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BIOSYNTHESIS AND STRUCTURE
OF CORN STARCH

by

Stig Robert Erlander

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Biophysical Chemistry

Approved

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I. INTRODUCTION

A study of the change in properties of corn starch during plant growth has been made. From this study information concerning the structure of starch and its relation to glycogen was obtained.

Some years ago glycogen was considered as a reserve polysaccharide of only the animal kingdom. Starch, a reserve polysaccharide for plants, was considered as being both chemically and physically different from glycogen. In the last 10 or 15 years, Meyer (1) and many others have proclaimed that neither starch nor glycogen is a chemical identity. Glycogen is found in the lower plants such as fungi and yeast and also in the higher plants such as Golden Bantam sweet corn.

Hehre, Hamilton and Carlson (2) observed that the polysaccharide synthesized by Neisseria perflava cultures has properties similar to those of amylopectin, the branched component of starch, while the polysaccharide synthesized by its cell-free enzyme system resembles that of glycogen. Barker, et. al. (3), using a different strain of Neisseria perflava, found that the polysaccharide synthesized by sucrose cultures had properties between glycogen and amylopectin. By changing the feed for starved rabbits from fructose or glucose to galactose or sucrose, the average chain length of the glycogen is changed from 12 glucose units to 18 (4). This latter example has been disputed (5). However, all of these examples

illustrate that a high degree of branching is not restricted to glycogen and a low degree of branching to amylopectin. This is most likely due to the changing of the relative activities of the branching and phosphorylase enzymes.

Starch consists of both a linear component called amylose and a branched component called amylopectin. Therefore the production of starch in sweet corn endosperm involves a three fold query: How can the highly branched glycogen, the lesser branched amylopectin, and the completely unbranched amylose be produced in the same cells and in the same vicinity in these cells? Cumulative evidence seems to point towards glycogen as being an intermediate in the synthesis of starch. Therefore the following sequence is proposed:



The conversion of glycogen into starch can best be explained by assuming an irreversible debranching of the glycogen followed by the connecting of these linear debranched chains to form amylose. The partially debranched glycogen is called amylopectin. The production of longer unbranched chains in the amylopectin allows this partially debranched glycogen and amylose to crystallize out of solution in the form of starch granules, the variation in the rate of crystallization producing the well-known granular rings.

The evaporation of water from the kernel appears to be connected with the synthesis of starch and the per cent amy-

lose in the starch (6). To study this, some corn ears were bagged with cellophane bags during maturation in order to retard the evaporation of water and others were husked in order to hasten it.

The mechanism proposed above involves a simultaneous production of amylopectin and amylose. Because the plant is subjected to diurnal variations, it is possible that amylose is produced during the day and amylopectin during the night. The twelve hour variations in yield and per cent amylose in corn starch were studied in order to determine if such a process occurs.

In order to study the effect of light on per cent amylose, potato plants were grown under three different conditions: a small amount of light, normal day light, and almost continuous lighting.

A study of how starch is produced also involves a study of the structure of the components of starch. A statistical model based on the random condensation of glucose units was developed (7). It is known that all of the 1,4-linkages in amylopectin and glycogen are acid hydrolyzed at the same rate. Acid hydrolysis would therefore be a reverse of a random condensation reaction. If amylopectin and glycogen are produced randomly as in a hypothetical condensation reaction instead of all branched molecules having the same structure, then they should behave as a statistical molecule during acid hydrolysis.

Acid degradation studies were therefore made on waxy, dent and sweet corn amylopectins and on sweet corn glycogen.

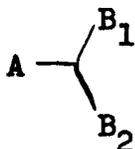
The high molecular weights of amylopectin obtained by light scattering methods have been subjected to much criticism. The statistical model predicts that the weight average molecular weight will be at least around 100 times the number average molecular weight. This would account for the discrepancies found in the literature. Some amylopectins were subjected to sedimentation measurements, centrifugation studies (8), variation in the solvent system, and variation in the method of dispersing the granules in order to show that this great difference is not due to the presence of a small amount of extremely large particles.

II. STRUCTURE AND BIOSYNTHESIS OF STARCH AND GLYCOGEN

A. Statistical Model

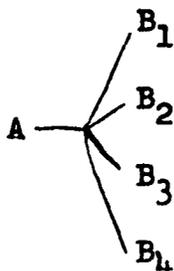
1. Development of statistical model

Erlander and French have developed a statistical model for a branched polymer built from a monomeric unit (such as glucose) which has two types of reactive groups (aldehydic and alcoholic). The treatment differs from that of Flory (9) in that the different B groups in the $A-R-B_{f-1}$ monomer have differing reactivities instead of equal reactivities. In applying this to carbohydrate chemistry glucose may be used to represent three different types of $A-R-B_{f-1}$ monomers. In each case the aldehydic group of glucose represents the A functional group and the alcohol groups represent the $f-1$ B-functional groups. Glucose may be represented as A-B if the functionality of the glucose monomer is two. When B represents the alcohol group on carbon atom number four, the condensation of these A-B units will give polymers resembling cellulose or amylose. When $f = 3$, glucose may be represented as an



monomer with B_1 and B_2 representing the alcohol groups on carbon atoms four and six. The condensation of such units would

give polymers resembling amylopectin and glycogen. Similarly for $f = 5$, glucose would be represented as



and the polymer obtained by the condensation of such units would resemble polyglucose (see Fig. 1).

Because of its length, the development of the statistical model, the number-, weight-, and Z- average degrees of polymerization, and the evaluation of the weight fraction of x -mer will not be given here. For the evaluation of these quantities one can refer to the paper by Erlander and French (7).

As noted above the glucose unit in starch or glycogen can be considered as an $A-R-B_{f-1}$ monomer when $f = 3$, the A group representing the potential aldehydic group and the B_1 and B_2 groups any two alcoholic groups on the glucose monomer. Since amylopectin and glycogen consist essentially of 1,4- and 1,6-linked glucose units, we can consider the glucose

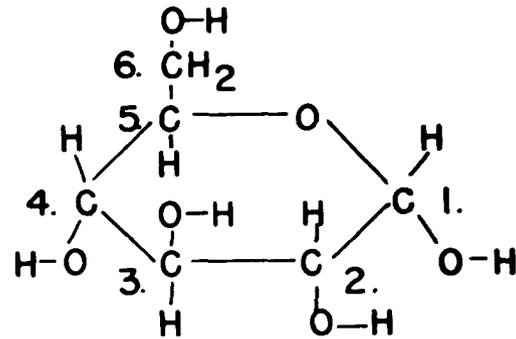
unit as an A $\begin{matrix} B_4 \\ \diagdown \\ B_6 \end{matrix}$ monomer having probabilities p_4 and p_6 for

forming a linkage at B_4 and B_6 , respectively. If there is

Fig. 1. Representation of glucose as various A-R-B_{f-1} monomers.

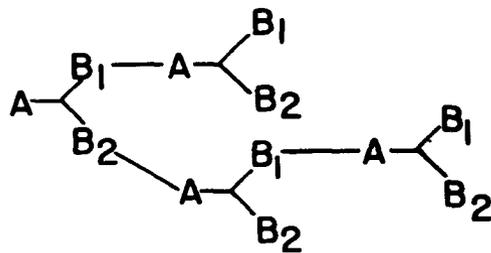
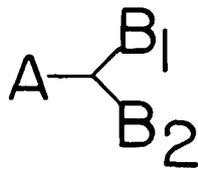
MONOMER

POLYMER

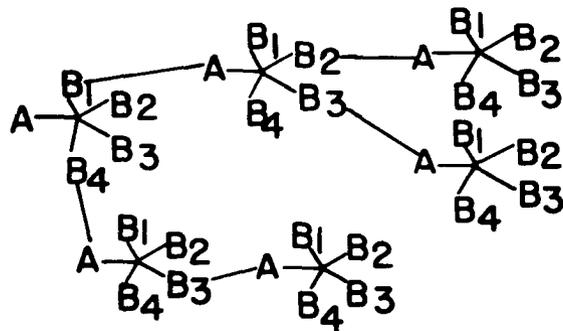
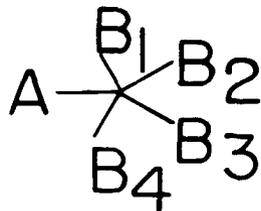


A-B

AB-AB-AB-AB-AB CELLULOSE, AMYLOSE.



AMYLOPECTIN,
GLYCOGEN.



POLYGLUCOSE.

branching other than 1, 6, it can be shown that the change (an increase) in weight-average molecular weight will be negligible. Also the weight or higher average molecular weights are not changed by the fact that the glucosidic bonds may be α or β or a mixture of α and β .

From the paper by Erlander and French (7) the molecular weights of amylopectin and glycogen are

$$\bar{M}_n = 162/(1-p_4-p_6)$$

$$\bar{M}_w = 162(1-p_4^2 - p_6^2)/(1-p_4-p_6)^2$$

$$\bar{M}_z = \frac{162 \left\{ (1-p_4-p_6)(1-p_4^2-p_6^2) - 2(1-p_4-p_6) [p_4^2(1-p_4) + p_6^2(1-p_6)] + 3(1-p_4^2 - p_6^2) [p_4(1-p_4) + p_6(1-p_6)] \right\}}{(1-p_4-p_6)^2 (1-p_4^2 - p_6^2)}$$

Here \bar{M}_n , \bar{M}_w , and \bar{M}_z are equal to the number-average, weight-average, and z-average molecular weights.

Using a different approach, Allen (10) has developed an equation for the mole fraction of the b_1, b_2 -mer in the condensation of A-R-B₂ units. His equation can be shown to be identical to the one developed in the statistical model and thus adds proof to the validity of the above equations.

2. Less random statistical models

Because starch and glycogen are produced by enzymes, there is a possibility that the neighboring branch points may be two

or more glucose units apart due to steric hindrance of the existing branch point. Two less random statistical models were therefore devised in order to determine whether they too would give a large difference between \bar{M}_w and \bar{M}_n . In the less random model V, one can consider the condensation of "monomer" units, each containing one branch point (1, 6 linkage) and having 25, 20, and 10 glucose units which represent 4, 5, and 10% branching, respectively (see Fig. 2). The letters A and B₄ have been attached to signify the aldehydic group and alcoholic groups that are capable of reacting. All 1,4-linkages are horizontal and the 1,6-linkage is vertical. Both B₄ groups form 1,4 linkages and their probability of reacting is α . The molecular weights for the condensation of such monomer units are:

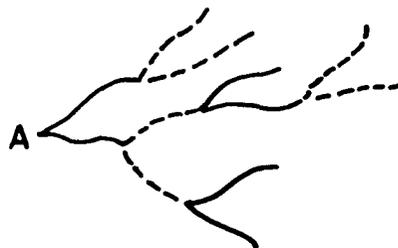
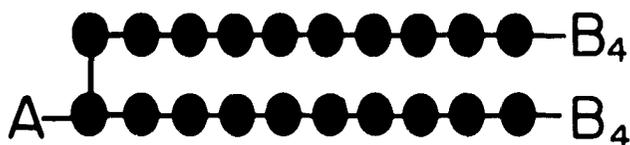
$$\bar{M}_n = 162 Y / (1 - 2\alpha) \quad \text{and} \quad \bar{M}_w = 162 Y (1 - 2\alpha^2) / (1 - 2\alpha)^2$$

where Y = the number of glucose units in a monomer unit. The structure of such a model built from these "monomer" units is also represented in Fig. 2. Alternate units in the polymer are distinguished one from the other.

The less random model I, as illustrated in Fig. 2, is made from "monomer" units that consist of all 1,4-linked glucose units. The B₄ and B₆ groups representing the 4 and 6 position on the last glucose unit having equal reactivity and their probability of forming a linkage is α . Each "monomer" unit in the polymer formed from such units is represented by

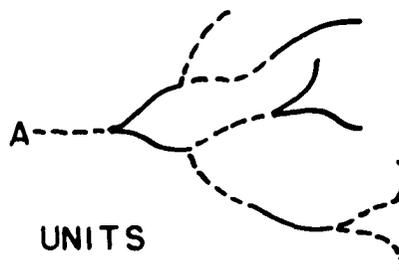
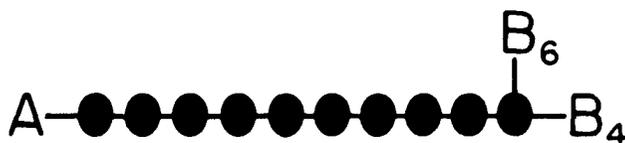
Fig. 2. Illustration of the two less random models and a comparison of their molecular weights with the statistical model.

LESS RANDOM MODEL V



"MONOMER" UNIT = 20 GLUCOSE
UNITS FOR 5% BRANCHING

LESS RANDOM MODEL I



"MONOMER" UNIT = 10 GLUCOSE UNITS
FOR 5% BRANCHING ; $P_4 = P_6$

MODEL	\bar{M}_w	\bar{M}_w/\bar{M}_n	
STATISTICAL MODEL	$35. \times 10^6$	140.	} $\bar{M}_n = 243,000.$ $\bar{X}_n = 1,500.$ 5% BRANCHING
MODEL I	18.5×10^6	76.	
MODEL V	9.4×10	39.	

a line or a dotted line. During polymerization of this theoretical "monomer", half of the condensations with the A group occur with the B₆ group and the other half with the B₄ group. Because a branch is formed only when the B₆ group reacts, then ten glucose residues instead of twenty as in model V are needed to give 5% branching. Therefore the difference between the two models is that the 5% branched less random model V always has a branch point every 10 glucose units while the 5% branched less random model I may have 10 or 20 or more glucose residues in a chain before a branch point is reached. The weight average molecular weights for the statistical and two less random statistical models for a given number average degree of polymerization and per cent branching are compared in Fig. 2. It is seen that the ratio \bar{M}_w/\bar{M}_n is still large even for the less random models.

3. Properties of the three statistical models and comparison with literature values

It has been thought for some time that the discrepancies between the molecular weights of amylopectin obtained by various methods were due to some physical factor such as degradation or aggregation or to inaccuracies of the chemical or physical methods used to determine these molecular weights. Literature values for the weight average molecular weights are about one hundred times larger than the number-average

molecular weights. For the Kuhn distribution (condensation of A-B units) (11) the ratio \bar{M}_w / \bar{M}_n approaches two with increasing size, but in randomly branched polymers (where f is greater than 2) \bar{M}_w / \bar{M}_n depends on the molecular size increasing without limit and in the range of amylopectin and glycogen may easily be in the hundreds.

To illustrate this, molecular weights for different degrees of polymerization have been calculated and are given in Table 1. The results show that, if corn amylopectin (4% branching), potato amylopectin (5% branching), or glycogen (10% branching) resemble a statistical model, then a large difference between weight- and number-average molecular weights is to be expected. It should be pointed out that \bar{M}_w increases as the degree of branching increases for a given \bar{M}_n , the number average degree of polymerization.

Table 1 illustrates the change in \bar{M}_w with a change in the degree of branching at constant \bar{M}_n , the change in \bar{M}_w with a change in \bar{M}_n at a constant degree of branching, and the change in \bar{M}_n with a change in the degree of branching at constant \bar{M}_w .

One can obtain the weight fraction for a molecule composed of x glucose units ("x-mer") if the number average degree of polymerization and per cent branching of all the molecules in the distribution are known. The weight fractions for various size molecules have been calculated and are listed

Table 1. Molecular weights for the statistical model
of amylopectin and glycogen

Per cent branching	\bar{X}_n	\bar{M}_n	\bar{M}_w
4	1000	162,000	12,800,000
5	1000	162,000	15,700,000
10	1000	162,000	29,500,000
4	1500	243,000	28,500,000
5	1500	243,000	35,100,000
10	1500	243,000	66,000,000
4	10,000	1,620,000	1,250,000,000
5	10,000	1,620,000	1,540,000,000
10	10,000	1,620,000	2,920,000,000
4	1675	271,000	35,000,000
5	1500	243,000	35,000,000
10	1100	178,000	35,000,000
5	550	89,100	5,000,000
10	400	64,800	5,000,000

in Table 2. In order to avoid the laborious calculations for each x-mer one can find the approximate weight per cent of all molecules between two x-mers by using the trapezoidal rule of integration. These "weight fractions" were found for the statistical and less random model V. Essentially the same distribution of x-mers for a given weight-average molecular weight was found for the two less random models and the statistical model. The discrepancies in the total percentage are due to the inaccuracy of the integration.

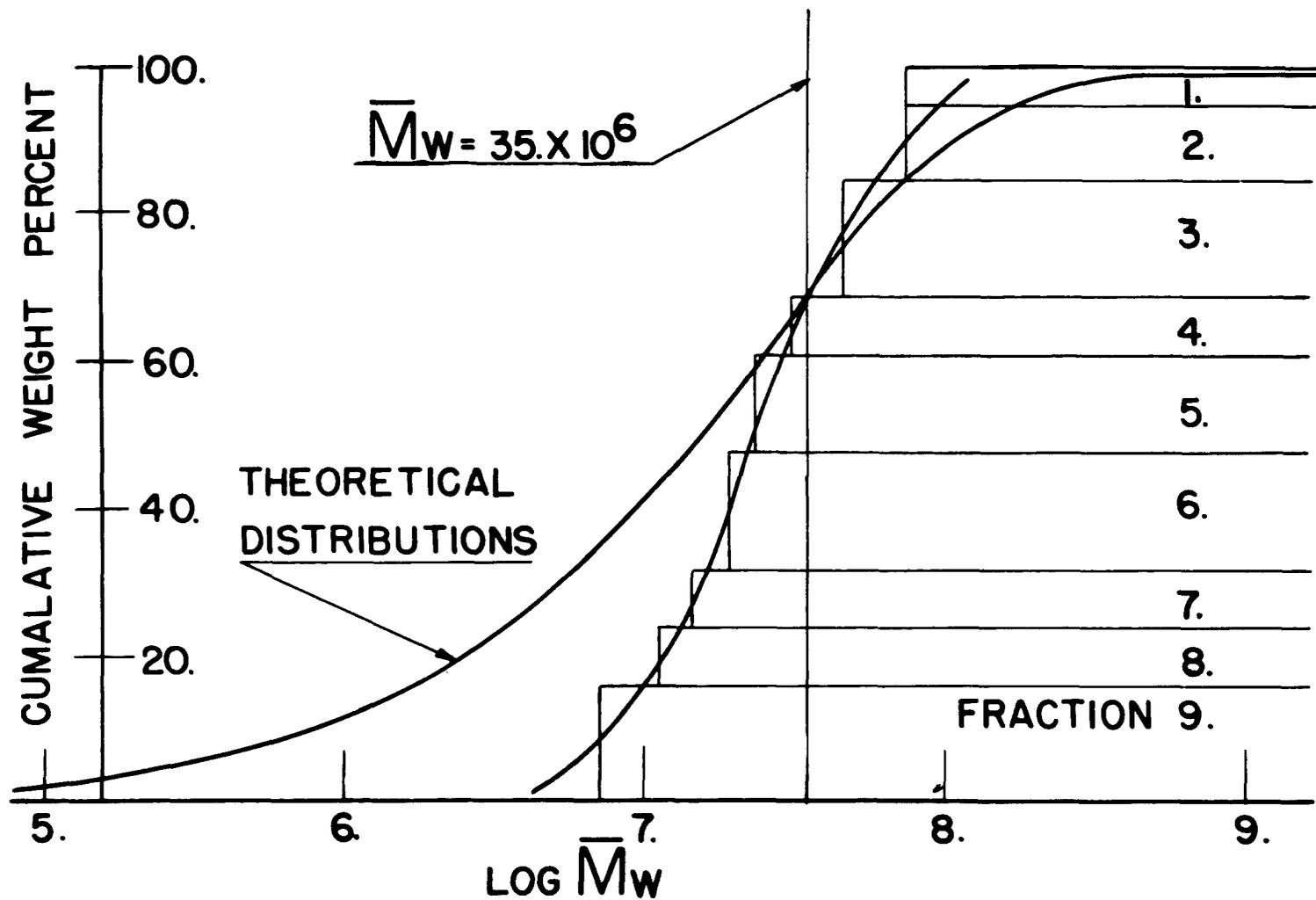
In Fig. 3 the normalized theoretical distributions for all three statistical models is compared to the "distribution"

Table 2. Weight per cent of "fractions" for the statistical model of amylopectin and for the less random model V

$\bar{X}_n = 1500$, $\bar{M}_w = 35 \times 10^6$, 5% branching, for statistical model
 $\bar{X}_n = 2900$, $\bar{M}_w = 35 \times 10^6$, 5% branching for less random model V
 The theoretical distribution obtained from these "fractions" are plotted in Fig. 3.

No. of glucose units in x-mer	Wt. fraction of x-mer $\times 10^7$	Wt. % of "fractions" for statistical model	Av. $MW \times 10^{-6}$ of "fraction"	Wt. % of "fraction" for less random model V
1×10^2	545	3.7	0.081	4.4
1×10^3	272			
1×10^4	84.6	16.5	0.89	16.1
5×10^4	34.5	23.8	4.85	24.0
1×10^5	21.7	14.0	12.2	14.2
2×10^5	12.2	16.9	24.3	17.1
3×10^5	7.87	10.0	40.5	11.0
4×10^5	5.40	6.6	57.7	7.6
5×10^5	3.83	4.6	73	4.6
6×10^5	2.77	3.3	89	3.3
7×10^5	1.74	2.3	105	2.4
1×10^6	0.84	3.9	138	4.3
2×10^6	0.059	4.5	243	4.5
3×10^6	0.00396	0.3	405	0.3
Total110.4%		113.8%

Fig. 3. Witnauer, Senti, and Stern's "distribution" is compared to essentially coincident distributions of the statistical and the two less random statistical models of potato amylopectin. $\bar{M}_w = 35 \times 10^6$ for the three models and for the potato amylopectin.



obtained from Witnauer, Senti and Stern's results (12). Their narrower distribution may be due to the overlapping of the fractions obtained by precipitation of potato amylopectin with alcohol. Or it may also be due to the chemical bonding of the starch molecules around a protein molecule. This will be discussed in more detail later.

An insight to the change in molecular weight from a loss of either high or low weight fractions can be obtained from Table 2. A weight-average molecular weight of 37×10^6 is obtained from the "fractions" by multiplying all of the average molecular weights of the "fraction" by their corresponding weight per cent, adding these calculations, and dividing by the total percentage. $\bar{M}_w = 37 \times 10^6$ is in close agreement with the actual \bar{M}_w of 35×10^6 obtained from the equation given previously for the statistical model. The effect of a loss of high or low molecular fractions is shown in Table 3 using the same type of calculation as described above. It has been seen that molecules having a molecular weight of less than one or two million have little effect on \bar{M}_w .

In Tables 4 and 5 are listed some literature values for amylopectin and glycogen. Also theoretical values for their corresponding \bar{M}_w or \bar{x}_n , assuming a statistical model, are included in these tables. These literature values for \bar{M}_n and \bar{M}_w are compared to the three models in Figs. 4, 5, and 6. According to Kerr *et. al.* (13), aggregation occurs in chloro-

Table 3. Change in molecular weight of the statistical model from a loss of high or low "fractions"

$$\bar{X}_n = 1500 \text{ and } \bar{M}_n = 243,000$$

MW range of fraction lost	Total wt. % lost	Resulting \bar{M}_w	$\Delta\bar{M}_w \times 10^{-6}$
None	None	37,000,000	0.0
<162,000	4	38,000,000	+1
<1,620,000	20	43,000,000	+6
>162,000,000	5	28,000,000	-9
>81,000,000	14	19,000,000	-18
<1,620,000 and >162,000,000	25	35,000,000	-2

form solutions of amylopectin acetate at concentrations around 0.5% or greater. It can be seen from Kerr's osmotic pressure plots that there is a change in direction of the experimental curve at concentration around 0.5% and thus an extrapolation from high concentrations would give a high molecular weight. In general the values for amylopectin agree quite well. Except for commercial glycogen the values for glycogen do not agree. They probably disagree because of errors (such as aggregation for osmotic pressure measurements) involved in obtaining \bar{M}_n and because of the extreme degradation involved

Table 4a. Some literature values for weight- and number - average molecular weights of amylopectin. Theoretical molecular weights based on the statistical model are included. Literature values for weight-average molecular weights of amylopectin.

Amylopectin	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n	Method
Potato (12)	36	1500	Light scattering
(12)	14	1000	Light scattering
(15)	0.92	250	Ultracentrifuge
Dent corn (16)	3 to 8	500 to 800	Ultracentrifuge
(17)	80	2500	Light scattering
(17)	43	1850	Light scattering
Easter lily (18)	250	4300	Light scattering

Table 4b. Literature values for number-average molecular weights of amylopectin

Amylopectin	Theor. $\bar{M}_w \times 10^{-6}$	\bar{X}_n	Method
Potato (19)	19	1100	Dinitrosalicylate
(20)	33	1450	Osmotic pressure
Dent corn (21)	12.8	1000	Dinitrosalicylate
(22)	>20	>1200	Methylation and terminal group assay
(13)	26	1440	Osmotic pressure
Waxy corn (21)	11.5	930	Dinitrosalicylate
Corn, wheat, Easter lily, tapioca, sago (23)	490 to 17,000	6200 to 37,000	Osmotic pressure
Tapioca (24)	88	2620	Colorimetric
(13)	21	1275	Osmotic pressure
Apple (25)	220	4200	Osmotic pressure

Table 5a. Some literature values for weight- and number-average molecular weights of glycogen. Theoretical molecular weights are based on the statistical model.

Literature values for weight-average molecular weights of glycogen.

Glycogen	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n	Method
Rabbit muscle, rabbit, cat, fetal sheep liver (26)	2.8 to 14.8	300 to 700	Light scattering
Rabbit liver, horse, rabbit, human muscle (27)	2.6 to 4.4	300 to 380	Sedimentation, dif- fusion
Rabbit liver, muscle (28)	6.3 to 18.5	450 to 800	Light scattering
Rabbit, dogfish hakefish, haddock liver, dogfish muscle (29)	1.0 to 3.6	200 to 350	Sedimentation, diffusion
Rabbit, cat liver rabbit muscle (30)	1 to 7	200 to 500	Turbidity
Rabbit liver fraction (31)	500	4100	Not known
Rabbit liver (32)	4	360	Sedimentation, diffusion
<u>Ascaris lumbrico-</u> <u>coides</u> (26)	8.8	550	Light scattering
(27)	0.77	150	Sedimentation, diffusion
Commercial (28)	3.6 to 5.8	350 to 450	Light scattering

Table 5b. Literature values for number-average molecular weights of glycogen

Glycogen	\bar{X}_n	Method
Rabbit liver (27)	3100 to 12,500	Osmotic pressure
Rabbit liver (33)	3000	Osmotic pressure
<u>Ascaris lumbricoides</u> (27)	620 to 3100	Osmotic pressure
Commercial (13)	500	Osmotic pressure
Dog liver (13)	5300	Osmotic pressure
Dogfish, haddock, hake liver (33)	1340 to 12,000	Osmotic pressure

Fig. 4. Literature values for corn amylopectin. Each horizontal or vertical line represents, respectively, a literature value for \bar{M}_n or \bar{M}_w as given in Table 2. Intersection of these lines illustrates how literature values compare to the three models and to polymers formed from A-B units.

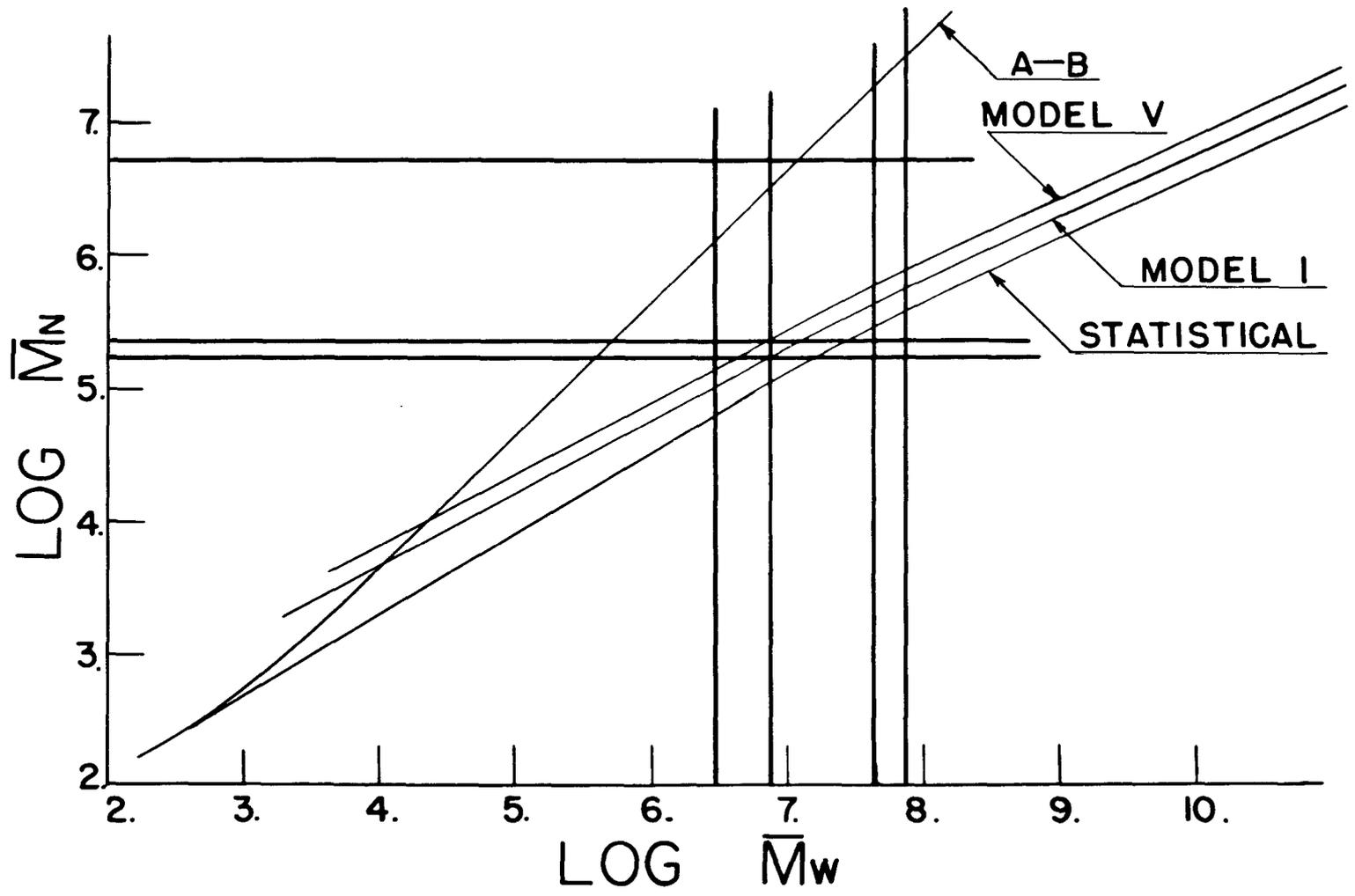


Fig. 5. Literature values for potato amylopectin. Each horizontal or vertical line represents, respectively, a literature value for \bar{M}_n or \bar{M}_w as given in Table 2. Intersection of these lines illustrates how literature values compare to the three models and to polymers formed from A-B units.

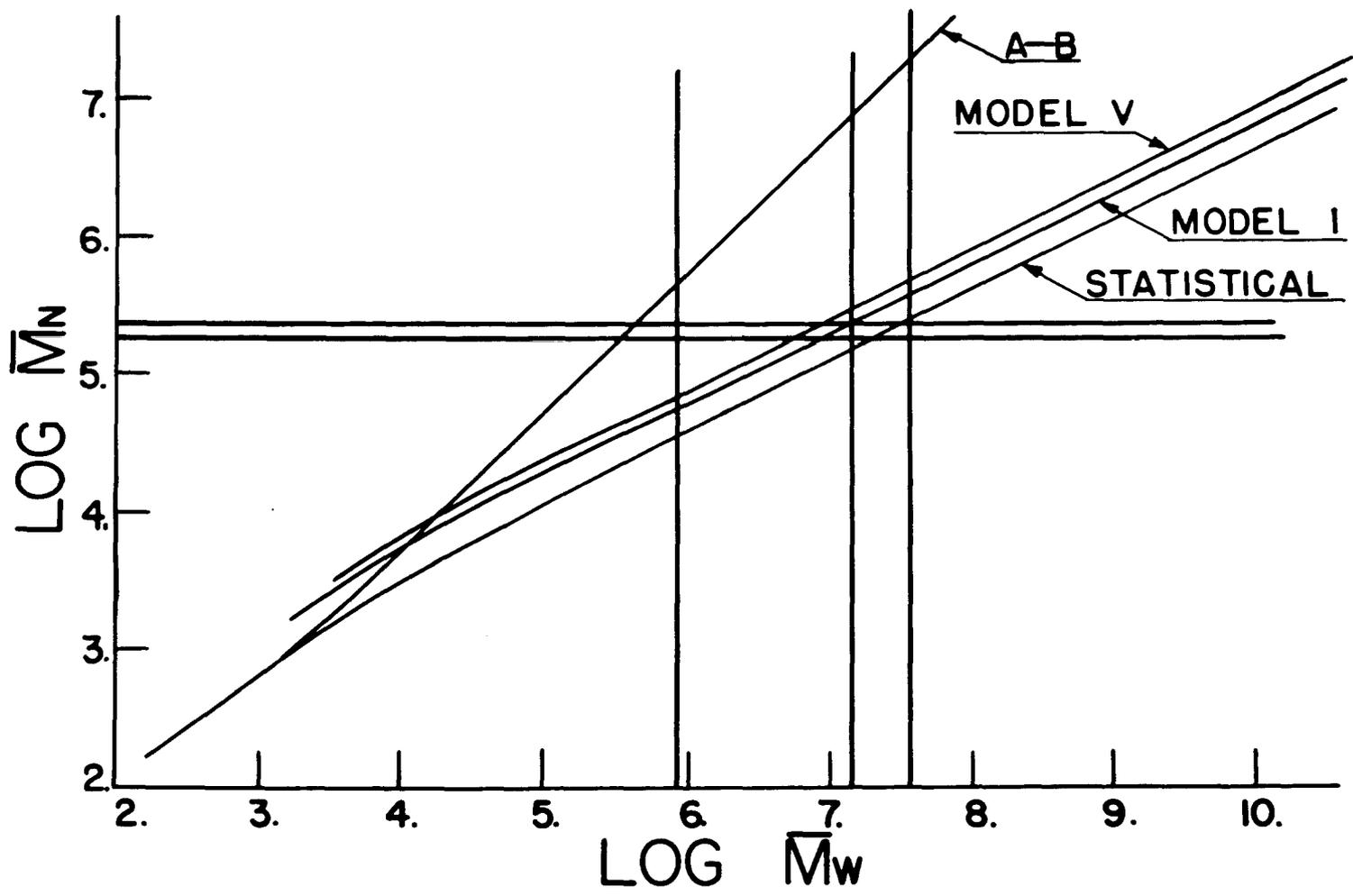
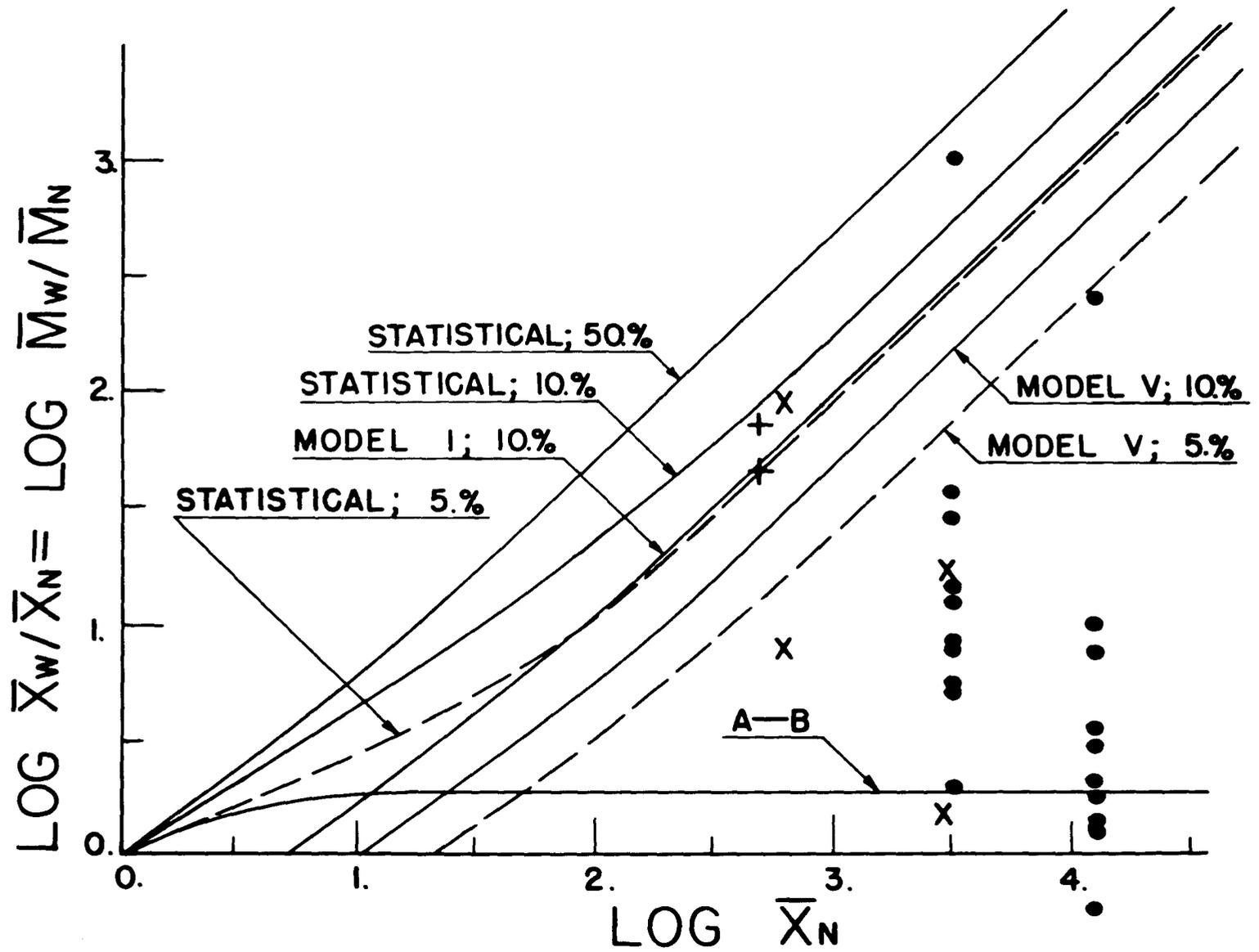


Fig. 6. Literature values for glycogen. (●) Rabbit liver glycogen; (+) commercial glycogen; (X) Ascaris lumbricoides glycogen. These points representing all possible \bar{M}_n and \bar{M}_w combinations of literature values as given in Table 3 are compared to the three models for 5 and 10% degree of branching. The 50% branched statistical model represents Flory's (9) derivation where $p_4 = p_6 = \alpha$. The model for linear polymers (condensation of A-B units) is included for comparison.



in isolating the animal glycogen. Recently a weight average molecular weight of 70.8×10^6 was obtained for rat liver glycogen by keeping degradation to a minimum (14). Such a high molecular weight from light scattering data agrees well with that predicted by the statistical model.

B. A Model for the Average Statistical Molecule

1. Development of the average statistical molecule

In order to consider what the average statistical molecule will look like, we have to consider what will be the average distance between branch points in the interior part of the molecule and the average distance from the outer most branches to the non-reducing end. Let us define a branch point as a glucose unit which has a linkage at position 6. Let the probabilities of forming linkages at carbon 6 and carbon 4 be p_6 and p_4 as above. Starting with any of the outermost branch points on any x-mer, the probability that a chain of n units, united by 1,4-linkages, is linked to the 4-position of this outer branch point is p_4^n ; that the last glucose unit has a free 4-position is $(1-p_4)$; and the probability that all the n units have their 6-position free is $(1-p_6)^n$. Thus the probability that a chain selected at random will be such a chain of length n is $p_4^n (1-p_6)^n (1-p_4)$.

The probability that a chain has $n + 1$ glucose units, united by 1,4-linkages, and is linked to the 6 position of

this branch point is $p_6 p_4^n$. And as above the probability of finding such a chain of length $n + 1$ is $p_6 p_4^n (1-p_6)^n (1-p_4)$.

With similar reasoning for the interior part of a molecule, the probability that a chain of x glucose units is linked to the 4-position of the branch point and that the x th glucose unit has a linkage at its 6-position is $p_4^x (1-p_6)^{x-1} p_6 (1-p_4)$ or $p_4^x (1-p_6)^{x-1} p_6 p_4$ depending on whether the last glucose unit has a linkage at its 4-position. Similarly for a chain of length $x + 1$ linked at the 6-position of the branch point we have

$$p_4^x (1-p_6)^{x-1} p_6 (1-p_4) \text{ or } p_4^x (1-p_6)^{x-1} p_6 p_4$$

The exact probability again depends on the fate of the last glucose unit.

If $p_4 \gg p_6$, the formation of the 1,6-linkage can be considered as an initiation step and is not involved in the rate of formation of the 1,4-linked chain which grows from it. When $p_6 = p_4$, both chains will grow at the same average rate from the initial glucose unit and thus their average length will be the same. When $p_4 \gg p_6$, both chains will grow at the same rate from the isomaltose unit and therefore the chain linked to the 6-position of the initial glucose unit will be one unit longer than the chain linked to the 4-position. For intermediate cases where $p_4 > p_6$, the chain linked to the 6-position will be p_6/p_4 units longer than the other. In the case of glycogen $p_4 \gg p_6$ and therefore the branch point can

be considered as an isomaltose unit with equal average chains growing from it.

If we now consider the branch point as an isomaltose unit and neglect the fate of the 4- and 6-position on the last glucose units, then the above probabilities for the exterior and interior chains become, respectively, $p_4^n (1-p_6)^{n-1}$ and $p_4^x (1-p_6)^{x-1}$. Since p_6 and p_4 are the same for both exterior and interior chains, then $x = n$ and the average length of the interior and exterior chains is the same. A model conforming to these specifications is shown in Fig. 7. A repeating unit is developed for this average molecule because (1) all average chain lengths are the same and (2) all 6-positions are available for branching and therefore the clustering of branch points in any part of the molecule is unlikely.

One can also approach this model by considering a b_4, b_6 -mer of a particular x -mer. In each b_4, b_6 -mer there will be b_4 1,4-linkages and b_6 1,6-linkages. A particular b_4, b_6 -mer will therefore exhibit its own p_4 and p_6 probabilities. All conceivable configurations of a particular b_4, b_6 -mer will exist and by the same reasoning above, the average for the interior and exterior chain lengths of the b_4, b_6 -mer will be the same. The average molecule for the b_4, b_6 -mer will have the same structure as the glycogen molecule in Fig. 7. The mole fraction of a particular b_4, b_6 -mer will depend on

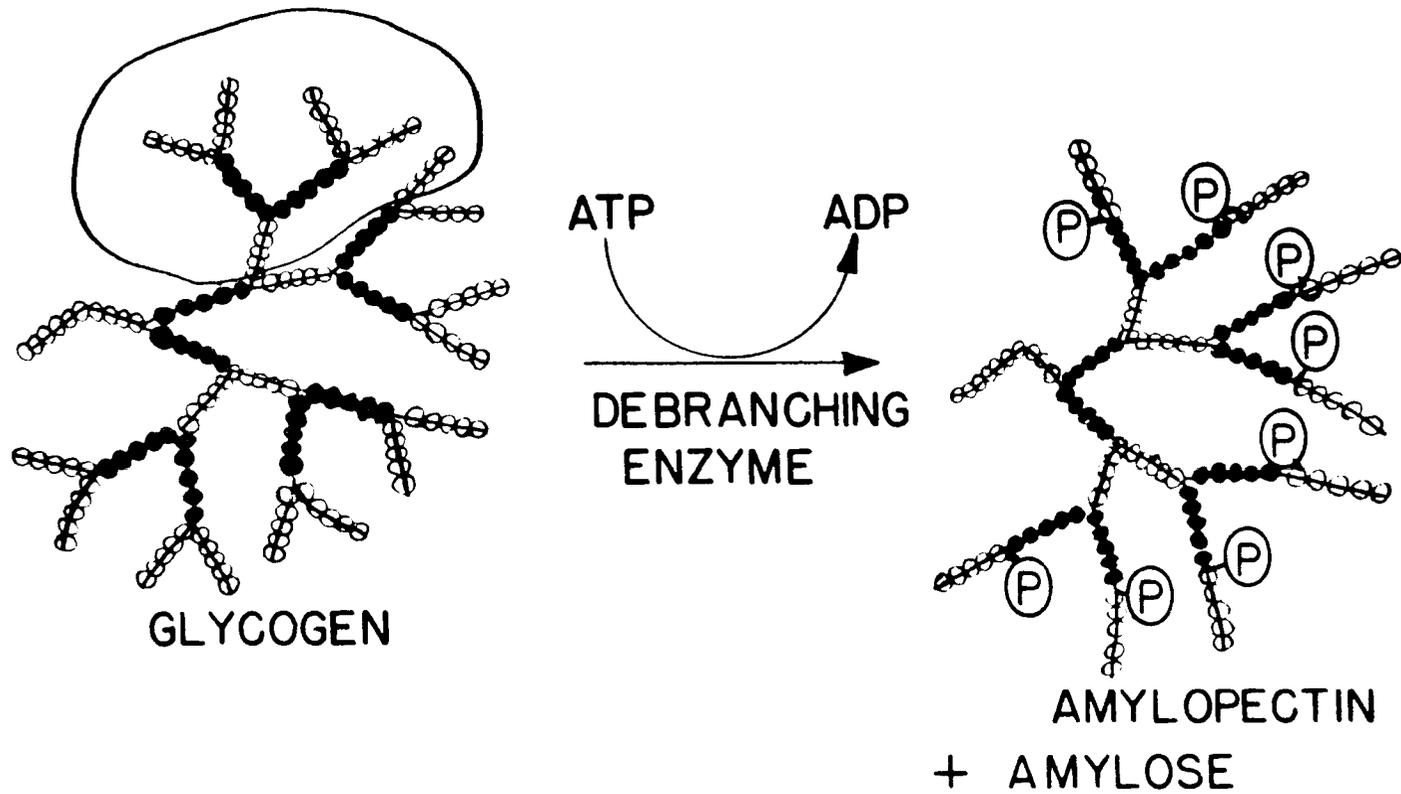


Fig. 7. The average statistical molecule. Proposed mechanism for debranching of glycogen.

its per cent branching and to what particular x-mer it belongs. For a given x-mer a maximum mole fraction will be obtained for that b_4 , b_6 -mer which has its per cent branching equal to that predicted by the probability p_6 . Starting at this maximum mole fraction, the mole fraction will decrease symmetrically in going towards more 1,4-linkages on the one hand and more 1,6-linkages on the other. Thus all the average molecules of the b_4 , b_6 -mers will have their counterpart. By summing over all the average molecules of the b_4 , b_6 -mers, we arrive at an average x-mer which is identical to the b_4 , b_6 -mer having the maximum mole fraction, that is, the one having its average interior and exterior chains in agreement with that predicted by p_6 . Consequently all the average x-mers will have the same type of configuration and spacing between branch points and therefore an average molecule can be visualized (see Fig. 7). If the weight-average molecular weight is obtained experimentally, then the average statistical molecule will have a molecular weight equal to this \bar{M}_w . Thus the size of the average statistical molecule will be equal to the average size of all the molecules. This size will vary depending on how the average size of all the molecules is obtained, that is, \bar{M}_w , \bar{M}_n , $\bar{M}(S,D)$, etc.

In order to avoid confusion the average chain length as described above for the distance between branch points will be called the average branch length, and the average chain

length for all 1,4-linked glucose units regardless as to whether this chain has a branch on it will be called the average chain length.

2. Properties of the average statistical molecule

It can be seen from Fig. 7 or reasoning that exactly 50% of all the glucose units in a statistical model will be in the exterior branches. If a molecule has 5% branching or an average chain length of 20, then one glucose unit in 20 will have a linkage at carbon atom 6. If one considers only the outside branches, each peripheral branch point is associated with two outside branches containing a total of 20 glucose units. That is, in removing the outside tier of branches, all of the 20 units must be removed in order that the remaining dextrin will have the same degree of branching.

Since the branch length starting at the reducing end of the molecule would be equal to only 1/2 the average chain length, in Fig. 7 it has been extended in order to make this branch length equal to the average chain length. When this is done the small glycogen molecule as pictured in Fig. 7 will have exactly 50% of the units in the outside tier, the same as in a large molecule.

Consider now an infinitely large statistical molecule. If all of the 1,6-linkages are cleaved but all of the 1,4-linkages are not, what will be the number-average degree of

polymerization for the resulting linear molecules? Every branch point will yield a single chain. Consequently, the \bar{x}_n of the resulting chains will be equal to the number of glucose units divided by the number of non-reducing ends. The degree of branching is the reciprocal of the average chain length. The average chain length is thus experimentally obtainable from the per cent of branching.

Further verification of this can be made using the average statistical molecule. Let us assume that the 1,6-linkages are cleaved tier by tier starting with the outside tier and working inward. It may be seen that 50% of the glucose units are on the outside tier and 50% of these are connected by a 1,6-linkage. Thus $(1/2)^2$ of the total number of original units would be removed the first time, $(1/2)^3$ the second and so on. The first chains removed will be equal in length to the average branch length, L , the second cleavage will give chains equal to $2L$ and so on. Solving for \bar{x}_n we have

$$\bar{x}_n = \frac{(1/2)^2 y L + 2(1/2)^3 y L + 3(1/2)^4 y L + \dots}{(1/2)^2 y + (1/2)^3 y + (1/2)^4 y + \dots}$$

where y , the number of units in the original molecule, is indefinitely large. Thus

$$\bar{x}_n = \frac{L \sum_{n=1}^{\infty} (n-1)(1/2)^n}{\sum_{n=2}^{\infty} (1/2)^n} = \frac{La^2 \frac{d}{da} \sum_{n=1}^{\infty} a^{n-1}}{\sum_{n=2}^{\infty} a^n}$$

where $a = 1/2$

and we have $\bar{x}_n = \frac{L}{(1/2)} = 2L =$ the average chain length of the molecule. If amylose were obtained by just cleaving the 1,6-linkages, then its \bar{x}_n would be 20 for 5% branching instead of the experimentally determined value of 200 or more. Consequently, if glycogen is converted into amylopectin plus amylose by debranching part of the glycogen, there must be some mechanism by which these debranched chains can be connected together in 1,4-linkages.

Bourne and Peat (34) proposed that glucose-1-phosphate is converted by phosphorylase to an intermediate having an average chain length of 20 units. This intermediate designated as pseudo-amylose was capable of being converted into either amylose by further use of phosphorylase or into amylopectin by means of an enzyme which would link the pseudo-amylose chains together through 1,6-linkages.

Following this proposal, the mechanism of the branching enzyme has been clarified showing that such a mechanism for the production of amylopectin and amylose is impossible (35, 36). However, if one considers the reverse of the above debranching mechanism for the conversion of the statistical molecule into short chains, then these chains would resemble the pseudo-amylose intermediate. If amylopectin were formed from pseudo-amylose as postulated by Bourne and Peat, the outside branches would contain 61% of the total units in the

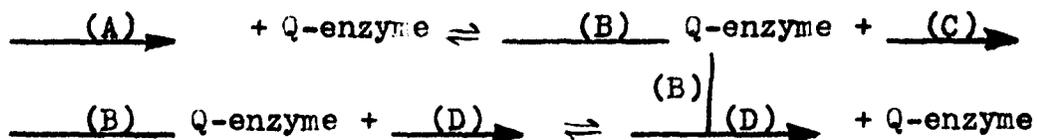
branched molecule. This would give an improbable localization of the branch units. The exact reverse of the above debranching reaction would call for either a complete equilibrium of all the pseudo-amylose chains or the production of an irreversible highly ordered system. Most likely, the longer amylose chains would aggregate and precipitate from the medium before complete equilibrium could be established. Therefore it is pointed out that without regard to the mechanism of the branching enzyme, the pseudo-amylose intermediate could not be converted into an amylopectin or glycogen of the statistical type.

C. Enzyme Synthesis and Breakdown of Starch

1. Properties of branching and debranching enzymes

A branched polysaccharide can be produced by the mixing of glucose-1-phosphate, phosphorylase, and a branching enzyme (37). Without the branching enzyme a linear 1,4-linked polymer resembling amylose is produced. The extent of branching depends on the relative activity of the branching and phosphorylase enzymes, a greater per cent branching resulting from an increase in the relative activity of the branching enzyme. These enzymes have the same reaction patterns regardless of the plant or animal source. However, they may differ slightly as to the size of the substrate that will react with the enzyme (37, 38). The mechanism of the conver-

sion of glucose-1-phosphate is shown below (37). The Q-enzyme represents any branching enzyme.



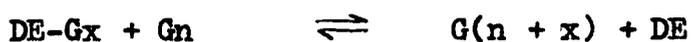
The (A) arrow represents the linear chain produced by phosphorylase or an already formed branched polymer. The arrow on each chain represents the reducing group. The (C) arrow represents the resulting dextrin after the removal of the linear chain (B) by the Q-enzyme. The chain complexed with the branching enzyme is then connected to the substrate (D) by an α -1,6-linkage. The receptor molecule (D) may be either (C), another linear chain, or a branched product formed earlier. The size of the receptor molecule is not important but it must have α -1,4-linked glucose units and cannot be cyclic. The branching enzyme is therefore a transglycosidase and requires no phosphate as is required by the phosphorylase enzyme. Larner (39) proved this mechanism beautifully by the use of radioactive glucose units. Under the conditions so far studied the branching enzyme does not seem to be reversible. However, Petrova (40) has found a branching enzyme in rabbit muscle which she claims is reversible.

Two types of debranching enzymes have been found (41). Both are irreversible and do not require phosphate in their mechanism. The R-enzyme obtained from the potato and broad-

bean can hydrolyze the external branches of amylopectin as shown by the increase in the β -amylolysis limits of amylopectin or β -dextrin after treatment with the R-enzyme. R-enzyme cannot hydrolyze the external branches of glycogen and therefore a steric effect is associated with the debranching of branched polysaccharides. Iso-amylase ("Amylosynthase") is a debranching enzyme found in Brewer's yeast and its action is similar to that of the R-enzyme. A second type of enzyme, amylo- α -1:6-glucosidase, can only remove single glucose units that are connected by an α -1,6-linkage to amylopectin or glycogen.

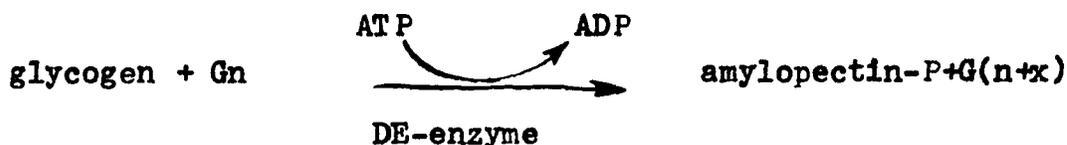
2. Proposed theory for the formation of starch from glycogen

Now that the important enzymes dealing with starch synthesis and breakdown have been reviewed, a postulation on how amylose and amylopectin may be produced from glycogen will be made. As noted above a mechanism must account for the large size of the amylose molecules since debranching of glycogen yields only small chains. Also since the 1,6-linkage is more stable than the 1,4-linkage (37) and the conversion of glycogen to starch is a conversion to a less random state, energy is required to form starch from glycogen. The following mechanism is therefore postulated:





or the overall mechanism would be



The phosphorylated debranching enzyme, DE-P, transfers its phosphate group to the branch point of the glycogen molecule and simultaneously becomes linked with the aldehydic group of the branch. This phosphate attached to the glycogen may be removed by a phosphatase if not sterically hindered to this enzyme. The complex DE-Gx between the enzyme and the debranched chain would be similar to that enzyme complex involved in the branching of glycogen. The DE-Gx complex can then transfer its chain to the non-reducing end of the receptor group Gn. The debranching enzyme after being phosphorylated by ATP can again attack a debranched glycogen or attack another glycogen molecule. It should be remembered that the average statistical molecule is an average of all of the possible configurations and a molecule having this configuration would be present only in very small amounts. Therefore some exterior and interior branch points will be more susceptible to the debranching enzyme because of the variation in distance between branch points. Postulates regarding this debranching mechanism are:

1. The debranching reaction would have to be irreversible in order to avoid addition of Gx to the many available 6-positions.

2. The glycogen molecule may be attacked more than once by the debranching enzyme.
3. Amylose reaches an optimum chain length as a receptor group. This optimum would depend on the average branch length of the glycogen precursor.
4. The transfer of a unit which has branches might result in the immediate debranching of this transferred unit because its removal from the parent glycogen would make its branch points more susceptible to attack.
5. The receptor group, Gn, must have an available non-reducing glucose group and could be of any size, glucose or maltose being the lower limit.
6. The receptor group would most likely be an unbranched molecule because of the steric hindrance involved in branched molecules. The lower the degree of branching, the greater is the probability of having long external chains. Therefore less highly branched glycogens would more likely act as receptor groups.
7. Steric hindrance such as displayed by the R-enzyme (41) would result in an attack by the DE-enzyme on the most susceptible branch points.
8. Glycogens that have low degrees of branching will have more susceptible branch points. Thus they will be debranched more randomly than the more highly branched glycogens. The more highly branched glycogens will therefore be more asymmetric.

9. Debranching produces longer external and internal chains.

10. The spherical and symmetrical glycogen molecules will be transferred into asymmetric amylopectin molecules.

11. Phosphate is left at carbon atom 6 of a glucose unit in the amylopectin molecule or in any amylose molecule when an internal branch is removed. This phosphate may be removed by phosphatase if not sterically hindered to this enzyme.

The production of longer and less sterically hindered chains giving asymmetric amylopectin molecules as postulated above would enable amylopectin to crystallize out of solution more readily than the original glycogen. In order to obtain a starch such as waxy maize one would have to have:

1. A lower degree of branching in the parent glycogen;
2. Relatively few acceptor groups in the medium in comparison with the available non-reducing ends of the parent glycogen;
3. A low activity of the debranching enzyme;
4. A debranching enzyme such as the R-enzyme instead of the DE-enzyme. By increasing the number of acceptor groups, such as maltose, the waxy maize endosperm should be able to produce amylose. One could produce more acceptor groups either by adding more glucose-1-phosphate or by inhibiting the production of glycogen and increasing the production of small acceptor groups. Amylose has been produced by waxy maize endosperm (42, 43, 44). Therefore (4.) would seem to be incorrect. In agreement with the above, it appears that starches which

have a low per cent of amylose have longer exterior chains (45).

Let us now consider the change in properties of the glycogen molecule after being debranched using the average statistical molecule as represented in Fig. 7. In doing this, two extremes have been considered. In the first case all of the external branches have been removed. In the second case one large internal branch and one external branch have been removed (those branches circled in Fig. 7). The small external branch is included in order to give an equal yield of amylose for the two cases. Table 6 compares the changes in properties.

If the per cent branching of the glycogen is high, there will be greater steric hindrance for the debranching enzyme and one would expect the properties of the amylopectin to be similar to the second case where an internal branch point is attacked (see postulates 7 and 8). If the per cent branching is low then the external branches are more available for debranching. An amylopectin having few branches would have properties resembling the first case where all the external branches are removed.

3. Examination of limit dextrans in light of proposed theory

(a) Literature values for limit dextrans. The β -amylolysis limits for various amylopectins and glycogens as reported in Manners' review (41) are listed in Table 7 and

Table 6. Changes in properties of the average statistical molecule after partial debranching

	Original glycogen	All external branches removed	Interior branch removed
Per cent units removed and converted into amylose	0	25%	25%
Per cent branching	10%	6.67%	9.16%
Average external branch length in glucose units	5	10	5.45
Per cent glucose units in external branches	50%	66.7%	50%
No. of phosphate groups per 100. glucose units	0	5	1.67
Postulated asymmetry	sphere	small asymmetry	large asymmetry

Table 7. Experimental* and predicted β -amylolysis limits for amylopectin

Amylopectin source	Per cent branching	Average branch length	β -limit	Maximum predicted β -limit
Easter lily	3.7	13.5	60	46.3
Potato	3.7	13.5	59	46.3
Barley	3.85	13	59	46.2
Maize	4.0	12.5	63	46.0
Waxy sorghum	4.0	12.5	52	46.0
Wheat	4.35	11.5	62	45.7
Tapioca	4.35	11.5	62	45.7
Sago	4.55	11	62	45.5
Waxy maize	4.55	11	53	45.5
Malted barley	5.7	8.75	44	44.3

*Data obtained from Manners (41)

Table 8. The predicted β -amylolysis limits for the glycogens and amylopectins assuming each behaves as a statistical model are also listed in these tables. In the above discussion on the properties of the average statistical molecule, it was pointed out that 50% of all the units make up the ex-

Table 8. Experimental* and predicted β -amylolysis limits for glycogen

Glycogen source	Per cent branching	Average branch length	β -limit	Maximum predicted β -limit
Rabbit liver	5.55	9.0	53	44.5
<u>Mytilus edulis</u>	5.88	8.5	47	44.2
Brewer's yeast	7.7	6.5	44	42.3
Rabbit liver	8.0	6.25	43	42.0
<u>Helix pomatia</u>	14.3	3.5	37	35.8
Baker's yeast	8.34	6.0	50	41.6
Sweet corn 1	8.35	6.0	47	41.6
Sweet corn 2	9.1	5.5	45	36.8

*Data obtained from Manners (41)

ternal branches. This 50% includes the isomaltose unit. The predicted β -limit is then equal to

$$\frac{\text{average chain length} - 2}{\text{average chain length}} \times 50\%$$

This is an upper limit since the chain will not be completely removed (46, 41). The actual β -limit for the glycogens is

slightly higher than this predicted limit but the difference is much greater for the amylopectins. Baker's yeast glycogen behaves as an ellipsoid with an axial ratio of 8:1 (47). Mussel glycogen behaves as a flat ellipsoid (48, 49, 50, 51). As noted above a debranching enzyme has been found in Brewer's yeast (41). It is therefore quite possible that the glycogens do not behave as a statistical model because they have been slightly debranched. Baker's yeast glycogen seems to be the only one that has been extensively debranched. The high β -amylase conversion limits with sweet corn glycogen may be due to the presence of amylose and a slight debranching of the glycogen. As in the case of Baker's yeast glycogen, the high yield of maltose in the β -amylolysis of the amylopectins can most logically be explained as due to the debranching of the amylopectin.

It can be shown that the maximum branching of the parent glycogen is 6% if the branching of the amylopectin is 4%. Therefore the amylopectins in Table 7 would be derived from glycogens having a lower degree of branching than the glycogens in Table 8.

If one compares the extensively debranched Baker's yeast glycogen and the amylopectins, it is seen that the β -amylolysis limits behave as predicted in Table 6 and its discussion. Because of their low per cent branching and low β -amylolysis limit, waxy maize and waxy sorghum appear to have been debranched to a lesser extent. This would indicate a lower activity of the

debranching enzyme and therefore conforms to the previous reasoning in explaining the absence of amylose in waxy starch.

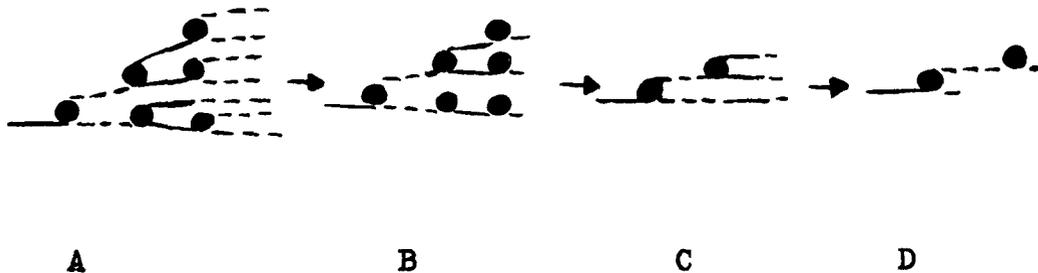
Cori, et. al. (52, 53), examined the fraction of glucose units in the four outer tiers of glycogen and the three outer tiers of amylopectin by the alternate action of phosphorylase and amylo- α -1,6-glucosidase. An examination of their results on glycogen shows that each new external tier approximately doubles the molecular weight as would be predicted by the statistical model. Only the external tiers of amylopectin are longer than those predicted by the statistical model.

It should be noted that the molecular weight can not be obtained solely from such data. Glycogen and amylopectin have very broad distributions and therefore it would be impossible to tell when the last tier corresponding to the correct \bar{M}_w had been reached. One could assume an infinitely high molecular weight and the same results would be obtained. Examination of the change in molecular weight would, however, add more evidence that this weight average molecular weight obtained by other means is correct (14, 17).

(b) Theories developed to account for literature values.

Two mathematical treatments, one by Beckmann (54) and the other by Myrbäck and Sillén (55), have been developed to account for the results of Larner, et. al. (52), and for the β -amylolysis limits. Beckmann concludes that in wheat amylopectin the fraction of "Q-chains" is 0.63 in the first tier (f_1) and 0.32 in

the second tier (f_2); for corn the fractions are 0.52 and 0.53. Consider A below, in which one exterior branch of a statistical molecule has been removed by a debranching enzyme. The black circles and horizontal lines represent glucose units connected by 1,6-linkages and by 1,4-linkages, respectively. Let A be degraded by phosphorylase giving B ($f_1 = 0.57$), then by α -1,6-amyloglucosidase giving C, and again by phosphorylase giving D ($f_2 = 0.33$). It appears that a selectively debranched statistical molecule could have f -values of 0.5, > 0.5 , or < 0.5 for any of the tiers. The observed deviation of f_1 and f_2 from the ideal value of 0.5 points to a steric hindrance or selectivity in the debranching mechanism. The more highly branched polysaccharides give the greatest deviations from 0.5.

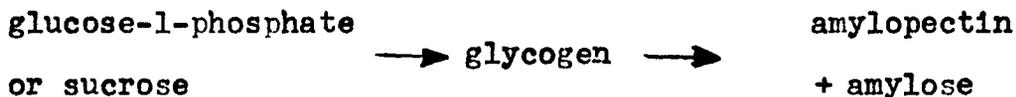


Myrbäck and Sillén (55) constructed an infinite statistical model for glycogen based on the condensation of glucose units and isomaltose units. It was shown above that the branch point in our statistical model can be considered as an

isomaltose unit. Their statistical model for the reversible synthesis of an infinite network therefore agrees with our statistical model. They concluded that in some cases the lengths of the external chains based on β -amylolysis limits were too long but in others glycogen behaved as if it is produced by a reversible synthesis. Amylopectin was in greater disagreement with their reversible synthesis than the extreme glycogen cases. This is in accord with the discussion of β -amylolysis limits presented above.

