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Investigations on factors influencing the properties of Irish Moss extracts, 1944.

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Introduction

It is known that low concentrations of gelose will help to hold insoluble materials in suspension. For example, as little as 0.03% gelose will hold approximately 37 gm. of chocolate suspended in 500 ml. of milk. Specimens vary in this power and some do not show it to any measurable extent. The degree to which it is shown is sometimes known as the "stabilizing power" of the sample.

Since a similar situation was observed in the case of certain insoluble salts, it was thought that it might be instructive to determine the factors that affected the amount of salt held in suspension and attempt to find a relationship between this amount and the concentration of the gelose solution. A comparison was made with the stabilizing power towards chocolate in milk, and it is suggested that a simple relationship exists between the two.

Attempts to filter and weigh barium sulphate and silver chloride precipitated in the presence of Chondrus extract proved very difficult, while the production of an oxalate and a determination of the portion sedimented by titration against $KMnO_4$ was relatively simple. The oxalate was formed by the interaction of calcium chloride and sodium oxalate. Barium oxalate, formed from barium hydroxide and oxalic acid, gave essentially the same results but was more difficult to use.

Procedure

Increasing quantities of a 0.50% gelose solution were pipetted into 15 ml. centrifuge tubes to give a series of known concentrations between 0.01% and 0.10% when diluted to 10 ml. The contents of each tube was then diluted to 8 ml. with distilled water. To ensure complete solution they were warmed at 60° and gently agitated. On cooling, 1 ml. of a 0.10 M calcium chloride solution was added and, after mixing, 1 ml. of a 0.10 M sodium oxalate solution was run in drop by drop with agitation and allowed to stand five to ten minutes. They were then centrifuged at approximately 2800 r.p.m. for a known length of time. The precipitate was washed twice with a 0.2% ammonia solution, centrifuging after each washing about five minutes. After draining on filter paper, 3 ml. of 5% sulphuric acid was added together with a short length of glass rod to facilitate stirring and breaking up the precipitate. The calcium oxalate was then titrated against N/50 potassium permanganate (31), in a micro-burette.

The amount of calcium oxalate held in suspension was obtained by subtracting this figure from a blank containing no gelose and was expressed as milligrams per milliliter, or in some cases as grams per gram of gelose.

Experimental

To ascertain what agreement in values might be expected on the repitition of a single determination, four tubes containing 0.05% gelose and four tubes containing 0.02% gelose were centrifuged five and twenty minutes each. The results are given in Tables 1 and 2. It will be seen that results are reproducible within 1%.

Table 1. Agreement in values after 5 minutes centrifugation.

Tube No.	Mg/ml. of CaC_2O_4 held in suspension at concentration of	
	0.05% Gelose	0.02% Gelose
1	6.72	4.67
2	6.66	4.64
3	6.72	4.61
4	6.66	4.67

Table 2. Agreement in values after 20 minutes centrifugation.

Tube No.	Mg/ml. of CaC_2O_4 held in suspension at concentration of	
	0.05% Gelose	0.02% Gelose
1	2.86	2.62
2	2.87	2.62
3	2.88	2.63
4	2.87	2.61

The manner of adding the sodium oxalate affected the results (Table 3). Higher values were obtained when the addition was made slowly with slight agitation. After standing five minutes the values obtained were then constant (Table 4). The tubes were centrifuged ten minutes.

Table 3. Effect of manner of addition of the sodium oxalate.

Procedure	Mg/ml. of CaC_2O_4 held in suspension at concentration of	
	0.05% Gelose	0.02% Gelose
Added slowly and allowed to stand 15 minutes	3.76	3.22
Stirred	3.22	2.88
Shaken	3.18	2.82

Table 4. Effect of standing after the addition of the sodium oxalate.

Time of standing (minutes)	Mg/ml. of CaC_2O_4 held in suspension at concentration of	
	0.05 Gelose	0.02% Gelose
0	4.35	3.64
5	3.76	3.22
10	3.75	3.21
15	3.76	3.22
30	3.75	3.22
10 hrs.	3.76	3.21

Sufficient quantities of the reagents could be added to cause a flocculent precipitate which was observed to settle without centrifugation. At a concentration of 0.05% gelose this occurred when over 2 ml. of each reagent was used (Table 5). Complete precipitation did not occur, but the amount remaining in suspension was decreased. It was associated with the partial precipitation of the gelose from solution, which occurred on the addition of the calcium chloride.

Table 5. Effect of concentration of reagents.

Quantity of reagents ml.	Mg/ml. of CaC_2O_4 held in suspension
0.5	3.74
1.0	3.75
1.5	3.74
2.0	3.76
2.5	3.20
3.0	1.25
4.0	1.50

Changes in temperature up to 60° had no measurable effect (Table 6). After the addition of the calcium chloride the tubes were heated for five minutes at the indicated temperature and the sodium oxalate added. After another five minutes they were centrifuged for fifteen minutes and the determination of calcium oxalate made as before. Above 60° irregular results were obtained.

Table 6. Effect of temperature.

Conc. of Gelose %	Mg/ml. of CaC_2O_4 held in suspension at 22° C.	56° C.	90°C.
0.10	6.60	6.71	6.56
0.06	5.10	5.08	5.13
0.04	4.04	4.06	3.98
0.02	3.08	3.10	3.06

The addition of sufficient quantities of NaCl, KCl and $CaCl_2$, in excess of that formed by the addition and reaction of the reagents, caused increased sedimentation. The salts were added before the sodium oxalate, and the tubes were centrifuged ten minutes (Table 7).

Table 7. Effect of added NaCl, KCl and $CaCl_2$.

Conc. of Gelose %	Additional conc. of salt. moles.	Mg/ml. of CaC_2O_4 held in suspension with added		
		NaCl	KCl	$CaCl_2$
0.05	0.005	3.74	3.75	3.77
	0.01	3.74	3.74	3.75
	0.02	3.73	2.26	2.68
	0.03	3.72	2.05	1.98
	0.04	3.82		1.28
0.01	0.005	1.60	1.61	1.62
	0.01	1.61	1.60	1.60
	0.02	1.60	1.22	1.34
	0.03	1.21		
	0.04	0.83		

A change in pH between 5.5 and 8.5 had little effect. Above and below this, however, the calcium oxalate held in suspension was decreased. Results are shown in Table 8. The pH was adjusted with sodium hydroxide and acetic acid. The exact value was measured by the glass electrode. The concentration of gelose was 0.05% and the centrifugation time eleven minutes.

Table 8. Effect of pH.

pH of solution	3.1	3.8	5.0	5.5	6.5	7.0	8.5	10.4	11.6
Mg/ml. of CaC_2O_4 held in suspension	1.92	2.43	2.50	3.71	3.74	3.74	3.78	2.45	1.92

The amount of calcium oxalate held in suspension decreased with prolonged centrifugation for all concentrations (Fig. 1). The curve was logarithmic and hence the logarithm of the time was linearly related to the number of grams of calcium oxalate held in

