

#2097 THE INSTITUTE OF PAPER CHEMISTRY  
(Physical Chemistry of Lignin)  
Project Reports (2)

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## THE PHYSICAL CHEMISTRY OF LIGNIN PREPARATION

*this is a book  
for Project 2421  
Pulp Mfg. no. League.*

### SUMMARY

A brief description of the methods of preparing organic solvent soluble lignins and diazomethane methylated lignosulfonic acid is given in this report.

Approximately a five-gram sample of lignin was accumulated by each of the following ways of treating sprucewood meal; vibratory ball-milled, gamma ray irradiated, and high temperature steam hydrolyzed.

The basic principles of the refractive index gradient measurement by diffusion experiment, dipping refractometer, and Rayleigh Interferometer are outlined and the results are compared and discussed.

Light scattering determinations were made, and the corrections for depolarization, fluorescence, and dissymmetry were applied in each case as was necessary.

Average molecular weights by light scattering of vibratory ball-milled lignin in benzyl alcohol and dimethyl sulfoxide were 140,000 and 101,000, respectively. Gamma ray irradiated lignin in benzyl alcohol had a lower molecular weight of 92,400. The diazomethane methylated lignosulfonic acid in the same solvent by the same technique had a molecular

weight as high as 110,577,000 without fluorescence correction. It was impossible to obtain molecular weights by the light scattering technique on the two high temperature steam hydrolyzed lignins because of the dark color of their solutions.

The ultracentrifuge sedimentation velocity method was also applied for molecular weight determination on these lignin materials. The average molecular weight of vibratory ball-milled (VBM) lignin in benzyl alcohol was 183,000. This was the best of the data obtained by this method as compared to 140,000 for the same material in the same solvent by light scattering. A much lower molecular weight of 63,900 was obtained for gamma irradiated (GI) lignin when dioxane was used as the solvent.

If possible, the separation of colored matter from the lignin materials prior to molecular weight determination is strongly recommended.

#### INTRODUCTION

This is Project Report Six for Project 2097. The project was established to prepare a diverse group of lignins soluble in organic solvents and to compare the physical-chemical properties of these lignins.

The methods chosen for separating the lignins from wood were the vibratory ball mill, gamma ray irradiation, and high temperature steam hydrolyzation.

The solubility of these lignins in a selected group of organic solvents was determined.

Light scattering and ultracentrifuge techniques were applied to these lignin materials for molecular weight determinations.

## II. PREPARATION OF DIAZOMETHANE AND METHYLATION OF LSA (1)

A mixture of 60 cc. of a 50% aqueous KOH solution and 200 cc. of ethyl ether was placed into a two-necked, 500 cc. round-bottom flask. The mixture was cooled to below 5°C. and 20 grams of nitrosomethyl urea were added with shaking. The appearance of an amber color in the supernate ether indicates the formation of diazomethane. The second neck of the flask is fitted with a condenser rigged for distillation. The lower end of the condenser carries a glass-stoppered adapter which dips below the surface of 40 cc. of ether placed in a 300 cc. side-arm, round-bottomed flask. This flask is cooled to below 0°C. with ice and salt. Into this ether is placed 2.7 g. LSA-64-dc. This material is not soluble to any great extent in ethyl ether, but the methylation by diazomethane takes place heterogeneously as well as in homogeneous solution. The ice bath is removed from the flask containing the ether-diazomethane supernate, and is replaced by a water bath at about 50°C. This bath is heated to the boiling point of ether. The ether is distilled, carrying the diazomethane with it, until it comes over colorless. This occurred when about 2/3 of the ether had been distilled. The collection flask was then set aside overnight to warm and react. The next morning the LSA had reacted, as could be seen from the color, i.e., straw yellow. The ether was allowed to evaporate. A sample was sent to the Analytical Group for a methoxy contents determination.

### III. SOLUBILITY TEST

About 5 cc. of organic solvent were introduced onto the lignin sample in a test tube. The color of the solution was observed after mixing.

### IV. LIGHT SCATTERING TECHNIQUE

#### 1. Refractive Index Gradient

##### A. Obtained from the diffusion experiments.

In the diffusion experiments, the Rayleigh fringe number had been determined as a function of concentration. If the size of the cell and the wavelength of the light are known, the difference in refractive index of the solvent from the solution is given by the equation,

$$\Delta\eta = \frac{\lambda J}{ch}$$

where  $\lambda = 5.461 \times 10^{-5}$  cm.

J = number of fringes

h = 1.204 cm.

##### B. By the Bausch and Lomb Dipping Refractometer

A reading can be taken simply by immersing the attached prism in the thermostated sample and observing the position of the achromatized critical boundary on a scale in the eyepiece of the telescope. The refractive index can be obtained from scale readings by interpolation in tables provided by the manufacturer.

C. By the Rayleigh Interferometer

With the reference solution in both chambers, the adjustments for obtaining sharp achromatic bands are made. The micrometer screw is turned until the upper achromatic band exactly coincides with the reference band. The lefthand cell is emptied and refilled with the unknown sample after rinsing with distilled water and the solution. The micrometer is turned until the bands again coincide. The difference of index of refraction of the reference and unknown solutions is given by

$$\frac{dn}{dc} = \frac{M \lambda}{L}$$

where  $M$  = the number of fringes shifted,  
 $\lambda$  = the wavelength of the light,  
 $L$  = the path length.

With a 1-cm. liquid cell, each 1/47 fringe shift corresponds to a refractive index change of one part per million.

2. Light Scattering Determination

The Brice-Phoenix Series 1000 light-scattering photometer was used for this investigation.

Prior to any measurements on polymer solutions, it is important that dust and other particles be completely removed. This may be done by filtration. The degree of filtration should be established by the investigator on the basis of one's knowledge of the properties of the material under study. In this study, a pressure filtration

funnel which was constructed entirely of stainless steel including base, collar, retaining ring, and funnel with a sinter stainless steel filter support was used. A 1500A Gelman filter paper which was conditioned in a 20% alcohol-water mixture was placed on the top of the sinter support. Prior to the filtration, the filter paper was washed a few times with distilled water. A small amount of solvent was poured through the filter paper and discarded. About 15 to 20 ml. of solvent, depending on the size of the cell used, were filtered and caught directly into a tared and cleaned semioctagonal dissymmetric cell. This cell was covered and set aside for turbidity examination.

On the same filter paper, the solution was filtered into a volumetric flask. This was repeated until the G45 and G135 measurements were almost equal. With the weights of the cell and the cell with solvent known, several cc. of the filtered solution were added to the solvent in the cell, the mixture was weighed again. The cell was then turned around manually in a centrifuge apparatus until the contents were well mixed. This solution was then ready for scattering ratios, depolarization, fluorescence and dissymetry measurements. These determinations were repeated at 5 to 6 concentrations on each sample. The calculation of  $H \frac{c}{\eta}$  and  $C$ . and the corrections for fluorescence and dissymetry were done by the 1620 computer.

## V. ULTRACENTRIFUGE TECHNIQUE

### 1. Partial Specific Volume Determination

#### A. Preparation of Water for Calibration

To each liter of the freshly distilled water were added .5 g. c.p. NaOH and 0.2 g. c.p. potassium permanganate. A third distillation may be made in the same way except that a few drops of  $H_2SO_4$  per liter was added instead of alkaline permanganate for 6th place accuracy work.

#### B. Density Measurement

A Lipkin pycnometer was used for this purpose

For experimental details of "weighing", "precautions in handling the pycnometer", "filling and adjusting", and "calculation of density" the reader is referred to reference (2).

After the densities of the solutions and solvent are determined the partial specific volume of the sample is then calculated by the following formula:

$$V = \frac{1}{d_0} - \frac{1}{c} \left( \frac{d - d_0}{d_0} \right)$$

where

$d_0$  = density of solvent,

$c$  = concentration of the sample, in g/ml;

$d$  = density of solution.

#### 2. Sedimentation Coefficient Determination

Sedimentation velocity method was chosen for this study. The experiments were performed by the Biochemistry Laboratory at the Institute in the Spinco Model E ultracentrifuge. The ultracentrifuge was operated at the speed of 56,100 r.p.m. Photographs were taken at



periodic intervals, depending on the rate of movement of the boundary. Photographs were read on a microcomparator which is connected to an IBM card punch. The photographic plates should be aligned in such a way that the meniscus is perpendicular to the cross motion of the comparator. Distances from the maximum ordinate to the reference hole were measured in a series of photographs. These distances on the plate were converted to the true distances in the cell by dividing them by the magnification factor of the camera lens. The converted distances were then increased by 5.72 cm. to give true distances from the axis of rotation to the boundary. Maximum ordinate sedimentation constants were determined by using the method given by Elias (3).

### 3. Diffusion Coefficient Determination

The free diffusion runs were done by the Biochemistry Group at the Institute, using the Spinco Model H and Rayleigh optics.

The photographic plates from the experiment were first aligned in the comparator so that the straight portions of the pattern are parallel to the motion of the comparator as judged by the cross hairs in the instrument. Starting at one end of the boundary with the plate carefully aligned, the screw of the comparator was turned so that the plate was moved and the number of "white" fringes crossed in traversing the boundary were counted and recorded. It was noticed that when the other end of the pattern was reached, the cross-hairs do not lie at the same level between the parallel lines as observed on the first side of the boundary. This displacement to the

separation between the parallel fringes is the fractional number of fringes. The total number,  $J$ , was the sum of these two measurements. The fractional fringe was done most readily from an early picture in which the width of the boundary was small. The method chosen for the calculation of diffusion coefficients was that of Longworth (4) by means of an IBM 1620 computer program (5). Molecular weight was calculated directly from the sedimentation and diffusion coefficients at infinite dilution by the following Svedberg equation,

$$M = \frac{RTS}{D(1 - v\rho)}$$

where

R = the gas constant,  $8.314 \times 10^7$  ergs/mole/degree

T = the absolute temperature,

M = the molecular weight.

## RESULTS AND DISCUSSION

### AMOUNT OF LIGNINS PREPARED AND THEIR SOLUBILITIES IN ORGANIC SOLVENTS

Recently many advances have been made in securing soluble lignin preparations. Of particular interest is the new vibratory ball mill technique which renders wood or isolated lignin material soluble in organic solvents. The amount of VBM and two high temperature steam hydrolyzed products extracted from dioxane, and percentage of yield based on the weight of wood meal are listed as follows:

	Amount Prepared, g.	Yield, %
VBM	5.2	5.09
2097-1858-86	6.0	7.19
2097-1858-92	4.8	6.22

For securing an uncharged derivative of LSA for molecular weight determination, Sample LSA-64-cd was diazomethane methylated. The methoxyl and moisture contents of LSA-64-cd and methoxylated LSA-64-cd are presented as follows:

Sample	Analysis	Method	Per Cent
LSA-64-cd	Moisture	IPC No. 18	.984
Methylated LSA-64-cd	"	"	.983
LSA-64-cd	Methoxyl		11.40
Methylated LSA-64-cd	"		22.20

The results of the solubilities of these prepared lignins and Indulin A in organic solvents are listed in Table I.

TABLE I

SOLUBILITIES OF GI, VEM, INDULIN A, METHYLATED LIGNIN AND STEAM HYDROLYZED LIGNINS IN ORGANIC SOLVENTS

Solvent	Gamma Ray Irradiated		VEM		Indulin A		Methylated Lignin		Steam Hydrolyzed		Steam Hydrolyzed	
	Soluble	Partial None	Soluble	Partial None	Soluble	Partial None	Soluble	Partial None	Soluble	Partial None	Soluble	Partial None
Toluene	x		x								x	
Xylene	x		x								x	
Carbon tetrachloride	x		x								x	
Benzyl alcohol	x		x		x		x		x		x	
Guaiacol	x		x		x		x		x		x	
Ethylene glycol	x		x		x		x		x		x	
Dimethyl sulfoxide	x		x		x		x		x		x	
P-dioxane	x		x		x		x		x		x	
Acetone	x		x				x				x	
Benzaldehyde	x		x				x		x		x	
Petroleum ether	x		x								x	
Acetic anhydride	x		x				x				x	
Amyl acetate	x		x								x	
Triethanolamine	x		x		x		x				x	
Dimethyl phthalate												
Isopropyl alcohol												
Cyclohexanone	x		x		x		x		x		x	
Dimethyl formamide	x		x		x		x		x		x	
g-Methoxyethanol	x		x		x		x		x		x	
Ethyl acetate	x		x									
Methyl ethyl ketone	x		x		x		x		x		x	
Ethyl alcohol												

<sup>a</sup> Soluble--complete solution occurs  
<sup>b</sup> Dark brown solution results  
<sup>c</sup> None--no color in the solvent

REFRACTIVE INDEX GRADIENT

The precision with which the absolute molecular weights can be determined depends greatly on the accurate measurement of the change in refractive index with concentration, because this appears as a squared term in the Debye equation. To illustrate this farther, for a 1% solution, the difference between the refractive index of the solution and that of the solvent is about 0.001, so that 1% accuracy in the difference requires the refractive index to be accurated in the fifth decimal. Great difficulties were encountered in determining this quantity, especially in our high temperature steam hydrolyzed and Indulin A samples.

The refractive index measurement by means of the dipping refractometer is based on the observation of the border line of total reflection between the substance being measured and the prism surface. It is frequently stated that the accuracy is about  $3.5 \times 10^{-5}$  in refractive index. For some unexplainable reason, in our samples the  $dn/dc$  values calculated from the refractometer readings were not nearly as accurate as they had claimed. The results are presented in Table II.

In an effort to secure more information about  $dn/dc$  values, the Rayleigh interferometer was applied. The Rayleigh Interferometer is a differential instrument. The temperature control is not as critical as with the previously mentioned refractometer; but for some problems the interferometer is invaluable; such as a means of analyzing solutions or of observing small changes in concentration. These results are much better than those obtained from the dipping refractometer.

The results of  $dn/dc$  values determined by the Rayleigh interferometer are shown in Table III.

TABLE II

dn/dc VALUES DETERMINED BY THE DIPPING REFRACTOMETER

Sample	Conc., g/ml.	Temp. <sup>o</sup> C.	Scale Division	Index	dn/dc
Benzyl alcohol		22.2	81.3	1.53842	--
VBM in benzyl alcohol	$2.8 \times 10^{-2}$	22.3	87.0	1.54021	.0640
" " " "	$1.3 \times 10^{-2}$	22.1	83.0	1.53895	.0408
" " " "	$8.4 \times 10^{-3}$	22.1	82.0	1.53864	.0262
Benzyl alcohol		19.5	85.0	1.53958	--
GI in benzyl alcohol	$4.3 \times 10^{-2}$	19.2	94.0	1.54240	.0656
" " " "	$1.7 \times 10^{-2}$	19.5	88.3	1.54062	.0612
" " " "	$8.6 \times 10^{-3}$	19.5	85.0	1.53958	--

TABLE III

dn/dc VALUES DETERMINED BY RAYLEIGH INTERFEROMETER

Sample	Conc., g/ml.	Temp., °C.	Drum Reading	dn/dc*
VBM in benzyl alcohol	$2.802 \times 10^{-3}$	21.5	170	.0698
" " " "	$4.206 \times 10^{-3}$	"	266	.0727
" " " "	$6.305 \times 10^{-3}$	"	439	.0801
" " " "	$8.406 \times 10^{-3}$	"	607	.0831
				av. .0764
GI in benzyl alcohol	$4.279 \times 10^{-3}$	21.5	211	.0568
" " " "	$8.558 \times 10^{-3}$	"	457	.0615
" " " "	$1.712 \times 10^{-3}$	"	962	.0646
				av. .0610
Indulin A in benzyl alcohol	$1.245 \times 10^{-3}$	21.8	-90	} .01443
"	$2.490 \times 10^{-3}$	"	-68	
"	$4.98 \times 10^{-3}$	"	-28	
"	$7.470 \times 10^{-3}$	"	-14	
Indulin A in dimethyl sulfoxide	$5.022 \times 10^{-5}$	23	-96	} .7926
"	$1.004 \times 10^{-4}$	"	86	
"	$2.511 \times 10^{-4}$	"	134	
"	$5.022 \times 10^{-4}$	"	507	
"	$7.533 \times 10^{-4}$	"	912	

\* See Appendix I for method of calculation

The  $dn/dc$  values of VBM and the two high temperature steam-hydrolyzed lignins in dimethyl sulfoxide were examined by the Beckman Model E ultra-centrifuge. The optical system of the Model E is a modified Rayleigh interferometer. The interference system measures the difference between the refractive indexes of solute and solvent contained in a double sector cell and records this difference by means of curved fringes. With an interference pattern, the change in refraction index can be determined by counting the number of fringes across the centrifuge cell with an optical microcomparator. The number of fringes can be counted to a precision within .01 of a fringe; thus, the system can measure concentration differences as small as 0.001%. Due to some unknown reasons, or sorption systems, the total number of fringes changed during the run. As a consequence, the values of  $dn/dc$  were also changed. The results of these values are presented in Table IV.

TABLE IV  
 $dn/dc$  VALUES DETERMINED BY BECKMAN MODEL E ULTRACENTRIFUGE

Sample	Conc., g/ml.	J	$dn/dc$
VBM in $(CH_3)_2SO$	$4.1 \times 10^{-3}$	6.6	0.0730
"	"	7.1	0.0711
			av. 0.0721
High temp. steam-hydrolyzed (86) in $(CH_3)_2SO$	$3.2 \times 10^{-3}$	7.2	0.1021
High temp. steam-hydrolyzed (92) in $(CH_3)_2SO$	$2.7 \times 10^{-3}$	6.6	0.1109
"	"	7.0	0.1176
"	"	7.1	0.1193
			av. 0.1159



Light Scattering Determinations

Scattering ratios, depolarization, fluorescence, and dissymmetry were determined at several concentrations of each sample in either dimethyl sulfoxide or benzyl alcohol.

Depolarization measurement is a means to measure the depolarization of unpolarized incident light. If the particles are isotropic, the light scattered is completely polarized. If the particles are anisotropic, some depolarization of the  $90^\circ$  scattered light may be detected and a correction must then be applied to the turbidity.

The correction for this effect has been related to the depolarization ratio,  $P_u$ , by Cabannes and Rocard (6) through the following ratio.

$$C_u = (6 - 7 P_u)/(6 + 6 P_u)$$

where  $C_u$  the Cabannes factor, is multiplied by the observed molecular weight, to obtain the true average molecular weight. The depolarization ratio is given by

$$(P_u)_c = \frac{(FG_{90}^H/G_0^H) \text{ solution} - (FG_{90}^H/G_0^H) \text{ solvent}}{(FG_{90}^V/G_0^V) \text{ solution} - (FG_{90}^V/G_0^V) \text{ solvent}}$$

where  $(P_u)_c$  is the value at a given concentration and must be extrapolated to zero concentration to obtain  $P_u$ . The superscripts  $H$  and  $V$ , respectively, refer to the horizontal and the vertical components of the scattered light.

In all of the samples under study, the solutions exhibit appreciable fluorescence. Because of this it is difficult to obtain a reliable depolarization value.

The ratio of  $I_{45}/I_{135}$  is known as dissymmetry. If the particles in solution are larger than the wavelength of the light, scattering will not be symmetric about  $90^\circ$ . There will be greater forward than backward scattering. In all the samples studied, we found only the methylated lignin had a ratio of 1.42 while the others are in the neighborhood of unity. In other words, they are all small particles.

The molecular weights were determined by plotting  $Hc/\eta$  vs.  $C$  for a number of solutions and extrapolating to zero concentration. The results of light scattering in various concentrations of VBM in benzyl alcohol and dimethyl sulfoxide are shown in Figures 1 and 2, respectively. The molecular weight of VBM is obtained as follows:

In benzyl alcohol

$$\text{Uncorrected } Hc/\eta = 1.12 \times 10^{-6} \text{ as } c \rightarrow 0$$

$$\text{Mol. wt.} = 892,800$$

$$\text{Fluorescence corrected } Hc/\eta = 2.0 \times 10^{-6} \text{ as } c \rightarrow 0$$

$$\text{Mol. wt.} = 500,000$$

Depolarization and fluorescence corrected mol. wt.

$$500,000 \times .28 = 140,000$$

(when  $C_u = .28$ )

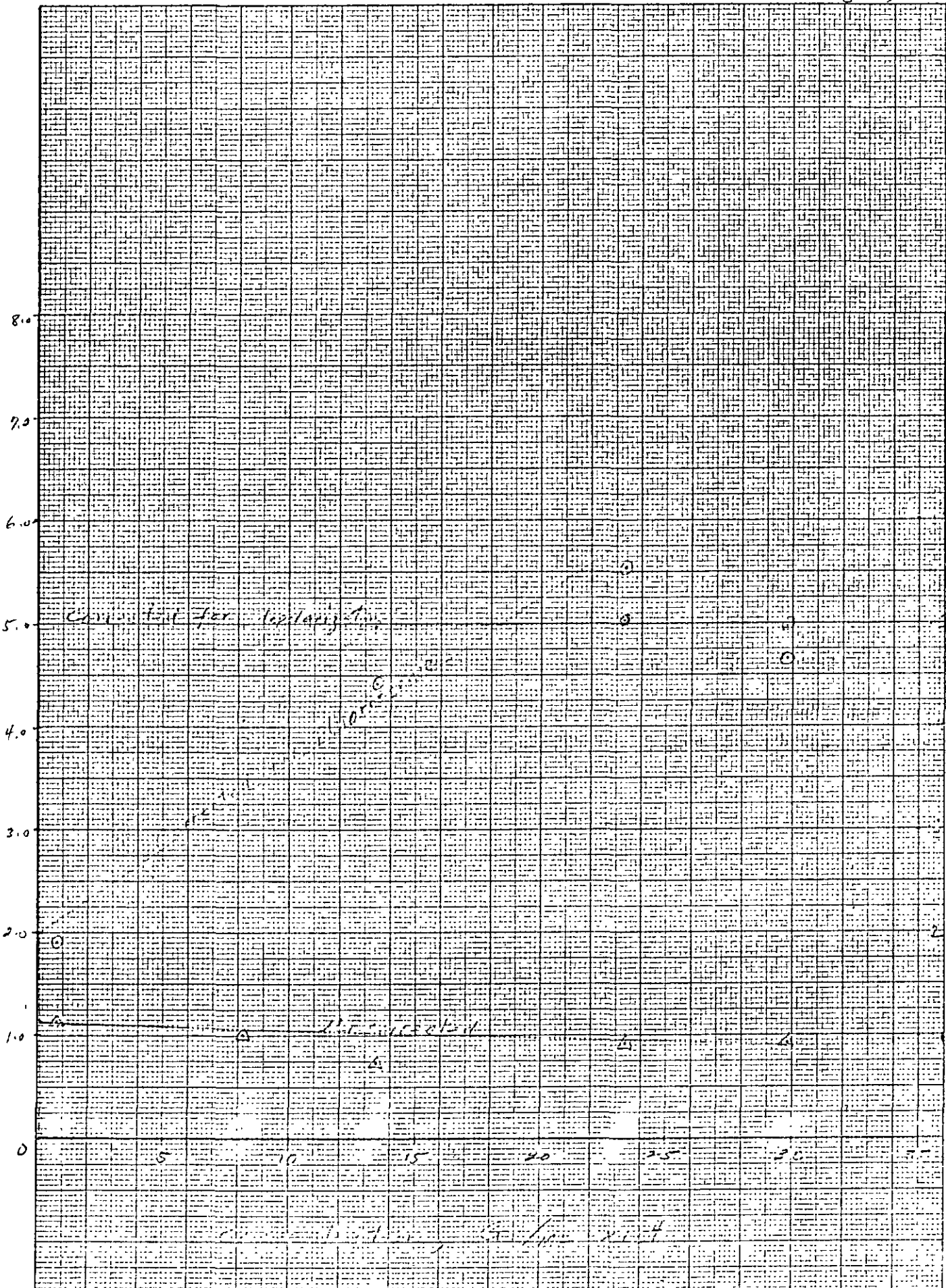
In dimethyl sulfoxide

$$\text{Uncorrected } Hc/\eta = 4.2 \times 10^{-7} \text{ as } c \rightarrow 0$$

$$\text{Mol. wt.} = 2,380,900$$

Heit x 10<sup>6</sup>

K&E 10 X 10 TO THE CENTIMETER 46 1510  
10 X 25 CM  
MADE IN U.S.A.  
KEUFFEL & ESSER CO



1.1

2

constant for (optical) time

K&W  
10 X 10 TO THE CENTIMETER 46 1510  
10 X 2 1/2 CM  
MADE IN U.S.A.  
KLUFFELL & ESSER CO.