4. Literature support for glycogen as an intermediate in the synthesis of starch

Points 1-14 below can be considered as support for the reactions:



In some cases it has not been established whether sucrose is converted into starch directly or via glucose-1-phosphate (42). The points supporting the proposed synthesis of starch will be discussed as they are brought up.

1. A generally distributed unorganized polysaccharide that stains red with iodine was found to be formed before starch in sugary waxy and sugary, high amylose starch endosperms (43). Amylose stains blue with iodine. Therefore such evidence

points toward the formation of a highly branched polysaccharide such as glycogen followed by the formation of amylose and amylopectin.

2. Using radioactive $^{14}\text{C}\text{O}_2$ for the production of starch in potato tubers, Badenhuizen and Dutton (56) found that the cytoplasm became active first (mostly due to sucrose), then at an intermediate stage amyloplasts became highly active, and finally the outer layers of starch granules slowly became active. This would correspond to the above mechanism if the amyloplasts are considered to contain glycogen.

3. A water soluble branched polysaccharide has been found in sweet corn. It has essentially the same properties as animal glycogen (57). It was thought previously that this glycogen was also composed of an intermediate (phytoamylose) (58) which had the properties of a slightly branched amylose molecule suggesting that amylose is produced first in the synthesis of starch. Later it was shown that phytoamylose is a mixture of amylose and the glycogen (59). The presence of this glycogen suggests that the above mentioned intermediates are also glycogens.

4. Wolf, et. al. (60), found that sweet corn starch granules lie embedded within globules of this glycogen. This suggested to them that glycogen acts as an intermediate in the synthesis of starch.

5. As little as 5 or 6 per cent amylose was found in immature starch granules (60). Mature starch granules have around 25

per cent amylose. As pointed out by Wolf, et. al. (60), one would expect the reverse of this if amylopectin is produced from amylose.

6. The priming activity of the straight 1,4-linked glucose chain for potato phosphorylase reaches an optimum at about 20 glucose units (37). If amylose were produced first, it would seem that the optimum priming activity should be at least 200 glucose units.

7. The ratio P/Q, the phosphorylase activity divided by the Q-enzyme activity, is less in dent than in waxy for samples obtained 12, 16, 20, and 25 days after pollination (61). Mature dent has 4.0% branching and mature waxy has 4.5% branching. One would expect the opposite of this from the above. However, as pointed out in Table 6, the per cent branching of the amylopectin is lower than its parent glycogen. Mature waxy has no amylose; mature dent has approximately 25% amylose. From a previous discussion on waxy, it was concluded that waxy is debranched to a lesser extent. Therefore the lower per cent branching in the dent amylopectin is probably due to a greater debranching of its parent glycogen.

8. By using iodine staining tests it was shown that incubated endosperm tissue of waxy maize produced amylose and the youngest leaves of Scilla ovatifolia Bak. produced a red-staining starch under the same conditions (42). Normally, waxy stains brown and Scilla stains blue due to its amylose. Production

of amylose by waxy maize under similar conditions was also noted by Fuwa (43). Apparently these results were brought about because of a change in the number of acceptor groups for the debranching enzyme. The red-staining starch in Scilla may either be due to the non-reducing ends or to shorter amylose chains acting as acceptors as in waxy.

9. In some cases it has been found that waxy maize starch granules stain blue at the nucleus of the granule. Lampe found that cells in the central region of the cap of waxy kernel contain granules without blue staining center spots but that blue staining center spots increase in size with distance away from this region (44). It therefore appears that the amylose in the center of the waxy maize granule is due to a variation in the amount of acceptor groups when the waxy granule is first being formed. More moisture would be lost at the cap of the kernel. This variation in loss of moisture may change the cell conditions and thus bring about the above results.

10. The diurnal variations of the carbohydrates in the leaves of corn have been studied. It has been found by different workers that the maximum sucrose content is reached at 4 p.m. (6, 62, 63), the maximum content of medium size cold water soluble dextrans at 4 p.m. and 7 p.m. (62), and the maximum starch content between 7 p.m. and 1 a.m. (63). If the dextrans that are soluble in cold water are considered as glycogen, then synthesis of starch occurs as indicated above.

11. There is no diurnal change in phosphorylase (64). Therefore the above sequence of carbohydrates is not due to a variation in the phosphorylase activity.

12. Phosphate is chemically bound to carbon atom 6 of the glucose units in amylopectin (65) but is not chemically bound to glycogen (48). This agrees with the idea that the synthesis of starch from glycogen involves the simultaneous transfer of a branch to the DE-enzyme and a phosphate group to the amylopectin.

13. Phosphate is located on glucose units at the linear parts of amylopectin (66). This agrees with the proposed reactions since those branches removed would be less sterically hindered.

14. Waxy maize has less phosphate bound to its starch than dent corn starch (67). This, however, may be due to the presence of more phosphatase instead of less debranching.

The above points have been interpreted in light of the proposed theory and other reasons may be given for these facts. However, these points can be explained by the proposed theory. The theory that amylose is converted into amylopectin cannot explain all of these points. It would seem impossible that glycogen with its almost spherical shape and short external branches, amylopectin with its large asymmetry and long external branches, and the linear amylose molecule could be synthesized by the same type of enzymes in the same cell and

in the same vicinity in that cell without having any correlation in their synthesis.

The predicted amount of phosphate attached to the amylopectin is much greater than that found (67). Phosphatase is present in some starch synthesizing cells but there appears to be no simple relationship between phosphatase and starch synthesis (42). Therefore the lack of a sufficient amount of phosphate on starch cannot be readily explained.

D. Experimental

1. Isolation of corn and potato starch samples

The variety of corn plants used including their endosperm geneology were Seneca Chief sweet corn su₁, su₁, su₁, Wx, Wx, Wx; Iowa 4297 dent corn Su₁, Su₁, Su₁, Wx, Wx, Wx; Iowax 5 hybrid waxy corn Su₁, Su₁, Su₁, wx, wx, wx. The sweet, dent and waxy varieties were hand pollinated, respectively, on July 20, 25, and 24 in 1955. All the samples of a given variety were pollinated at the same time. Samplings were made on all varieties on the 13th morning, 13th night, 14th morning, 20th morning, 20th night, and 21st morning after pollination and mature samples. The immature kernels were collected between 5:45 and 6:30 in the morning and between 5:45 and 6:30 in the evening. Each ear was first husked while on the plant, then picked, and then immediately shelled with a knife and frozen on solid carbon dioxide in order to keep enzyme degradation

to a minimum. All immature samples were kept frozen on dry ice until they were processed for starch (no longer than four days after picking).

In order to study the effect of water evaporation from the kernel, some samples that were picked on the 13th morning and 20th morning were (1) covered with cellophane bags and (2) husked about the third or fourth day after pollination. Two cellophane bags, tied on by an elastic band, covered the ear. The outer bag contained a small amount of water in order to retard evaporation. These samples were collected as above.

The starch was isolated from the kernels in a room held at 4°C. The frozen kernels were ground with a small amount of iced distilled water (60) for 3 minutes in a Waring Blendor. The slurry was screened through a number 17 nylon bolting cloth. The magma was then squeezed as dry as possible. The press cake was ground for another 3 minutes with fresh water, then screened as before. The combined extracts were centrifuged in an International centrifuge. Microscopic examination of the separated granules indicated negligible granule damage. The gluten in the starch was removed according to the method of Schoch (68). The above starch-gluten mixture was shaken with a 20% Pentasol mixture for three or four 15 minute periods. Each period was followed with centrifuging, discarding the supernate, and adding fresh 20% Pentasol solution. The final starch was washed with 95% ethanol and Soxhlet-

extracted for 48 hours with 95% ethanol to remove the associated fatty acids. The starches were then air-dried and kept in closed containers. The mature samples were isolated in the same way without SO₂ treatment.

Certified Irish Cobbler potatoes were planted 6/1/55 in three plots which had been equally fertilized and were located 3 to 5 feet apart. Four plants were planted in each plot. In the first plot the plants were exposed to natural sunlight throughout the day and to a fluorescent lamp at night. The light was held a few inches above the plant and was turned off for two hours each night. The second plot was exposed to the regular diurnal variations in light. The third plot was shaded so that the plants were exposed to sunlight for approximately two hours every afternoon. The potatoes grown in the shade were fewer in number but comparable in size to the normal potatoes. The potatoes grown under the light were quite small in size.

The mature potatoes were ground in a meat grinder. Water was added and the starch slurry was passed through number 17 nylon bolting cloth. The same procedure as described above for separation of protein and fat was then followed.

2. Iodine titrations of starch samples

The per cent amylose in all the samples was determined according to the method of Lansky, Kooi, and Schoch (69) which

is a modification of the method by Bates, French, and Rundle (70). The data, however, was graphed in a different manner. A plot of mg. of iodine bound per 100 mg. sample versus the logarithm of mg. of free iodine per 100 ml. of solution was made. The intercept of the two lines gives the mg. of iodine bound per 100 mg. sample.

All of the samples were corrected for moisture content by drying representative samples in a vacuum oven at 60°C until constant weight had been obtained. It was assumed that the amylose in all of the starches bound 19 mg. of iodine per 100 mg. sample. The per cent amylose in the starch was calculated from this. The small error involved in this assumption will be an absolute error in all of the results.

3. Results on iodine titrations and yield of starch samples

(a) Corn starch samples. As described above, three samples for each variety of starch were picked 12 hours apart starting with the 13th morning. Three more samples which were also 12 hours apart were picked starting with the 20th morning, one week later. The per cent amylose obtained from Figs. 9-14 and yields on a dry basis of the defatted sweet, dent, and waxy samples are listed in Tables 9, 10, 11, 12, 13, and 14. Yields were expressed as grams per ear since this gives roughly the amount of starch per plant.

Table 9. Comparison of experimental and predicted diurnal variations in per cent amylose assuming daytime production of amylose for immature dent corn starch

Sample	Per cent amylose in sample	Predicted per cent assuming amylose is produced during the daytime and amylopectin during the night			
		From daily yields	From weekly yields-0th order reaction	From weekly yields-1st order reaction	From weekly yields-2nd order reaction
13th morning	17.90	17.90 ± .06	17.90 ± .06	17.90 ± .06	17.90 ± .06
13th night	17.90	22.1 ± 1.6	23.3 ± 1.7	21.2 ± 1.5	20.1 ± 1.5
14th morning	18.70	18.7 ± .06	18.60 ± .06	18.60 ± .06	18.60 ± .06
20th morning	22.35	22.35 ± .35	22.35 ± .35	22.35 ± .35	22.35 ± .35
20th night	23.15	27.4 ± 2.0	24.8 ± 1.8	26.1 ± 1.9	30.0 ± 2.2
21st morning	23.60	23.60 ± .35	23.05 ± .35	23.05 ± .35	23.05 ± .35

Table 10. Comparison of experimental and predicted diurnal variations in per cent amylose assuming daytime production of amylose for immature sweet corn starch

Sample	Per cent amylose in sample	Predicted per cent assuming amylose is produced during the daytime and amylopectin during the night			
		From daily yields	From weekly yields-0th order reaction	From weekly yields-1st order reaction	From weekly yields-2nd order reaction
13th morning	16.05	16.05 ± 1.0	16.05 ± 1.0	16.05 ± 1.0	16.05 ± 1.0
13th night	18.40	24.5 ± 2.1	26.5 ± 2.3	20.1 ± 1.7	18.1 ± 1.6
14th morning	18.30	18.30 ± 1.0	16.80 ± 1.0	16.80 ± 1.0	16.80 ± 1.0
20th morning	23.05	23.05 ± .40	23.05 ± .40	23.05 ± .40	23.05 ± .40
20th night	22.50	33.4 ± 2.3	26.1 ± 1.8	28.0 ± 1.9	37.4 ± 2.5
21st morning	22.90	22.90 ± .40	23.80 ± .42	23.80 ± .42	23.80 ± .42

Table 11. Twelve hour period yields for dent corn starch and their iodine binding capacities

Sample	No. of ears	Starch yield (dry, defatted) in gms per ear	% starch produced during daytime	Mg. I ₂ bound per 100 mg. sample
13th morn	10	6.67		3.40
13th night	10	6.27	-19.6%	3.40
14th morn	10	8.31		3.55
20th morn	3	12.60		4.25
20th night	3	11.86	17.6%	4.40
21st morn	3	12.97		4.48

Table 12. Twelve hour period yields for sweet corn starch and their iodine binding capacities

Sample	No. of ears	Starch yield (dry, defatted) in gms per ear	% starch produced during daytime	Mg. I ₂ bound per 100 mg. sample
13th morn	11	1.16		3.05
13th night	11	1.33	29.8%	3.50
14th morn	11	1.73		3.48
20th morn	3	5.76		4.38
20th night	3	5.98	5.59%	4.28
21st morn	4	9.70		4.35

Table 13. Twelve hour period yields for waxy corn starch

Sample	No. of ears	Starch yield (dry defatted) in gms per ear	% starch produced during daytime
13th morn	8	5.27	≤ 34.2%
13th night	8	8.6	
14th morn	8	≥ 15	
20th morn	2	26.7	36.3%
20th night	2	30.8	
21st morn	2	38.0	

Table 14. Results on per cent amylose for bagged and husked ears of corn

Variety	Dent	Dent	Sweet	Sweet	Waxy	Waxy
Days after pollination	13	20	13	20	13	20
Husked	17.7	24.1	16.7	21.9	1.1	.87
Bagged	12.5	19.6	12.7	15.2	3.1	.84
Control	17.9	22.4	16.1	23.1	.92	.68

Calculations were made assuming that amylose is produced during the daytime and amylopectin during the night since most of the starch is produced during the night and most of the starch consists of amylopectin. By subtracting the 13th morning yield from the 14th morning yield, the amount of starch produced in one day per ear is obtained. Multiplying this by the fraction of amylose in the 14th morning sample which was found by iodine titrations, we obtain $A\Delta 1$, the number of grams of amylose produced in one day. Multiplying the 13th morning yield by its corresponding fraction of amylose gives us $A13$, the number of grams of amylose in the 13th morning sample. The total number of grams of amylose produced by the 13th night will then be this weight plus the weight of amylose produced during the 13th day. The predicted per cent amylose for the 13th night assuming only amylose is produced during the day is then equal to

$$\frac{A\Delta 1 + A13}{13\text{th morning starch yield} + A\Delta 1} \times 100$$

This predicted percentage was not obtained using the 13th night yield because of the small increase in yield during the daytime. The 20th morning and 21st morning data are treated in the same manner. The results are listed in Tables 9 and 10 under the heading "from daily yields".

Similar calculations are made from weekly yields because of the possibility of a large error in the daily yields. Cal-

ulation of the amount of starch produced during the 13th day and during the 20th day were made assuming zero, first, and second order production rates of starch. The rate constants were obtained using three points: the 13th morning to the 20th morning; the 13th night to the 20th night; and the 14th morning to the 21st morning starch yields. The daily increases were then obtained from the calculated rate constant using the 13th morning and the 20th morning yields as the initial yields. The weekly increase in per cent amylose was obtained by using the same three points described above. The daily increase was calculated from this assuming zero order kinetics. Only a small error is involved in this assumption because of the small weekly increase in per cent amylose during this period.

We now have the predicted daily yields for the 13th and 20th day samples and the predicted per cent amylose for the 14th morning and 21st morning based on weekly increases. The same type of calculations as made above using the daily yields are then repeated. The results from the weekly yields are listed under their assumed rate of reaction.

The arithmetical mean was used in all cases to find the average result and the standard deviation to find the error in this result (71). The error in the yield was calculated assuming that

$$\frac{20\text{th morn. yield}}{13\text{th morn. yield}} = \frac{20\text{th night yield}}{13\text{th night yield}} = \frac{21\text{st morn. yield}}{14\text{th morn. yield}} = K$$

where K is equal to a constant. The error in each of the yields was taken as being equal to the error in K. The calculated error for the results obtained from the weekly yields assumes that in each case the order of reaction holds. The predicted per cent amylose and its relative error along with the experimental per cent amylose are listed in Tables 9 and 10. This error is only relative because the iodine adsorption capacity for pure amylose was considered as 19 in all cases. It was also assumed that any protein impurities present in the starch bound iodine to the same extent in all the starches.

From Tables 9 and 10 it is seen that the predicted increase in amylose during the daytime is much higher than that actually found. Therefore it is concluded that amylose and amylopectin are not produced separately during this time period. This conclusion cannot be made for other diurnal variations. However, since these other diurnal variations are less severe, the above results are in harmony with the postulate that amylose and amylopectin are produced at the same time from glycogen.

The results on the percentage of starch produced during the daytime are given in Tables 11, 12, and 13. It is seen that most of the starch is produced during the night. This is in agreement with the results obtained for the diurnal

variation of starch in the corn leaf (63). Because both are produced during the same period, the production of starch during the night is not due to a delayed translocation of sucrose.

The results on the per cent amylose for the bagged and husked samples along with the control values are listed in Table 14. The husked samples show no marked variation from the control. They were, however, exposed to the sunlight and their pericarps became extremely hard. This may have prevented a rapid loss of moisture. The per cent amylose in the bagged samples for dent and sweet corn are much lower than the per cent amylose in the control samples. The per cent amylose in the 13th day bagged waxy sample is much higher than the control. This sample stains blue with iodine while the control stains brown. The blue color intensity is about the same as that found in the immature dent and sweet corn starch samples. There is a possibility that the amylose is due to impurities from the ovary or pericarp. However, such impurities should also manifest themselves in the husked and control samples.

This per cent amylose increase in waxy and decrease in dent and sweet is similar to the results found by Badenhuizen for Scilla and waxy endosperm tissue which were described previously (42, 43). Both the decrease and increase in per cent amylose may be attributed to an increase in receptor

groups. In sweet and dent an increase in the number of receptor groups would lower the molecular weight of the amylose and therefore its iodine binding capacity. In waxy the increase in receptor groups would make it possible for the debranched chains to form amylose instead of adding to the non-reducing ends of the amylopectin.

Since the per cent amylose increases with maturity, the smaller amount of amylose in the bagged ears of dent and sweet may be due to retarding the growth of the kernel. Therefore the above point is open to criticism. However, it does suggest how amylose can be produced by waxy maize and how Scilla produces no detectable amylose.

(b) Potato starch samples. The samples which were exposed to a light at night had 20.25% amylose; those in the shade had 21.65% amylose; and the control samples had 20.95% amylose. All of the sample sizes used in the iodine titrations were within 0.4% of each other. The moisture in all of the samples was determined under the same conditions at the same time. Therefore any large error will be an absolute error and not a relative error.

The occurrence of small tubers in the plants exposed to a light at night indicates that starch was not produced during this period, but the sugars normally used for this purpose were used for plant growth. If amylose is produced during the daytime, one would expect a greater percentage than that

found for this sample since production of amylopectin during the night is hindered. If on the other hand the small tubers are due to the production of amylose instead of amylopectin during the night, one would again expect an increase in the per cent amylose. Thus the percentages in these samples indicate that amylose is not produced during the daytime.

4. Determination of alkali and ferricyanide numbers

The alkali number (alk. no.) and ferricyanide number (f. no.) were obtained as described by Kerr (72). The number average degree of polymerization, \bar{x}_n , was obtained according to the following equations (13):

$$\bar{x}_n = (6700/\text{alk. no.}) - 30 \quad \text{and} \quad \bar{x}_n = 600/\text{f. no.}$$

The alk. no. is obtained by heating a 0.1 N NaOH starch solution for one hour in a hot water bath and measuring the loss of NaOH. The f. no. is obtained by heating a sodium carbonate, potassium ferricyanide, starch solution for 15 minutes in a hot water bath and determining the amount of ferricyanide that has been reduced. Both methods depend on the alkaline degradation of the reducing chain. As soon as a branch point is reached, this degradation stops (73). The alkaline degradation in determining the alk. no. is much more severe than in the f. no. determinations. Therefore the alk. no. will be subjected to a larger error for samples that have been acid hydrolyzed or have a high degree of branching.

The alk. no., f. no., and light scattering molecular weight were all obtained on the same starch solutions in the acid hydrolysis experiments.

5. Determination of per cent branching and results

The per cent branching was determined according to the method of Rankin and Jeanes (74). The amylopectin solutions were prepared as described in the next section.

Approximately 0.5 grams of amylopectin solution was pipetted into a glass stoppered flask which had been cleaned with chromic acid and flushed with CO₂-free nitrogen. A 0.3 molar solution of NaIO₄ was added. The ratio of the number of moles of anhydrous glucose units to the moles of NaIO₄ was approximately 2.5. Enough CO₂-free double distilled water was added to dilute the solution to exactly 100 ml. The flask was stoppered and allowed to stand in the dark. Three 10 ml. aliquots were withdrawn at 72 hours and three more at 96 hours. The aliquots were pipetted into glass stoppered flasks which had previously been flushed with CO₂-free nitrogen. Two ml. of ethylene glycol was added to each aliquot and the stoppered flask was allowed to stand in the dark for one hour. The ethylene glycol had previously been vacuum distilled over solid NaOH. The formic acid in the aliquots was then titrated with 0.01 N NaOH to the first stable pink of the phenolphthalein indicator. The NaOH was standardized against weighed

portions of standard 0.2 N H₂SO₄. Blanks were included in each run and subtracted from the result. A correction for the presence of the reducing group was made on all samples having an \bar{X}_n of less than 1000 as determined by the f. no. The results are listed in Table 15.

It should be pointed out that the per cent branching for "immature", "mature", and "overmature" sweet corn glycogen samples is 9.25%, 8.4% and 7.58%, respectively (75, 68).

6. Light scattering

(a) Preparation of solutions. Starch granules were dispersed by using either 6 or 8 molar lithium bromide or buffered n-amyl alcohol solutions.

In dispersing with LiBr, a concentrated LiBr solution was placed in a 500. ml three-necked glass stoppered flask. The LiBr solution was first clarified by passing it through a medium porosity sintered glass filter. The flask was equipped with a thermometer and an inlet for helium gas, a glass stirrer, a reflux condenser, and a heating mantle. The atmosphere was kept inert by bubbling helium gas through the concentrated LiBr solution. A starch slurry was added when the temperature was approximately 90°C. The resulting solution was 6 or 8 M in LiBr and approximately 2 or 3 per cent in starch.

The dispersion of the starch granules was followed by withdrawing samples at various times into a stoppered bottle,

Table 15. Per cent branching of various amylopectins and sweet corn glycogen

Sample	Calculated per cent branching		Average
	Reaction time		
	72 hrs	96 hrs	
13th day waxy	5.74	5.88	5.8
Mature* waxy	-	-	4.5
14th day dent	6.75	6.90	6.8
Mature dent	4.05	3.80	4.0
14th day sweet	7.56	8.56	8.0
20th day sweet	7.36	9.01	8.2
Mature sweet	5.95	6.34	6.1
Glycogen	8.47	8.88	8.67

*Per cent branching of mature waxy maize was obtained from Rankin and Jeanes (74).

cooling these samples by swirling the bottle under the water tap, and diluting the cooled solution twenty-five fold into a light scattering cell. The pipet used in the dilution was rinsed several times with the diluted solution in order to insure complete delivery. The solution was held near its refluxing temperature (113°C for 6 M LiBr and 125°C for 8 M LiBr) until a steady 90° intensity (i_{90}) and dissymmetry reading (Z) were obtained.

In the case of immature dent and sweet corn starch, the hot lithium bromide solution hydrolyzed the starch samples. It is also believed that the mature dent sample was slightly hydrolyzed. The hydrolysis appears to be due to the presence of phosphate on the amylopectin. The pH of a 6 or 8 M LiBr solution is changed from an almost neutral solution to a solution having a pH of about 1.5 when a phosphate buffer which normally buffers at $\text{pH} = 6.4$ is added to the LiBr solution. In waxy starch there appears to be less phosphate and consequently no noticeable hydrolysis occurred. In order to remove acidity in the starch, one could either dialyze a mixture of starch granules and cold 6 M LiBr against 6 M LiBr using a sintered glass filter for separating the two, or one could develop a buffer system in the 6 M LiBr that would keep hydrolysis to a minimum.

The use of concentrated LiBr was suggested by Harrington who with Schellman found it to be an excellent solvent for

silk and other proteins. According to Harrington and Schellman (76), the LiBr tends to produce more hydrogen bonds between the protein groups by lowering the activity of water. The higher the activity of the LiBr, the lower is the activity of the water. The helical form of the protein has the greatest number of hydrogen bonds and therefore is the most stable in the concentrated lithium bromide solutions. Thus intermolecular hydrogen bonds are broken to form intramolecular hydrogen bonds and the protein goes into solution.

The dispersion of starch granules can also be thought of as breaking intermolecular hydrogen bonding and forming intramolecular bonds. The complexing of the lithium bromide with the starch chains is probably similar to the iodine complexing, the negative bromide ion being located in the center of the helix and the highly hydrated lithium ions adhering to the external side of the helix. Any salt that has a high activity and a large anion could act as an excellent solvent. LiBr was chosen because of its higher activity coefficients (77). Lithium thiocyanate would appear to be a good solvent, but its activity in aqueous solutions has not been studied. 15% $MgCl_2$ which also has a high activity coefficient was found by Staudinger (78) to be a good solvent for glycogen and was later used by Stetten, *et. al.* (14) as a medium for light scattering measurements on glycogen. The effect of various salts in dispersing starch has been reviewed.

In the immature waxy samples, dispersed with LiBr as described above, the solution was dialyzed until silver nitrate tests were negative. In all other LiBr dispersions, the hot solution was added with vigorous stirring to 95% ethanol. The precipitated starch was then washed three or four times with 80% ethanol and finally with methanol. The starch was brought back into solution by adding water and shaking. The small amount of methanol remaining in the solution was removed by vacuum distillation between 25°C and 40°C.

The amylose in the case of dent and sweet corn samples was removed by reheating the above solution to its refluxing temperature under a helium atmosphere, adding n-amyl alcohol, cooling slowly with stirring to room temperature, centrifuging off the precipitated amylose in an International centrifuge and filtering through a millipore filter of sufficient size. The amylopectin solution was then precipitated and washed with alcohol, redissolved, and vacuum distilled as before. It was found that if the n-amyl alcohol is vacuum distilled off instead of using the above precipitation method, the solution becomes acid.

Lansky, Kooi, and Schoch's method (69) was used for the dispersion of starch granules in buffered n-amyl alcohol solutions. The pH changed from 6.4 to 6.1 during the 24 hour refluxing period. The same apparatus and method for dispersing starch granules in concentrated LiBr solutions was used in

dispersing starch in buffered n-amyl alcohol solutions. After 24 hours of refluxing the amylose was removed as described above. The resulting amylopectin solution was dialyzed to remove the phosphate buffer. The starch was then precipitated and washed with alcohol, redissolved, and vacuum distilled as described above.

The resulting 2 to 3 per cent amylopectin solutions from the LiBr and n-amyl alcohol dispersion methods were centrifuged at 20,000 rpm for one hour or at 42,000 rpm for 15 minutes in a Spinco preparatory centrifuge. The solution was gently poured off, the impurities adhering to the side of the centrifuge tube. In waxy maize samples I, II, and IV, the upper three-fourths of the solution was withdrawn slowly with a hypodermic syringe. This latter precaution was found to be unnecessary and was eliminated.

Weighed portions of the above solutions were diluted with double distilled water for ultracentrifuge studies.

In the acid hydrolysis experiments, the above resulting solution was heated to its refluxing temperature under a helium atmosphere in a 250 ml. three-necked glass-stoppered flask equipped with a thermometer, refluxing condenser, heating mantle and magnetic stirrer. The buffer was then added. A 0.05 M potassium acid phthalate solution was used to hydrolyze the 13th day waxy samples and a 0.05 M acetic acid plus 0.009 M sodium acetate solution was used for the other samples.

The calculated pH for the phthalate (79) and acetate (80) buffered solutions at the refluxing temperature was 4.24 and 4.22, respectively. Samples were extracted at various time intervals and immediately precipitated by adding the hot solution to 95 per cent ethanol with vigorous stirring. They were washed three or four times with 80 per cent ethanol and then with methanol, redissolved and vacuum distilled as described above.

The concentration of the above solutions was determined by evaporation of a weighed portion to dryness under a heat lamp, followed by drying in a vacuum oven at 95°C to a constant weight (approximately 12 hours). Weighed portions of the resulting amylopectin solutions were diluted with double distilled water to approximately 100 ml. and weighed again on a Sartorius balance.

These diluted solutions were then prepared for light scattering by using HA (4500 A pore size) and AA (8000 A pore size) millipore filters. A picture of the apparatus used is shown in Fig. 77. The size of the filter used depended on the molecular weight of the sample. It was found by optical rotation that passing the solution through the filter did not change the concentration. In cases where the molecular weight was over approximately 100 million, the solutions were not passed through the filter but only the water used in diluting these samples was clarified.

In making the light scattering measurements, 50.00 ml of HA filtered water was added to a Brice-Phoenix cylindrical cell. The above diluted starch solutions were added to the cell in increments of 1, 5, 15 and 50 ml. This procedure enables one to obtain an accurate blank and avoids the cumulative contamination obtained in going from concentrated to more dilute solutions. This is extremely important since the concentrations used were very low, the lowest being 9.7×10^{-7} gm./ml. For the first three additions of starch solution to the light scattering cell, the cell was gently swirled to obtain a homogeneous solution. After the last addition it was tipped back and forth. It was found that although a magnetic stirrer can be used to stir the solution, it was more cumbersome and did not give as good results. Light scattering measurements were made over the angle range from 21.6° to 135° .

The amyloses from 20th day and mature sweet corn starch were obtained from the above buffered n-amyl alcohol dispersions. They were also dialyzed and precipitated and washed with alcohol as described above. They were put into solution by using a boiling water bath before determining their f. no.

The sweet corn glycogen obtained from Schoch is readily soluble in water. Amylose impurities were removed by precipitating the amylose as described above. The glycogen after being precipitated, washed, and redissolved was clarified by passing the approximately 2% glycogen solution through an HA

millipore filter instead of centrifuging the solution as above. By removing the amylose impurities it was found that the molecular weight of the glycogen had increased from 14.6×10^6 to 18.5×10^6 .

(b) Theory of light scattering technique. There are numerous reviews on light scattering (81, 82, 83, 84, 85). Therefore, except for essential parts, the theory and its applications will not be reviewed here.

In obtaining the light scattering data, one passes a narrow monochromatic beam of light through an optical glass cell containing the solution. The intensity of the light scattered at various angles from the exiting beam is measured, the light scattered by the solvent being subtracted. The intensity of the scattered light for a given concentration and molecular weight depends on the width of the cell and the distance of the light to the photometer. Consequently the intensity is only relative and must be calibrated with solutions of known turbidity.

The molecular weight can be obtained from the following equations:

$$KC/R_{\theta,C} = HC/T_{\theta,C} = 1/\bar{M}WP_{\theta} + 2BC + \dots$$

where

$R_{\theta,C}$ = reduced intensity or Rayleigh's ratio corrected for volume scattering and the use of unpolarized light

$$K = 2\pi^2 n_0^2 (dn/dc)^2 / N \lambda^4$$

$T_{\theta,c} = (16\pi/3) R_{\theta} =$ turbidity of the solution

$H = (16\pi/3) K$

$\bar{M}_w =$ the weight average molecular weight

$P_{\theta} =$ correction factor for intraparticle interference

For the constants H and K, $n =$ refractive index of the solution; $n_0 =$ refractive index of the solvent; $N = 6.023 \times 10^{23}$; $\lambda =$ wave length of light in vacuum.

Two methods are usually employed for obtaining the molecular weight: the dissymmetry and the Zimm methods. In the dissymmetry method the turbidity is obtained at 90° . $HC/T_{90^\circ,c}$ is plotted against the concentration and extrapolated to zero concentration according to the above equation. In order to correct \bar{M}_w for the intraparticle interference, equations for rods, spheres, and poly- and mono-disperse coils have been derived relating P_{90° to the dissymmetry at zero concentration (86). The dissymmetry at zero concentration is found by plotting the intensity at 45° divided by the intensity at 135° versus the concentration.

In the Zimm method the molecular weight (87) is obtained by plotting $KC/R_{\theta,c}$ versus $\sin^2 \theta/2 + kC$ where the constant k is chosen so that kC will be approximately the same order of magnitude as $\sin^2 \theta/2$. By extrapolating to zero angle and zero concentration, we obtain a molecular weight which is independent of the shape of the molecule since $P_{\theta} = 1$ at $\theta = 0^\circ$.

From the Zimm plot the radius of gyration can be obtained using the initial slope of the zero concentration line and the intercept (88, 89, 17):

$$\bar{R}_z^2 = \frac{3 \lambda^2 (\text{initial slope})}{16 \pi^2 (\text{intercept})}$$

Equations have been derived relating the radius of gyration to various models (81). If the polymer is heterogeneous, as with amylopectin and glycogen, the Z-average radius of gyration will be obtained (89).

The light scattering instrument and the refractometer used have been described previously (90, 91). Measurements were made at $\lambda = 4358 \text{ \AA}$ using a Brice-Phoenix cylindrical cell (92) with plane entrance and exit faces. At this wave length dn/dc was found to be equal to 0.156 ml./ gm. for both glycogen and amylopectin solutions using water as the solvent ($n_0 = 1.34$ for water). The calculated value of K was found to be $K = 3.97 \times 10^{-7}$.

Witnauer, et. al. (12), found that the measured depolarization for potato amylopectin was approximately 2%. Therefore it was assumed that the optical anisotropy for the samples studied was also small.

The scattering correction (93) for the reflexion of the exit beam in the light scattering cell was not made. A 5.3% correction was made, however, on the mature dent sample and it is seen in Graph 42 that for this molecular weight, the

scattering correction is negligible. It may, however, be more predominant at lower molecular weights.

The calibration of the light scattering instrument was made according to the method of Northrup and Sinsheimer (90). The constants A and σ were found to be 10.70 cm-per cent and 4.37×10^3 cm./per cent, respectively, by the method of least squares. Data given by Stamm (94) for the refractive indices of NaCl solutions at $\lambda = 4358$ A were used to calibrate the refractometer.

(c) Calculation of rate constants. The rate constants were calculated from the slope of the line in the $1/\bar{X}_n$ versus time graphs by the following methods.

Consider the hydrolysis of an infinitely large statistically branched polymer. The degree of hydrolysis is equal to $\frac{x}{n}$ where x is the number of linkages hydrolyzed (the number of free aldehyde groups), and n is the total number of glucose units. The degree of hydrolysis is therefore equal to $1-p_A$ where p_A equals the probability of having a linkage at the aldehyde group on a glucose unit. Hence

$$1-p_A = 1-p_4-p_6 = 1/\bar{X}_n = \frac{x}{n}$$

In this large polymer containing n glucose units there will be essentially n linkages. Thus

$$dx/dt = k(n - x) \sim k n$$

Assuming that the number of linkages equals the number of

glucose units, we have $d(1/\bar{X}_n)/dt = k$. And upon integrating

we have $(1/\bar{X}_n)_{\text{final}} - (1/\bar{X}_n)_{\text{initial}} = k \Delta t$

where Δt equals the change in time in seconds. If we start with an infinitely large polymer, then $(1/\bar{X}_n)_{\text{initial}}$ equals zero. However, since an infinitely large polymer is not involved, $(1/\bar{X}_n)_{\text{initial}}$ is not zero. This equation will hold for large values of \bar{X}_n as encountered in the hydrolysis experiments, but it will not hold for extreme hydrolysis. When $\bar{X}_n = 100$, there will only be a one per cent error in assuring that the number of glucose units equals the number of linkages. Acid reversion of the hydrolyzed products will also be negligible in these experiments (95).

If the statistical model applies to amylopectin and glycogen, the rate of change of $1/\bar{X}_n$ obtained from \bar{M}_w versus time should be linear. From the light scattering results on acid hydrolyzed amylopectins, it will be shown that this is true. The rate of hydrolysis can therefore be obtained from these resulting linear graphs. As described previously, \bar{X}_n can also be obtained from the f. no. and alk. no.

The rates of hydrolysis calculated from the acid hydrolysis experiments are listed in Table 16. The true rate of hydrolysis calculated from experiments done by French (96) and Calamari (97) on mature dent amylopectin and mature waxy maize for acid concentrations of around 0.1 to 0.2 N HCl and H_2SO_4 were found to be equivalent to $k = 6.7 \times 10^{-8} \text{ sec}^{-1}$ and

Table 16. Rate constants obtained from
acid hydrolysis studies

Amylopectin	$k \times 10^8 \text{sec}^{-1}$ (from \bar{M}_w)	$k \times 10^8 \text{sec}^{-1}$ (from f. no.)	$\frac{k \text{ (f. no.)}}{k (\bar{M}_w)}$
Mature dent (4% branching)	2.41	7.05	2.92
Mature dent (6% branching)	3.02	7.05	2.33
Mature dent (6% branching \bar{x}_n from $\bar{M}_w/4$)	6.27	7.05	1.12
13th day waxy IV (98°C)	2.82	4.62	1.64
13th day waxy II (99°C)	3.33	-	-
20th day sweet (from \bar{M}_w)	3.17	3.02	0.952
20th day sweet (from $\bar{M}_w/4$)	6.56	3.02	0.460
14th day sweet	4.43	1.39	0.314
Glycogen	1.018	0.667	0.655

$9.8 \times 10^{-8} \text{ sec}^{-1}$ for a pH of 4.22 and a temperature of 99.5°C . The hydrogen ion concentration for H_2SO_4 was approximated. These rates were obtained by the copper reduction method. The rate constant for the hydrolysis of mature dent amylopectin obtained from the f. no. agrees well with these rate constants.

The errors that may occur in obtaining the rates from \bar{X}_n are the following:

1. The rate of hydrolysis is a function of both the hydrogen ion concentration and the temperature. Therefore a change in refluxing temperature because of a change in atmospheric pressure will change the rate of hydrolysis.
2. The change in the equilibrium constant of the buffer with a change in the refluxing temperature will change the hydrogen ion concentration.
3. Phosphate on the amylopectin or protein impurities may change the pH of the buffer.
4. The rate from the f. no. may be increased if long exterior chains are present. The rate from the alk. no. would not be changed as much since small molecules do not appreciably affect the alk. no. (13). This error exists because linear molecules have a higher f. no. and alk. no. than branched molecules.
5. The rates from the f. no. and alk. no. may be decreased if the amylopectin or glycogen has a high percentage of branch points. The alkaline degradation of the reducing chain in both cases will be stopped at a branch point. A much greater

error will be encountered in the rates obtained from the alk. no.

6. An error in per cent branching will alter the rate of hydrolysis obtained from \bar{M}_w .

7. It has been found that the rate of hydrolysis of the 1,4-linkage is approximately four times as fast as the rate of hydrolysis of the 1,6-linkage (98). Since p_6 is less than p_4 , then the hydrolysis of the 1,6-linkage should occur at a faster rate in order for the hydrolysis to act as a reverse of the synthesis of a statistical model. A slower rate will be obtained because of the stability of the 1,6-linkage. The hydrolyzed amylopectin or glycogen should still act as a statistical molecule since the degree of hydrolysis in these experiments is not severe.

8. The rate of hydrolysis from \bar{M}_w may be increased if the molecules exist as chemically bound aggregates.

Variations in the rate due to temperature, per cent branching, and obtaining \bar{X}_n from $\bar{M}_w/4$ instead of \bar{M}_w are included in Table 16. The maximum per cent branching of the glycogen precursor for the 4 per cent branched nature dent amylopectin is 6 per cent. Therefore values using this percentage are included. Except where indicated, the \bar{X}_n was obtained from \bar{M}_w instead of $\bar{M}_w/4$ and from the experimental degree of branching. The rates for 14th day sweet corn amylopectin and gly-

cogen were obtained from the linear part of their curves (see Figs. 45 and 55).

The results in Table 16 indicate that the errors described in (1), (2), and (6) will be small. The differences in rate constants between the sweet corn amylopectins and glycogen may be due to (3) because the protein was separated from the starches and glycogen by different methods (68) and glycogen may have a smaller amount of phosphate. The error described in (4) will be large if long exterior branches are present. Preliminary data on mature waxy maize starch show that this error is quite large indicating the presence of a few extremely long exterior chains. It may be that some of the longer exterior chains in the waxy starch are being used as acceptors in the debranching mechanism. The results on the acid hydrolysis of mature waxy starch are not reported here. In the case of mature dent amylopectin, the error due to its longer exterior chains is believed to be negligible since the rate obtained from the f. no. agrees quite well with the rates found by French (96) and Calamari (97). However, this could be checked by obtaining the rate of hydrolysis of its β -amylase limit dextrin. This experiment was not done.

The rate obtained from the f. no. gradually decreases with an increase in the experimental degree of branching. This may be due to either (4), (5), or (7). It was concluded above that there is not great error due to (4). Since there

is no detectable change in the rate obtained from \bar{M}_w with a change in the experimental degree of branching, then the error described in (7) is most likely negligible. Thus the decrease in the rate obtained from the f. no. is most likely due to (5).

The correlation between the rates obtained from \bar{M}_w and $\bar{M}_w/4$ will be discussed below.

(d) Light scattering results. The results on the unhydrolyzed samples of the waxy, dent, and sweet corn amylopectins and the sweet corn glycogen are listed in Table 17. The table includes the weight-average molecular weight, Z-average radius of gyration and the predicted number-average degree of polymerization assuming the statistical model is valid and assuming that the experimental per cent branching is correct. For comparison, the \bar{X}_n obtained from the f. no. and alk. no. is included. The method of dispersion - either by 6 or 8 M LiBr or n-amyl alcohol - is given. The results on the acid hydrolysis of each sample are listed in the Appendix. The Z-average radii of gyration for samples of various amylopectins having the same molecular weight are compared in Table 18.

From these results the following points will be considered:

1. The \bar{X}_n obtained from the \bar{M}_w of immature and mature waxy remains essentially constant, the variation in \bar{M}_w being due

Table 17. Molecular weights of various amylopectins,
radii of gyration are included.

Theoretical \bar{x}_n obtained from the statistical model

Sample and method of * dispersing	$(\bar{R}_z^2)^{\frac{1}{2}}$	$\bar{M}_w \times 10^{-6}$	Theor. \bar{x}_n	Observed** \bar{x}_n
13th day waxy (LiBr)	4680 A	168	3070	6160 (f. no.) 8960 (alk.no.) 8650 (alk.no.)
Mature waxy (LiBr)	4301 A	125	2980	3990 (alk.no.)
14th day dent (alcohol)	3420 A	168	2880	2670 (alk.no.)
Mature dent (LiBr)	2302 A	45	1890	3090 (alk.no.)
14th day sweet (LiBr)	1893 A	15.8	810	260 (f. no.) 524 (alk.no.)
20th day sweet (alcohol)	2456 A	43	1320	717 (f. no.) 702 (alk.no.)
Mature sweet (alcohol)	3160 A	76	2015	596 (f. no.) 1050 (alk.no.)
"Mature" glycogen	580 A	18.5	843	533 (f. no.)
14th day sweet (extrapolated)***	-	3.93	400	-
"Mature" glycogen (extrapolated)***	-	4.58	416	-

* Dispersed either by refluxing in 6 or 8 M LiBr or buffered n-amyl alcohol solutions

** Obtained from alkali and ferricyanide numbers (13)

*** See Figs. 45 and 55

Table 18. Primary specificity of phosphorylase

Data obtained from Kunyi Tanaka's paper (99)

Per cent phosphate liberated using phosphorylases obtained from corn endosperms and potato

Priming substance	Waxy	Dent	Sweet	Potato
None	10	10	16	3
Sweet corn glycogen	30	25	21	58
Corn amylose	27	21	27	47
Corn amylopectin	42	30	24	17
Soluble starch	14	15	27	34
Reaction time of column	4110 min.	4345 min.	3960 min.	4300 min.
<u>Amylopectin</u> amylose	1.56	1.43	.890	.36
<u>Glycogen</u> amylose	1.11	1.19	.78	1.23

to the variation in per cent branching.

2. The \bar{M}_w and \bar{X}_n for sweet amylopectin increases with maturity.

3. The relative phosphorylase activity (for amylopectin as a primer) in the three varieties is waxy > dent > sweet (99).

This predicts that the \bar{X}_n will vary in the same manner.

4. Acid hydrolysis studies show that the amylopectin and glycogen behave as statistical models and as if these molecules exist in aggregates of four.

5. The results on glycogen and 14th day sweet corn amylopectin give further evidence that glycogen is a precursor to starch.

6. The comparison of the radii of gyration for various amylopectins indicates that the amylopectins have been subjected to a debranching enzyme.

Since the \bar{X}_n of waxy remains essentially constant, one would expect that the glycogen precursor of dent amylopectin would also remain constant. The per cent branching for 14th day and mature dent amylopectin is 6.8% and 4.0%, respectively. Assuming that the \bar{X}_n for 14th day dent amylopectin equals the \bar{X}_n for the mature amylopectin, the predicted \bar{M}_w for mature amylopectin will be 10^4 million. A larger predicted \bar{M}_w would be obtained if the \bar{X}_n of the parent glycogen was used. The molecular weight as listed in Table 17 is 45 million. Stacy and Foster (17) obtained molecular weights of 43, 80, and 100 million. Assuming that glycogen is debranched to form amylo-

pectin and amylose, the molecular weight of the mature dent amylopectin should be lower than that predicted. This is because the per cent amylose and therefore the amount of de-branching increases with maturity. Both the \bar{X}_n and the \bar{M}_w should decrease in dent amylopectin and the \bar{X}_n of its parent glycogen should remain the same.

The increase in \bar{M}_w for sweet amylopectin with maturity is most likely due to the formation of a limiting molecular weight because of the high per cent branching and the steric hindrance associated with these non-reducing ends being too close together. If glycogen is a precursor to starch, then the molecular weight of glycogen should also increase with maturity. Schoch (68) found an increase in the intrinsic viscosity of glycogen with maturity. Therefore his results agree with those found for the amylopectin.

The data from Tanaka's paper (99) show how dent, sweet, waxy and potato phosphorylase act towards different primers. The amount of phosphate liberated indicates how much glucose-1-phosphate has reacted with the primer. The greater the amount of phosphate, the greater is the catalyst activity of the enzyme for that primer. Because amylose is a linear molecule having no possible steric hindrance, one can assume that the activity of all the phosphorylases for the amylose primer is the same. The relative activities for the amylopectins are obtained by dividing the amount of phosphate liberated when

amylopectin is the primer by the amount when amylose is the primer (see Table 18). In treating Tanaka's results in this manner, one finds that waxy, dent and sweet corn have phosphorylases which act with different speeds toward amylopectin. The results on glycogen can not be used in comparing the phosphorylase rates because of the possible steric hindrance associated with the branching in the glycogen. This rate would only be valid for the sweet corn phosphorylase.

Waxy phosphorylase has the greatest activity for amylopectin followed by dent and finally by sweet corn phosphorylase. Since the number average and not the weight average molecular weight of waxy remains constant during plant growth, only the \bar{X}_n can be compared. This variation in phosphorylase activity predicts that the \bar{X}_n of waxy, dent, and sweet will decrease in that order. This has been found to be true using either the \bar{X}_n obtained from \bar{M}_w or from the f. no. and alk. no. A limiting molecular weight due to higher per cent branching as discussed above may be a factor here. It is not an important factor because even though immature dent amylopectin has a greater per cent branching than the mature sweet, the immature dent amylopectin has the larger molecular weight.

As pointed out previously the rate of change of $1/\bar{X}_n$ with time should be constant if the amylopectin or glycogen behaves as a statistical model. The branched components of mature dent, 20th day sweet, 14th day sweet, "mature" glyco-

gen and four different preparations of 13th day waxy were acid hydrolyzed. A straight line from a plot of $1/\bar{X}_n$ versus time was obtained for all amylopectins except in the initial part of the 14th day sweet and glycogen studies. The less random models also give straight lines (see Figs. 45 and 55). The amylopectins therefore behave as statistical models. As pointed out previously all of the statistical models have the same broad distribution.

Witnaur, et. al. (12), observed from ultracentrifuge studies that an amylopectin fraction behaved as other high polymers of "similar" nature. They concluded that the ratio \bar{M}_w/\bar{M}_n would be 1.5 to 2 for this fraction as in A-B condensation polymers. If this factor is applied to all their fractions as suggested by them, only a small difference in \bar{M}_w and \bar{M}_n is obtained. They also observed that Potter and Hassid's (23) \bar{M}_n values (1 to 6 million) for amylopectins were similar to their \bar{M}_w value of approximately 10 million. For high molecular weights of A-B condensation polymers, \bar{M}_w is essentially equal to $2\bar{M}_n$. In Fig. 60, \bar{x}_n was obtained from $\bar{M}_w = 2\bar{M}_n$. It can be seen from these hydrolysis curves that the amylopectins and glycogen do not behave as A-B condensation polymers. Potter and Hassid's high \bar{M}_n is probably due to high concentration effects in their osmotic pressure measurements.

The 14th day sweet corn amylopectin and the "mature" glycogen behave similarly toward acid hydrolysis. The 14th day sweet corn was at the edible stage. Both samples were therefore obtained at approximately the same stage of maturity since in sweet corn terminology "mature" refers to the edible stage. From the initial and extrapolated values of glycogen and 14th day sweet corn amylopectin we have:

$$\text{Glycogen: } \frac{\bar{M}_w \text{ (original)}}{\bar{M}_w \text{ (extrapolated)}} = \frac{18.5 \times 10^6}{4.58 \times 10^6} = 4.04$$

$$\text{14th day sweet corn amylopectin: } \frac{\bar{M}_w \text{ (original)}}{\bar{M}_w \text{ (extrapolated)}} = \frac{15.8 \times 10^6}{3.93 \times 10^6} = 4.02$$

Thus exactly the same amount of aggregation exists in both cases. If one considers that this glycogen is converted into the amylopectin and amylose as described previously, the predicted per cent amylose from the initial and from the extrapolated weight-average molecular weights is 14.3% and 14.6% amylose, respectively. It was found from iodine titrations that 14th day sweet starch has 18.3% amylose. Both the glycogen and the amylopectin behave as statistical models after these aggregates of four have been broken up. The above evidence shows strongly that glycogen may be a precursor to starch.

Consider that glycogen consists of 1,3-, 1,4-, and 1,6-linked glucose units and that $p_3 = p_6$. It can then be assumed that the \bar{X}_n obtained from the extrapolated value is correct

and that the initial hydrolysis is due to the breaking of "weaker" 1,3-linkages. The \bar{M}_w predicted from this \bar{X}_n assuming an equal amount of 1,3- and 1,6-linkages before hydrolysis is 4.68×10^6 . This is much smaller than the original 18.5×10^6 . It was found that if glycogen is refluxed 24 hours in n-amyl alcohol, the resulting molecular weight is 7.8×10^6 . This is much greater than the extrapolated molecular weight of 4.58×10^6 . Therefore it is concluded that the initial hydrolysis is not due to the breaking up of physical aggregates or the presence of 1,3- or 1,2-linkages in the polymer, but it is due to the chemical bonding of four glycogen molecules to a protein molecule or some other point source.

In Table 19 the Z-average root mean square radius of gyration for different amylopectins and glycogen illustrates the following:

1. When the molecular weights are comparable, it is found that the greater the per cent branching the greater is the asymmetry. The debranching enzyme would encounter more steric hindrance the higher the per cent branching. This result therefore agrees with the postulate made previously regarding the mechanisms of the debranching enzyme.
2. The asymmetry of the 20th day sweet amylopectin is greater than the 14th day sweet amylopectin even though both have essentially the same per cent branching. Because of the short branch lengths, the debranching enzyme would be sterically

Table 19. Comparison of radii of gyration
with per cent branching

Amylopectin	% branching	$\bar{M}_w \times 10^{-6}$	$(\bar{R}_z^2)^{\frac{1}{2}}$
13th day waxy	5.8	168	4680A
14th day dent	6.8	168	3194A
Mature sweet	6.1	76	3160A
13th day waxy	5.8	83.5	2786A
Mature dent (17)	4.0	80	2050A
20th day sweet	8.2	43	2456A
13th day waxy	5.8	45	2350A
Mature dent	4.0	43	2350A
14th day dent (in \underline{N} KOH)	6.8	28.6	2344A
14th day dent (in H_2O)	6.8	24.4	2145A
14th day sweet	8.0	15.8	1893A
13th day waxy	5.8	21.5	1640A
13th day waxy	5.8	15.8	1460A
Potato fraction (12)	5.0	23	1370A
Potato (12)	5.0	13.6	1010A
Sweet corn glycogen	8.67	18.5	580A
20th day sweet	8.2	6.0	1149A
13th day waxy	5.8	6.02	1003A
Mature dent	4.0	6.50	912A
Potato fraction (12)	5.0	7	820A
20th day sweet	8.2	2.80	863A
Mature dent	4.0	2.23	660A
Sweet corn glycogen	8.67	3.50	434A
20th day sweet	8.2	1.22	613A
14th day sweet	8.0	1.48	600A
13th day waxy	5.8	1.48	585A
Sweet corn glycogen	8.67	2.53	416A

hindered as previously postulated. Therefore a greater asymmetry is obtained with a greater amount of debranching since 14th day sweet corn starch has 18% amylose and 20th day sweet corn starch has 23% amylose. In the case of mature dent and potato the per cent branching is probably low enough so that steric hindrance is no great factor.

3. Initially waxy has a larger radius than 13th day dent amylopectin even though this dent starch has 6.8% branching and 18% amylose. Some of the longer non-reducing ends in waxy maize starch may act as acceptors in the debranching mechanism giving greater asymmetry. Potato amylopectin may have been equilibrated more with the debranching enzyme in order to give it less asymmetry.

4. In 14th day dent amylopectin the radius of gyration is greater in N KOH than in H_2O . This may be attributed to a spreading out of the molecule due to the adhering negative hydroxyl groups. The exact opposite was found for the highly phosphated potato amylopectin. Witnauer, *et. al.* (12), found that the decrease in radius for the potato amylopectin in NaOH was similar to that found for the amylopectin in a salt solution.

Again, as in previous sections, the results have been interpreted assuming that glycogen is a precursor to starch. It is considered that the results are in good agreement with this theory.

III. VALIDITY OF LIGHT SCATTERING RESULTS

A. Introduction

Since light scattering results are actually the basis for most of the conclusions made in this thesis, then substantiating these results by other chemical or physical methods gives added force to the conclusions.

There are two major factors that may give erroneous light scattering results:

1. Physical aggregation due to hydrogen bonding. These aggregates may either give a broad distribution such as that found in the statistical model or they may present as a mixture of high molecular weights and low molecular weights.
2. The presence of a small amount of extremely large particles. These particles may be dust, partially undissolved granules or the like. They are differentiated from the above by being present in only a minute amount and being much larger than the amylopectin molecules.

Aggregation involving primary bonds (chemical aggregation) will not be considered here because the dispersion of such aggregates would involve the oxidation or hydrolysis of these bonds.

The points used to prove that these two factors are not present are the following:

- a. **Consistency of results.** The method for a given dispersing agent may be varied with regard to time and temperature. Undissolved granules would be most affected by this and would vary in size depending on the conditions. Consistency of the results would rule out foreign particles such as dust since it would be extremely improbable that one would obtain the same size dust particles in every experiment.
- b. **Variation in dispersing agent.** The disaggregation of some particles may not occur as fast or not at all with some dispersing agents.
- c. **Variation in solvent used in light scattering measurements.** Some solvents may cause aggregation even though the particles were initially dispersed.
- d. **Obtaining the weight average molecular weight by some other method.**
- e. **Centrifugation studies.** Extremely large particles would be immediately centrifuged out of the solution, and a large change in the molecular weight will result.
- f. **Acid hydrolysis experiments.** If the branched molecules are present as entanglements, then the acid hydrolysis of these molecules and their entangled branches should result in a drastic reduction of their molecular weight. The acid hydrolysis experiments were described previously and therefore will be omitted here. The fact that the molecules behave as a statistical polymer strongly indicates that entanglements are not present.

g. Examination of the structure of the amylopectin molecule by chemical methods in order to determine whether a statistical model exists.

The above points will now be discussed with regard to the literature and present experimental findings.

a. Consistency of results. The literature values of \bar{M}_w and \bar{M}_n reviewed earlier are in agreement with the statistical model.

Also light scattering results on potato amylopectin measured at 75°C and 25°C gave the same result (12).

b. Variation in dispersing agent. Amylopectin initially separated from the amylose with a heated aqueous solution and Pentasol was heated for two hours in 0.5N NaOH in the absence of oxygen. The light scattering results agreed within 10% (12).

c. Variation in solvent. Formamide, water, N KOH, anhydrous ethylenediamine, ethylenediamine hydrate give essentially the same molecular weights for corn amylopectin (17). A high molecular weight for glycogen was obtained using 15% MgCl₂ (14).

d. \bar{M}_w from other methods. A molecular weight of 3 to 8 million was obtained by using the ultracentrifuge (16). This is much lower than the light scattering results. The amylopectin may have been hydrolyzed. A \bar{M}_w of 14×10^6 was obtained for glycogen from sedimentation and diffusion constants (32).

e. Centrifugation studies. Fractions obtained by centrifuging glycogen solutions had molecular weights from 10 to 159 million (14). The glycogen had an original \bar{M}_w of 70 million. Glycogen therefore has a broad distribution.

g. Examination of structure. Enzymatic hydrolysis studies on amylopectin and glycogen were reviewed previously. They showed that the distribution of branches occurs as in the statistical model. Isomaltotriose was found to be present in glycogen showing that two branch points can exist next to each other (100). This indicates lack of or at least only a small amount of steric hindrance in the branching mechanism. The result would be the formation of a broad distribution of molecules. The change in \bar{M}_w in the β -amylolysis of glycogen or amylopectin agrees with the yield of maltose (14, 17). Therefore the removal of the long exterior chains does not destroy any aggregates that may be present.

B. Experimental

1. Lithium bromide dispersion of granules

The method for dispersing with LiBr was described previously. It was concluded at that time that the constant dissymmetry and 90° intensity readings indicated that the starch had been completely dispersed. Four different dispersions of the 13th day waxy maize sample showed no essential difference

in the extrapolated molecular weights. These samples were:

Sample	LiBr conc.	Refluxing temperature	Hours refluxed
I	6 <u>M</u>	113°C	9
II	6 <u>M</u>	113°C	2
III	8 <u>M</u>	125°C	6
IV	8 <u>M</u>	125°C	6

2. Pentasol dispersion of granules

The 14th day dent amylopectin was dispersed by refluxing in unbuffered and buffered Pentasol solutions according to Schoch's method using a nitrogen atmosphere. The nitrogen was passed through a vanadyl sulfate train (101). Despite this precaution, it was found that the amyl alcohols were oxidized and the solution became acid. The acid hydrolysis was followed by using consecutive experiments. The \bar{M}_w for the amylopectin after 3 hours in unbuffered Pentasol is comparable to that of the \bar{M}_w after 20 hours in buffered Pentasol (see Table 31 and Figs. 64-71 in Appendix).

3. Molecular weights using various solvents

The \bar{M}_w of the amylopectins obtained from the starch samples dispersed with Pentasol are listed in Table 20. Both N KOH and H₂O were used as solvents. The constant $K = 3.305 \times 10^{-7}$ used in obtaining the \bar{M}_w in N KOH solutions was de-

rived from values by Stacy and Foster (17), and Witnauer, et. al. (12), for $dn/dc = 0.146$ and from $n_0 = 1.35$. The amylopectins were in the N KOH for approximately four to five hours before light scattering measurements were made. The molecular weights obtained in alkali are higher than those in H_2O . The discrepancy is probably due to an error in n_0 for N KOH. However, the results show that if aggregates are present, they are not broken up by N KOH.

The \bar{M}_w of the 13th day dent amylopectin obtained by refluxing 20 hours in buffered Pentasol was also studied using 6 M LiBr as a solvent. No detectable change in the \bar{M}_w was observed.

Since N KOH was used as a solvent, the degradation of the amylopectin in N KOH was studied under different conditions (8). The results are listed in Tables 20 and 21.

4. Fractionation of amylopectin by centrifugation

In order to determine whether the large molecular weights in light scattering are due to a small number of extremely large particles or to a broad distribution of molecules, a dilute solution of amylopectin was fractionated by centrifugation. A sample of potato amylopectin was dispersed in N KOH, neutralized with N acetic acid, and centrifuged for various lengths of time at 20,000 g in a Servall centrifuge. The upper $3/4$ portion of solution was then pipetted from the

Table 20. The effect of oxygen upon amylopectin dispersed in one normal alkali for 54 days

Sample	Conditions	Z	$\bar{M}wP_{90^\circ}$
Waxy maize starch	air, room temp.	1.07	0.15×10^6
Potato amylopectin	air, room temp.	1.13	0.19×10^6
Potato amylopectin	oxygen, r. t.	1.02	0.14×10^6
Waxy maize starch	oxygen, r. t.	1.10	0.95×10^6
Waxy maize starch	nitrogen 4°	5.2	8.5×10^6
Waxy maize starch	nitrogen, r. t.	4.8	9.5×10^6

Table 21. The effect of oxygen upon potato amylopectin dispersed in one normal alkali

Time of standing (hours)	Sample 1 (conc., 6.06×10^{-4})		Sample 2 (conc., 6.72×10^{-4})	
	Z	$\bar{M}wP_{90^\circ}$	Z	$\bar{M}wP_{90^\circ}$
5	5.8	10.3×10^6	3.41	6.8×10^6
29	5.6	9.9	3.16	6.2
53	4.7	7.5	3.0	5.55
77	3.8	6.5	2.22	4.36
101	2.5	5.6	2.0	3.45
149	2.2	4.6	1.81	3.13

centrifuge tube. The concentration, determined by optical rotation, dropped from 0.056% to 0.035% in three hours; the corresponding drop in turbidity was from 0.0129 to 0.0089 with the dissymmetry remaining nearly constant. The estimated drop in molecular weight, using a polydisperse coil as a model, is only from about 80 to 70 million. Two runs gave the same result within experimental error.

The experiment above was repeated using waxy maize starch. It was found that the concentration decreased sharply during the first $1\frac{1}{2}$ hours (0.06 down to 0.001%) and then leveled off. Molecular weights remained in the millions. Thus even after removal of 98% of the material by centrifugation, particles (molecules) remaining in the supernatant are still very large.

These centrifugation studies rather conclusively demonstrate the absence of undispersed entanglements of extremely high particle weight, and convincingly demonstrate that this is not the cause of the discrepancy between number- and weight- average molecular weights. The molecules therefore have a broad distribution as predicted by the statistical model.

5. Sedimentation constants and the corresponding molecular weights

The sedimentation constants were obtained for the 13th day waxy I and III (both dispersed with LiBr) and 14th day

dent amylopectin (dispersed 20 hours in buffered Pentasol). In the immature waxy, it was found that there were two components (see Fig. 8). The sedimentation coefficients for the two components of immature waxy and for immature dent amylopectin are listed in Tables 32 and 33 in the Appendix. The extrapolation of these coefficients to zero concentration is shown in Figs. 75 and 76 in the Appendix.

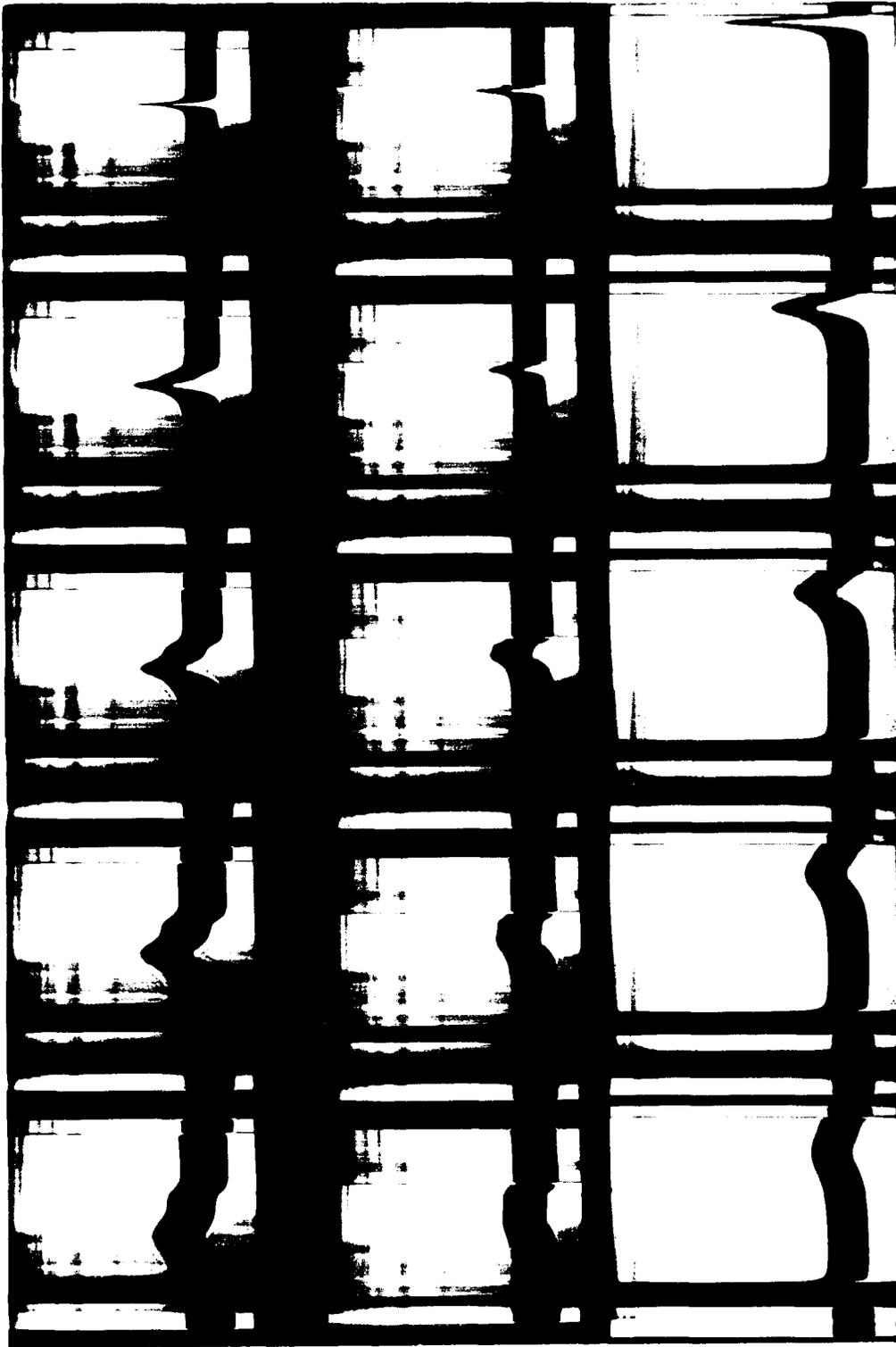
In calculating the molecular weight, Bridgman's values (32) on glycogen for the partial specific volume and diffusion coefficients were used. The diffusion coefficient was assumed to be equal to 1.1×10^{-7} for the slow component. For the fast component and for the dent amylopectin, the diffusion coefficient was assumed to be equal to 0.5×10^{-7} . The partial specific volume was considered as being equal to 0.65 in all cases. Both the immature dent and the waxy have an \bar{M}_w of 168×10^6 from light scattering. The fast component and the dent amylopectin have an \bar{M} (S,D) between 80×10^6 and 120×10^6 . The results therefore show that the light scattering \bar{M}_w has the correct order of magnitude. The slow component seems to have a \bar{M} (S,D) of 1×10^6 to 6×10^6 .