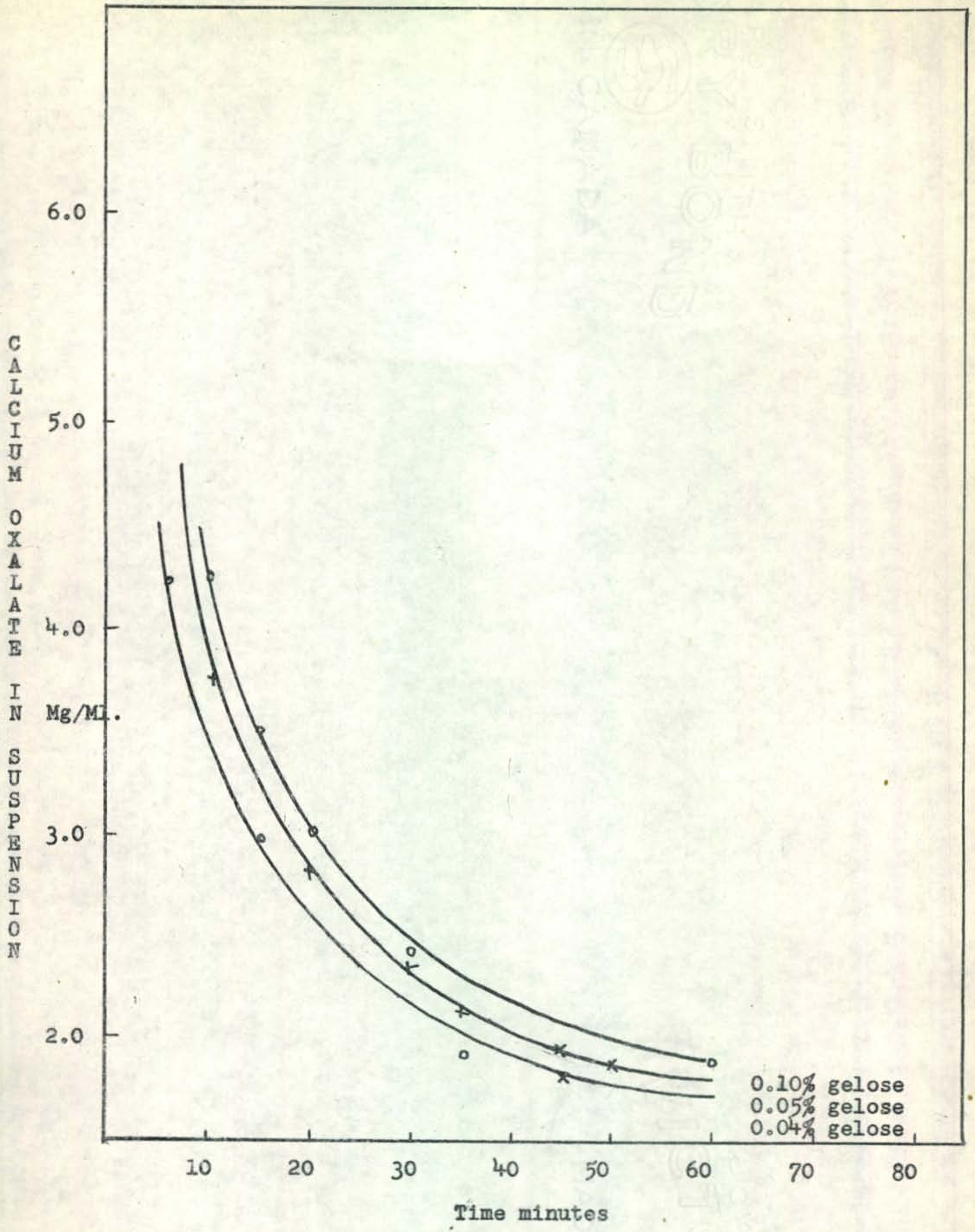


Figure 1. Effect of time of centrifugation on the calcium oxalate in suspension.

suspension per gram of gelose at the particular concentration (Fig. 2).

The same logarithmic relationship was observed in regard to the amount of calcium oxalate held in suspension at various concentrations (Figs. 3 & 4). The angle of the line as well as the value at zero concentration varied with individual samples.

Barium hydroxide and oxalic acid were used to form barium oxalate. The results were parallel to those obtained with calcium oxalate (Table 9). It will be noted that in this case no sodium chloride is formed by the reaction between the reagents.

Table 9. Comparison BaC_2O_4 and CaC_2O_4.

Conc. of Gelose %	BaC_2O_4 Mg/ml.	CaC_2O_4 Mg/ml.	$\frac{BaC_2O_4}{CaC_2O_4}$ Mg/ml.
0.10	2.75	3.06	0.89
0.08	2.71	3.01	0.90
0.06	2.58	2.87	0.89
0.04	2.50	2.77	0.90

Since the concentration of gelose and the number of grams of calcium oxalate held in suspension per gram of gelose varied logarithmically and the same was true as regards the variation in time of centrifugation, we may write the relationship as $m = K/tPc^n$, where m is the number of grams of calcium oxalate held in suspension per gram of gelose at a concentration c after centrifuging a time t. K, p and n are constants in which p and n would be equal to the tangent of the angles made by the lines plotted for t and p respectively. K can be obtained by substitution at any concentration where the calcium oxalate gm/gm gelose is known. When this was done the following agreement between observed and calculated values was obtained.

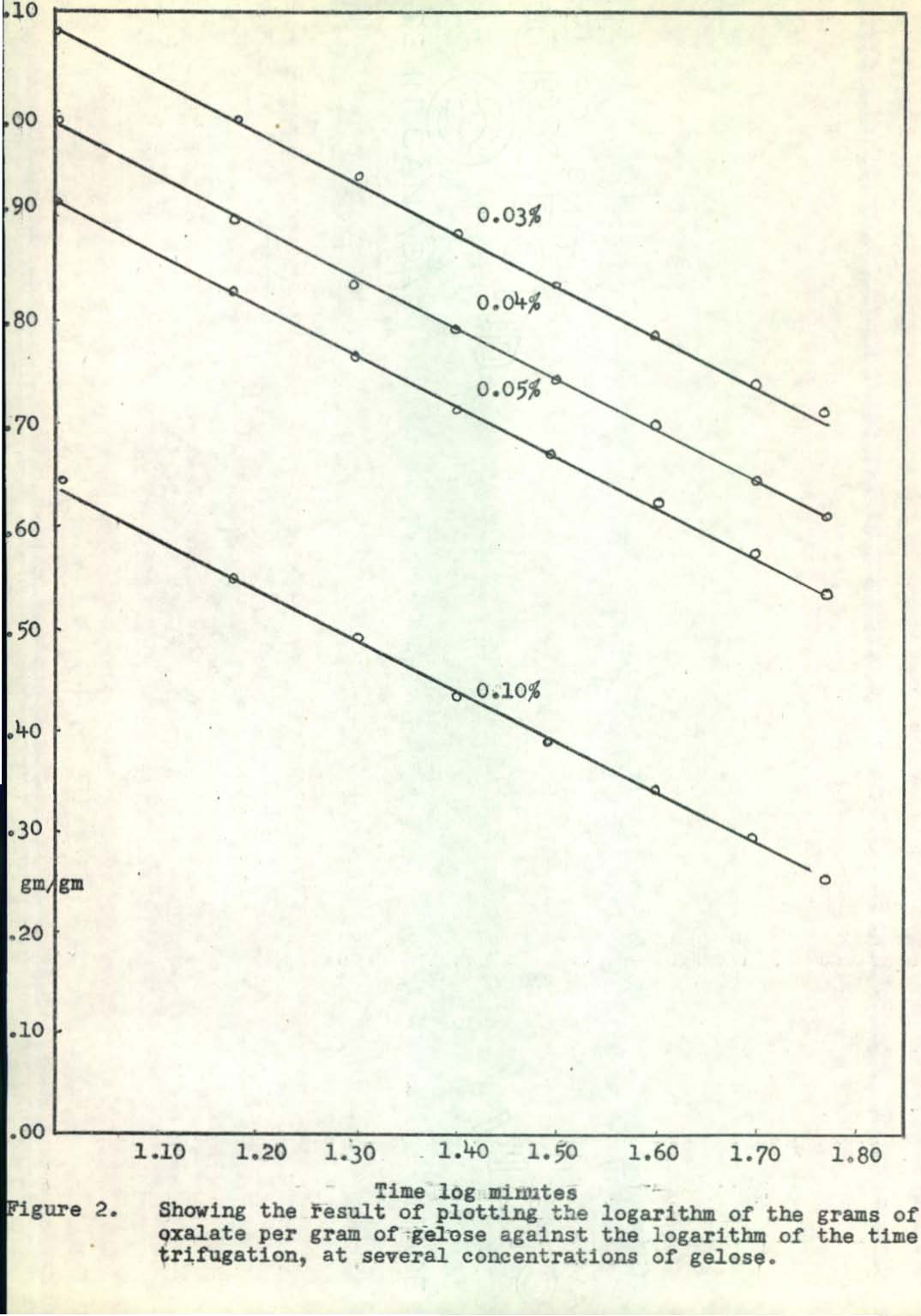


Figure 2. Showing the result of plotting the logarithm of the grams of calcium oxalate per gram of gelose against the logarithm of the time of centrifugation, at several concentrations of gelose.

