept as constant as possible due to the fact that viscosity changes with time.



The salt was made up in concentrations of, 0.500 molar, 0.250, 0.0625, 0.0156, 0.0029 and 0.00097 molar. These solutions were then brought to the pH to be studied. A solution with no electrolyte was also run, too.

To 0.400 grams of gelatin twenty millieliters of distilled water was added. The mixture was heated to sixty degrees and stirred until a clear solution was obtained. Acid was added to the solution until it was brought to the desired pH. To this twenty-five millieliters of the previously prepared electrolyte were added and the contents mixed and brought to a total volume of fifty millieliters. The pH was again checked on the Beckman pH meter.

The fifty millieliters of solution were then placed in the constant temperature bath and allowed to come to thirty degrees.

The viscometer was cleaned and dried before each run. The reservoir was then filled with five millieliters of the solution. The liquid was then drawn into the upper reservoir and the time for it to run out was taken with a stopwatch. A like run was made with distilled water.

The liquid was allowed to run through the capillary five times. The average time was recorded and used in figuring the relative viscosity.

The viscometer was then cleaned and dried and made ready for the next run.

$$\text{Relative viscosity} = \frac{\text{time for gelatin}}{\text{time for water alone}}$$

## Data

HCl added	Conc. of salt	time	relative viscosity
at pH 4.0	with NaCl	, HCl used 0.109 normal	
1.70 ml.	0.500M	136.4sec.	1.470
1.67	0.250M	137.4	1.480
1.70	0.0625	148.6	1.593
1.70	0.0156	156.2	1.878
1.71	0.0029	182.4	1.968
1.70	0.00097	223.5*	2.038
1.71	pure (no salt)	193.8	2.088
—	water	92.7	—
	* water	109.0	
at pH 4.4	with NaCl		
1.70	0.500	151.2sec.	1.445
1.62	0.250	153.2	1.464
1.63	0.0625	155.6	1.490
1.65	0.0156	160.2	1.531
1.47	0.0029	190.0	1.818
1.60	0.00097	192.9	1.845
1.70	pure	211.4	2.025
at pH 4.7	with NaCl		
1.10	0.500	153.6	1.454
1.00	0.250	153.9	1.467
1.11	0.0625	154.4	1.470
1.00	0.0156	156.0	1.481
0.90	0.0029	169.2	1.610
0.90	0.00097	177.4	1.670
0.96	pure	184.6	1.755
***	water	104.9	***



Data(continued)

HCl used in ml.	Conc. of salt molar	time in seconds	relative visc.
at pH 5.2. with NaCl			
0.20	0.500	151.4	1.458
0.20	0.250	153.6	1.477
0.20	0.0625	154.3	1.482
0.20	0.0156	156.4	1.503
0.30	0.0029	161.5	1.552
0.20	0.00097	166.2	1.596
0.20	pure	167.5	1.600
---	water	104.0	---

Up to here it was believed that the isoelectric point of gelatin to be at a pH 4.7, but the data at pH 5.2 made this doubtful. Viscosity measurements at various pH's were run with 0.80% gelatin solutions with no salts to find the isoelectric point.

pH	time	relative viscosity
4.0	193.4	2.088
4.4	211.4	2.025
4.7	184.6	1.755
5.2	167.4	1.600
5.3	157.0	1.461
5.5	151.4	1.455 ← isoelectric point
5.6	152.4	1.463
5.7	158.0	1.512



Viscosity of electrolytes used:

Sodium chloride	time	concentration	relative visc.
	115.0	0.500	1.095
	108.6	0.250	1.032
	106.6	0.100	1.018
	105.4	0.050	1.000
	105.2	water	-----
Sodium acetate	121.0	1.000	1.175
	112.2	0.500	1.087
	108.1	0.250	1.050
	106.2	0.050	1.030
	103.0	0.010	1.000

time	Conc. in salt	ml. of NaOH	relative visc.
164.4	0.500	0.30	1.640
160.1	0.250	0.32	1.520
154.8	0.0625	0.30	1.471
148.4	0.0156	0.30	1.412
150.0	0.0029	0.28	1.431.
151.0	0.00097	0.25	1.420
151.4	pure	0.26	1.442
***104.0	water	***	***

Above at pH 5.5 with NaCl.

## Data (con't)

ml. HAc	Conc. of salt	time	relative viscosity
1.80	0.500	163.1	1.519
1.70	0.250	164.2	1.528
1.78	0.0625	173.8	1.612
1.80	0.0156	183.4	1.702
1.80	0.0029	187.8	1.728
1.80	0.00097	191.0	1.760
1.70	pure	192.6	1.775
---	water	108.5	----

Above at pH 4.7 with NaAc.

1.10	0.500	158.8	1.522
1.30	0.250	161.2	1.548
1.40	0.0625	163.1	1.578
1.20	0.0156	170.4	1.580
1.10	0.0029	175.0	1.612
1.20	0.00097	182.0	1.691
1.00	pure	183.4	1.705

Above at pH 5.2 with NaAc.

0.50ml NaOH	0.500	167.2	1.540
0.51	0.250	163.1	1.505
0.49	0.0625	162.0	1.492
0.52	0.0156	161.8	1.489
0.50	0.0029	155.8	1.435
0.56	0.00097	163.2	1.505
0.50	pure	164.2	1.512
---	water	104.0	---

Above at pH 5.5 with NaAc.