C. Discussion of Results

The points made previously will now be discussed in light of the above results.

Fig. 8. Sedimentation picture showing two components in immature waxy maize and one in immature dent amylopectin.

The sedimenting pictures read from right to left. The three frames from top to bottom were taken from studies on waxy maize I starch (.50% conc.), waxy maize I starch (.200% conc.), and 13th day dent amylopectin (.529% conc.).



- a. Consistency of results. Waxy maize was subjected to a variation in temperature and time in dispersing with 6 and 8 M LiBr. The resulting molecular weights were the same.
- b. Variation in dispersing agents. The immature dent amylopectin dispersed with Pentasol had a molecular weight of the same order of magnitude as the immature waxy dispersed with LiBr. Also the molecular weight for mature dent amylopectin dispersed with LiBr has the same order of magnitude as that found by Stacy and Foster (17). Since in all cases the \bar{M}_w is large, then any aggregates if present are not dispersed by either agent.
- c. Variation in solvent. Water, 1 N KOH, and 6 M LiBr, amylopectin solutions all give the same order of magnitude in molecular weight.
- d. Molecular weight by other methods. The molecular weight obtained by sedimentation and diffusion measurements is slightly smaller but has the same order of magnitude as that obtained from light scattering.
- e. Centrifugation studies. Fractions obtained by centrifugation indicated that amylopectin has a broad distribution and that its large molecular weight is not due to the presence of a small amount of extremely large particles.

The above results together with those in the literature prove that no physical aggregation exists in the amylopectin molecules. They also show that starch does not exist as an

infinite molecule in the starch granule. If this were true, then the molecular weight would depend on the method of dispersion. However, chemical aggregates may exist as indicated previously.

IV. DISCUSSION AND SUMMARY

Results of β -amylolysis studies in the literature show that glycogen does not behave as a statistical model but it has longer exterior branches. A debranching enzyme called iso-amylase has been found in Brewer's yeast (41). It is therefore proposed that these discrepancies occur because the glycogens have been subjected to a debranching enzyme. The glycogen having the largest discrepancy (Baker's yeast glycogen) also deviates the most from the spherical shape (47). This indicates debranching has occurred.

The amylopectins differ more from the statistical model than the glycogens with respect to β -amylolysis limits. Also a review of the literature indicates that glycogen may be a precursor to starch. A debranching mechanism is therefore proposed for the formation of amylose and amylopectin from glycogen.

The per cent branching of immature and mature sweet corn amylopectins is only slightly less than the per cent branching of their corresponding glycogens. A greater asymmetry is found for these highly branched amylopectins than for the glycogens or lesser branched amylopectins. Therefore these properties suggest that inner more available branches are being removed instead of exterior branches. These properties correspond to those predicted by the average statistical molecule which was developed previously. One would expect

that the β -amylolysis limit on sweet corn amylopectin would be closer to the statistical model as found with the highly branched malted barley amylopectin.

The results on diurnal variations in yield and in per cent amylose indicate that amylose and amylopectin are not produced at a different time of the day but they are produced at the same time as proposed. A review of the literature shows that a glycogen type polysaccharide is first formed from sucrose followed by the production of starch at night. The above experiment therefore agrees with previous experiments.

The potato plants exposed to light at night, shade, and regular diurnal variations showed no appreciable difference in amylose. This also indicates that amylose is not produced during a separate time of the day.

Less amylose was found in the starches obtained from the bagged samples of dent and sweet corn ears. Amylose was found in the starch obtained from the immature bagged samples of waxy. These results are similar to those found by Badenhuizen for immature Scilla leaves and waxy endosperm (42). In sweet and dent an increase in the number of receptor groups would lower the molecular weight of the amylose and therefore its iodine binding capacity. This may account for the above results. In waxy the increase in receptor groups such as maltose would make it possible for the debranched chains to form amylose instead of adding to the non-reducing ends of the amylopectin.

The per cent branching of the glycogen decreases with maturity. The per cent branching of the amylopectin was also found to decrease with maturity. Both are therefore subjected to the same change in relative activities of the branching and phosphorylase enzymes. It was found that the f. no. of mature and 20th day sweet corn amyloses are 2.79 and 4.17, respectively. Their approximate degrees of polymerization estimated from other studies (102) are:

$\bar{X}_n = 270$ for mature amylose and

$\bar{X}_n = 140$ for 20th day amylose.

From Kerr (102) the f. no. of mature dent amylose is 1.43 and its corresponding \bar{X}_n is 455. This would indicate an increase in the \bar{X}_n of amylose with a decrease in per cent branching of the glycogen or amylopectin for a particular plant. If glycogen acts as a precursor, shorter chains on the glycogen should give shorter amylose chains.

Ultracentrifuge data (see Fig. 8) indicates that there are two components in immature waxy maize starch but not in immature dent amylopectin. Only one component was found in mature waxy maize starch. This smaller component in immature waxy starch may be due to the presence of either molecules that have not been linked with a protein molecule or amylose or amylopectin impurities from the pericarp or ovary.

From acid hydrolysis experiments rate constants were determined from the linear plot of $1/\bar{X}_n$ versus time. These

\bar{X}_n were obtained from f. no., from \bar{M}_w assuming a statistical model, from \bar{M}_w assuming less random models, and from $\bar{M}_w / 4$ assuming that after the statistical polymer is produced, it becomes chemically bonded to protein molecules. It was seen that the rate constants obtained from the f. no. decrease with per cent branching. It was concluded that this is due to the stopping of the alkaline degradation of the reducing chain by a branch point.

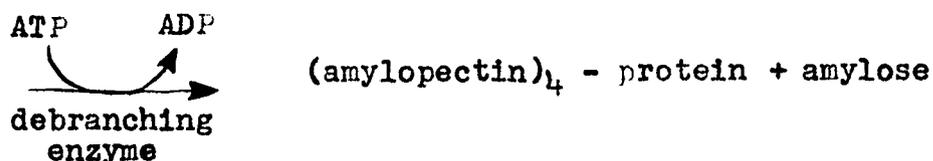
The 13th day and 20th day sweet amylopectins and the corresponding glycogen all have approximately the same high degree of branching. Because of this one would expect the ratio of their hydrolysis rates obtained from the f. no. and \bar{M}_w to be the same. It was found that 14th day sweet amylopectin and glycogen behave as if their molecules existed initially in aggregates of four. The ratio of the rates for the 20th day sweet amylopectin obtained from the f. no. and $\bar{M}_w / 4$ agree with those obtained from 13th day sweet amylopectin and glycogen after their chemical aggregates have broken up. Therefore it is concluded that 20th day sweet amylopectin behaves as if four molecules are chemically bound around a protein molecule.

In mature dent amylopectin the f. no. has been correlated with the \bar{X}_n obtained from osmotic pressure (13). These \bar{X}_n can therefore be considered as correct while those from the higher branched amylopectins in sweet and immature waxy cannot. It was found that the rate from the f. no. did correspond

to an absolute rate obtained by using higher acid concentrations. Because of this the rates obtained from \bar{M}_w and the f. no. should be the same. They are not the same. If the rate is obtained from $\bar{M}_w / 4$ instead of \bar{M}_w they are almost the same. Dent amylopectin therefore behaves as if its statistically produced molecules are chemically bound to a protein molecule.

The amylopectin and glycogen molecules also behave as if produced from less random models. From the above discussion one can assume that the \bar{X}_n obtained from the f. no. of mature dent amylopectin is correct and that the \bar{X}_n from the f. no. of glycogen is a little high. If these assumptions are made, then it can be shown that the actual model for the amylopectin and glycogen molecules is between the statistical and less random model I.

The glycogen and all the amylopectins behave as if chemically bound to a protein molecule. Since the 13th day sweet amylopectin behaves as if it is produced from glycogen, one can conclude that the following sequence of reactions occurs in the production of all corn starches:



If any number of molecules can be bound in chemical aggregates, the statistical model will not hold because the \bar{M}_w will be shifted in favor of the larger aggregates. It can be shown that most of the molecules which influence the weight average molecular weight in the statistical model have approximately the same radius. Therefore it is concluded that the molecules that affect \bar{M}_w the most exist as exactly four molecules chemically aggregated together.

The statistical distribution will become narrower as a result of this chemical aggregation. Protein molecules which initially have large glycogen molecules attached to them will tend to bind themselves to smaller glycogen molecules because of the steric hindrance of the large glycogen molecules. The narrower distribution found by Witnauer, *et. al.* (12), can therefore be due to both the above factor and to an incomplete alcohol fractionation. It should be noted that the glycogen molecules will remain spherical if four molecules are chemically linked in this manner. The existence of chemical complexes between synthetic or natural glycogens and protein has been found (103).

The above reasonings as to why these chemical aggregates should be exactly four and why they act as statistical molecules are not on solid ground. It is believed, however, that a mathematical explanation for this can be derived using the following definition of \bar{M}_w :

$$\bar{M}_w = \frac{\sum M_1 w_1}{\sum w_1} = \frac{\sum M_1 n_1 v_1}{\sum n_1 v_1}$$

where M_1 , w_1 , n_1 , and v_1 are the molecular weight, total amount (in weight), number of moles, and molecular volume of the i^{th} species. This definition assumes the density does not depend on the size of the molecule and that it does not vary with the distance from the center of the molecule to the periphery.

All of the amylopectins and the glycogen were hydrolyzed to the same extent yet only the immature sweet and glycogen aggregates were broken up. It may be that bonds which are more resistant to acid hydrolysis are present in the stable aggregates.

V. CONCLUSIONS

1. Starch is synthesized from glycogen.
2. A debranching enzyme removes part of the more available exterior and interior branches on glycogen and combines them together to form amylose. A source of energy, perhaps ATP, is required.
3. The greater the degree of branching of the parent glycogen, the greater the asymmetry of the amylopectin.
4. The degree of branching will be less on the amylopectin than on the parent glycogen. The greater the degree of branching, the smaller the difference. Therefore a high degree of branching is not restricted to glycogen, but the degree of branching of the amylopectin depends on the degree of branching of its parent glycogen.
5. The greater the degree of branching of the parent glycogen, the lower the β -amylolysis limit for the amylopectin.
6. The greater the degree of branching of the amylopectin, the lower the molecular weight of the amylose in corn starch.
7. Amylose and amylopectin are produced at the same time of the day in the starch synthesizing cells.
8. It is postulated that waxy maize endosperm does not normally produce amylose because of the lower degree of branching in its starch and a lower activity of the debranching en-

zyme. Amylose can be produced by changing the conditions in the cell. It appears that this production of amylose is brought about by increasing the number of receptor groups such as maltose.

9. The \bar{X}_n of waxy starch and the parent glycogen of dent starch remains essentially constant during plant growth, the variation in \bar{M}_w being due to the variation in degree of branching.

10. The \bar{M}_w and \bar{X}_n for sweet corn amylopectin increases with maturity. It is postulated that the high degree of branching in the immature glycogen sterically hinders the phosphorylase enzyme giving a limiting molecular weight for the resulting amylopectin and glycogen.

11. A comparison of the \bar{X}_n for the amylopectins shows that waxy > dent > sweet. This agrees with the phosphorylase activities when amylopectin is the primer (99).

12. The structure of amylopectin and glycogen is between the statistical model and the less random model I (7). These statistical molecules seem to be chemically linked to a protein molecule. The chemical aggregate seems to exist in groups of four.

13. Sedimentation studies show that two components are present in immature waxy maize starch. The nature of the slower component is not known. However, it disappears during maturation and does not appreciably influence the light scattering results. Its molecular weight is in the millions.

14. Centrifugation studies, sedimentation measurements, variation in solvent, and variation in method and dispersing agent show that no physical aggregates are present in amylopectin solutions. The high \bar{M}_w is due to the broad distribution and to chemical aggregates, each aggregate existing as four molecules tied together, perhaps to a protein nucleus.

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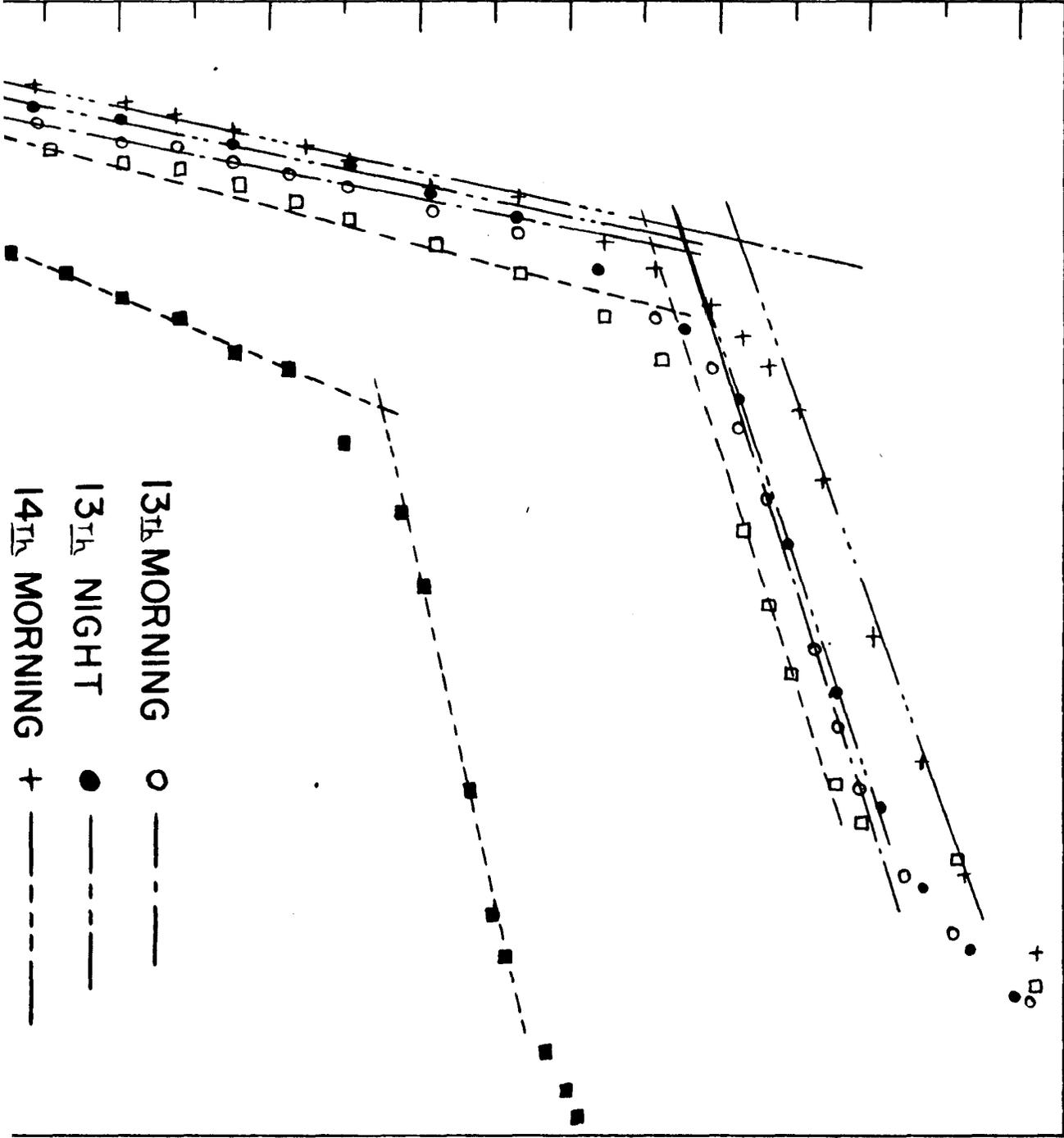
VIII. VITA

Stig Robert Erlander was born in Minneapolis, Minnesota, May 24, 1928, the second of three children born to Elvira (Anderson) and Erland Hartvig Erlander. After graduating from West High School in Minneapolis, he enlisted in the U. S. Navy. He received training in radio and automatic telephone and later was a draftsman in the Seabees while stationed at Adak, Alaska. After being discharged in 1948 he entered the University of Minnesota and in 1951 received the degree of B.A. with a major in Chemistry and a minor in Mathematics. After graduating, he was recalled into the Navy as a draftsman. He attended graduate night classes at Drexel Institute of Technology while stationed in Philadelphia, Pa. After being discharged in 1952 he entered Iowa State College where he held teaching and research assistantships. His study on starch was under the direction of Dr. J. F. Foster until 1954 and for the remaining period was under the direction of Dr. Dexter French. In 1952 he was married to Leatrice Gloria Kurtyka. They have one child, Stig Paul, who was born August 9, 1955.

IX. APPENDIX

PERCENT OF IODINE BOUND / 100. MILLIGRAM SAMPLE

1.5 2.0 2.5 3.0 3.5 4.0 4.5



13TH MORNING ○ — — — —
13TH NIGHT ● — — — —
14TH MORNING + — — — —

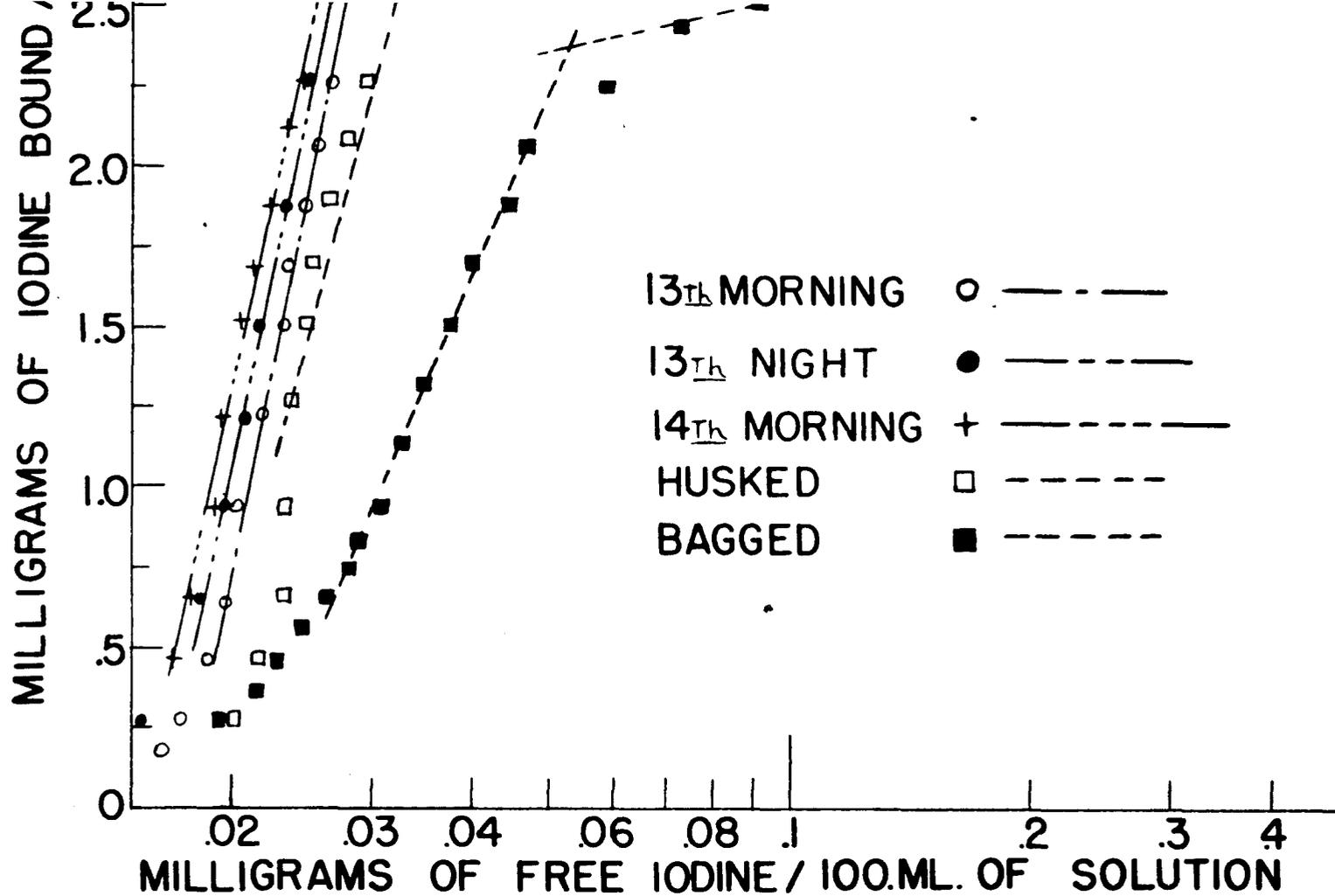
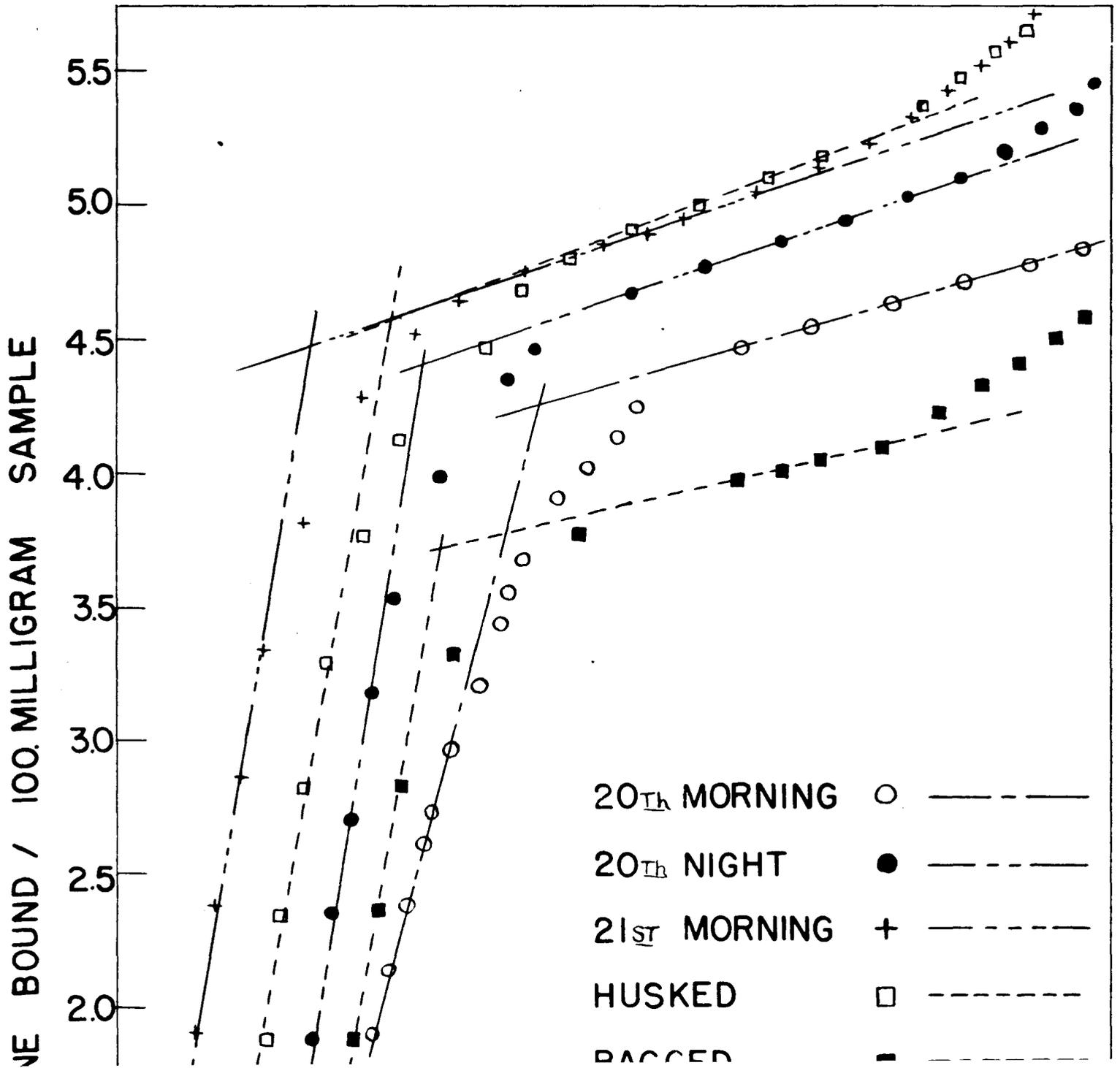


Fig. 9. Iodine titration curves for 13th and 14th day dent corn starch samples.



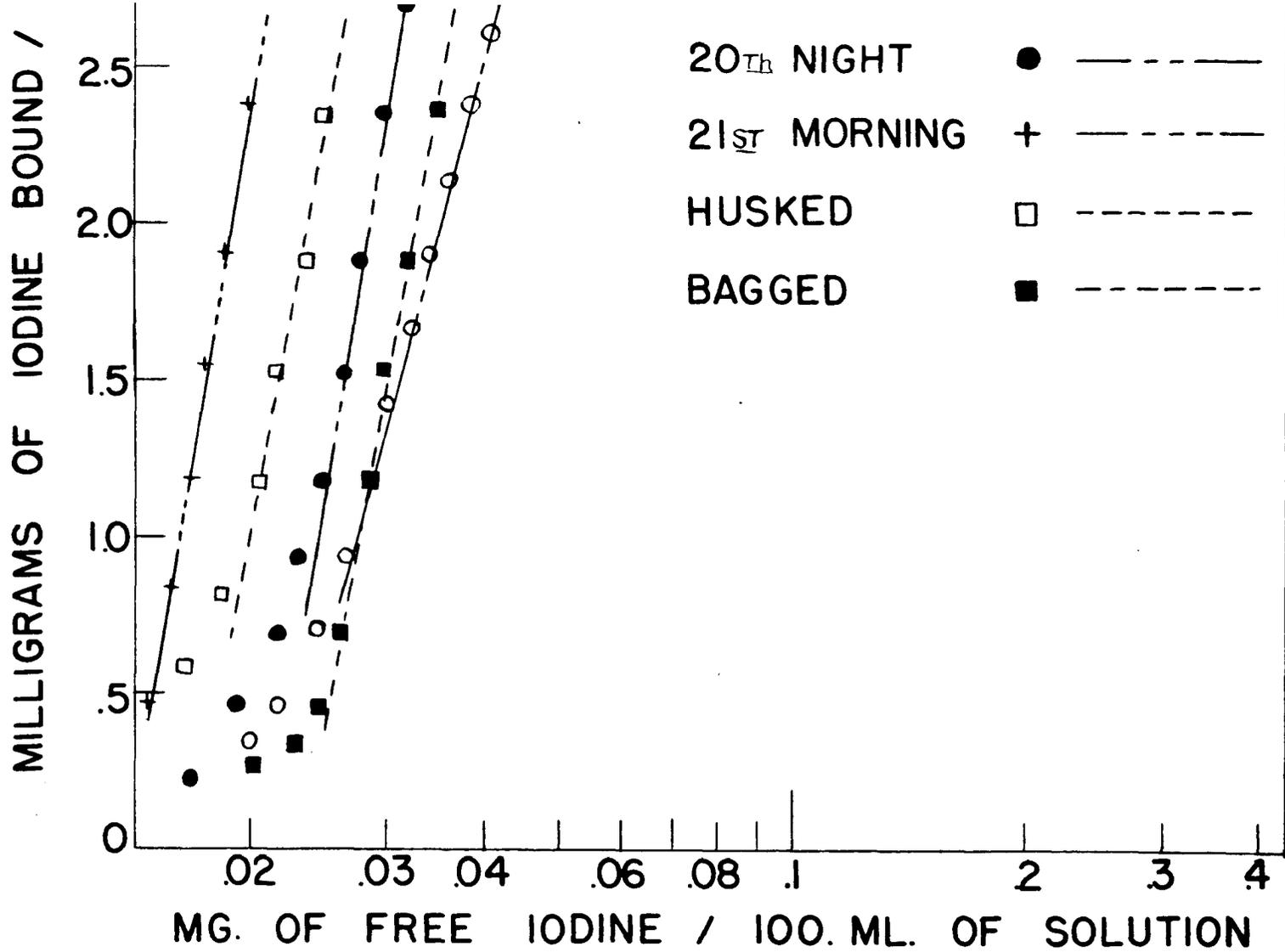
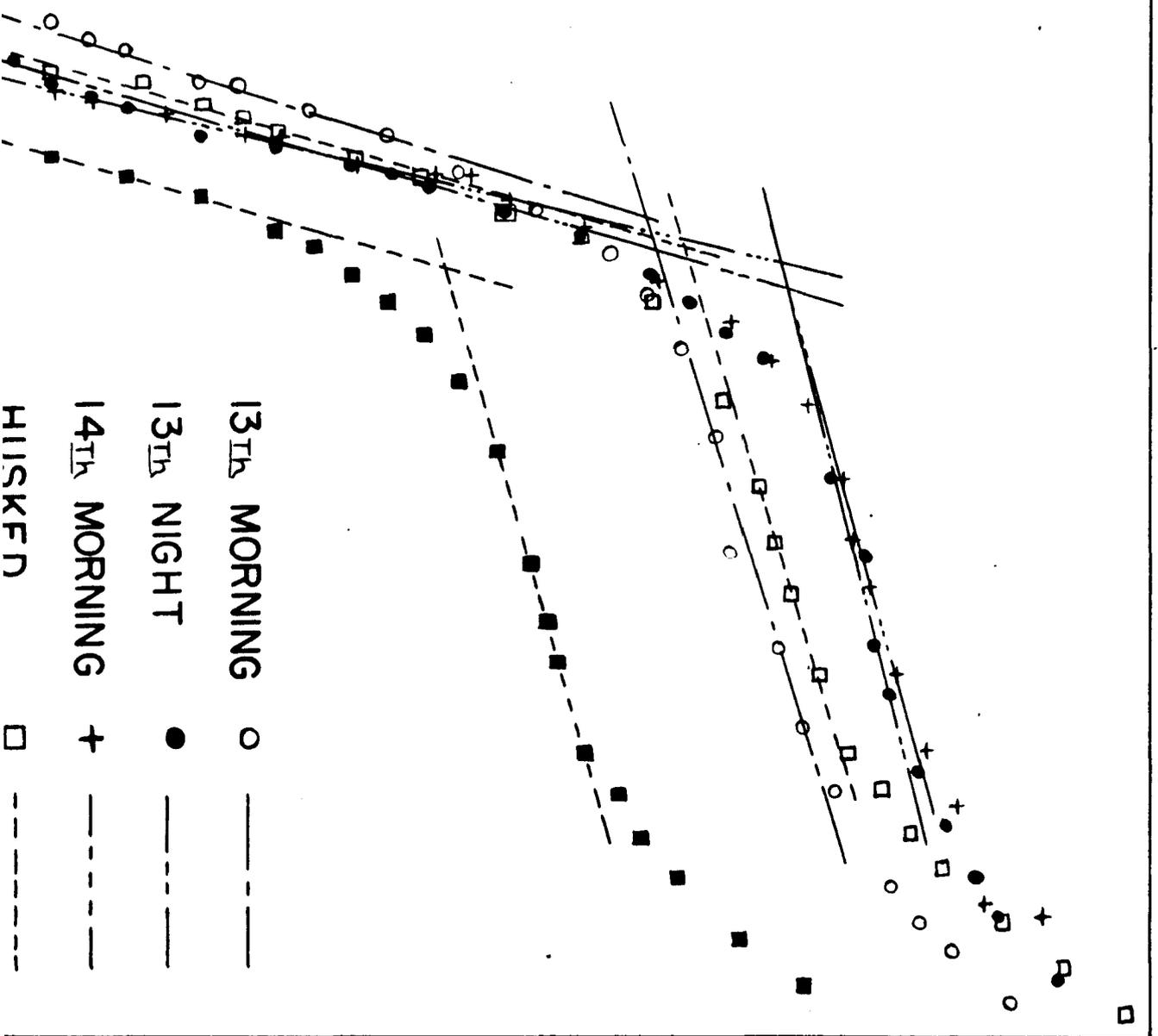


Fig. 10. Iodine titration curves for 20th and 21st day samples of dent corn starch.

OF IODINE BOUND / 100. MILLIGRAM SAMPLE

1.5 2.0 2.5 3.0 3.5 4.0 4.5



13TH MORNING ○ —
13TH NIGHT ● - - -
14TH MORNING + - · - · -
HUSKED □ - - -

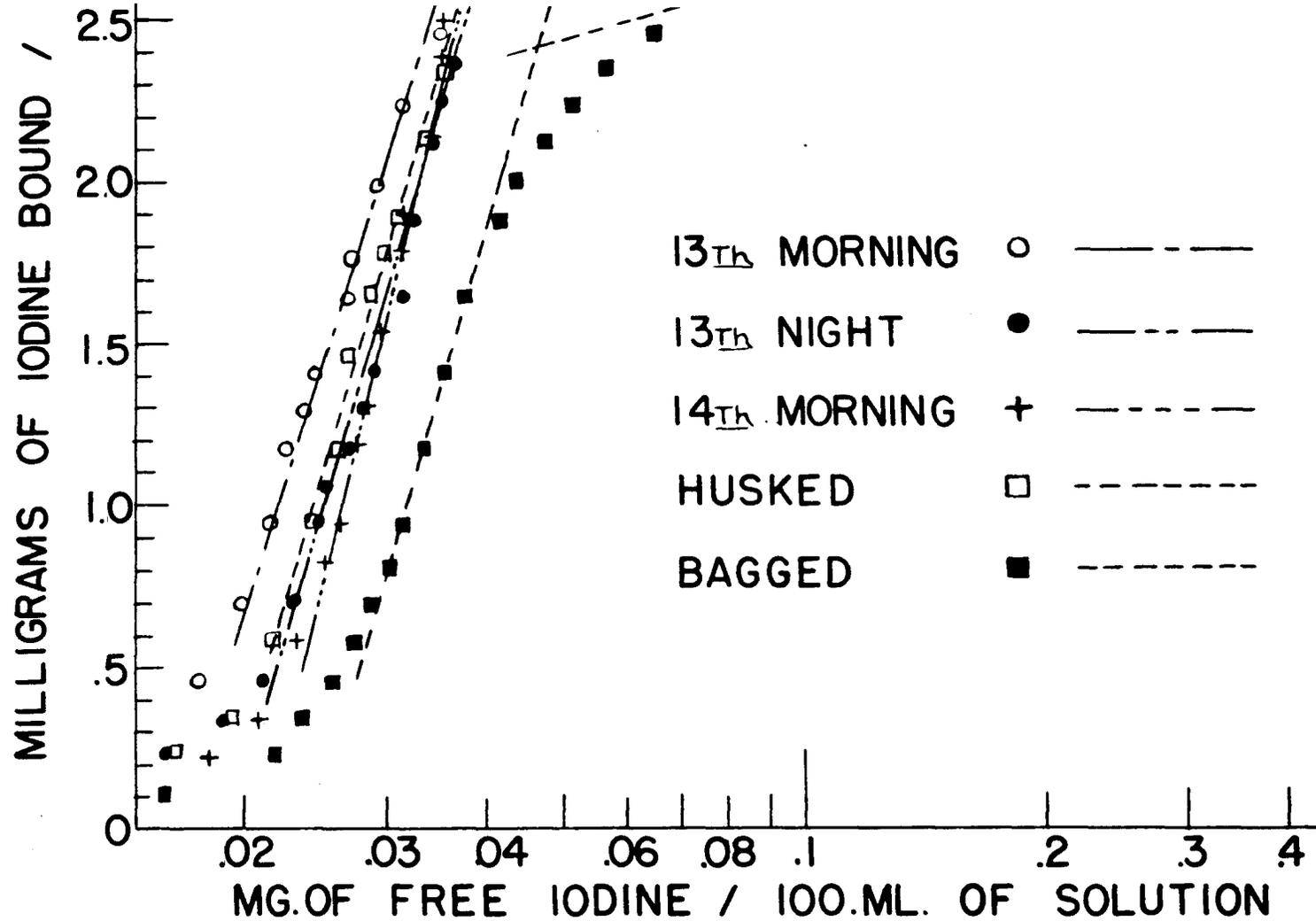
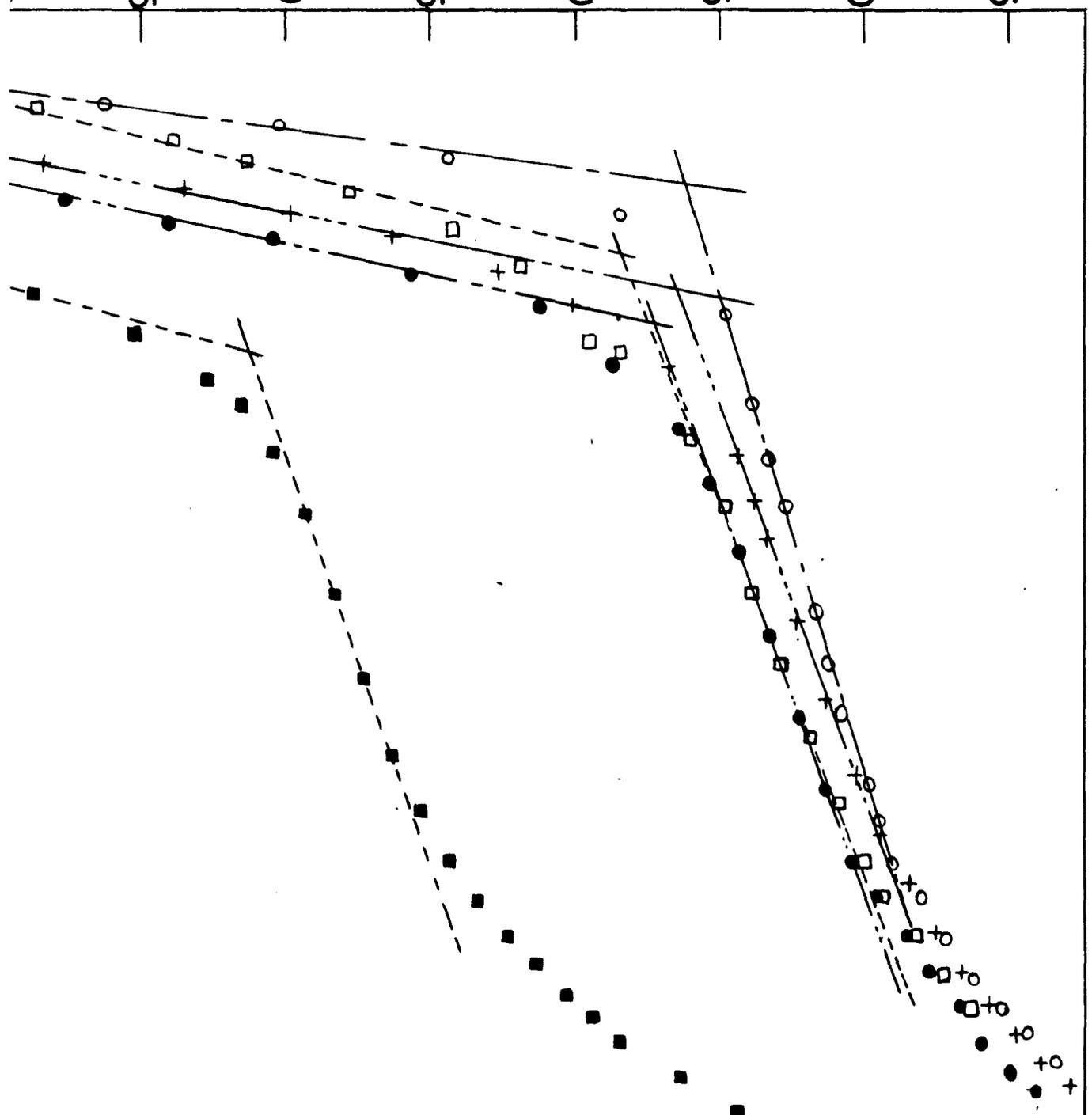


Fig. 11. Iodine titration curves for 13th and 14th day sweet corn starch samples.

BOUND / 100. MILLIGRAM SAMPLE

2.5 3.0 3.5 4.0 4.5 5.0 5.5



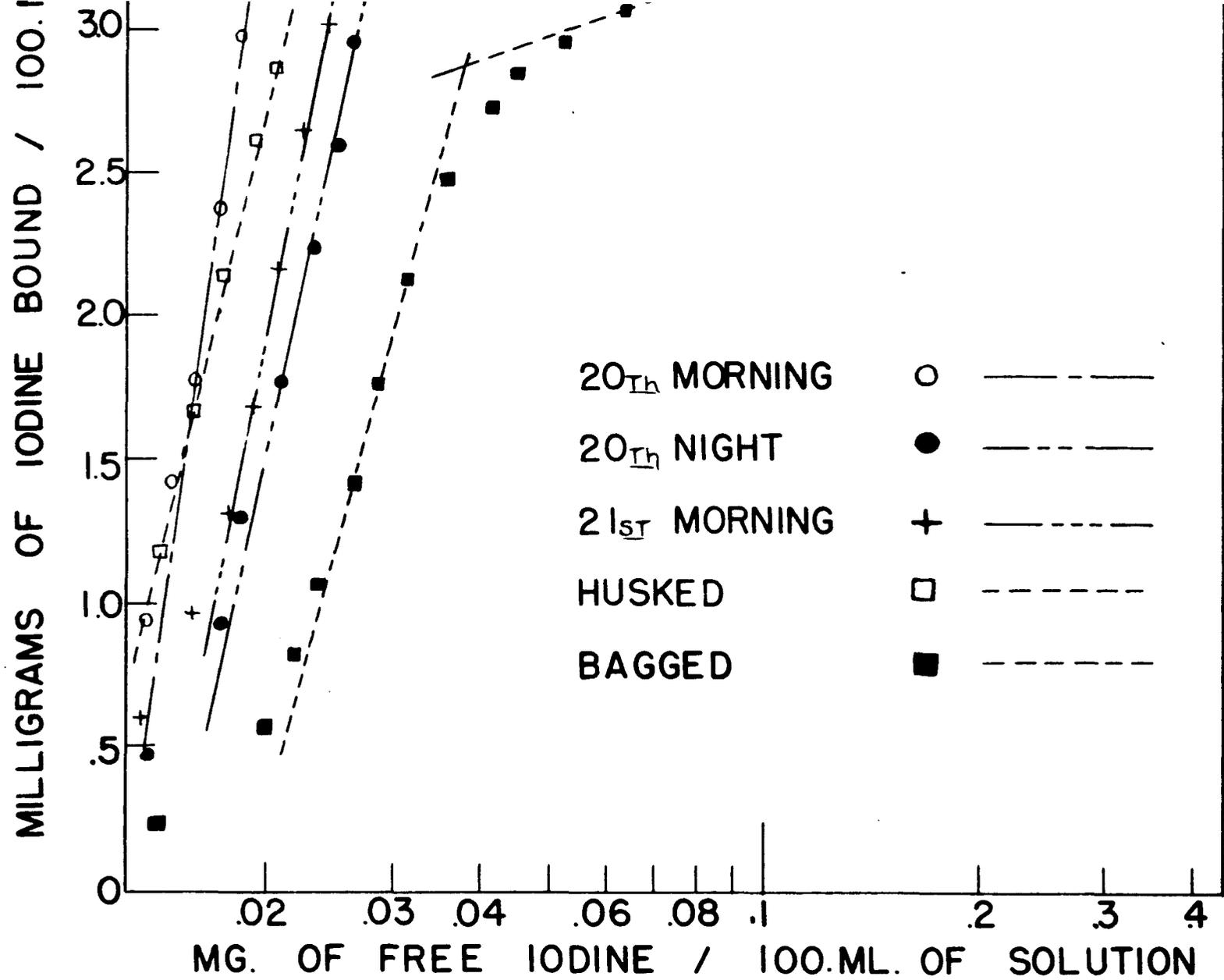
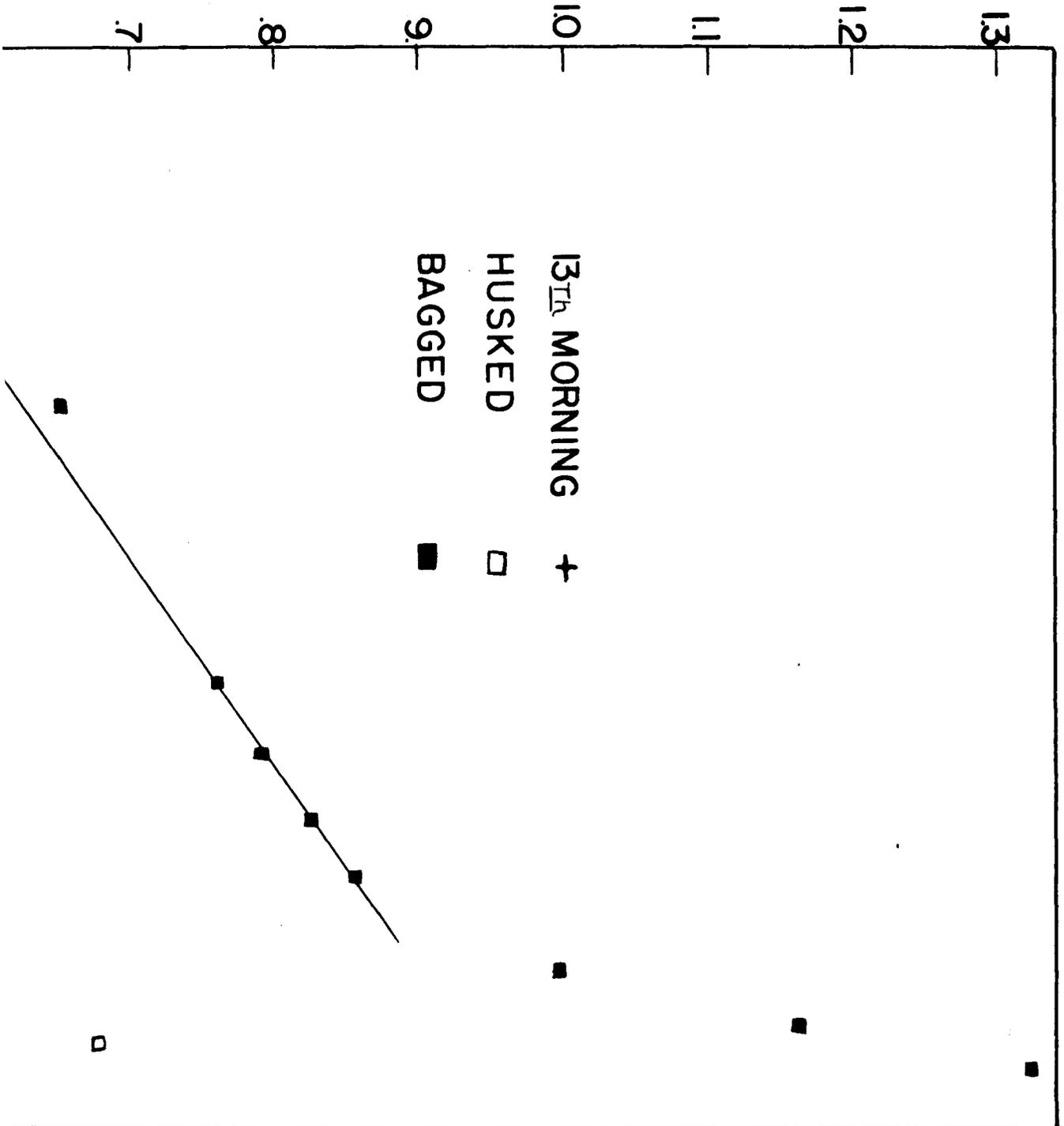


Fig. 12. Iodine titration curves for 20th and 21st day samples of sweet corn starch.

/ 100. MILLIGRAM SAMPLE



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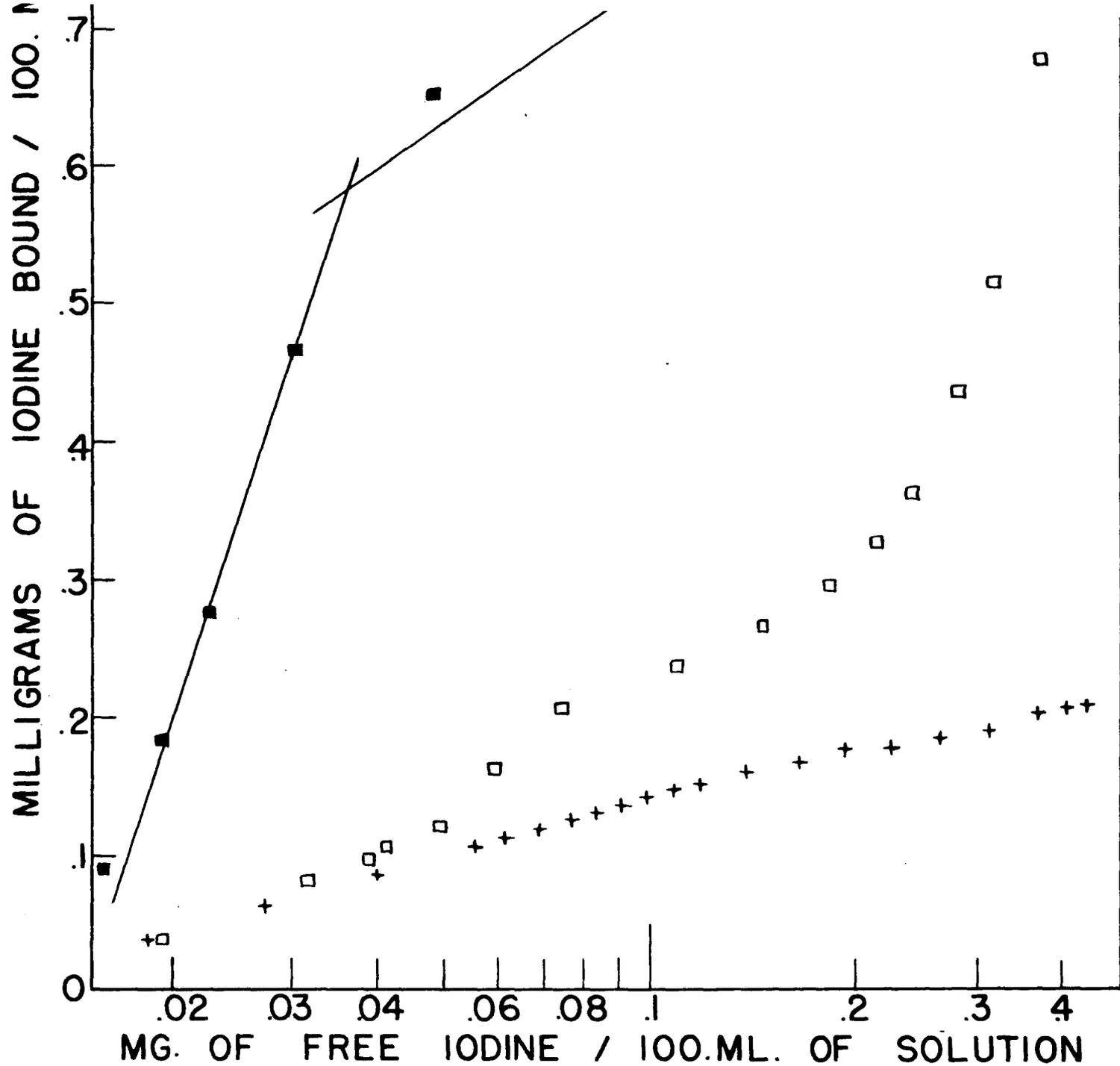
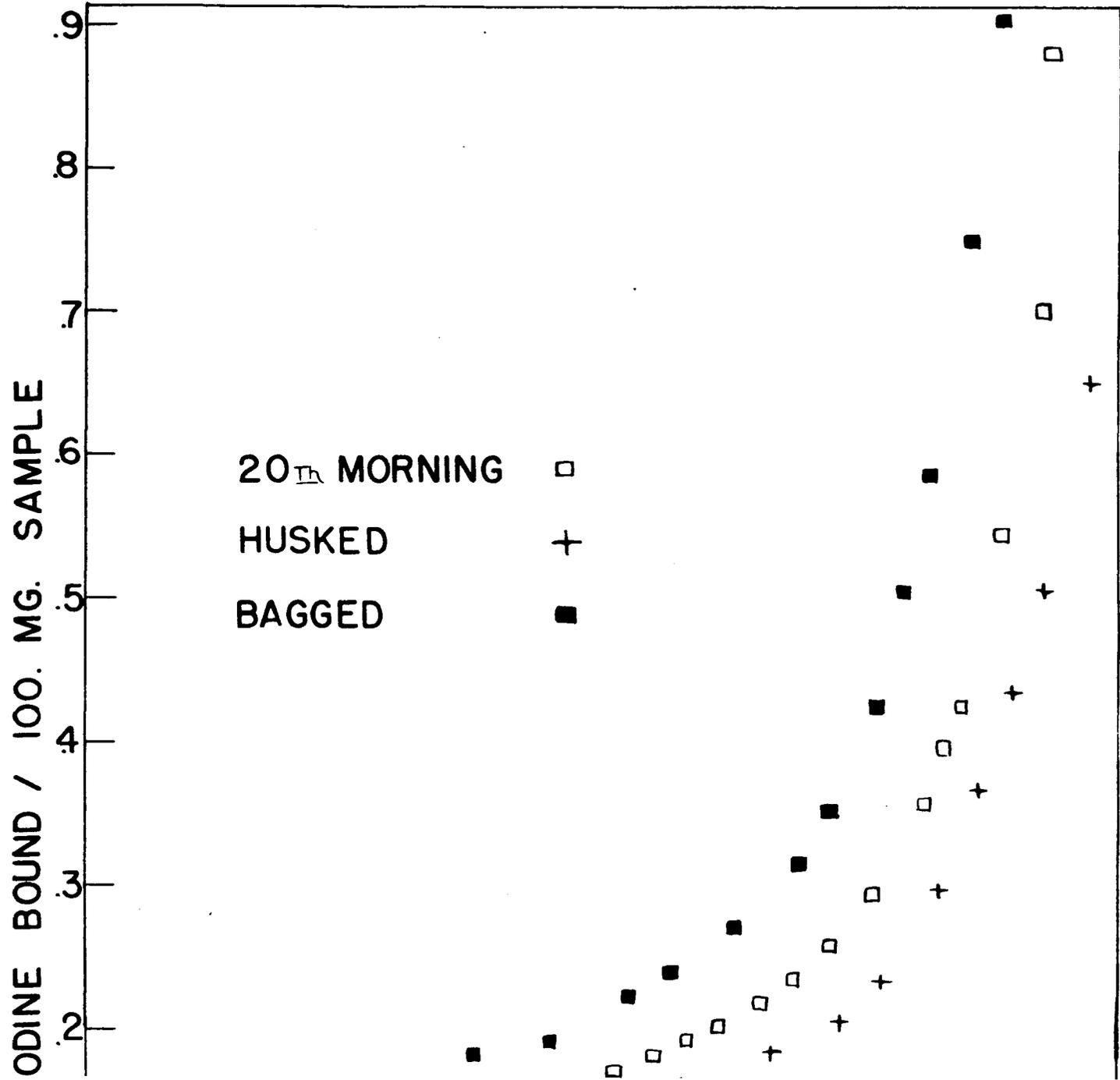


Fig. 12. Iodine titration curves for 13th day samples of waxy maize starch.



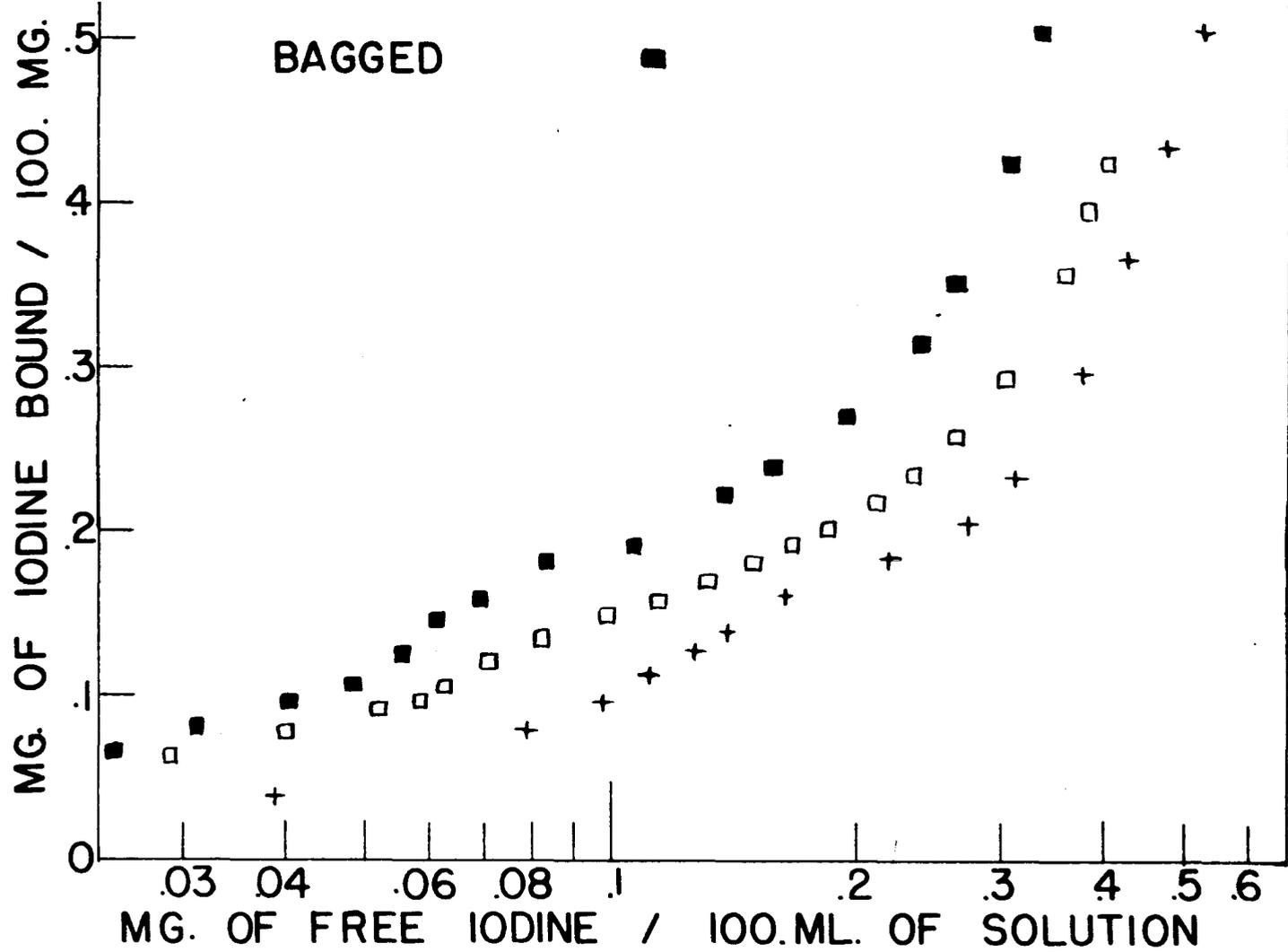
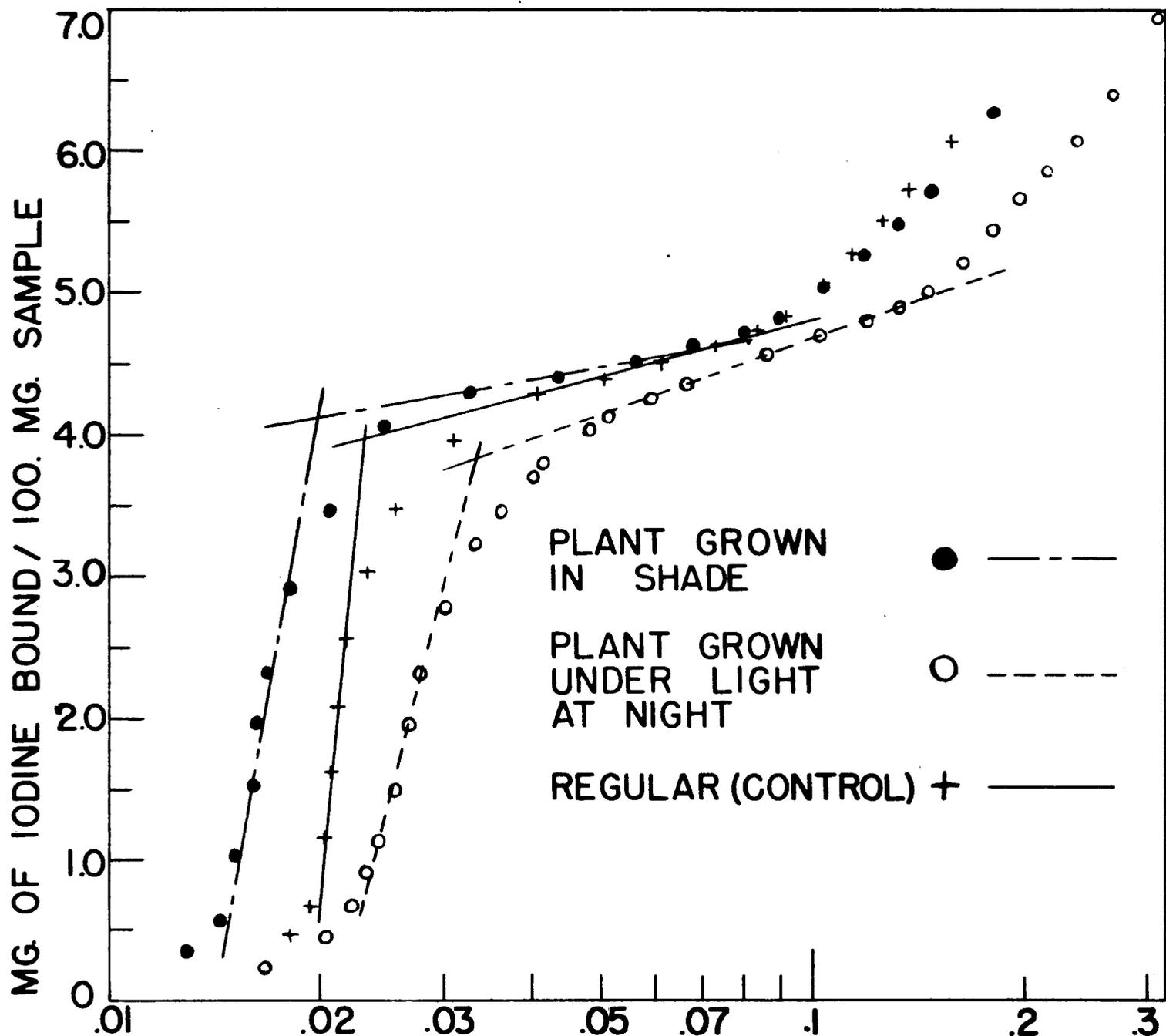


Fig. 14. Iodine titration curves for 20th day samples of waxy maize starch.



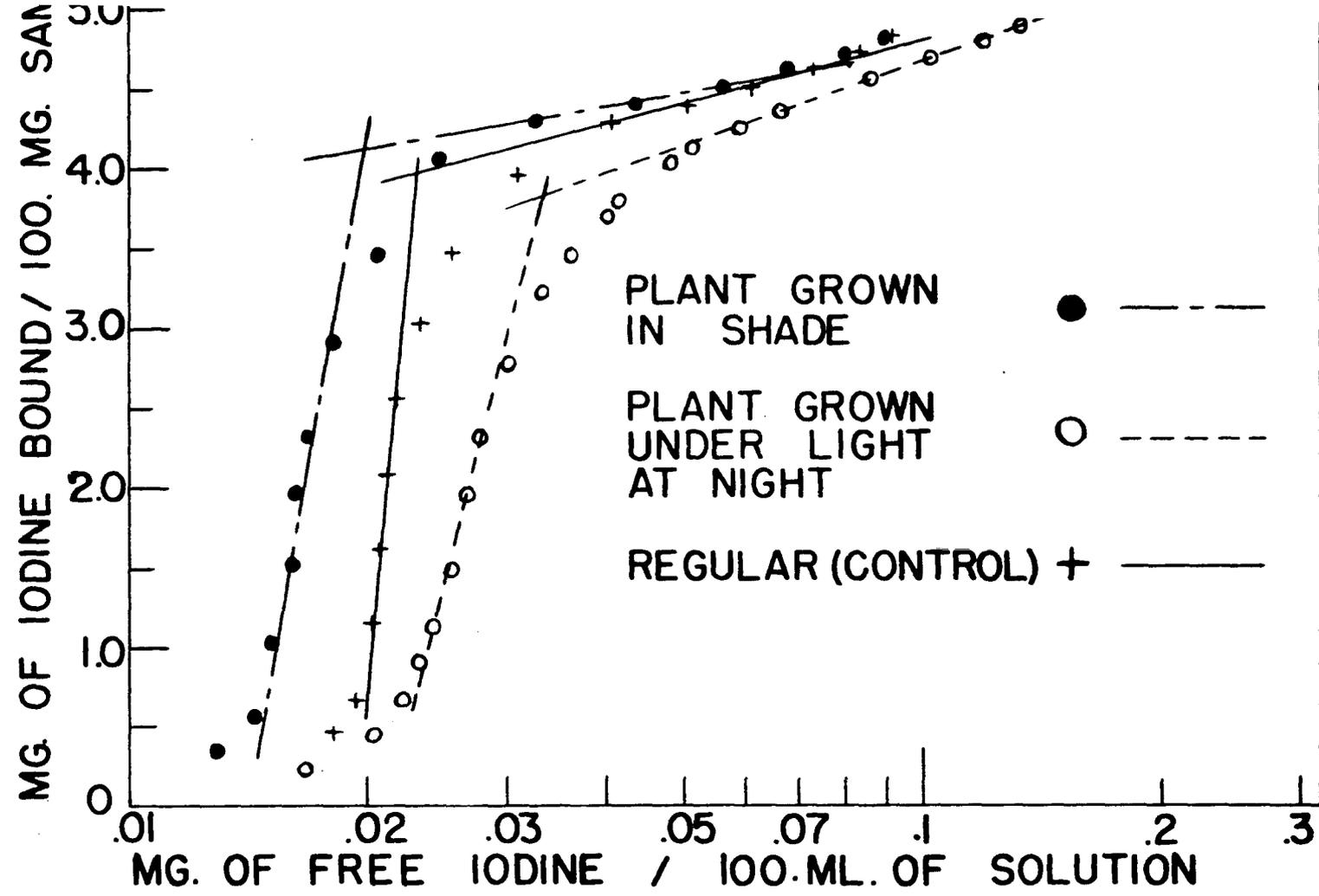


Fig. 15. Iodine titration curves for samples of mature potato starch produced under various conditions.