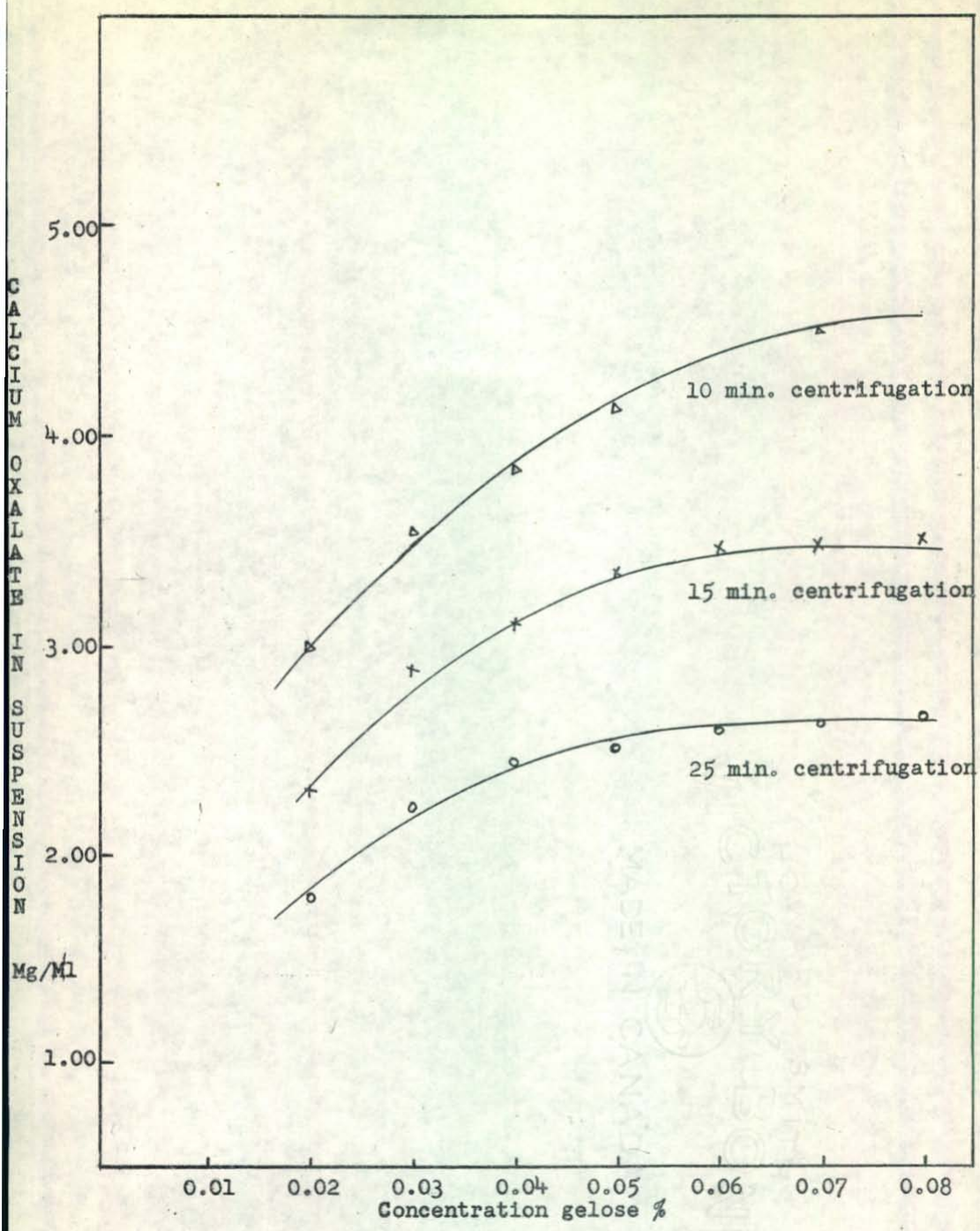
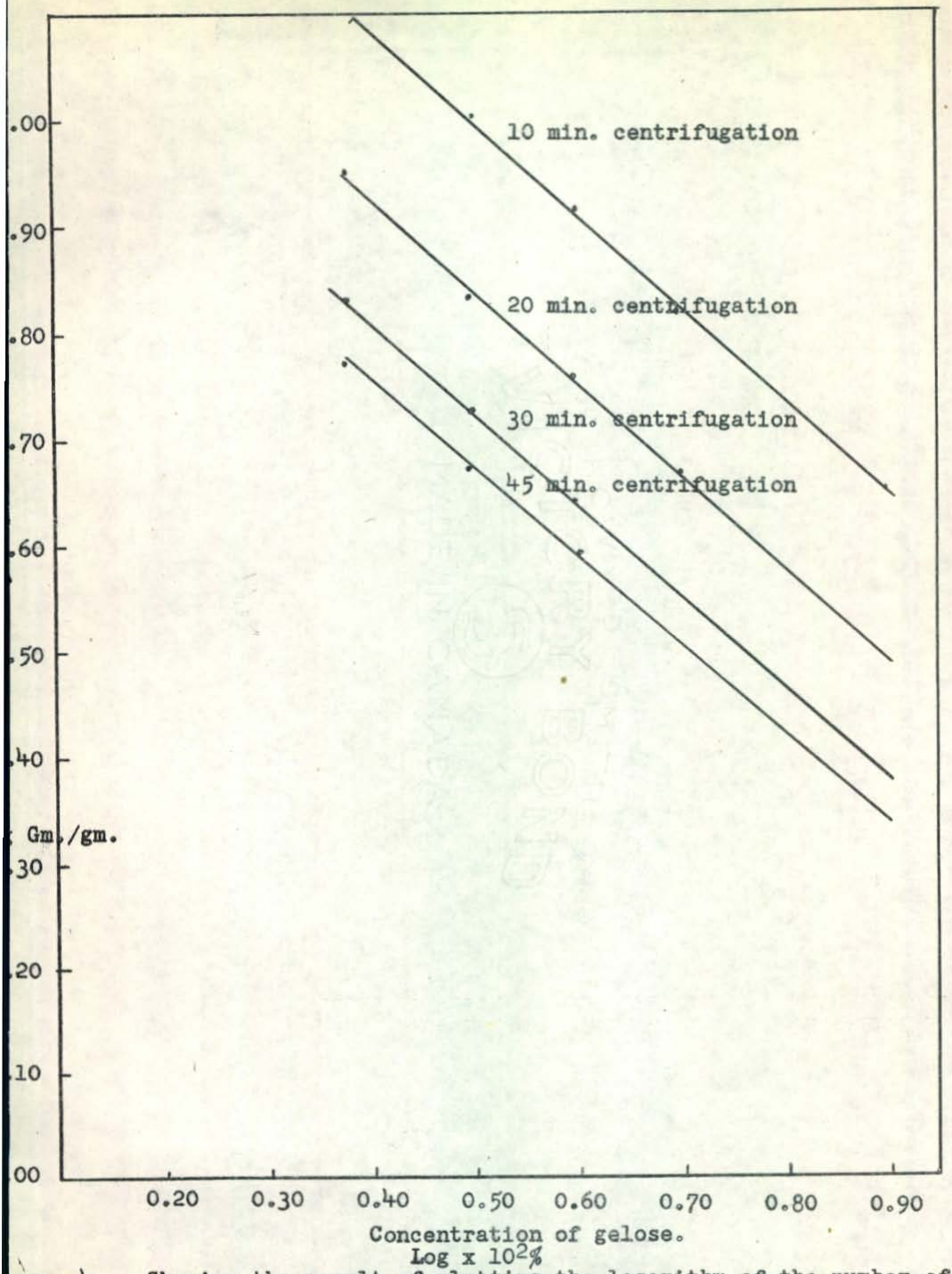


Figure 3. Effect of concentration and time of centrifugation on suspended calcium oxalate.



gure 4. Showing the result of plotting the logarithm of the number of grams of calcium oxalate suspended per gram gelose, against the logarithm of the concentration %. Also the effect of increasing the time of centrifugation.

Table 10. Agreement in observed and calculated values.

Time of centrifugation (minutes)	Conc. of gelose %	CaC_2O_4 grams per gram gelose	
		calculated	observed
20	0.03	893	892
20	0.05	575	576
10	0.03	1190	1210
15	0.05	671	670
35	0.05	421	428
10	0.10	457	452

Samples of gelose were used to compare the amount of calcium oxalate held in suspension with their stabilizing power towards chocolate in milk. The lowest concentration necessary to stabilize 36.6 gm. of chocolate in 500 ml. of milk testing 2% butter fat was taken as an index of stabilizing power. The results are given in Table 11. The constants for the samples of gelose are given. It will be seen that K appears to be related to the ability to hold chocolate in suspension.

Table 11. Comparison of constants obtained by CaC_2O_4 and concentration necessary to hold 36.6 gm. chocolate in 500 ml. of milk.

Sample No.	K	p	n	Conc. gelose necessary to stabilize chocolate
1	did not give usual results 1.17 gm/l held at 0.05%.			>0.30
2	211	0.525	0.860	0.045
3	202	0.254	0.748	0.050
4	7.3	0.217	0.140	>0.30
5	4.1	0.179	0.326	>0.30
6	247	0.101	0.467	0.040
7	329	0.428	0.325	0.030
8	177	0.152	0.620	0.060
9	215	0.314	0.208	0.045

Since K represents the amount of calcium oxalate held in suspension at unit concentration of gelose and after a unit time of centrifugation, we might expect K to be related to the amount of chocolate held in suspension in a given quantity of milk within the unavoidable 10% error made in the determination of stabilizing power by means of chocolate. Table 12 shows a linear relationship within 7% on four samples.

Table 12. Relation between K and chocolate held in suspension per gram of gelose.

K	Conc. gelose holding chocolate in suspension %.	Chocolate held in suspension per gram gelose.	K/chocolate held in suspension per gram gelose.
202	0.050	146.5	1.38
211	0.045	162.8	1.30
247	0.040	183.0	1.35
329	0.032	244.0	1.35
177	0.060	106.7	1.60

Discussion

In 1930 Ewe (28) showed that methylene blue would precipitate a solution of gelose and claimed the reaction was quantitative. He suggested that the dye was adsorbed by the carrageen, forming an insoluble adsorption complex.