All sodium acetate solutions adjusted with 0.100 normal acetic acid.



## Data(cont)

ml.HNO <sub>3</sub>	conc.of salt	time	relative viscosity
At pH 4.7 with NaNO <sub>3</sub>			
0.60	0.500	171.0	1.630
0.62	0.250	173.2	1.650
0.60	0.0625	178.8	1.702
0.61	0.0156	188.6	1.795
0.65	0.0029	192.2	1.830
0.60	0.00097	214.0	2.039
0.63	pure	218.0	2.079
***	water	105.0	***
At pH 5.2 with NaNO <sub>3</sub>			
0.10	0.500	160.1	1.525
0.12	0.250	161.6	1.530
0.10	0.0625	163.4	1.550
0.11	0.0156	171.8	1.634
0.10	0.0029	182.0	1.742
0.13	0.00097	187.0	1.781
0.10	pure	189.6	1.810
***	water	105.0	***
At pH 5.5 with NaNO <sub>3</sub>			
0.41ml NaOH	0.500	163.8	1.580
0.40	0.250	163.0	1.571
0.40	0.0625	158.8	1.532
0.42	0.0156	156.5	1.511
0.41	0.0029	154.0	1.490
0.40	0.00097	154.8	1.495
0.40	pure	155.6	1.505
---	water	105.0	---



## Data (con't)

Concentration	time	relative viscosity
0.500	220.0	had coagulated--
0.250	240.0	2.196
0.0625	252.6	2.399
0.0156	278.9	2.648
0.0029	300.0	2.958
0.00097	several minutes	-----
water	104	-----

Above NaCl at pH 3.5, adjusted to pH with two ml. of one normal HCl.

Below NaAc at pH 3.5 adjusted with glacial acetic acid.

0.500	257.0	had coagulated
0.250	286.5	2.443
0.0625	300.2	2.861
0.0156	325.4	3.070
0.0029	360.2	3.430
0.00097	several minutes	-----
water	105.0	-----

In both solutions, gelatin with NaCl and NaAc, there were what appeared to be semisolid masses formed. Due to this and to the coagulation which occurred to some extent in all the solutions the results are probably not exactly reproducible.

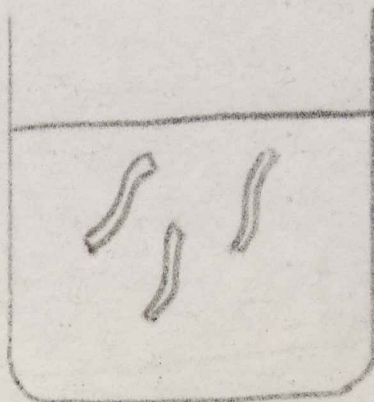


Table of Relative Viscosities for Electrolytes Used

pH	Salt	Concentrations of Electrolytes in moles					
		0.500M	0.250	0.0625	0.0256	0.0029	0.00097
pH 4.7	NaCl	1.454	1.467	1.471	1.481	1.620	1.690
	NaAc	1.519	1.528	1.612	1.702	1.728	1.760
	NaNO	1.630	1.650	1.702	1.795	1.830	2.039
pH 5.2	NaCl	1.458	1.477	1.482	1.503	1.552	1.596
	NaAc	1.522	1.548	1.578	1.580	1.612	1.691
	NaNO	1.525	1.530	1.550	1.634	1.742	1.781
pH 5.5	NaCl	1.640	1.520	1.471	1.430	1.410	1.442
	NaAc	1.540	1.505	1.492	1.489	1.435	1.505
	NaNO	1.580	1.571	1.532	1.511	1.490	1.495

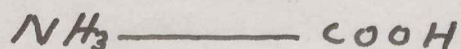


## Discussion

Salts produce a depressing effect on the viscosity of gelatin solutions. Both Loeb and Hofmeister agreed on this. The extent of this depression was the disagreement occurred. Loeb claimed that the depression was due to the valence of the ion involved while Hofmeister said each ion depressed the viscosity a distinct degree.

In running this experiment it was decided to work close to the isoelectric point of the gelatin solutions because it was believed that at this point any small variations in viscosity caused by the ion would be more pronounced and more easily noticed than at a lower pH where the concentration of the hydronium ion would tend to mask small differences.

All salts used were sodium salts because below a pH 5.5 gelatin is at the acid side of its isoelectric point, which means it has a positive charge on it.



The ion which affects the solution in this case is the anion.

Gelatin is a complex combination of several amino acids. Among them are:

Glycine	33%
Proline	17.5%
Hydroxyproline	14%
Serine	3%

Gelatin is characterized by large number of polar groups within its structure. Fourteen percent of the amino acids which compose it are polar, of these fifteen percent are basic and ten percent are acid amino acid groups. The polar portion of the molecule is the hydroxyproline part.



In general, protein solutions have a high viscosity.

The theories for this are briefly:

A hydration effect. The protein ion is a typical colloid micelle according to this theory. The particle has a water surrounding it which makes the whole particle larger and thus gives it a high viscosity. The thickness of this water layer depends on the charge on the protein, thus explaining why solutions at lower pH's have higher viscosities.