Table 22. Acid hydrolysis of 13th day waxy maize I starch

Refluxed at 99.0°C and pH = 4.24. Theoretical \bar{X}_n based on statistical model.

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n
0.5	105	2270
1.0	83.5	2160
2.0	53.7	1731
3.0	37.6	1450
5.0	21.5	1093
7.0	15.8	936

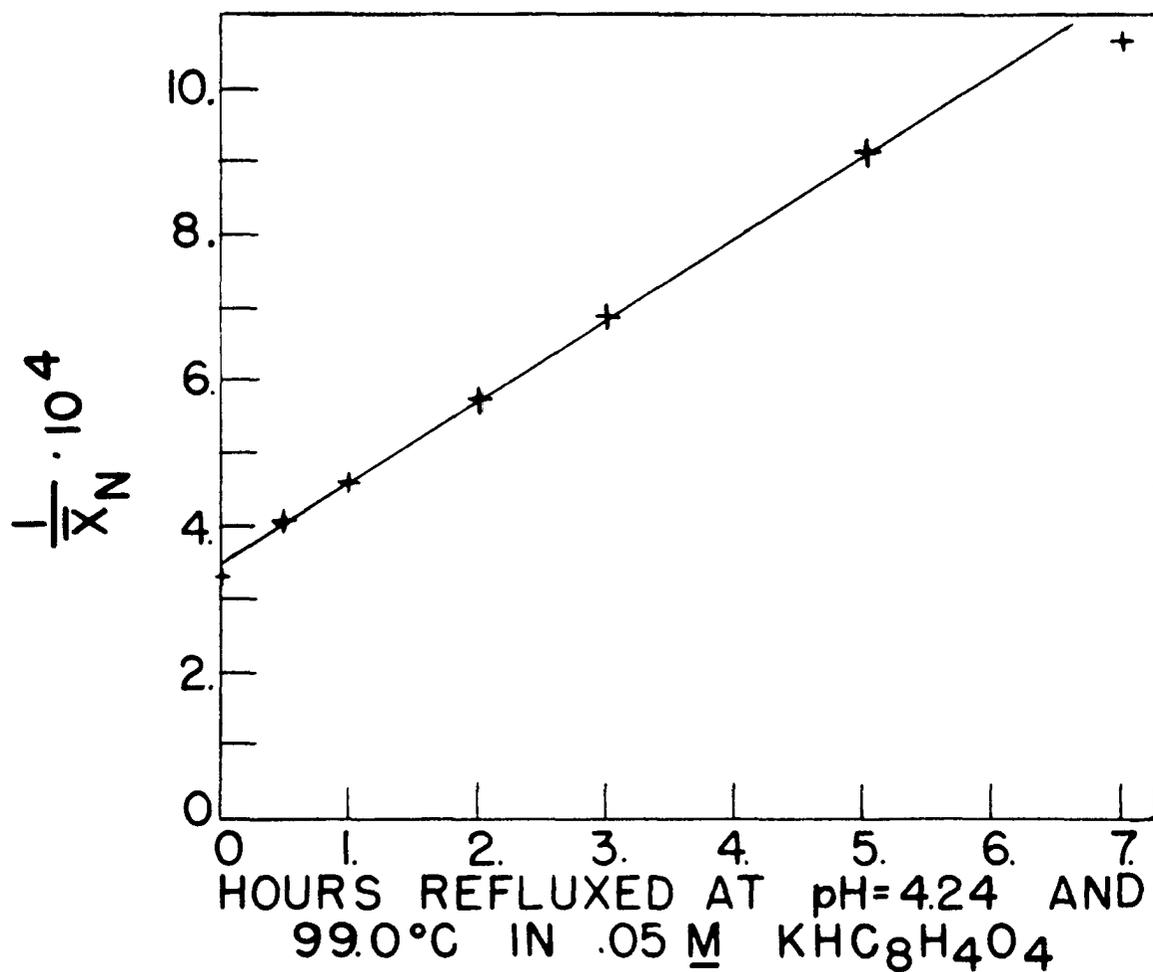
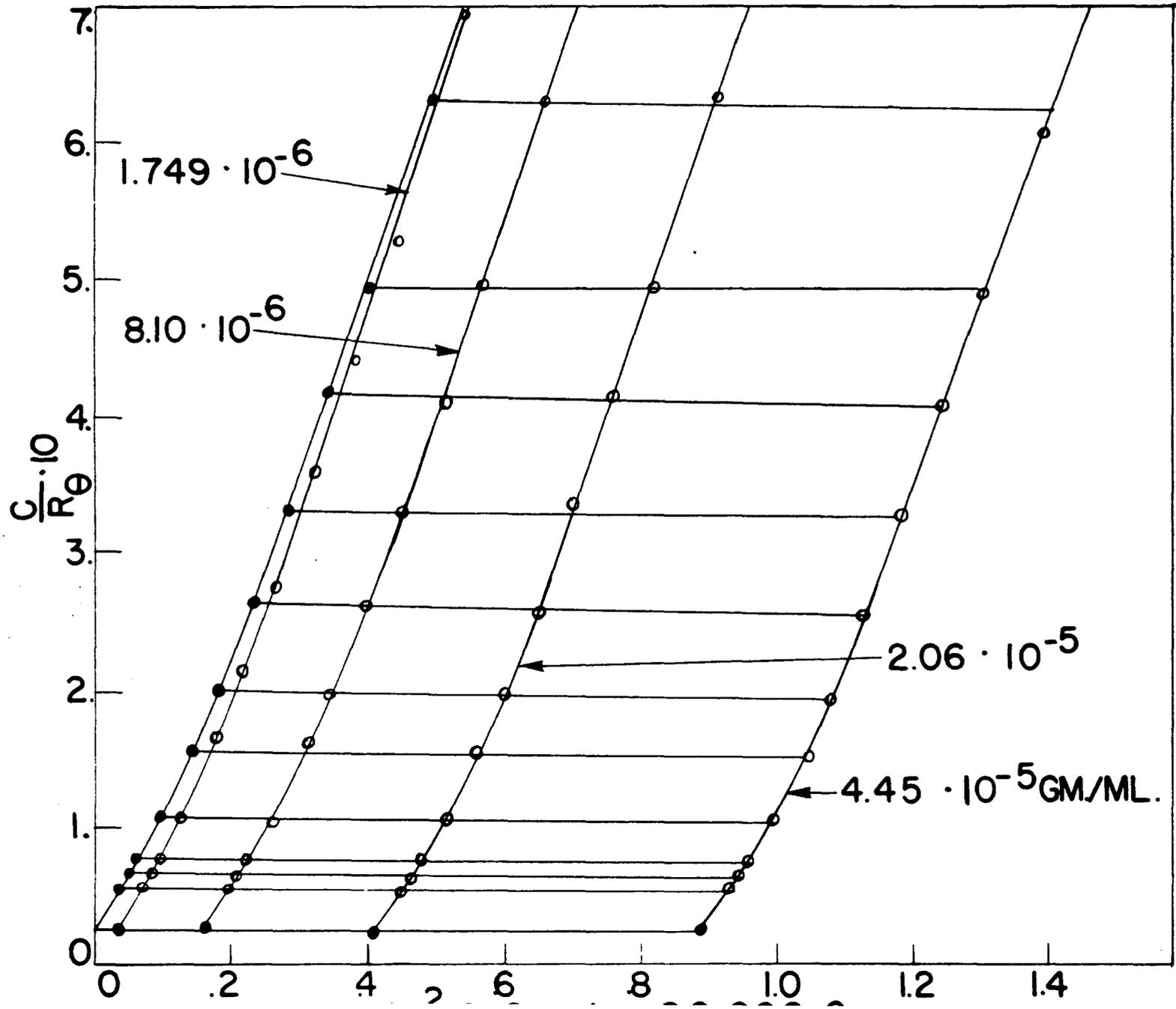


Fig. 16. Acid hydrolysis of 13th day waxy maize starch, sample I. Points were obtained from \bar{M}_w using 5.8% branching for the statistical model.



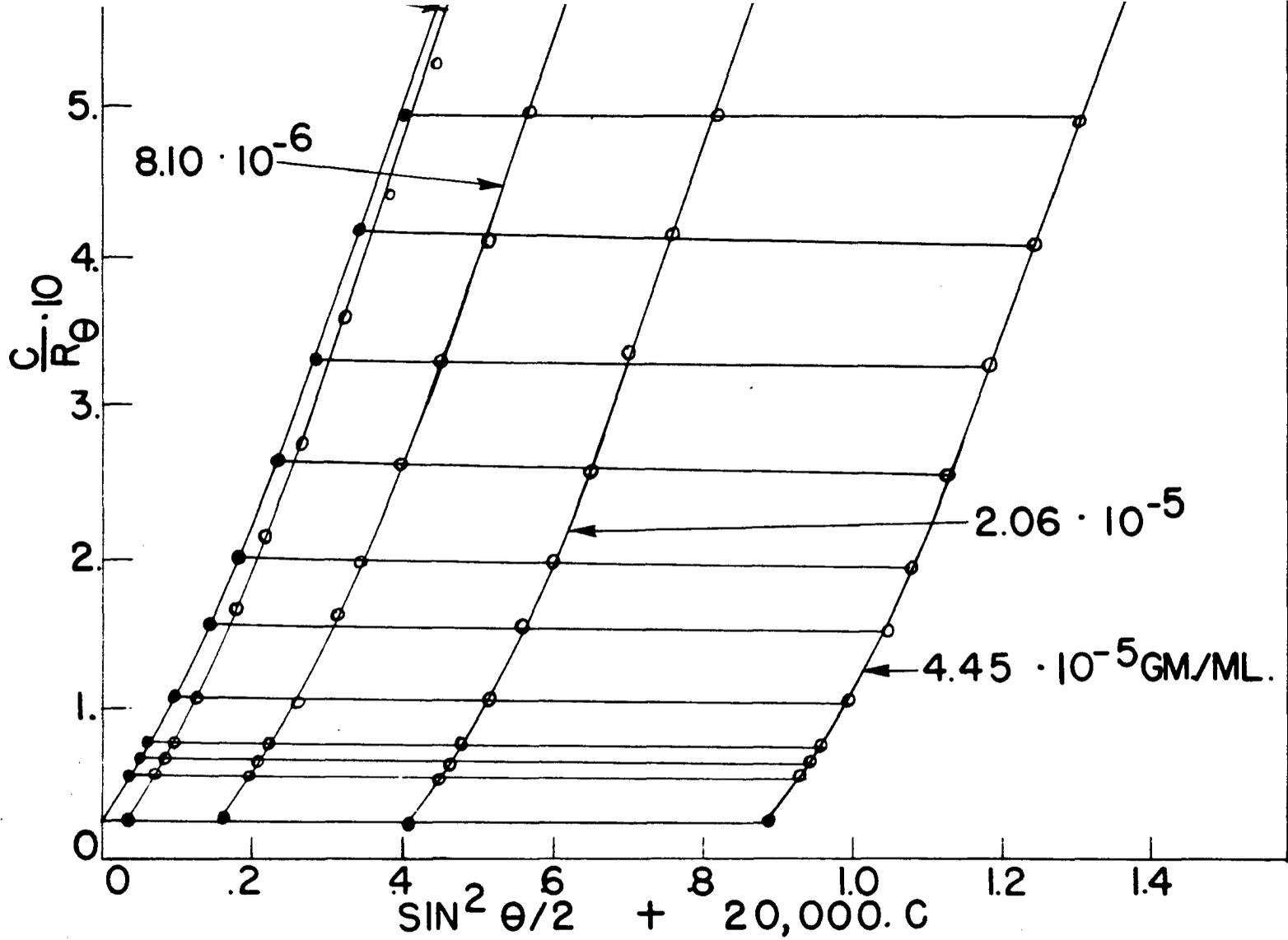
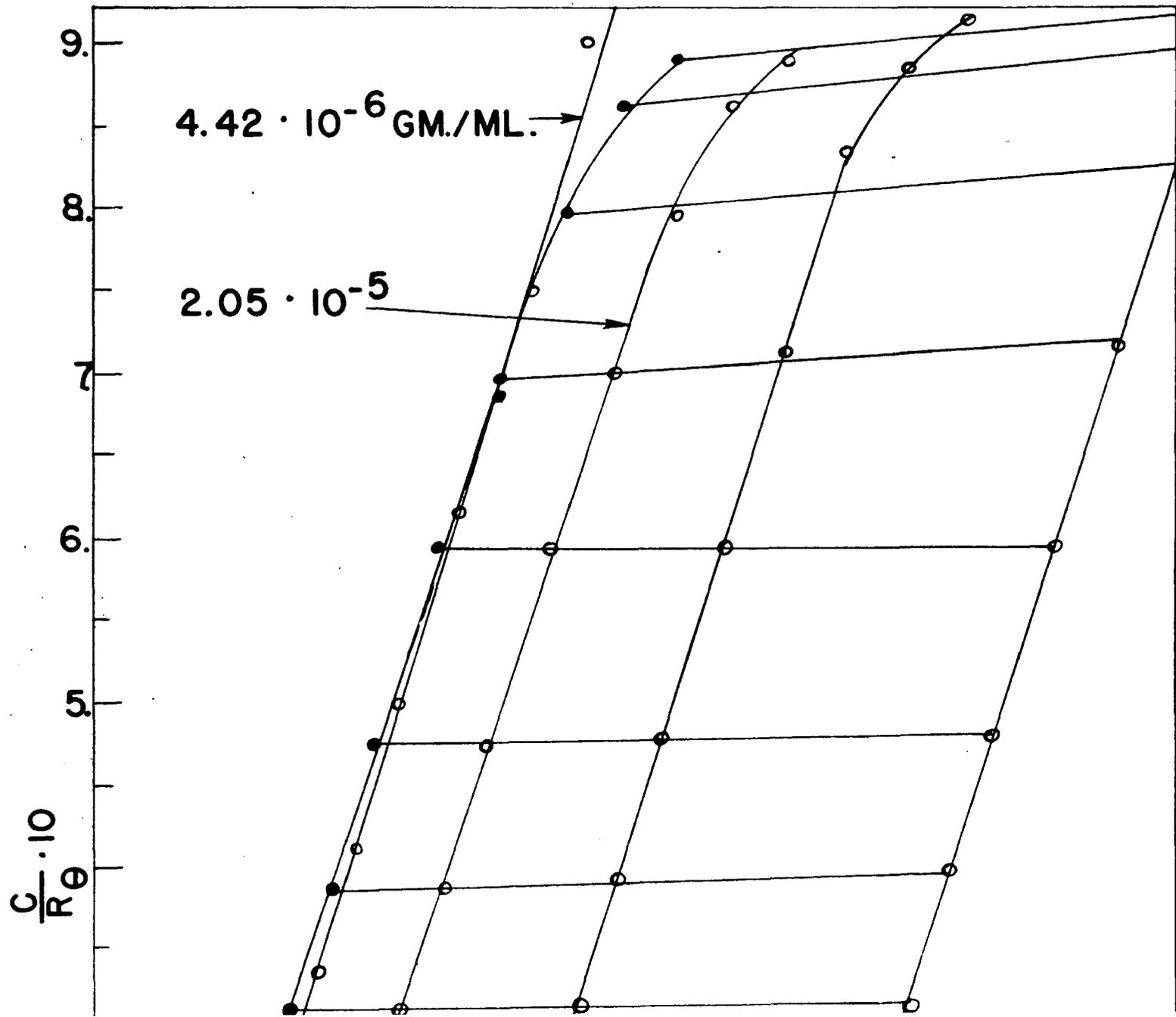


Fig. 17. The 0.5 hour sample of waxy maize I starch. $\bar{M}_w = 105 \times 10^6$.



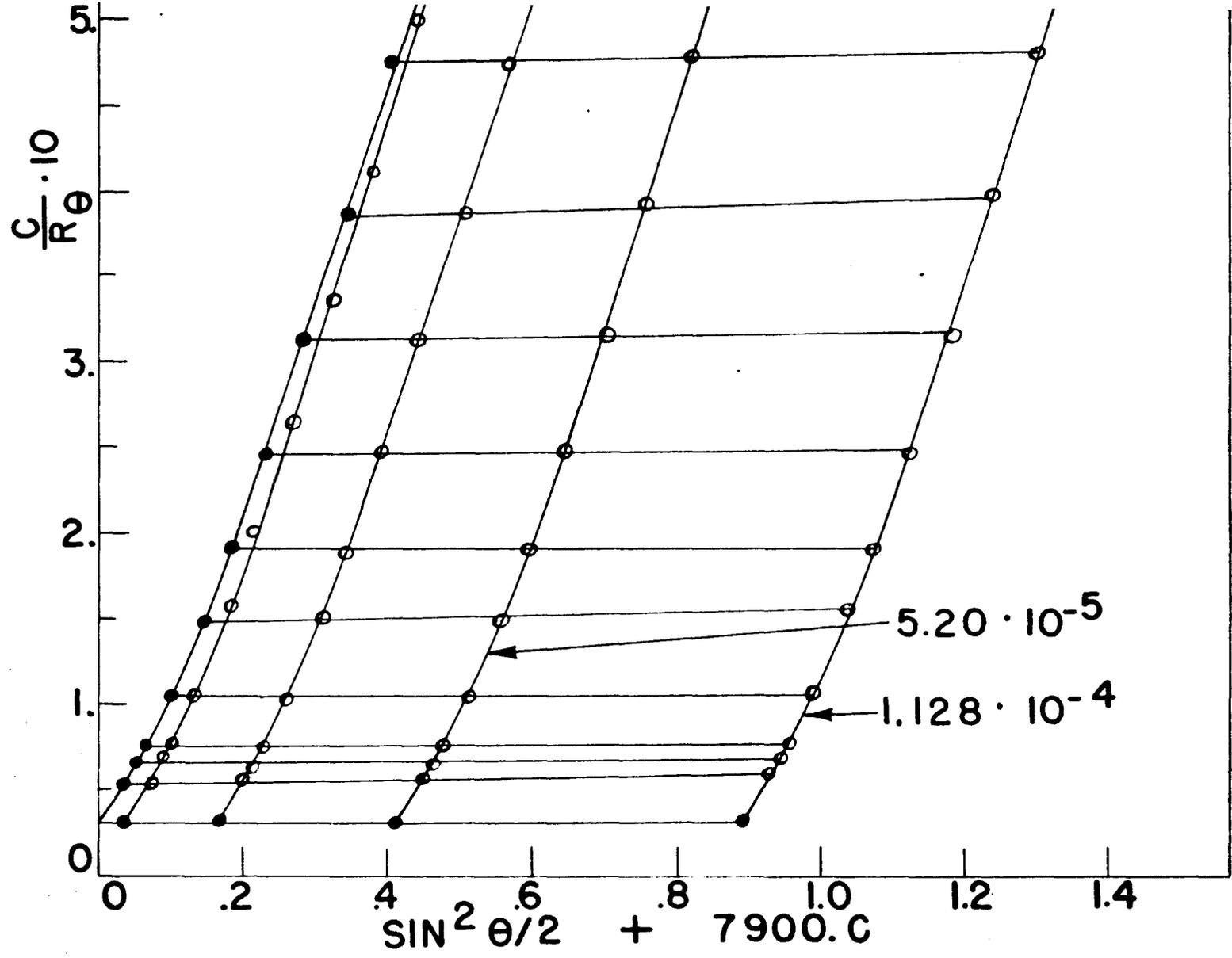
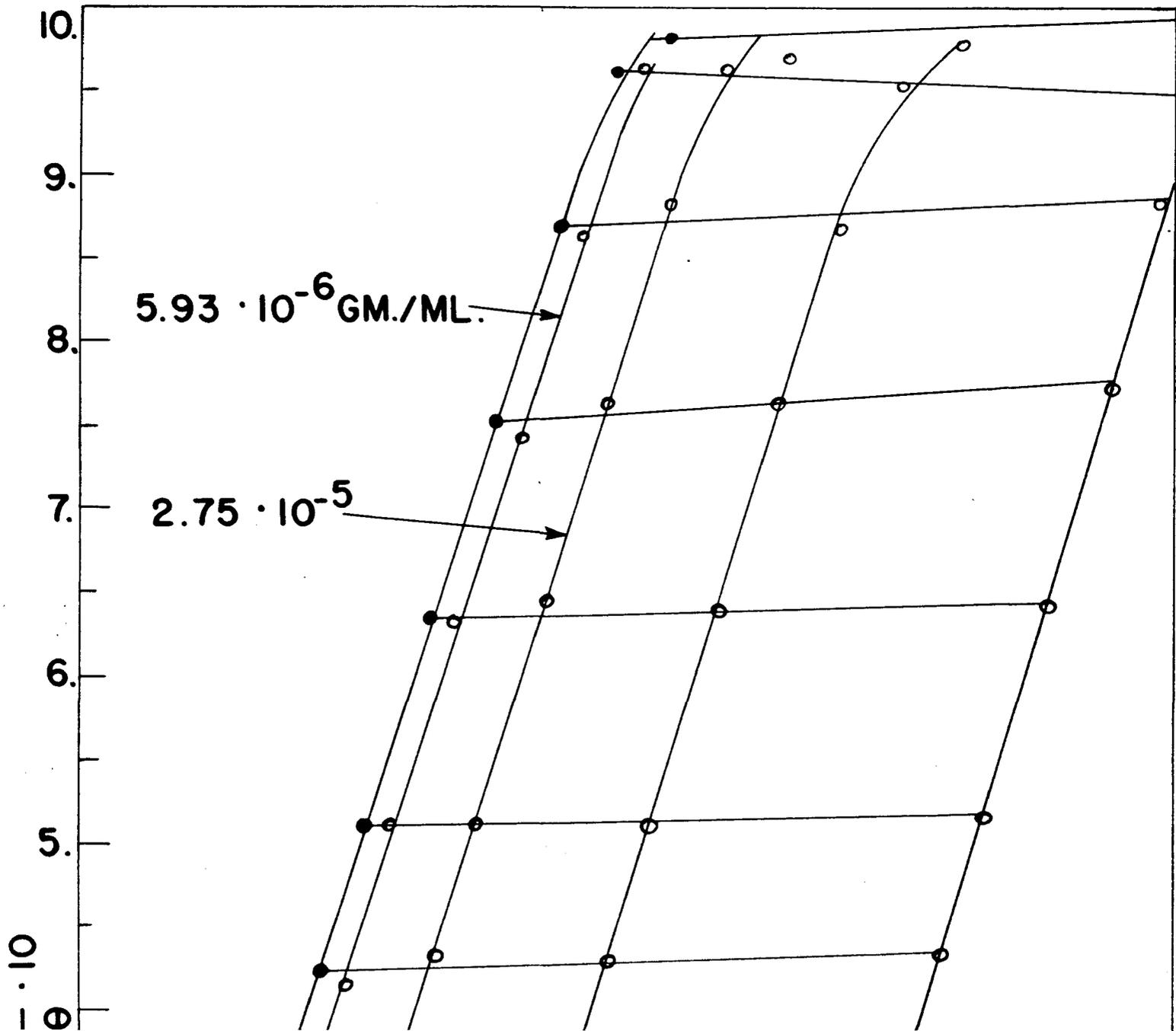


Fig. 18. The 1.0 hour sample of waxy maize I starch. $\bar{M}_w = 83.5 \times 10^6$.



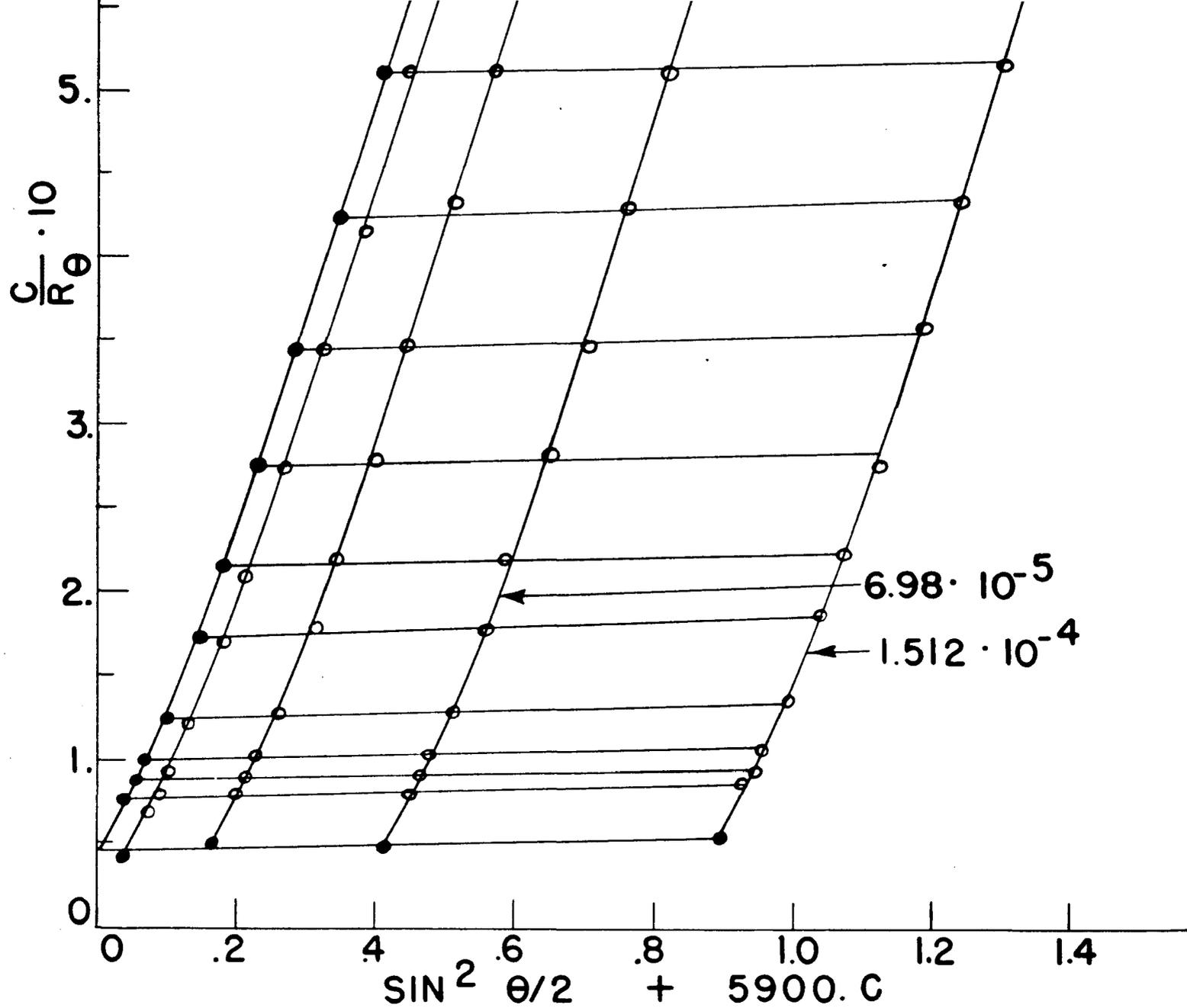
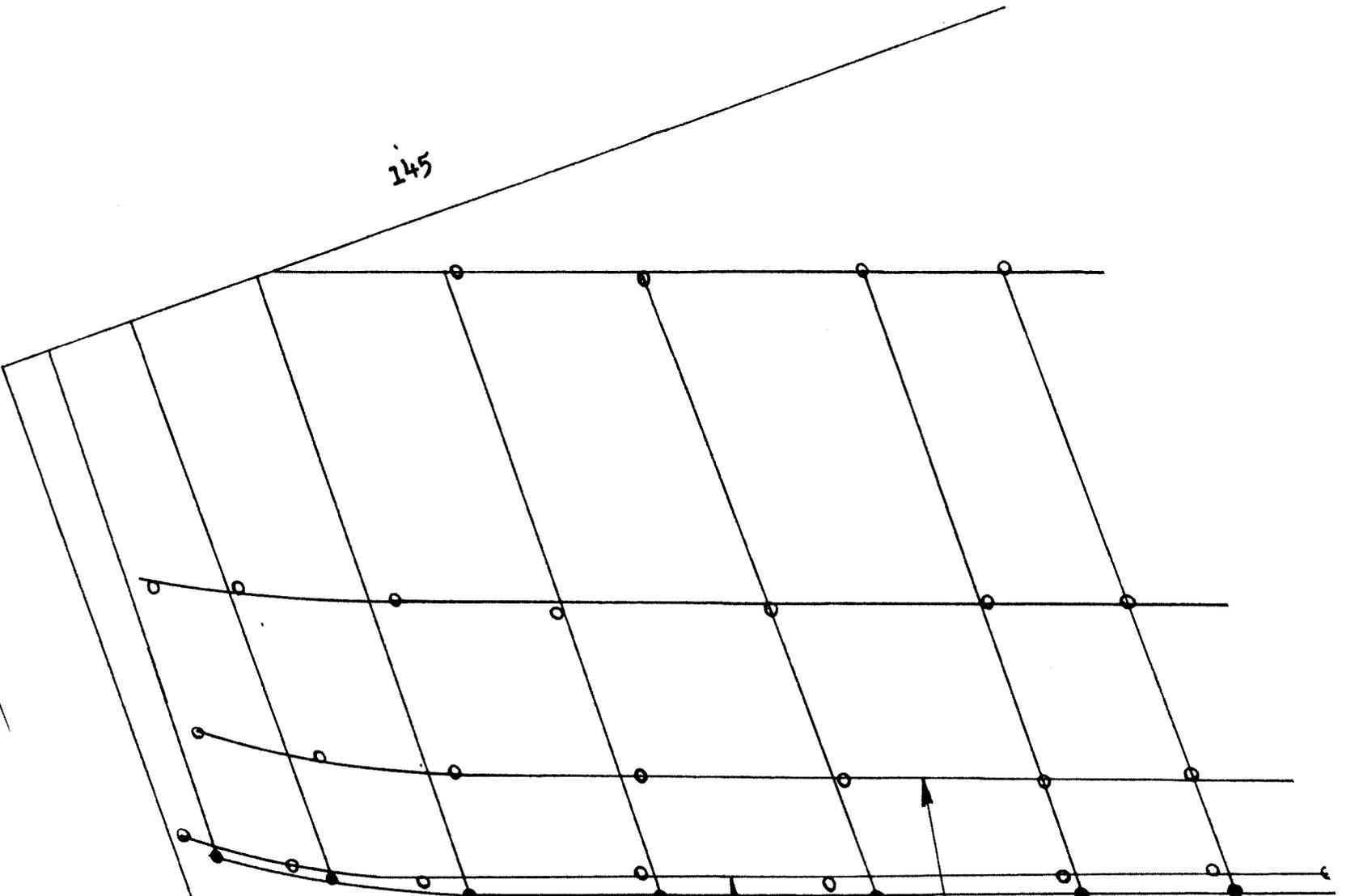


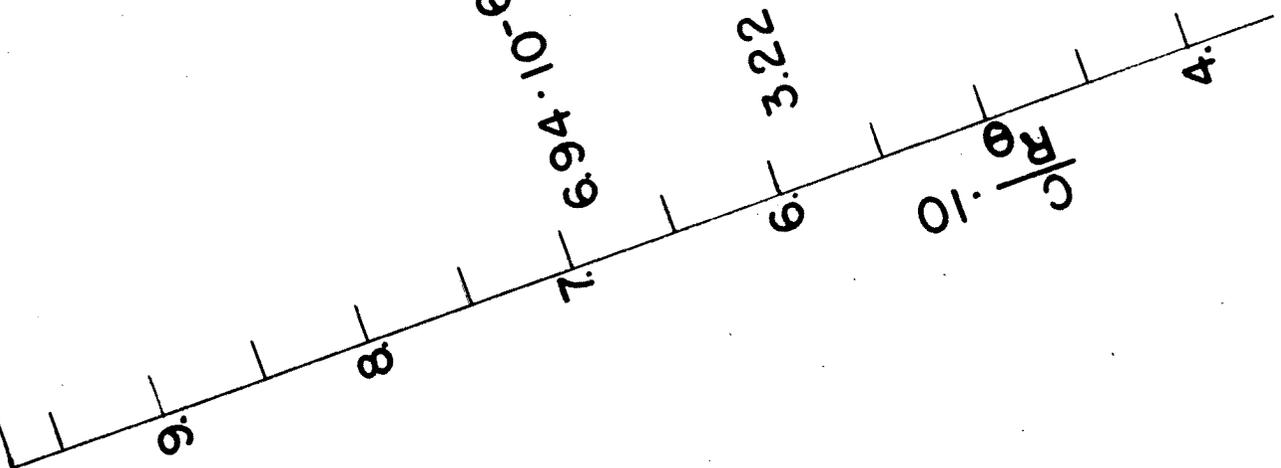
Fig. 19. The 2.0 hour sample of waxy maize I starch. $\bar{M}_w = 53.7 \times 10^6$.

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$6.94 \cdot 10^{-6} \text{ GM./ML.}$

$3.22 \cdot 10^{-5}$



$\frac{\theta}{c} \cdot 10$

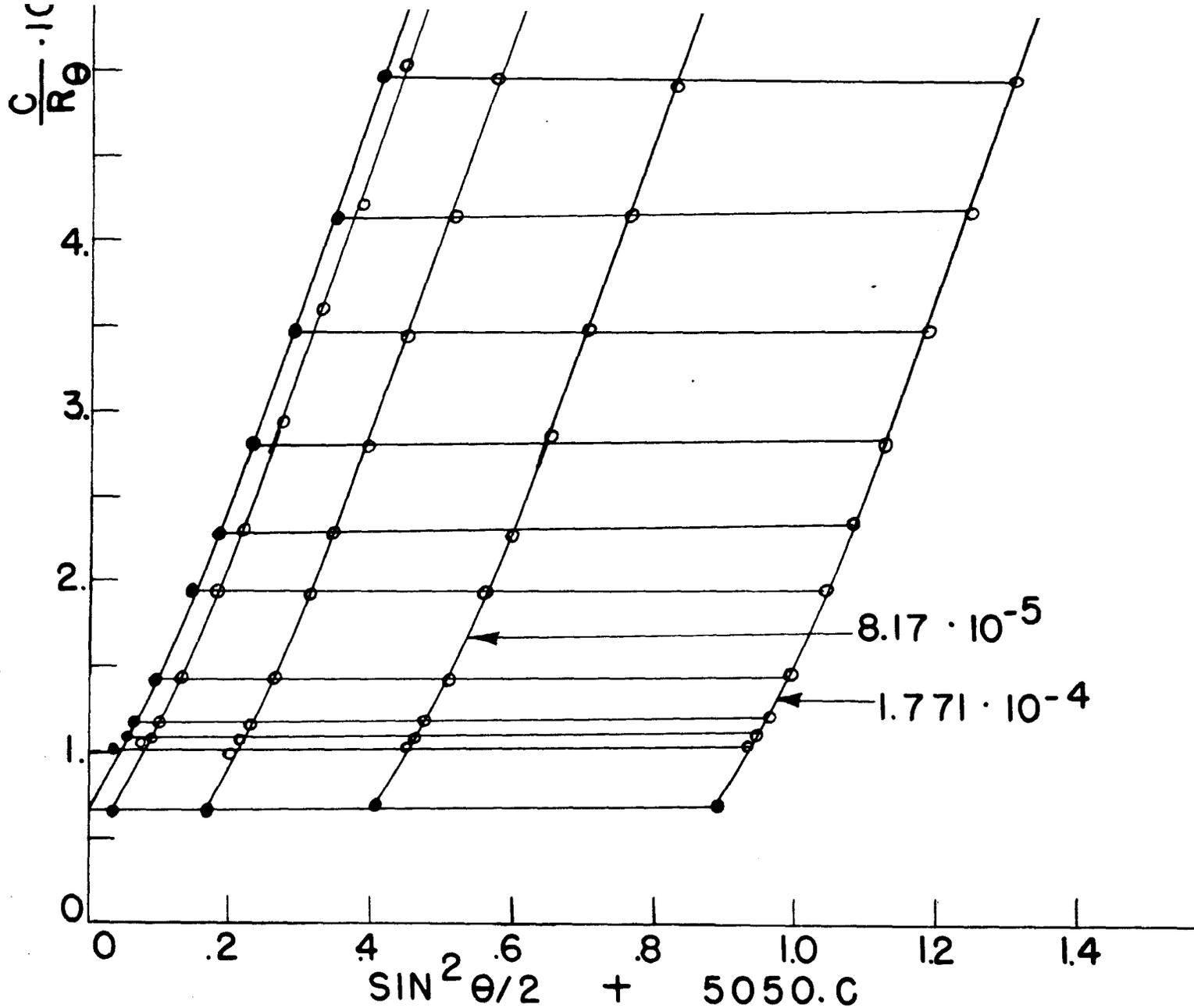


Fig. 20. The 3.0 hour sample of waxy maize I starch. $\bar{M}_w = 37.6 \times 10^6$.

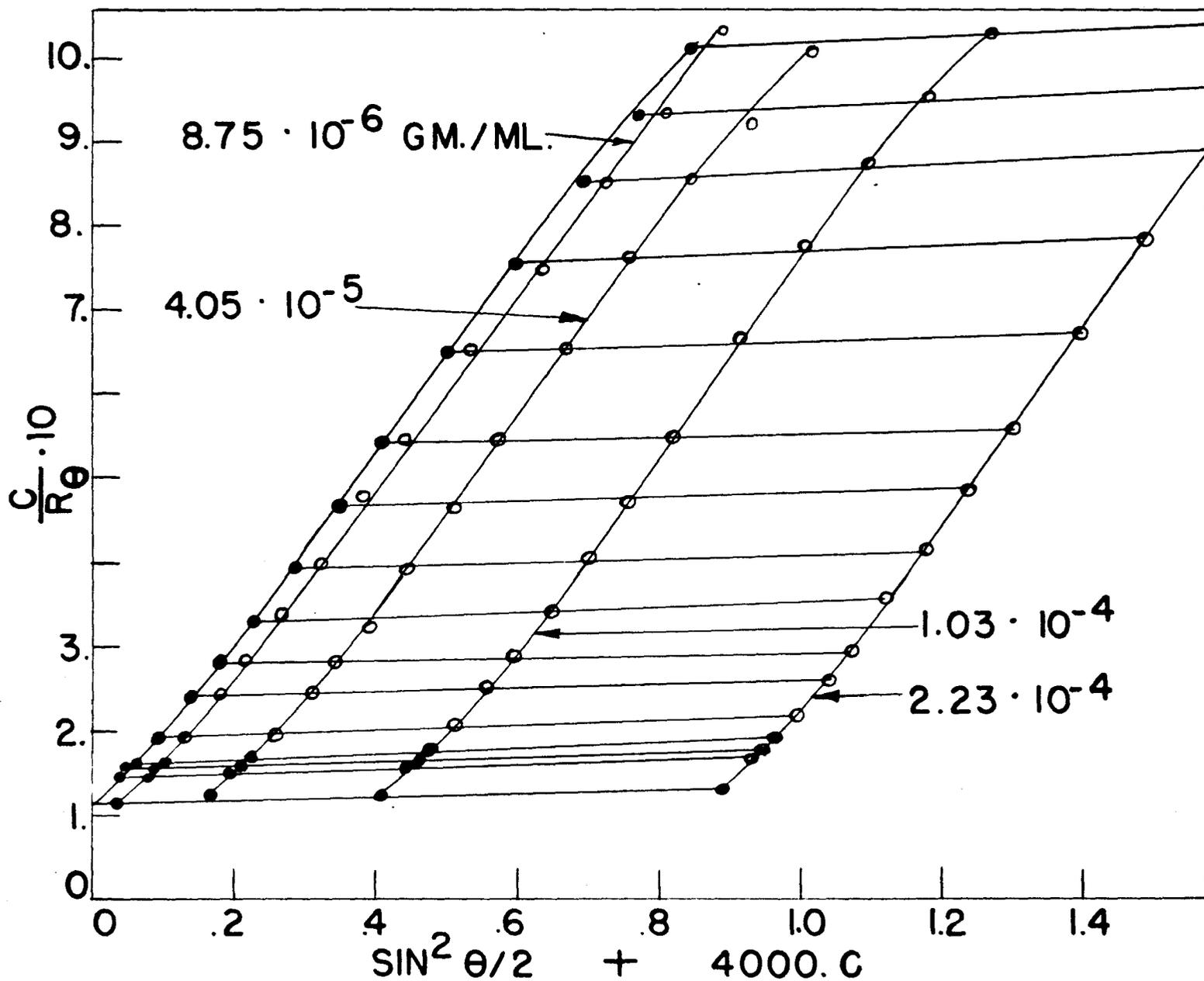


Fig. 21. The 5.0 hour sample of waxy maize I starch. $\bar{M}_w = 21.5 \times 10^6$.

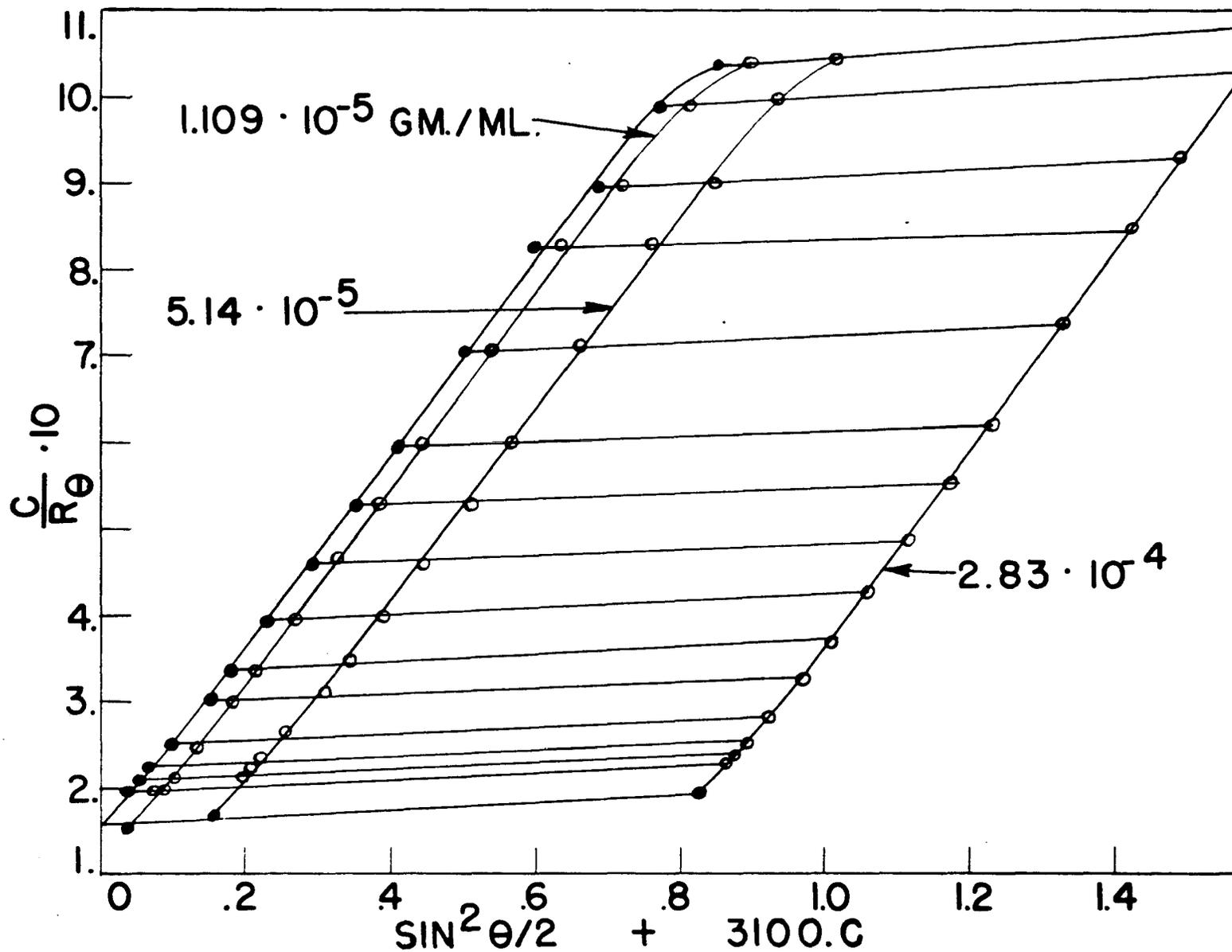


Fig. 22. The 7.0 hour sample of waxy maize I starch. $\bar{M}_w = 15.8 \times 10^6$.

Table 23. Acid hydrolysis of 13th day waxy maize II starch

Refluxed at 99.0°C and pH = 4.24. Theoretical \bar{X}_n based on statistical model.

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n
2.0	55.6	1765
5.0	18.8	1021
7.0	13.3	858
10.0	7.91	660
13.0	6.02	575
16.0	3.67	447
27.0	1.48	281

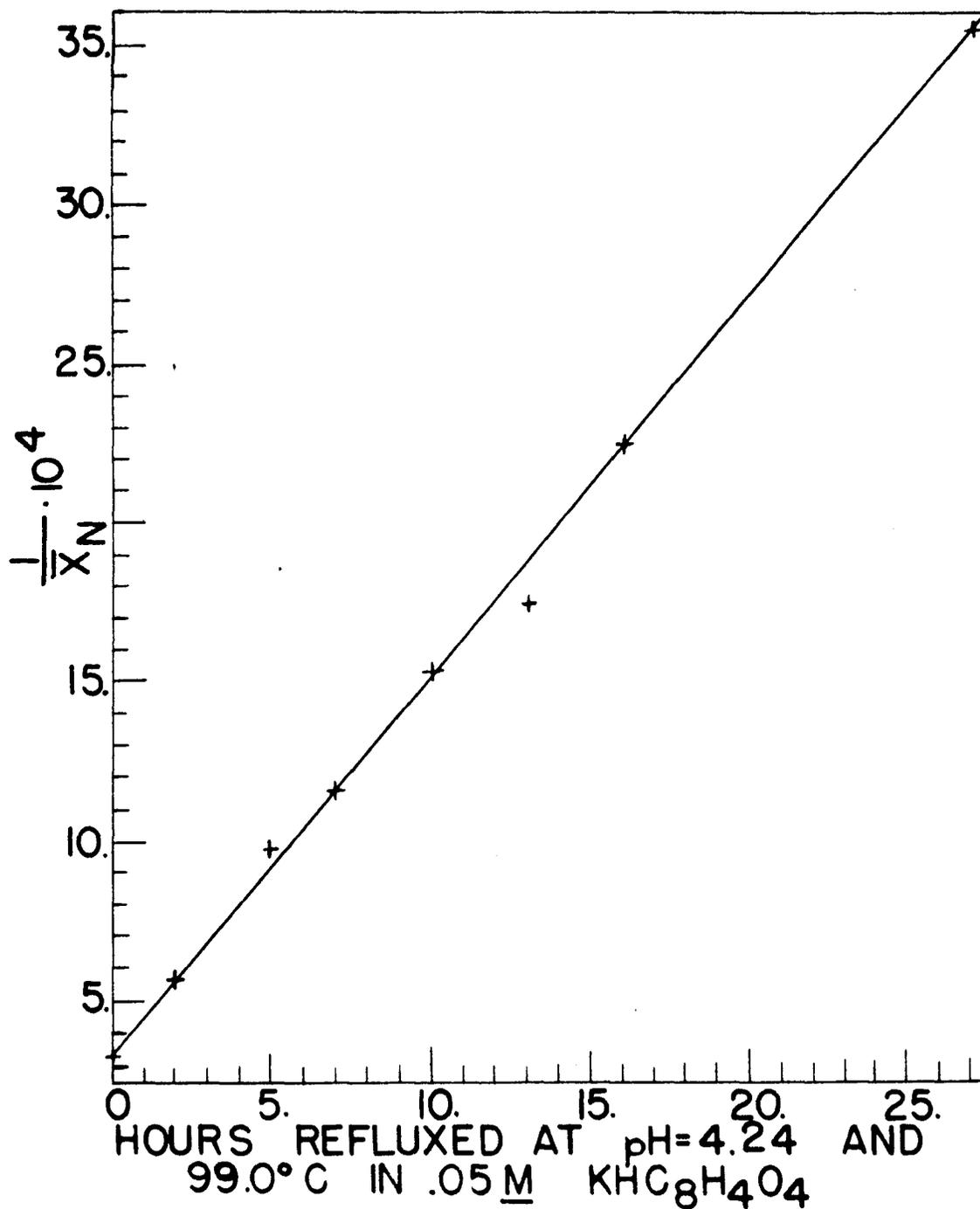


Fig. 23. Acid hydrolysis of 13th day waxy maize starch, sample II. Points were obtained from \bar{M}_w using 5.8% branching for the statistical model.

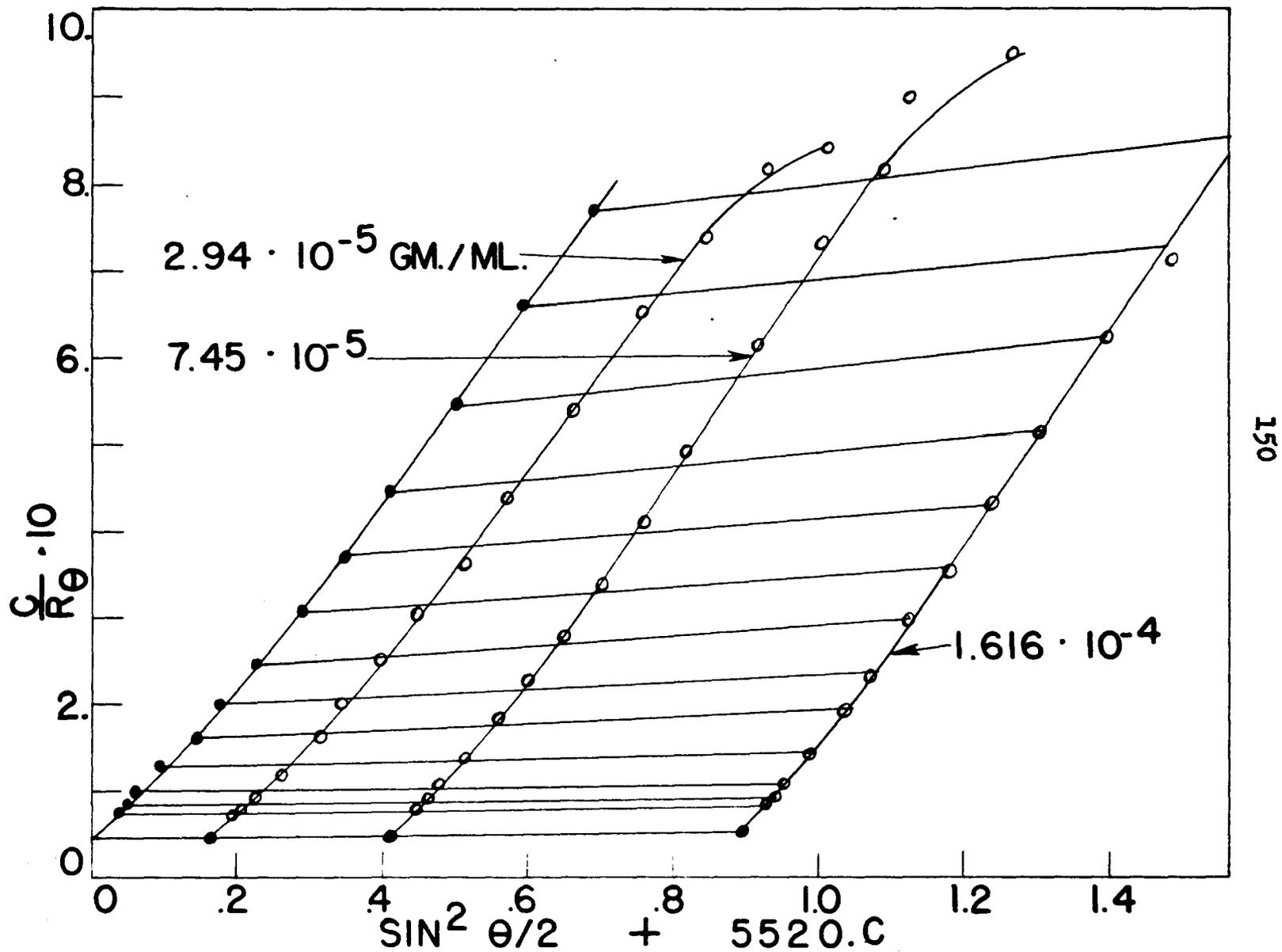


Fig. 24. The 2.0 hour sample of waxy maize II starch. $\bar{M}_w = 55.6 \times 10^6$.

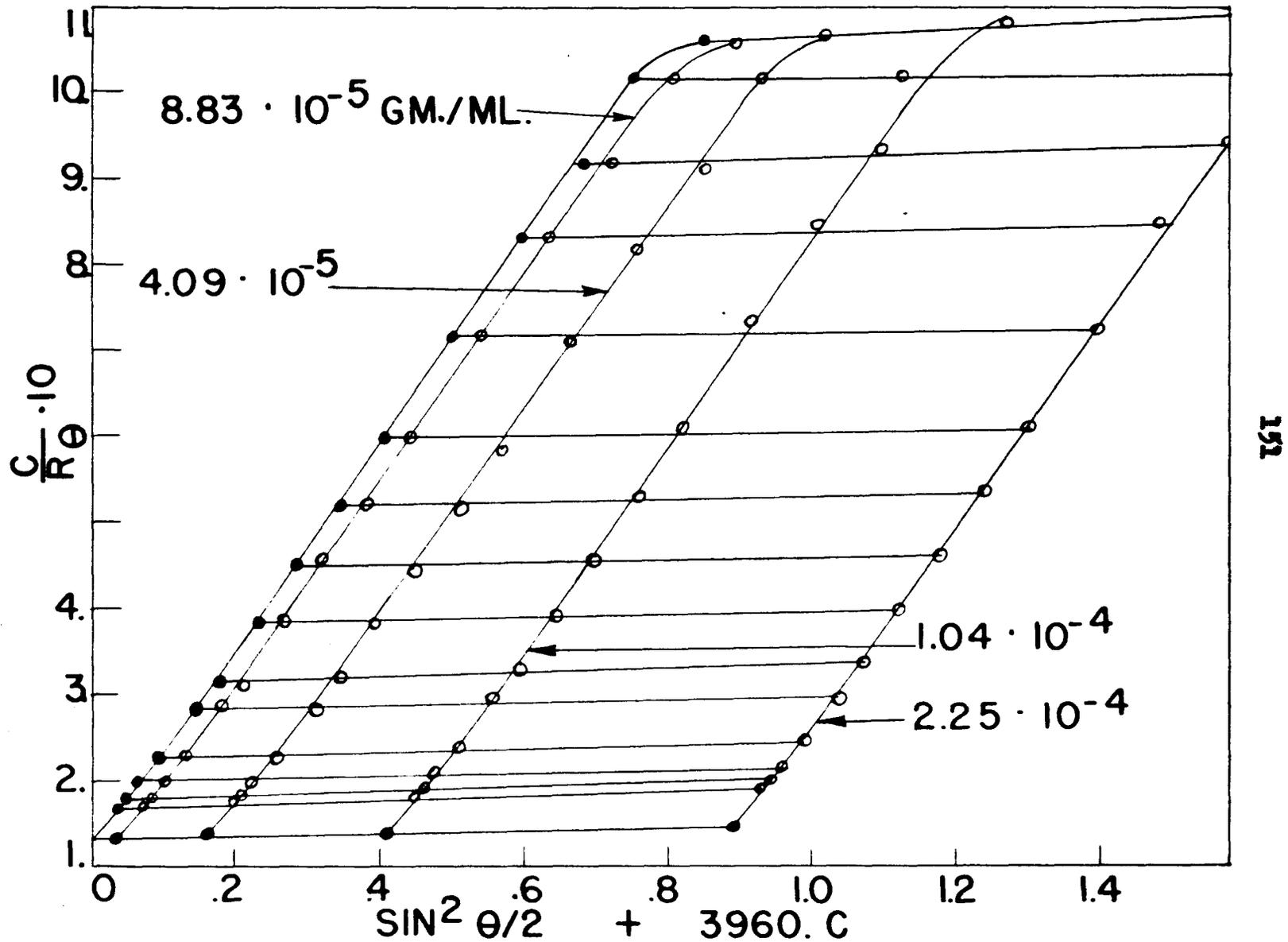


Fig. 25. The 5.0 hour sample of waxy maize II starch. $\bar{M}_w = 18.8 \times 10^6$.