Adsorption has been found to follow a certain rule in regard to the quantity taken up by a given weight of adsorbent. This is usually stated as $a = \alpha c^{1/n}$, (the Freundlich adsorption isotherm) (29). a is the amount adsorbed per unit quantity of adsorbent; c the equilibrium concentration in the solution; α and 1/n are constants. If the logarithms of this equation are taken we get $\log a = \log\alpha + 1/n \log c$. The two sides of this equation bear a linear relationship to each other. The similarity between this and the effect noted for calcium oxalate in gelose solution is evident.

Mason and Weaver (30) showed that "All suspensions should sediment under the influence of gravity" and "This system should show a logarithmic gradient of concentration". We might

assume that the suspended material would therefore be thrown out of suspension logarithmically under the influence of centrifugal force and hence the material in suspension would be logarithmically depleted.

It is believed that these suggestions might account for the observed stabilizing power relationships in gelose solutions.

Summary

1. An insoluble salt, calcium oxalate, was found to be retained in suspension by gelose solution in such a way that the quantity held in suspension per gram of gelose bore a logarithmic relationship to the concentration of gelose. When centrifuged, the quantity in suspension per gram varied logarithmically with the time.

2. Results could be reproduced within 1%. Temperatures below 60° had little effect, as well as a variation in pH between 5.5 and 8.5. The quantity and manner of addition of the reagents could cause increased sedimentation. The addition of sufficient quantities of KCl, NaCl and $CaCl_2$ also lessened the stabilizing power.

3. The equation $m = K/tPc^n$ was developed and checked, where m is grams CaC_2O_4 held in suspension per gram gelose at concentration c and centrifuged time t,- K, p and n are constants which depend on the sample. K is shown to be related to the ability to stabilize chocolate in milk.

4. Stabilizing power is interpreted as an adsorption phenonemon.

2. Effect of acid on stabilizing power and viscosity

An attempt was made to determine whether it was possible to treat highly viscous samples of gelose with acid and to reduce its viscosity without decreasing its stabilizing power.

For this purpose four samples were used which had been dried and bleached in Prince Edward Island during the summer of 1943.

Procedure

900 cc. of a solution containing 5 gm. of gelose in 0.10 N H_2SO_4 was divided into ten samples of 90 cc. each. The first was immediately neutralized with 1.0 N NaOH using alk-acid paper as an indicator, and made up to 100 cc. with distilled water. This gave a final concentration of 5.0 gm. per 100 cc. The samples were heated at 60° C. and at intervals of 15 minutes one was cooled in ice and analyzed.

90 cc. of the solution were used for the determination of viscosity with a Stormer viscosimeter, while the remaining 10 cc., after dilution to 100 cc. were used to determine the stabilizing power. (Section 1.)

Results

The effect of acid on the viscosity and stabilizing power.

Time Minutes	Sample Number I		II		III		IV	
	Rel. Visc.	Stab. Powr.	Rel. Visc.	Stab. Powr.	Rel. Visc.	Stab. Powr.	Rel. Visc.	Stab. Powr.
0	1.25	410	1.46	380	1.50	211	2.10	182
15	1.12	408	1.39	245	1.46	208	1.52	181
30	1.06	395	1.21	180	1.20	195	1.20	106
45		104		36		76	1.15	62
60		82		7		12		
120	1.02	9	1.01	5	1.03	8	1.04	12

Conclusion

The results show that while the viscosity is decreased by treatment with dilute acid much more rapidly than the stabilizing power, it is impossible to consistently find a point where the viscosity has been decreased without the loss of stabilizing power.

Tests for reducing power were all negative with the exception of a very slight positive on samples II and IV at the end of two hours. This indicated that the viscosity could be decreased without causing measureable hydrolysis into free sugars.

II THE EFFECT OF TEMPERATURE AND SOLVENT ON THE VISCOSITY AND GEL-FORMING PROPERTIES OF CHONDRUS EXTRACTS.

Introduction

Considerable experimental and theoretical work has been done in recent years on the physical properties of high polymers in solution. It has been found (16) that a relation exists between the intrinsic or specific viscosity and the concentration of the polymer. In very dilute solutions this relationship is almost linear. More accurately it may be written that $\eta_{sp} = ac + bc^2$ where a and b are constants, c the concentration and η_{sp} the specific viscosity. Various factors determine the values of these constants. They are influenced considerably by temperature and solvent, and the exact values in each case must be found emperically.

Alfrey, Bartovics and Mark (17) have explained the effect of temperature and solvent on polymers such as polystyrene, rubber and cellulose acetate in terms of the mean molecular shape of the molecule. They consider that a linear molecule instead of remaining linear in solution takes on a number of random shapes. The mean of these is determined by the "energetically favourableness" of the solution, which is related to the change in entrophy, occurring when the substance dissolves. They have shown, as is expected theoretically, that the specific viscosity is high in a good solvent and low in a poor solvent or solvent-non-solvent mixture. In a series of solvent-non-solvent mixtures the quadratic term b in the equation ($\eta_{sp} = ac + bc^2$) is reduced linearly more than the linear term a by the presence of the non-solvent. At the upper limit of solubility, the polymer will form a gel particularly in a poor solvent, since an energetically unfavourable solvent will favour polymer-polymer contacts between different chains. "An energetically favourable solvent will stand a higher concentration of the polymer and yet give a fluid stable condition."

Since gelose may be considered as a polymer made up of carbohydrate units, it was thought that a similar situation might exist in a gelose solution where the addition of salts would cause a decrease in solubility, and hence increasing quantities would produce a progressively less soluble medium. This might behave in the same way as the solvent-non-solvent mixture does towards synthetic high polymers.

Experimental

The material used was prepared by either (a) drying the crude extract or (b) drying after precipitation with alcohol (7) or (c) drying after alcohol precipitation and dialyzing. The dried material was ground in a Wiley mill to a fine powder. The required amount of gelose, after drying in a pistol desiccator over phosphorus

pentoxide at 62°, was dissolved in distilled water. Where salts were used, they were added to this solution.

The viscosity determinations were made by means of a Stormer viscosimeter after the instrument had been calibrated (18). The weight used was adjusted so that the time measured by a stop-watch reading to 0.1 seconds was of the order of 30-40 seconds. The solutions were maintained at a temperature of 30° in a constant temperature bath.

Measurements were made over a series of concentrations from 0.1 to 1.2 gm. per 100 ml. of solution at approximately 0.05 gm/100 ml. intervals. Variations in procedure are given in the succeeding paragraphs.

Results and Discussion

It is known that the addition of any one of a number of salts will decrease the viscosity of Irish Moss solutions (13) and that their addition, particularly KCl, will cause an increased hardness of the gel (4).

The viscosities of four extracts were determined over a range of concentrations. The extracts were prepared from the same crude material and would differ in only the salt content inherent in their preparation. The first three were as given above (a, b and c), and the fourth was prepared by adding 0.25% KCl to (b). Results are shown in Fig. 1.

If the equation $\eta_{sp} = ac + bc^2$ is true for gelose solutions η_{sp}/c plotted against c should give a straight line. This is shown well within the range of experimental error. From their preparation these extracts would contain varying amounts of naturally occurring salts, which would dissolve in the medium. Ash determinations were made and assuming (c) to contain no inorganic material the calculated salt concentration of the other solutions were found to be: (b) 0.022%; (a) 0.028%; and the fourth extract 0.27%. On being twice precipitated in 80% alcohol the fourth extract gave its original viscosity measurements.

If we assume the gelose material in each sample to be identical, the added salts must account for the observed variations in the viscosity as indicated by the changed values of the constants a and b, which are progressively decreased. Assuming a decreasing solubility of gelose in salt solutions (since it is quite insoluble in a cold 3% sodium chloride solution and can be precipitated by the addition of salts (6)) the results are compatible with those observed for other polymers in solvent-non-solvent mixtures.