The micelle looks like this:



In other words, proteins form a hydrophilic sol upon addition of water. In order to coagulate such a system the particle must first be dehydrated, then the zeta potential lowered below the critical zeta potential.

Due to the necessary dehydration a much higher concentration of salt is needed to coagulate the solution.

The other theory considers gelatin solutions to be a highly associated system. The molecules aggregate together to form large masses and hence increase the viscosity of the solution. Water is taken up and held (~~and held~~) in these aggregates.

Coagulation occurs when these particles grow so large that the solvent can no longer hold them in solution.

In either case the effect of adding anions or cations to such ~~to~~ a system is one of hydration and competition for the available water present.

When an ion is added to a protein solution it



immediatly takes up a certain portion of the water to form a hydration shell around it. The fact that electrolytes themselves have a relative viscosity greater than one shows this to be true.

Thus it stands that the higher the concentration of ions added to the gelatin solution, the more water that will be pulled from the protein micelle or aggregate and the lower the viscosity will become.

This is limited to concentrations below which all the water will be pulled from the protein and coagulation will occur.

Loeb did most of his work around pH's of 3.0, 3.3 and 3.8. In an attempt to reproduce this work it was not possible to get any results similar to what he reported. Using acetic acid which has the same anion as the salt being measured it was found that concentrations of salt above 0.250 molar caused coagulation. Less concentrated solutions had visible particles in them which caused some inaccuracy in the measurements. The particles seemed to be semi-solid.

Graph seven contains the data reported by Loeb and the data obtained during the previous years work. Loeb's data does not coincide with this work. The results conform more closely with Hofmeister's work.

Loeb lowered the pH of his solutions to 3.0 by adding hydrochloric acid even though he was supposed to be studying the effect of the acetate ion.

The following table may explain in part why he got the results he did.



\* Millieliters of HCl added to get pH 3.8

Ml. of 0.5 molar HCl

NaCl	0.2
NaNO <sub>3</sub>	0.3
NaAc	425.0

In his solution of sodium acetate he had more chloride ion than acetate ion.

To summarize the results of this work; Hofmeister's series is correct at a pH of 3.5 as well as at higher pH's, as will be seen later. Loeb's contention that the only significant factor is the pH of the solution does not hold.

The experiment has shown that the chloride ion has the strongest depressing effect of those measured. It has the  $\mu$  highest hydration power and therefore pulls more water from the protein.

The depressing power:

	<sup>t</sup>	
Cl	acetate	NO <sub>3</sub>

This is shown in graphs four, five and six. At a pH of 4.7 the difference is very marked.

At pH 5.2 the difference is present although less marked.

At the isoelectric point, pH 5.5, the same general tendency is still present. The general form of the curves is similar except to the extremes to which the higher concentrations of salts cause variations. This rise in viscosity

\*Reference one, page 143

may be due in a large part to the hydration of the electrolyte and subsequent rise in viscosity of the solution. Chloride is more highly hydrated than acetate and nitrate so therefore has a higher viscosity. It is more highly hydrated as proved by the fact that it depresses the viscosity of the protein the most. At the isoelectric point the protein particles should be the least hydrated and any change in viscosity, assuming the gelatin stays exactly at its isoelectric point, would come from the solution added. Some variation may be due to a pH change caused by the salt or carbon dioxide in the air. Or perhaps salts may actually change the isoelectric point. In this respect note that in graph eight the viscosity minimum shifts from a pH 4.4 with 0.5009 molar sodium chloride to 4.7 with 0.0625 molar. Other concentrations give different minimum points. Below 0.0156 molar the minimum is at pH 5.5. If the isoelectric point is at the point of minimum viscosity then it has been shifted by the addition of salt.



## Summary

At pH's of 4.7 and 5.2 sodium chloride, sodium acetate and sodium nitrate lower the viscosity of the gelatin solution. The relative viscosity starts high in each case as more salt is added it is decreased. The high viscosity at low salt concentrations is due to the hydrated protein cation. the effect of the salt is to dehydrate this cation thus lowering the viscosity.

At a pH 5.5, the relative viscosity starts low. This is possibly due to the smaller extent of hydration of the zwitterion. The relative viscosity decreases a small amount as the salt concentration increases. As further salt is added the viscosity increases an amount approximately equal to the viscosity increase of the salt alone for sodium chloride and sodium acetate. There is an abnormal increase for sodium chloride.

This work has proven that Loeb was incorrect when he claimed the Hofmeister series was not valid. It seems that the order  $\text{Cl}^- > \text{acetate}^- > \text{NO}_3^-$  for the depressing power of the anions holds. Why Loeb's work was not confirmed is probably due the way he adjusted his pH's with HCl regardless of the salt used. He was in reality always measuring the effect of the chloride ion.

The principle reason for the viscosity depression by an electrolyte is a hydration factor. The salt pulls water away from the protein micelle and thus reduces their size and viscosity.