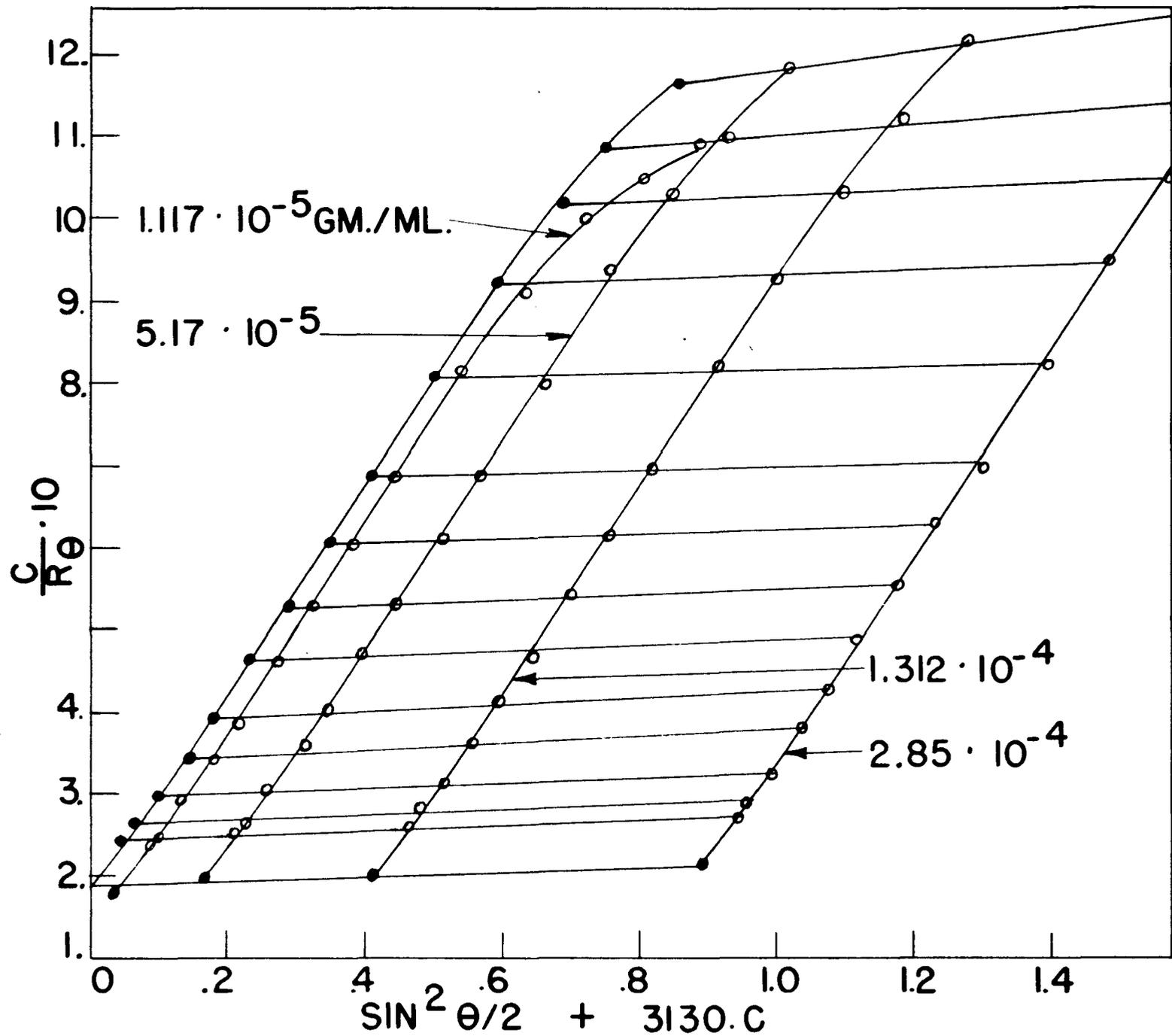


Fig. 26. The 7.0 hour sample of waxy maize II starch $\bar{M}_w = 12.2 \cdot 10^6$

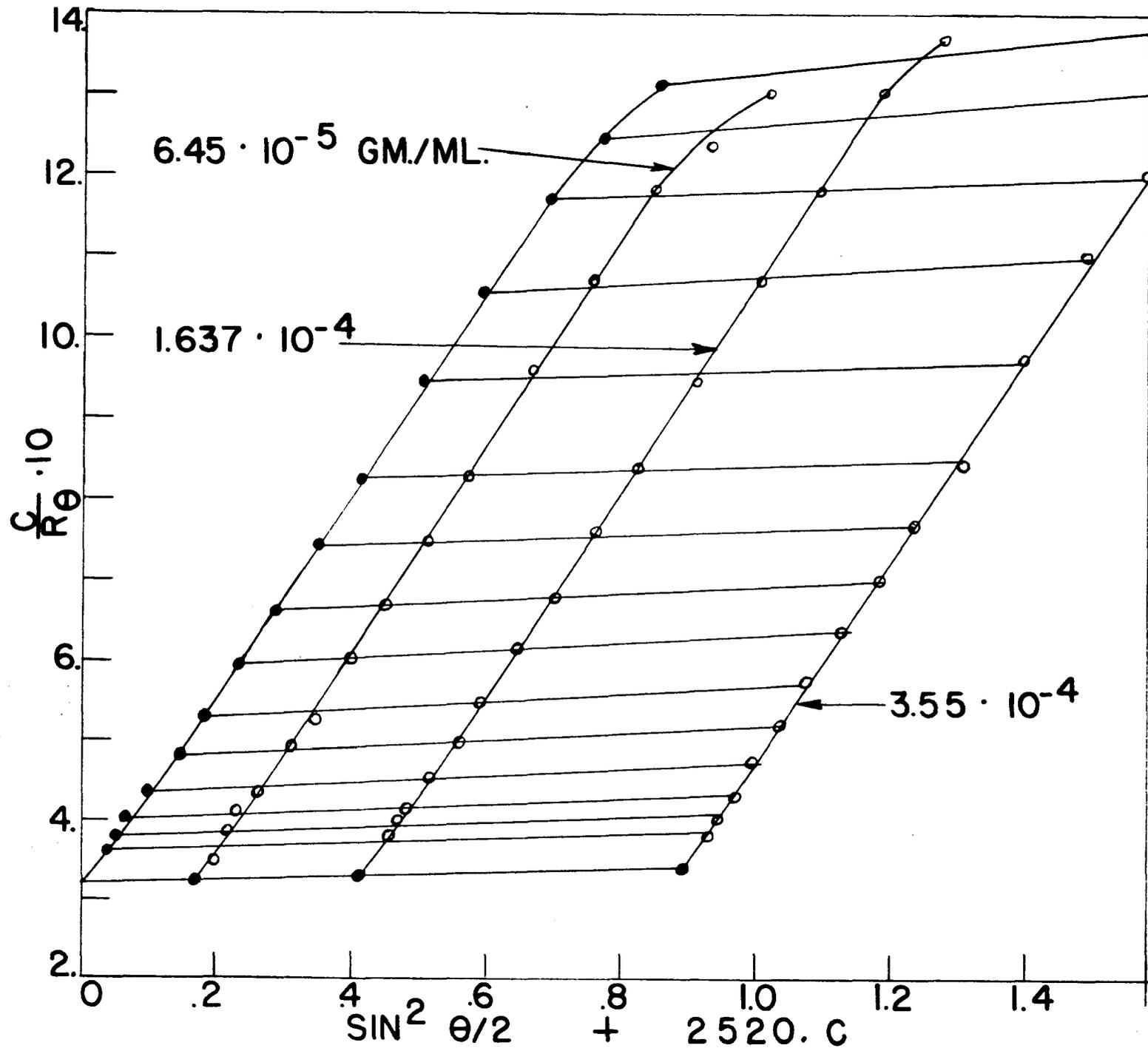
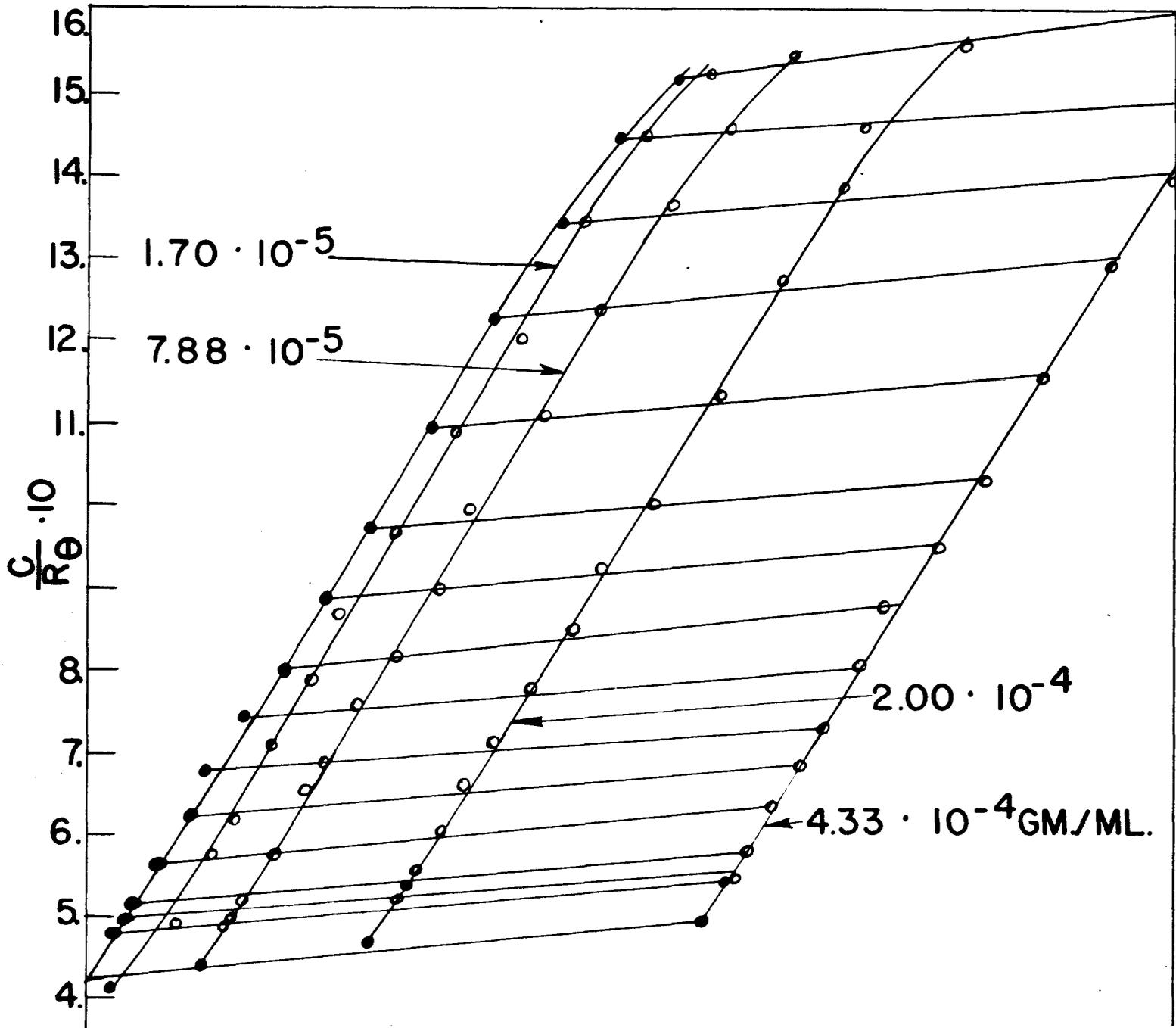


Fig. 27. The 10.0 hour sample of waxy maize II starch. $\bar{M}_w = 7.01 \cdot 10^6$



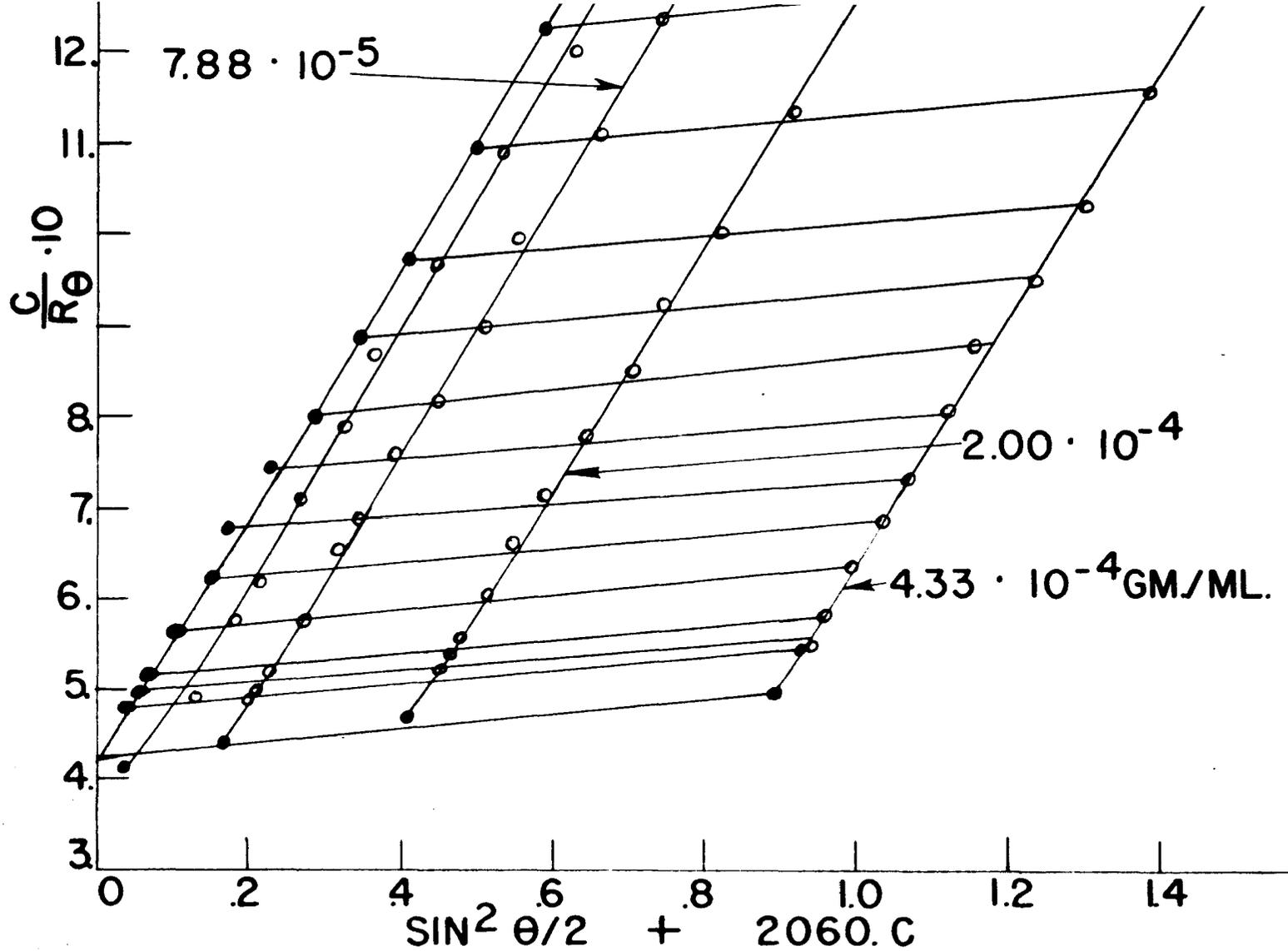
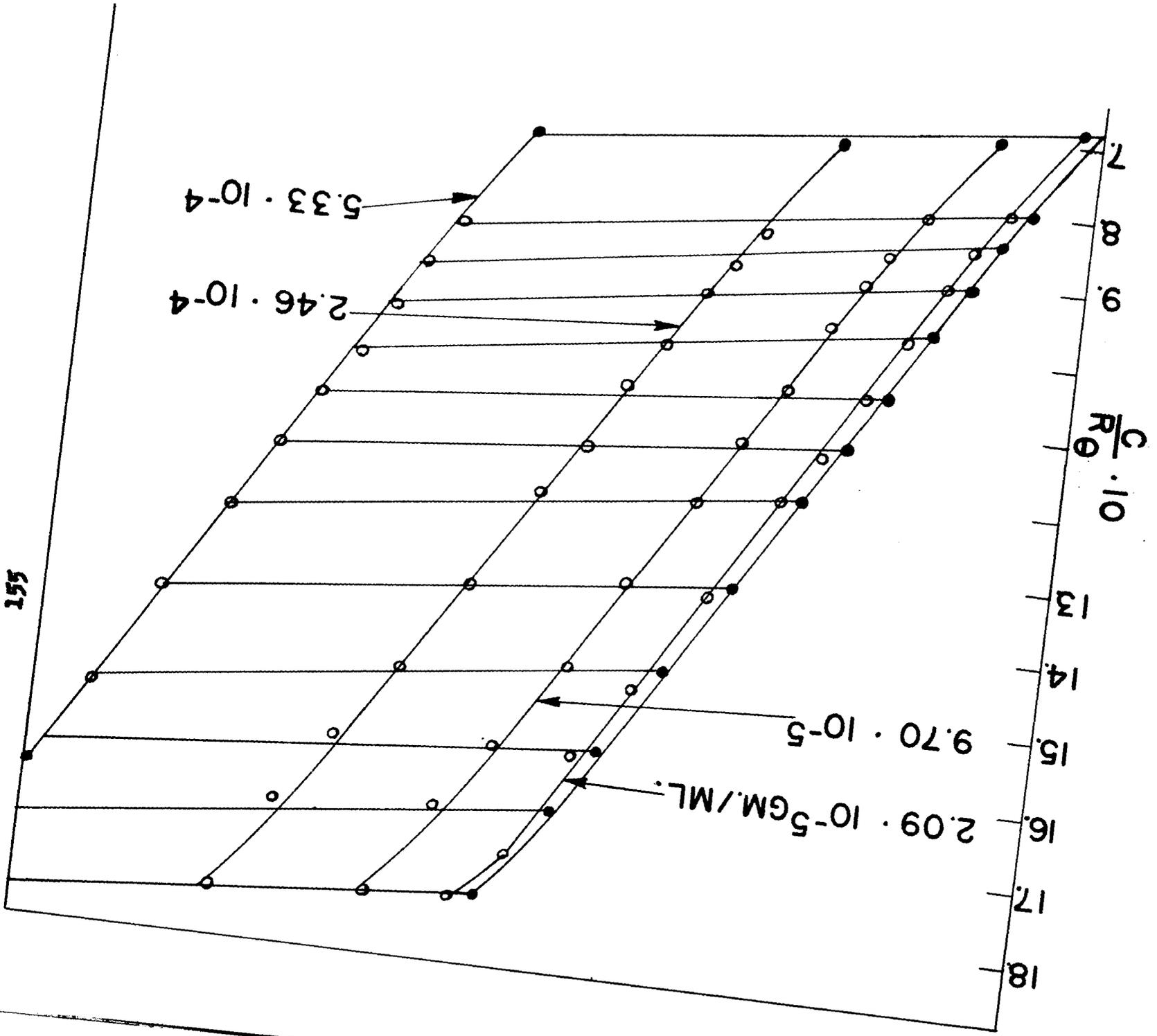


Fig. 28. The 13.0 hour sample of waxy maize II starch. $\bar{M}_w = 6.02 \times 10^6$.



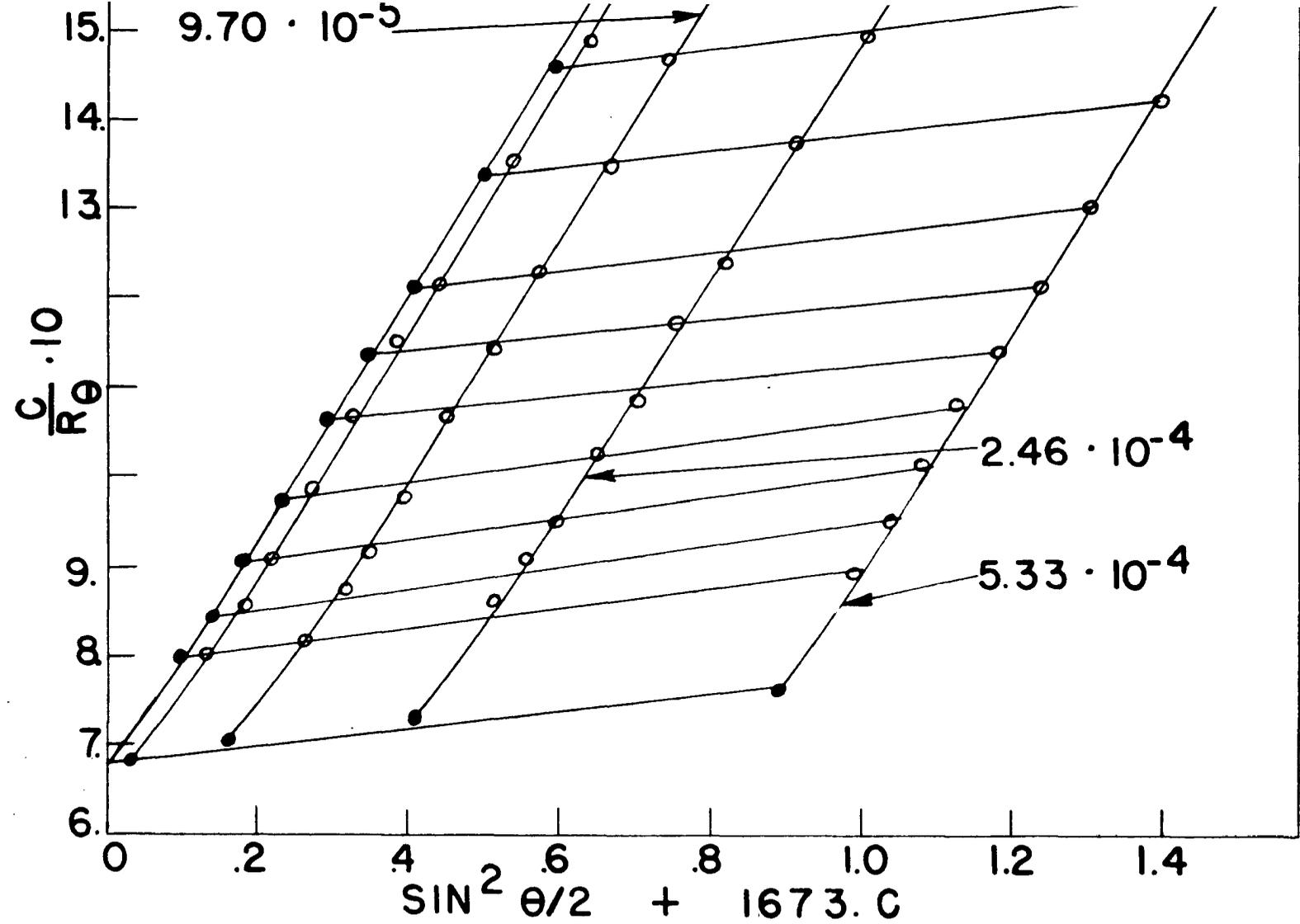


Fig. 29. The 16.0 hour sample of waxy maize II starch. $\bar{M}_w = 3.67 \times 10^6$.

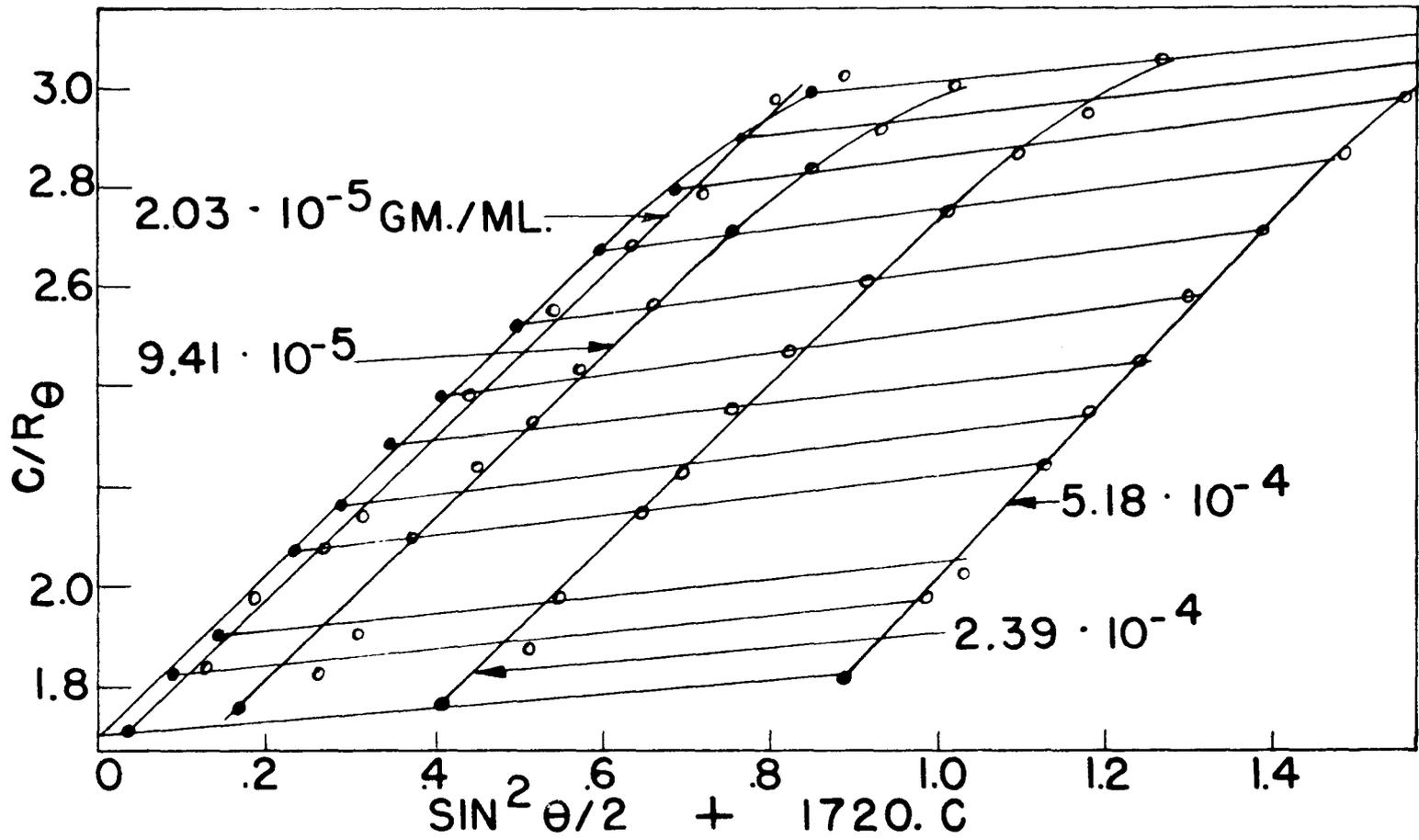


Fig. 30. The 27.0 hour sample of waxy maize II starch. $\bar{M}_w = 1.48 \times 10^6$.

Table 24. Acid hydrolysis of 13th day waxy maize III starch.

Refluxed at 99.0°C and pH = 4.24. Theoretical \bar{X}_n based on statistical model.

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n
0	168	3070
3.0	43.0	1550
6.0	18.3	1009
10.0	9.1	709
14.0	5.25	536

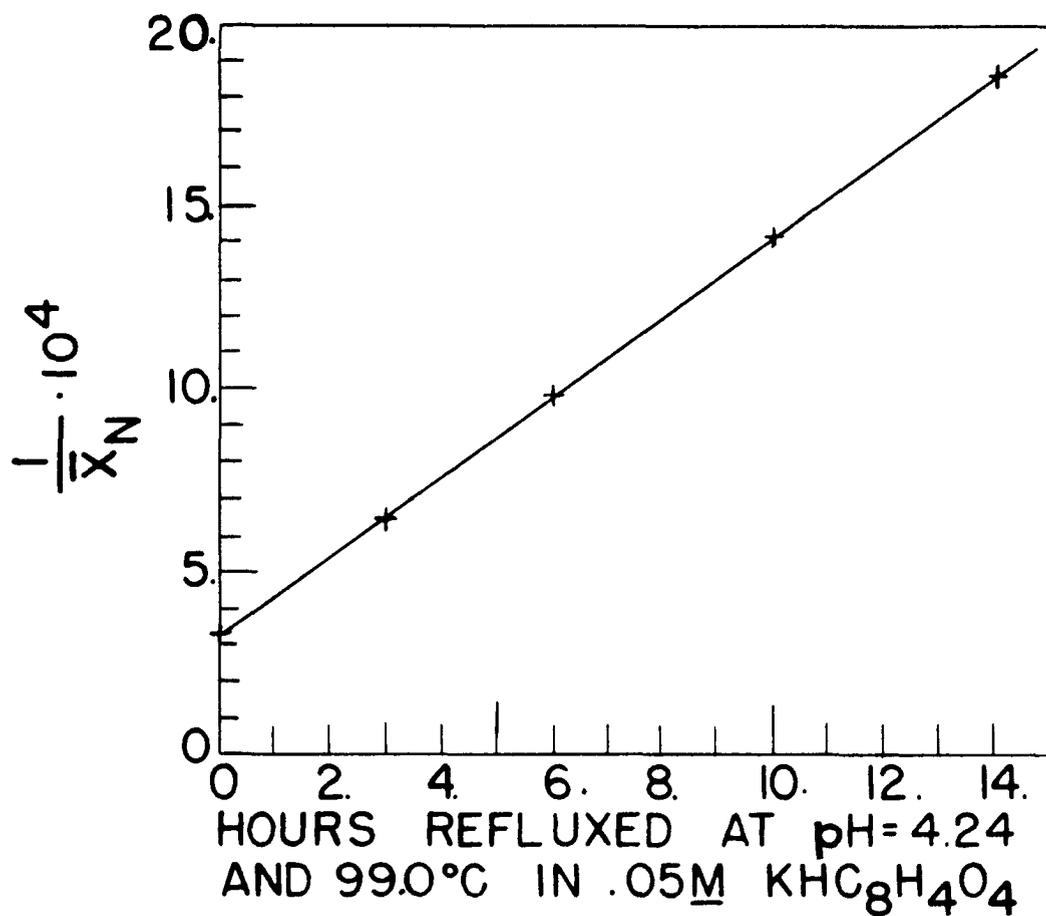
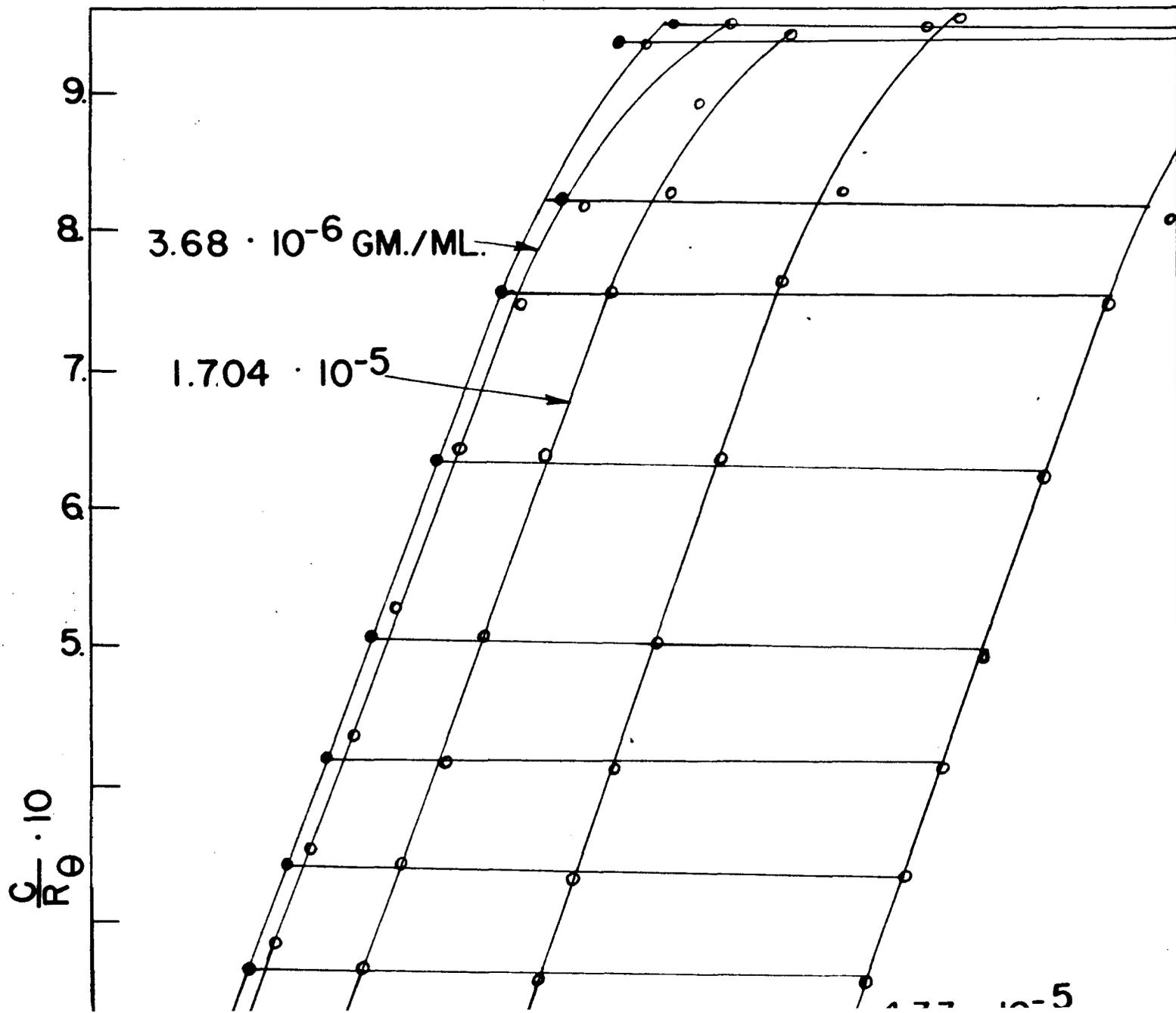


Fig. 31. Acid hydrolysis of 13th day waxy maize starch, sample III. Points were obtained from \bar{M}_w using 5.8% branching for the statistical model.



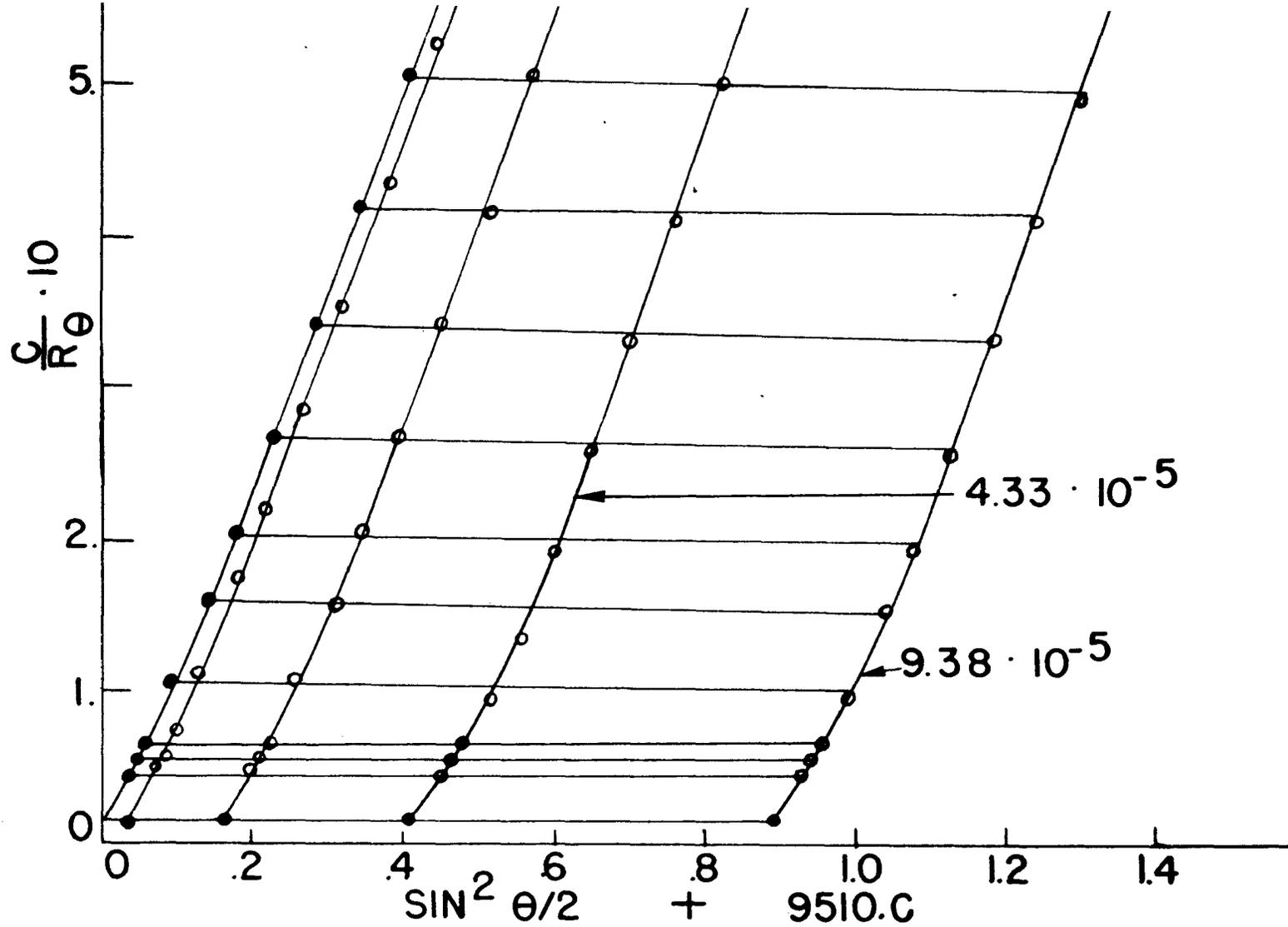


Fig. 32. Waxy maize III starch. $\bar{M}_w = 168. \times 10^6$.

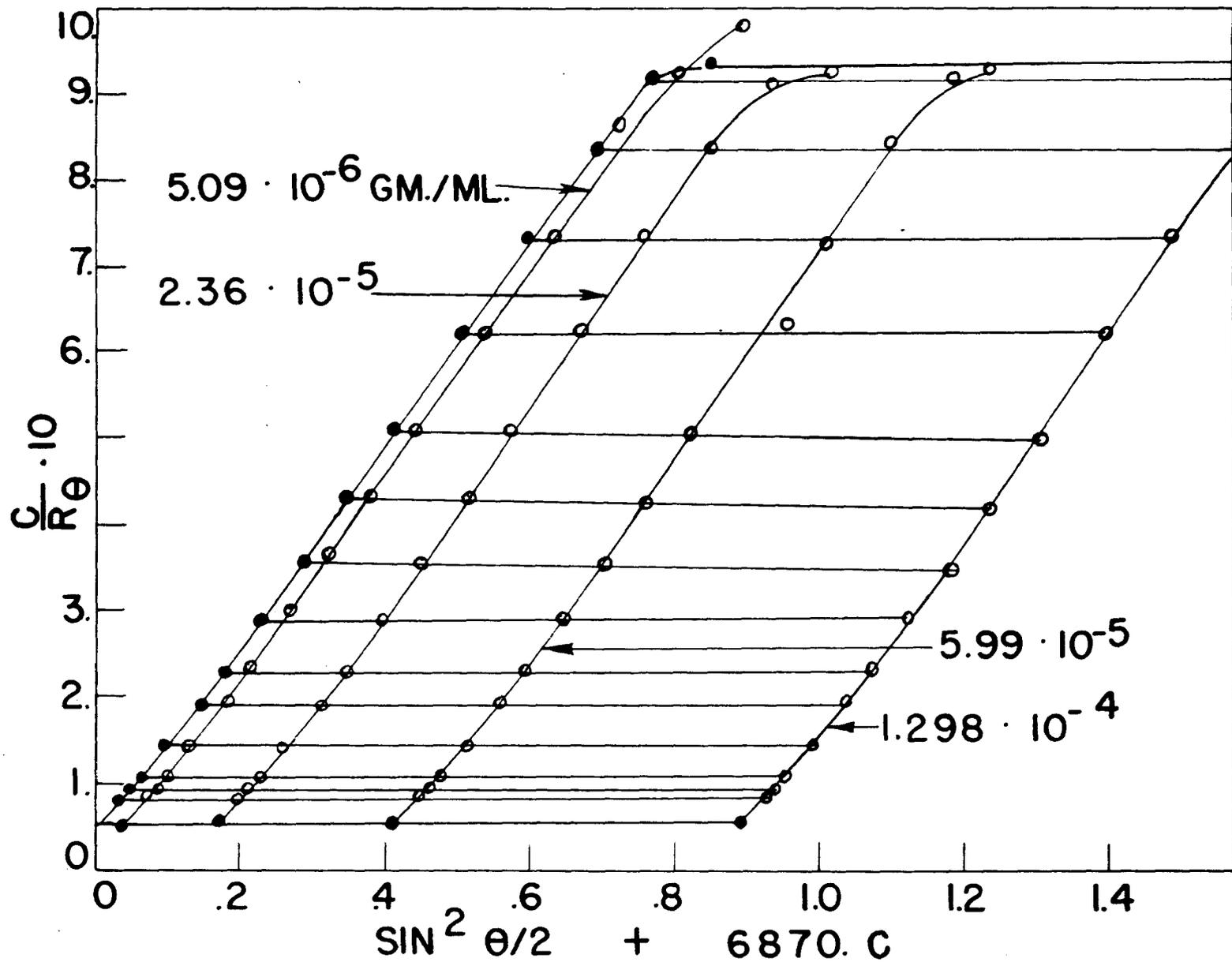


Fig. 33. The 3.0 hour sample of waxy maize III starch. $\bar{M}_w = 43 \times 10^6$.

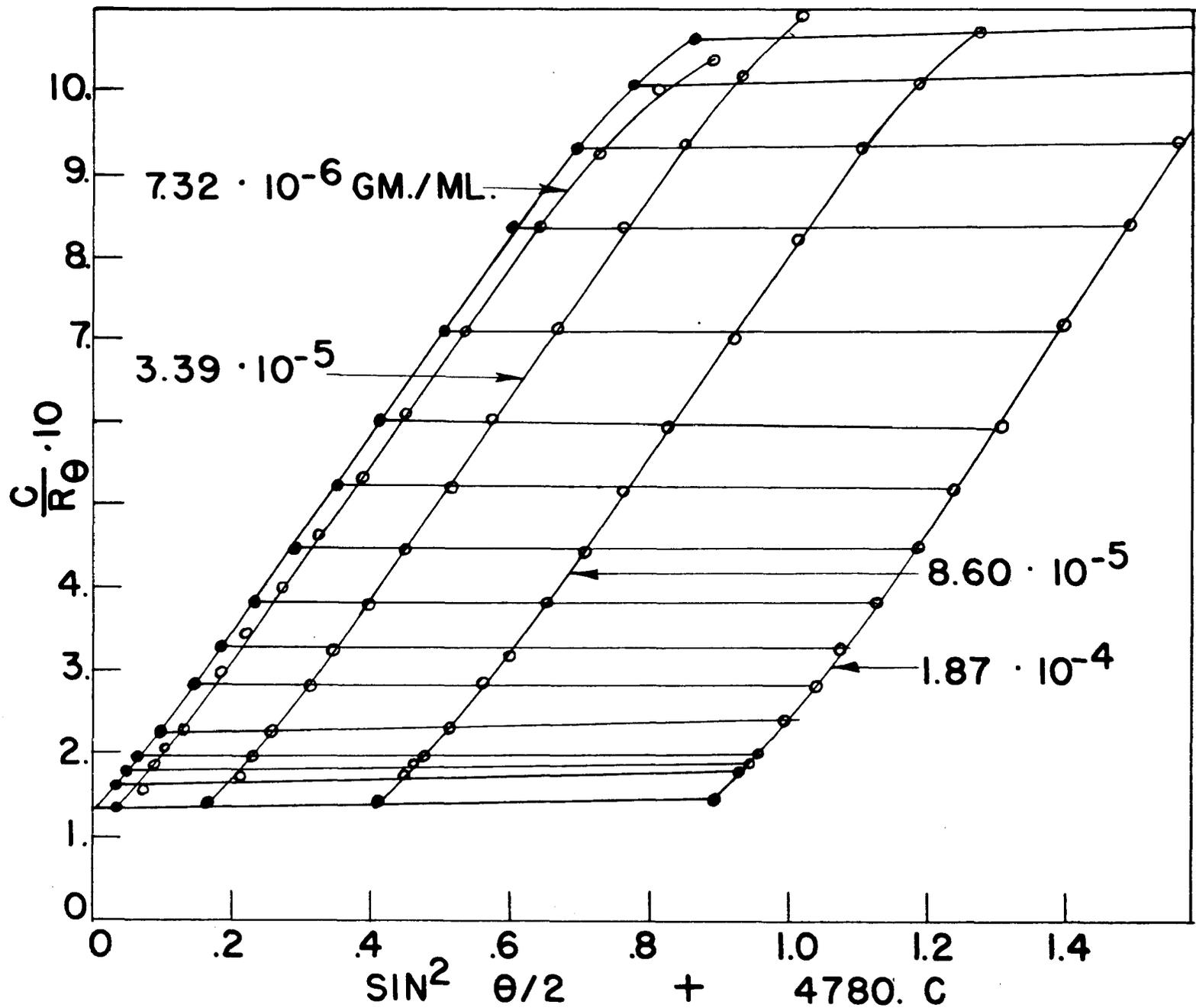


Fig. 34. The 6.0 hour sample of waxy maize III starch. $\bar{M}_w = 18.3 \times 10^6$.

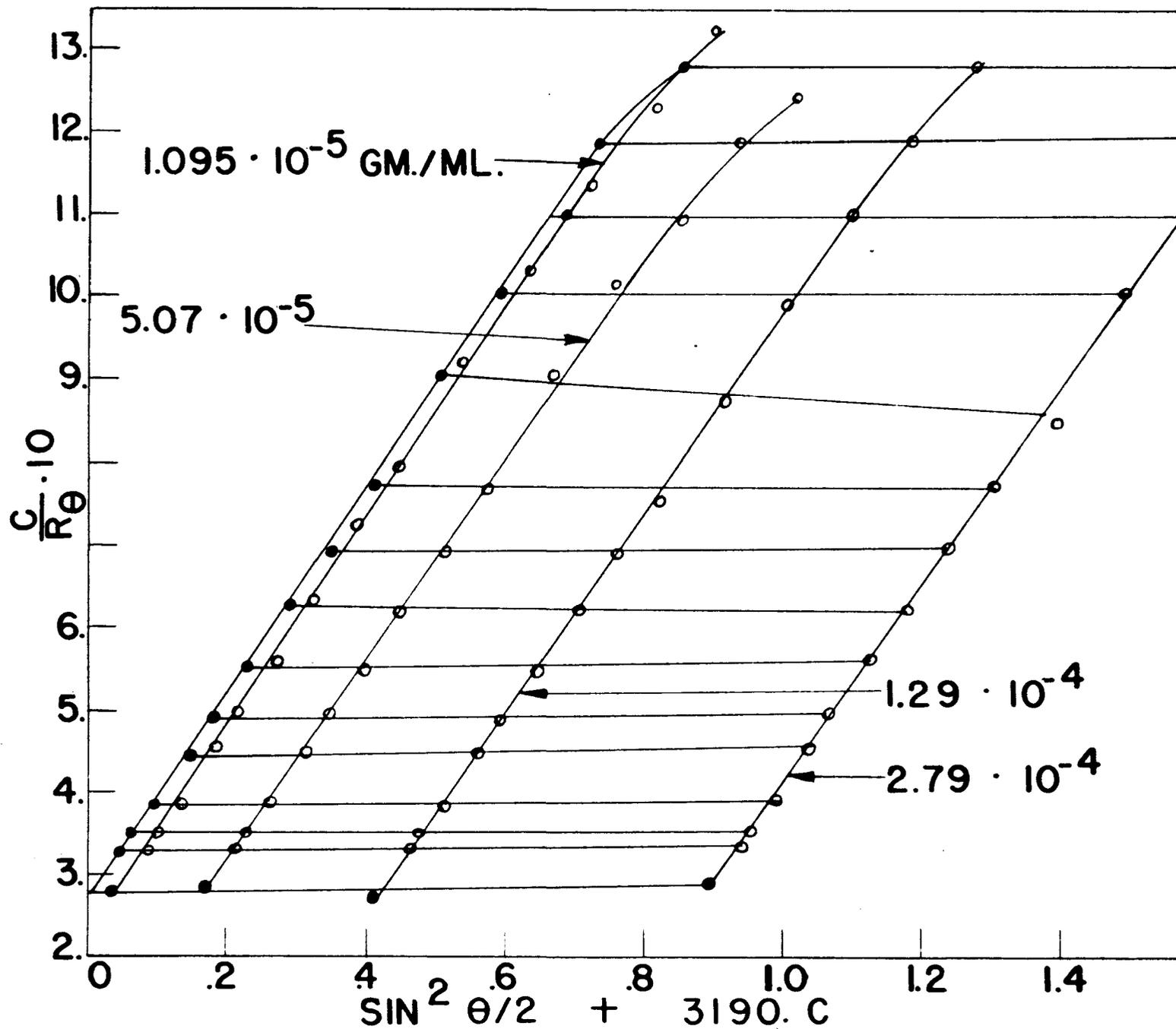


Fig. 35. The 10.0 hour sample of waxy maize III starch. $\bar{M}_w = 9.1 \times 10^6$.

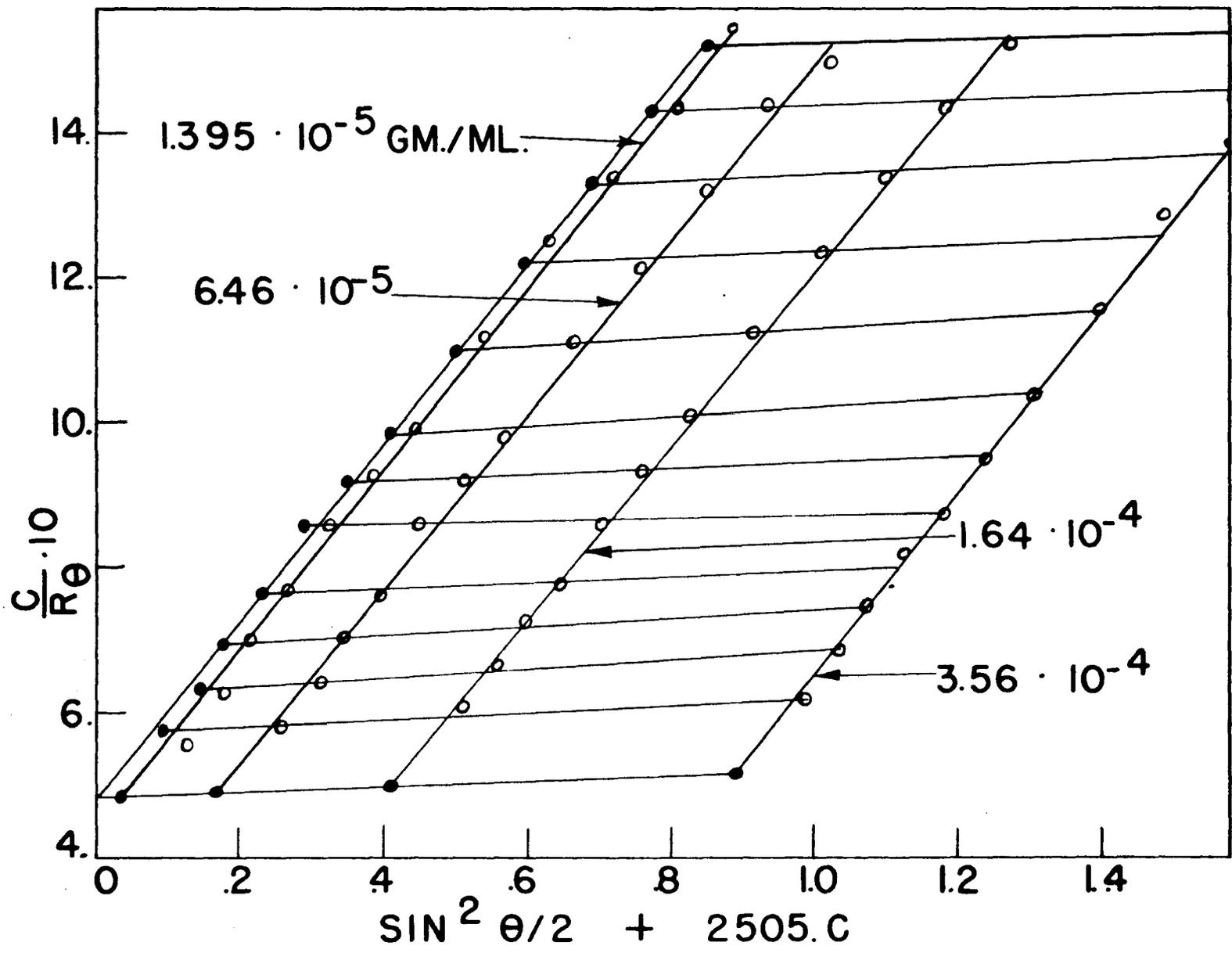


Fig. 36. The 14.0 hour sample of waxy maize III starch. $\bar{M}_w = 5.25 \times 10^6$.

Table 25. Acid hydrolysis of 13th day waxy maize IV starch.

Refluxed at 98.0°C and pH 4.23. Theoretical \bar{X}_n based on statistical model. Observed \bar{X}_n obtained from f. no. and alk. no. according to Kerr et. al. (13).

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n	\bar{X}_n (f.no.)	\bar{X}_n (alk.no.)
0	168	3070	6160	8960 8650
8.0	14.0	881	760	859
19.0	3.76	452	276	493
32.0	1.46	279	182	360

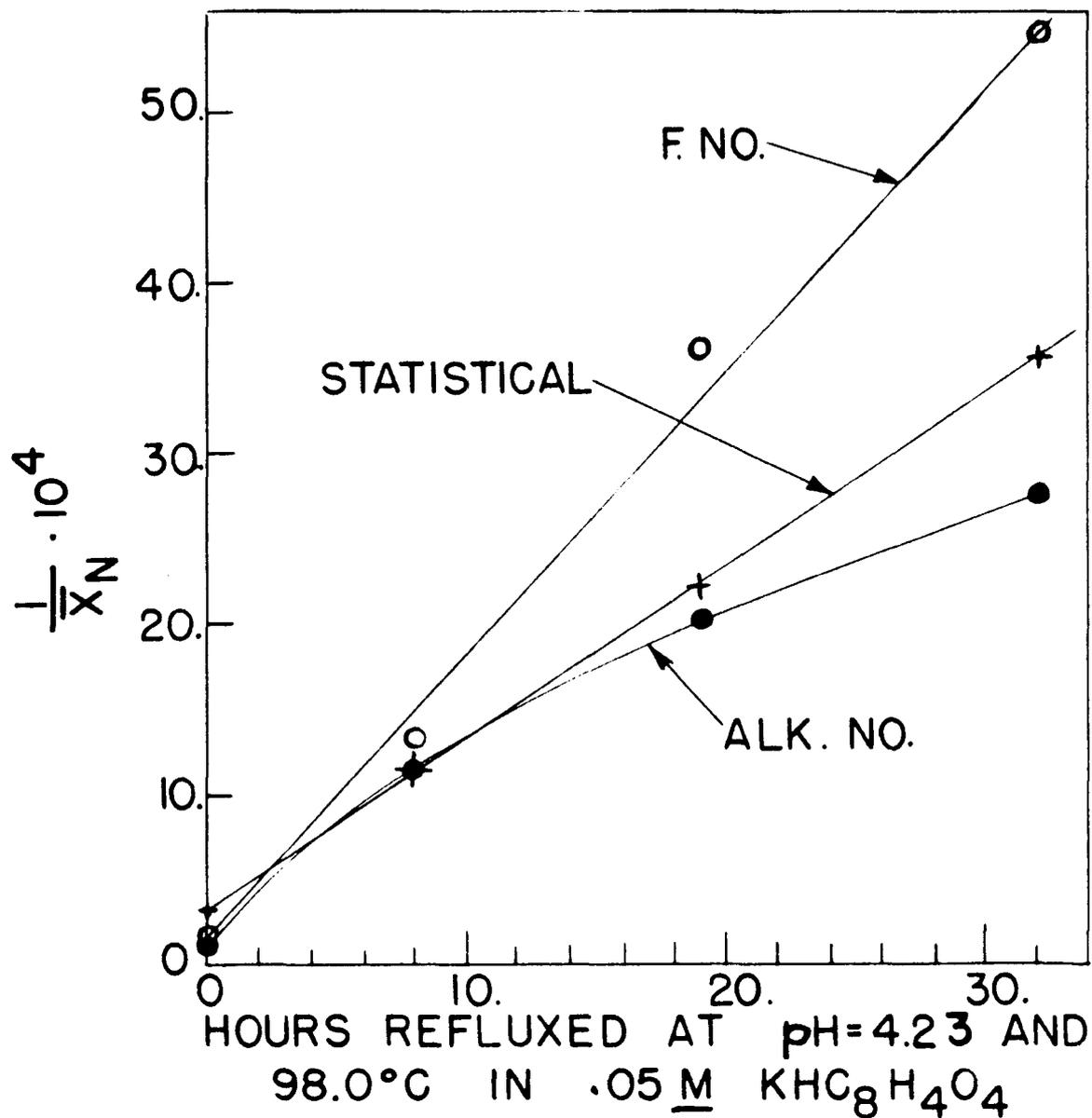
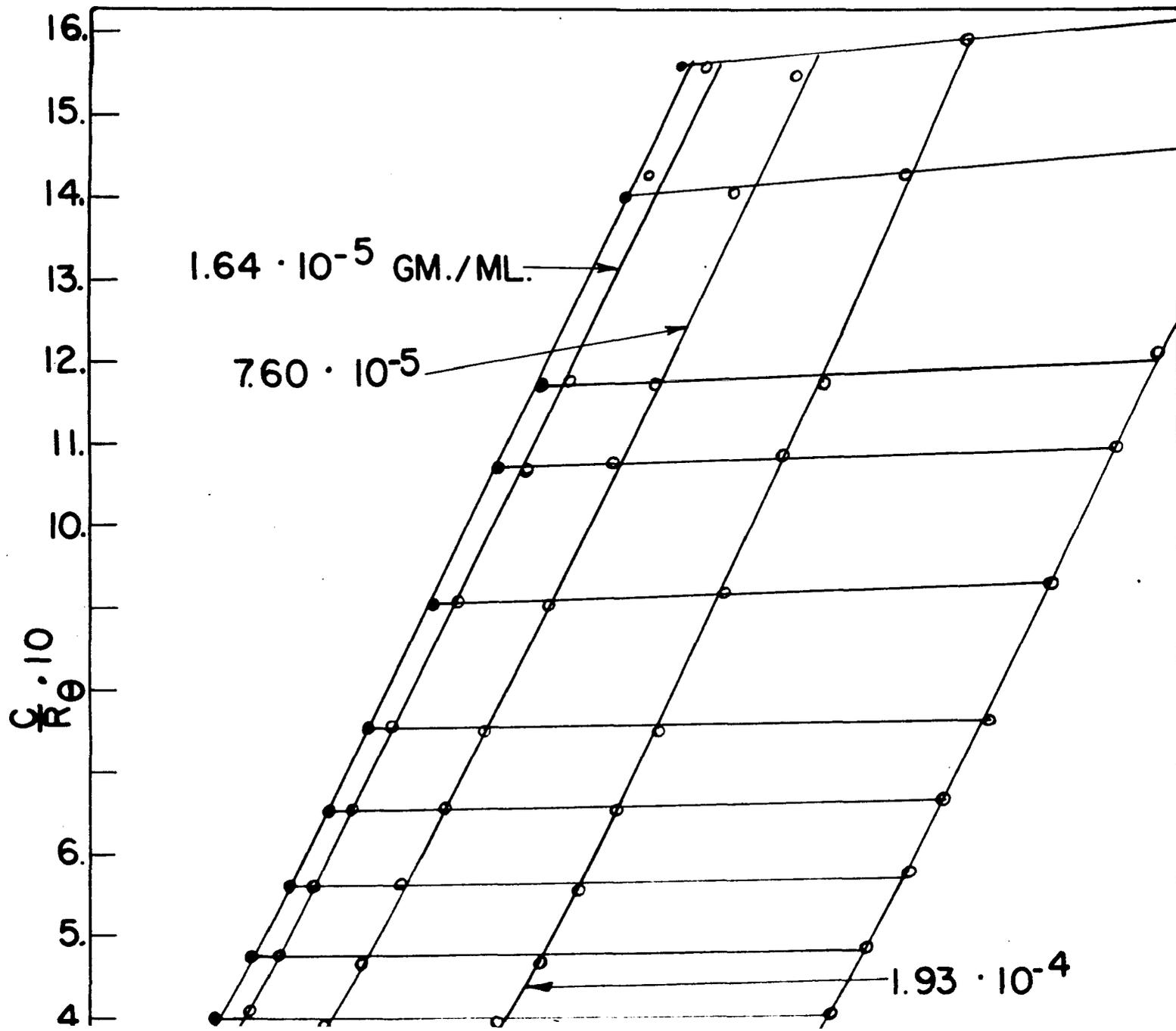


Fig. 37. Acid hydrolysis of 13th day waxy maize starch sample IV. (O) \bar{X}_n obtained from f. no. (●) from alk. no. (+) from light scattering using 5.8% branching for the statistical model.



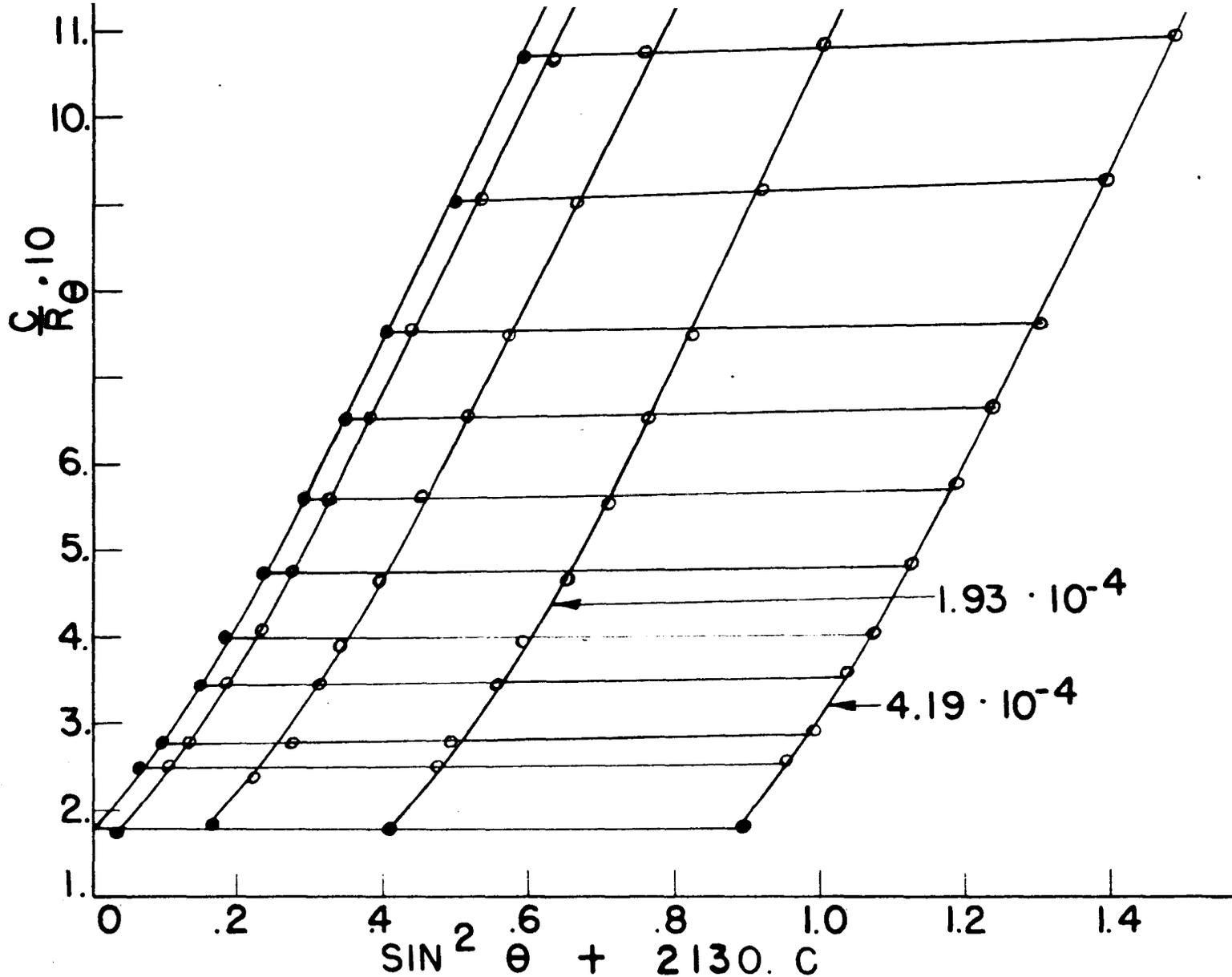


Fig. 38. The 8.0 hour sample of waxy maize IV starch. $\bar{M}_w = 14.0 \times 10^6$.



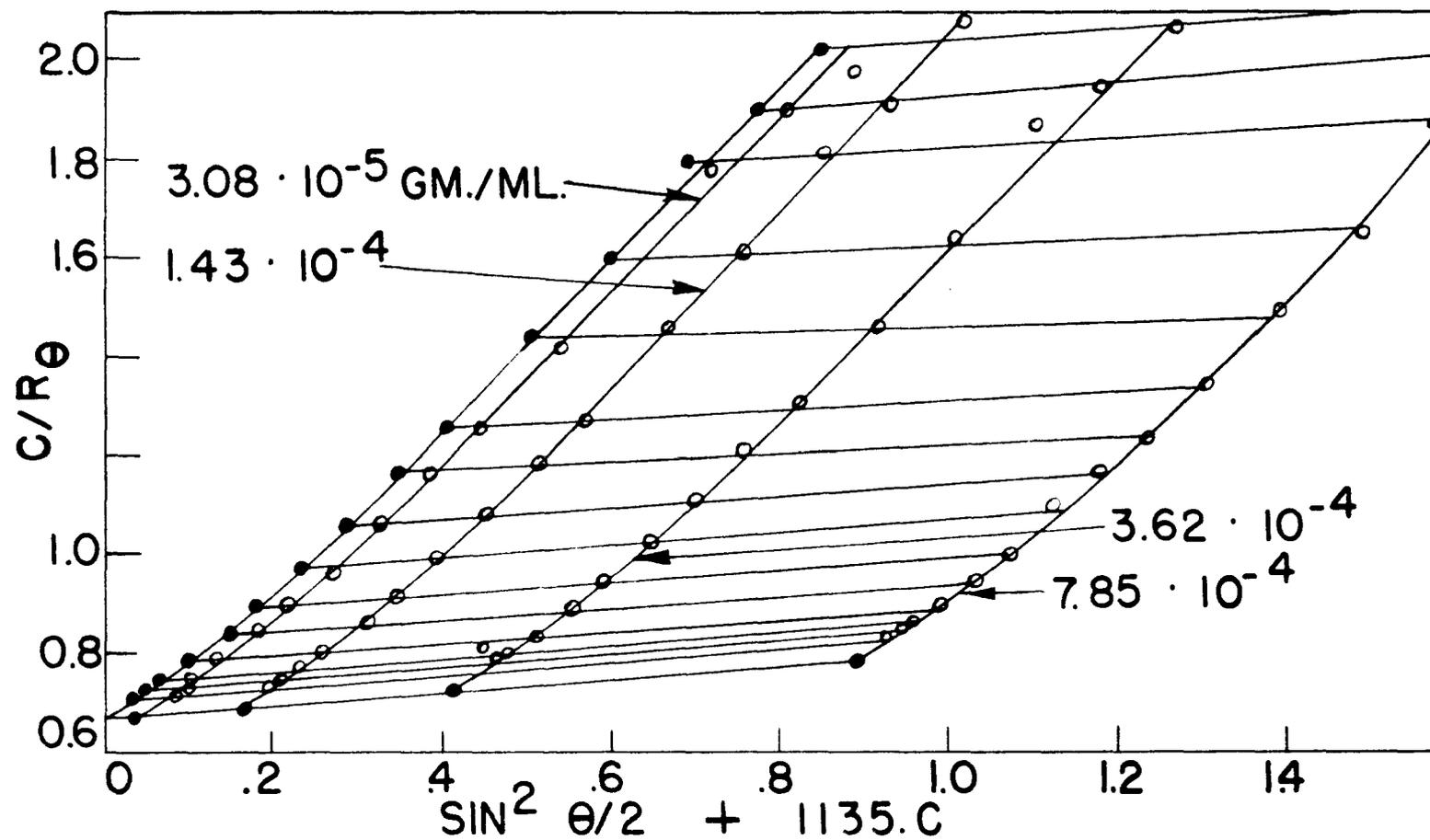


Fig. 39. The 19.0 hour sample of waxy maize IV starch. $\bar{M}_w = 3.76 \times 10^6$.

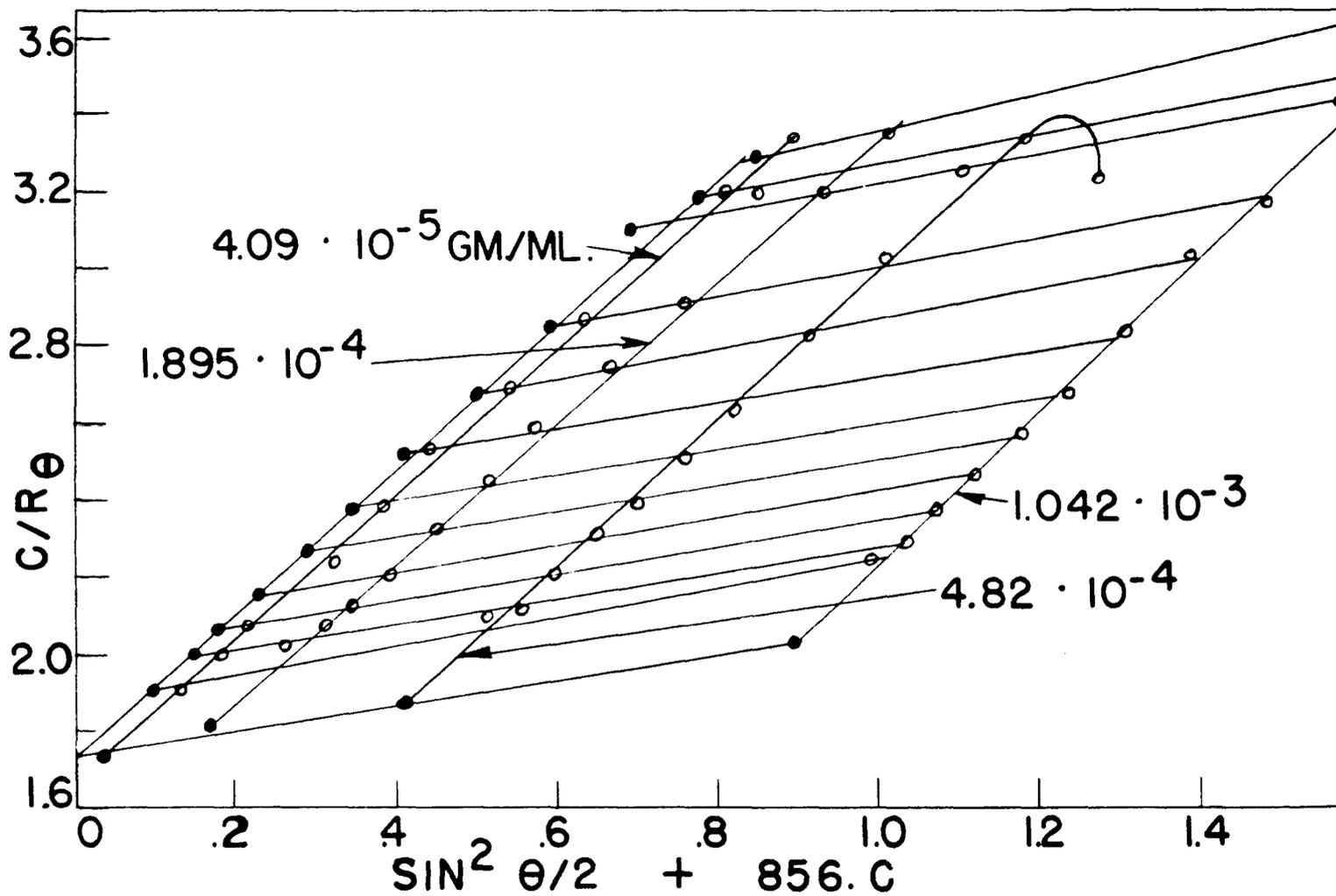


Fig. 40. The 32.0 hour sample of waxy maize IV starch. $\bar{M}_w = 1.46 \times 10^6$.

Table 26. Acid hydrolysis of mature dent corn amylopectin.

Refluxed at 99.5°C and pH = 4.22. Theoretical \bar{X}_n based on statistical model assuming 4%, 6%, and 50% branching. Observed \bar{X}_n obtained from alk. no. and f. no. according to Kerr *et. al.* (13).