That the above viscosity changes are due to salt concentration is further indicated by the fact that the addition of various amounts of potassium chloride (Fig. 2) to a dialyzed extract gave essentially the same result. A comparison with the sodium salt (Fig.3)

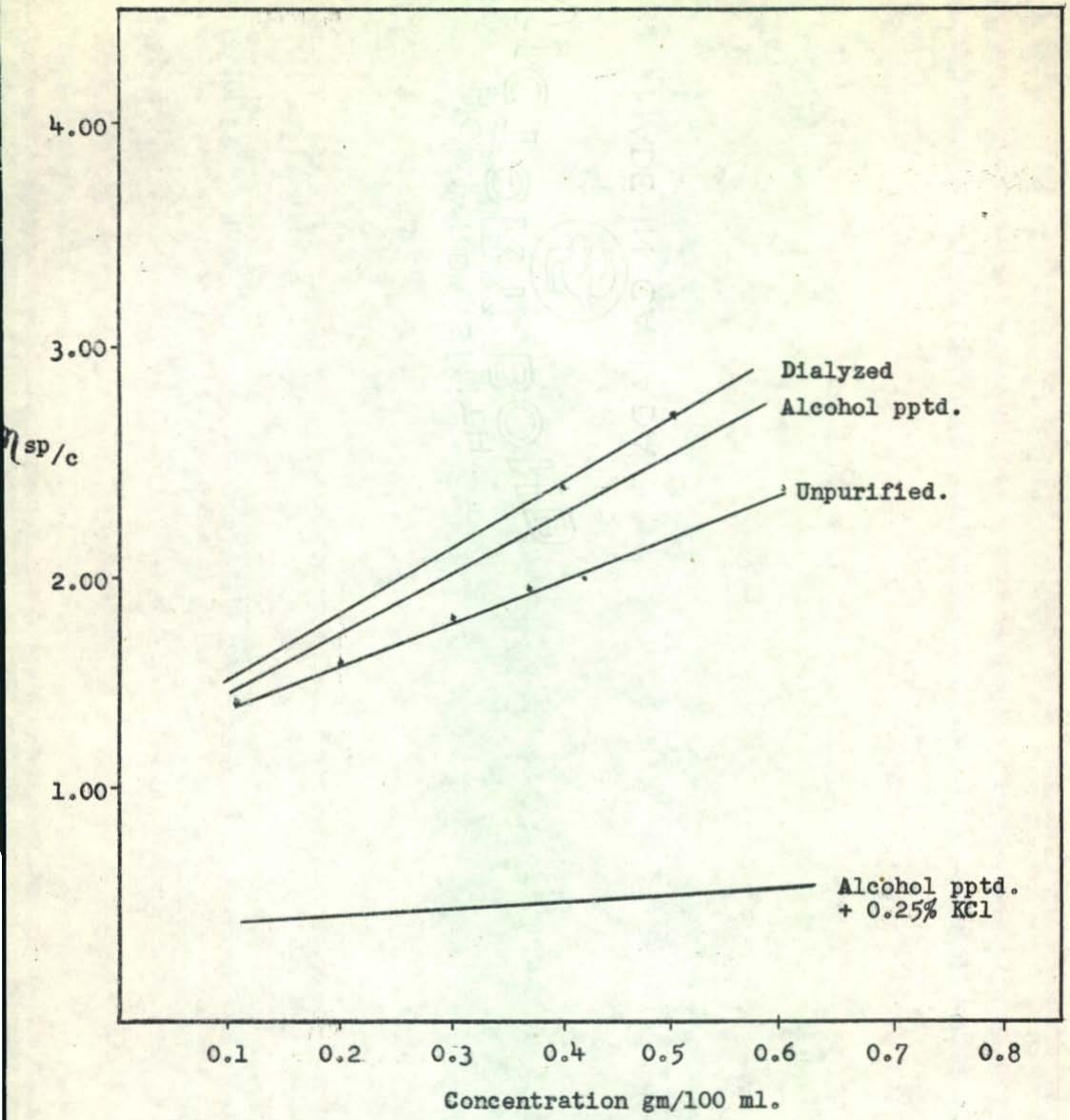


Figure 1. Effect of increasing salt concentration on η_{sp}/c and c relationship.

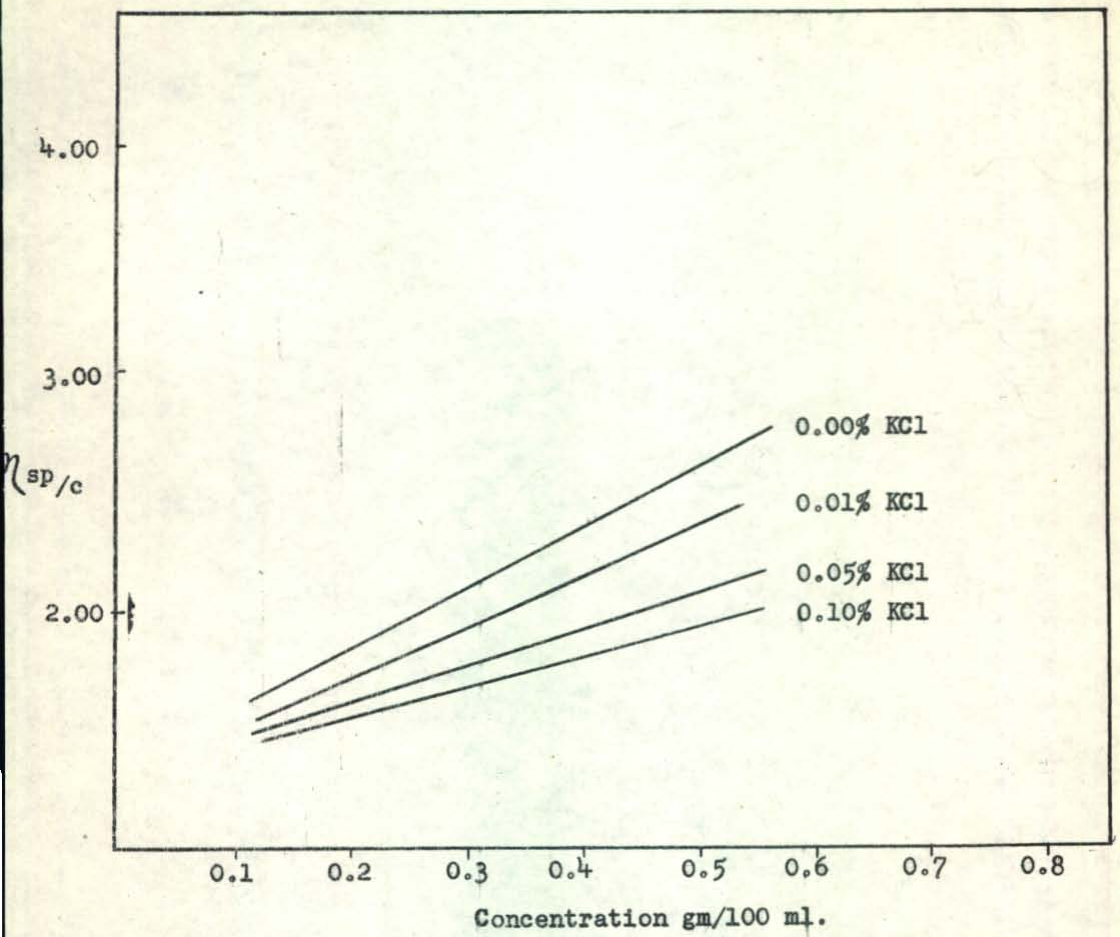


Figure 2. Effect of increasing quantities of KCl on the viscosity of gelose solutions.