$H_c / r \times 10^7$

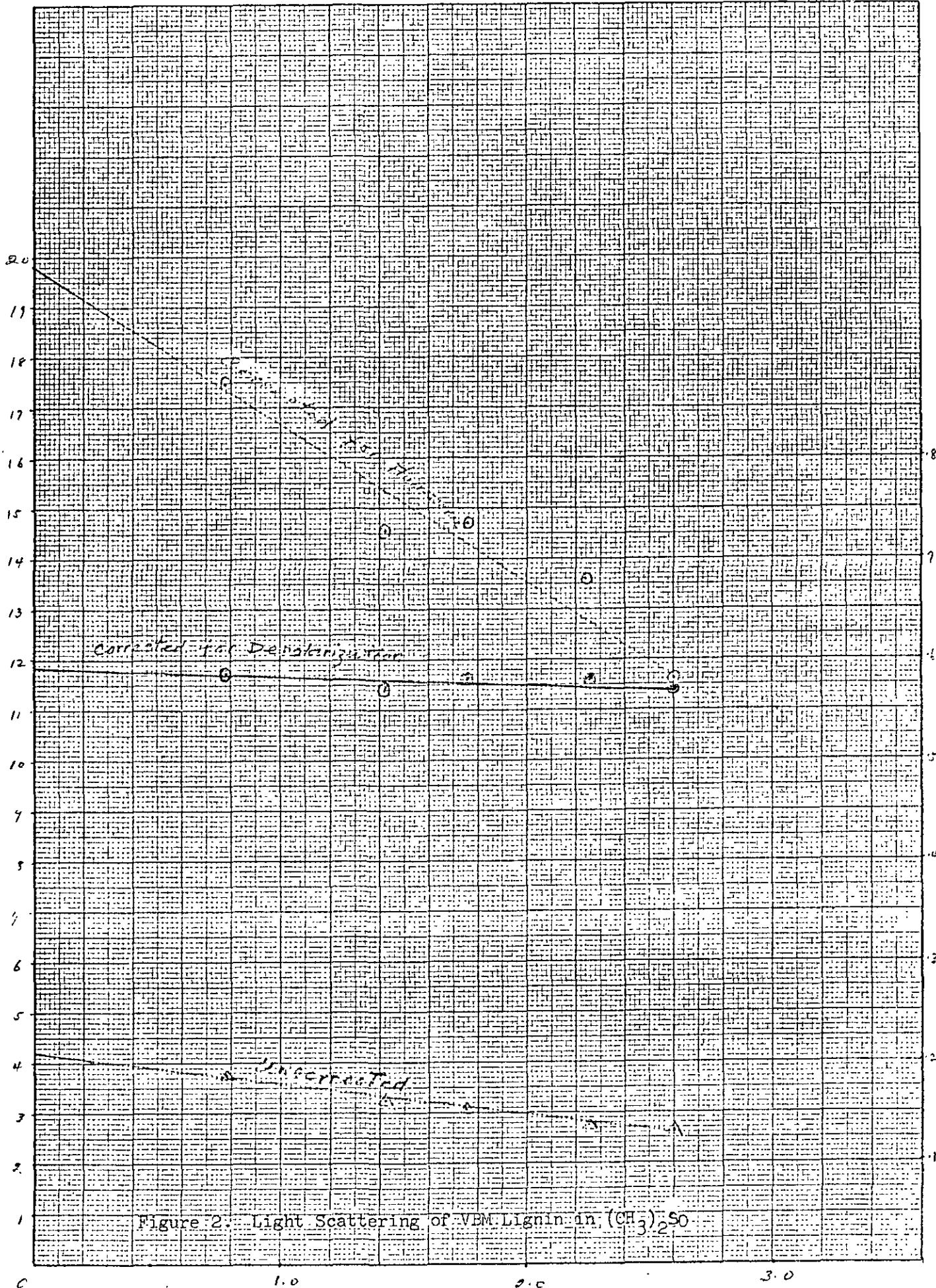


Figure 2. Light Scattering of VBM Lignin in  $(CH_3)_2SO$

Fluorescence corrected  $H_c/$  =  $19.8 \times 10^{-7}$  as  $c \rightarrow 0$

Mol. wt. = 505,050

Depolarization and fluorescence (when  $C_u = .20$ ) corrected molecular weight =  $505,050 \times .20 = 101,010$ .

It is noted that both the fluorescence and depolarization corrections are of considerable size, plus the difference in the nature of polymer-solvent interaction. One would understand why the results are not in good agreement; however, they are in the same order. The dissymmetry values in both cases are small. There is no need for correction. The result of the light scattering of gamma ray irradiated lignin in benzyl alcohol, the fluorescence and depolarization correction are shown in Figure 3. The depolarization and fluorescence corrected molecular weight is 92,400. There is no dissymmetry correction necessary. The results of light scattering of Indulin A in benzyl alcohol and dimethyl sulfoxide are shown in Figures 4 and 5. It is seen that to make any extrapolation to zero concentration is impossible. Deviation from a straight line was assumed by many. Tanford (7) explained it from charge fluctuation. Bump (8) stated that it was due to molecular association. Neurath, Greenstein, and Putnam (9) suggested that the aggregation of the textile dyes in their light scattering experiments was due to the formation of the hydrogen bond. The presence of a large colloidal impurity in the sample is another possibility. However, the writer believes that the most important thing for these highly colored samples is to get rid of the color or purify them by some technique.

It is unfortunate that the data for fluorescence correction was so irrational. Therefore, the linear extrapolation of methylated lignin, shown in Figure 6, is possible only for uncorrected. The depolarization corrected

K&E 10 X 10 TO THE CENTIMETER 46 1510  
10 X 25 CM KEUFFEL & ESSER CO.

$He/\lambda \times 10^6$

4.0  
3.0  
2.0  
1.0  
0

6  
5  
4  
3  
2  
1

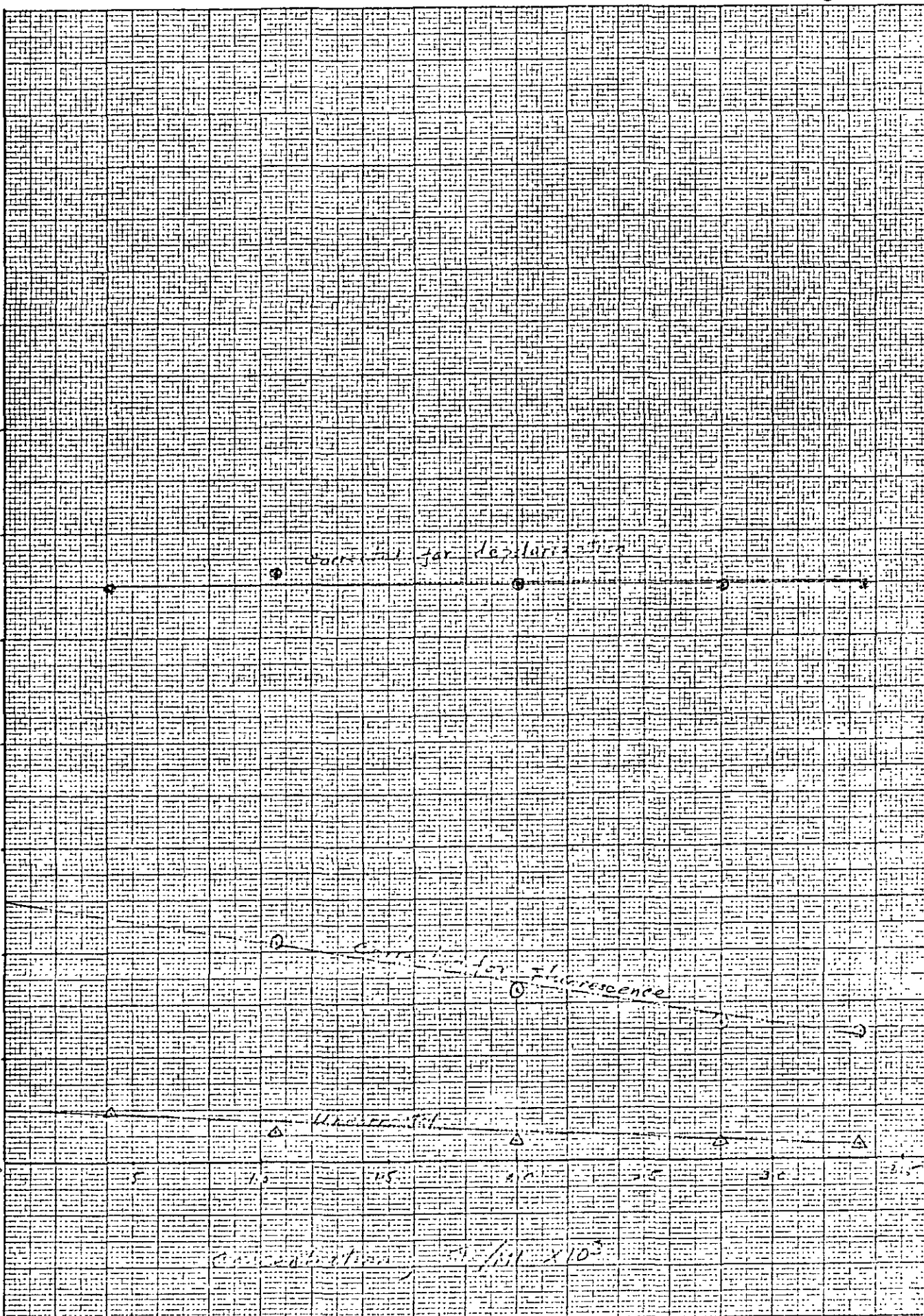


Figure 3. Light Scattering of GI Lignin in Benzyl Alcohol



Figure 4. Light Scattering of Indulin A in  $C_6H_5CH_2OH$

K&E  
10 X 10 TO THE CENTIMETER 45 1510  
MADE IN U.S.A.  
KLEINFELDER & ESSER CO.

$H_c / r \times 10^{10}$

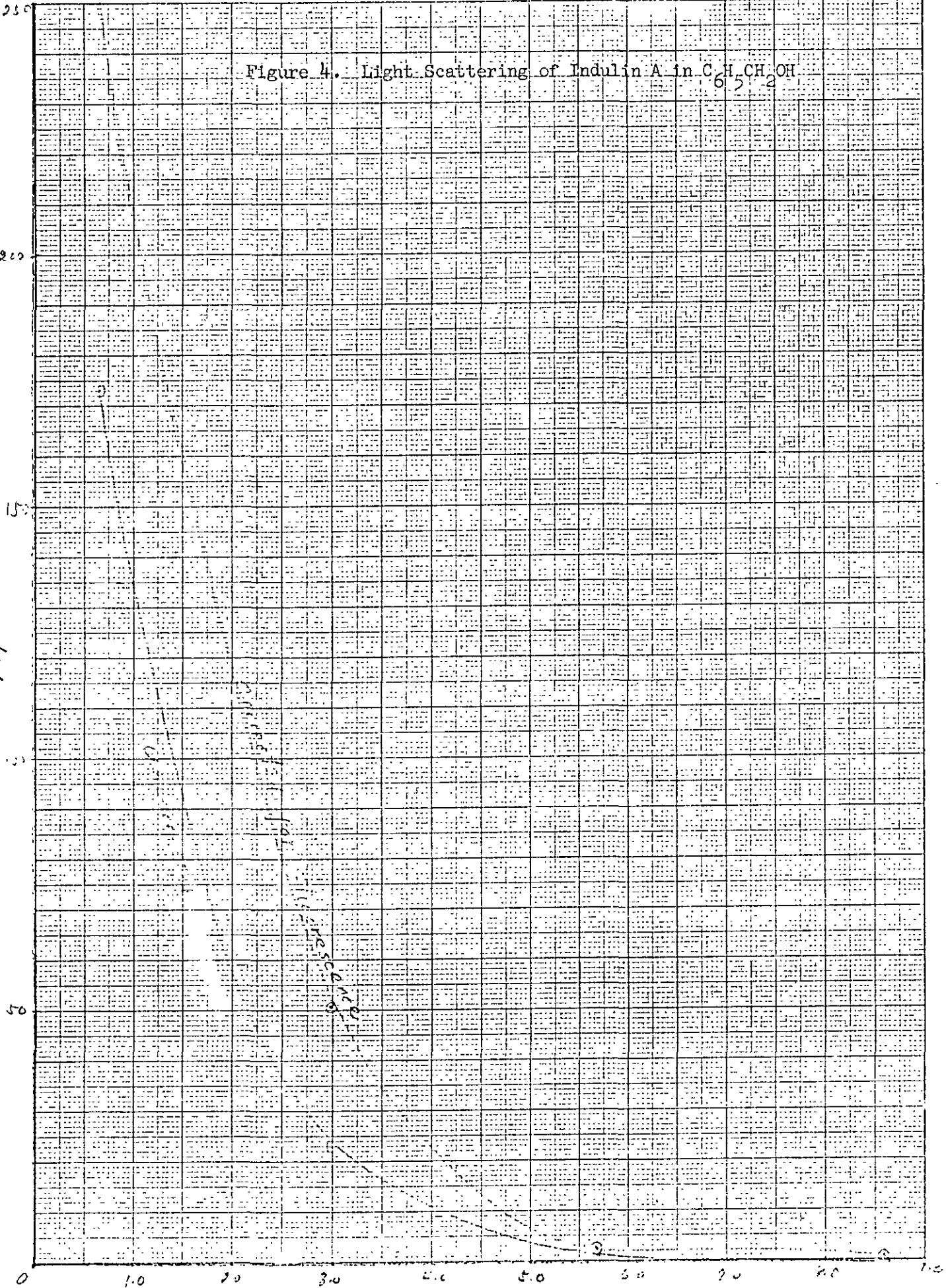


Figure 5. Light Scattering of Indulin A in  $(CH_3)_2SO$

40

30

20

10

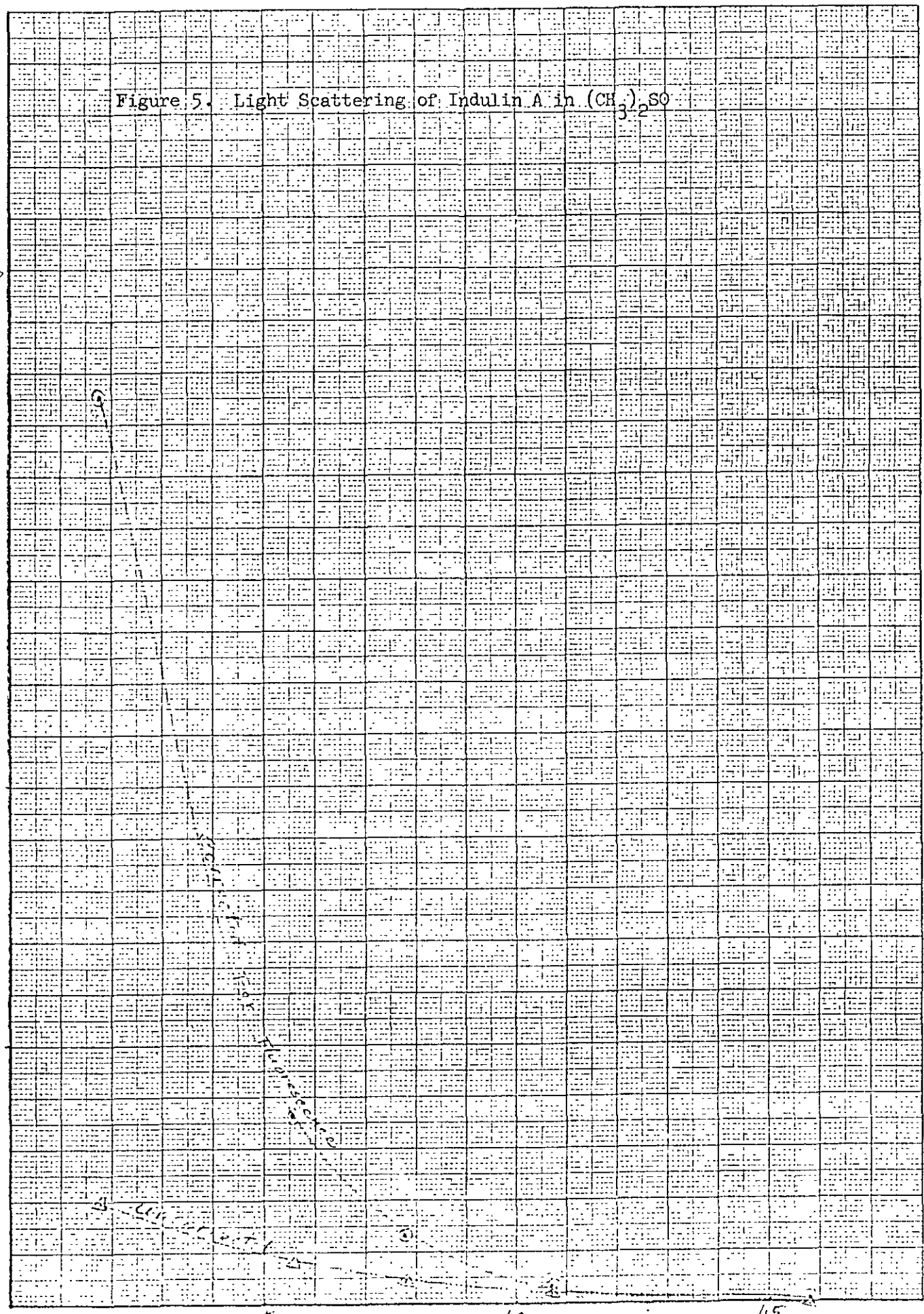
25

10

15

H<sub>2</sub>O x 106

K&E 10 X 10 TO THE CENTIMETER 46-1510  
KLEINFELDER G. GIBSON CO.





K&E 10 X 10 TO THE CENTIMETER 46 1510  
MADE IN U.S.A.  
KEUFFEL & ESSER CO.

1014 x 107

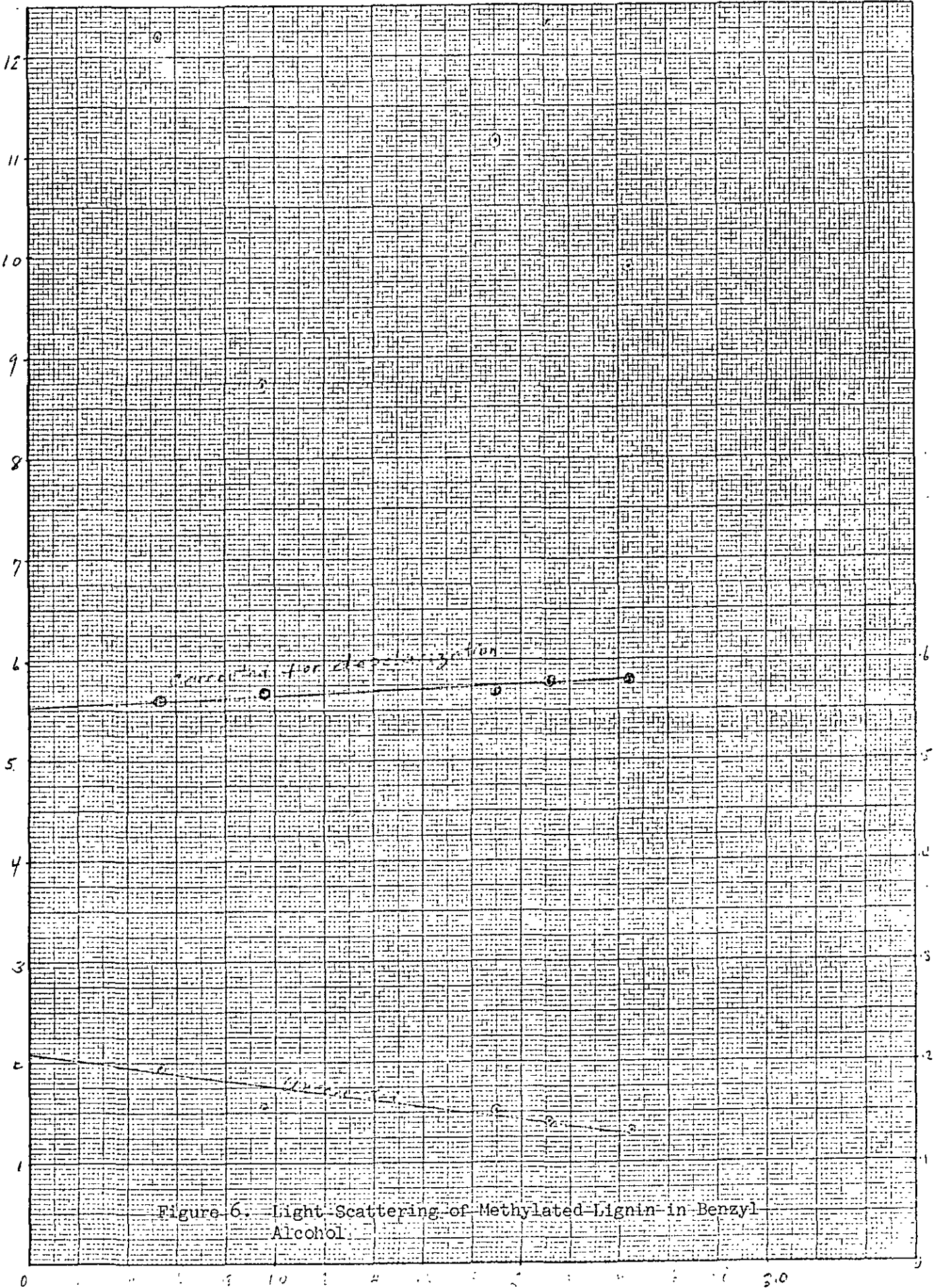


Figure 6. Light Scattering of Methylated Lignin in Benzyl Alcohol.

0 1 2 3 4 5 6 7 8 9 10 11 12

Concentration of Lignin

corrected molecular weight is 110,577,100 when

$$Hc/\tau = 2.08 \times 10^{-9} \text{ as } c \rightarrow 0$$

and  $C_u = .23$ .

The dissymmetry ratio of this sample is 1.42 but this information alone is not enough to know the size and shape of this particle. Further technique such as Jimm's plot should be explored.

The two high temperature, steam-hydrolyzed lignins, namely -86 and -92, are very dark in color. The light scattering patterns are similar to those of Indulin A. The lines shown in Figures 7 and 8 are far from linear. Therefore, zero extrapolation is impossible. Frank and Ullman (10) observed an effect of light absorption in color solutions during dissymmetry measurement, and suggested that it must be corrected. According to them, the correction is a function of optical path length, width of incident beam, width of aperture at the light receiver, and absorption coefficient.

The results of uncorrected and corrected molecular weight from light scattering experiments are summarized in Table V.