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n	Theor. \bar{X}_n	Theor. \bar{X}_n	\bar{X}_n (f. no.)	\bar{X}_n (alk. no.)
0	45	1890	1560	745	-	3090
10.0	6.50	710	588	282	342	420
22.0	2.23	411	338	165	169	386

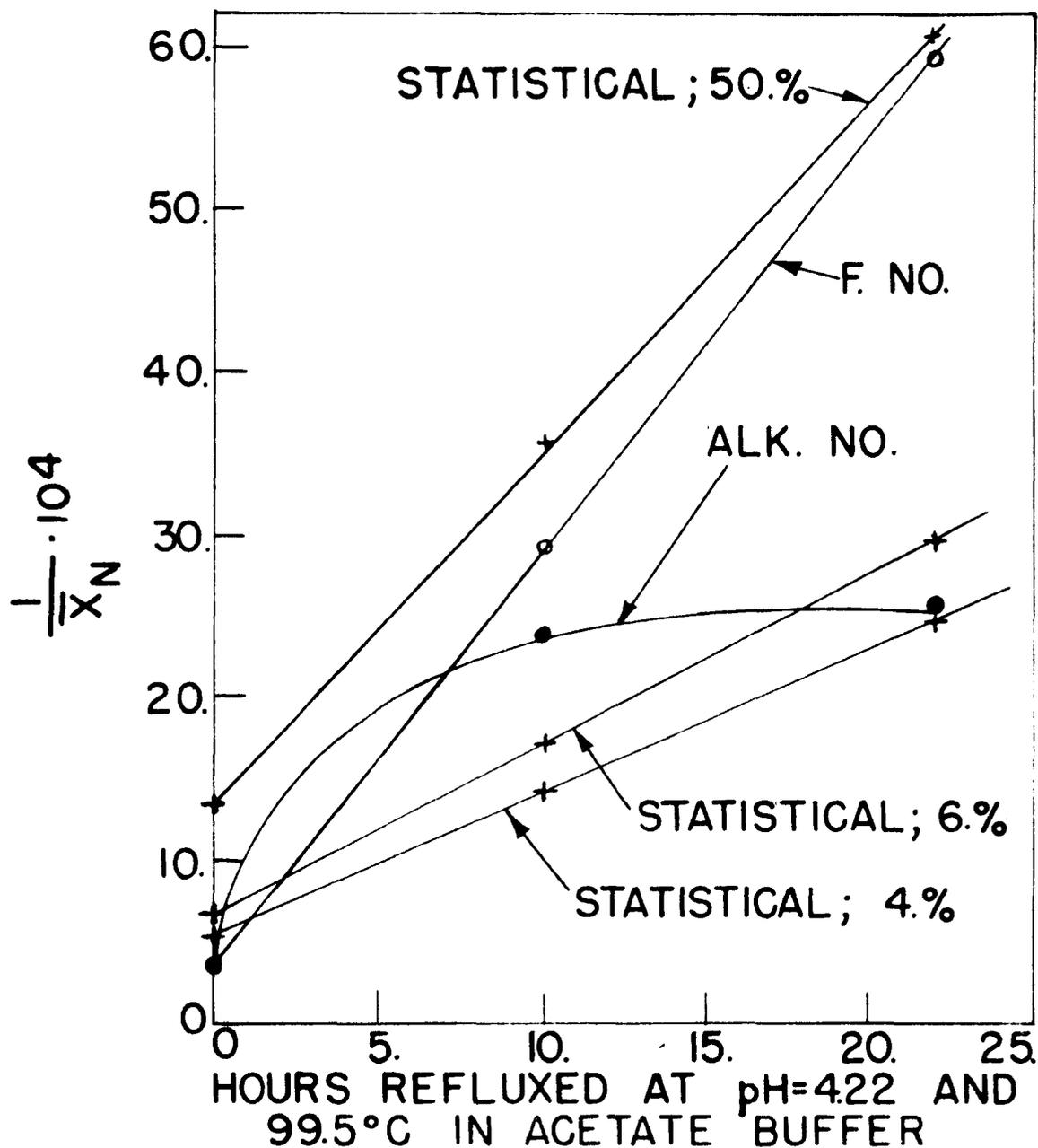
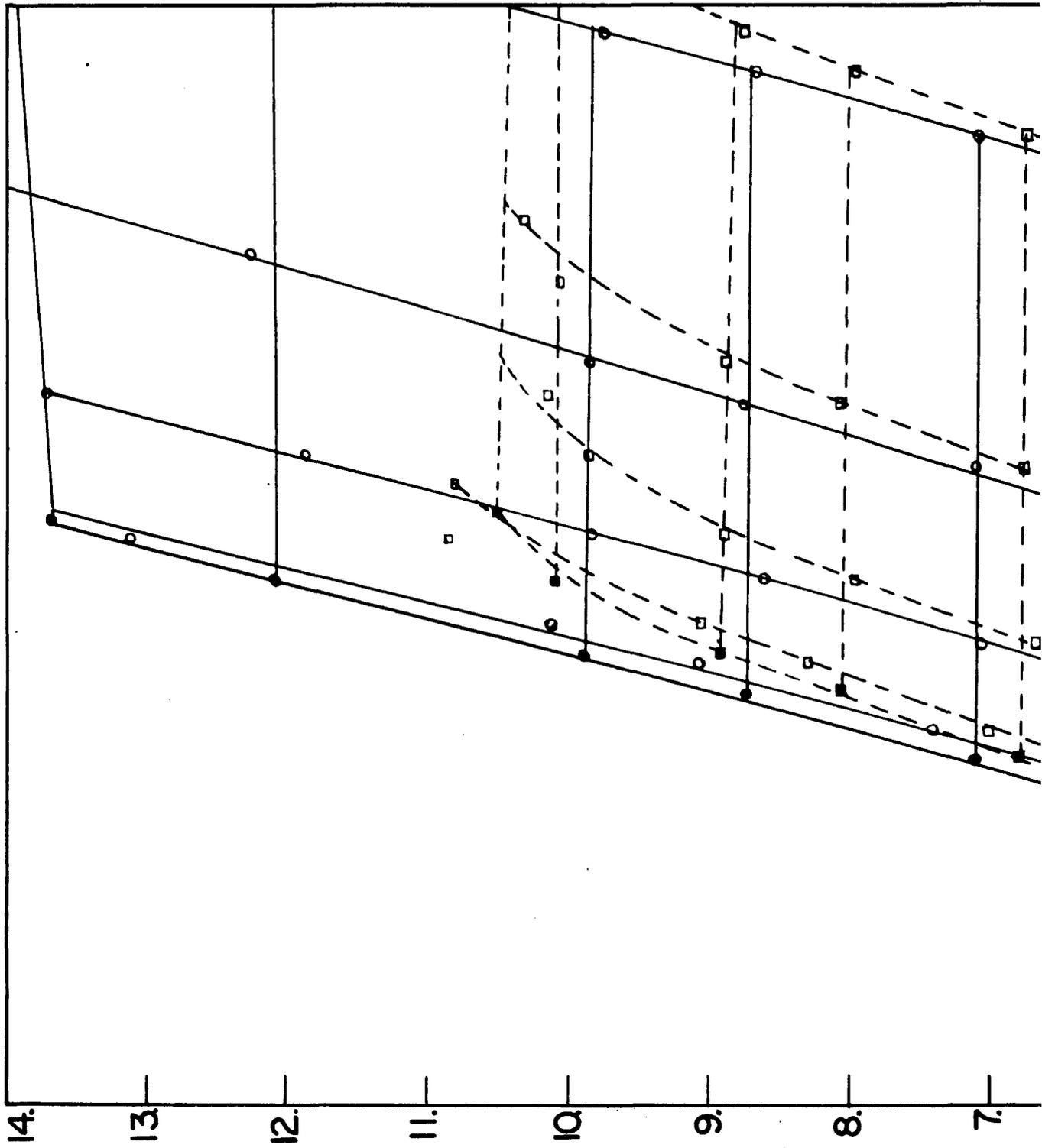


Fig. 41. Acid hydrolysis of mature dent corn amylopectin.
 (o) \bar{X}_n from f. no.; (•) \bar{X}_n from alk. no.; (+) \bar{X}_n from
 light scattering using 4%, 6%, and 50% degree branching
 for the statistical model.





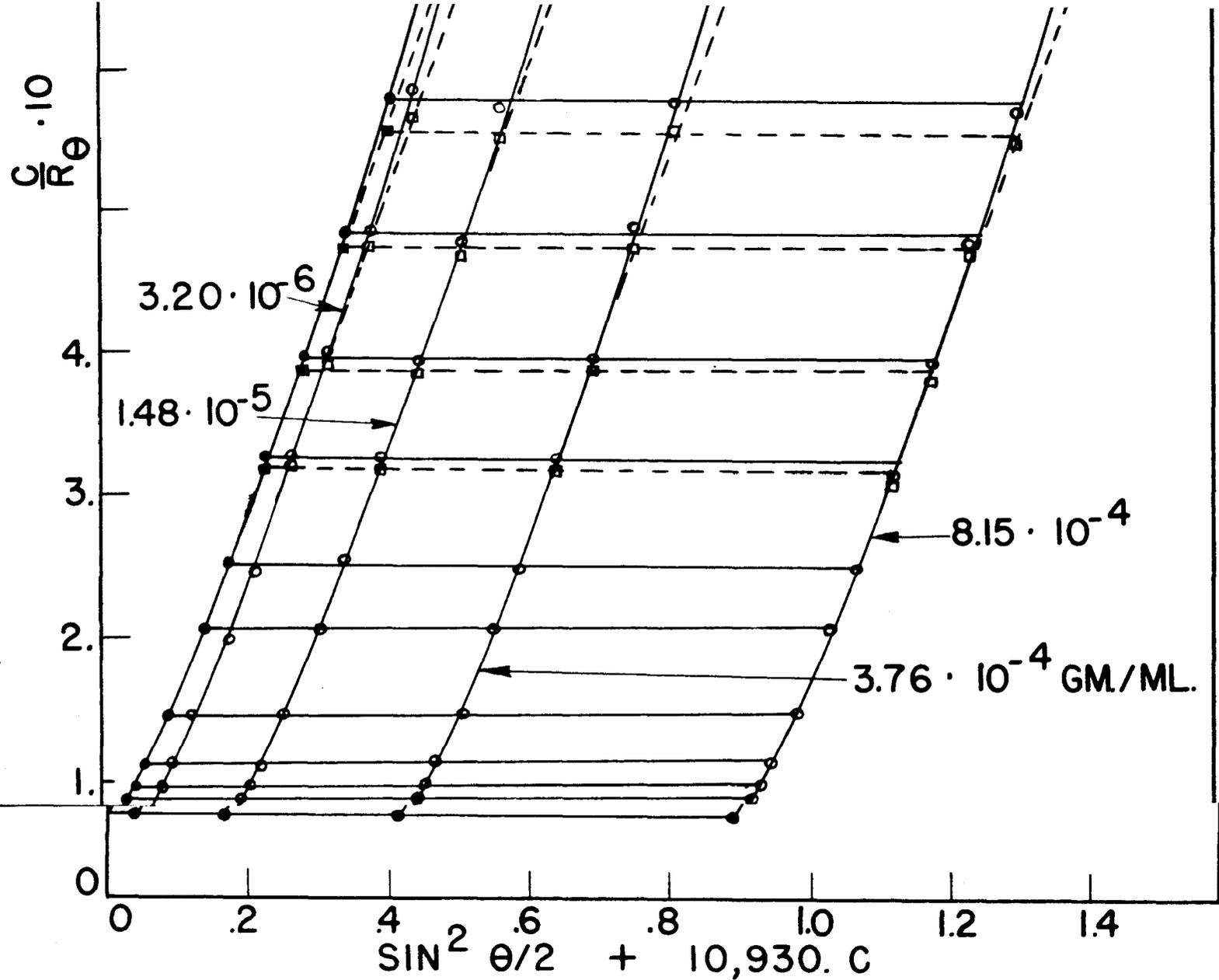
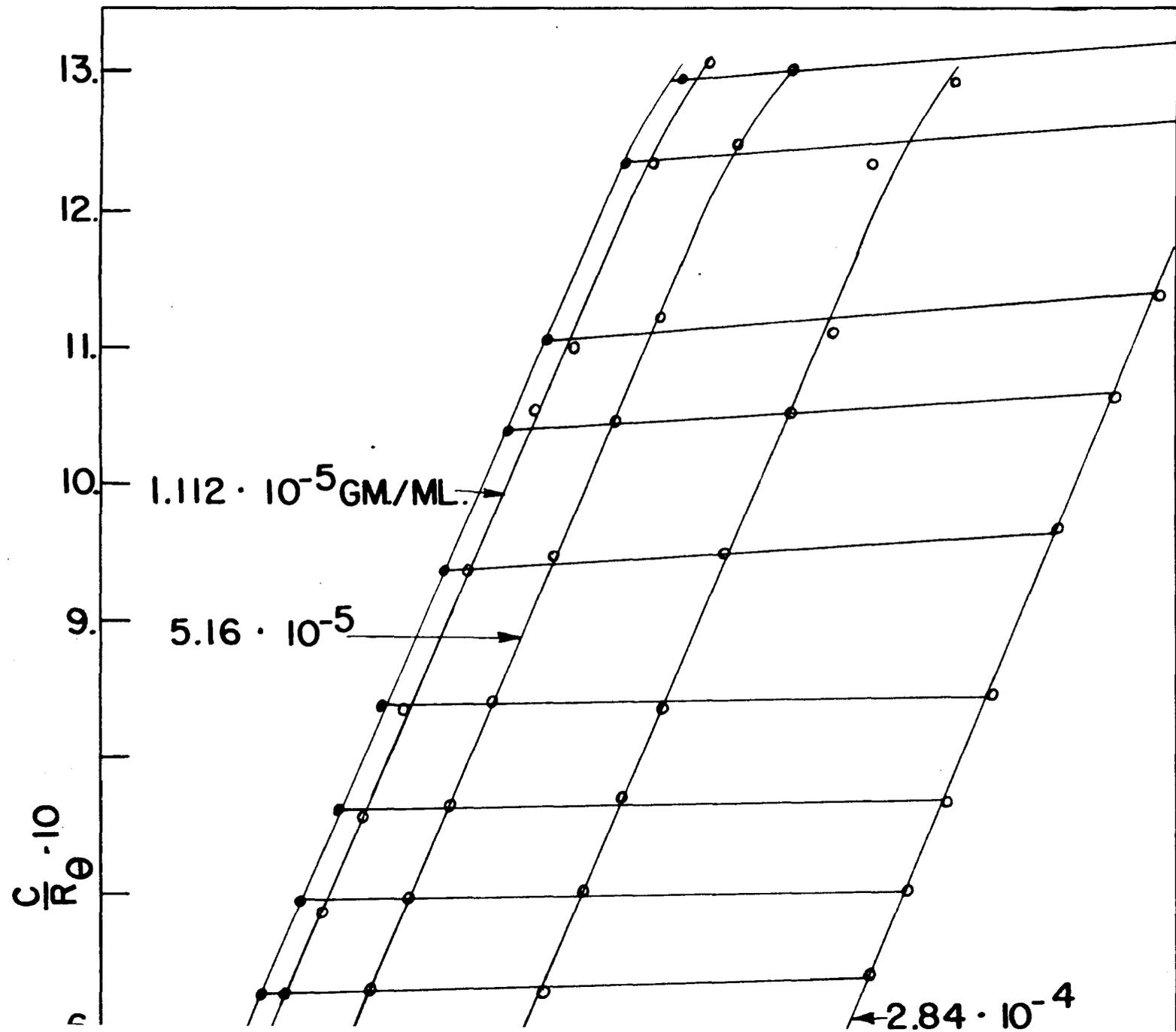


Fig. 42. Mature dent corn amylopectin. $\bar{M}_w = 45 \times 10^6$. Solid and dotted lines represent reflection corrected and uncorrected Zimm plots, respectively.



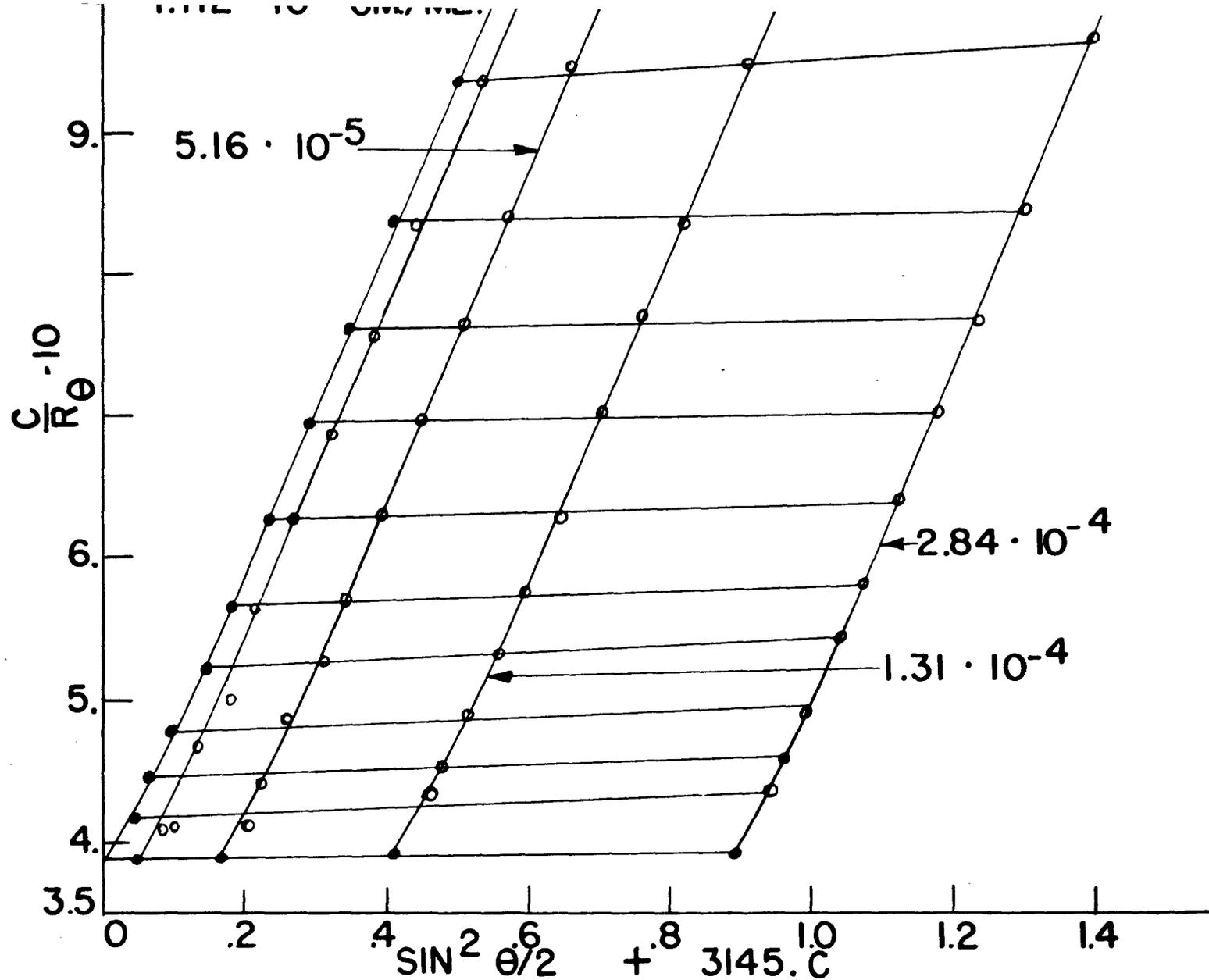


Fig. 43. The 10.0 hour sample of mature dent corn amylopectin. $\bar{M}_w = 6.50 \times 10^6$.

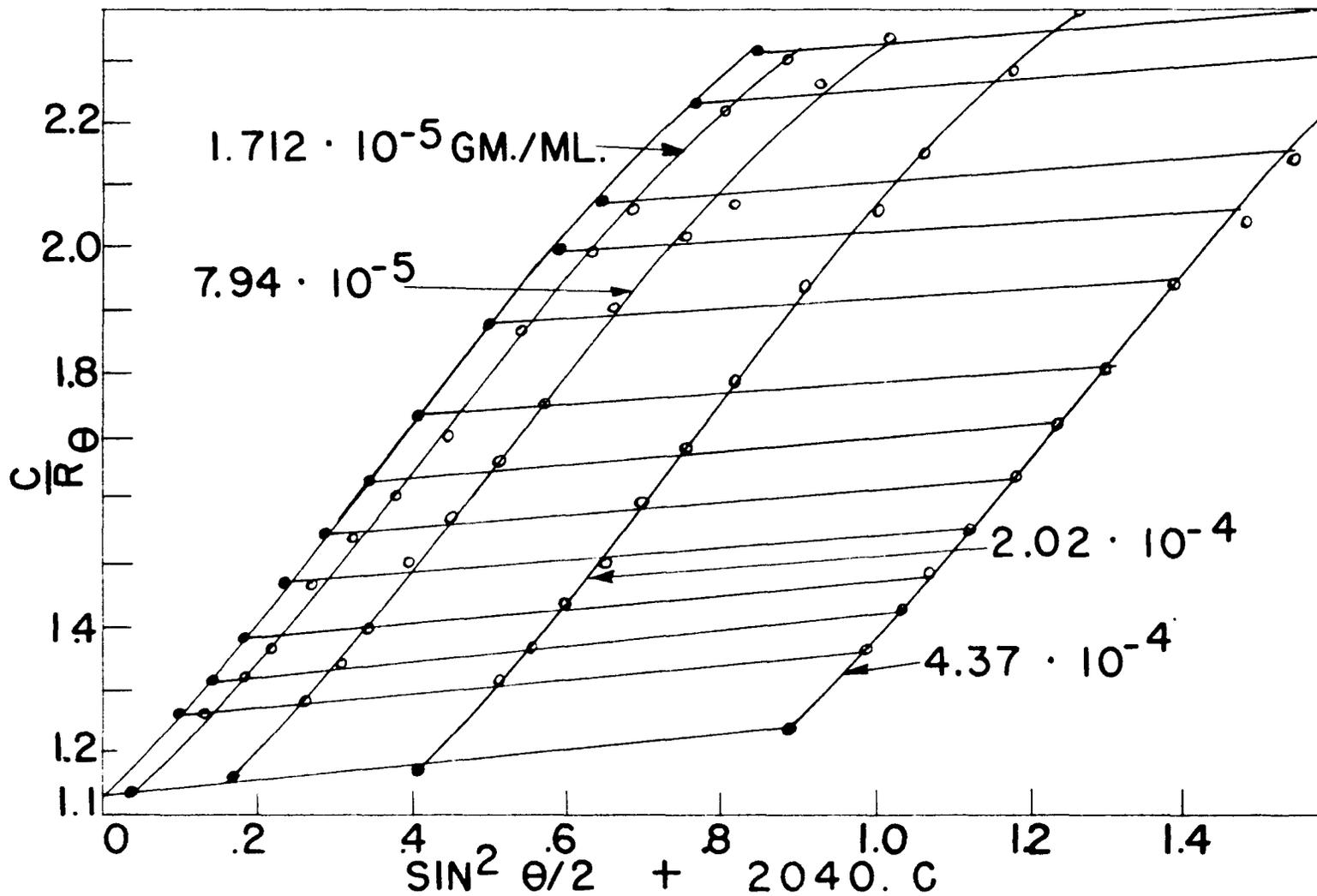
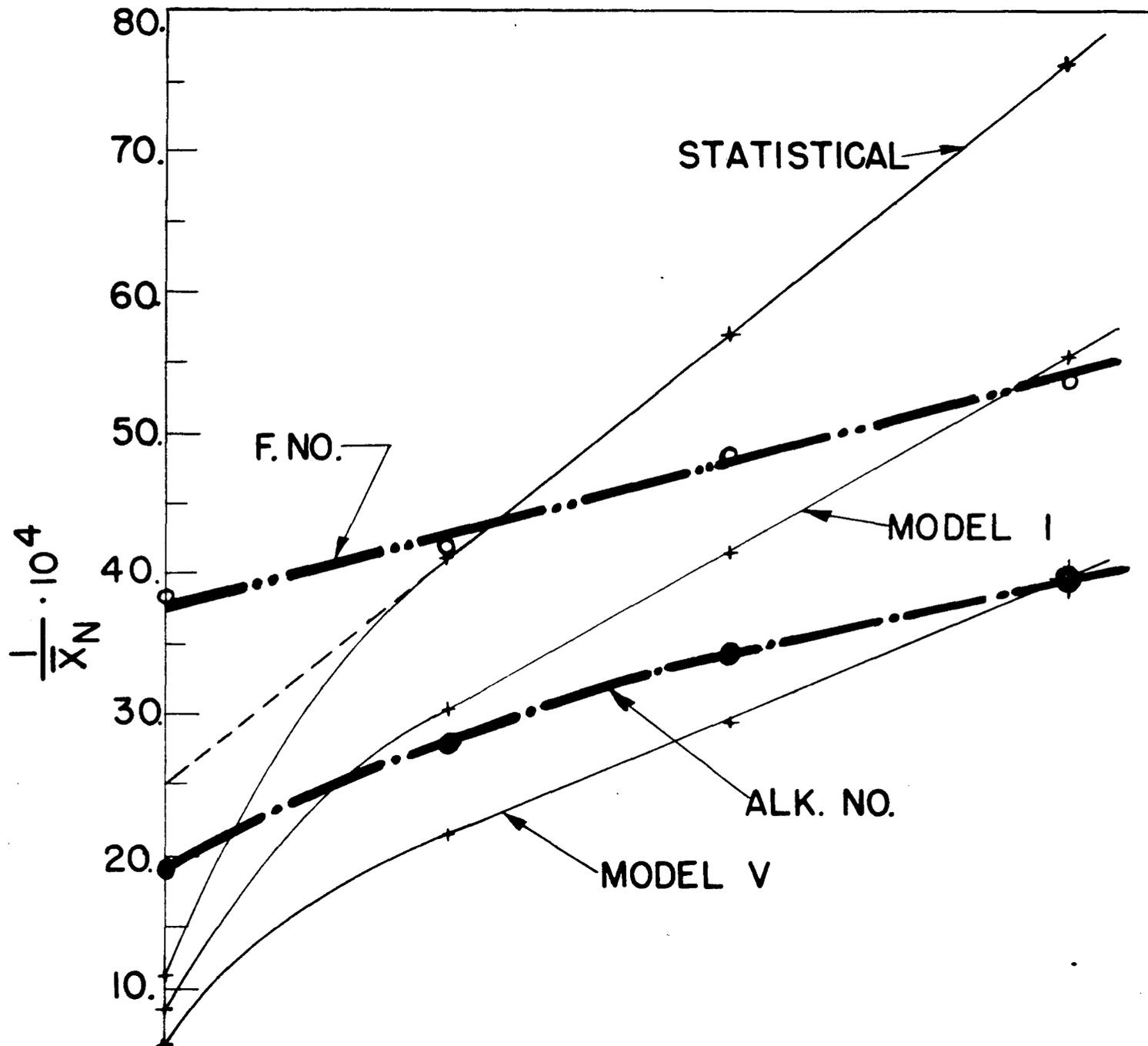


Fig. 44. The 22.0 hour sample of mature dent corn amylopectin. $\bar{M}_w = 2.23 \times 10^6$.

Table 27. Acid hydrolysis of 14th day sweet corn amylopectin.

Refluxed at 99.5°C and pH = 4.22. Theoretical \bar{X}_n based on statistical and less random models I and V. Observed \bar{X}_n obtained from f. no. and alk. no. according to Kerr et. al. (13).

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	\bar{X}_n (Stat.)	\bar{X}_n (Model I)	\bar{X}_n (Model V)	\bar{X}_n (f. no.)	\bar{X}_n (alk. no.)
0	15.8	810	1100	1575	260	524
10.0	1.48	243	332	466	240	361
20.0	0.795	176	242	339	207	291
32.0	0.451	131	181	252	186	252



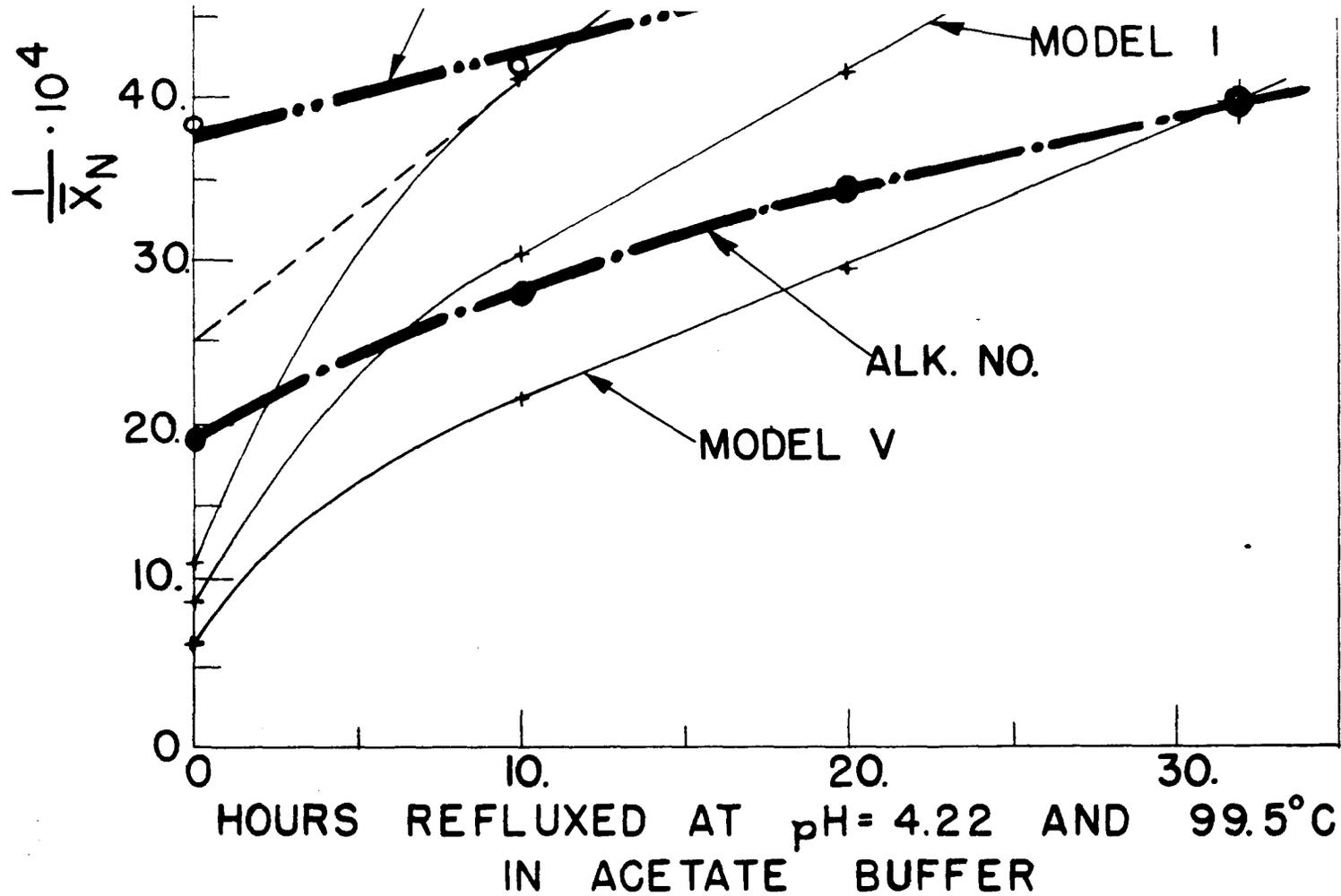
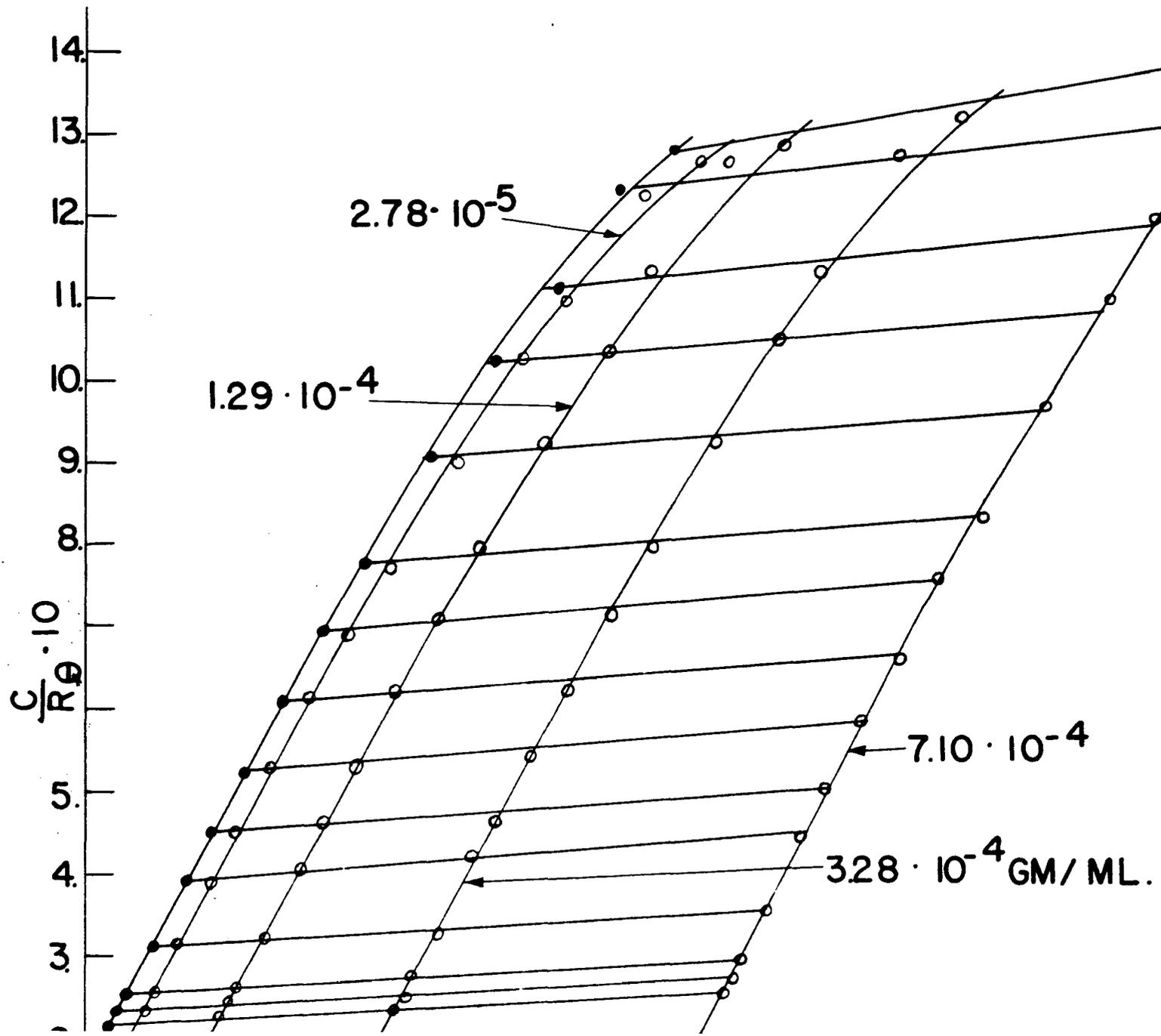


Fig. 45. Acid hydrolysis of 14th day sweet corn amylopectin. (O) \bar{X}_n from f.no.; (●) from alk. no.; (+) from light scattering using 8.0% branching for the statistical and two less random statistical models.



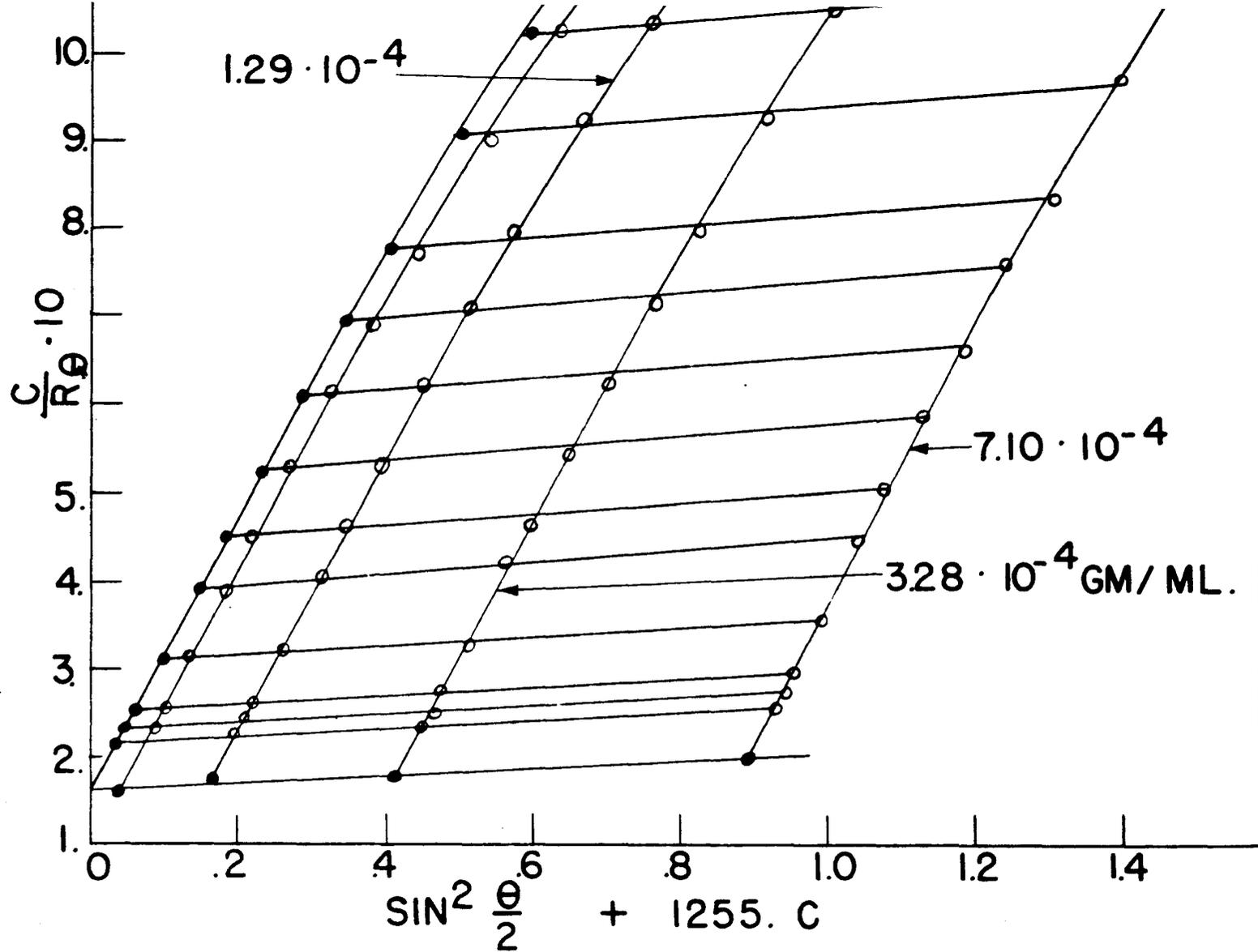


Fig. 46. The 14th day sweet corn amylopectin. $\bar{M}_w = 15.8 \times 10^6$.

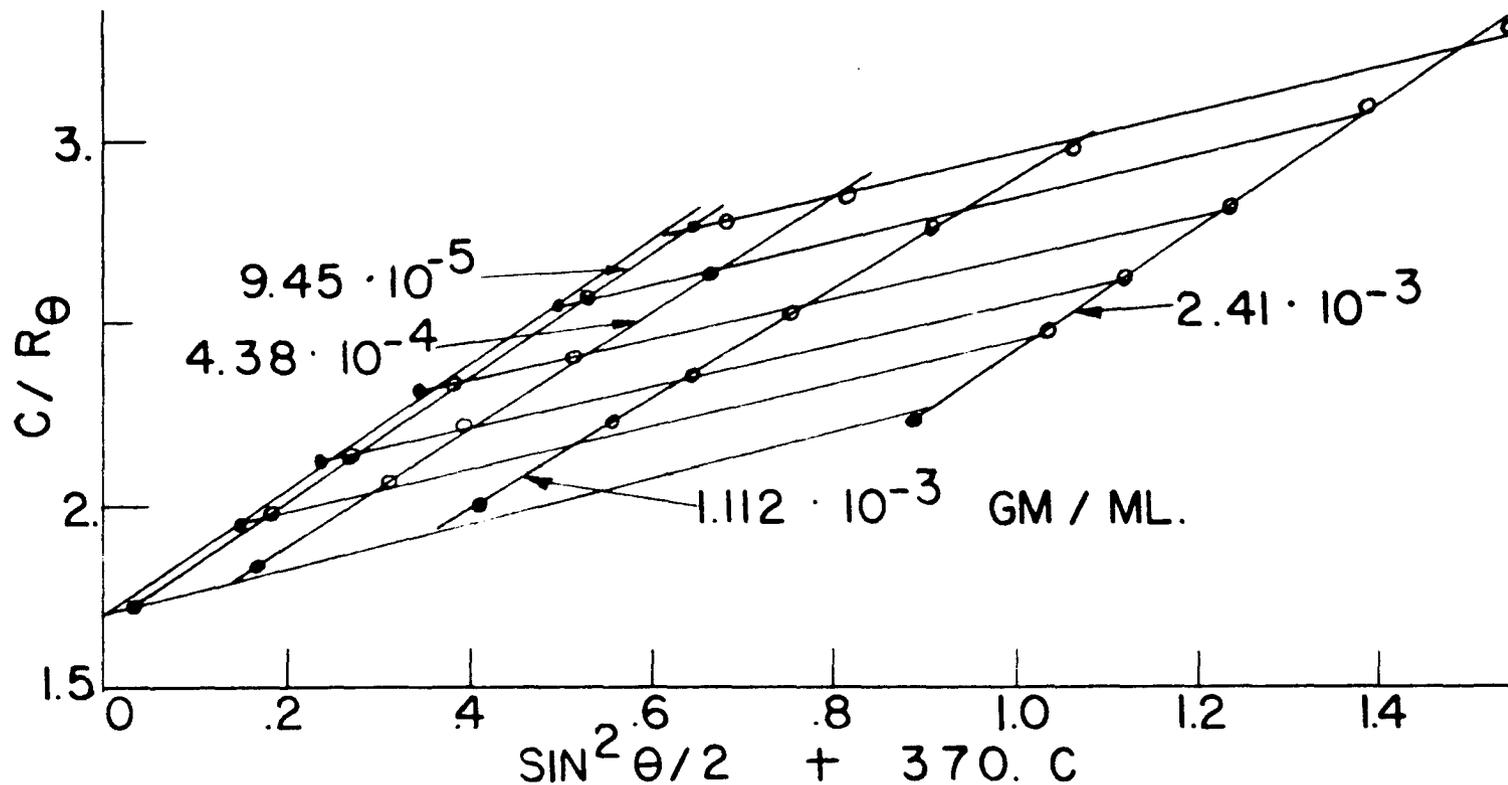


Fig. 47. The 10.0 hour sample of 14th day sweet corn amylopectin. $\bar{M}_w = 1.48 \times 10^6$.

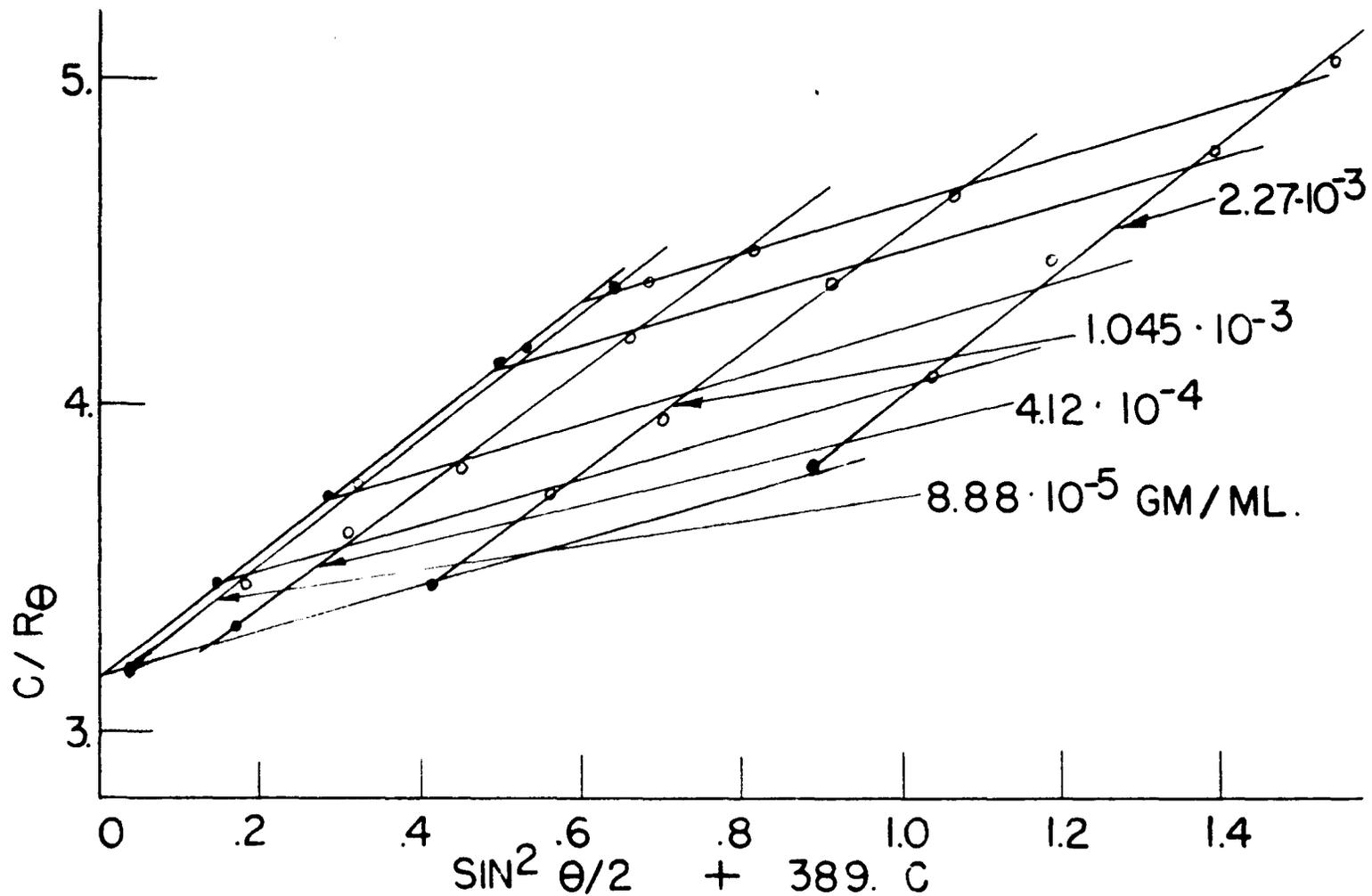
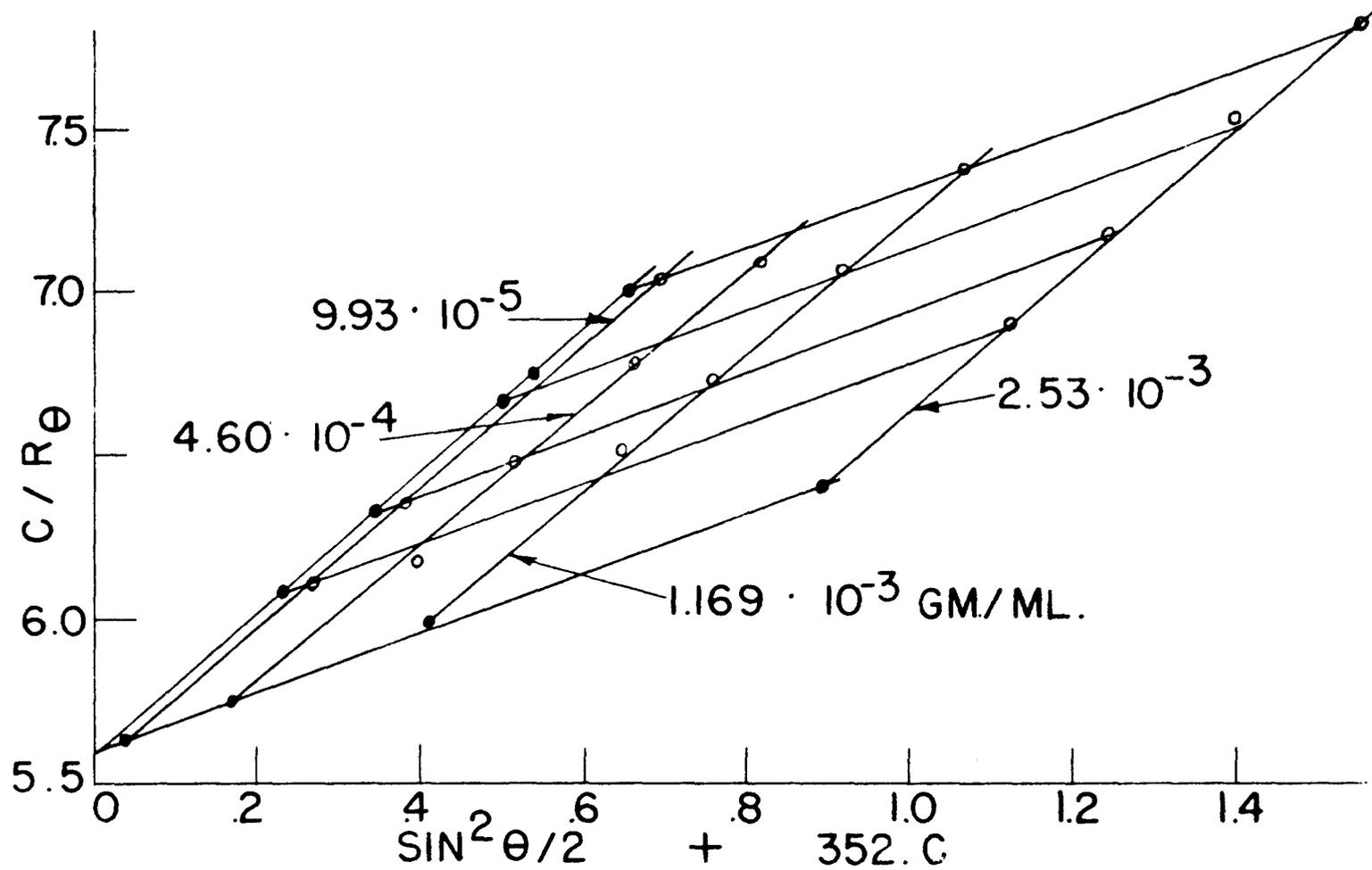


Fig. 48. The 20.0 hour sample of 14th day sweet corn anylopectin. $\bar{M}_w = 795,000$.



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Fig. 49. The 32.0 hour sample of 14th day sweet corn amylopectin. $\bar{M}_w = 451,000$.

Table 28. Acid hydrolysis of 20th day sweet corn amylopectin.

Refluxed at 99.8°C and pH = 4.22. Theoretical \bar{X}_n obtained from \bar{M}_w and $\bar{M}_w/4$ assuming the statistical model. Observed \bar{X}_n obtained from alk. no. and f. no. according to Kerr *et. al.* (13).

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	\bar{X}_n (\bar{M}_w)	\bar{X}_n ($\bar{M}_w/4$)	\bar{X}_n (f. no.)	\bar{X}_n (alk. no.)
0	43	1320	659	717	702
10.57	6.0	490	242	406	742
20.25	2.80	332	164	278	268
33.5	1.22	218	106	243	248

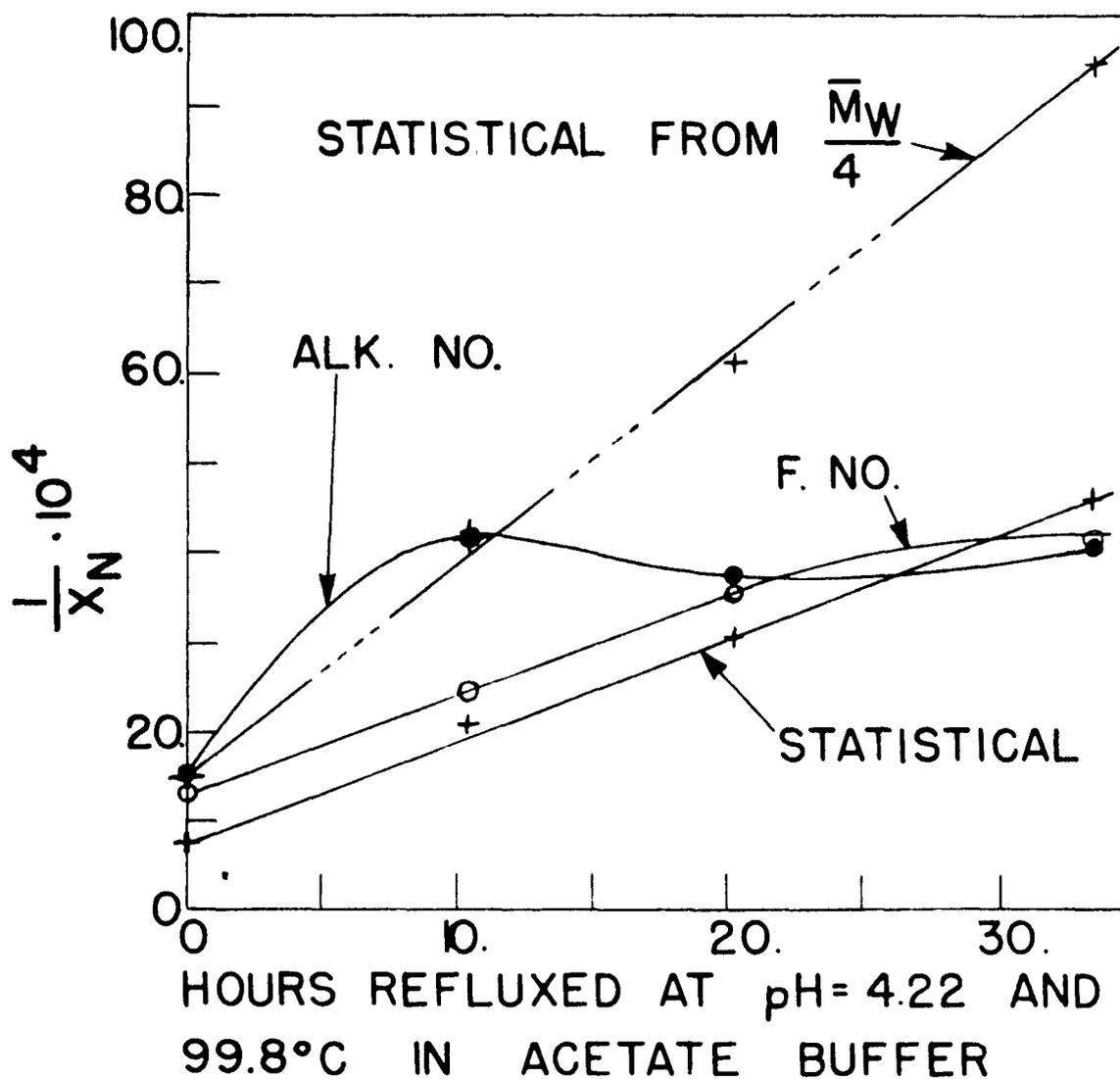
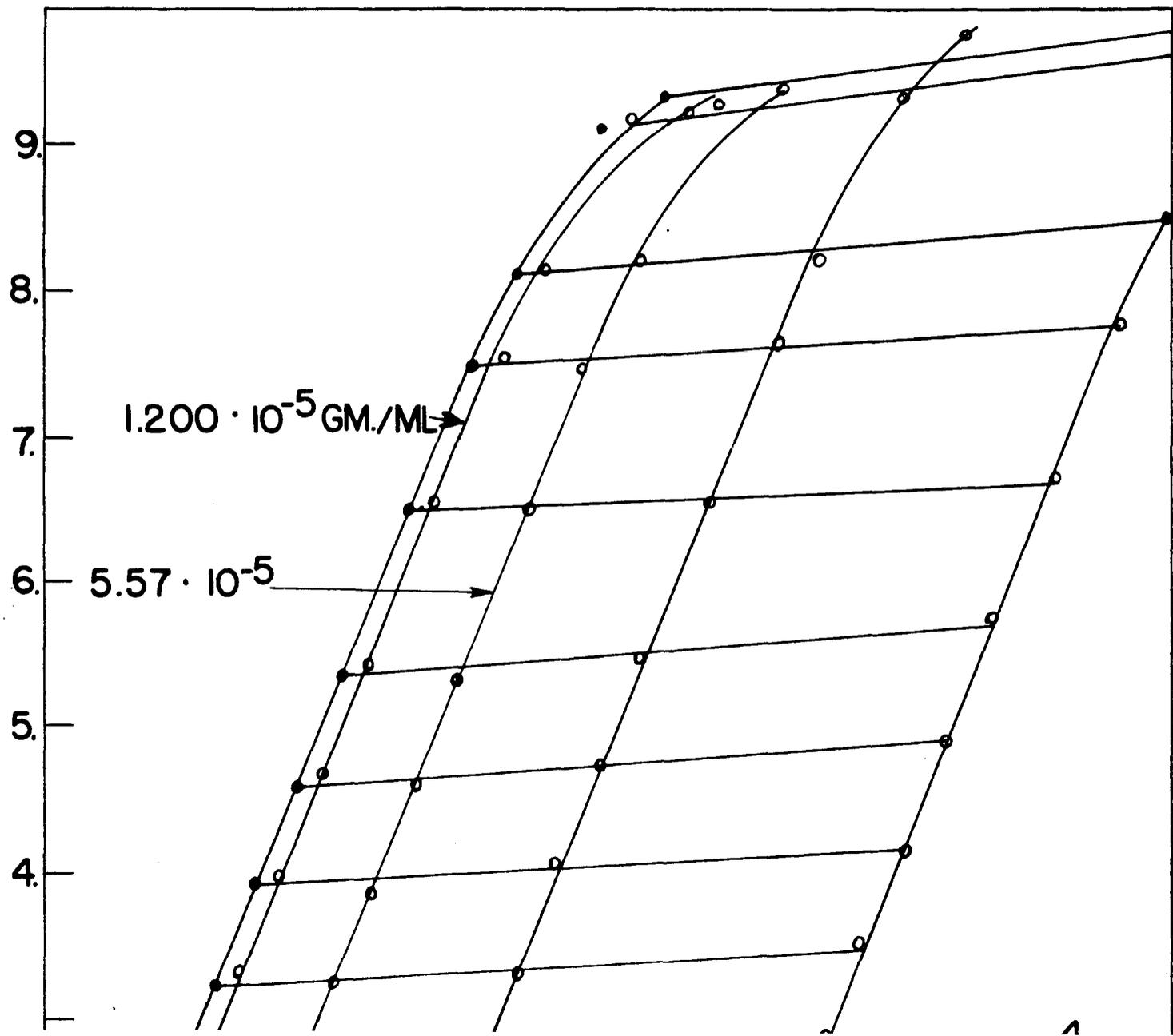


Fig. 50. Acid hydrolysis of 20th day sweet corn amylopectin.
 (O) \bar{X}_n obtained from f. no.; (●) from alk. no.; (+)
 from light scattering \bar{M}_w and $\bar{M}_w/4$.



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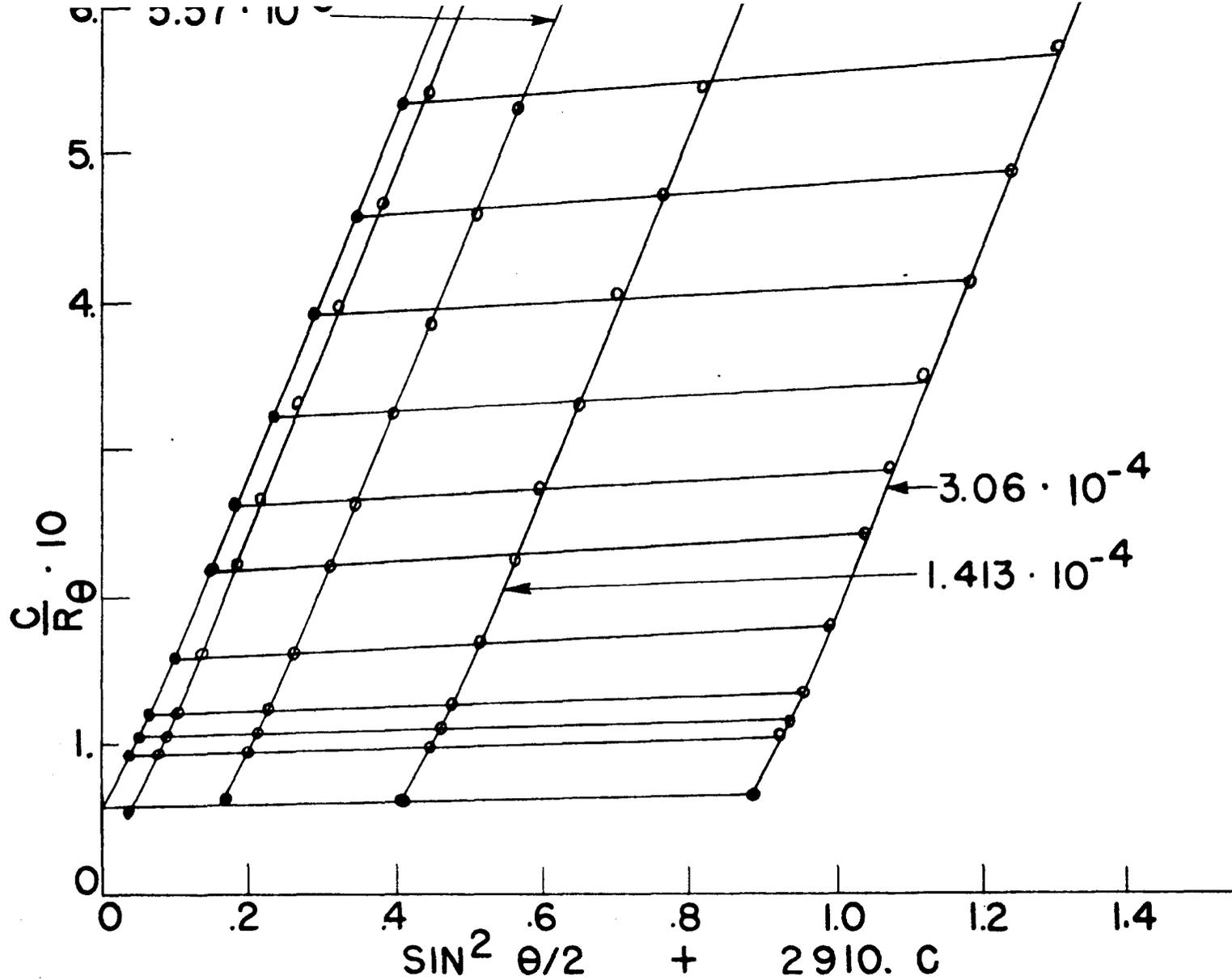


Fig. 51. The 20th day sweet corn amylopectin. $\bar{M}_w = 43 \times 10^6$.

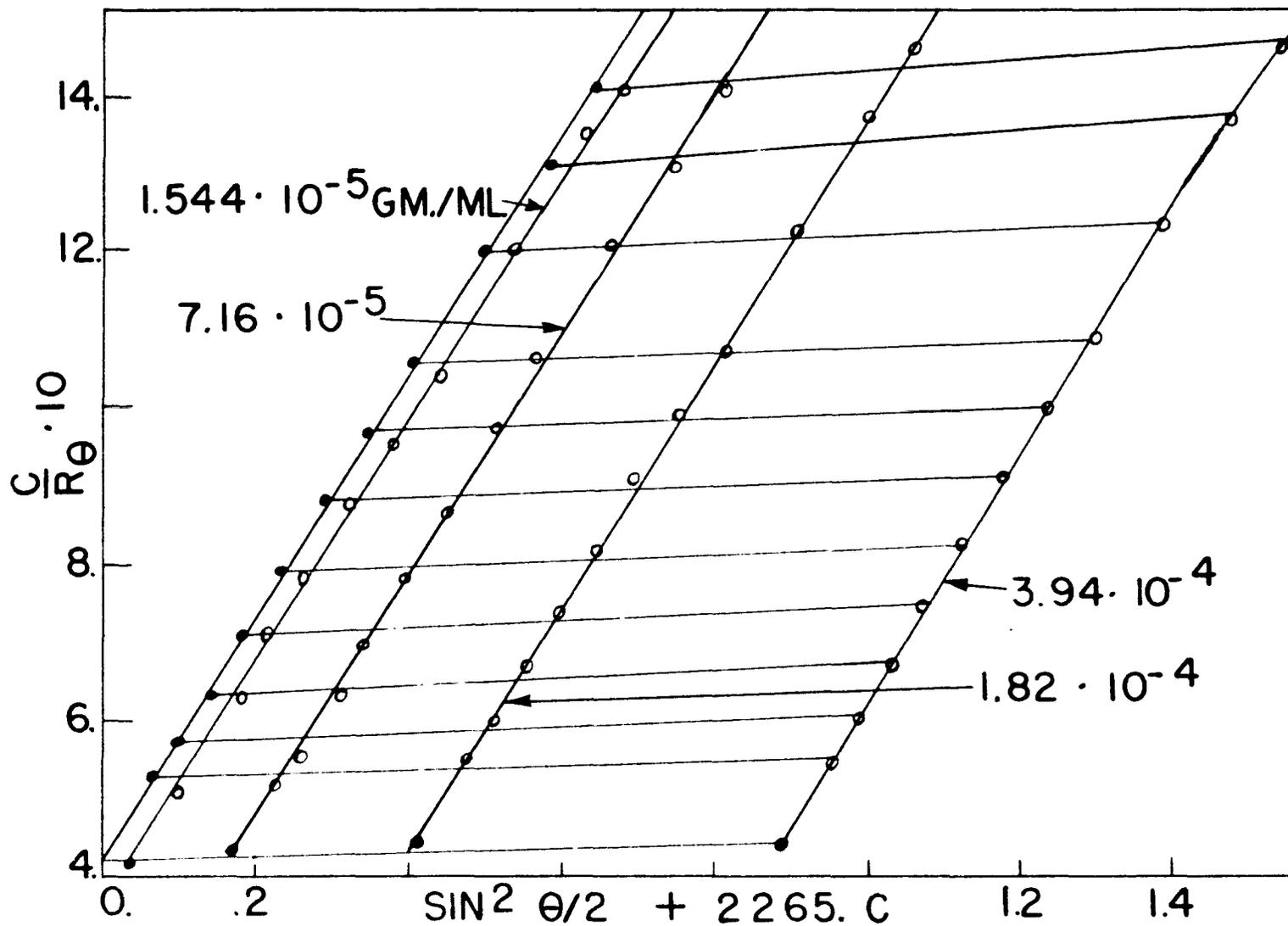


Fig. 52. The 10.57 hour sample of 20th day sweet corn amylopectin. $\bar{M}_w = 6.0 \times 10^6$.