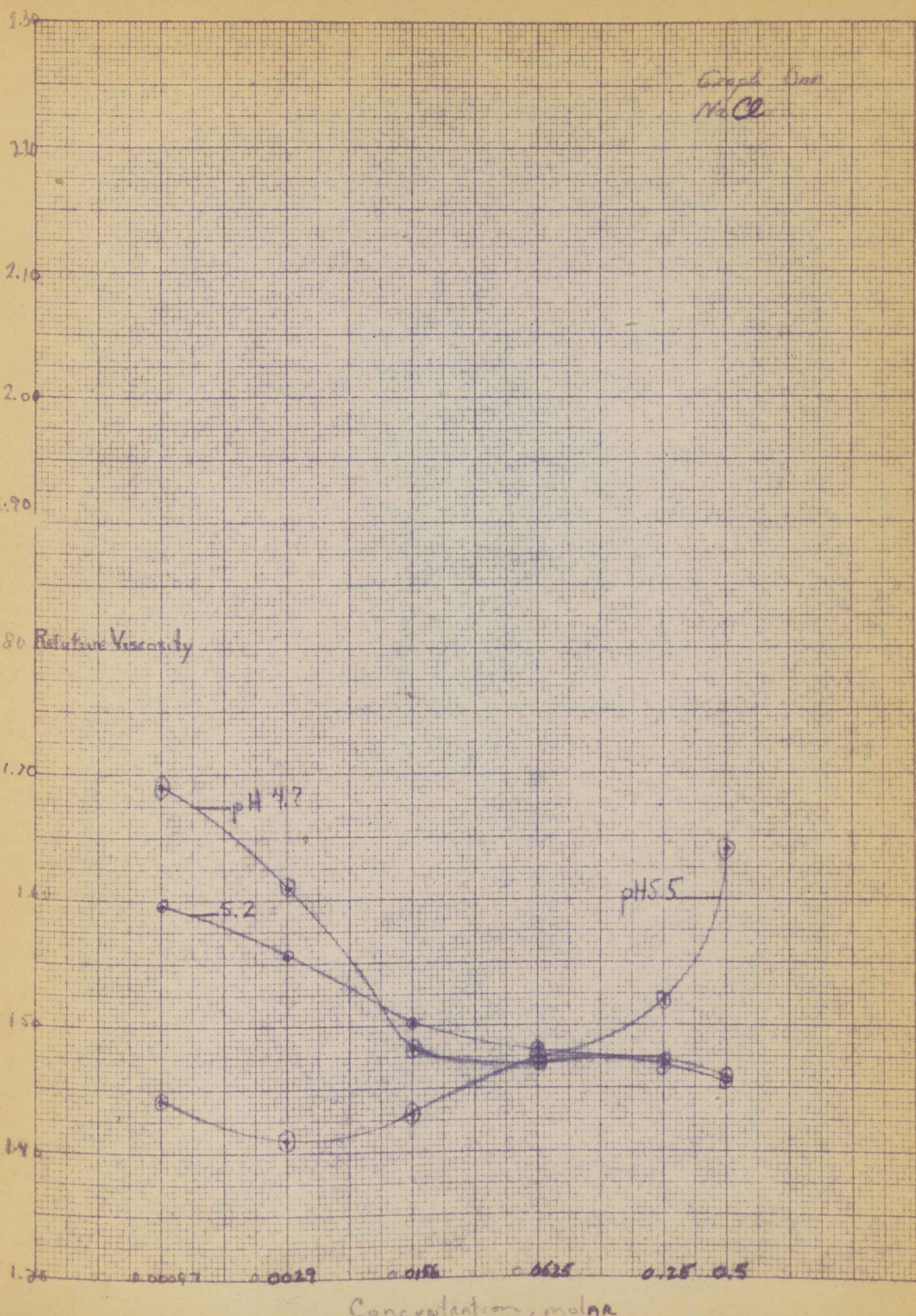
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GRAPHS