TABLE V

UNCORRECTED AND CORRECTED MOLECULAR WEIGHTS OBTAINED  
 FROM LIGHT SCATTERING MEASUREMENTS AT 4358 Å

Lignin	VBM	VBM	GI	Methylated LSA
Solvent	$C_6H_5CH_2OH$	$(CH_3)_2SO$	$C_6H_5CH_2OH$	$C_6H_5CH_2OH$
Uncorrected*	892,800	2,380,900	2,000,000	480,770,000
Fluorescence corrected*	500,000	505,050	400,000	--
Depolarization corrected*	140,000	101,010	92,400	110,577,100

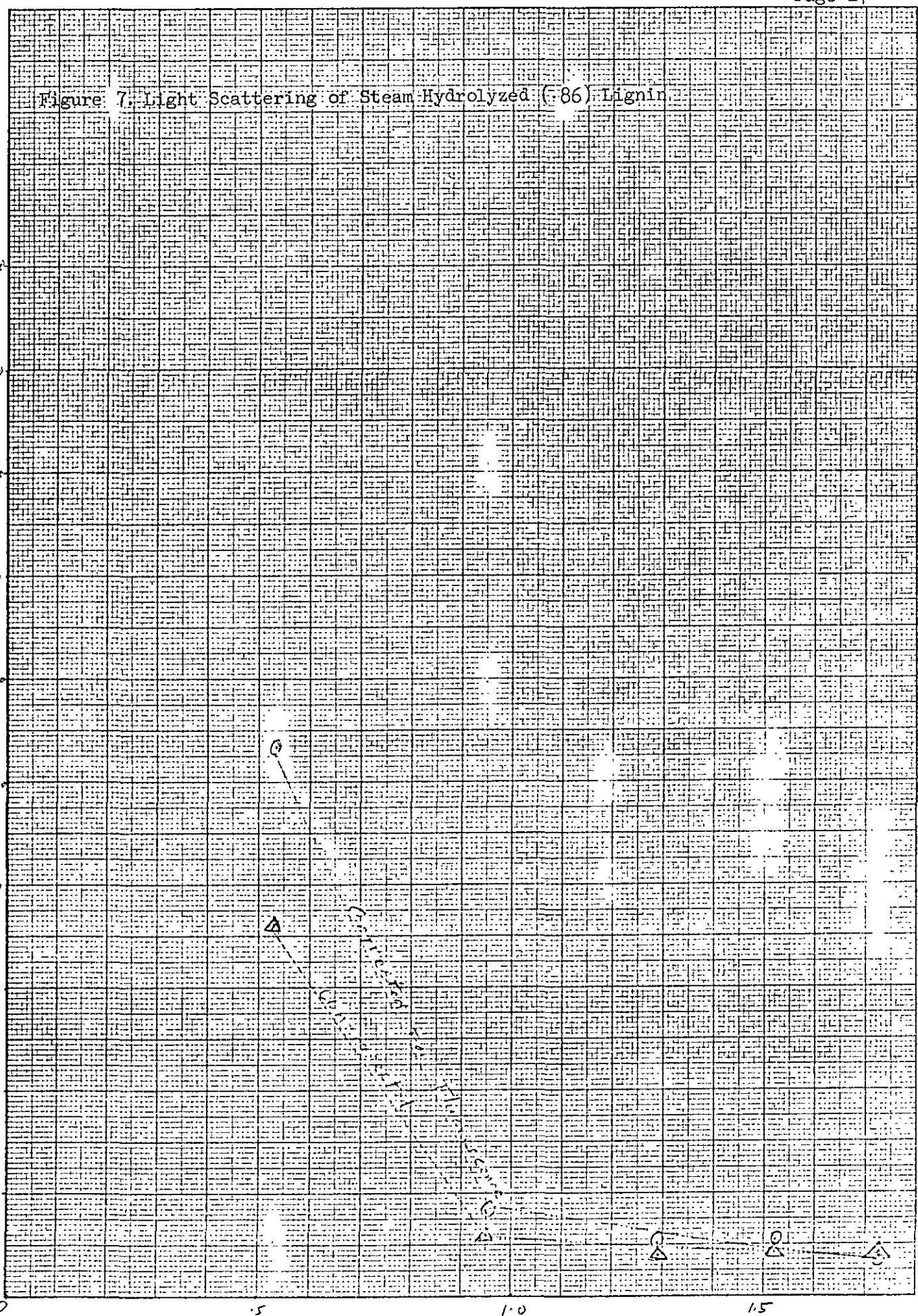
\*Data for  $Hc/\tau$  vs. concentrations, depolarization corrections and fluorescence corrections can be found in Appendix II.

Figure 7. Light Scattering of Steam Hydrolyzed (.86) Lignin

K&E 10 X 10 TO THE CENTIMETER 46 1510  
1 1/2 X 2 1/2 CM  
KLUFFEL & ESSER CO.

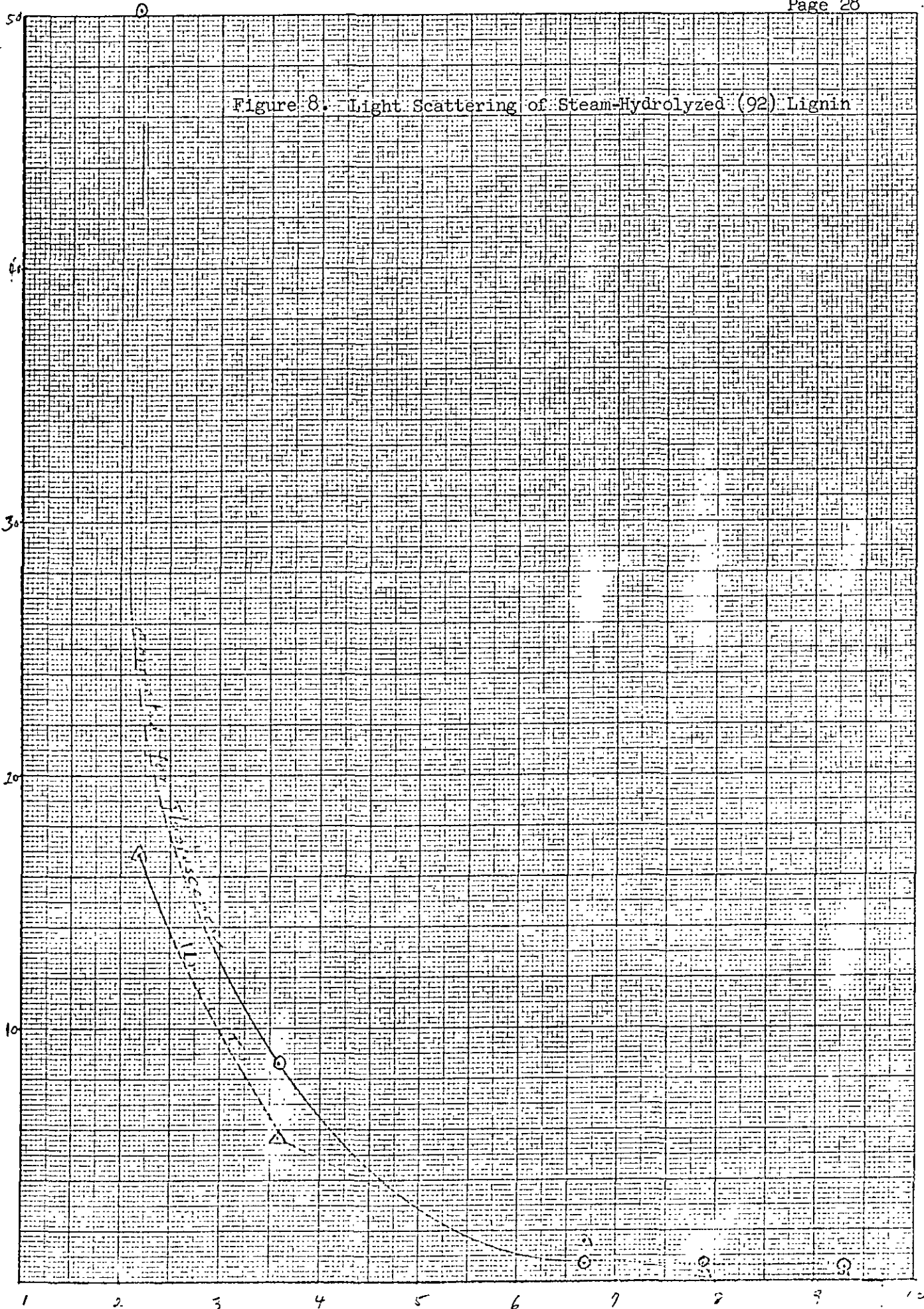
$Hc/R \times 10^9$

10.0  
9.0  
8.0  
7.0  
6.0  
5.0  
4.0  
3.0  
2.0  
1.0  
0



0.5 1.0 1.5  
1.1 1.3

Figure 8. Light Scattering of Steam-Hydrolyzed (92) Lignin



K & W  
10 X 10 TO THE CENTIMETER 46 1510  
MADE IN U.S.A.  
KLUFFEL & ESSER CO.

$Hc/K \times 10^9$

1 = 2

## ULTRACENTRIFUGATION

### Sedimentation Velocity Analysis

The sedimentation velocity method chosen for this work is used to study the velocity of sedimentation of lignin molecules through a solution under the influence of a centrifugal field, and is based on observations, by optical methods, of the movement of the boundary between solvent and solution. The sedimentation coefficient, velocity of the sedimenting molecules per unit field, is calculated according to the equation:

$$S = \frac{1}{\omega^2 x} \frac{dx}{dt}$$

where  $x$  is the distance of the boundary in centimeters from the axis of rotation,  $t$  is the time in seconds, and  $\omega$  is the angular velocity in radians per second.

### Diffusion

Diffusion is the transfer of material from a region of certain concentration of a given species to a region of lower concentration. The diffusion coefficient,  $D$ , is a measure of the mass of solute transported across a plane of known cross section in a given period of time under the influence of a known driving force.

At first a mixture of 90% dioxane and 10% water was used as the solvent for samples of Indulin A and GI lignin for sedimentation and diffusion runs. Due to some unexplainable reasons, the concentrations of these solutions in the cell during the run were changing. It seems that we were working with an absorbing system. A few points during the sedimentation and diffusion run were chosen and the respective sedimentation coefficient, diffusion coefficients and molecular weights were calculated.

Pure solutions of 100% dioxane and benzyl alcohol were used as solvents for VBM lignin and Indulin A in the later runs. No interaction was encountered. The results are summarized in Table VI.

TABLE VI  
 SUMMARY OF MOLECULAR WEIGHTS OBTAINED FROM  
 SEDIMENTATION AND DIFFUSION MEASUREMENTS

Lignin	Solvent	Time, min.	Partial Specific Volume*	Diffusion Coefficient	Sedimentation Coefficient	Molecular Weight
Indulin A	90% dioxane 10% water	14	.7768	$7.2675 \times 10^{-7}$	$8.9628 \times 10^{-13}$	153,978
	"	22		$7.5545 \times 10^{-7}$	$8.1824 \times 10^{-13}$	52,594
	"	32		$5.2500 \times 10^{-7}$	$1.9060 \times 10^{-12}$	453,268
Gamma Ray Irradiated Lignin	"	21	.7043	$9.9225 \times 10^{-7}$	$1.1441 \times 10^{-12}$	104,756
	"	29		$9.2915 \times 10^{-7}$	$2.8945 \times 10^{-12}$	283,013
	"	39		$9.1815 \times 10^{-7}$	$9.3929 \times 10^{-13}$	92,945
VBM	100% dioxane		.7125	$8.3924 \times 10^{-7}$	$5.7219 \times 10^{-13}$	58,228
Indulin A	"		.7241	$4.7823 \times 10^{-7}$	$1.1853 \times 10^{-12}$	220,614
VBM	100% benzyl alcohol		.7237	$2.4360 \times 10^{-7}$	$4.3510 \times 10^{-13}$	156,346

\*The densities of solvents and solutions and partial specific volume of these lignins in various solvents are listed in Appendix III.

## RECOMMENDATIONS

The recommendations which may be drawn from this research are:

1. If possible, action should be taken to decolorize or purify the sample prior to application of light scattering and sedimentation techniques in order to eliminate this interference. Techniques such as Sephadex separation or modified disk electrophoresis should first be explored.
2. It is known that the diazomethane method (under anhydrous conditions) only methylates acids and phenolic hydroxyl groups. Therefore, it would be better to methylate the sample further by dimethyl sulfate which methylates both the aliphatic and phenolic hydroxyl groups, and then study this totally unchanged and expanded particle by light scattering and sedimentation techniques.
3. It would be desirable to use the hydrogen bond breaking agents, such as urea or phenol, on purified lignin samples to test the hypothesis of hydrogen bond formation in light scattering experiments.
4. Green light (546 m ) should be used for light scattering experiments because the presence of extraneous particles are less important for the light of longer wavelength and because it will match our  $dn/dc$  values which were determined at 546 m .

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APPENDIX I

dn/dc Sample Calculation Determined by Rayleigh Interferometer

Sample VBM

$$\begin{aligned}\Delta n &= \frac{M}{L} = \text{drum reading} \frac{5.46 \times 10^{-5}}{47.4} \\ &= \text{drum reading} \times 1.15 \times 10^{-6} \\ \Delta n_1 &= 170 \times 1.15 \times 10^{-6} = 1.955 \times 10^{-4} \\ \Delta n_2 &= 266 \times 1.15 \times 10^{-6} = 3.059 \times 10^{-4} \\ \Delta n_3 &= 439 \times 1.15 \times 10^{-6} = 5.049 \times 10^{-4} \\ \Delta n_4 &= 607 \times 1.15 \times 10^{-6} = 6.981 \times 10^{-4}\end{aligned}$$

$$\text{Therefore, } \frac{dn_1}{dc} = \frac{1.955 \times 10^{-4}}{28.02 \times 10^{-4}} = .06980$$

$$\frac{dn_2}{dc} = \frac{3.059 \times 10^{-4}}{42.06 \times 10^{-4}} = .07270$$

$$\frac{dn_3}{dc} = \frac{5.049 \times 10^{-4}}{63.05 \times 10^{-4}} = .08008$$

$$\frac{dn_4}{dc} = \frac{6.981 \times 10^{-4}}{84.06 \times 10^{-4}} = .0831$$

$$\text{av.} = \frac{dn}{dc} = .07647$$

Sample Indulin A in Benzyl Alcohol

Test No.	Conc. (X)	Drum Reading (Y)	(XY)	(X <sup>2</sup> )
1	12.45 x 10 <sup>-4</sup>	-90	-.1125	1.55 x 10 <sup>-6</sup>
2	24.90 x 10 <sup>-4</sup>	-68	-.169	6.20 x 10 <sup>-6</sup>
3	49.80 x 10 <sup>-4</sup>	-28	-.139	2.48 x 10 <sup>-5</sup>
4	74.70 x 10 <sup>-4</sup>	-14	-.104	5.58 x 10 <sup>-5</sup>
	$\Sigma X = .0162$	$\Sigma Y = -200$	$\Sigma XY = -.5245$	$\Sigma X^2 = 8.835 \times 10^{-5}$

$$\Sigma x \times \Sigma Y = .0162 \times (-200) = -3.24$$

$$(\Sigma X)^2 = (.0162)^2 = 2.62 \times 10^{-4}$$

$$\begin{aligned} \text{Slope} &= \frac{n \Sigma XY - \Sigma X \cdot \Sigma Y}{n \Sigma X^2 - (\Sigma X)^2} = \frac{4(-.5245) - (-3.24)}{4(8.835 \times 10^{-5}) - (2.62 \times 10^{-4})} \\ &= \frac{1.142}{91 \times 10^{-6}} \end{aligned}$$

$$\text{Therefore, } \frac{dn}{dc} = \frac{1.142}{91 \times 10^{-6}} \times 1.15 \times 10^{-6} = .01443$$

APPENDIX II

MOLECULAR WEIGHT CALCULATION FROM LIGHT-SCATTERING  
MEASUREMENTS AT 4358 Å

1. VBM in Benzyl Alcohol

$$\text{Uncorrected } Hc/\tau = 1.12 \times 10^{-6} \quad \text{as } c \rightarrow 0$$

$$\text{Molecular weight} = 892,800$$

$$\text{Fluorescence corrected } Hc/\tau = 2.0 \times 10^{-6} \quad \text{as } c \rightarrow 0$$

$$\text{Molecular weight} = 500,000$$

Fluorescence and depolarization corrected

$$\text{Molecular weight} = 140,000 \text{ when } P_u = .5 \quad C_u = .28$$

2. Gamma Ray Irradiated in Benzyl Alcohol

Uncorrected .

$$Hc/\tau = 5.0 \times 10^{-7} \quad \text{as } c \rightarrow 0$$

$$\text{Molecular weight} = 2,000,000$$

$$\text{Fluorescence corrected } Hc/\tau = 2.5 \times 10^{-6} \quad \text{as } c \rightarrow 0$$

$$\text{Molecular weight} = 400,000$$

Fluorescence and depolarization corrected

$$\text{Molecular weight} = 92,400 \text{ when } P_u = .55, C_u = .23$$

3. Indulin A in  $C_6H_5CH_2OH$

Zero concentration extrapolation is impossible.

4. Indulin A in  $(CH_3)_2SO$

Zero concentration extrapolation is impossible.

5. Methylated Lignin in  $C_6H_5CH_2OH$

Uncorrected

$$Hc/\tau = 2.08 \times 10^{-7} \text{ as } c \rightarrow 0$$

Molecular weight = 480,770,000

Depolarization corrected molecular weight = 110,577,100

$$\text{when } P_u = .55 \quad C_u = .23$$

6. -86

Zero extrapolation is impossible.

7. -92

Impossible

8. VBM in  $(CH_3)_2SO$

Uncorrected

$$Hc/\tau = 4.2 \times 10^{-7} \text{ as } c \rightarrow 0$$

Molecular weight = 2,380,900

Fluorescence corrected

$$Hc/\tau = 19.8 \times 10^{-7} = 1.98 \times 10^{-6} \text{ as } c \rightarrow 0$$

Molecular weight - 505,050

Fluorescence and depolarization corrected

Molecular weight = 101,010

$$\text{when } P_u = .59, C_u = .20$$

VBM 209  
2097-1704-71 IN BENZYL ALCOHOL

{ 1.1383874E-06	, 7.3084171E-05
.13157328	
1.9025862E-06	
{ 1.0041819E-06	8.2128190E-04
.47855015	
1.8914141E-05	
{ 7.3814956E-07	1.3410078E-03
.49791576	
4.4249207E-06	
{ 9.1654451E-07	2.3333713E-03
.50586564	
5.5120470E-06	
{ 9.2549230E-07	2.9840311E-03
.49708322	
4.6583214E-06	

1.1084217	9.2232459
1.0283556	35.266402
.87963825	-8.3082838
.99828083	-581.66589
.98444535	-64.289250

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/\tau$ ,  $C$ ,  
 DEPOLARIZATION, AND  $H_c/\tau$  CORRECTED FOR FLUORESCENCE

Date: Oct. 9, '64

Name: C. Y. Chew

Project: 2097

Polymer: 2097-<sup>VBM</sup>1704-71,75,76 Sample:  
 .49479%

Solvent: Benzyl Alcohol

(1) H	$2.10 \times 10^{-6}$	(8) Solvent filter factor G-90/G0	$50 \times 10^{-4}$
(2) 4360 Å : 1.14 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>	.2097	(9) Solvent V90	95.0
(3) 5460 Å : 1.21 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>		(10) Solvent V0	87.0
(4) Grams of Polymer in (5)	.0061	(11) Solvent filter factor V90/V0	$10.65 \times 10^{-4}$
(5) Ml's. of solution	1.	(12) Solvent H90	43.5
(6) Solvent G90	95.0	(13) Solvent H0	39.0
(7) Solvent G0	75.5	(14) Solv. filt. factor H90/H0	$50 \times 10^{-4}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	16.5673

Concentration	1	2	3	4	5	6	7	8	9	10
---------------	---	---	---	---	---	---	---	---	---	----

	Scattering Ratios									
Filter factor for G-90/G0	$50 \times 10^{-4}$	$12.5 \times 10^{-3}$	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$2.66 \times 10^{-2}$					
G90	95.0	95.0	95.0	95.0	95.0					
G0	68.5	82.0	48.5	79.5	65.5					
Wt. Sol'n. (solv.) added	.2009	2.3767	2.0908	5.5948	5.6026					

	Depolarization									
Filter fact. for V90/V0	$12.5 \times 10^{-3}$	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$					
V90	95.0	95.0	95.0	95.0	95.0					
V0	94.0	46.0	65.0	48	87.0					
Filter fact. for H90/H0	$12.5 \times 10^{-3}$	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$					
H90	44.0	46.0	47.5	49.0	48.0					
H0	95.5	46.5	66.0	48.5	89.0					

	Fluorescence									
uv aux. filter	7	13	20.5	22.0	24.0					
uv aux. filter	9	25.5	32.0	34.5	36.5					
Filter fact. for GH/GV	1	1	1	1	1					

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/Z-1)

Date: Oct. 9, '64 Solvent: Benzyl Alcohol Name: \_\_\_\_\_ Project: 2097

Polymer: 2097-1704-71, 75, 76 Sample: .4947

- (1) Number of concentrations 5
- (2) Solvent G45 71.0
- (3) Solvent G0 75.5
- (4) Solvent G135 63.0
- (5) Solvent filter factor for G-90/G0  $5.0 \times 10^{-4}$
- (6) Solvent filter factor for G45  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	72.0	68.0	62.5	62.5	62.5					
G0	68.5	82.0	48.5	79.5	65.5					
G135	64.0	63.50	66.5	61.0	62.0					
Filter fact. for G-90/G0	$5.0 \times 10^{-4}$	$12.5 \times 10^{-3}$	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$2.66 \times 10^{-2}$					
Filter fact. for G45	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					
Filter fact. for G135	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					

Wt. of tare + solvent 81.8891  
 Wt. of tare 65.3218  
 Wt. of solvent 16.5673

Wt. of tare + solvent + soln.	(1)	(2)	(3)	(4)	(5)
Wt. of tare + solvent	82.0880	84.4647	87.5551	93.1499	98.7525
Wt. of soln.	81.8871	82.0880	84.4643	87.5551	93.1499
	<u>.2009</u>	<u>.23767</u>	<u>2.0908</u>	<u>5.5948</u>	<u>5.6026</u>

Wt. of tare + 2 c.c. soln.	(1)	(2)
Wt. of tare	19.2840	19.8629
Wt. of 2 c.c. soln.	19.2718	19.8507
	<u>21.0122</u>	<u>21.0122</u>
	<u>.0061</u>	<u>.0061</u>

AV. .0061 g/c.c.

49075008  
3.7528628E-07      7.7935724E-04  
.58893853  
1.7472410E-06  
3.2393827E-07      1.4229621E-03  
.56782727  
1.4530967E-06  
3.0380975E-07      1.7540478E-03  
.58318631  
1.4687732E-06  
2.7594545E-07      2.2477458E-03  
.57617083  
1.3597367E-06  
2.0230826E-07      2.6037947E-03  
.56845206  
1.1613180E-06

VBM in  $(\text{CH}_3)_2\text{SO}$

1.1388143      7.2038687  
1.1422063      7.0320372  
1.1350133      7.4066777  
1.1129305      8.8550037  
1.1180715      3.4694443

VBM

7. ...  
1.4 ...  
1.7 ...  
2. ...  
3. ...



LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/\tau$ ,  $C$ ,  
 DEPOLARIZATION, AND  $H_c/\tau$  CORRECTED FOR FLUORESCENCE

Date: Oct. 14, 1965 Name:

Project: 2097

Polymer: VBM Lignin Sample:

Solvent:  $(CH_3)_2SO$

(1) $H$	$5.54E-6$	(8) Solvent filter factor G-90/G0	$50 \times 10^{-4}$
(2) $4360 \text{ \AA} : 1.14 (n^2 R_w/R_c) a r/r^2$	$2.039$	(9) Solvent V90	95.0
(3) <del><math>5460 \text{ \AA} : 1.21 (n^2 R_w/R_c) a r/r^2</math></del>	$0.0054$	(10) Solvent V0	76.0
(4) Grams of Polymer in (5)	1.	(11) Solvent filter factor V90/V0	$10.65 \times 10^{-4}$
(5) Ml's. of solution	95.0	(12) Solvent H90	43.0
(6) Solvent G90	68.5	(13) Solvent H0	77.5
(7) Solvent G0		(14) Solv. filt. factor H90/H0	$10.65 \times 10^{-4}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	<del>17.7000</del> 17.6994

Concentration	1	2	3	4	5	6	7	8	9	10
---------------	---	---	---	---	---	---	---	---	---	----

Scattering Ratios										
filter factor for G-90/G0	$12.5 \times 10^{-2}$	$2.66 \times 10^{-2}$	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$5.88 \times 10^{-2}$					
G90	95.0	95.0	95.0	95.0	95.0					
G0	52.5	63.0	50.0	81.6	68.2					
Wt. Sol'n. (solv.) added	2.9648	3.3174	2.1588	4.0529	3.8011					

Depolarization										
filter fact. for V90/V0	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$5.88 \times 10^{-2}$	$2.13 \times 10^{-1}$	$10 \times 10^{-2}$					
V90	95.0	95.0	95.0	42.0	95.0					
V0	61.2	78.0	62.2	71.8	62.0					
filter fact. for H90/H0	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$5.88 \times 10^{-2}$	$2.13 \times 10^{-1}$	$10 \times 10^{-2}$					
H90	52.6	54.0	54.3	24.0	54.0					
H0	62.5	80.0	63.2	73.0	63.2					

Fluorescence										
aux. filter	35.6	28.0	27.0	13.0	30.0					
V aux. filter	33.0	36.8	38.0	17.5	40.0					
filter fact. for GH/GV	1	1	1	1	1					

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/Z-1)

Date: Oct 14, 1965 Solvent:  $(CH_2)_2SO$  Name: c. y. chu Project: 2097

Polymer: UBM Lignin Sample:

- (1) Number of concentrations 5
- (2) Solvent G45 57.0
- (3) Solvent G0 68.5
- (4) Solvent G135 50.0
- (5) Solvent filter factor for G-90/G0  $50 \times 10^{-4}$
- (6) Solvent filter factor for G45  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	65.5	66.8	67.0	65.8	<del>65.5</del> 58.5					
G0	52.5	63.0	50.0	81.6	68.2					
G135	57.5	58.5	59.0	59.0	58.5					
Filter fact. for G-90/G0	$12.5 \times 10^{-2}$	$2.6 \times 10^{-2}$	$2.6 \times 10^{-2}$	$5.8 \times 10^{-2}$	$5.8 \times 10^{-2}$					
Filter fact. for G45	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					
Filter fact. for G135	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					

Wt. of solvent + cup 95.2635  
 Wt. of cup. 77.5641  
 Wt. of solvent. 17.6994

	(1)	(2)	(3)	(4)	(5)
Wt. of solution + s.c.	98.2283	101.5457	103.7045	107.7574	111.5585
Wt. s.c.	95.2635	98.2283	101.5457	103.7045	107.7574
Wt. of solution	2.9648	3.3174	2.1588	4.0529	3.8011

Wt. of sample per ml. =  $\frac{0.2716}{50} = 0.005432 \text{ g. (unfiltered)}$

GI

2097-1858-53 IN BENZYL ALCOHOL

4.7676120E-07	4.1662557E-04
.54774232	
-1.2799404E-06	
2.2945912E-07	1.0619621E-03
.55592473	
2.0839027E-06	
1.3831736E-07	2.0096995E-03
.54871167	
-1.6307244E-06	
1.5715541E-07	2.8094160E-03
.54742945	
1.3032705E-06	
1.1428514E-07	3.3460949E-03
.54705330	
1.0667232E-06	

.68095613	-3.1343648
.96157713	-26.026145
.92705762	-13.709447
.92495907	-13.326048
.94586098	-18.470938

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/\tau$ , C,  
DEPOLARIZATION, AND  $H_c/\tau$  CORRECTED FOR FLUORESCENCE

August 5, 1968  
Page 44

Date: Oct. 8. '64

Name: C. Y. Chau

Project: 2097

Polymer: 2097-1958-53  
(G2)  
(.49509%)

Sample:

Solvent: Benzyl alcohol

(1) H	$1.74 \times 10^4$	(8) Solvent filter factor G-90/G0	$50 \times 10^{-4}$
(2) $4360 \text{ \AA}$ : 1.14 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>	.2097	(9) Solvent V90	95.0
(3) $5460 \text{ \AA}$ : 1.21 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>	.0064	(10) Solvent V0	85.0
(4) Grams of Polymer in (5)	1.	(11) Solvent filter factor V90/V0	$10.65 \times 10^{-3}$
(5) Ml's. of solution	95.0	(12) Solvent H90	47.0
(6) Solvent G90	70.0	(13) Solvent H0	87.0
(7) Solvent G0		(14) Solv. filt. factor H90/H0	$10.65 \times 10^{-3}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	14.5238

Concentration	1	2	3	4	5	6	7	8	9	10
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Scattering Ratios										
Filter factor for G-90/G0	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$10 \times 10^{-2}$	$10 \times 10^{-2}$					
G90	95.0	95.0	95.0	95.0	95.0					
G0	96.0	69.5	74.5	78.5	49.0					
Wt. Sol'n. (solv.) added	1.0113	1.8781	3.7590	4.7156	4.5494					

Depolarization										
Filter fact. for V90/V0	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$10 \times 10^{-2}$	$2.13 \times 10^{-1}$	$2.13 \times 10^{-1}$					
V90	95.0	95.0	95.0	95.0	95.0					
V0	91.0	93.0	72.0	93.5	66.3					
Filter fact. for H90/H0	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$10 \times 10^{-2}$	$2.13 \times 10^{-1}$	$2.13 \times 10^{-1}$					
H90	50.5	52.0	53.0	52.5	51.5					
H0	93.0	94.0	74.0	95.0	66.0					

Fluorescence										
ix. filter	23	27	30	31	32.0					
ix. aux. filter	53	42	47.5	49.0	50.5					
Filter fact. for GH/GV	1	1	1	1	1					

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/2-1)

Date: Oct. 8. '64 Solvent: Benzyl Alcohol Name: \_\_\_\_\_ Project: 2097

Polymer: 2091-1858-53 Sample: \_\_\_\_\_  
 (1.49505%)

- (1) Number of concentrations 5
- (2) Solvent G45 73.0
- (3) Solvent G0 70.0
- (4) Solvent G135 62.0
- (5) Solvent filter factor for G-90/G0  $50 \times 10^{-4}$
- (6) Solvent filter factor for G45  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	70.0	65.0	61.2	61.0	58.2					
G0	96.0	69.5	74.5	78.5	49.0					
G135	78.0	65.0	64.5	65.0	61.0					
Filter fact. for G-90/G0	$12.5 \times 10^{-3}$	$2.66 \times 10^{-2}$	$5.88 \times 10^{-2}$	$10 \times 10^{-2}$	$10 \times 10^{-2}$					
Filter fact. for G45	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					
Filter fact. for G135	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$					

Wt. of tare + solvent 79.8520  
 Wt. of tare 65.3282  
 Wt. of solvent 14.5238

	(1)	(2)	(3)	(4)	(5)
Wt. of tare + solvent + soln.	80.8544	82.7357	86.5940	91.3088	95.8565
Wt. of tare + solvent	79.8471	80.8576	82.7350	86.5932	91.3071
Wt. of soln.	1.0113	1.8781	3.7590	4.7156	4.5494

	(1)	(2)
Wt. of tare + 2cc. soln.	18.9458	18.6884
Wt. of tare	18.9336	18.6759
Wt. of 2cc. soln.	$\frac{21.0122}{.0061}$	$\frac{21.0135}{.0067}$

AV. .0064 %/c.c.

C

LOAD DATA	
1.9581415E-09	5.3644032E-04
.56347339	
1.2175326E-08	
1.5202887E-09	9.4877588E-04
.56500279	
8.7331325E-09	
1.5088697E-09	1.8984959E-03
.56855114	
1.1150285E-08	
1.8098434E-09	2.1122532E-03
.57777774	
1.2310453E-08	
1.2979513E-09	2.4437085E-03
.55732447	
9.8894209E-09	

*Methylated Liquin  
in C<sub>6</sub>H<sub>5</sub>OH*

*disinfecting*

1.4522753	2.2110426
1.4297520	2.3269234
1.4063081	2.4611864
1.4180096	2.3922895
1.4161351	2.4030657

*Methylated Liquin*

*e*  
5.514 x 10<sup>-4</sup>  
1.457 x 10<sup>-3</sup>  
1.2 x 10<sup>-3</sup>  
2.112 x 10<sup>-3</sup>  
2.443 x 10<sup>-3</sup>

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/T, C,$   
DEPOLARIZATION, AND  $H_c/T$  CORRECTED FOR FLUORESCENCE

Project 2091  
August 5, 1968  
Page 47

Date: 5/28/65 Name: E. Y. Chew  
Polymer: methyl lignin Sample:  
Solvent: Benzyl alcohol

Project: 2097

(1) H	1.19416	(8) Solvent filter factor G-90/GO	$50 \times 10^{-4}$
(2) 4360 Å : 1.14 ( $n^2$ Rw/Rc) $\frac{a}{r/r^1}$	1.254	(9) Solvent V90	95.0
(3) 5460 Å : 1.21 ( $n^2$ Rw/Rc) $\frac{a}{r/r^1}$	90.5	(10) Solvent V0	81.0
(4) Grams of Polymer in (5)	1	(11) Solvent filter factor V90/V0	$12.5 \times 10^{-3}$
(5) Ml's. of solution	95.0	(12) Solvent H90	42.0
(6) Solvent G90	60.6	(13) Solvent H0	81.8
(7) Solvent G0		(14) Solv. filt. factor H90/H0	$12.5 \times 10^{-3}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	17.8945

Concentration	1	2	3	4	5	6	7	8	9	10
Scattering Ratios										
Filter factor for G-90/GO	$12.5 \times 10^{-3}$	$2.0 \times 10^{-2}$	$5.58 \times 10^{-2}$	$5.58 \times 10^{-2}$	$5.58 \times 10^{-2}$					
G90	95.0	95.0	95.0	95.0	95.0					
G0	58.0	69.0	84.8	82.6	59.0					
Wt. Sol'n. (solv.) added	2.1506	2.0402	6.7625	2.1354	4.0174					

Depolarization										
Filter fact. for V90/V0	$5.58 \times 10^{-2}$	$10 \times 10^{-2}$	$10 \times 10^{-2}$	$2.13 \times 10^{-1}$	$2.13 \times 10^{-1}$					
V90	54.0	53.6	49.0	47.0	49.0					
V0	91.0	86.6	48.0	89.4	75.2					
Filter fact. for H90/H0	$5.58 \times 10^{-2}$	$10 \times 10^{-2}$	$10 \times 10^{-2}$	$2.13 \times 10^{-1}$	$2.13 \times 10^{-1}$					
H90	28.0	29.0	27.5	27.0	27.4					
H0	92.4	87.6	49.0	91.5	97.2					

Fluorescence										
GH aux. filter	11.0	14.2	14.0	13.8	14.2					
GV aux. filter	<del>15.6</del>	19.8	20.2	20.0	20.8					
Filter fact. for GH/GV	1	1	1	1	1					

$a = .07065$

$\frac{R}{R^1} = 1.22$

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/Z-1)

Date: 5/28/65 Solvent: Benzyl alcohol Name: C. G. Chou Project: 2097  
 Polymer: Methylcellulose Sample:

- (1) Number of concentrations \_\_\_\_\_
- (2) Solvent G45 67.0
- (3) Solvent G0 60.6
- (4) Solvent G135 57.0
- (5) Solvent filter factor for G-90/G0  $5.0 \times 10^{-4}$
- (6) Solvent filter factor for G45 ~~4.7~~  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	84.0	86.4	88.0	78.0	81.0					
G0	58.0	69.0	84.5	72.6	59.0					
G135	62.0	62.6	58.0	56.0	58.0					
Filter fact. for G-90/G0	$1.25 \times 10^{-3}$	$2.66 \times 10^{-2}$	$5.55 \times 10^{-2}$	$5.55 \times 10^{-2}$	$5.85 \times 10^{-2}$					
Filter fact. for G45	.47	.47	.47	.47	.47					
Filter fact. for G135	.47	.47	.47	.47	.47					

(width over)  
 Wt. of tare + solvent 95.4688  
 wt. of tare 77.5743  
 Wt. of solvent 17.8945

Wt. of tare + solvent + solution	(1)	(2)	(3)	(4)	(5)
Wt. of tare + solvent	97.6194	99.6596	106.4224	108.5578	112.575
Wt. of solution	95.4688	97.6194	99.6596	106.4224	108.5578
	2.1506	2.0402	6.7628	2.1354	4.0174

Conc. of filtered solution (5 C.C.)

A.	33.6665	B.	32.3361
	33.6365		32.3058
	<u>5.0300</u>		<u>5.0303</u>
	.0060		.0061



INDULIN A IN BENZYL ALCOHOL

1.7345109E-08	6.1632353E-04
.47535840	
2.6058396E-08	
2.2639124E-09	2.9509616E-03
.41361643	
5.0722989E-09	
2.4559756E-10	5.6648305E-03
.23344237	
2.6478010E-10	
.6.4670693E-11	8.5963254E-03
.15782372	
.8.4696171E-11	

.89277419	-9.3261049
.81925214	-5.5325677
.60372040	-2.5234708
.30003453	-1.4286413

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/r$ ,  $C$ ,  
 DEPOLARIZATION, AND  $H_c/r$  CORRECTED FOR FLUORESCENCE

Date: Nov. 6, '64 Name: C. Y. Chu Project: 2097  
 Polymer: Indulin A Sample:  
 Solvent: Benzyl alcohol

(1) H	$7.49 \times 10^{-8}$	(8) Solvent filter factor G-90/GO	$12.5 \times 10^{-3}$
(2) $4360 \text{ \AA} : 1.14 (n^2 R_w/R_c) a r/r^1$	1.2097	(9) Solvent V90	45.0
(3) <del><math>5460 \text{ \AA} : 1.21 (n^2 R_w/R_c) a r/r^1</math></del>		(10) Solvent VO	80.6
(4) Grams of Polymer in (5)	0.0249	(11) Solvent filter factor V90/VO	$12.5 \times 10^{-3}$
(5) Ml's. of solution	1.	(12) Solvent H90	49.6
(6) Solvent G90	95.0	(13) Solvent HO	82.4
(7) Solvent GO	53.0	(14) Solv. filt. factor H90/HO	$12.5 \times 10^{-3}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	14.6847

Concentration	1	2	3	4	5	6	7	8	9	10
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	Scattering Ratios									
Filter factor for G-90/GO	$2.66 \times 10^{-2}$	$4.7 \times 10^{-1}$	/	/						
G90	95.0	95.0	95.0	95.0						
GO	92.0	91.5	11.5	2.0						
Wt. Sol'n. (solv.) added	0.7727	1.6016	2.3504	3.4182						

	Depolarization									
Filter fact. for V90/VO	$5.88 \times 10^{-2}$	/	/	/						
V90	95.0	95.0	95.0	95.0						
VO	91.0	90.0	4.0	1.0						
Filter fact. for H90/HO	$5.84 \times 10^{-2}$	/	/	/						
H90	52.0	40.8	22.2	15.0						
HO	92.0	92.5	4.0	1.0						

	Fluorescence									
GH aux. filter	26.2	25.9	9.5	3.0						
GV aux. filter	38.0	37.8	14.5	4.5						
Filter fact. for GH/GV	/	/	/	/						

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/2-1)

Date: Nov. 6, 64 Solvent: Benzyl alcohol Name:

Project: 2097

Polymer: Inulin A Sample:

- (1) Number of concentrations 4
- (2) Solvent G45 67.6
- (3) Solvent G0 53.0
- (4) Solvent G135 62.3
- (5) Solvent filter factor for G-90/G0  $12.5 \times 10^{-3}$
- (6) Solvent filter factor for G45  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	63.0	45.0	37.0	8.0						
G0	72.0	91.5	11.5	2.0						
G135	62.0	54.0	61.0	26.5						
Filter fact. for G-90/G0	$2.66 \times 10^{-2}$	$4.7 \times 10^{-1}$	1	1						
Filter fact. for G45	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	all out	all out						
Filter fact. for G135	$4.7 \times 10^{-1}$	$4.7 \times 10^{-1}$	all out	all out						

Wt. of tare + solvent 75.2919  
 Wt. of tare 60.6072  
14.6847

	(1)	(2)	(3)	(4)
Wt. of tare + solvent + soln.	75.6646	77.2662	79.6166	83.0346
Wt. of " "	75.2919	75.6646	77.2662	79.6166
	<u>.3727</u>	<u>1.6016</u>	<u>2.3504</u>	<u>3.4180</u>

$$\frac{dy}{dc} = -0.1443$$

INDULIN A IN DIMETHYL SULFOXIDE

3.9962301E-06	1.7352514E-04
.63252895	
3.5143654E-05	
1.5870911E-06	5.5749134E-04
.60499270	
7.2960658E-06	
8.7346330E-07	7.7204317E-04
.55114608	
2.7695721E-06	
3.3201440E-07	1.0713867E-03
.45519959	
6.4593872E-07	
7.5286757E-08	1.5805019E-03
1.32965741	
1.0126606E-07	

1.0334444	29.900371
1.0038349	257.40688
.97214750	-35.903419
.88073258	-8.3845138
.62474067	-2.6648233

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/T, C,$   
DEPOLARIZATION, AND  $H_c/T$  CORRECTED FOR FLUORESCENCE

Date: Jan. 14. '65 Name: C. Y. Chu Project: 2097  
 Polymer: Indulin A Sample:  
 Solvent:  $(CH_3)_2SO$   
 (with normal light)

(1) H	$1.84 \times 10^{-4}$	(8) Solvent filter factor G-90/G0	$29.4 \times 10^{-4}$
(2) 4360 Å : 1.14 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>	.1220	(9) Solvent V90	60.5
(3) 5460 Å : 1.21 ( $n^2$ Rw/Rc) a r/r <sup>1</sup>	$n^2 = 0.05022$	(10) Solvent V0	74.0
(4) Grams of Polymer in (5)	→ 1.	(11) Solvent filter factor V90/V0	$12.5 \times 10^{-4}$
(5) Ml's. of solution	95.0	(12) Solvent H90	26.3
(6) Solvent G90	56.4	(13) Solvent H0	75.6
(7) Solvent G0		(14) Solv. filt. factor H90/H0	$12.5 \times 10^{-4}$
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	16.9686

Concentration	1	2	3	4	5	6	7	8	9	10
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Scattering Ratios										
Filter factor for G-90/G0	$5.88 \times 10^{-2}$	$4.7 \times 10^{-1}$	1	1	1					
G90	95.0	95.0	95.0	95.0	95.0					
G0	99.3	83.5	71.0	19.5	3.0					
Wt. Sol'n. (solv.) added	.6073	1.5116	.9636	1.5193	3.1910					

Depolarization										
Filter fact. for V90/V0	$2.13 \times 10^{-1}$	1	1	1	1					
V90	54.6	54.0	62.4	72.5	91.0					
V0	91.8	56.5	23.5	6.5	1.2					
Filter fact. for H90/H0	$2.13 \times 10^{-1}$	1	1	1	1					
H90	37.0	33.2	34.8	33.0	25.0					
H0	94.0	57.6	23.8	6.5	1.0					

Fluorescence										
H aux. filter	18.0	20.0	20.2	19.0	12.0					
V aux. filter	23.0	25.0	25.0	23.0	13.8					
Filter fact. for GH/GV	1	1	1	1	1					

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/Z-1)

Date: Jan. 14 '65 Solvent:  $(CH_3)_2SO$  Name: C. Y. Chu Project: 2097  
 Polymer: Indulin A Sample:

- (1) Number of concentrations 5  
 (2) Solvent G45 72.4  
 (3) Solvent G0 56.4  
 (4) Solvent G135 54.0  
 (5) Solvent filter factor for G-90/G0  $29.4 \times 10^{-4}$   
 (6) Solvent filter factor for G45  $4.7 \times 10^{-7}$   
 (7) Solvent filter factor for G135  $4.7 \times 10^{-7}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	64.0	60.0	55.0	49.5	31.0					
G0	79.3	83.5	71.0	19.5	3.0					
G135	60.8	59.6	56.5	48.8	49.6					
Filter fact. for G-90/G0	$5.88 \times 10^{-7}$	$4.7 \times 10^{-7}$	1	1	1					
Filter fact. for G45	$4.7 \times 10^{-7}$	$4.7 \times 10^{-7}$	$4.7 \times 10^{-7}$	all out	all out					
Filter fact. for G135	$4.7 \times 10^{-7}$	$4.7 \times 10^{-7}$	$4.7 \times 10^{-7}$	$4.7 \times 10^{-7}$	all out					

Wt. of tare + solvent 91.8555  
 Wt. of tare 74.8869  
 Wt. of solvent 16.9686

$a = 0.521$

$\frac{r}{r_1} = 0.993$

	(1)	(2)	(3)	(4)	(5)
Wt. of tare + solvent + soln.	92.4628	93.9744	94.9380	96.4573	99.6483
Wt. of tare + solvent	91.8555	92.4628	93.9744	94.9380	96.4573
Wt. of solution	0.6073	1.5116	0.9636	1.5193	3.1910

$\frac{dn}{dc} = 0.7926$

3.6070143E-09     5.6519410E-04  
.29171080  
5.3413855E-09  
5.9688122E-10     9.5043283E-04  
.24200631  
8.0805834E-10  
4.0555089E-10     1.2918014E-03  
.18941468  
5.0030915E-10  
4.3054076E-10     1.5238101E-03  
.17047013  
5.1072312E-10  
4.3354299E-10     1.7262987E-03  
.12469440  
3.6117416E-10

Steam Hydrolyzed -86

.92093093     -12.647165  
.69829253     -3.3144684  
.41789832     -1.7179128  
.32135816     -1.4735311  
.23126522     -1.3008387

--5'6

5.652 x 10<sup>-4</sup>  
9.504 x 10<sup>-4</sup>  
1.2918 x 10<sup>-3</sup>  
1.5238 x 10<sup>-3</sup>  
1.726 x 10<sup>-3</sup>

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/T, C,$   
DEPOLARIZATION, AND  $H_c/T$  CORRECTED FOR FLUORESCENCE

Project 2097  
August 5, 1968  
Page 56

Date: 9/30/65

Name: C. Y. Chiu

Project: 2097

Polymer: Steam hydrolyzed Lignin (2097-1858-86) Sample:

Solvent:  $(CH_3)_2SO$  (normal light)

(1) H	3.04 E-6	(8) Solvent filter factor G-90/G0	50 x 10 <sup>-4</sup>
(2) 4360 Å : 1.14 (n <sup>2</sup> R <sub>w</sub> /R <sub>c</sub> ) a r/r <sup>1</sup>	.2039	(9) Solvent V90	95.0
(3) 5460 Å : 1.21 (n <sup>2</sup> R <sub>w</sub> /R <sub>c</sub> ) a r/r <sup>1</sup>	.004714	(10) Solvent V0	69.0
(4) Grams of Polymer in (5)	1.	(11) Solvent filter factor V90/V0	12.5 x 10 <sup>-3</sup>
(5) Ml's. of solution	95.0	(12) Solvent H90	50.0
(6) Solvent G90	50.0	(13) Solvent H0	70.5
(7) Solvent G0		(14) Solv. filt. factor H90/H0	12.5 x 10 <sup>-3</sup>
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	19.1609

Concentration	1	2	3	4	5	6	7	8	9	10
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Scattering Ratios

Filter factor for G-90/G0	1	2	3	4	5	6	7	8	9	10
G90	95.0	95.0	95.0	95.0	95.0					
G0	40.5	4.0	2.0	1.8	1.6					
Wt. Sol'n. (solv.) added	2.6103	2.2285	2.3940	1.9195	1.9189					

Depolarization

Filter fact. for V90/V0	1	2	3	4	5	6	7	8	9	10
V90	95.0	95.0	95.0	95.0	95.0					
V0	19.5	2.0	1.0	0.9	<del>2.0</del> 0.6					
Filter fact. for H90/H0	1	2	3	4	5	6	7	8	9	10
H90	28.5	23.0	18.0	16.2	15.8					
H0	20.0	2.0	1.0	0.9	<del>2.0</del> 0.8					

Fluorescence

GH aux. filter	1	2	3	4	5	6	7	8	9	10
GH aux. filter	21.0	10.0	5.0	3.6	3.0					
GV aux. filter	25.2	10.8	5.0	3.5	2.4					
Filter fact. for GH/GV	1	2	3	4	5	6	7	8	9	10
Filter fact. for GH/GV	1	1	1	1	1					



LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/Z-1)

Date: 9/30/65 Solvent: (C13) 50 Name: C. Y. Chu Project: 2097  
 Polymer: 2017-1858-86 Sample:

- (1) Number of concentrations 5
- (2) Solvent G45 57.0
- (3) Solvent G0 50.0
- (4) Solvent G135 ~~57~~ 51.0
- (5) Solvent filter factor for G-90/G0  $50 \times 10^{-4}$
- (6) Solvent filter factor for G45  $4.7 \times 10^{-1}$
- (7) Solvent filter factor for G135  $4.7 \times 10^{-1}$

Concentration	1	2	3	4	5	6	7	8	9	10
G45	76.8	32.0	9.0	5.8	3.6					
G0	40.5	4.0	2.0	1.8	1.6					
G135	83.3	45.8	21.5	18.0	15.5					
Filter fact. for G-90/G0	1	1	1	1	1					
Filter fact. for G45	1	1	1	1	1					
Filter fact. for G135	1	1	1	1	1					

Wt of cup + solvent. 96.7247  
 Wt of cup. 77.5638  
 Wt. of solvent. 19.1609

	(1)	(2)	(3)	(4)	(5)
Wt. of solv.	$\frac{99.3350}{96.7247}$	$\frac{101.5635}{99.3350}$	$\frac{103.9575}{101.5635}$	$\frac{105.8770}{103.9575}$	$\frac{107.7959}{105.8770}$
	2.6103	2.2285	2.3940	1.9195	1.9189