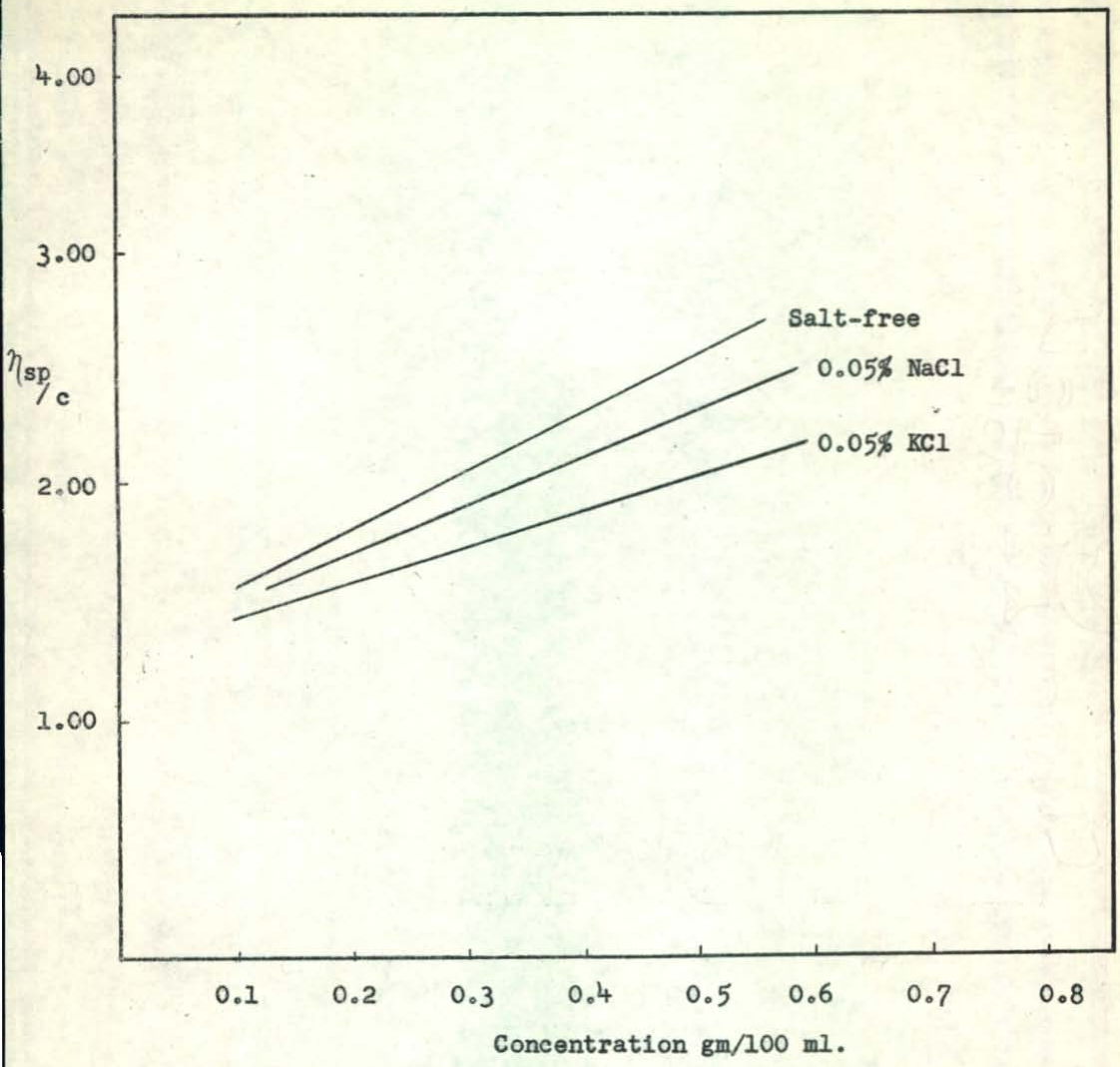


Figure 3. Comparison of the effect of KCl and NaCl on viscosity.

showed the latter to be less effective. It will be shown later that a lower concentration is required for precipitation with potassium chloride than with sodium chloride.

Measurements of the change in viscosity with increasing temperature were made, and it was found that while the salt-free extracts had initially a higher viscosity than those containing salt, the rate of decrease, with an increase (Fig. 4) in temperature, was much greater. The initial viscosity, as well as the rate of decrease progressively diminished as the concentration of salt increased. As postulated before, if the viscosity of a gelose solution depends on the mean molecular shape, this effect is to be expected theoritically since (17) "the change in viscosity with temperature should increase in a good or energetically favourable solvent at a greater rate than in a less favourable solvent".

The equation $\eta_{sp} = ac + bc^2$ is known to be true only at comparatively low concentrations. In the case of gelose solutions the departure from the rule occurs at about 0.5 gm/ 100 ml. (Fig. 5). If the concentration is increased the solution will as a rule form a stiff gel at about 2%. The exact concentration at which this takes place and the hardness of the gel is not however solely dependent on salt concentrations. Dialyzed extracts differ considerably and this variation (Fig. 7) is not associated with the method of preparation.

When potassium chloride is added to a solution of gelose a decrease in the specific viscosity of the solution is observed and initial gelation occurs at a lower concentration (Fig. 6) than in the salt-free extract. An extract which normally formed a gel at a concentration of 1.9% at about 1.1% in a 0.06% KCl solution, and at 0.7% in a 0.25% KCl solution. The firmness of the gels, however, were progressively decreased. The hardness of the gel at 1.9% was noticeably greater after the addition of the salt. No attempt was made to determine this increase.

Extracts with different concentration-viscosity relationships were also found to differ markedly in the concentration at which a gel could be formed by the addition of potassium chloride (Fig. 7). The higher viscosity extracts formed gels at a lower concentration on the addition of equal amounts of potassium chloride. The addition of sufficient potassium chloride to a dilute extract would cause precipitation rather than gelation. This was observed at a concentration of 0.5% or less, depending on the extract.

Solutions of gelose were precipitated when sufficiently dilute by a lower concentration of potassium chloride than of sodium chloride. In a 0.7% solution 10% of KCl was required in contrast to 16% of NaCl to cause precipitation (Table 1). It is

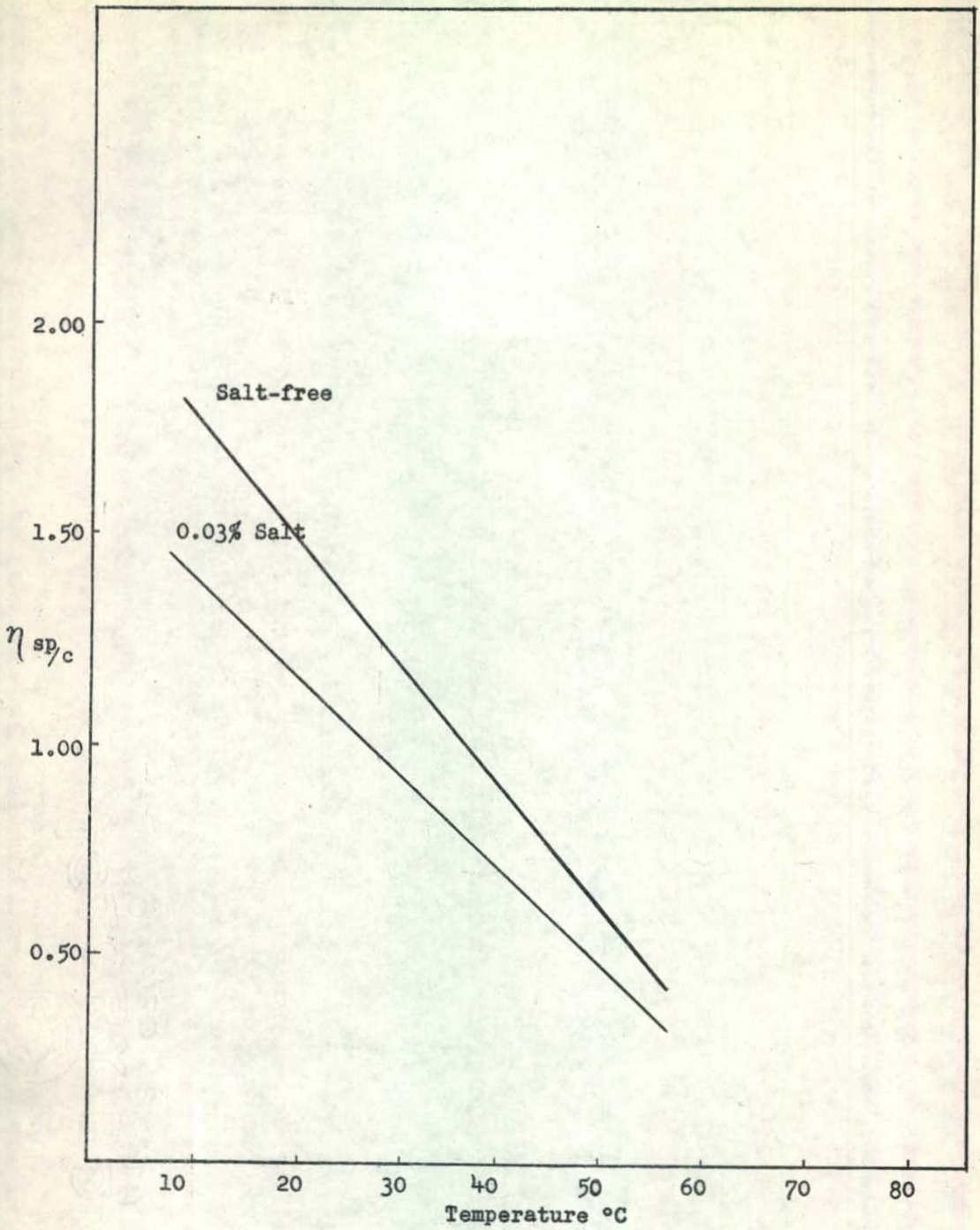


Figure 4. Change in viscosity with an increase in temperature in a 0.10% gelose solution with and without the addition of salt.