Graph Two  
Na Acetate

2.10

2.00

1.90

Relative Viscosity

1.80

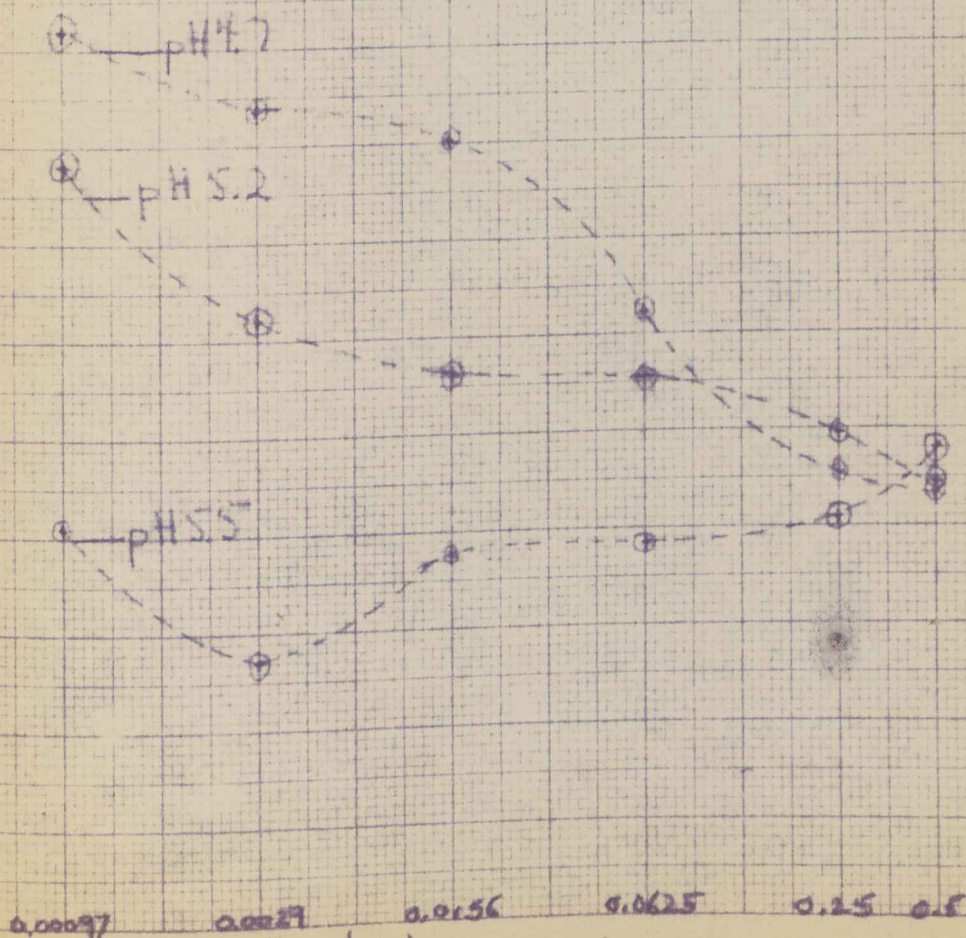
1.70

1.60

1.50

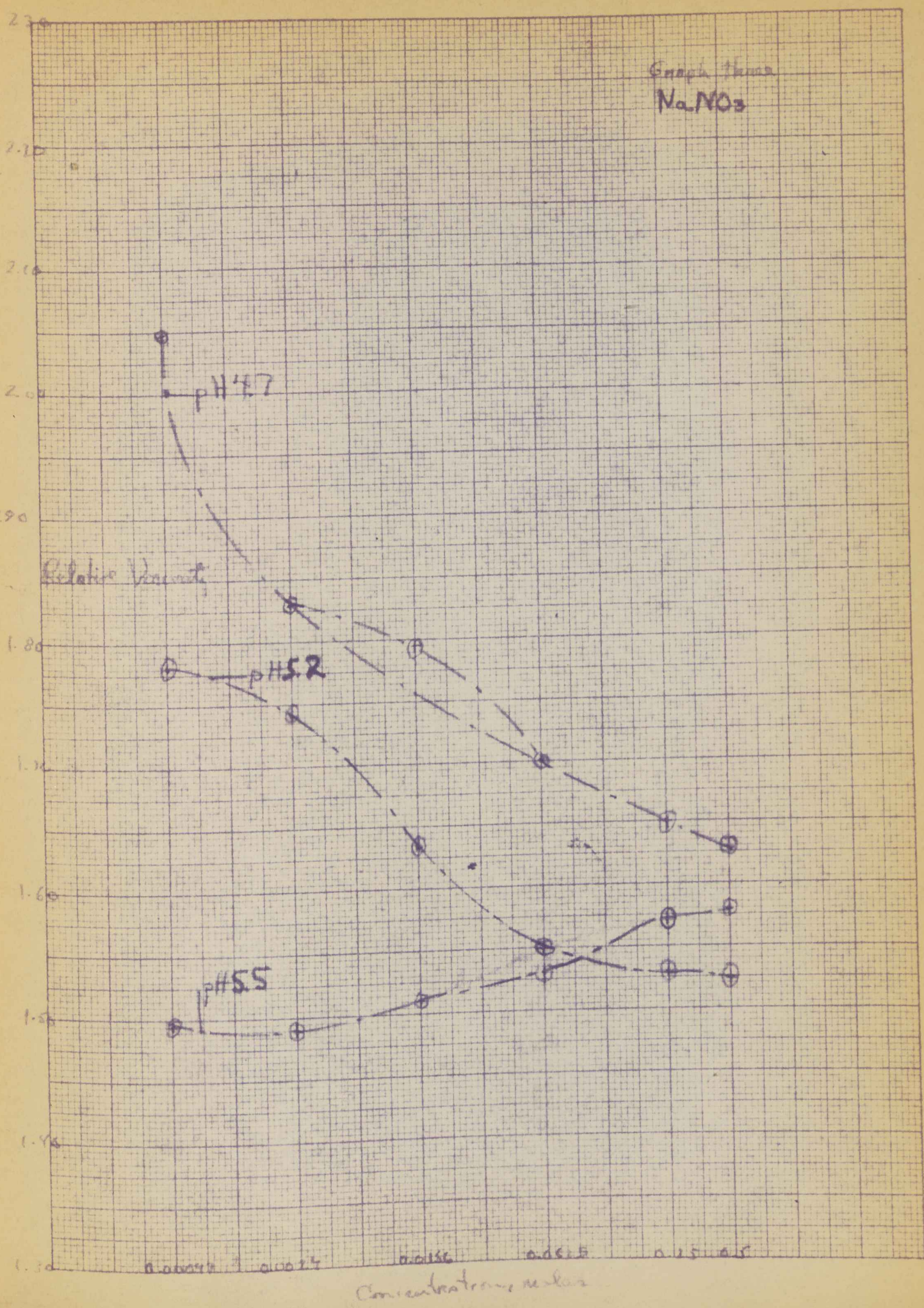