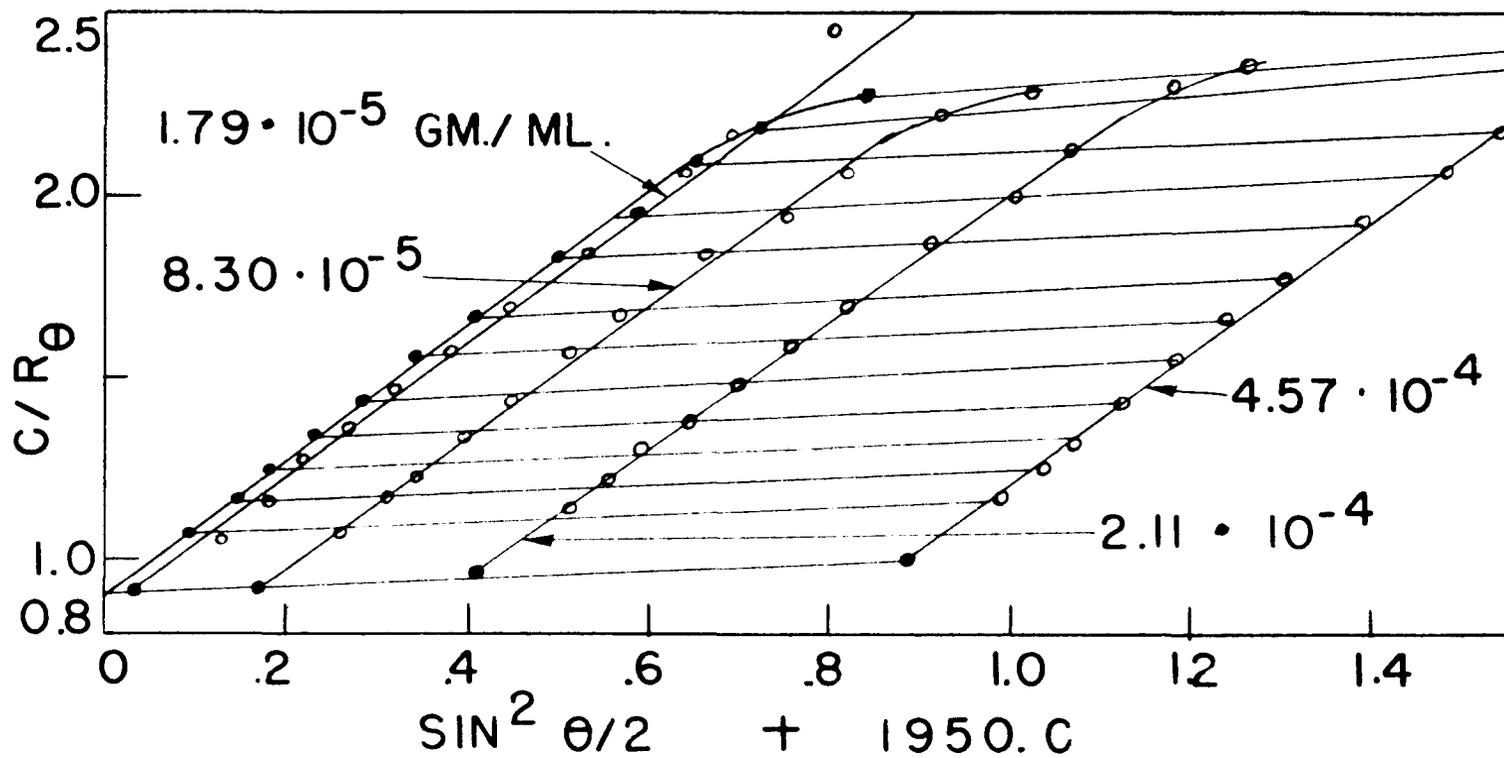


Fig. 53. The 20.25 hour sample of 20th day sweet corn amylopectin.

$$\bar{M}_w = 6.0 \times 10^6.$$

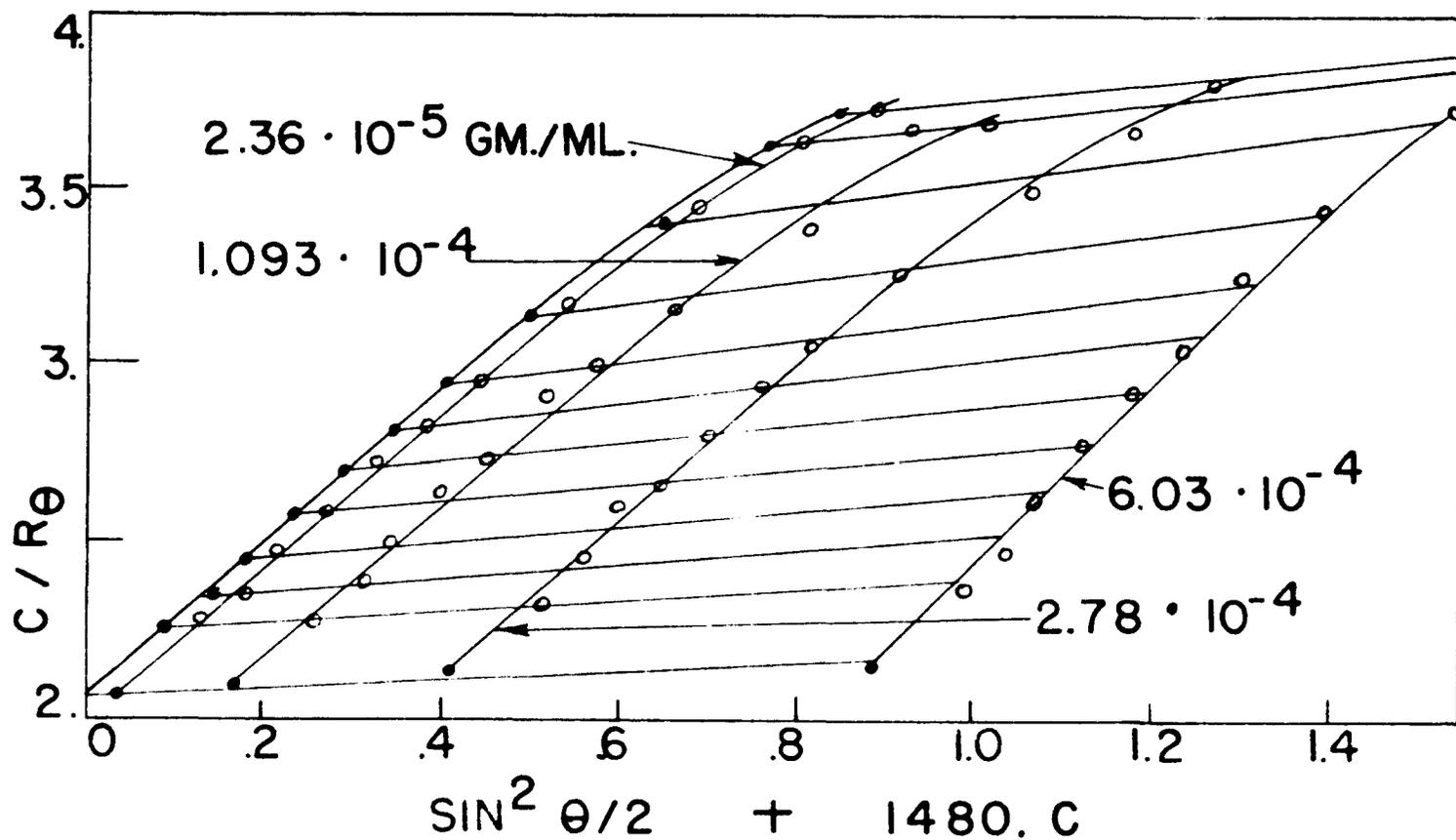
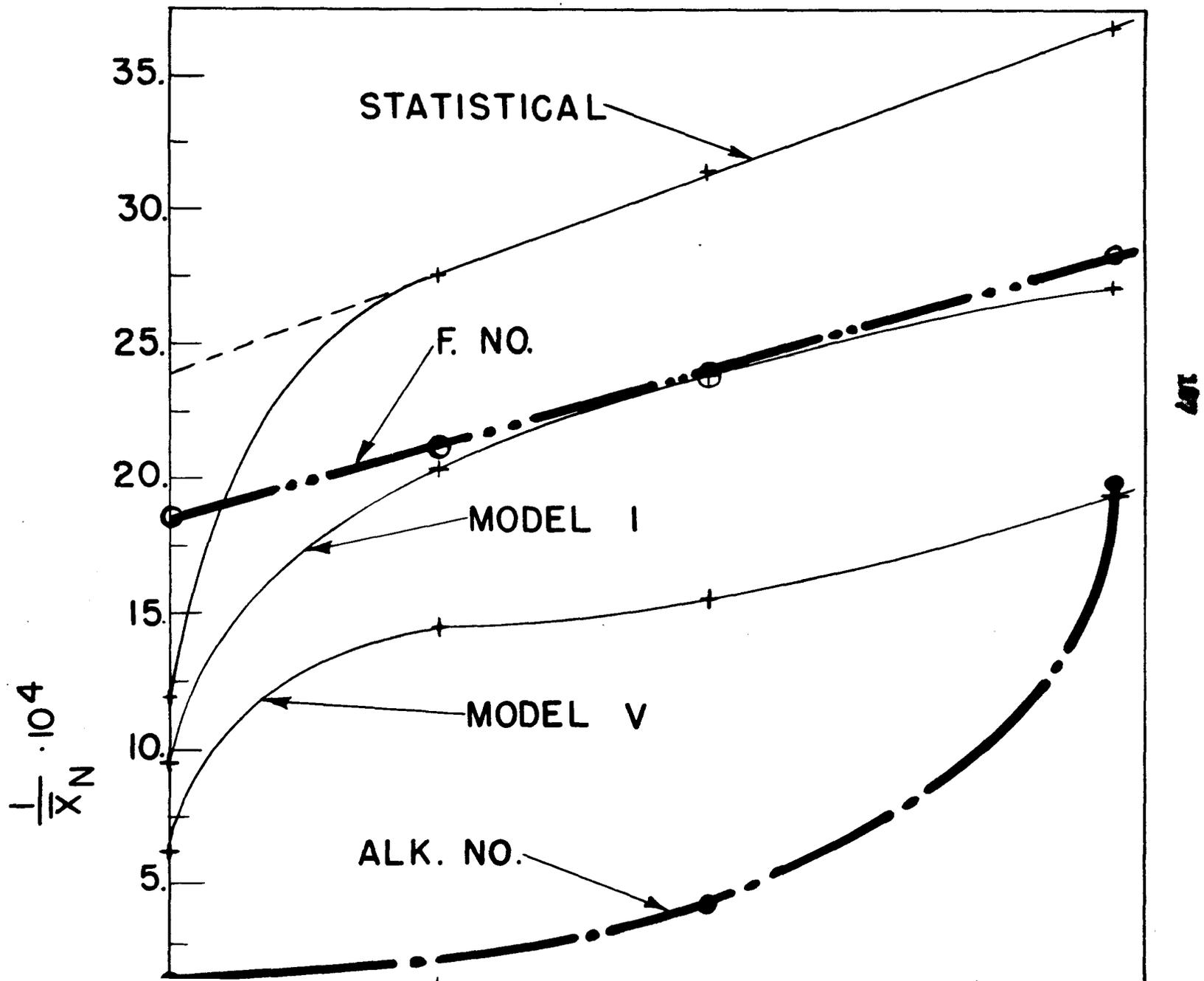


Fig. 54. The 33.5 hour sample of 20th day sweet corn amylopectin.
 $\bar{M}_w = 1.22 \times 10^6$.

Table 29. Acid hydrolysis of "mature" sweet corn glycogen.

Refluxed at 99.8°C and pH = 4.22. Theoretical \bar{X}_n based on statistical and less random models I and V. Observed \bar{X}_n obtained from f. no. and alk. no. according to Kerr *et. al.* (13).

Hrs. refluxed	$\bar{M}_w \times 10^{-6}$	\bar{X}_n (Stat.)	\bar{X}_n (Model I)	\bar{X}_n (Model V)	\bar{X}_n (f. no.)	\bar{X}_n (alk. no.)
0	18.5	843	1140	1610	533	7530
10.0	3.50	364	493	691	471	10,190
20.0	2.53	318	419	645	419	2470
35.0	1.97	272	369	519	370	504



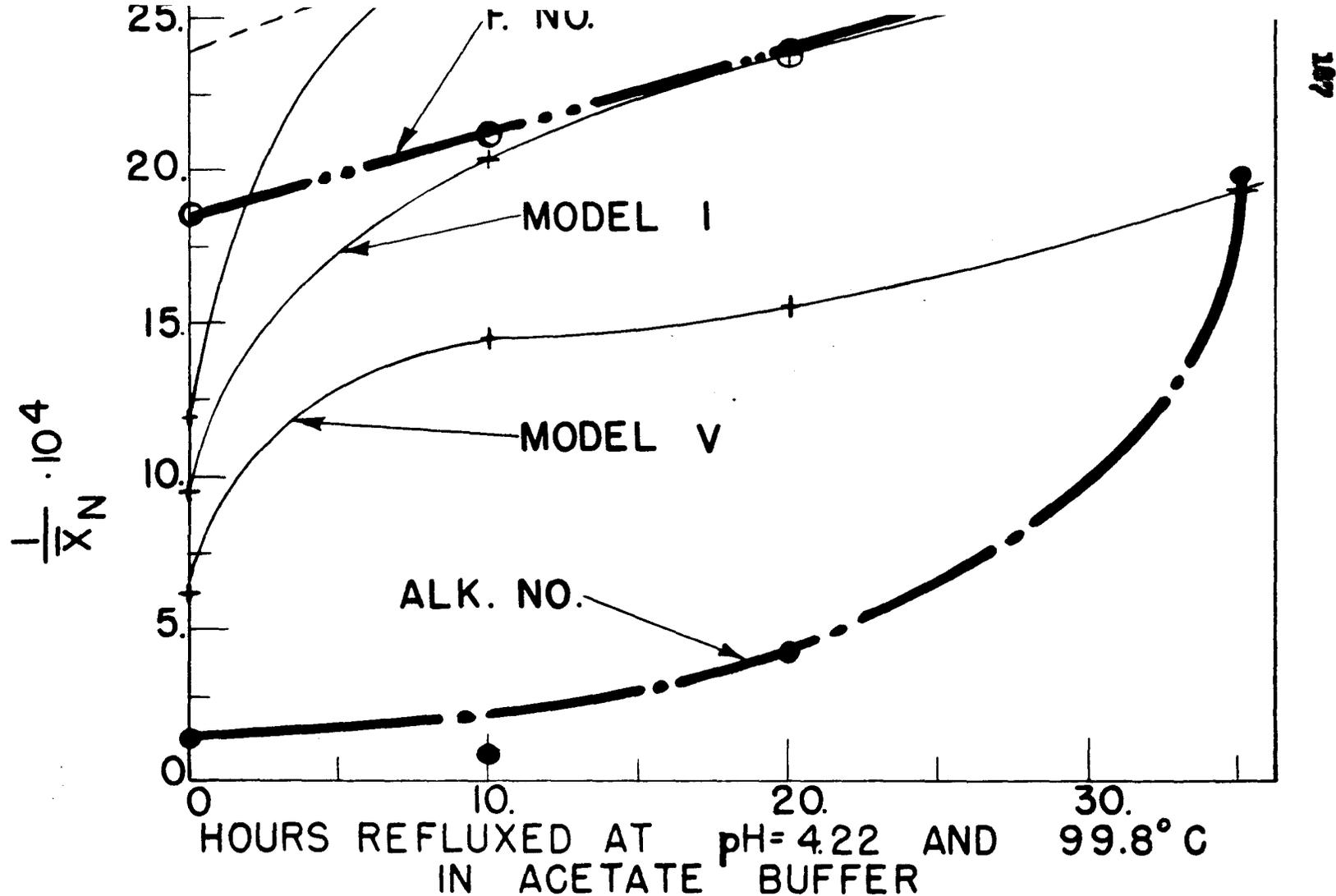


Fig. 55. Acid hydrolysis of sweet corn glycogen. (O) \bar{X}_n from f. no.; (●) from alk. no.; (+) from light scattering using 8.67% branching for the statistical and two less random statistical models.

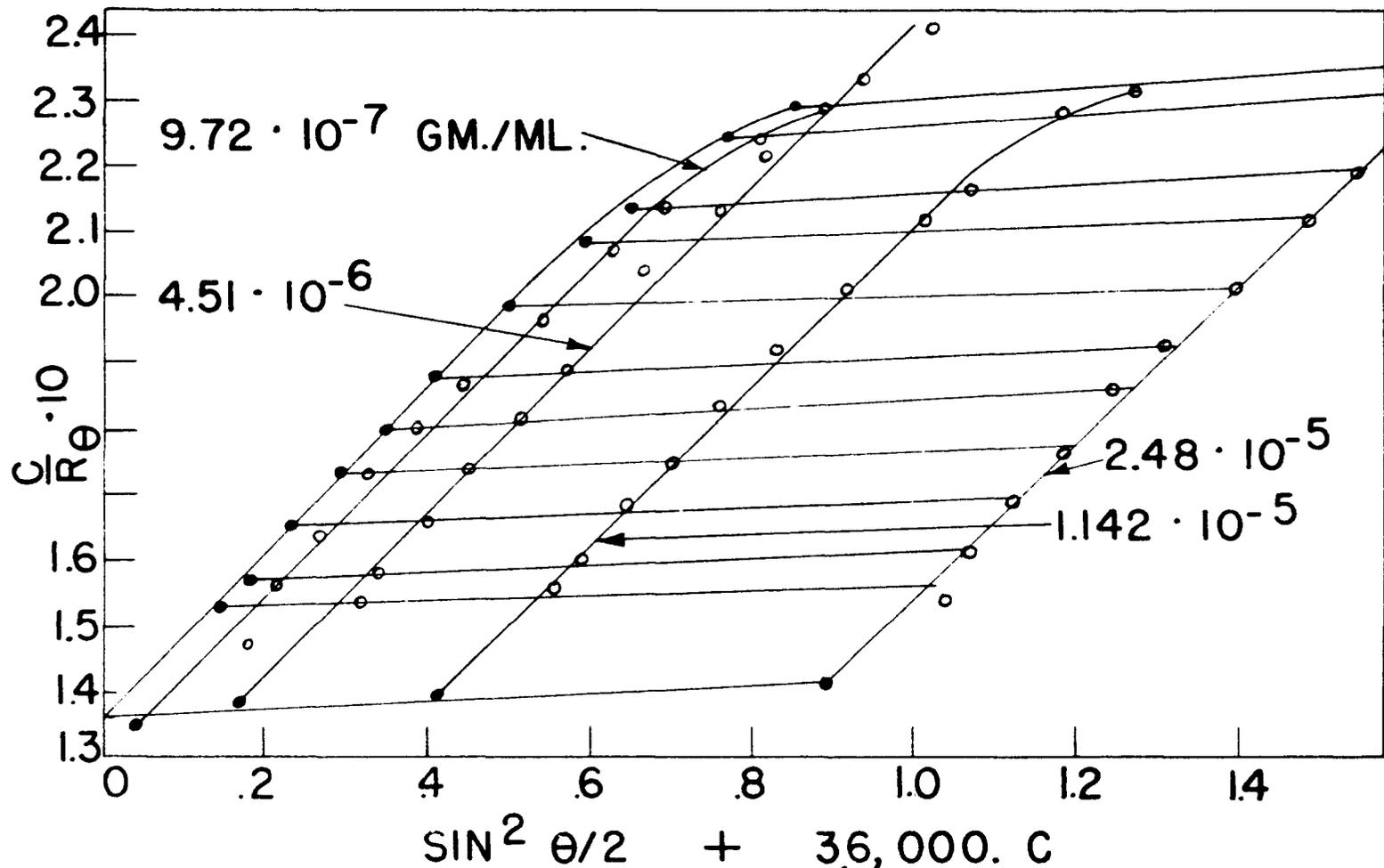


Fig. 56. Amylose-free glycogen. $\bar{M}_w = 18.5 \times 10^6$.

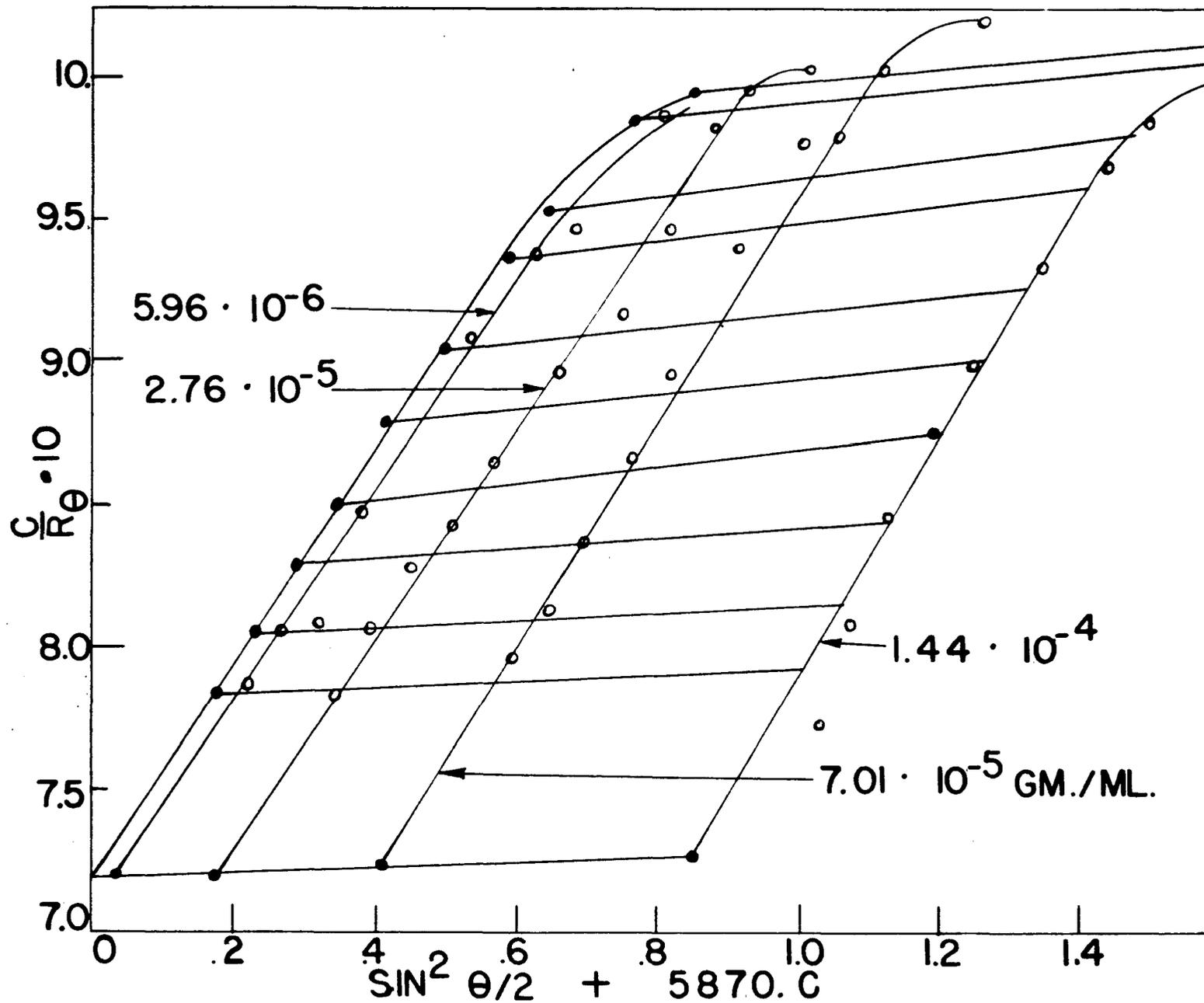


Fig. 57. The 10.0 hour sample of glycogen. $\bar{M}_w = 3.50 \times 10^6$.

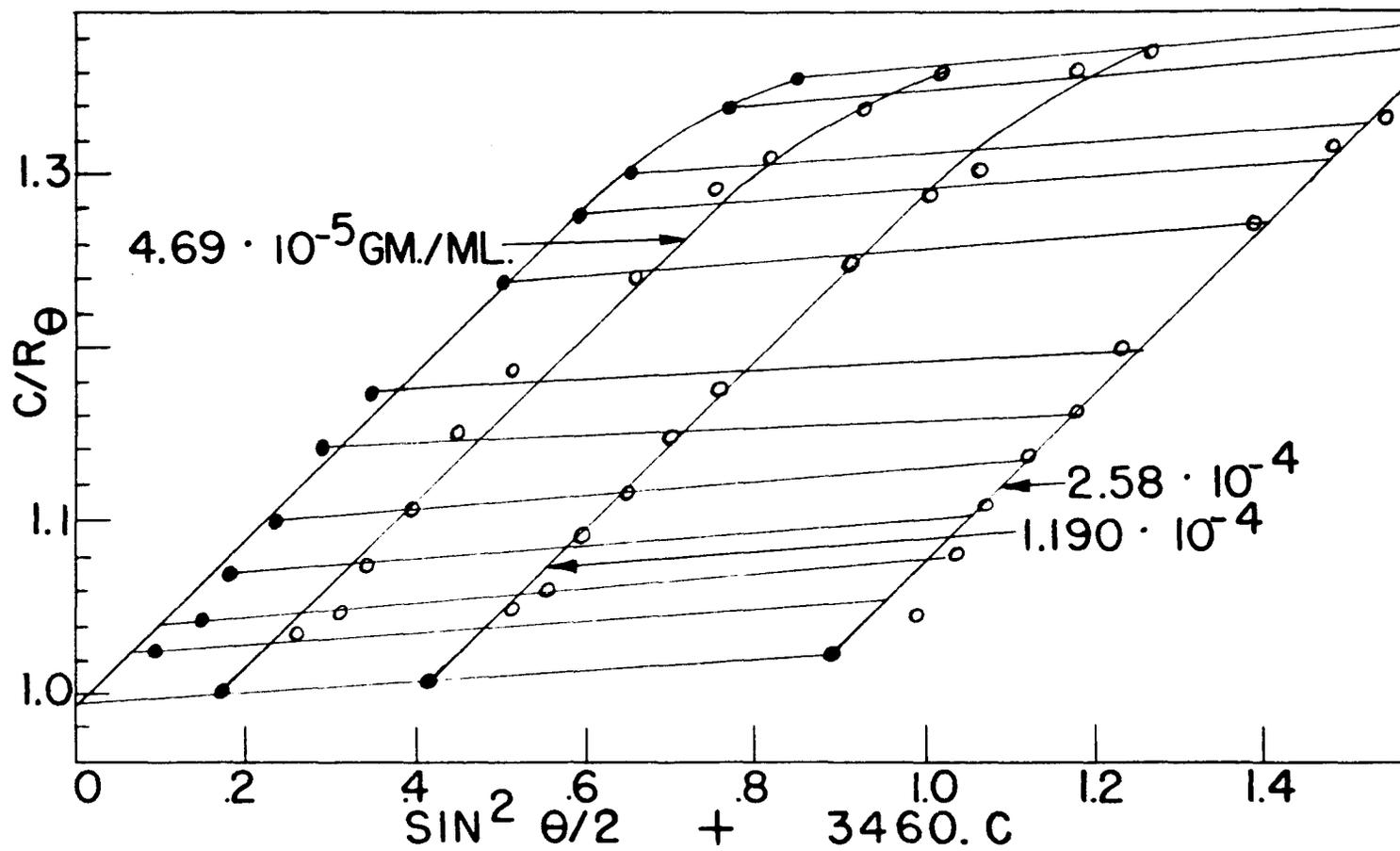


Fig. 58. The 20.0 hour sample of glycogen. $\bar{M}_w = 2.53 \times 10^6$.

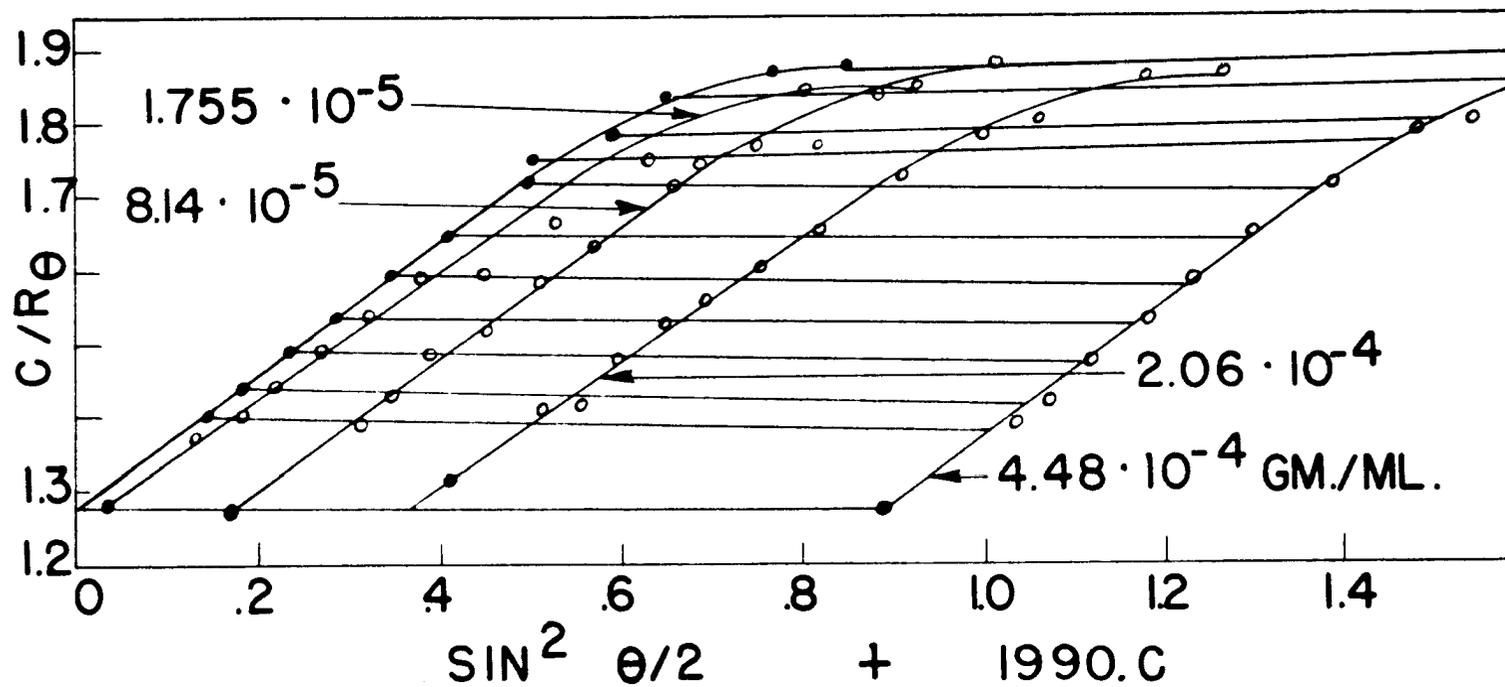


Fig. 59. The 35.0 hour sample of glycogen. $\bar{M}_w = 1.97 \times 10^6$.

Table 30. Acid hydrolysis of corn amylopectin and glycogen.

Points were obtained from \bar{M}_w assuming the glycogen and amylopectin behave as linear polymers ($\bar{M}_w \sim 2\bar{M}_n$). Refluxed at pH = 4.2.

14th day sweet amylopectin		13th day waxy IV amylopectin		Sweet corn glycogen	
Hrs. refluxed	\bar{X}_n	Hrs. refluxed	\bar{X}_n	Hrs. refluxed	\bar{X}_n
0	48,700	0	519,000	0	57,000
10	4,570	8	43,200	10	10,800
20	2,450	19	11,600	20	7,800
32	1,390	32	4,500	35	6,080

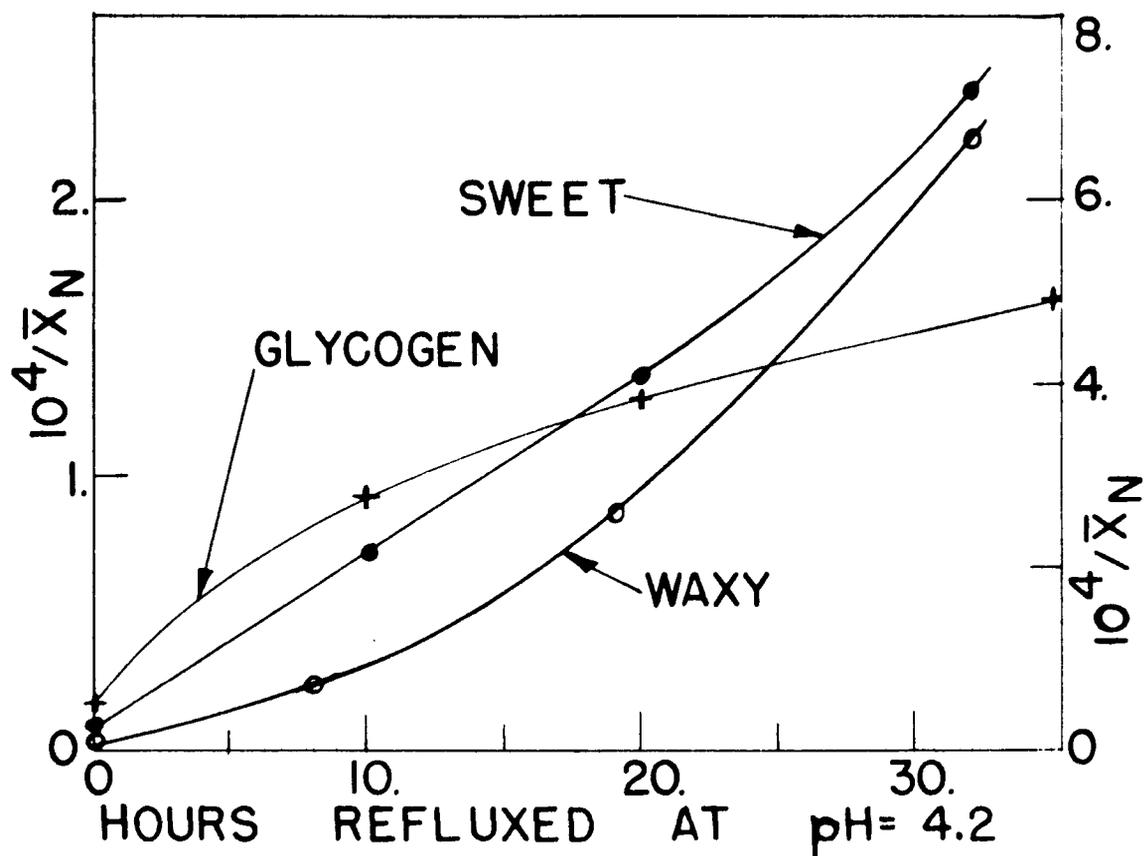


Fig. 60. Acid hydrolysis of 14th day sweet corn amylopectin (●); 13th day waxy starch sample IV (○); and sweet corn glycogen (+). Points were obtained from \bar{M}_w assuming the samples behave as linear polymers ($\bar{M}_w = 2 \bar{M}_n$ for large \bar{M}_n). The right hand ordinate pertains only to the sweet corn amylopectin.

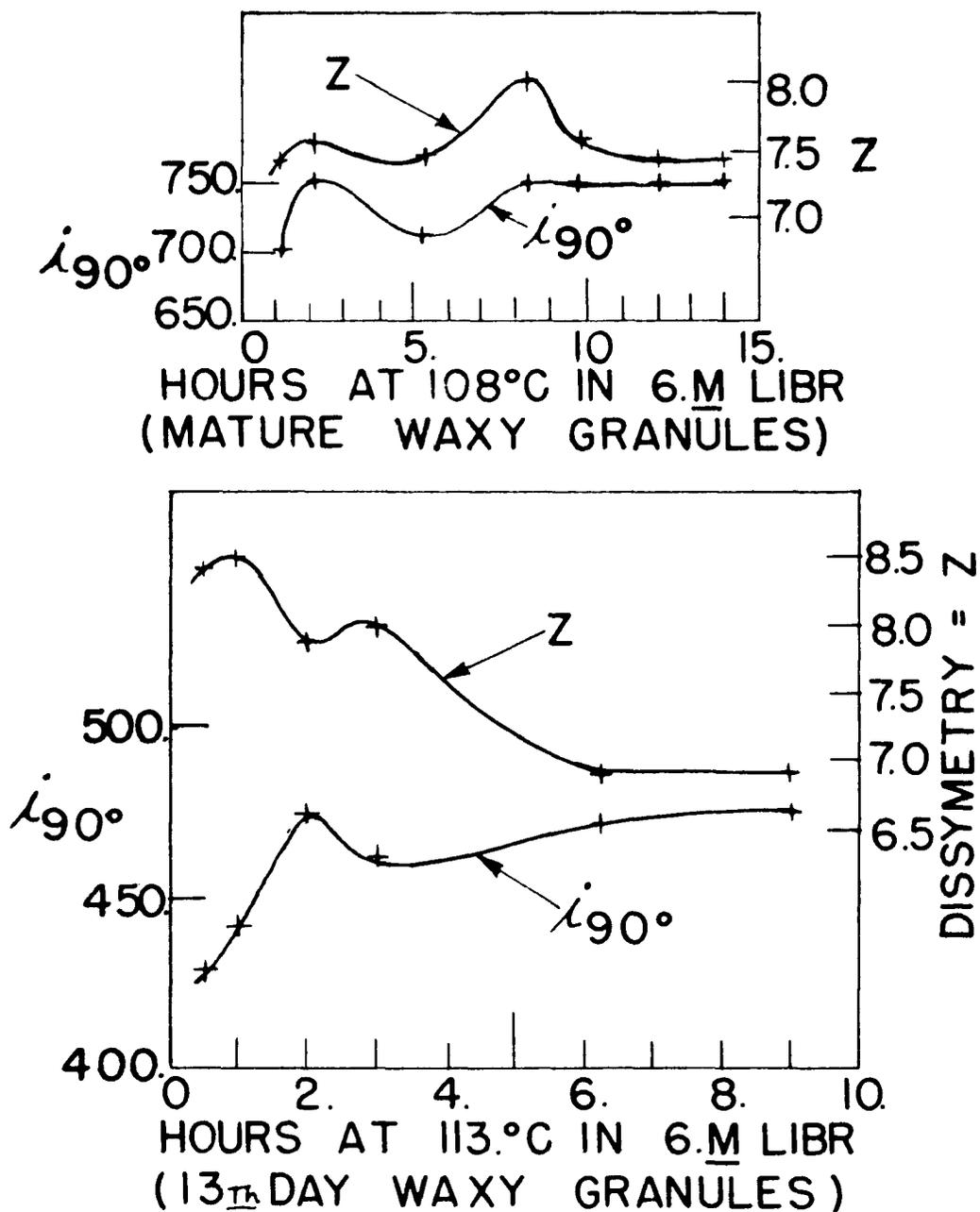


Fig. 61. Lithium bromide dispersion studies on starch granules as followed by light scattering. Mature and 13th day waxy starch samples.

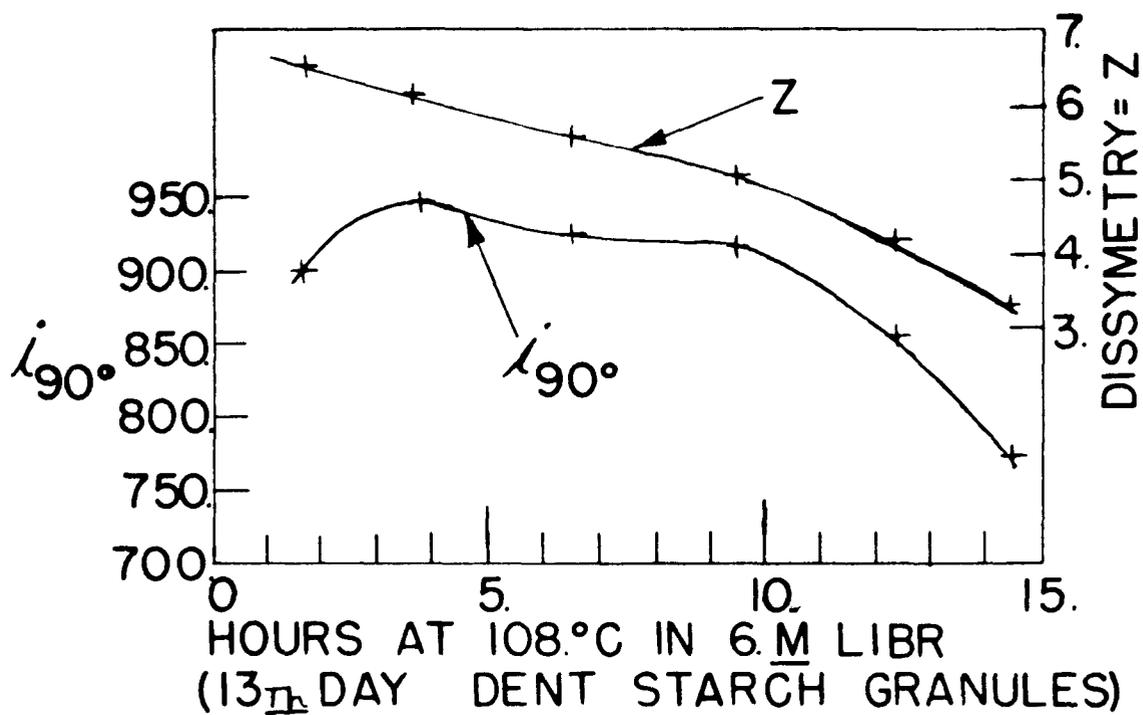
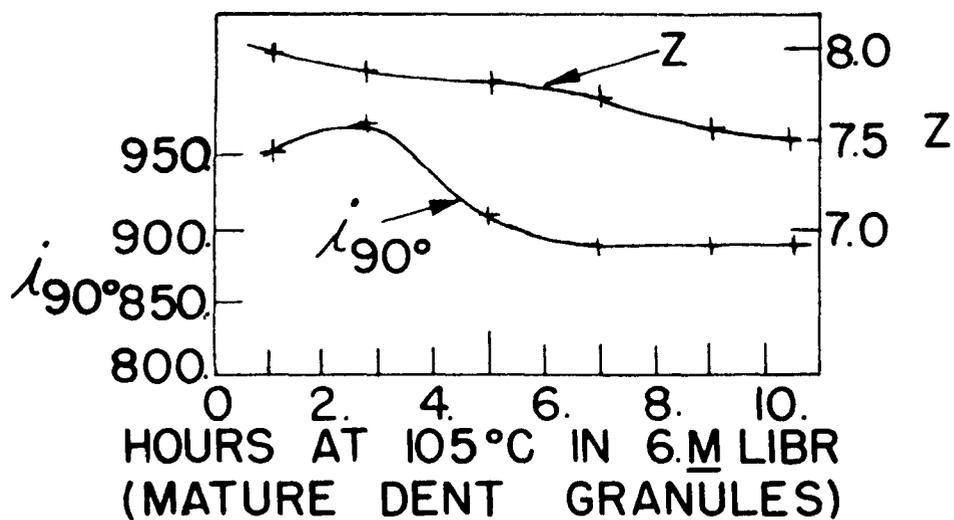


Fig. 62. Lithium bromide dispersion studies on starch granules as followed by light scattering. Mature and 14th day dent corn starch samples.

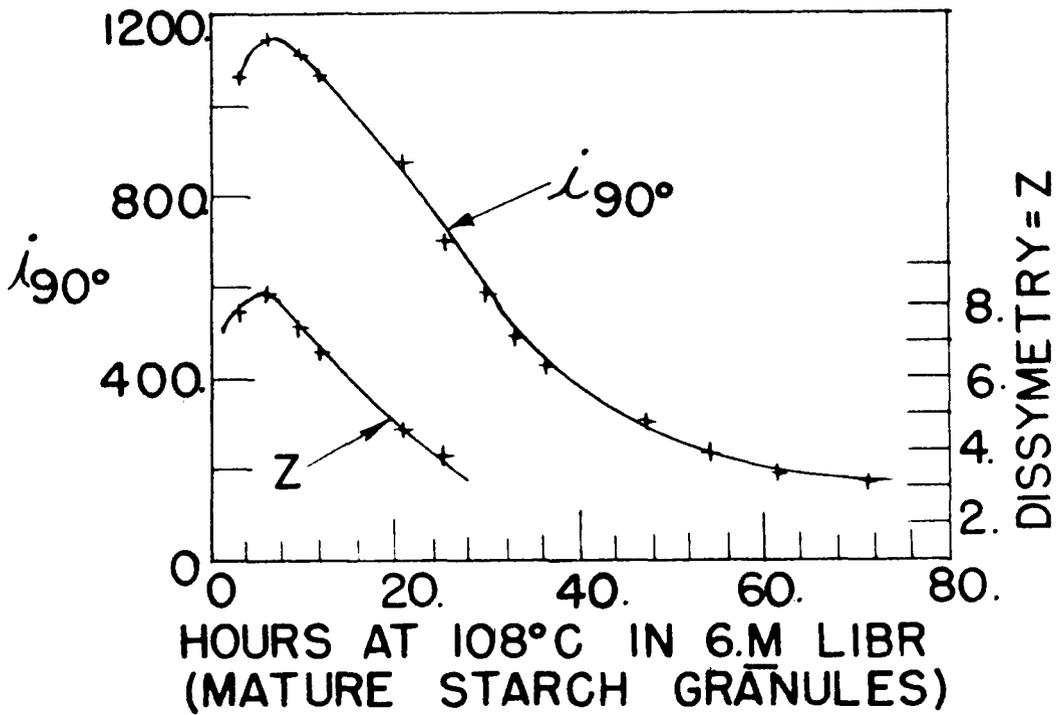
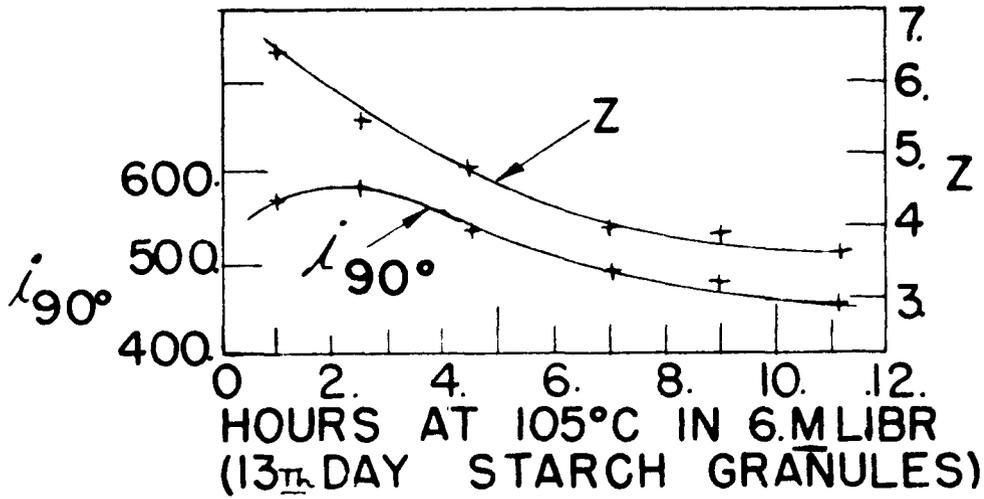


Fig. 63. Lithium bromide dispersion studies on starch granules as followed by light scattering. Mature and 14th day sweet corn starch samples.

Table 31. Pentasol dispersion of 14th day dent starch granules.

Refluxed in unbuffered and buffered Pentasol solutions. Molecular weights of the resulting amylopectins.

Theoretical \bar{X}_n based on the statistical model. Observed \bar{X}_n obtained from the alk. no. according to Kerr *et. al.* (13).

Amylopectin sample	Solvent	$\bar{M}_w \times 10^{-6}$	Theor. \bar{X}_n	\bar{X}_n
20 hrs., buffered	H ₂ O	168	2880	-
3 hrs., unbuffered	<u>N</u> KOH	168	2880	2670
6 hrs., unbuffered	<u>N</u> KOH	118	2390	1305
6 hrs., unbuffered	H ₂ O	98	2180	1305
8 hrs., unbuffered	<u>N</u> KOH	28.6	1170	801
8 hrs., unbuffered	H ₂ O	24.4	1081	801
10 hrs., unbuffered	H ₂ O	12.2	764	740
14 hrs., unbuffered	H ₂ O	3.97	432	392

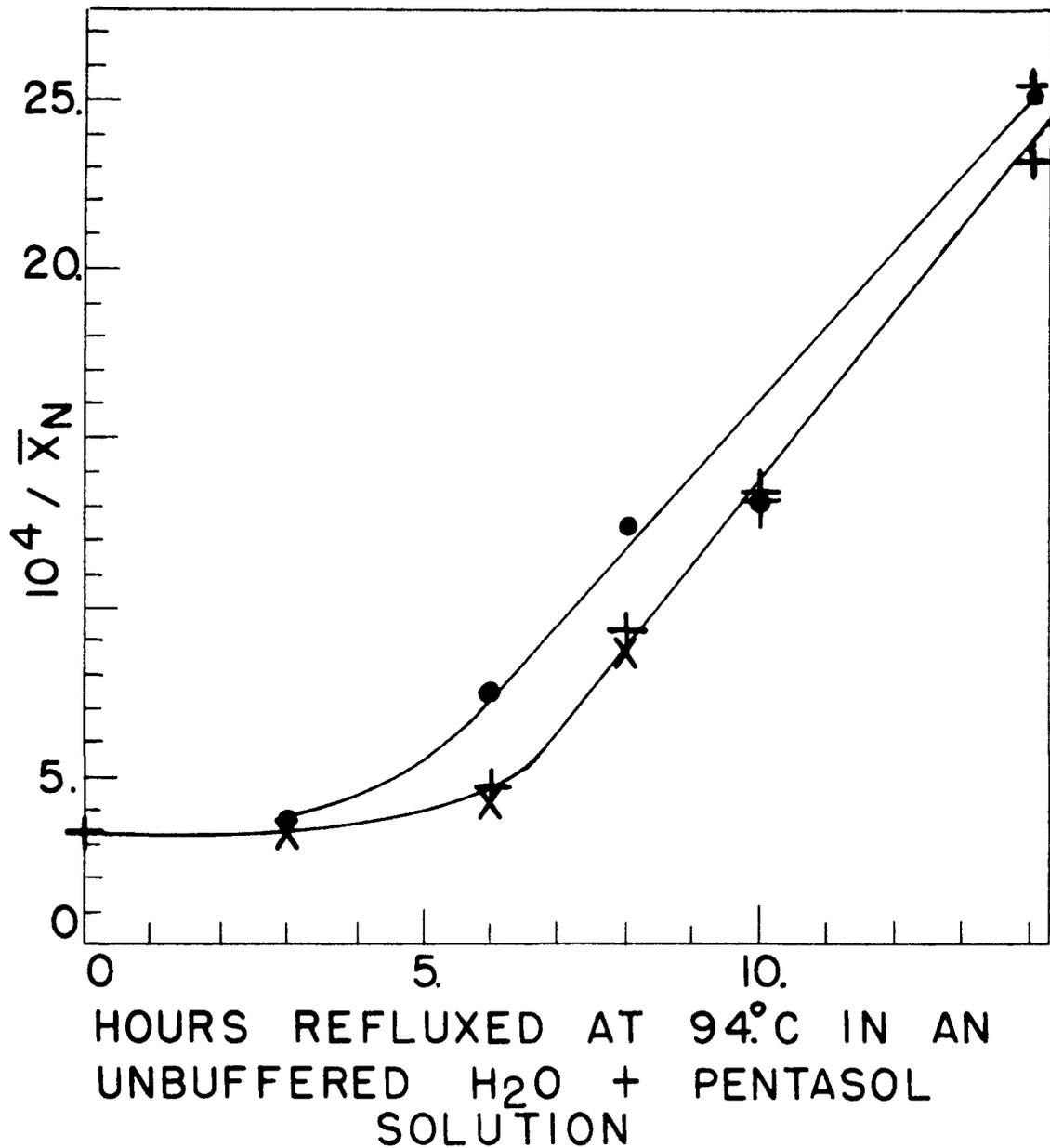
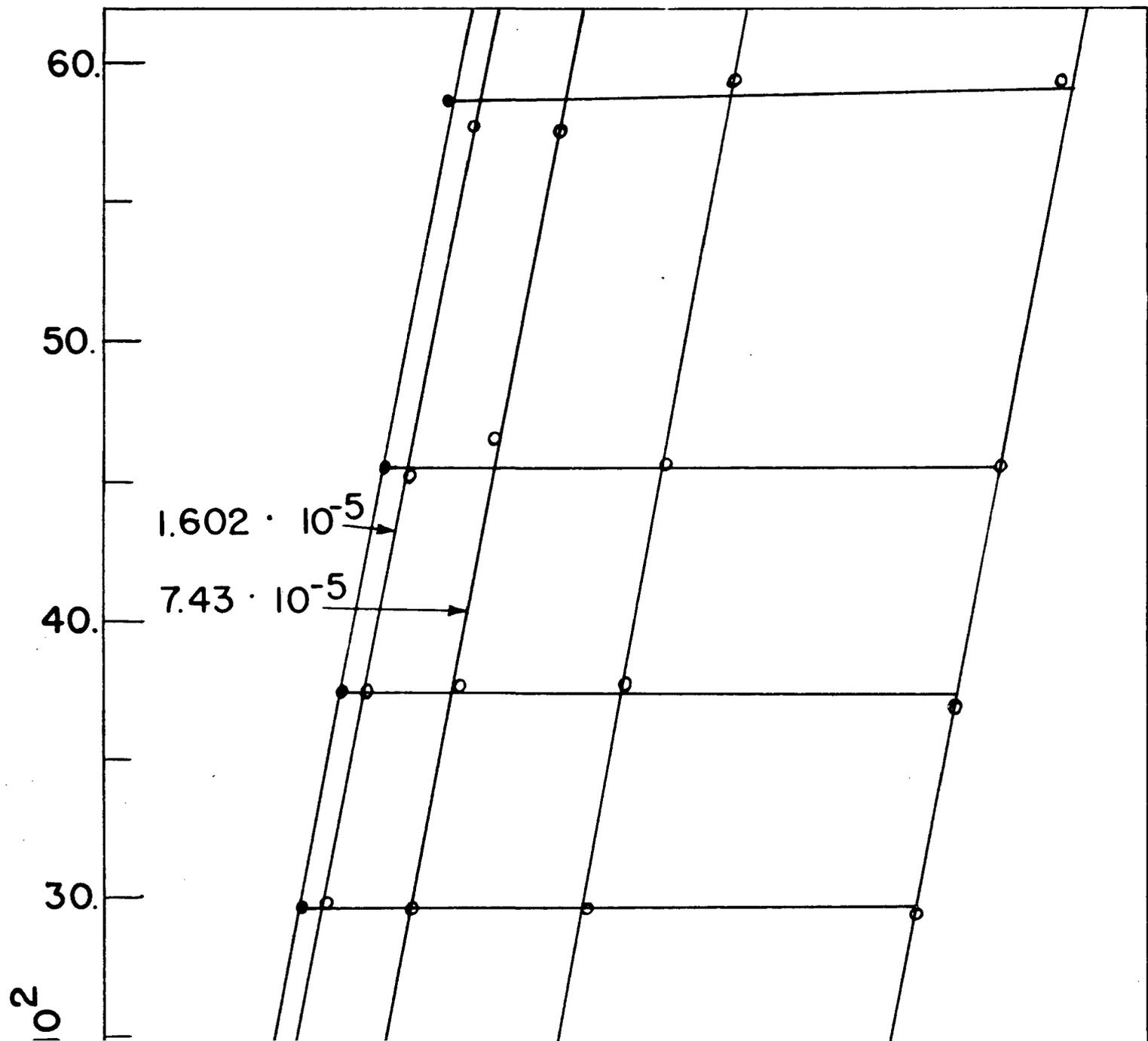


Fig. 64. Pentasol dispersion of 14th day dent starch granules. The above points were obtained from the branched component. (+) \bar{X}_n obtained from light scattering using H₂O as solvent; (X) from light scattering using 1 N KOH as solvent; (●) from alk. no.



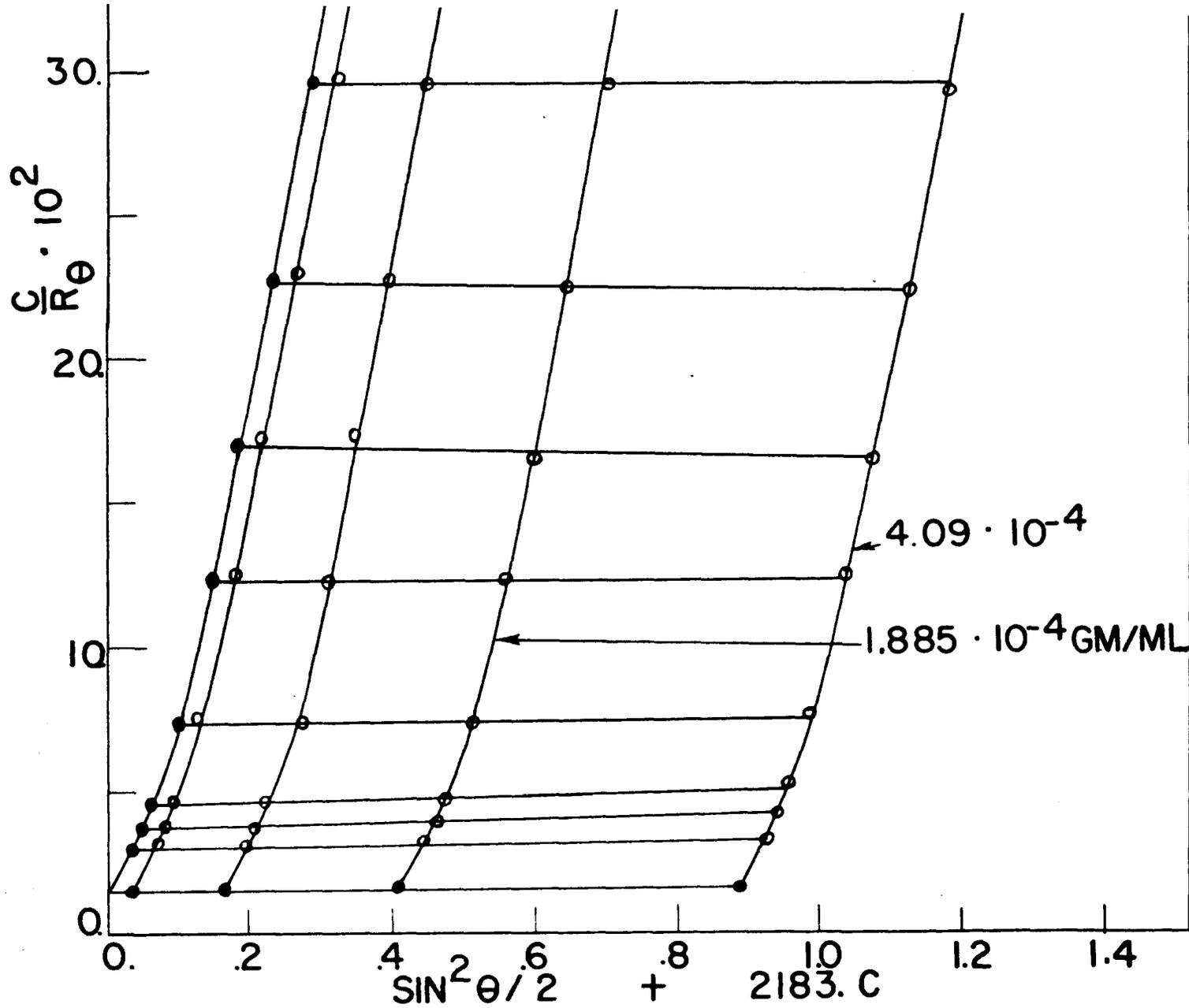
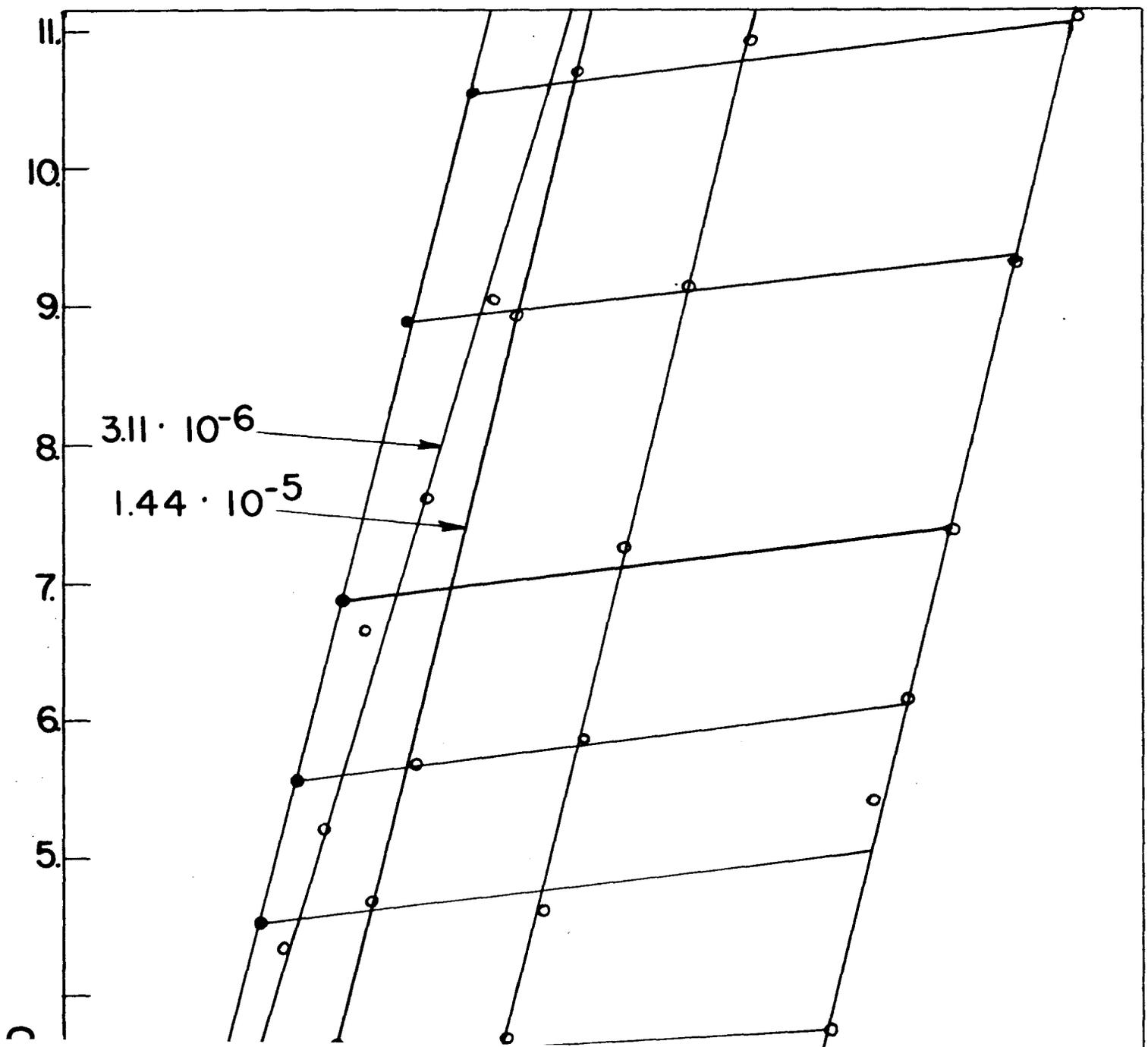
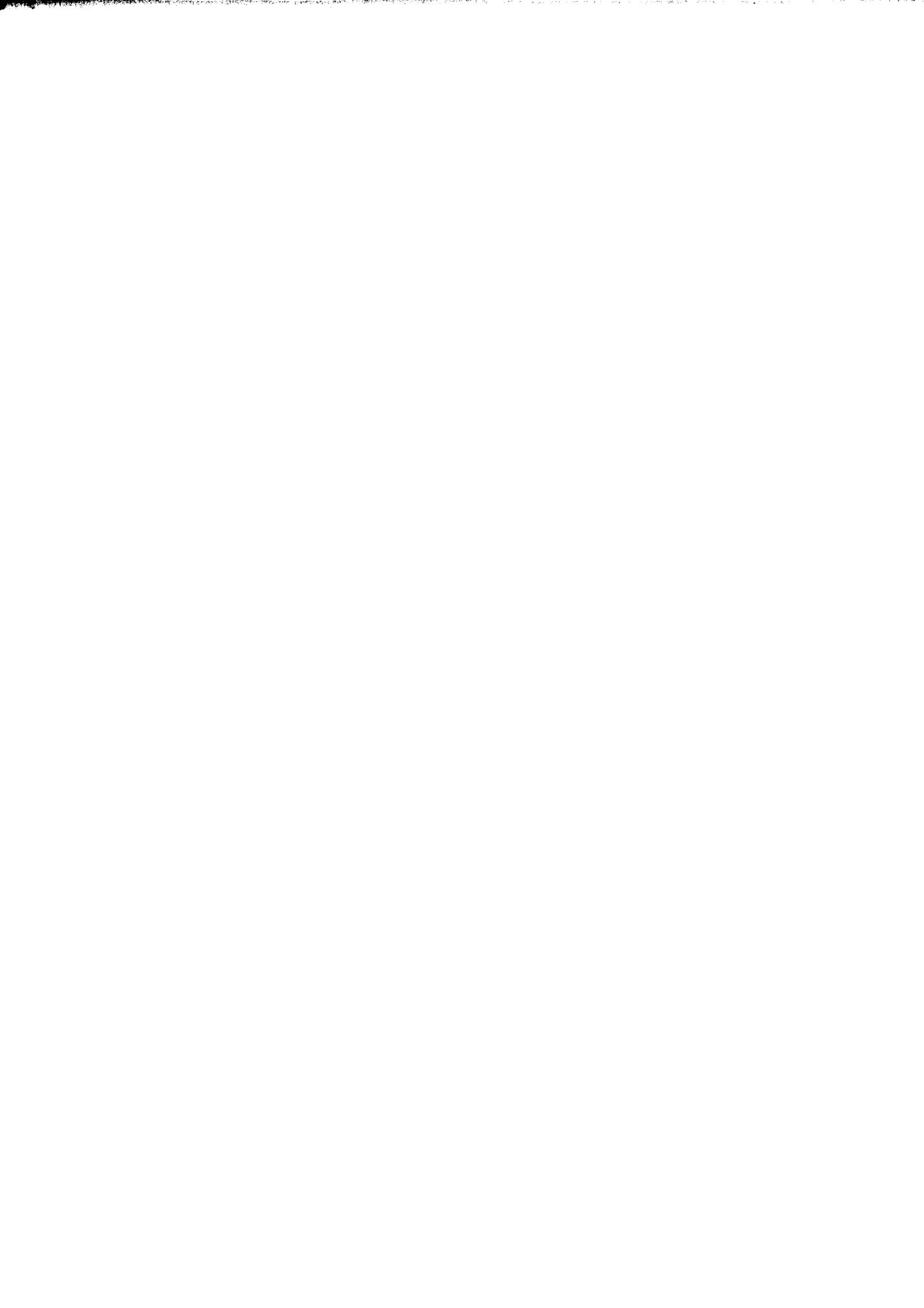


Fig. 65. The 14th day dent corn amylopectin. Refluxed 20 hours in buffered Pentasol. $\bar{M}_w = 168 \times 10^6$.