Wt. of sample per ml (unfiltered)  
 $1.2357 \div 50 = 0.004714 \text{ g/cc.}$

SR

1.6878920E-08	2.1785920E-04
.50584468	
5.0348751E-08	
5.6364893E-09	3.5864616E-04
.37984699	
8.5982620E-09	
4.0649592E-10	6.6770126E-04
.13404896	
2.7246435E-10	
3.1968074E-10	7.8771194E-04
.10177365	
2.7265557E-10	
2.8293448E-10	9.2959206E-04
5.9819005E-02	
1.2483663E-10	

Steam Hydrolyzed -92

*Asymmetry*

.97786520	-45.177729
.94989113	-19.956534
.60794102	-2.5506365
.44451826	-1.8002389
.28921778	-1.4069005

- 92

$2.179 \times 10^{-4}$   
 $3.526 \times 10^{-4}$   
 $6.677 \times 10^{-4}$   
 $7.877 \times 10^{-4}$   
 $9.296 \times 10^{-4}$

LIGHT-SCATTERING DATA FOR COMPUTING  $H_c/I$ ,  $C$ ,  
DEPOLARIZATION, AND  $H_c/I$  CORRECTED FOR FLUORESCENCE

Project 2097  
August 5, 1968  
Page 59

Date: 10/7/65 Name: *e. y. Chu* Project: 2097  
 Polymer: 2097-1858-92 Sample:  
 Solvent:  $(CH_3)_2SO$

(1) H	3.93 E-6	(8) Solvent filter factor G-90/G0	50x10 <sup>-4</sup>
(2) 4360 Å : 1.14 (n <sup>2</sup> Rw/Rc) a r/r <sup>1</sup>	1.2039	(9) Solvent V90	95.0
(3) 5460 Å : 1.21 (n <sup>2</sup> Rw/Rc) a r/r <sup>1</sup>	.00297	(10) Solvent V0	84.6
(4) Grams of Polymer in (5)	1.	(11) Solvent filter factor V90/V0	12.5x10 <sup>-3</sup>
(5) Ml's. of solution	95.0	(12) Solvent H90	<del>86.6</del> 45.1
(6) Solvent G90	64.5	(13) Solvent H0	86.6
(7) Solvent G0		(14) Solv. filt. factor H90/H0	12.5x10 <sup>-3</sup>
		(15) Wt. Solvent in cell (omit if solv. added to sol'n.)	16.9505

Concentration	1	2	3	4	5	6	7	8	9	10
---------------	---	---	---	---	---	---	---	---	---	----

Scattering Ratios										
Filter factor for G-90/G0	2.13x10 <sup>1</sup>	1	1	1	1					
G90	95.0	95.0	95.0	95.0	95.0					
G0	79.0	77.0	3.0	2.0	1.5					
Wt. Sol'n. (solv.) added	1.7418	.9862	2.5879	1.2025	1.6041					

Depolarization										
Filter fact. for V90/V0	4.7x10 <sup>1</sup>	1	1	1	1					
V90	95.0	95.0	55.0	62.0	61.5					
V0	93.0	32.0	.50	.50	.20					
Filter fact. for H90/H0	2.13x10 <sup>1</sup>	1	1	1	1					
H90	49.0	36.8	11.80	10.1	9.2					
H0	43.0	32.6	.80	.80	.50					

Fluorescence										
GH aux. filter	29.6	20.0	3.8	3.0	1.8					
GV aux. filter	37.5	24.0	4.0	3.0	2.0					
Filter fact. for GH/GV	1	1	1	1	1					

LIGHT SCATTERING DATA FOR COMPUTING Z AND (1/2-1)

Date: 10/7/65 Solvent: (CH<sub>2</sub>)<sub>2</sub>SO Name:

Project: 2097

Polymer: 2097-1858-92 Sample:

- (1) Number of concentrations 5
- (2) Solvent G45 58.0
- (3) Solvent G0 79.0
- (4) Solvent G135 50.0
- (5) Solvent filter factor for G-90/G0 ~~2.13 x 10<sup>-1</sup>~~ 5.0 x 10<sup>-4</sup>
- (6) Solvent filter factor for G45 4.7 x 10<sup>-1</sup>
- (7) Solvent filter factor for G135 4.7 x 10<sup>-1</sup>

Concentration	1	2	3	4	5	6	7	8	9	10
G45	45.0	78.0	16.0	8.9	4.0					
G0	79.0	77.0	3.0	2.0	1.5					
G135	45.8	82.0	26.3	20.0	13.8					
Filter fact. for G-90/G0	2.13 x 10 <sup>-1</sup>	1	1	1	1					
Filter fact. for G45	4.7 x 10 <sup>-1</sup>	1	1	1	1					
Filter fact. for G135	4.7 x 10 <sup>-1</sup>	1	1	1	1					

Wt. of cup + solvent 94.5145  
 Wt. of cup 77.5640  
 Wt. of solvent 16.9505

	(1)	(2)	(3)	(4)	(5)
Wt. of soln. + cup + solvent	95.8570	96.8432	99.4311	100.6336	102.2777
Wt. of cup + solvent	94.5182	95.8570	96.8432	99.4311	100.6336
Wt. of Soln.	1.3418	.9862	2.5879	1.2025	1.6041

0.1488g in 50.1 ml

∴ 0.00297 g/ml (unfiltered)

4. Molecular Weight Calculation ( $M = \frac{RTS}{(1-VP)D}$ )

A. Indulin A in 90% dioxane and 10% water at 14 minutes:

$$M = \frac{8.315 \times 10^7 \times 303 \times 8.9628 \times 10^{-13}}{[1 - (.7768 \times 1.027605)] \times 7.2675 \times 10^{-7}} = 153,978$$

At 22 minutes:

$$M = 1.2485 \times 10^{11} \times \frac{3.1824 \times 10^{-13}}{7.5545 \times 10^{-7}} = 52,594$$

At 32 minutes:

$$M = 1.2485 \times 10^{11} \times \frac{1.9060 \times 10^{-12}}{5.25 \times 10^{-7}} = 453,268$$

B. G. I. in 90% dioxane and 10% water,

At 21 minutes:

$$M = \frac{8.315 \times 10^7 \times 303 \times 1.1441 \times 10^{-12}}{[1 - (.7043 \times 1.026129)] \times .9923 \times 10^{-6}} = 104,756$$

At 29 minutes:

$$M = 9.0855 \times 10^{10} \times \frac{2.8945 \times 10^{-12}}{.9292 \times 10^{-6}} = 283,013$$

At 39 minutes:

$$M = 9.0855 \times 10^{10} \times \frac{9.3929 \times 10^{-13}}{9.1815 \times 10^{-7}} = 92,945$$

C. VBM in 100% dioxane

$$M = \frac{8.315 \times 10^7 \times 303 \times 5.7219 \times 10^{-13}}{[1 - (.7125 \times 1.026129)] \times .8392 \times 10^{-6}} = 63,883$$

D. Indulin A in 100% dioxane

$$M = \frac{8.315 \times 10^7 \times 303 \times 1.1853 \times 10^{-12}}{[1 - (.7243 \times 1.026129)] \times .4782 \times 10^{-6}} = 243,180$$

E. VBM in 100% benzyl alcohol

$$M = \frac{8.315 \times 10^7 \times 303 \times 4.351 \times 10^{-13}}{[1 - (.7237 \times 1.041673)] \times 2.436 \times 10^{-7}} = 182,843$$

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PROJECT NO. Project 2097  
COOPERATOR Institute of Paper Chemistr  
REPORT NO. 4  
DATE July 2, 1964  
NOTE BOOK 1704, p. 58-60, 67, 70-71  
PAGE 1858, p. 44-53  
SIGNED *Edward J. Jones*  
Edward J. Jones

## GAMMA IRRADIATED WOOD MEAL LIGNIN (Supplement to Reports 2 and 3)

Samples of gamma irradiated wood meal lignins were prepared following the general teachings of Garnett and Merewether (Reference 15, Report Two.

Air-dried black spruce wood meal from the same benzene-ethanol extracted batch described in Report Two was sealed in ten No. 2 tin cans and irradiated in the spent fuel rod facility at Argonne National Laboratory to the levels indicated in Table I.

TABLE I

Can No.	Dosage, rads
1	$10^{8.31}$
2	$10^{8.42}$
3	$10^{8.41}$
4	$10^{8.29}$
5	$10^{8.00}$
6	$10^{6.90}$
7	$10^{7.00}$
8	$10^{6.97}$
9	$10^{6.84}$
10	$10^{6.58}$
Ave. (1-4)	$10^{8.36}$
Ave. (6-9)	$10^{6.93}$

The contents of Cans 1, 2, 3, and 4 were mixed and coded 2097-GI-45-1.

The contents of Cans 6, 7, 8, and 9 were mixed and coded 2097-GI-45-2.

Extraction procedures for lignins were similar to those used for milled wood lignin (Report Two). Allowing for losses, the lignin yield for 2097-GI-45-2 was 2.2% of total lignins. The lignin yield for 2097-GI-45-1 was estimated to be 6.0%.

Identification of samples available from work in this report:

Samples 2097-1858-26-VBM spruce	-30
- "	" -31
27- "	" -32
- "	" -33
28- "	" -35
30- "	" -36

Series run with 2097-1704-60 black spruce meal extracted, 60-mesh Wiley mill, dried over  $P_2O_5$ .

- 30. VBM equipped with new shaft and bearings. Eccentric at extreme. 7 mm. amplitude. No toluene. 5 g. for 16 hr. 11.63% soluble lignin.
- 31. Repeat of (30) with toluene. 3.34% soluble lignin.
- 32. 48-hr. milling in toluene. 11.76% soluble lignin.
- 33. 3 g. milled dry 16 hr. 23.22% soluble lignin.
- 34. 2 g. milled dry 16 hr. 33.95% soluble lignin.
- 35. 1 g. milled dry 16 hr. 27.62% soluble lignin.
- 36. 2 g. milled dry 16 hr. 26.22% soluble lignin.

Sample 2097-1704-156-P&G/T

Received from Mr. Norman A. Bates, Proctor and Gamble (letter to Jones, Nov. 13, 1959).

Black spruce wood meal twice through 60-mesh Wiley mill, dried over  $P_2O_5$ . Milled in their VBM with toluene for 48 hrs. and shipped to us under toluene. Found to be completely soluble in 2N NaOH. Extracted. 17.5% soluble lignin.

Sample 2097-1704-55-VBM Runs 1 and 2 - Aspen

Aspen wood meal, EtOH-benzene extracted, 5.23% MeO, obtained from Busche (1727-25-B). Dried over  $P_2O_5$ . Run in toluene in Lampen mill. 5.5-g. charges run in VBM in toluene. Combined and extracted. MeO lignin - 20.72%.

Sample 2097-1704-111-VBM-19 (For comparison with VBM-24-28)

6.20 g. dried, unextracted wood meal milled in toluene with original VBM mount, full eccentric (2-3 mm. amplitude) for 25 hr., 2.42% soluble lignin.

Samples: 2097-1704-131-VBM-24  
          -140-VBM-25  
          -135-VBM-26  
          -151-VBM-27  
          -155-VBM-28

This represents a series in which the spring mounting of the VBM was changed to give increased amplitude and meal was run for different treatment times.

On VBM, leaf springs were reduced to two each side and suspension spring added, with rubber tubing damper. Amplitude 6-7 mm.



Milling without toluene:

- VBM-24. 2097-1704-60 extracted black spruce. 6 g. air-dry run 8 hr. Increased but not complete solubility in 2N NaOH. Run in VBM for additional 8 hr. (Total 16 hr.) Completely soluble in NaOH. 7.58% soluble lignin.
- VBM-25. 5.01 g. desiccator dried and 60-mesh Wiley milled meal, milled 8 hr. 5.30% soluble lignin.
- VBM-26. 5 g. as (-25), milled 16 hr., 12.54% soluble lignin.
- VBM-27. 5 g. as (-25), milled 32 hr., 27.84% soluble lignin.
- VBM-28. 5 g. as (-25), milled 48 hr., 29.06% soluble lignin. Also sample of unextracted meal.

	<u>% MeO</u>
-VBM-25	15.42
-26	15.60
-27	15.32
-28	14.90
-1704-60W.M. meal	5.20
-VBM-28 meal (before extrac.)	4.93

Samples 2097-1704-128-22 )  
-23 )  
-FPL-AD ) Residual meals after dioxane extraction  
-FPL-DD )  
-FPL-M/T )

Comparison set VBM our lab and Forest Products Laboratory, all from extracted spruce 2097-1704-60.

- 22. Mill eccentric at extreme position, 3 mm. amplitude. 5.73 g. meal dry. No toluene. Milled 8 hr., product cloudy and yellow in 2N NaOH. 2.08% soluble lignin.

- 23. 5.03 g. dry meal through 60-mesh Wiley mill. Run without toluene, 3 mm. amplitude, 8 hr. Product cloudy and yellow in 2N NaOH. 2.58% soluble lignin.
- FPL-AD. Milled air dry by Mr. Pew in FPL VBM without toluene, 9 mm. amplitude, (?)hr., clear amber solution in 2N NaOH. 8.49% soluble lignin.
- FPL-DD. Desiccator dried by Mr. Pew over magnesium perchlorate. Milled same. Clear solution in 2N NaOH. 19.96% soluble lignin.
- FPL-M/T. Dry (?) meal milled 8 hr. under toluene. NaOH solubility as 22 and 23. 2.30% soluble lignin.

Two small samples labeled -22 and -FPL-M/T may be soluble lignin.

Sample Ex.M-3-4-2097-1704-73

Residual wood meal after extraction of soluble lignin, air dried.

Lignin yield-3.75% (total lignin)  
Extraction with aqueous dioxane.

VBM runs - Lampen mill product divided into two parts (3 and 4). Each placed in VBM with 3080 g. SS balls and jar 80% filled with redistilled toluene. Run for 48 hr. (p. 62). Amplitude of jar later measured at 3 mm. (p. 72).

Lampen mill run - approximately 10 g. (p. 71) dried, extracted spruce wood meal run with 800 ml. redistilled toluene in Lampen mill for 48 hr., using pulp gasket coated with PVA1. (p. 61).

Wood meal - black spruce (Picea Mariana), 31-yr <sup>log</sup> (p.58),  
barked, reduced to sawdust, air dried. Wiley milled to 20-mesh.

610 g. extracted in large Soxhlet with 1:2 ethyl alcohol:benzene,  
followed by 95% EtOH. Washed with distilled H<sub>2</sub>O. Air dried.

2097-1704-60. Portions to be milled dried in vacuum desiccator  
over P<sub>2</sub>O<sub>5</sub>.

Analysis (p. 65):

Methoxyl -	5.26%
Klason lignin -	28.00%
Moisture -	6.56%

Sample 2097-1858-40

VBM spruce lignin, combined Runs 37-50

Sample "Combination of Lignin from VBM Runs 3-8"

2097-1704-71-VBM spruce	3-4
" " " " "	5-6
" " " " "	7-8

Preparation and extraction of black spruce meal, Lampen and  
48-hr. VBM milling as described for Sample Ex.M.-3-4-2097-1704-73.

Extraction of lignin all as for 2097-1704-71-3~~4~~: (p. 67).

Toluene decanted off meal

Standing in 100:4 aqueous dioxane overnight, centrifuged [50 cc.  
to 2.5 g. meal].

Fresh lots aqueous dioxane 2 days

" " " " 5 days

Extracts combined and taken to dryness in a Bong evaporator at  
50-60°C.

Residue dissolved in 20 ml. 90% acetic acid, ppt. into 200 ml. distilled  $H_2O$ , centrifuged, air dried. Dissolved in 20 ml. 2:1 1,2 dichloroethane-95% ethanol. Ppt. into 200 ml. ethyl ether. Centrifuged. Washed 3x ethyl ether, 1x in L.B. pet. ether. Vacuum dried over  $P_2O_5$  and paraffin.

	Yield	Wood Lignin, %
Run 3-4	0.1062 g.	3.75
5-6	0.0744	(Part lost)
7-8	<u>0.1353</u>	3.97
	0.3159	

Sample Ex. M-5-6-2097-1704-73

Same process as EX.M-3-4

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PROJECT NO. 2097  
COOPERATOR Institute of Paper Chemistry  
REPORT NO. 5  
DATE November 4, 1965  
NOTE BOOK I858  
PAGE 42, 71-3, TO 80-88, 92  
SIGNED *S. C. Nagel*  
S. C. Nagel

## PHYSICAL CHEMISTRY OF LIGNIN PREPARATION

### PREPARATION OF EXTRACTED WOOD MEAL

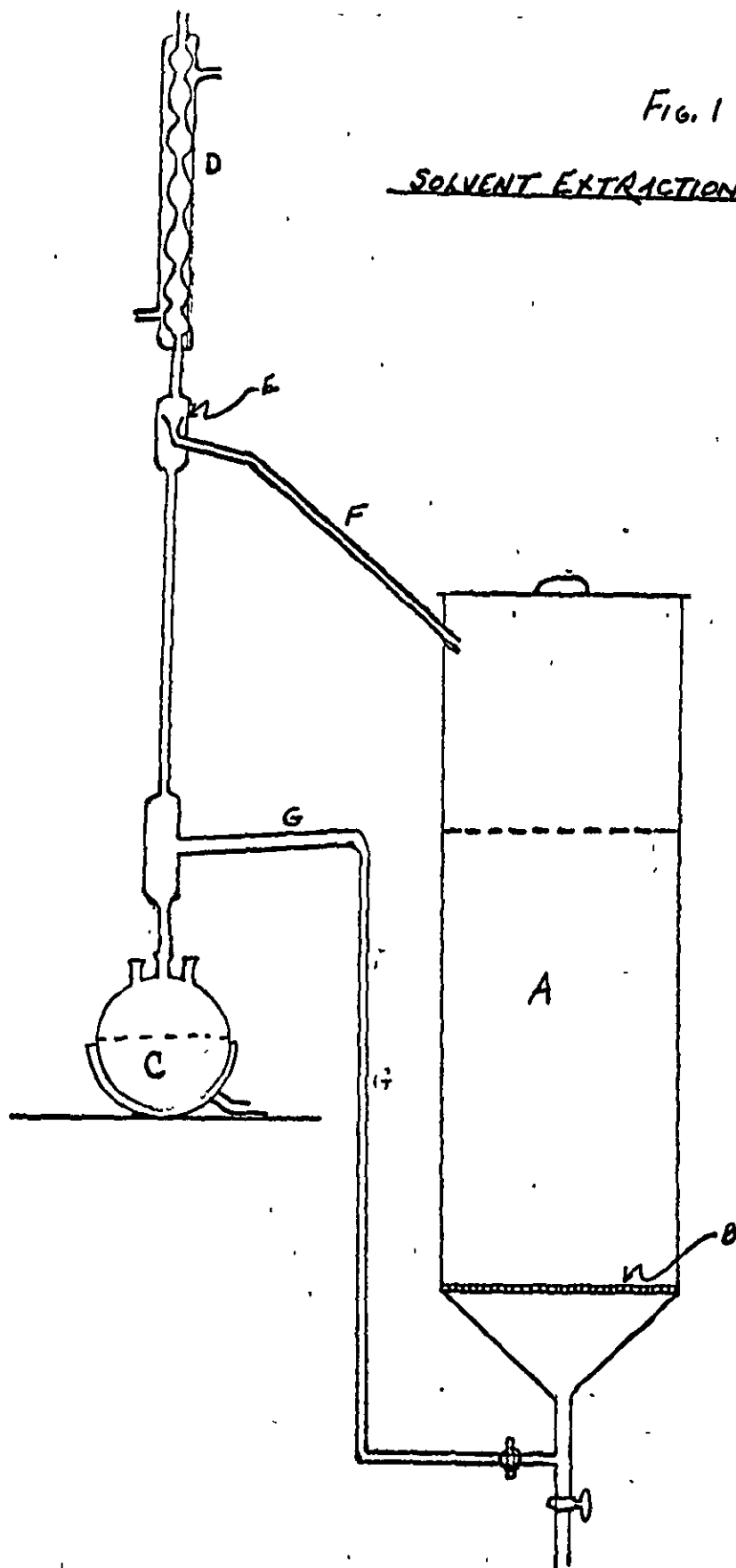
Source of the wood was a 30-year old black spruce (*Picea mariana*) tree\*. Two-foot logs were debarked and freed of knots. The bare logs, 10-1/2 to 12 cm. in diameter, were chipped in the Carthage chipper. The chips were air dried one to two weeks and put through a Wiley mill having a screen plate with 0.040-inch holes. The black spruce wood meal was air dried one week and stored in closed containers.

The wood meal (6592 grams) was extracted 45 hours (after 1.5 hours' warmup) in a continuous extractor as shown in Fig. 1 with a 2 to 1 by volume mixture of benzene and 95% ethyl alcohol (15 gallons total). The benzene-alcohol solvent was drained from the meal and otherwise removed from the equipment. A second extraction was made, this time 22 hours with 95% ethyl alcohol (12 gallons). The solvent was drained from the wood meal and replaced with fresh ethyl alcohol (7 gallons). The suspension was mixed in the extractor with a paddle and the solvent again removed. Suspension washing was repeated four times using distilled water. The extracted meal was air dried eight days (95% nonvolatile at 100°C.) and stored in closed containers having polyethylene film liners. Portions to be milled were dried in a vacuum desiccator over calcium chloride. Portions to be hydrolyzed at elevated temperatures were used without the additional drying.

\*Collected April 18, 1960 by Dr. R. E. Kremers and Miss B. Reeder from the Jennie Webber fire lane, Rhinelander Paper Company experimental forest, 5 to 10 miles north of Rhinelander, Wisconsin.

FIG. 1

SOLVENT EXTRACTION APPARATUS



- A. STAINLESS STEEL EXTRACTOR
- B. POROUS SEPTUM
- C. 12-L. THREE-NECKED FLASK
- D. CONDENSER
- E. SPECIAL ADAPTOR
- F. SIDEARM
- G. SIDEARM

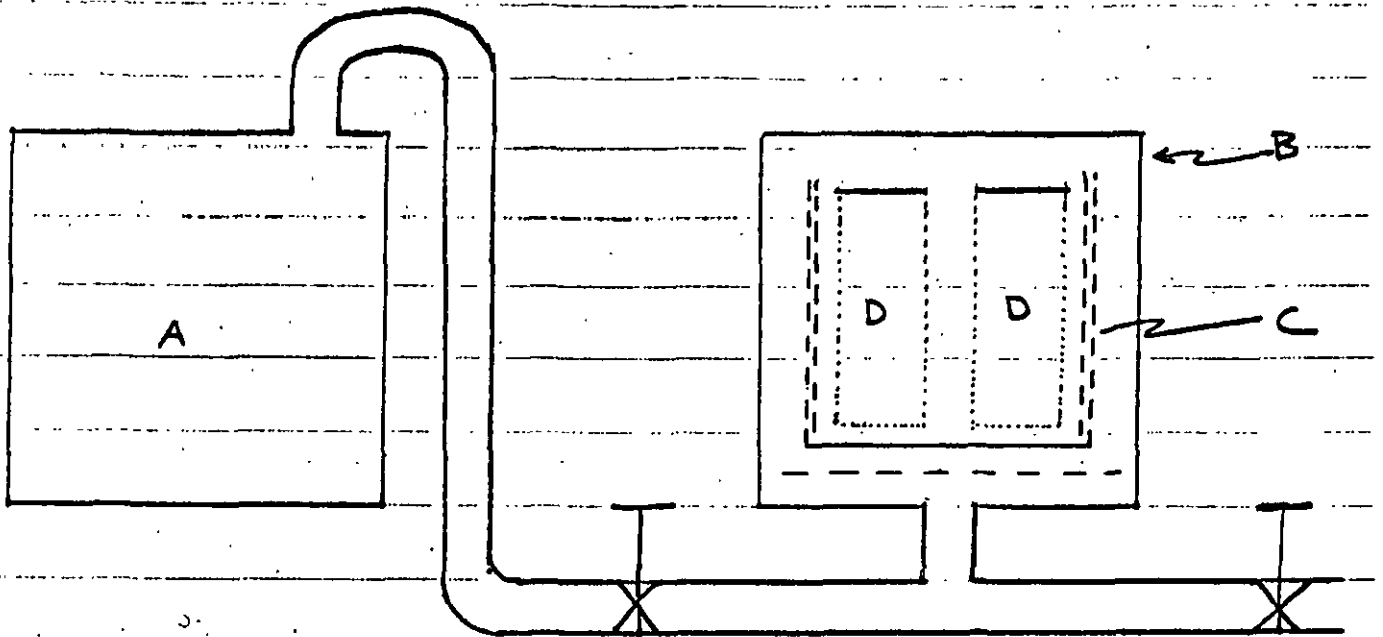
#### VIBRATION BALL MILLING

The Institute of Paper Chemistry vibratory ball mill (1) was charged with 3000 g. 1/4-inch stainless steel balls and the eccentric plate adjusted to give 6 to 7 mm. amplitude vibration. Three grams of dried wood meal were charged and processed 16 hr. in a room maintained at 40°F. ( $\pm 5^\circ$ ). The balls and milled meal were poured in small lots onto a screen over a pan and the meal separated as much as possible with a brush. The vibratory ball-milled black spruce wood meal was stored in a glass container and kept dry over calcium chloride in a vacuum desiccator.