1.40

1.30



Concentration, molar

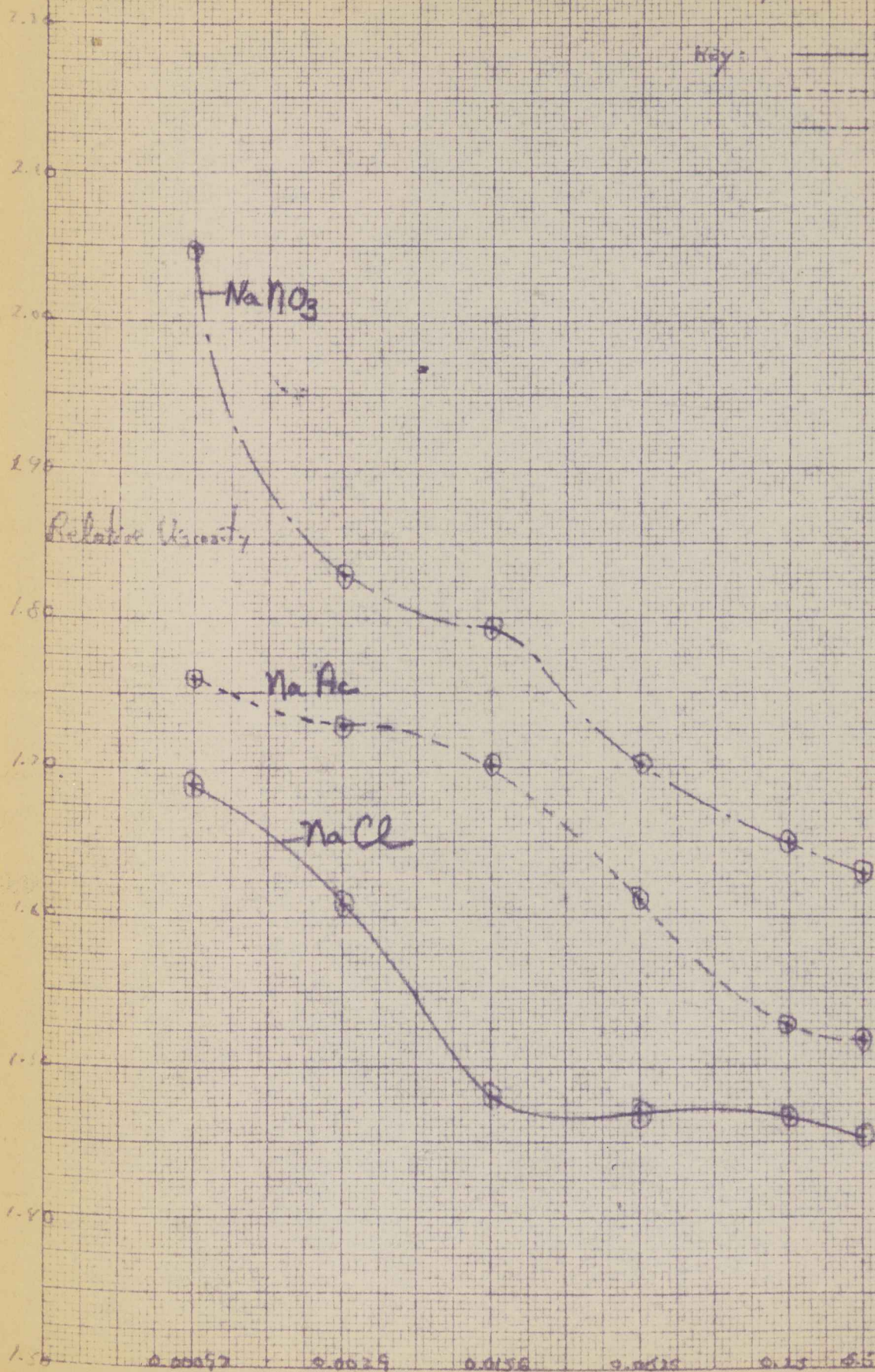




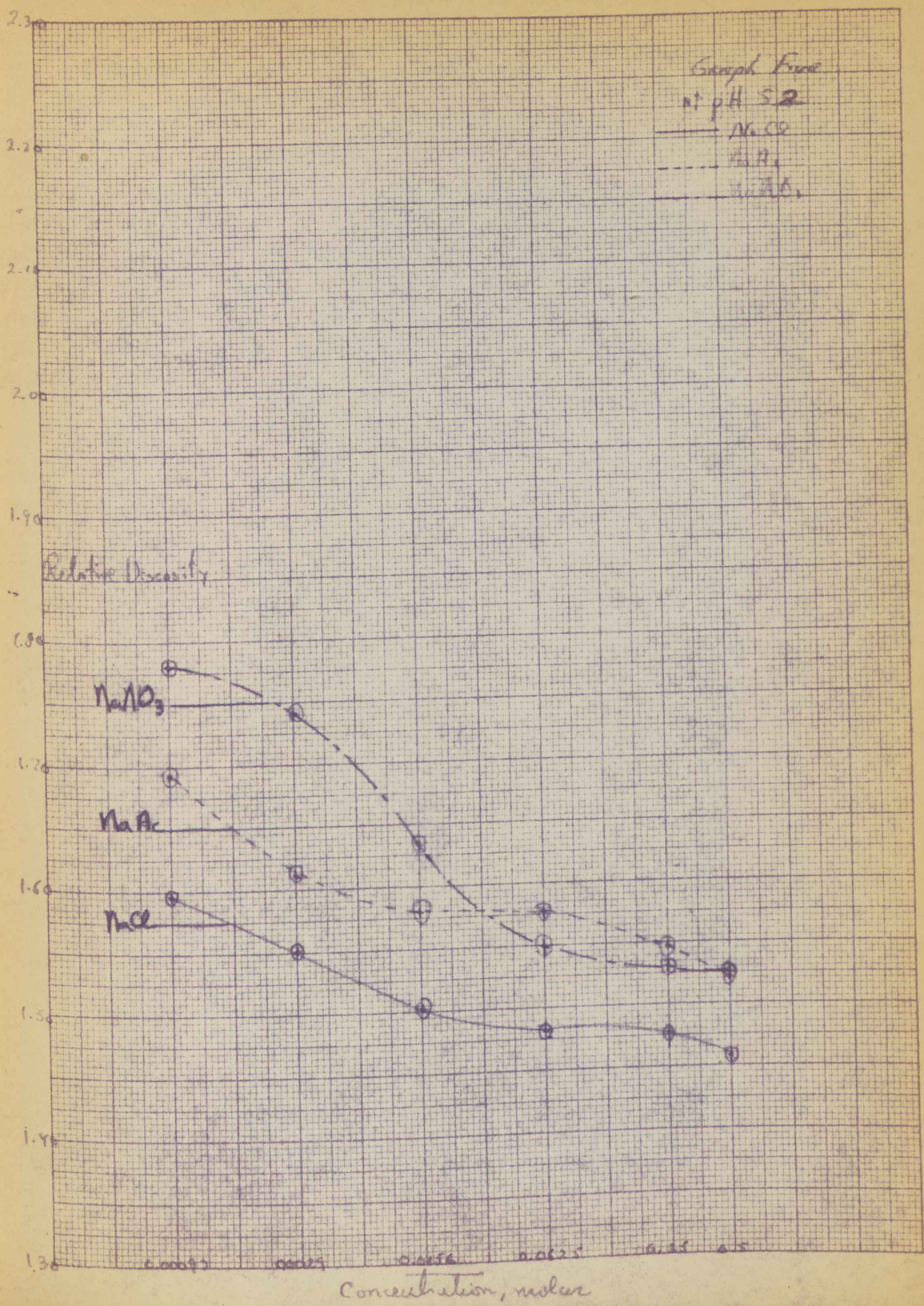