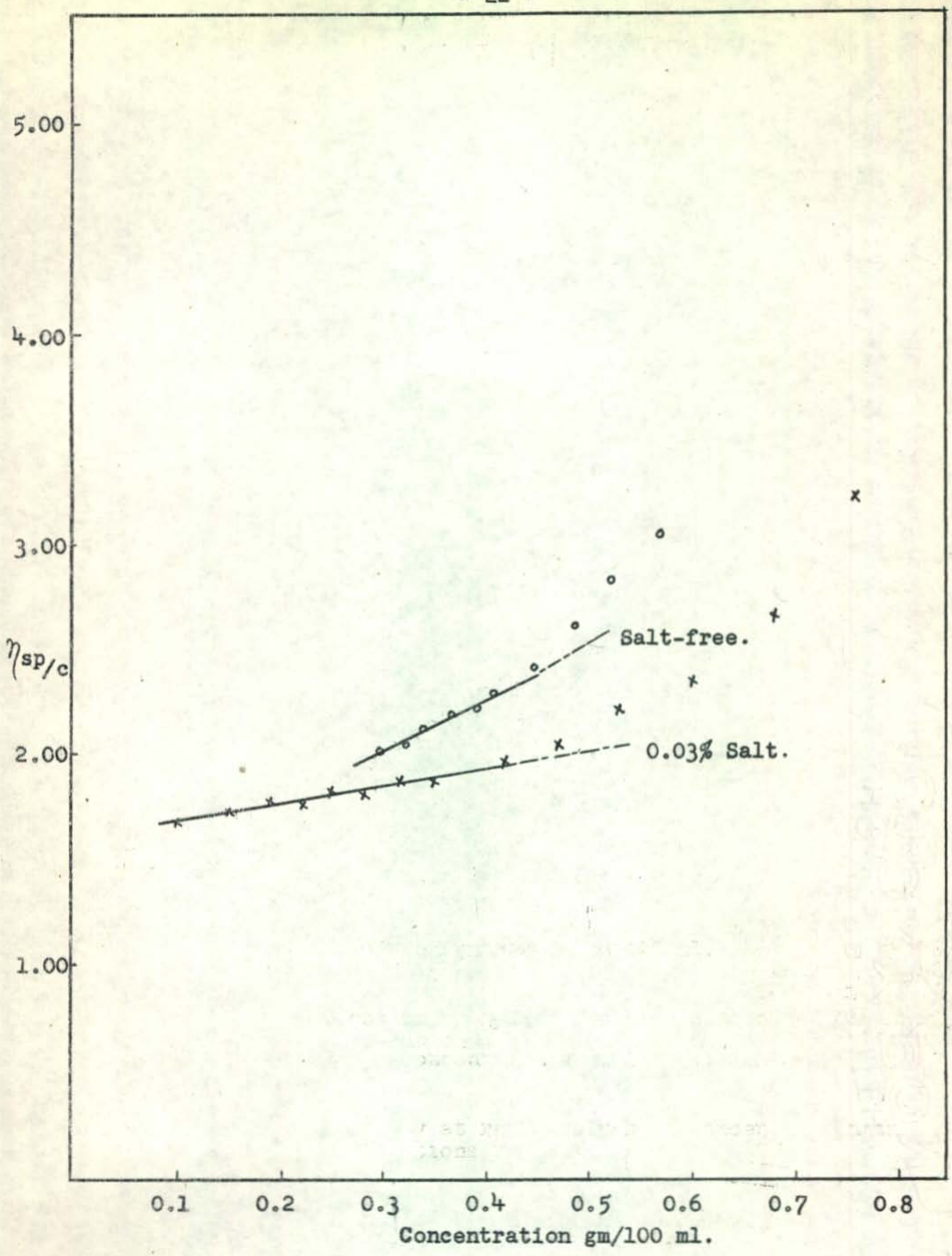


Figure 5. Change in viscosity at relatively high concentration in salt-free and salt solutions.

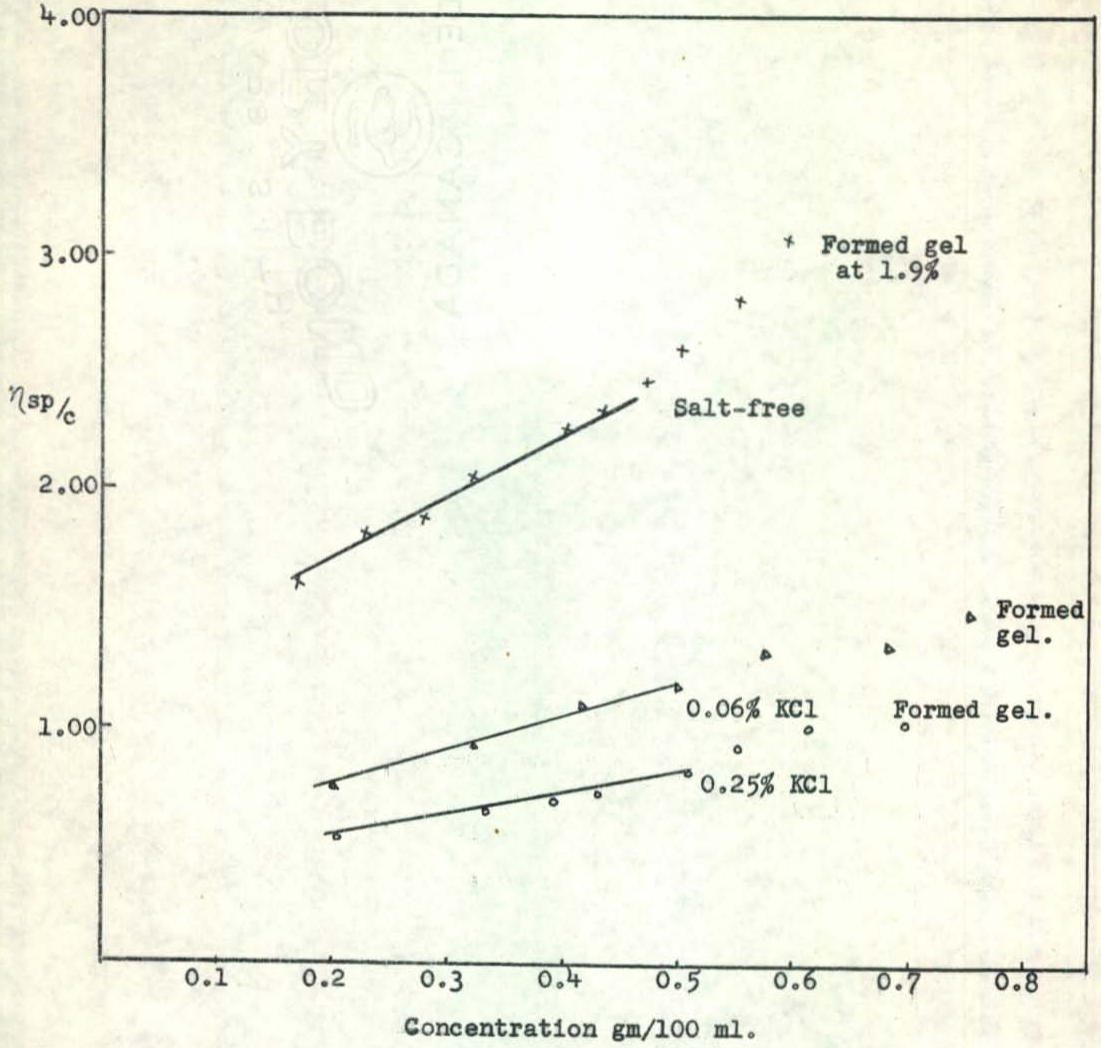


Figure 6. Effect of increasing quantities of KCl on gel formation.

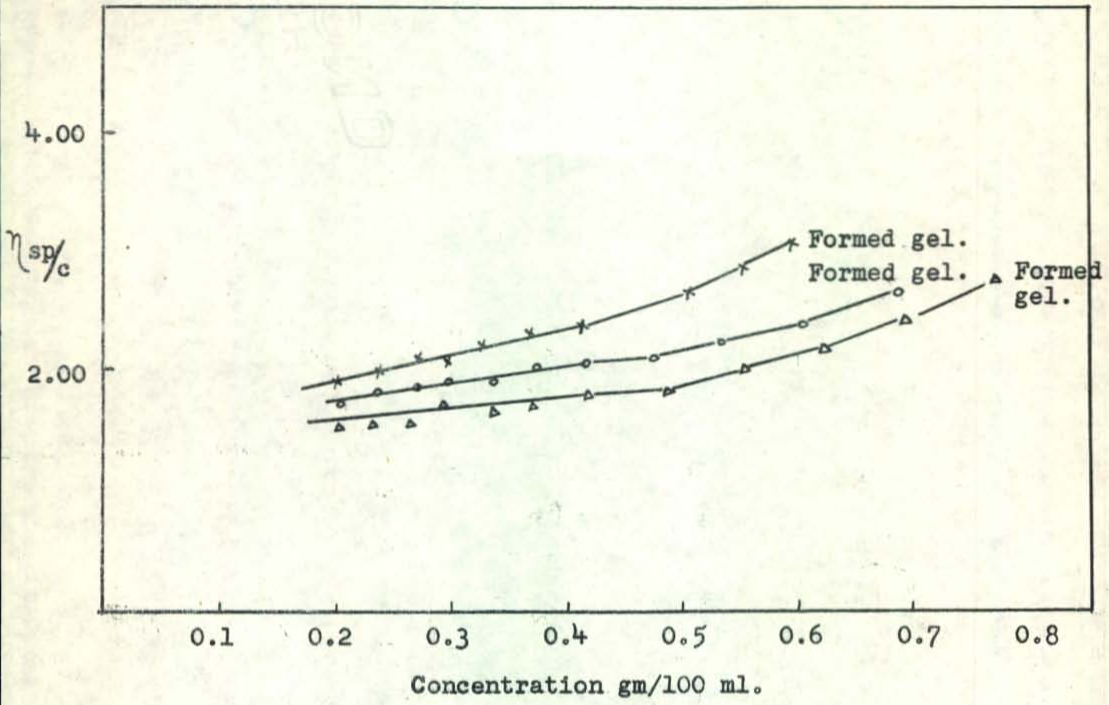


Figure 7. Effect of the addition of 0.1% on the formation of a gel by extracts with different viscosities.

suggested that this is related to the increased effect on viscosity and gelation of KCl over NaCl.