105



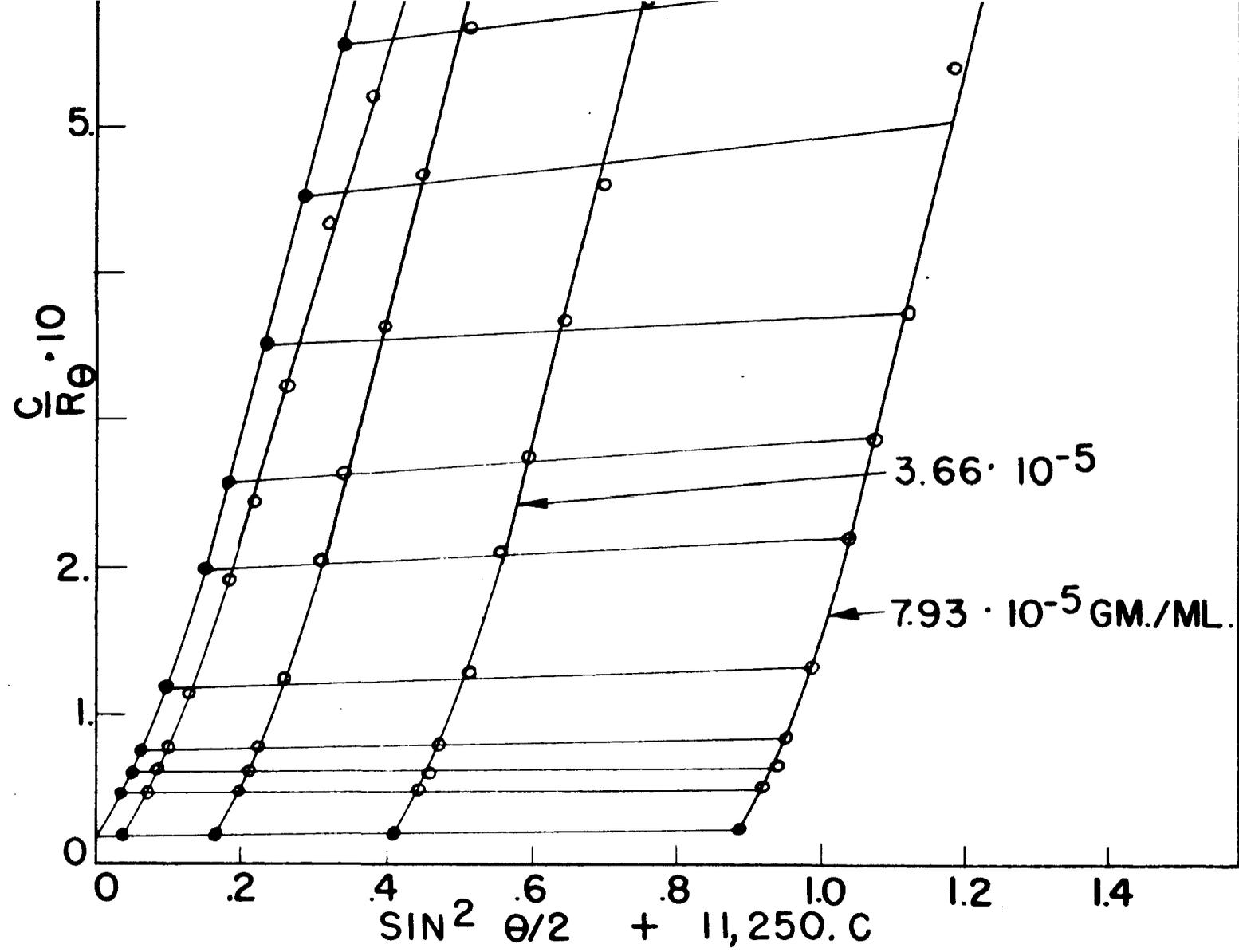
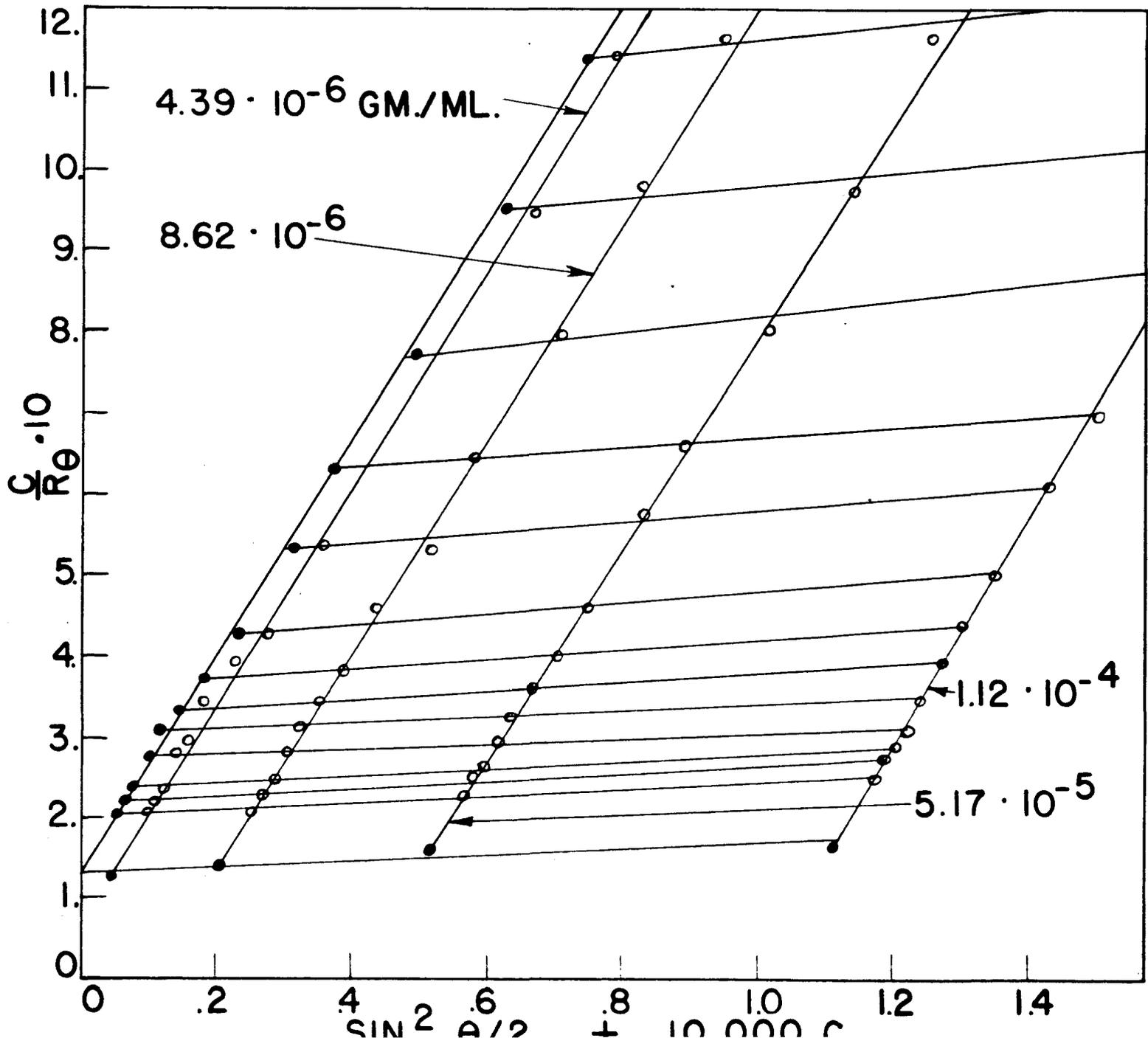


Fig. 66. The 14th day dent amylopectin. Refluxed 3.0 hours in buffered Pentasol. $\bar{M}_w = 168 \times 10^6$ using 1 N KOH as solvent.



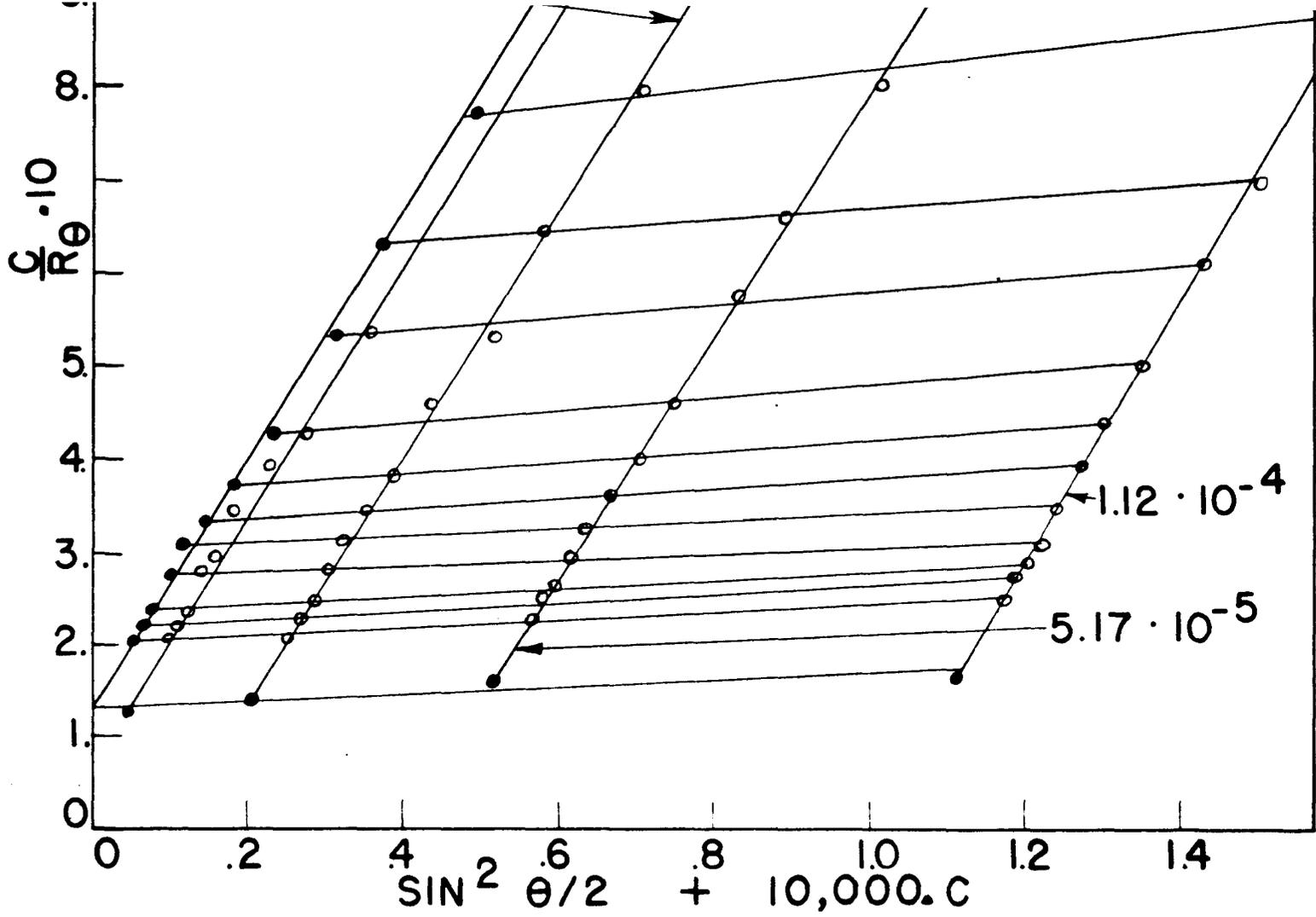
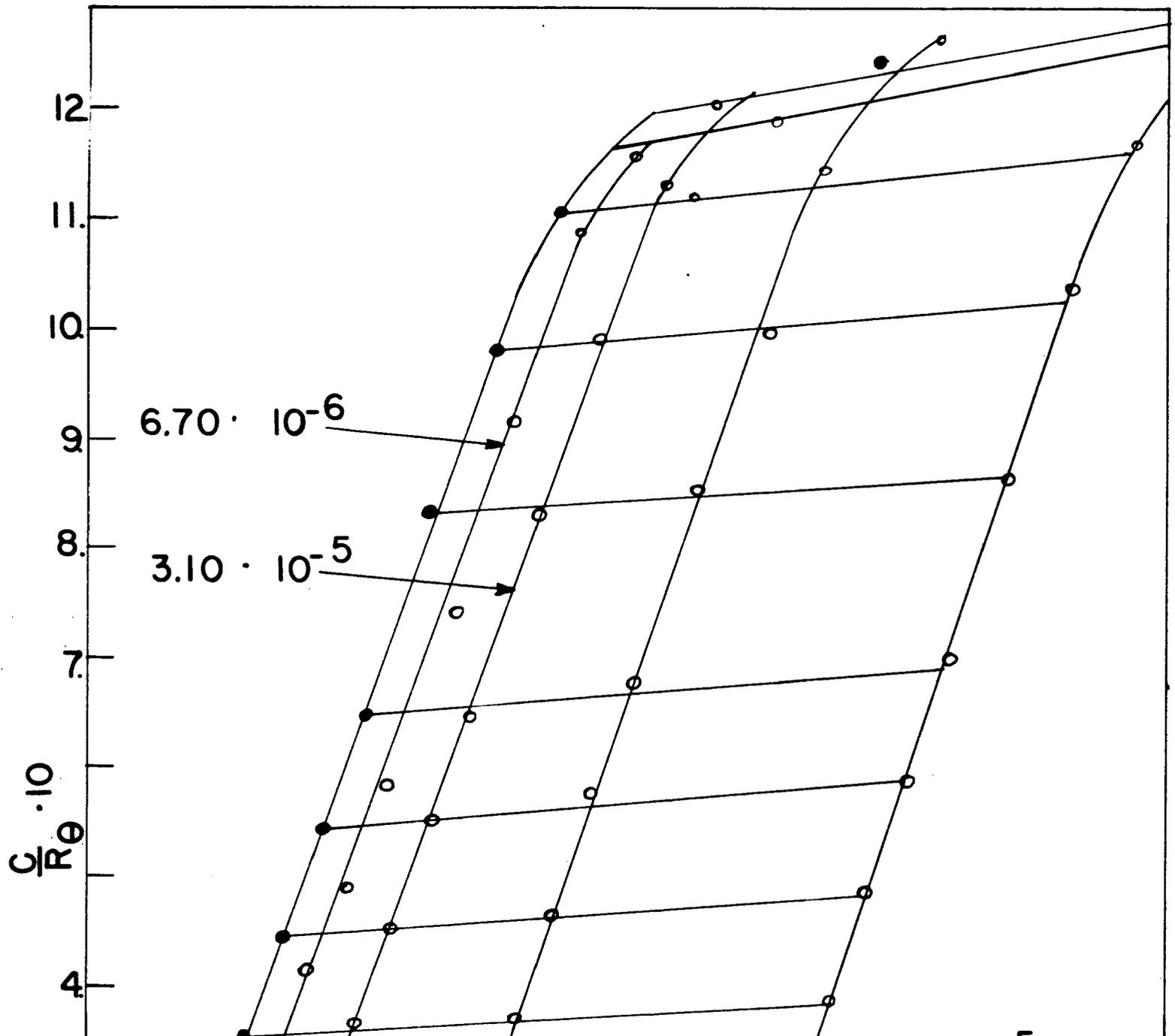


Fig. 67. The 14th day dent amylopectin. Refluxed 3.0 hours in unbuffered Pentasol with rapid stirring. $\bar{M}_w = 22.6 \times 10^6$ using 1 N KOH as solvent.



100

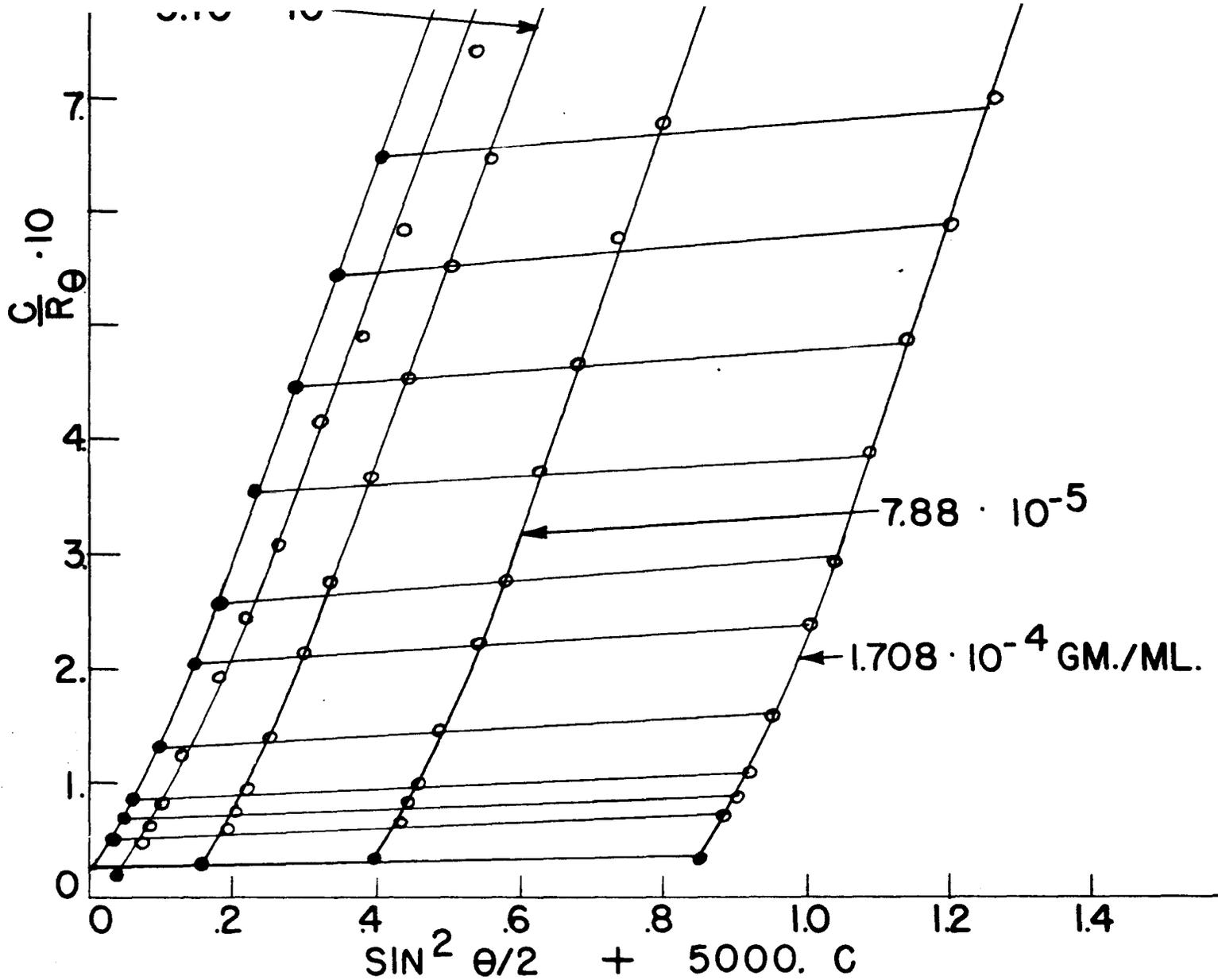
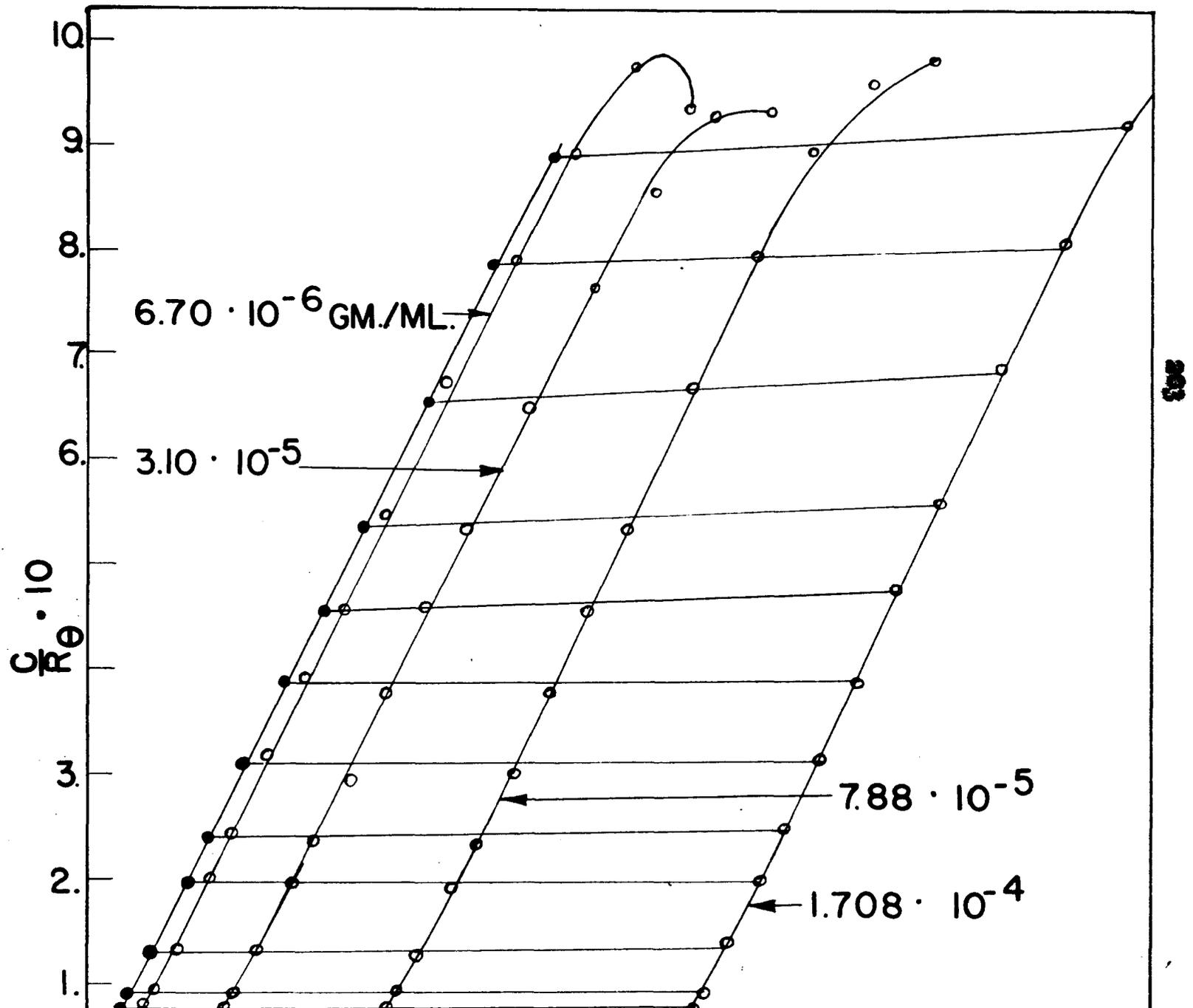


Fig. 68. The 14th day dent amylopectin. Refluxed 6.0 hours in unbuffered Pentasol.
 $\bar{M}_w = 118 \times 10^6$ using 1 N KOH as solvent.



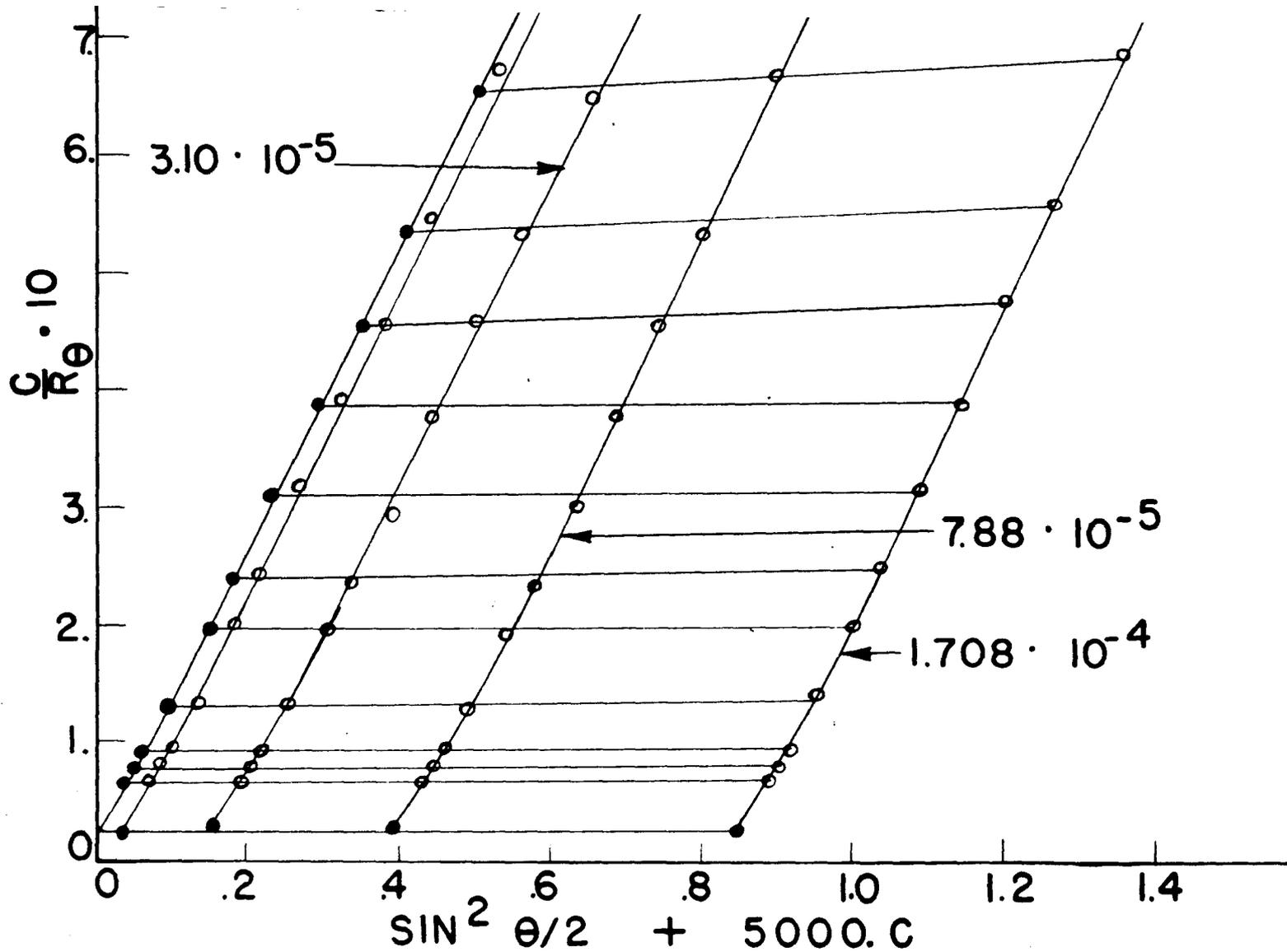
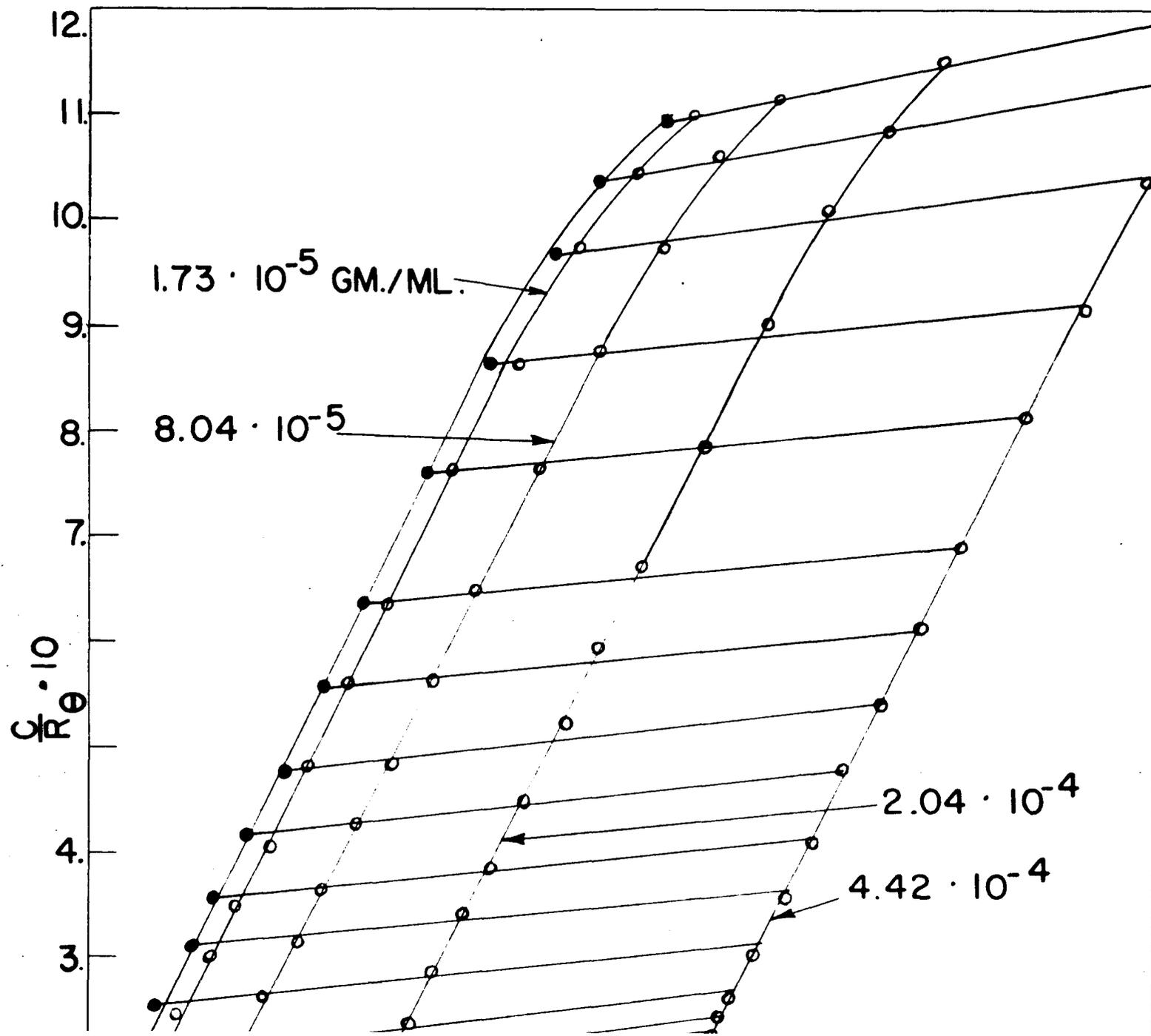


Fig. 69. The 14th day dent amylopectin. Refluxed 6.0 hours in unbuffered Pentasol. $\bar{M}_w = 98 \times 10^6$ using H_2O as solvent.







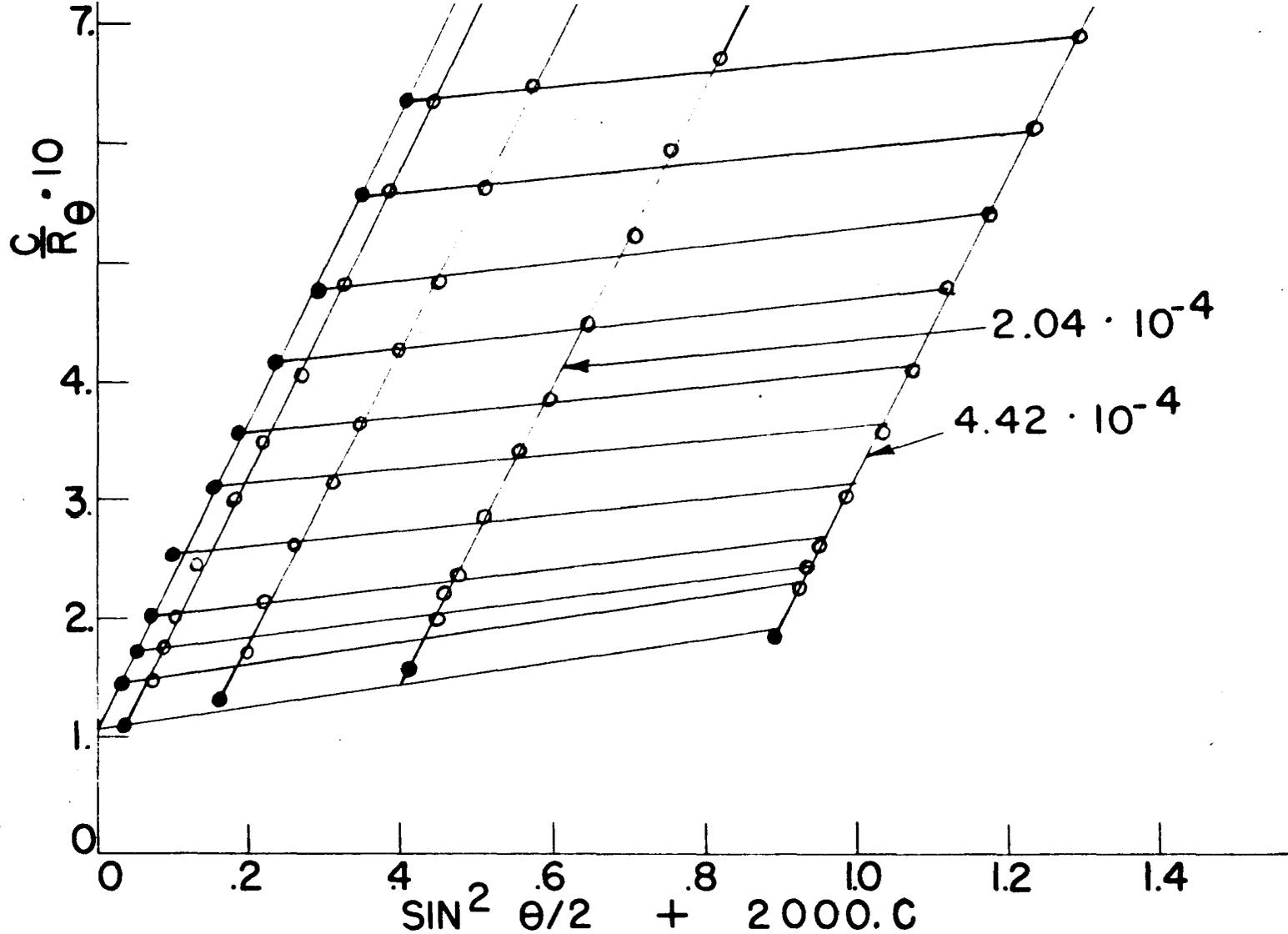


Fig. 70. The 14th day dent amylopectin. Refluxed 8.0 hours in unbuffered Pentasol. $\bar{M}_w = 24.4 \times 10^6$ using H_2O as solvent.

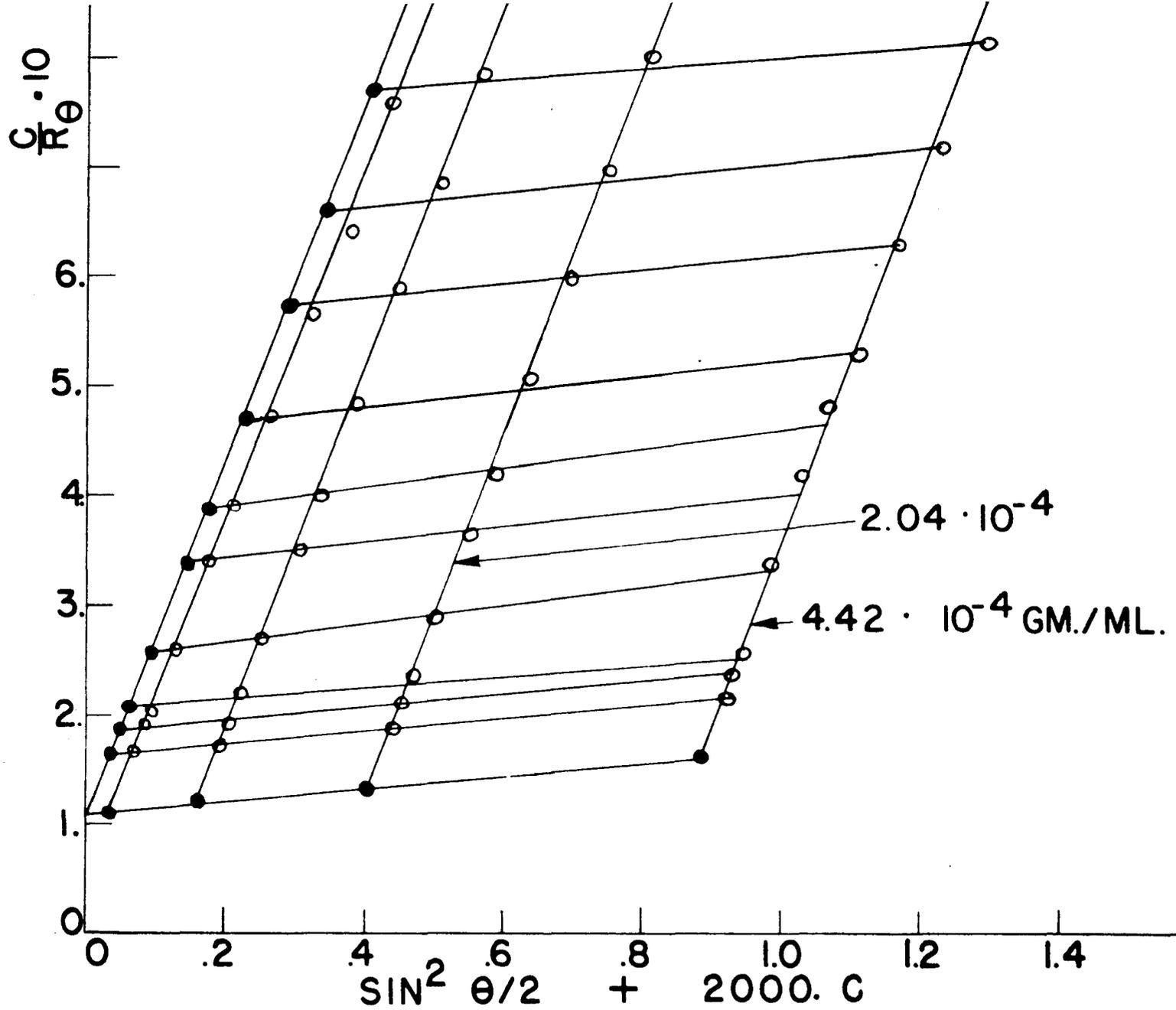
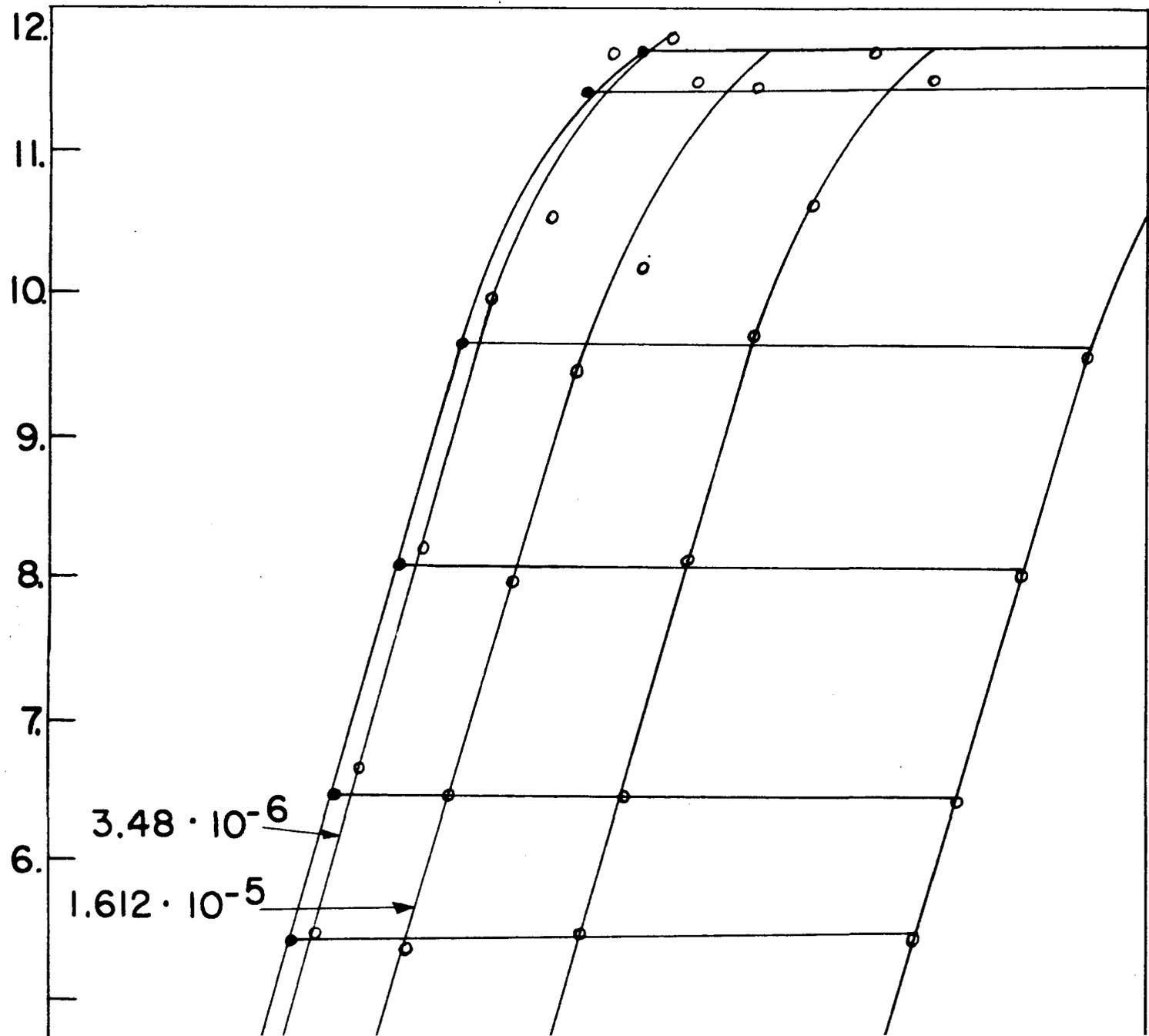


Fig. 71. The 14th day dent amylopectin. Refluxed 8.0 hours in unbuffered Pentasol. $\bar{M}_w = 28.6 \times 10^6$ using 1 N KOH as solvent.



204



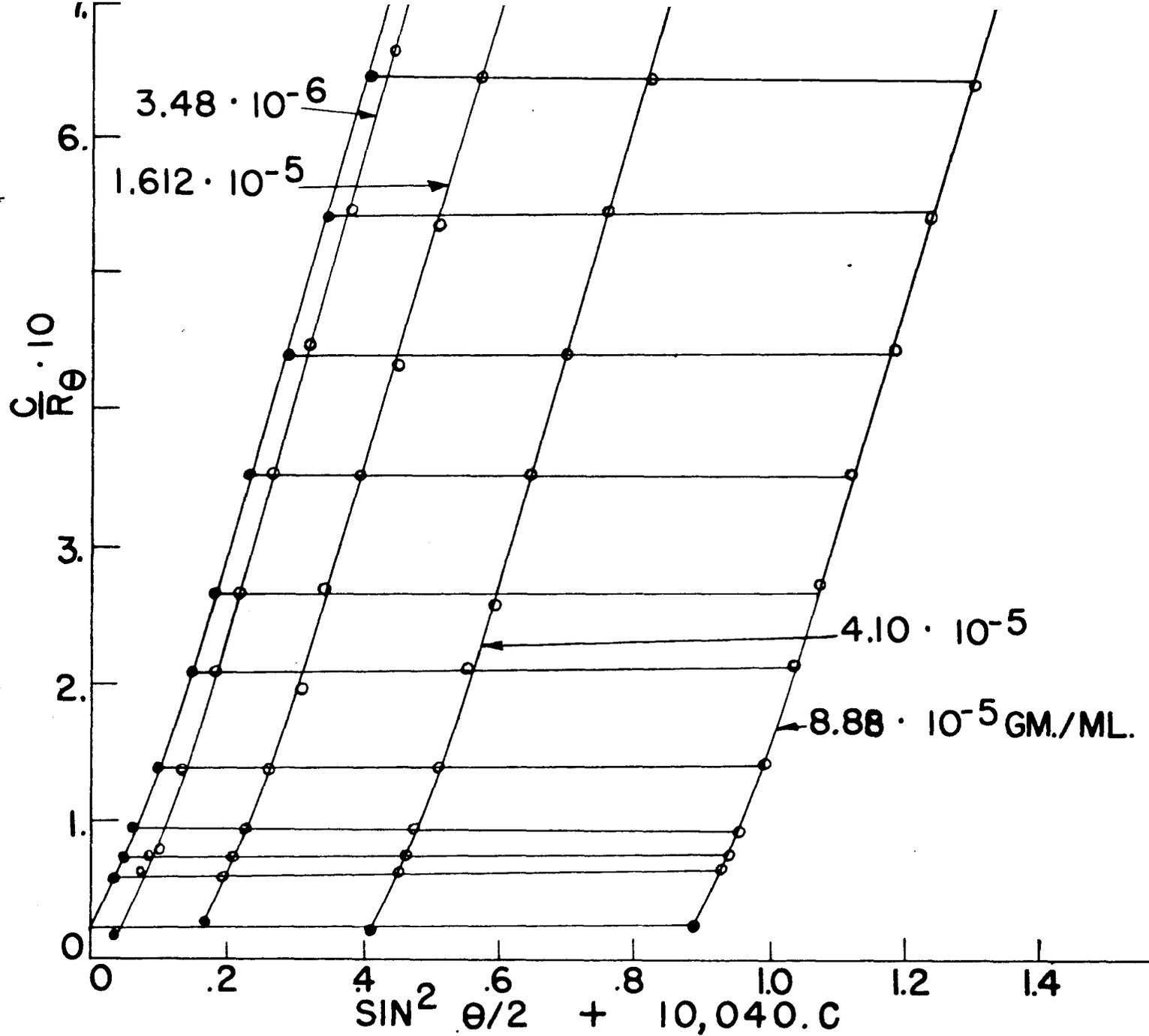
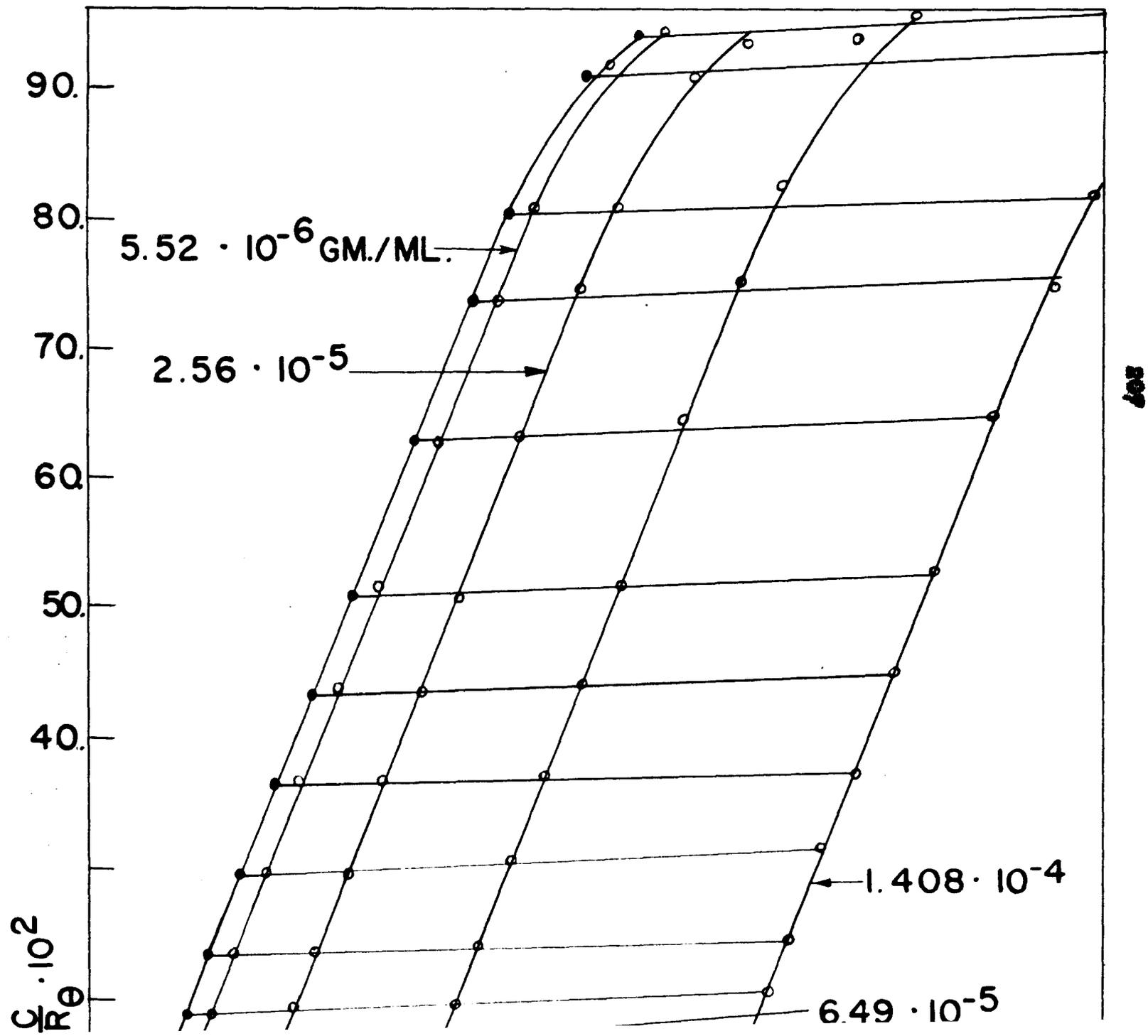


Fig. 72. Mature waxy starch. Dispersed in 6 M LiBr. $\bar{M}_w = 125 \times 10^6$.





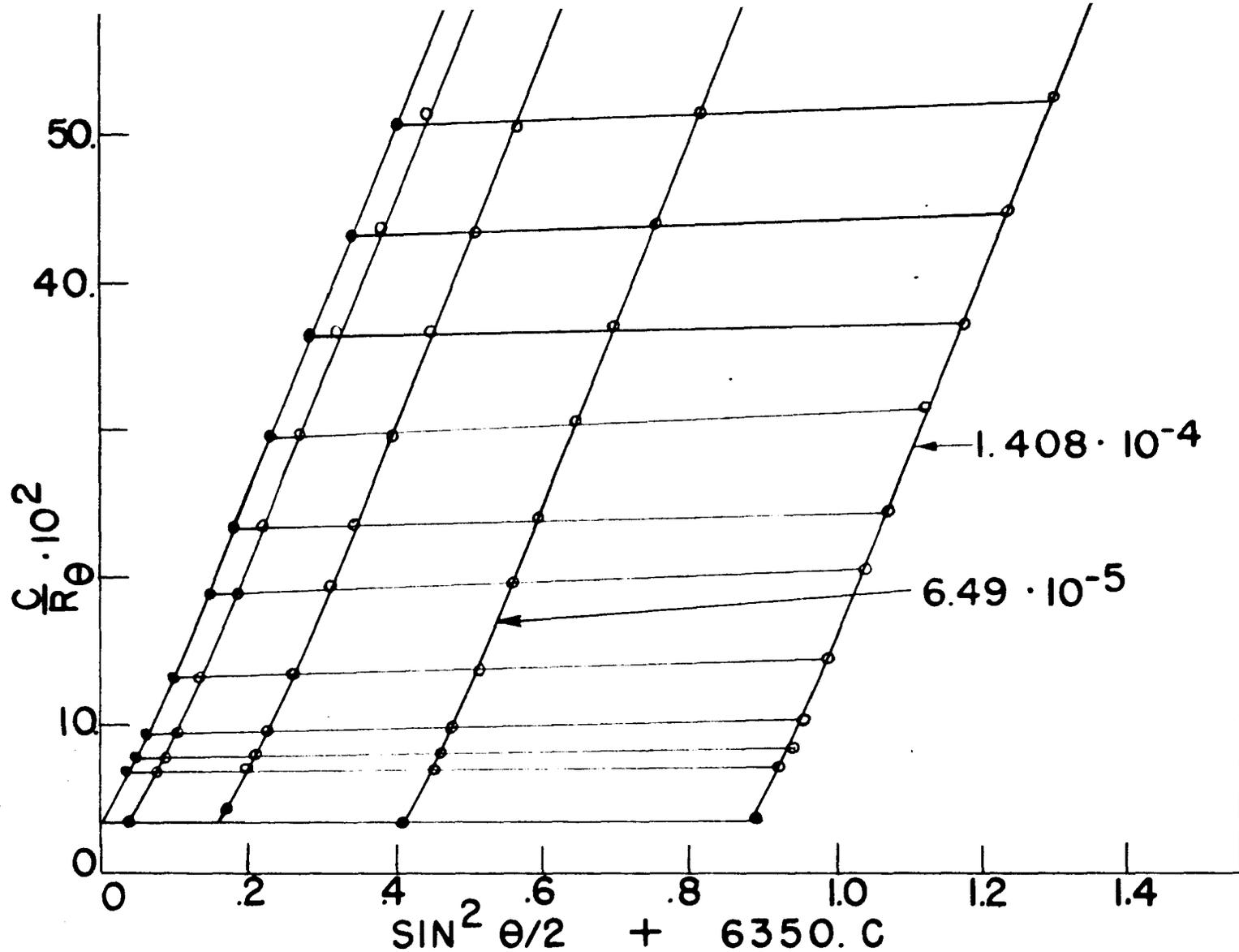


Fig. 73. Mature sweet corn amylopectin dispersed 24 hours in buffered n-amyl alcohol solution. $\bar{M}_w = 76 \times 10^6$.

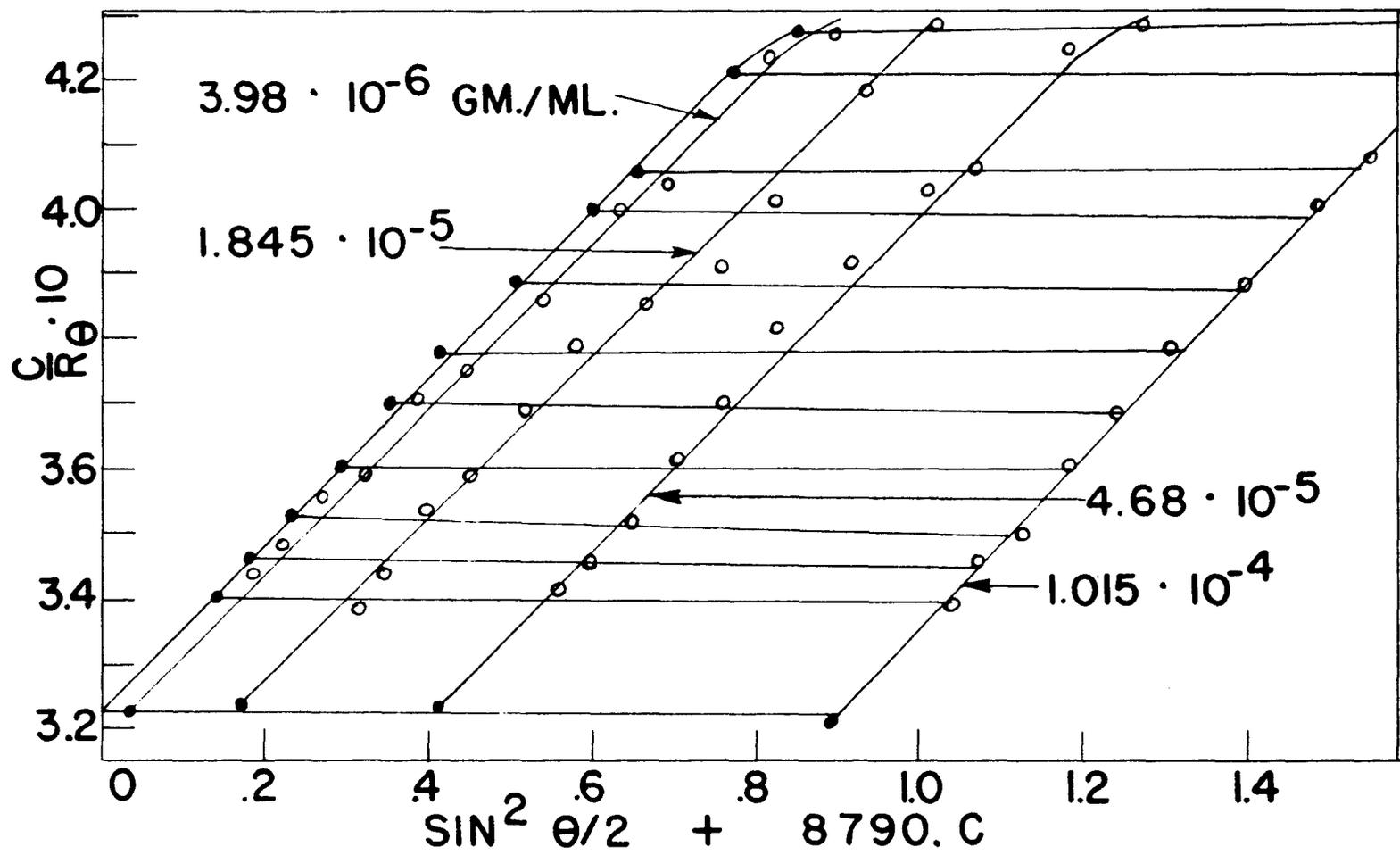


Fig. 74. Glycogen. Refluxed 24 hours in buffered n-amyl alcohol solution.
 $\bar{M}_w = 7.81 \times 10^6$.

Table 32. Sedimentation coefficients for 14th day dent corn amylopectin.

Amylopectin was dispersed for 20 hours in buffered Pentasol solution. Bridgmann's values of $\bar{V} = 0.65$ and $D_{20} = 0.5 \times 10^{-7}$ were used to calculate the molecular weight.

$C \times 10^2$ (gm./ml.)	1.586	0.529	0.1586
S_{20} (in Svedburgs)	36.2	106	257
Extrapolated value: $s_o^{20} = 625$ s			
$\bar{M}(S,D) = 87 \times 10^6$			

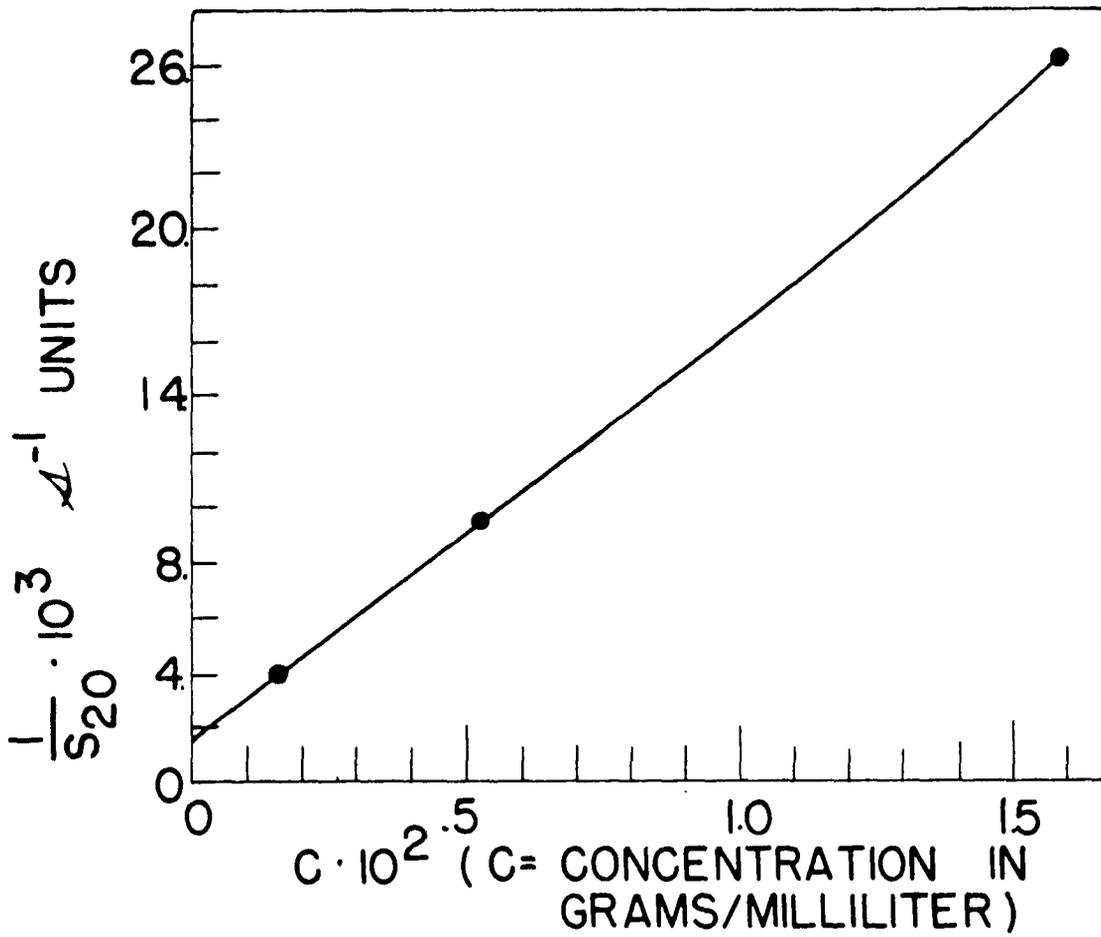


Fig. 75. The 13th day dent amylopectin. Extrapolation of sedimentation coefficients to zero concentration.

Table 33. Sedimentation coefficients for 13th day
waxy maize starch.

The 13th day waxy maize I and III starch samples were dispersed at 113°C in 6 M LiBr for 9 hours and at 125°C in 8 M LiBr for 6 hours, respectively. Bridgmann's values of $\bar{V} = 0.65$ and $D_{20} = 0.5 \times 10^{-7}$ and 1.1×10^{-7} for fast and slow components, respectively, were used to calculate the molecular weight.

Waxy I starch (fast component)

$C \times 10^2$ (gm./ml.) 1.008 0.501 0.454 0.200 0.0454 0.0454

S_{20} (in Svedburgs) 95 141 223 296 331. 552

Extrapolated value: $S_o^{20} = 720$ S

\bar{M} (S,D) = 100×10^6

Waxy III starch (fast component)

$C \times 10^2$ (gm./ml.) 0.574 0.491 0.1648 0.0404

S_{20} (in Svedburgs) 114 131 386 610

Extrapolated value: $S_o^{20} = 870$ S

\bar{M} (S,D) = 120×10^6

Waxy I starch (slow component)

$C \times 10^2$ (gm./ml.) 1.008 0.501 0.200

S_{20} (in Svedburgs) 17 20 94

\bar{M} (S,D) = 1.26×10^6 for $S_o^{20} = 20$ S

\bar{M} (S,D) = 5.9×10^6 for $S_o^{20} = 94$ S

Table 33. (Continued)

Waxy III starch (slow component)		
$C \times 10^2$ (gm./ml.)	0.574	0.491
S_{20} (in Svedburgs)	42	44

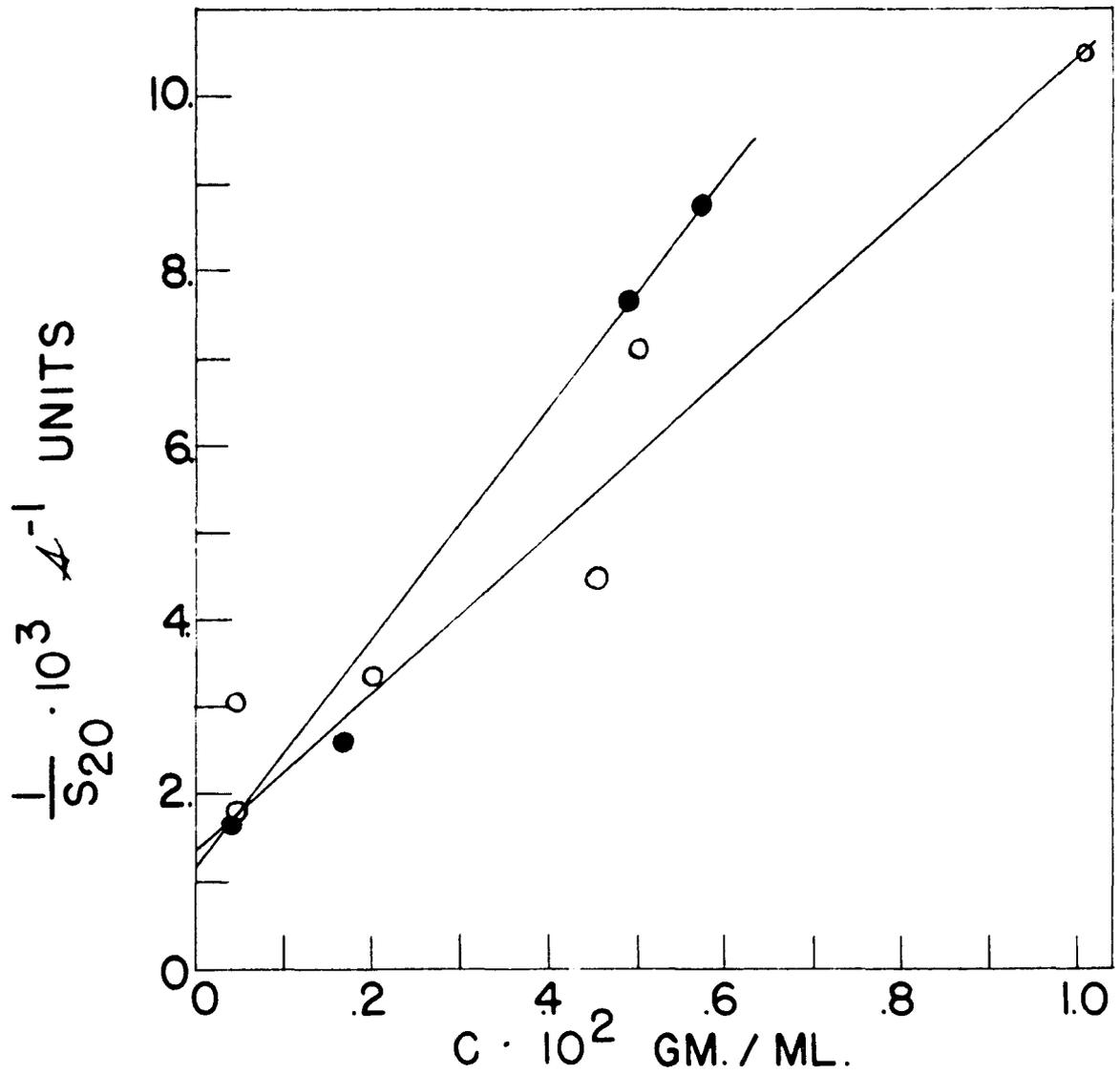


Fig. 76. (●) 13th day waxy maize starch sample III; (○) 13th day waxy maize starch sample I. Extrapolation of sedimentation coefficients to zero concentration.

Fig. 77. Picture showing light scattering clarification method.