Graph Four  
AT pH 4.7

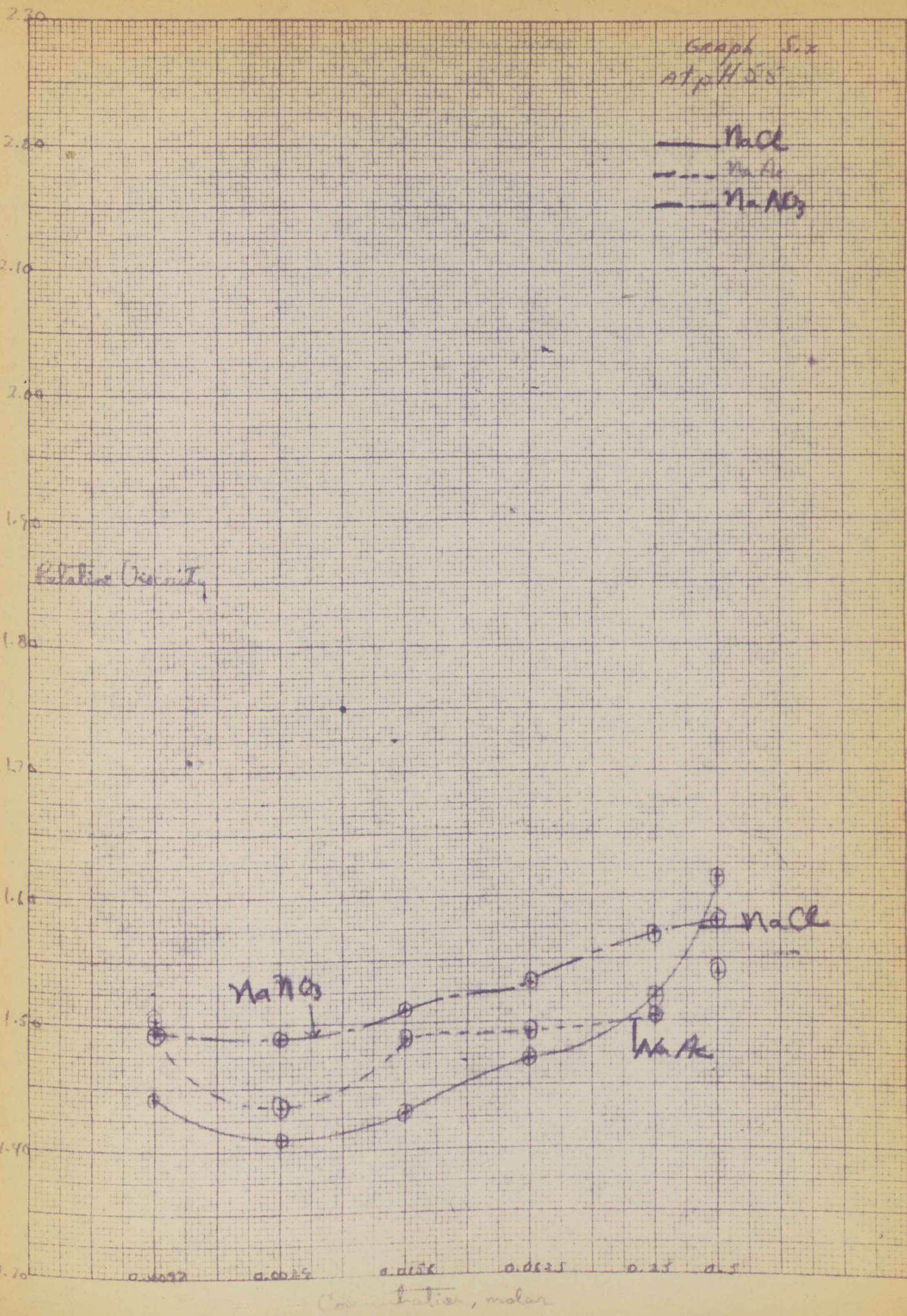
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--- NaAc  
--- NaNO<sub>3</sub>