#### STEAM HYDROLYSIS OF EXTRACTED WOOD MEAL

The Institute of Paper Chemistry isothermal digester (2, 3) was modified so that steam instead of superheated liquid was blown from preheater to digester. (Schematically shown in Fig. 2.) Two 100-mesh stainless steel wire baskets were made to slip into the existing shielded basket. Wire basket dimensions were 2-1/4 by 4-1/2 inches oval cross section by 12 inches high. The 200-g. charge of extracted wood meal used in each basket filled it to within two inches of the top.

Before each cook, vacuum was applied to the digester and the walls heated for a limited time to minimize preheating the wood meal. At the start steam from the preheater was blown to the digester. During hydrolysis additional steam was metered in to maintain temperature. At the end steam pressure was discharged from the digester and the baskets removed. Volatiles in the hydrolyzed wood meal were reduced by air drying.



- A PREHEATER (BOILER)
- B DIGESTER
- C SHIELDED BASKET
- D 1.00 MESH WIRE BASKETS

FIG 2 APPARATUS FOR STEAM HYDROLYSIS  
OF EXTRACTED WOOD MEAL



Four steam hydrolysis cooks were performed on the extracted black spruce wood meal, Table I. Of these 2097-1858-86 and -92 were the best. In each cook the lower temperatures and pressures occurred near the end of the heating cycle as the available steam in the preheater was giving out. The hydrolysis materials all had a scorched odor and were brown in color.

TABLE I  
OPERATING CONDITIONS FOR STEAM HYDROLYSIS COOKS  
OF EXTRACTED BLACK SPRUCE WOOD MEAL

Code 2097- 1858-	Duration, min.	Temperature, <sup>a</sup> °C.	Pressure, p.s.i.	Remarks
84-1	9	217	295-310	Partially washed by cooling water
86	2.5	228-243	450-500	
88	10	ca. 190	150-260	Many problems
92	10	226-235	375-410	

<sup>a</sup>Thermocouple located between baskets

#### LITERATURE CITED

1. Busche, L. R. The Klason lignin determination as applied to aspenwood with special reference to acid-soluble lignin. Doctor's Dissertation. Appleton, Wisconsin, The Institute of Paper Chemistry, 1960; Tappi 43, no. 12:961-70, 970-4(Dec., 1960).
2. Walters W. Z. The effect of elevated temperatures on the neutral sulfite pulping process. Doctor's Dissertation. Appleton, Wisconsin, The Institute of Paper Chemistry, 1959.
3. Walter W. Z. and May, M. N., Tappi 43, no. 11:887-8(Nov., 1960).

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PROJECT NO. Project 2097  
COOPERATOR Institute of Paper Chem  
REPORT NO. 3  
DATE August 29, 1960  
NOTE BOOK 1704 p.58-60,67,70-71  
PAGE 1858 p.44-53  
SIGNED John D. Scribner  
John D. Scribner

## ISOLATION OF BLACK SPRUCE LIGNIN BY GAMMA IRRADIATION

### SUMMARY

This is Project Report Three on Project 2097 concerned with the physical chemistry of lignin. The first two reports have been concerned with the isolation of lignin using a vibratory ball mill, and osmotic pressure determinations of the molecular weight of this milled wood lignin.

In the present experiment, two portions of 20-mesh extracted black spruce wood meal were irradiated at average dosages of  $10^{6.36}$  rads and  $10^{6.93}$  rads. After extraction with dioxane and subsequent purification, the yield of lignin from the heavily dosed meal was about 100 times that obtained from the meal with lesser dosage. These results confirm previous data concerning the effect of high-intensity irradiation of wood on the extractable lignin. The quantity of lignin obtained was not nearly that obtained in another similar experiment, but demonstrated that a certain dosage must be reached before the effect on the extractable lignin is appreciable.

The lignin yield from the heavily dosed meal was comparable to the milled wood lignin yield for only those milling runs made at the beginning of the project. The yield is only about one-fifth of the milled wood lignin obtained when milling was standardized at optimum conditions.

## REVIEW OF PREVIOUS WORK

It is quite generally known that cellulose is profoundly affected by ionizing radiation (3). Lawton, Balwit, and Bueche (7) found that cellulose is degraded by high-energy electrons. In other experiments, Lawton, Bellamy, et al. (8) found that, whereas the cellulose in natural wood is almost completely resistant to the enzymatic action of rumen microbes, the cellulose in wood irradiated by high-energy electrons (dosage of  $10^{8.52}$  "röntgen units) is digestible by rumen contents to about the same degree as hay. They found also that irradiated ball-milled filter paper was 89% soluble in hot water, and noted that, at this stage, the cellulose was probably degraded to molecules approximately six  $C_6H_{10}O_5$  units long. Lawton, Millett, and Saeman (9) found that cotton linters were 44% decomposed at  $10^{8.7}$  "röntgens, and were made water soluble. Blouin and Arthur (2) noted a number of effects on purified cotton while working with dosages from  $10^5$  -  $10^8$  "röntgens. Among these were: Formation of carbonyl group, formation of carboxyl groups, and chain scission in the approximate ratio of 20:1:1; increased solubility in water and dilute alkali; decrease in tensile strength of fibers.

Although much data has been collected on irradiated cellulose, little is known about the effects of comparative dosages on the lignin in the wood. Lawton, Bellamy, et al., (8) noted during their experiments on irradiated wood that the effect on the Scholler lignin seemed to be very slight, if not negligible. They also noted that the behavior of the wood seems to confirm the theory that lignin is linked chemically with the carbohydrates in wood, and is not an "incrusting substance" as thought by some (4). The effect of irradiation is to break this bond, freeing the lignin for extraction or the cellulose for attack by biochemical agents, as in their experiments.

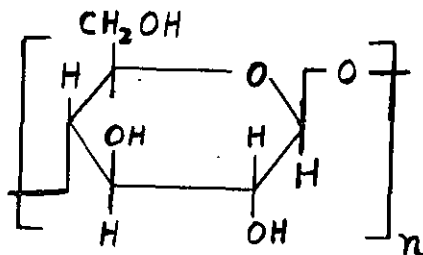
Recently, Garnett and Merewether (6) attempted the isolation of lignin from Eucalyptus regnans by gamma irradiation. Little radiation effect was noted at or below  $10^7$  rads. At maximum yield ( $10^{8.25}$  rads), the soluble lignin was 16.8% of the total wood; about half of this was extracted with ethanol, and the remainder with 0.12N NaOH.

#### GENERAL EFFECTS OF IRRADIATION

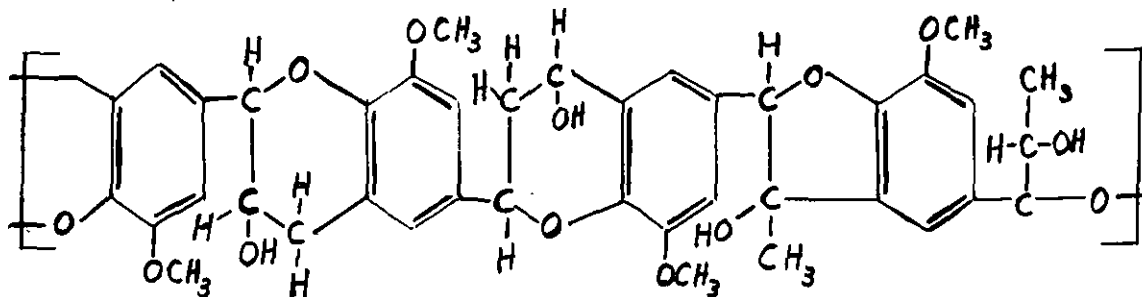
Little (10) found that the initial effect of pile and Van de Graaff radiation on linear high polymers is the breakage of bonds, forming free radicals. Further effects depend on whether these radicals form: (In oxygen) low molecular weight compounds and carboxyl end groups; (in vacuo) different end groups and low molecular weight compounds; the original bonds; end-linked units; cross-linked chains. It was found that samples exposed to the air generally are scissioned; whereas reactions in vacuo have resulted in formation of covalent or dipolar bonds for greater polymer stability. Little found that the presence or formation of unsaturated rings is a factor in preventing the scission expected for a given dosage. This was demonstrated by Barton and Patrick (5) for small molecules by radiolysis of a mixture of cyclohexane and benzene- $d_6$ . By measurement of the yields of  $H_2$ , HD, and  $D_2$ , it was shown that the benzene- $d_6$  actually protects the excited cyclohexane from decomposition, the mechanism of protection being energy transfer and dissipation. Alexander and Charlesby (1) demonstrated this effect for polymers by comparing the irradiation effects on unsubstituted polymethyl methacrylate and the polymer with 10% substitution of alkyl thiourea, aniline, or dimeta-tolyl-thiourea, finding that the energy required for one main chain break increased from 61 electron volts to 143, 152, and 227 e.v., respectively. It was also found that, whereas polyiso butylene required 17 e.v. for<sup>a</sup> main chain break, a copolymer of 20% styrene and 80% iso-butylene required 32 e.v.

### APPLICATION OF RADIATION DATA TO WOOD

These findings should be kept in mind while comparing the structures of cellulose and lignin, cellulose being generally accepted as:



Brauns (4) notes that although many structures have been proposed for lignin, a generally accepted building stone is the phenyl-propane unit, incorporated below into one possible structure:



It is easily seen that if the data concerning aromatic groups in irradiated material can be applied to lignin and cellulose, it should be possible to separate the lignin from wood, quite unchanged, by extraction with neutral solvents after the wood has been subjected to high intensity radiation in order to degrade the cellulose and break the lignin-carbohydrate bonds.

## EXPERIMENTAL PROCEDURES

### PREPARATION OF WOOD MEAL

A 31-year-old black spruce tree (Picea mariana) with a base diameter of 8.6 - 9.2 cm. was cut, peeled, and sectioned. It was then ground to sawdust and air dried six days, after which it was Wiley-milled to pass a 20-mesh screen. The meal was then extracted for 45 hours in a large Soxhlet extractor over 1:2::ethanol:benzene, after which it was extracted for 20 hours over 95% ethanol. This was followed by one washing with ethanol and four washings with distilled water, after which the meal was air-dried for six days before storage in polyethylene bags until further use.

### IRRADIATION DATA

The extracted wood meal used in this experiment was sent to Argonne National Laboratory, Lemont, Illinois, to be processed in the gamma irradiation facility there. The amounts of irradiation received by the various portions of meal are noted in Table I. After the meal was received following treatment, the No. 2 cans in which the meal had been irradiated were opened and the meal weighed. The meal from cans No. 1, 2, 3, and 4 was combined in a 3-l. beaker (Meal A), and that from cans No. 6, 7, 8, and 9 was mixed in a second 3-l. beaker (Meal B). Cans No. 5 and 10 were not included in this experiment, because the dosages received by these portions of meal were significantly below those received by the other portions in their respective batches. Rather, they were emptied into separate glass jars and stored, after a moisture determination was made on each portion with the following results: Can No. 5--7.95%; can No. 10--7.22%.

TABLE I  
DOSAGES RECEIVED BY PORTIONS OF BLACK SPRUCE WOOD MEAL

Can No.	Weight, g.	Dosage, rads	Can No.	Weight, g.	Dosage, rads
1	140.642 <sup>a</sup>	10 <sup>8.31</sup>	6	79.794	10 <sup>6.90</sup>
2	79.539	10 <sup>8.42</sup>	7	82.883	10 <sup>7.00</sup>
3	79.763	10 <sup>8.41</sup>	8	72.005	10 <sup>6.97</sup>
4	74.945	10 <sup>8.29</sup>	9	78.750	10 <sup>6.84</sup>
5	80.870	10 <sup>8.00</sup>	10	69.959	10 <sup>6.58</sup>

Av. dosage rec'd by No. 1-4 = 10<sup>8.36</sup>. Av. dosage rec'd by No. 6-9 = 10<sup>6.93</sup>

<sup>a</sup>The meal in this can was extremely wet, as indicated by approximate weighings of the meal following extraction and air drying

	<u>Meal A</u>	<u>Meal B</u>
Approximate weight before extraction	374 g.	313 g.
" " after "	<u>299 g.</u>	<u>307 g.</u>
Apparent weight loss during extraction	75 g.	6 g.

The appearance of Meal A was a uniform yellow color, except for dark brown agglomerations in Can 1. After extraction and air drying, a small portion of Meal A was placed in a test tube and covered with concentrated HCl. To this was added a few drops of K<sub>3</sub>Fe(CN)<sub>6</sub> solution, giving a bluish haze. When the same treatment was applied to large flakes of the dark brown material, originally from Can 1, which had settled to the bottom of the extraction beaker, a heavy blue precipitate was formed. These tests indicated that the dark material in Can 1 was iron deposits in some form, and not carbonized meal as thought at first.

The appearance of Meal B seemed to be no different from that of wood meal which had not been irradiated.

## EXTRACTION AND PURIFICATION OF LIGNIN

The procedure for extracting and purifying the lignin was much the same as that described in Project Report Two (11), with the only significant difference being in the quantity of wood meal extracted. Approximately 2300 ml. of 96% dioxane was poured into each of the two beakers containing the irradiated meal. The thick slurries were thoroughly mixed three or four times a day, and at three-to four-day intervals the supernatant liquids were poured into Erlenmeyer flasks, and fresh dioxane was poured into the beakers. The extraction of Meal B was discontinued after about 4400 ml. of dioxane had been added. About 3500 ml. of this reclaimed, with the rest being lost through evaporation, absorption by the wood meal, samples removed for analysis, and one spillage. All of the dioxane solution was centrifuged (2000 r.p.m. for 40 min.) of suspended wood meal before being evaporated to dryness on a Borg rotary evaporator. About 7100 ml. of dioxane was used for Meal A (extraction was continued longer because of higher yield) with about 6000 ml. of this being reclaimed, cleared of suspended matter, and evaporated.

Only enough 90% acetic acid was used to just dissolve the evaporator residues, and 20 ml. of the solutions were precipitated at a time with mechanical stirring into 200 ml. of distilled water. The material from Meal B was dissolved in 20 ml. of HAc, while that from Meal A required 200 ml. for solution. The precipitate in each case was dissolved in 1:2:95% ethanol:1,2 dichloroethane, with this solution being precipitated into diethyl ether. After two washings with diethyl ether, the lignin was washed with reagent grade petroleum ether (B.P. 30-60°C.) and dried under a gentle stream of dried air before being placed in a vacuum desiccator over  $P_2O_5$  and paraffin shavings. In the precipitations of the lignin from Meal A, all precipitations at each point of procedure were made into the same centrifuge bottle to minimize the lignin loss on eventual transfer to a weighing bottle. One precipitation at each point was required for the lignin from Meal B.



PENETRATION OF SOLVENTS

The solvents used were purified as described in Project Report Two (11).

LIGNIN YIELD

The amounts of lignin obtained are listed in Table II. The percentage of Klason lignin is given on a basis of Klason lignin being 28% of the total wood.

TABLE II  
 LIGNIN ISOLATED BY GAMMA IRRADIATION

Source	Weight, g.	Klason Lignin, %	Total Wood, %
Meal A	4.8935	5.8 (% of 85 g.)	1.6 (% of 305 g.)
Meal B	0.0246	0.028	0.0079
Corrected Meal B <sup>a</sup>	0.048	0.055	0.016

<sup>a</sup>Corrections were made for losses of lignin through spilling. After the precipitation of the acetic acid solution into water, the centrifuge bottle was upset, and a large portion of the suspension was lost. The corrections were made as follows:

$$\text{Corrected yield} = (\text{weighed lignin}) \left( \frac{\text{amount in centrifuge bottle before spilling}}{\text{" " " " after " "}} \right)$$

$$\left( \frac{\text{total dioxane reclaimed}}{\text{dioxane reclaimed minus spilled}} \right) = 0.0246 \text{ g.} \left( \frac{230 \text{ ml.}}{135 \text{ ml.}} \right) \left( \frac{4000 \text{ ml.}}{3500 \text{ ml.}} \right) =$$

approximately 0.048 g.

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# PROJECT REPORT FORM

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Edward J. Jones

## THE PHYSICAL CHEMISTRY OF LIGNIN PREPARATIONS

### EXTRACTION OF LIGNIN FROM BLACK SPRUCE WOOD MEAL

This report covers work done under Project 2097 approximately four years ago. Pressure of other programs has prevented its issuance until this time. However, we are actively engaged in physical chemical studies of the products secured and this report is essential to identification of the materials being used. The following report was largely prepared by James Modeen.

#### SUMMARY

Black spruce wood meal was solvent extracted and milled in the vibratory ball mill to obtain lignin samples for molecular weight determinations. Numerous conditions were changed on the ball mill and modifications were made accordingly for more efficient operation. The lignin yield was significantly improved after the modifications were made and by using constant conditions, lignin was accumulated for future studies.

Wood meal solubility in alkali greatly increased with increasing amplitude (vibratory action) of the mill. Methoxyl content, an indication of the lignin present, decreased with increased milling time, while the lignin yield increased, indicating extraction of carbohydrate material in addition to the lignin. A decrease in charge weight also improved the yield indicating a critical mass is required in the mill for optimum operation.

The osmometry work detailed in Project Report No. 1 was partially extended. The data collected was inconclusive, possible because of variations in the constant temperature bath. Polyvinyl alcohol films were cast to be used as osmometer membranes but have not yet been evaluated.

#### INTRODUCTION

This is Project Report Two on the physical chemistry of lignin preparations. Lignin has been studied quite extensively over the past half century but the exact nature of the material is still rather speculative. The uncertainty of lignin chemistry arises from attempts at isolation of the lignin through chemical means which invariably alters the basic structure of the unit. Thus, different lignins are obtained depending on the method used in the isolation. Likewise, since the exact structure is somewhat questionable, synthesis of the molecule is equally difficult. The difficulty in obtaining lignin is explained by some as being due to strong chemical bonds between the lignin and carbohydrates, or from a very high molecular weight polymer having a three dimensional network within the wood, or possibly by weaker forces such as hydrogen bonding (such forces making cellulose insoluble in water, for example).

One means of obtaining the original, or protolignin, is by dissolution in neutral solvents. One of the early successful attempts was made by Brauns (10) who extracted a small percentage of the available lignin from wood meal with ethyl alcohol. Since the percentage was small, it was argued that the product was probably not representative. The small

yield may have been due to inaccessibility of the solvent to the lignin and means to disintegrate the wood have been contemplated or attempted. Among such methods are the vibratory ball mill (especially at low temperatures when the wood is brittle), "explosion" of the wood after building up pressure, biochemical decomposition, ultrasonics, and thermophilic fermentation (4). Recently, gamma irradiation methods have been used to render lignin more soluble in ethanol and alkali (15).

The scope of this project is to carefully establish the adaptability of such techniques as light scattering, osmotic pressure, spectrographic, and ultracentrifugation to lignin materials, and the application of these methods to lignin preparations. The possibility of securing a lignin sample by one of the methods mentioned previously renders the study of such a preparation by physical means of great interest and importance. This phase of the project, then, was concerned mostly with obtaining a representative sample of lignin.

The method chosen for obtaining the lignin was the vibratory ball mill. This machine was used for student thesis work (11) and was similar to equipment described in other reports (4, 13). The procedures used in the milling of the meal, and the extraction and purification of the lignin followed the methods used by Björkman (4). Unfortunately, parts of the report were rather inexplicit or possibly erroneous. Because of this, additional work was necessary and a change in concept of the original milling intentions had to be made. However, the series of articles published (3-8) describe the procedures used and the results obtained so that comparative work can be done and the methods evaluated.

The effect of the vibratory ball mill is quite different from regular ball mills. In the process of milling, the methoxyl content decreases (indicating an increase in the carbohydrate content), the decrease being dependent on the milling conditions. The possibility of a lignin-carbohydrate complex is raised with the subsequent question of the nature of the lignin-carbohydrate bond in wood. The lignin-carbohydrate complex was investigated by Björkman (5-7) and by Lindgren (24). A literature survey of recent investigations on the lignin-carbohydrate bond has been made by Pearl and Rowe (25) and a literature review of the isolation and structure of lignin has been made by Kremers (23).

It is believed by some (6) that protolignin can be categorized into two main fractions (with respect to its association with hemicellulose). The first fraction has few or no bonds with hemicellulose and is located in the middle lamella where the lignin content is high. This material may be extracted from coarse meal under rather mild conditions and is the substance known as "milled wood lignin". The remaining fraction is more rigidly bonded to the hemicellulose and is isolated only by rather drastic measures. This material is the so-called lignin-carbohydrate complex. Apparently, there is no distinct boundary between the two fractions.

The lignin obtained by milling wood meal is referred to in this report as milled wood lignin (M.W.L.) as differentiated from ethanol-extracted lignin commonly known as Brauns' native lignin (B.N.L.). The lignin-carbohydrate complexes (L.C.C.) constitute some undertermined ratio of lignin and carbohydrate materials. The yields of lignin obtained in this laboratory are calculated as a percentage of the 29% Klason lignin in the wood meal expressed as total lignin. The yields have been corrected for loss, **wherever** possible.

## EXPERIMENTAL

### PREPARATION OF WOOD MEAL FOR MILLING

A 31-year old black spruce tree (Picea mariana) was freshly cut by Dr. Kremers near the Jennie Webber fire lane, east of Highway 17, north of Rhinelander. The log was sectioned, peeled, and ground to sawdust. The sawdust was air-dried and put through a Wiley mill to reduce the size to 20-mesh. The first portion passed through the 20-mesh sieve so no further attempts were made to process the remaining sawdust.

Approximately 600 g. of the meal were placed in a large Soxhlet extractor and extracted with a 2:1 ratio of benzene:95% ethanol for 45 hours. The solvent siphoned at the slow rate of about once an hour so distillation was interrupted daily at a point that would keep the meal immersed in solvent overnight. After the benzene-alcohol process, the meal was extracted for 20 hours with 95% ethyl alcohol, washed in fresh ethyl alcohol and then in distilled water. The meal was filtered on a large Büchner funnel and the water washings repeated four times. The solvent-extracted meal was finally air dried for approximately one week before storage in closed containers.

### LAMPEN MILLING

For the first few vibratory ball mill runs the meal was ground in the Lampen mill. After thorough drying over phosphorous pentoxide in vacuo, 6 g. of the meal were placed in the Lampen mill. To this 800 to 850 ml. of toluene were added and the contents then milled continuously for 48 hours. The meal was recovered by carefully tilting the chamber to catch the slurry in a pail and then removing the brass ball. The chamber

and ball were wiped with glass wool and rinsed with toluene. The slurry was centrifuged at 2000 r.p.m. for 10 minutes in a No. 2 International Centrifuge, (Model V, Head No. 277), and the excess toluene decanted.

#### MODIFICATION OF THE VIBRATORY BALL MILL

The ball mill used was a modified version of the mill designed by the National Bureau of Standards (13). Certain changes were made in the original design to accomodate work proposed in a graduate thesis (11). Essentially, the mill consists of a milling jar rotating freely in a housing containing an adjustable eccentric weight. The weight is on a shaft connected with a flexible coupling to a constant speed, direct-drive motor. The housing is suspended by means of leaf springs connected to the base of the machine. When fully loaded, and with eccentric in the extreme outer position, up to 10 mm. amplitude in the front part of the mill jar was possible under certain conditions. A more normal condition gave 6 to 7 mm. amplitude. At an intermediate position, the jar would vibrate 3 to 4 mm.