Table 1. Concentration of KCl and NaCl required to precipitate gelose.

Conc. gelose %	Conc. req'd to ppt. with KCl%	NaCl%
0.7	10.2	17.1
0.5	8.7	12.9
0.3	7.6	

(10 ml. of gelose solution was used and a saturated solution of the salt was added from a microburette until precipitation occurred. Results were calculated.)

Summary

1. Extracts of Irish Moss are found to have the same viscosity-concentration relationship as observed in other high polymers.

2. The addition of salt decreases the viscosity in such a way as to decrease the value of the constants a and b in the equation $\eta_{sp} = ac + bc^2$. This corresponds to observations on polymers in progressively poor solvent-non-solvent mixtures.

3. Increasing the temperature decreases the viscosity. The ratio η_{sp}/c: c decreases more rapidly in salt-free solutions.

4. The effect of KCl on viscosity and gelation is more pronounced than NaCl. This is related to the solubility of gelose in the respective salt solutions.

5. The addition of salt initiates gelation at a lower concentration than would normally occur. The exact concentration is, however, dependent on the intrinsic viscosity of the salt-free extract.

III PREPARATION AND EXAMINATION OF SAMPLES

1. Preparation of commercial samples

The following procedures were used on samples shipped to the Kraft and Krimco Companies.

At Yarmouth the work was done at the Kraystay Factory. The moss was spread on a board floor to such a depth that there was approximately one pound per square foot. It was impossible at the time to obtain any large uniform sample of green moss so a large well mixed sample of black moss was used.

The samples shipped to Kraft Cheese Co. were:

1. Initial weight 175 lbs. After being soaked for two hours in fresh water the moss was spread on the floor at 12 noon and allowed to bleach and dry for 4 hrs. At the end of that time the moss had become partly dry but was still sticky to touch in spots. The moss had been turned by rake at the end of two hours and was now wet with sea water for an hour by means of a hose. The following five days the moss was kept wet by light continuous sea water sprinkling. This was discontinued each day at 5 p.m. and resumed in the morning at about 10 a.m. There was no rain and the moss was not covered at night. After one day drying it was baled and had a final weight of 200 lbs.

2. Weight 175 lbs. Treated as No. 1 but bleached for only 2 days instead of 5 days. Final weight 154 lbs.

3. Weight 175 lbs. Soaked 2 hrs. in salt water and then after bleaching 4 hrs. as in No. 1 was bleached as before for 5 days with sea water. After a two-hour soaking in fresh water the sample was dried for one day. Final weight 136 lbs.

4. Weight 100 lbs. Soaked for two hours in sea water and then bleached as in No. 3 omitting the final fresh water wash. Final weight 85 lbs.

5. Weight 130 lbs. The initial soaking was carried out with fresh water acidified with HCl (1 pint to 45 gal.) and then continued as No. 1 except that the period of bleaching was shortened to one day. Final weight 62 lbs.

6. Weight 45 lbs. treated as No. 5 with the exception that the initial washing was with the already used acid solution (from 5).

Samples were also shipped to Kraft Cheese Company from Tignish, P.E.I. These were prepared from green moss obtained at North Cape. Black moss from Rustico was also used. The green

moss was first dried and weighed samples bleached. In drying 600 lbs. of moss yielded 208 lbs. of black moss. The following samples were prepared.

1. Weight 150 lbs. Soaked in fresh water 2 hrs. and kept wet with sea water by pouring water over the spread moss by means of a bucket. The sample was allowed to bleach for five days as in the previous sample No. 1.

2. Weight 150 lbs. Wet initially by soaking 2 hrs. in salt water and maintained wet by salt water while bleaching for two days. Fresh water was then used as a wetting agent for a further period of two days when the moss was stored after drying for one day. The fresh water wetting was aided by numerous showers and the weather was very dull. The moss was bleached however by the end of the fourth day. Final weight 50 lbs.

3. Weight 150 lbs. Treated as No. 2, except that sea water was alone used. The weather was dull but the moss received no rain.

4. Weight 150 lbs. Sample of black moss from Rustico, treated as No. 1. Final weight 110 lbs.

Samples shipped to the Krimco Company were prepared as follows:

1. Weight 150 lbs. Prepared as No. 4.

2. Weight 150 lbs. Sample of west side moss dried without wetting.

3. Sample from North Cape. Weight 150 lbs. bleached with sea water four days.

4. Sample from west side. Weight 150 lbs. bleached with sea water four days.

The samples for Krimco were collected by their agent at Rustico.

The weather at Tignish was on the whole poor for both bleaching and drying. Most of the time the sky was overcast and for two days there were light rains. This rain was taken advantage of to determine the effect of light rain and dull weather for bleaching on the quality of the Chondrus extracts. (Reports from the two companies.)

There was some difference in the mode of turning the moss. That at Yarmouth was turned by means of a rake at approximately one and a half to two hour intervals. At Tignish turning was done principally by hand and was not so efficient, taking a much longer period to completely turn the sample.

2. Examination of commercial samples

About 40 grams of each of the samples prepared as explained in the previous section were extracted in 2 l. of boiling water. After 12 hours extraction 100 cc. was withdrawn, filtered and evaporated to dryness. After grinding in a Wiley mill 0.50 gm. was weighed and dissolved in 100 cc. of distilled water. The viscosity and stabilizing power was determined as given in preceding sections.

The following results were obtained on the samples examined:

Treatment			
Moistening solution	Time of bleaching days ?	Relative viscosity 0.5% gelose	Stabilizing power 'K'
fresh water	5	1.28	358
salt water	5	2.06	326
fresh water	2	1.41	198
salt water	2	2.32	196
fresh water	5	1.36	262
salt water	5	1.78	231

It would seem that prolonged bleaching increases the ability of the gelose to hold insoluble materials in suspension.

The opposite is true of the viscosity and consequently the gelling power of the extract. Treatment with fresh water appears to lower the viscosity with, if anything, an increase in stabilizing power.

It must be remembered however that only a few samples were investigated and any statement is therefore of necessity in the nature of a postulate.

3. Examination of stabilizing power of samples prepared in the summer of 1943.

Several samples prepared during the summer of 1943 were examined for stabilizing power.

It was found that gelose which had been prepared previously by precipitation from aqueous solution with ethanol and stored for

ten months possessed no measureable stabilizing power. Extracts however which were prepared without precipitation with ethanol retained their stabilizing power. This was also true of extracts prepared fresh from the stored plant.

The stabilizing power was determined with and without pasturization. Gelose was added to milk testing 2% butterfat to make samples containing 0.02, 0.03, 0.04, 0.045, 0.05 and 0.06 gm./ 100 cc. Chocolate was added in the proportion of 36.6 gm. per 500 cc. of solution and after thorough mixing allowed to stand 48 hours in the ice box. After a further 48 hours at room temperature the bottles were examined for signs of precipitation. In only one case was the chocolate not completely precipitated under these conditions. This occurred in a sample which had been bleached with frequent washing with fresh water over a period of three weeks.

Similar samples were prepared with the addition that they were pasturized for 15 minutes at 60° C. before being placed in the ice box. The chocolate was then held in suspension in the samples investigated. No correlation however was found in the conditions necessary to produce an extract with high stabilizing power.

The following are typical results:

Effect of the mode of bleaching on the stabilizing power

Mode of bleaching	Stabilizing power without pasteurization	Stabilizing power after pasteurization
fresh water bleached		
black	0.0	0.06
1 day	0.0	0.05
2 day	0.0	0.06
2 day	0.0	0.05
5 day	0.0	0.03
salt water bleached		
black	0.0	0.06
1 day	0.0	0.06
2 day	0.0	0.04
5 day	0.0	0.03
5 day	0.0	0.05

The stabilizing power is given as the grams of gelose per 100 cc. of milk required to hold the chocolate in suspension.

As before there is an increase in stabilizing power with prolonged bleaching.