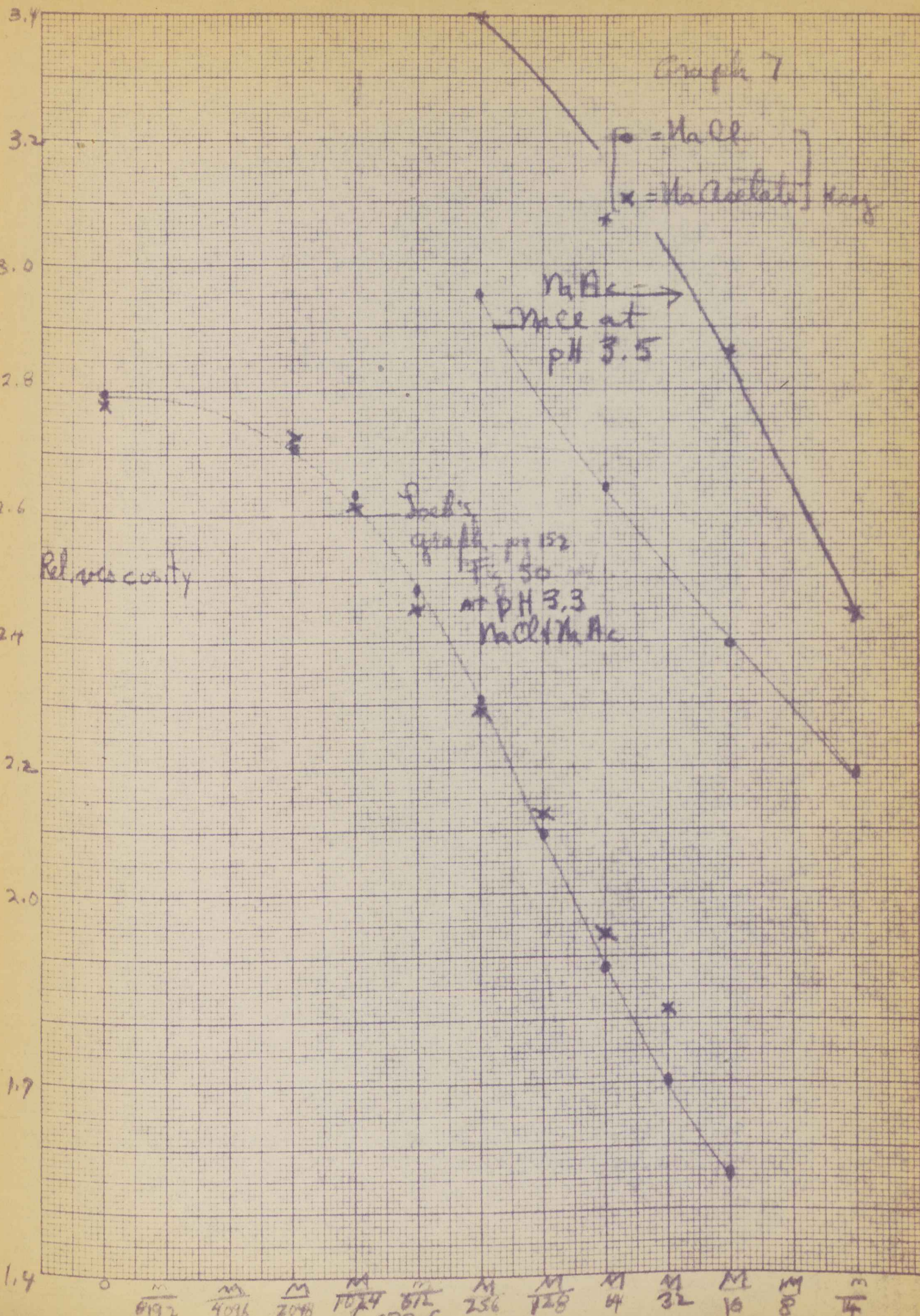














Chapter 8