The changes mentioned consisted of equipping the milling jar with a jacket for external cooling. This, in turn, necessitated changing the original clamping device. The eccentric was also changed in size and weight from the original dimensions. The extra inertial mass gained through modification distributed the vibration not only to the jar but also to the other parts of the machine and placed an overload on the shaft bearings. The normal life of the bearings as expected under average use was thereby reduced. If the bearings were not replaced in time, a certain



amount of damage to the motor, housing, or shaft often occurred. After numerous attempts to rectify the situation, it was found that an additional hardened steel jack shaft placed in two outboard pillow-block bearings between the motor and flexible coupling that was connected to the housing shaft prevented excessive vibration on the motor. Removing some of the leaf springs and suspending the housing by a coil spring also aided in isolating the vibration to the jar. Heavy-duty, preset bearings were placed in the housing.

#### MILLING THE WOOD MEAL

The first milling trials used 6 g. of the dried meal with toluene added to within one inch of the top of the 1-liter jar. Approximately 3000 stainless steel, 1/4-inch diameter balls were used in the jar. The length of time for milling was 48 hours. A fan was used for external cooling of the jar.

In later trials the toluene was omitted and the meal was milled dry. The milling time was also reduced to 16 hours and the charge weight to 3 g. In all cases, the meal was recovered by dumping the contents of the jar onto a No. 10 screen, catching the material in a pan beneath the screen. The screen was vigorously shaken to free the meal adhering to the balls. The jar was wiped down with glass wool (if toluene was used) or with a brush (if the meal was milled dry). When the dry procedure was used the inside of the jar was cleaned with sodium hydroxide; the average loss of meal was 0.42 g./3 g. charge (loss was not determined when using toluene).

#### EXTRACTION OF THE LIGNIN

The meal and toluene from the ball mill was centrifuged at 2000 r.p.m. for 10 minutes and the toluene poured off. Aqueous dioxane solution, 50 ml., was added to each of two separate portions of the ground meal in 250-ml. centrifuge bottles and allowed to stand overnight. The contents were centrifuged the following day under the same conditions and the dioxane collected. The process was repeated for a second extraction of two days' length, and for a third extraction of an additional five days. The combined extracts were then evaporated to dryness on a Borg rotary evaporator using the laboratory line vacuum and a water bath at 50 to 60°C.

#### PURIFICATION OF THE LIGNIN

The dioxane residue was dissolved in 20 ml. of 90% acetic acid. The acid was added dropwise, with stirring, to distilled water in a 250-ml. centrifuge bottle. The lignin precipitated out at this step in a light tan-colored floc. With stirring this changed to a powder which was centrifuged at 2000 r.p.m. for 40 minutes. The water was decanted and the lignin spread on the sides of the bottle before drying with a stream of dry air (two hours or longer). The dried lignin was then dissolved in 20 ml. of a 2:1 ratio of 1,2-dichloroethane:95% ethanol. The solution was added in a fine stream, with stirring, to ether in a 250-ml. centrifuge bottle. Again, the lignin precipitated out in a light tan floc which was collected by centrifuging at 2000 r.p.m. for 40 minutes. The ether was decanted and the lignin suspended in fresh ether and allowed to stand overnight. The washing was repeated with ether and once with petroleum

(low boiling, 30 to 60°C.), centrifuging at the same speed and standing overnight in each case. The purified lignin was then dried by a stream of dry air and set over phosphorous pentoxide and paraffin shavings, in vacuo, for a period of about five days.

#### PURIFICATION OF SOLVENTS

The toluene used was refluxed with sodium for three hours and then distilled over sodium. The p-dioxane (1, 4-dioxane) was refluxed with sodium for at least 11 hours before distilling over sodium. Distilled water was added to the dioxane immediately after distillation to give a 4:100 water:dioxane dilution ratio, by volume. The diethyl ether, U.S.P., was distilled over sodium without refluxing. The b.p. range in each case was within 0.3 to 1.0°C., depending on the solvent being distilled. In later distillations, the solvents were fractionally distilled through a Vigreux column. The 1,2-dichloroethane, petroleum ether, benzene, and 95% ethyl alcohol were used without further purification.

#### OSMOMETRY

Preliminary work on the use of Zimm-Myerson osmometers, the techniques, and the membranes used were described in an earlier report (28). The unreliability of single readings made it desirable to determine the best method for taking osmotic pressure measurements. Readings were taken in a number of ways; at each capillary, at the bright line of the meniscus, at the bottom of the meniscus, and by focusing on each capillary. It was found that an average of five consecutive readings on one capillary,

turning the telescope on its spindle and then refocusing on the other capillary before taking five more measurements resulted in more reliable data.

For the molecular weight determinations, lignin was weighed to the nearest 0.1 mg. and dissolved in 90% dioxane. Successive dilutions of the stock solution resulted in concentrations of approximately 0.1 to 0.4 g. lignin/kg. solution. Leakage tests and osmotic pressure runs were made using the first two solutions of lower concentration in osmometers having polyvinyl alcohol, "super-dense", and "swollen super-dense" membranes (cf. 28).

#### POLYVINYL ALCOHOL FILMS

Du Pont "Elvanol" 71-30, a medium-viscosity polyvinyl alcohol, was made up into a solution by dispersing the powder in cold water, heating to 95°C. with stirring, and maintaining this temperature for 25 minutes. Different percentage solutions were prepared to study the effect of solution viscosity on film thickness. The solutions were cast on polyethylene or glass plate surfaces by using a 0.012" clearance Bird bar, by "puddling", or by using a Gardner Ultra applicator at different clearance settings. The glass or polyethylene surfaces were thoroughly scrubbed with Alconox, rinsed with distilled water, rinsed with absolute ethanol, and left to dry before casting the solution.

The most satisfactory method was found to consist of casting a 6% solution on the clean glass plate using the Gardner applicator with a clearance of 0.040". Strips of Scotch tape were placed on three sides of the pool to form a rectangular area to contain the solution. After drying,

the films were stripped from the plate and 1-1/4" diameter circles were cut from the films for use in the osmometers. The circles and scraps were stored in atmospheres of 75.5, 53.5, 43.9, and 32.9% R.H., prepared by making saturated solutions of sodium chloride, magnesium nitrate, potassium carbonate, and magnesium chloride, respectively. The film was allowed to condition at the different relative humidities for a period of at least five days and then sent to the Analytical Group for Karl Fisher moisture determination. Because of the results on the first samples, the films of 43.9% were allowed to condition for a longer period of time and the moisture will be determined at some future date. The caliper of the films before conditioning varied from about 1.2 to 2.0 mils, averaging about 1.6 mils.

## RESULTS AND CONCLUSIONS

### THE EFFECT OF MOISTURE ON MILLING WOOD MEAL

A considerable amount of time was spent in obtaining a satisfactory method for milling the wood meal because the first trials of milling in toluene produced extremely low yields (2 to 4%) by comparison with published data (30 to 50%) (6). Consideration was first given to the drying of the meal since great stress was placed on this subject by others (4). The presence of moisture or other wood swelling agents during milling supposedly swelled the wood and reduced the milling efficiency.

There was 5 to 6% moisture in the black spruce meal before drying. By spreading the meal in a thin layer in large Petri dishes over phosphorous pentoxide in a vacuum desiccator, the meal dried sufficiently in a period of

three weeks if the surfaces of the meal and the desiccant were renewed occasionally. The desiccator was first evacuated by using the laboratory line vacuum, but later the vacuum was improved by using a vacuum pump. There was no further loss in weight of the meal with continued drying or by heating in an oven at 105°C.

After no apparent improvement in lignin yield, consideration was then given to the solvents used. Solvents were initially distilled by ordinary methods, but as an extra precaution the solvents were fractionally distilled. Toluene was finally stored over sodium to preclude any possibility of moisture. At the same time, the ball mill jar and steel balls were stored over phosphorous pentoxide, in vacuo before using, to remove any moisture from that source. Such precautions failed to increase the yield appreciably. The lignin obtained, however, was often gummy on removal of dioxane rather than flaky if moisture was present during the milling. The extent of moisture regarded as detrimental may be of the magnitude of 5 to 6%. An indication of the effect of moisture at this level may be seen from the first two samples in Table II.

The moisture content, then, was not entirely responsible for the low yields obtained. Milling of aspen meal, dry, (11) produced higher yields (approximately 10%) on the same machine with the same eccentric setting. By comparison, the yields from the first few runs are given in Table I.

TABLE I

YIELD OF LIGNIN OBTAINED FROM MILLING BLACK SPRUCE WOOD MEAL\*

Run No.	Yield, %
3-4	3.75
5-6	2.23
7-8	3.97
9-10	3.48
11-12	2.12
15-16	3.33
17	5.89

\* All runs were made in toluene, 48 hr. milling time with 3 mm. jar amplitude. Runs 3 through 12 were Lampén milled prior to ball milling. Runs 15-15 used fractionally distilled toluene. Run 17 was made with 6 mm. jar amplitude.

THE EFFECT OF THE MILLING MEDIUM

It was apparent that for a given set of conditions, milling in toluene hampered the vibratory action in this modified mill and that the high yields reported in the literature could not be practically attained. Inspection of a mill located at the Forest Products Laboratory (F.P.L.) at Madison, Wisconsin also made it evident that the machine at the I.P.C. was not performing at highest efficiency. Samples of wood meal milled at the F.P.L. (27) and at the Proctor and Gamble Laboratories (P&G)(2) were extracted and compared to the results obtained at this laboratory. The results are tabulated in Table II.

TABLE II

YIELD OF LIGNIN FROM MEAL MILLED AT OTHER LABORATORIES

Sample	Description	Wt. of Meal, g.	Yield, %
FPL-AD	Air-dried meal milled at FPL, dry 8 hr.	5.17	8.49
FPL-DD	Desiccator-dried (magnesium perchlorate) meal milled at FPL, dry, 8 hr.	3.19	19.96
FPL-M/T	Desiccator-dried (magnesium perchlorate) meal milled at FPL, in toluene, 8 hr.	3.90	2.30
P&G-M/T	Wiley-milled, 60 mesh, desiccator- dried meal milled at P&G; in toluene, 48 hr.	6.0(est.)	17.50

Magnesium perchlorate may be a more efficient drying agent than phosphorous pentoxide but it would not totally explain such a large increase in yield (FPL-DD). The difference between the first two FPL samples is partly explained by the difference in charge weight used. The high yield of the second sample is due to the more efficient action of the mill on the dry meal when toluene is not used. The third sample (FPL-M/T) bears out the findings at I.P.C.; that high yields were not possible when milling in toluene. The high value from the P&G sample must be attributed to some other factors, probably a very efficient, high amplitude, milling action. The low yield reported by Bland (9) is, undoubtedly, due to much of the same kind of milling trouble experienced at I.P.C.

The milling medium, then, obviously affects the vibratory action to a very large extent. Björkman investigated at length, the effect of the milling medium on yield but reported little on the effect of the medium on the milling action (4, 6). It was Björkman's contention that (1) grinding



would be promoted in a suitable, low viscosity liquid, (2) the temperature increase would be reduced by the liquid, and (3) the atmosphere of oxygen greatly excluded. Theoretically, the milling medium should not swell the wood as the swelling supposedly hindered milling action. Toluene was selected as a liquid for milling because it was satisfactory under the conditions in use. Other liquids were better but the corrosive action of some prevented their use. The best yield was obtained when the meal was ground first in toluene and then in dioxane, but this combination was not used because the methoxyl content in the lignin was lower. Surprisingly, milling in water gave Björkman a much higher lignin yield than anticipated. This, by itself, would raise some doubt as to the validity of his milling theories.

The effect of temperature and oxidation caused by milling in a dry atmosphere was studied by others (13). Dry milling of cellulose caused a reduction in the chain length with a rapid conversion to amorphous cellulose. The oxidation was negligible, however. Infrared analysis revealed no structural changes although there was a small increase in carboxyl content. It was therefore concluded that the degradation was hydrolytic rather than oxidative. A similar conclusion was reached by Grohn, et al (16-18). The degradation of cellulose in the ball mill during milling was also studied by others (20,21). In a study of the efficiency of the milling method, Batel (1) claimed that air currents created by the throwing movements would push the meal particles from between the balls and reduce the grinding action. Operating in a vacuum was said to improve the process 100%.

THE EFFECT OF PARTICLE SIZE AND AMPLITUDE

From the results of the above runs, consideration was given to milling without toluene and further reducing the size of the meal before milling. The results of these effects are given in Table III.

TABLE III  
YIELD OF LIGNIN FROM SAMPLES MILLED DRY

Run	Description	Wt. of Meal, g.	Yield, %
22	Coarse meal milled dry, 8 hr. 3 mm. amplitude	5.46	2.08
23	Wiley-milled meal, 60-mesh, milled dry, 8 hr., 3 mm. amplitude	4.41	2.58
24	Coarse meal milled dry, 16 hr., 6 to 7 mm. amplitude	3.94	7.58

Grinding in the Lampén mill before milling did not improve the yield (see Table I). As seen from the above table, size reduction of the meal in the Wiley mill did not help increase the yield to any extent. Except for Run 24, however, meal was ground in the Wiley mill to keep this factor constant in future extractions. A screen analysis was made on the Wiley-milled material and the data is reported in Table IV. Actually, a large portion of the meal was finer than 60-mesh.

TABLE IV  
SCREEN ANALYSIS OF WILEY-MILLED WOOD MEAL

<u>On Screen</u>	<u>%</u>
50-mesh	0.0
100 "	17.9
200 "	45.5
pan	36.6

Between Runs 23 and 24 the ball mill was modified as previously described; i.e., leaf springs were removed, the jar suspended from a spring coil, and the eccentric placed in an extreme position to give greater amplitude. The increase in yield between Runs 23 and 24 is partly due to this increase in amplitude.

Another effect of amplitude (and milling action) was noticed in the alkali solubility (26). Samples from meal milled in toluene at 3 mm. amplitude (Runs 22 and 23) were not soluble in 2 N sodium hydroxide whereas Samples FPL-M/T and P&G-M/T, Table II, were immediately soluble. Run 24, Table III, on the other hand, was partially soluble after 8 hours milling but completely soluble after 16 hours. Microscopic examination of the ground meal by the Fiber Microscopy group failed to distinguish significant differences between different samples of meal of varying alkali solubility (see Institute File Nos. 183527/530 and 183667). Björkman (6) reported particle sizes from 1 to 60 sq.  $\mu$  using the electron microscope. However, the experimental techniques used, such as passing the meal through a colloid mill, may have affected the actual particle size.

A variable not studied in this investigation was the frequency of rotation of the motor. The motor used had a constant speed of 1750 r.p.m. Some motors in use in other laboratories are equipped with a variable speed attachment and may be run at different speeds which would affect the vibratory action of the mill. Björkman used a motor having a speed of 1410 r.p.m. (4).

THE EFFECT OF MILLING TIME

Run 24 indicated as did Björkman's work (4, 6), that an increase in milling time would increase the lignin yield. It was reported that 48 hours milling of a 1-g. sample gave a 50% yield in only 14 days extraction time, while 12 hours milling of the same weight gave only a 20% yield after 3 months extraction. Similarly, the effect of milling time can be seen in Table V.

TABLE V\*

MILLED WOOD LIGNIN (M.W.L.) OBTAINED FROM  
TWO MILLINGS OF PICEA ABIES (6 g. wood)

Time, days	M.W.L., mg. est. from A at 280 mm <sup>5</sup>	Purified M.W.L.	
		mg.	%MeO
2	630	460	15.04
12	930	550	14.92

\*Data obtained from Reference 6.

In order to substantiate the findings in Table V, runs were made for different milling periods. The yields are shown in Table VI.

TABLE VI

EFFECT OF MILLING TIME ON LIGNIN YIELD\*

Run	Milling Time, hr.	Yield, %
25	8	5.76
26	16	13.63
27	32	31.03
28	48	29.06

\*Wiley-milled meal, 6 to 7 mm. amplitude, milled dry.

The decrease in the percentage of yield of Run 28 was possibly due to interruption of the milling at the beginning of the period because of bearing trouble. The amount of "play" in the machine at the end of Run 27 evidently increase the amplitude and gave a higher yield than would normally be obtained. Likewise, the new bearings installed at the beginning of Run 28 decreased the amplitude (as compared to Run 27) and lowered the yield.

The meal and the lignin obtained were analyzed and compared. The results are given in Tables VII and VIII.

TABLE VII

ANALYSIS OF WOOD MEAL

Sample	MeO, %	Ash, %
Meal, 20-mesh	5.26	--
Meal, 60-mesh	5.20	0.16
Meal from Run 28 before dioxane extraction	4.93	0.68

TABLE VIII

METHOXYL CONTENT OF M.W.L.

Sample	MeO, %
Lignin from Run 25	15.42
" " " 26	15.60
" " " 27	15.32
" " " 28	14.90

The methoxyl content of the 20- and 60-mesh samples is virtually the same. The decrease in methoxyl with corresponding increase in ash after 48 hours milling, on the other hand, indicates a certain amount of degradation of the meal, as well as the presence of some metal from the machine. The lower percentages of methoxyl in the lignin milled for longer periods also indicates the extraction of larger amounts of L.C.C.

The effect of milling time was also demonstrated by milling a 5 g. charge in toluene under conditions similar to Runs 25 through 28. The yield of lignin obtained (Table IX) was greatly increased over the runs previously made in toluene and probably demonstrates milling efficiency more dramatically than the effect of milling time.

TABLE IX

EFFECT OF MILLING TIME ON LIGNIN YIELD

Run	Time, hr.	Lignin Yield, %
31	16	3.64
32	48	11.76

THE EFFECT OF CHARGE WEIGHT

The amount of meal charged to the jar was shown (6) to affect the yield of M.W.L. obtained. After Run 28 when the final modification of the ball mill had been made, the relationship of charge weight to yield was determined. All other factors were kept constant by milling 16 hours without toluene at 7 mm. amplitude. The results are given in Table X.

TABLE X  
THE EFFECT OF CHARGE WEIGHT ON LIGNIN YIELD

Run	Charge Wt., g.	Yield, %
26	5.0	13.63
33	3.0	23.22
34	2.0	33.95
35	1.0	27.62

The milling efficiency evidently reached a maximum at a charge weight of 2 g. This was in contrast to reported values (6) in which the highest yield was obtained at a charge weight of 1 g. (See Table XI. In this table, the yield increased progressively with decreasing charge weight. The large difference between the estimated and the actual percentage of lignin was due to removal of L.C.C. during the purification process.)

TABLE XI  
INFLUENCE OF AMOUNT OF WOOD ON MILLING

Charge, g.	Extracted Lignin, Est. from A <sub>S</sub> at 280 mm $\mu$ .	Kason Lignin Weighed After Purification, %
25	9	5
5	44	33
1	92	54

\*Data taken from Reference 6. The meal was milled for 48 hours.

The large difference in yield for a 1-g. sample between Tables X and XI lies partly in increased milling time (Table XI), but mainly on greater vibratory action.

#### SELECTION OF OPTIMUM CONDITIONS FOR MILLING

Weighing four or five samples of meal after milling showed that the average loss amounted to 0.42 g./3 g. charge. Because of this loss, too small a charge weight would not produce a sufficient quantity of lignin, even though the yield would be higher. It was also apparent that greater degradation would occur as well as increased extraction of L.C.C. From the data obtained (Tables V and IX) it was felt that a 3.0-g. charge weight milled without toluene for 16 hours would produce a representative lignin sample in sufficient quantity and without excessive degradation. Under these conditions, 2 g. of the M.W.L. was accumulated, ground and blended in an agate mortar, and set aside for future use. The amount of lignin obtained in each run is shown in Table XII. The gradual increase in yield with the later runs may indicate more "play" due to bearing wear. The static in the lignin was overcome during blending by using the steam cone in a hood to supply a moist atmosphere. The lignin was then dried again over phosphorous pentoxide in vacuo.

TABLE XII

#### YIELD OF M.W.L. UNDER CONSTANT MILLING CONDITIONS

<u>Run</u>	<u>Yield, %</u>
37-38	23.32
39-40	23.13
41-42	24.27
43-44	23.65
45-46	26.26
47-48	28.93
49-50	28.51



#### PURIFICATION AND EXTRACTION OF LIGNIN

The purification and extraction procedures were standardized and no variation from Björkman's method (4) was attempted, although the solvent is known to affect the amount of L.C.C. extracted (6). The length of dioxane extraction changed by a few days on occasion, but the greater part of the extraction was completed with the first few days. The extract was a deep amber color, becoming lighter as the solvent was replaced and the extraction time continued. A visual observation of the first days extract would enable one to estimate a high or low yield. When milling dry the liquid that condensed in the safety flask, on evaporation of the dioxane was essentially clear and colorless, but when toluene was used in the milling, this condensed liquid was light-yellow to amber in color. Phloroglucinol tests, however, indicated that lignin was not being carried over in the condensate and it was assumed that the color was due to coloring matter only.

The liquid above the precipitated acetic acid lignin was also often light-yellow to amber in color and undoubtedly, some of the lignin complexes mentioned by Björkman (5) were present. No attempt at this point was made to analyze the solution or recover any of the lignin present. The lignin obtained after purification was a light tan colored powder containing a great deal of static electricity after drying that made it quite difficult to handle without some loss.

## MOLECULAR WEIGHT STUDIES

The work with the osmometers for molecular weight determinations has been previously reported (28). The work described was continued, in part, as time permitted. Leak tests were run again on the osmometers containing polyvinyl alcohol, super-dense, and swollen super-dense membranes. These curves were similar to the ones presented in Report 1 and have not been included here. Molecular weight determinations were made using 0.1 and 0.2 g./kg. lignin solutions. The data obtained was erratic and inconclusive, possibly due to fluctuations in the water bath temperature. Time did not permit further evaluation of the membranes at different lignin concentrations.

The literature (12) and previous work (28) indicate that the membrane used will greatly affect the molecular weight calculations. Too permeable a membrane will allow small solute molecules as well as solvent to diffuse through the membrane. Up to the near present, osmotic pressure molecular weight determinations on polymers having a molecular weight below 15,000 have been discouraged because of the permeability of most membranes to molecules in this size range. Osmotic pressure determinations reported for B.N.L. from Picea mariana gave  $\bar{M}_n$  values from 2,800 to 6,700 with 4200 as a value for the unfractionated sample (19). A method has been described for determining osmotic pressure of a solute which can diffuse through the membrane (14). Such a method may overcome some of the difficulties encountered with osmotic pressure determinations.

The work completed in Report 1 of this project showed that "very dense" membranes were not suitable for lignin determinations. On the other hand, the long time required for some determinations makes it desirable to use a membrane that will allow quick results. It has been reported that polyvinyl alcohol membranes attain equilibrium in a matter of hours (22). They are also reported to be suitable for polymers with molecular weights as low as 2000. The "speed" of the membrane varies with the relative humidity at which they are conditioned. The values of the per cent water retained (an indication, inversely of the speed) at various relative humidities are reported in Table XIII.

TABLE XIII

MOISTURE DETERMINATION OF POLYVINYL ALCOHOL MEMBRANES

Reference 22		I.P.C.	
R.H., %	H <sub>2</sub> O, %	R. H., %	H <sub>2</sub> O, %
75	8.86	75.5	12.14
44	2.5	53.5	7.75
32	1.5	32.9	6.08

The favorable data in Reference 22 indicated that faster determinations could be made without sacrificing passage of the small solute molecules through the membrane. The reason for the large differences between reported values and I.P.C. moisture figures has not yet been determined, but may be caused from moisture pickup during analysis of the sample. Others have reported satisfactory results with "Ultrafein" membranes and it has been claimed that the more selective of these are capable of retaining molecules of greater than 1500 M.W. (29).

The viscosity of the polyvinyl alcohol solution played a major role in the casting of the films. With too thin a solution it was impossible to obtain any control over the fluid and this invariably ended up as a "puddle" on the glass plate. A thick solution cast more favorably but the presence of air bubbles made it less desirable than a solution of lower viscosity. The film thickness was also dependent on the solution viscosity as well as on the depth of the cast solution. The depth, in turn, would affect the permeability. The adaptability of these membranes for lignin molecular weights will be determined in future experiments.

The solvent also has an effect on the molecular weight determination. The trials in Report 1 were made using 100% dioxane. Differences in moisture content between the solvent and the solution would be accentuated and adversely affect the results. To counteract this effect, the dioxane was diluted to 90% with distilled water. As stated before, however, temperature or other variables probably overshadowed the solvent factor and a true value was not determined. Solvents other than dioxane may possibly be used in future experiments.

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## THE PHYSICAL CHEMISTRY OF LIGNIN PREPARATION

### Determination of Molecular Weight of Milled Wood Lignin by Osmotic Pressure

#3  
.15

#### INTRODUCTION

The purpose of this project is twofold; to establish the applicability of various physical chemistry tools to finding the molecular structure of milled wood lignin, and to use these tools in determination of the molecular weight of milled wood lignin.

#### PREVIOUS WORK

Before attempting to establish the applicability of osmometry to the determination of the molecular weight of lignin, an investigation was made into the results of other experimenters who worked with variously prepared lignins.

Previously determined molecular weights vary widely according to the methods used to prepare the lignin. Bjorkman and Person (1) found from their observations that molecular weights for Brauns' "native" lignin were less than half of those found for "milled wood lignin". Hess (2) made similar observations when he found molecular weights of from 2800 to 6700 for fractionated samples of lignin (Picea mariana) prepared according to Brauns' procedure, with an average for the unfractionated lignin of 4200. Bjorkman's value for "milled wood lignin" (Picea abies) was 11,000. Hess also gives a number of other experimenters with their results.

## CALCULATIONS

The calculation of molecular weight from osmotic pressure depends on the following equation:  $\frac{\pi}{c_0} = \frac{RT}{M d_1 g}$  (Van't Hoff's law), where  $M$  = number average molecular weight,  $R$  = gas constant,  $T$  = absolute temperature,  $\pi$  = osmotic pressure in g./sq. cm.,  $c$  = concentration of solute in g./cc. of solvent,  $d_1$  = density of the solvent,  $g$  = gravitational constant, with all figures in CGS units. The subscript (o) indicates that  $\frac{\pi}{c}$  is to be taken at infinite dilution, i.e., when  $c \rightarrow 0$ . This can be done by plotting  $\frac{\pi}{c}$  vs.  $c$  for various concentrations and extrapolating to zero concentration.

By using  $H$ , the osmotic head in cm., and  $w$ , the concentration in g./kg., the above equation may be simplified to  $\frac{H}{w_0} = 25,700/M$  for 30°C.  $\text{Lim } \frac{H}{w}(w \rightarrow 0)$  may be plotted in the same manner as plotting  $\frac{\pi}{c}$  vs.  $c$ .

A more accurate explanation of this theory is given by Doty and Spurlin (3).

## MEMBRANES

Part of the program of this project is to evaluate the membranes used in making the osmotic pressure determinations, since the entire success of any osmometry hinges on the selection of satisfactory membranes. In general, work in osmometry with polymers having molecular weights below 15,000 is discouraged in the literature. The reason given is that most membranes are permeable not only to solvent but also to solute of the size mentioned or less. Two factors offset this objection.



The first is that a slow rate of diffusion of solute through the membrane can be compensated for by plotting the decay in the osmotic height after it has reached a maximum value and extrapolating the best straight line back to the time of filling. This is explained more thoroughly by Doty and Spurlin (3). Of course, if the desired equilibrium plot of zero slope is attained, this is unnecessary.

The second factor is that new methods of reducing the average pore diameter have been devised in order to render membranes more nearly impermeable to low molecular weight polymers. Doty and Spurlin (3) note that "Ultracellafilter allerfeinst", supplied by Membranfilter Gesellschaft, Gottingen, Germany, gives satisfactory results with well-fractionated polymers down to 10,000 molecular weight. The membrane referred to is the same as that called "super dense" in this experimentation.

Hess (2) determined his values for "native" lignin using 300PT62 and 600PT cellophane membranes, supplied by E. I. du Pont de Nemours and Co., Inc., after swelling them in various media, among them 1:1::EtOH:H<sub>2</sub>O and 10% by weight KOH solution.

Doty and Spurlin also note that membranes made from polyvinyl alcohol have given excellent results with molecular weights down to 2000. It might be worthwhile to attempt to make such a membrane and work with it.

## EXPERIMENTAL MATERIALS

### LIGNIN

The material used in this experimentation was lignin taken from black spruce (Picea mariana) which was first ground to sawdust after peeling and then to 20-mesh meal. The meal was then extracted for 45 hours in a 1:2::EtOH: benzene mixture. This was followed by a 20-hour extraction in 95% EtOH, followed in turn by generous washings with distilled water. It was then air dried for six days and placed in dessicators over  $P_2O_5$ . The meal was then ground for 48 hours in a Lampen mill followed by 48 hours in a vibratory ball mill. The lignin was then extracted by repeated centrifuging and redissolving in aqueous dioxane. The lignin finally placed in a dessicator over  $P_2O_5$  was a light cream color.

### OSMOMETER

The osmometers used were of the Zimm-Myerson type (see Fig. 1). Their chief virtue is simplicity of construction and assembling. Each is an all-glass, one-piece unit, thereby making it impossible for air bubbles to go unnoticed and eliminating any crevices from assembling.

Each unit consists of a cylindrical glass cell (A) with a volume of about four ml., to which are attached a filling tube (B) and the capillary tube (C) for measurement of the osmotic head. A reference capillary (D) of the same bore, with open ends, is attached to the primary capillary. The osmotic head is the difference in levels of the menisci in the two capillaries.

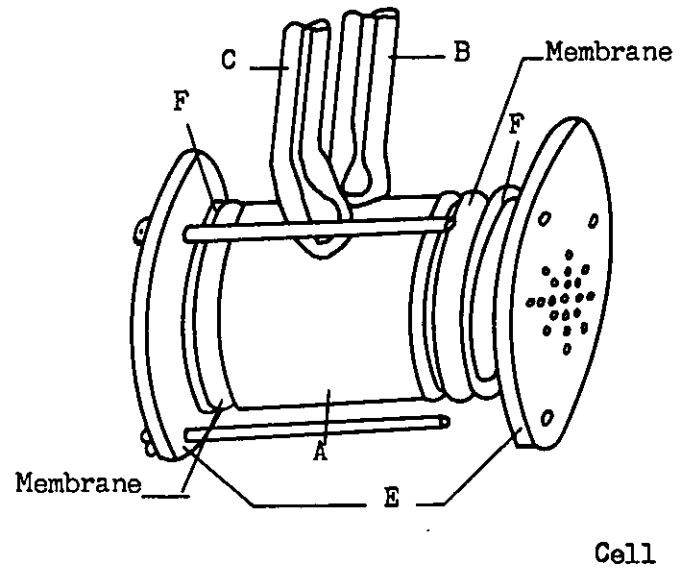
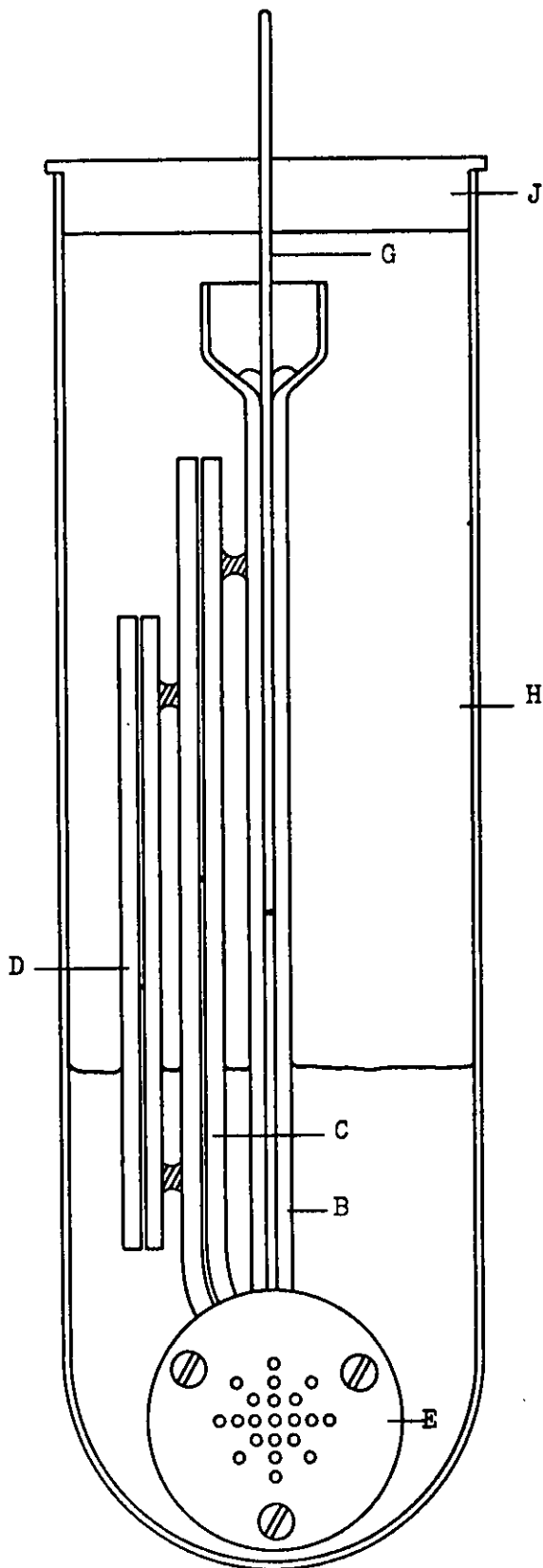


Fig. 1. Zimm-Myerson Osmometer

The membranes are attached to the osmometer by means of two perforated brass plates (E) which are fitted with Teflon gaskets (F). Filling is easily done without creating air bubbles by using a hypodermic syringe with a 30 cm. needle which is inserted through the filling tube almost to the bottom of the cell.

After the osmometer is filled, a tight-fitting stainless steel rod (G) is inserted into the filling tube. By manipulation of this rod the level of the meniscus in the capillary can be adjusted. The mouth of the filling tube is then sealed with a few drops of mercury, and the entire assembly is set into a large culture tube "jacket" (H) filled with pure solvent to a depth of about five or six cm. (so that the lower end of the reference capillary is below the surface). The "jacket" is fitted with a polyethylene stopper (J) with a hole for the stainless steel rod, and is placed in a firm mount in a bath thermostatted to  $\pm .005^{\circ}\text{C}$ .

#### Polishing the Osmometer Surfaces

To minimize the possibility of leaks through the interface between the cell and the membrane, the faces of all the osmometers were polished. When the osmometers were received, the faces of the cells were in a medium coarse ground glass state. By using emery (275 mesh) on a polishing lap of paraffin poured over plate glass, the cell faces were polished to near transparency; i.e., using a 10 X lens, no continuous pitting could be seen across the polished faces.

## SOLVENTS

The solvent used in all work described in this report was p-dioxane, refluxed over sodium and distilled. Future work will be done also with methyl Cellosolve and dimethyl sulfoxide.

## PREPARATION OF THE MEMBRANES

The membranes used were "ultracellafilter" brand, grades "very dense" and "super dense", and were supplied by Membranfilter Gesellschaft.

### Conditioning the Membranes

The "very dense" membranes had already been cut to size (1-1/16 in. diam.) and conditioned to pure acetone before commencement of this project. They were then conditioned to dioxane by successive dioxane-acetone washings of 25, 50, 75, and 100% dioxane. After conditioning they were kept in a dessicator in a dish of dioxane over dioxane.

Six "super dense" membranes were cut from a 15-cm. diameter sheet which had been kept in an EtOH-water mixture of unknown proportions. After cutting, the membranes were immersed in distilled water and then conditioned to dioxane by step-wise conditioning through water-dioxane mixtures. A better procedure might have been to first condition them to acetone and then to dioxane.

Another six "super dense" membranes were cut and immersed in distilled water. They were then immersed for one-half hour in 10% by weight KOH solution in an attempt to tighten the pores. This procedure was followed by gradual dilution to distilled water through successive one-half hour washings in increasingly dilute solutions until they were immersed in

distilled water. After repeated washings in distilled water to remove any traces of adsorbed alkali, the membranes were conditioned to dioxane as before.

However, it was found that the swelling procedure reduced the diameter enough to make it extremely difficult to fit the membranes to the osmometers without large leaks. Consequently, a new set was prepared, cut to 1-1/4 inch diameter and conditioned as before.

## EXPERIMENTAL PROCEDURES

### LEAK TESTS

The assembled osmometers were tested for leaks by filling with solvent and adjusting the meniscus head to about six or seven cm. Periodic readings were taken to determine the decay in  $\Delta H$  (the pressure head of the osmometer). The log of  $\Delta H/\Delta H_0$  was plotted vs. time on semilog paper (subscript (o) indicates initial  $\Delta H$ ). A straight line over a 20 to 30 hour period was evidence of negligible leakage.\*

The results of the leak are shown in Appendix B.

### Bath Temperature

The temperature of the thermostatted bath was kept at  $30^{\circ}\text{C.} \pm .005^{\circ}\text{C.}$  for the leak tests and the ensuing osmotic pressure runs. This control was necessary since the osmometer is essentially a sensitive thermometer because of its large cell volume compared to the volume of the capillary. (See also "Observations--Osmotic Heights" in section following.)

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\* Due to the worker's inexperience in osmometry at the beginning of this project, he erred by not taking readings of  $\Delta H$ , but rather of the absolute height of the primary capillary. This resulted in erroneous plottings for the first four leak tests.

### Selection of Membranes

In this work, as in work reported by other experimenters, an unexplainable phenomenon, the "membrane correction", or "membrane constant" occurred. A finite equilibrium height was noted, even with solvent on both sides of the membrane. In this experimentation, some membranes which exhibited too large a "membrane constant" were replaced. For further elaboration, see "Observations--Evaluation of Membranes".

Data concerning the membranes used will be found in Appendix B with the leak test data.

### PREPARATION OF SOLUTIONS

After estimating the solution strength needed to attain the desired osmotic head, by assuming the material to have a molecular weight of 5000, a bulk solution was prepared, and aliquots were taken from it and diluted to the various concentrations desired. Details are given in Appendix C.

### CONDITIONING ASSEMBLED OSMOMETERS

After the osmometers were leak tested and the membrane constants established, it was necessary to condition them to the solution used for making an osmotic pressure run. This was done according to the procedure outlined by Alvang and Samuelson, (4).

1. Three rinsings of osmometer with solution to be used
2. Fill osmometers with new solution and let stand in jacket for four hours.
3. Refill with new solution and let stand overnight
4. Fill jacket with fresh solvent; refill osmometer with new solution and begin run.

## OBSERVATIONS

### EVALUATION OF MEMBRANES

The observations made both during the leak tests and the osmotic pressure runs bear out the general tone of the literature concerning various types of membranes.

No evident differences in slope could be noted during the leak tests (Appendix B). However, during the first two osmotic pressure runs (Figures 2 and 3), the difference of diffusion rate of the lignin through the "very dense" and "super dense" membranes became apparent. It can be seen that the extrapolated lines plotted on the "super dense" membranes have much less of a slope than those lines plotted on the "very dense" membranes. One can note that, in the second osmotic pressure run, the diffusion of solute through the "very dense" membranes became so great that a negative osmotic height was observed on one osmometer; the second osmometer was well on its way to a like reading.

As a consequence of these data, it may safely be said that membranes of the "very dense" type are valueless when used with polymers of the same molecular weight as lignin. These "very dense" membranes were removed from the osmometers after the second osmotic pressure run.

### MEMBRANE CORRECTION

The membrane correction proved to be very real as can be seen by comparing the extrapolated values of osmometers no. 4 and no. 7 in all osmotic pressure runs. No absolute criteria were set to determine which membranes



Conc.-- .1936 g./kg.  
 Temp.-- 30°C. ± .01°C.

Osmometers

- No. 2 (○) = very dense
- No. 3 (●) = very dense
- No. 4 (△) = super dense
- No. 7 (▲) = super dense

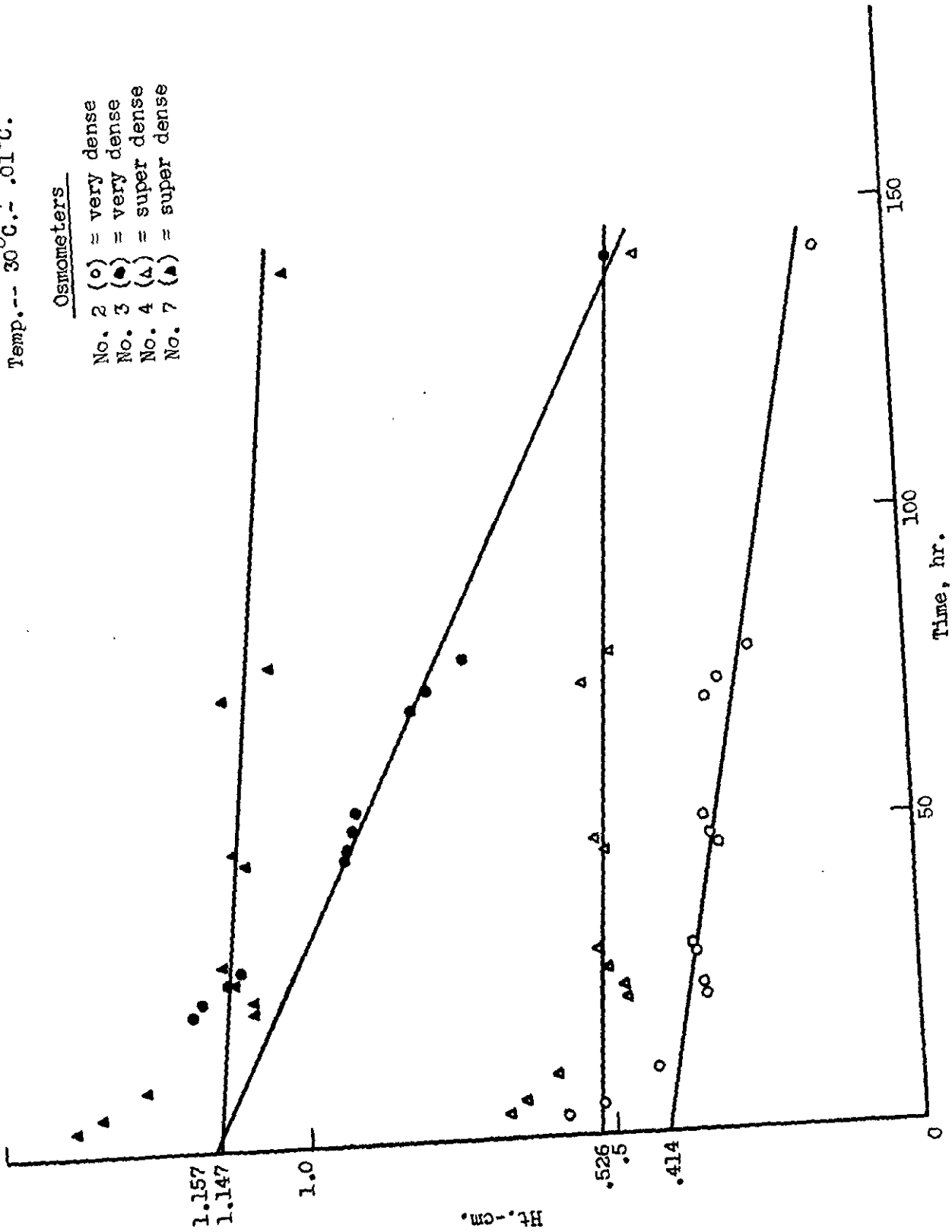


Fig. 2. Osmotic Pressure Determinations, Spruce Milled Wood Lignin

Conc. --- .1936 g./kg.  
 Temp. --- 30°C. ± .01°C.

- No. 2 = very dense
- No. 3 = very dense
- △ No. 4 = super dense
- ▲ No. 7 = super dense

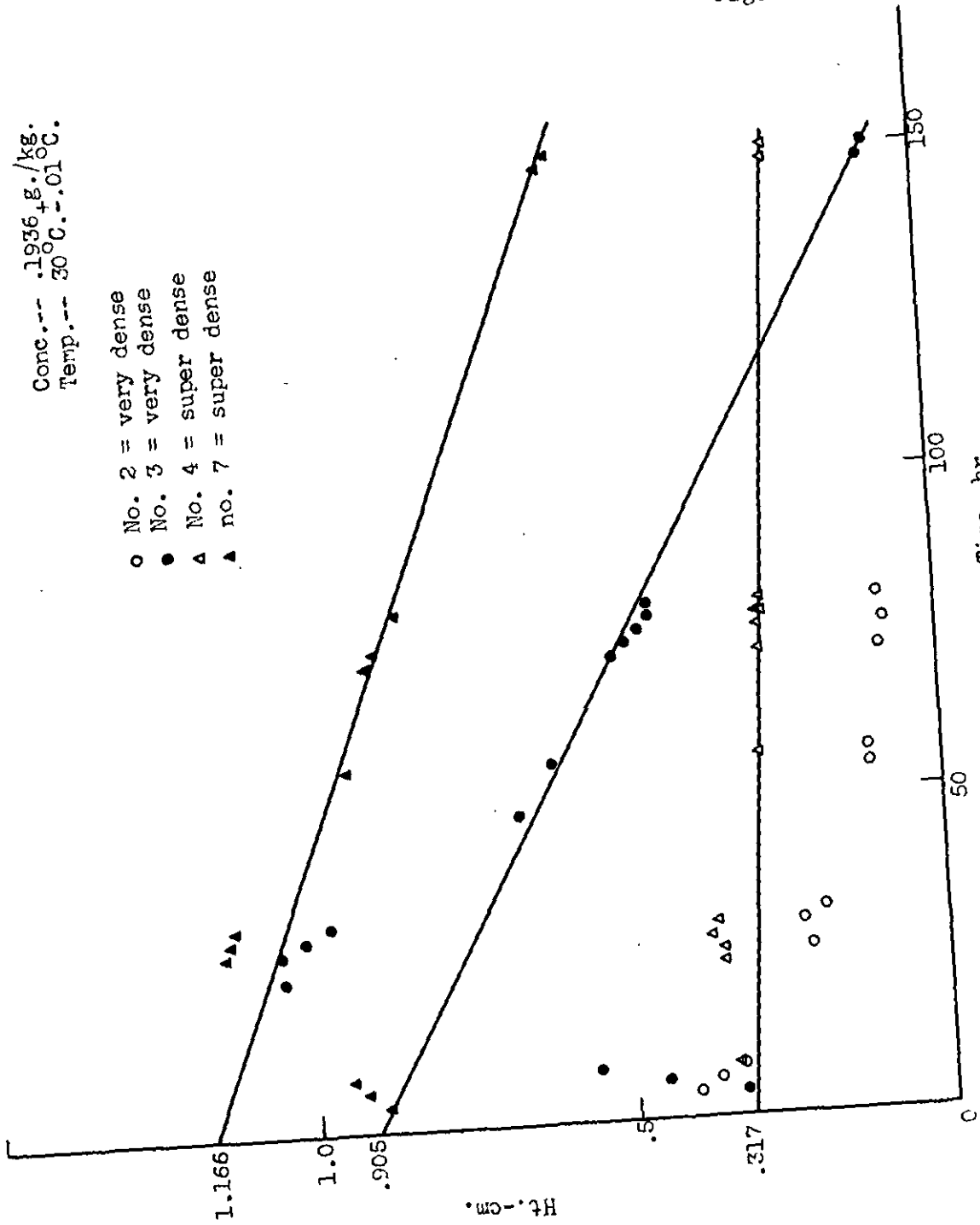


Fig. 3. Osmotic Pressure Determinations, Spruce Milled Wood Lignin

would be replaced during leak testing. Originally, "very dense" membranes were placed on osmometers no. 2, 3, 4, and 7. After the membrane constants were established with those membranes, the membranes on osmometers no. 4 and 7 were replaced with "super dense" membranes. Membrane constants were established for these units and the osmotic pressure runs were begun. No changes were made of membranes after this point unless a hole was detected in one of them. Swollen "super dense" membranes were placed on osmometers no. 1 and 4a when it became apparent that the "very dense" membranes were giving poor data. After the membrane constant for osmometer no. 4a was established, the unit was accepted for use. The membrane constant for osmometer no. 1 exceeded one cm., and the osmometer was not used.

It is possible that unreasonable membrane constants are caused by absorption of water by the solvent in the sleeve. It would be well to determine this when work on this project is resumed.

#### OSMOTIC HEIGHTS\*

There were two factors having an important effect on the extrapolated values of the osmotic heights. The first of these was diffusion of the lignin through the membranes.

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\* Osmotic pressure run no. 3 was cut off after 20 hours when apparent leakage effects were observed.

To adequately correct for such diffusion, it was necessary that the best straight line be fitted to a portion of the data and extrapolated to the time of filling. Which portion of data to be extrapolated from depended largely upon the judgment of the observer.

The extrapolations made on runs no. 4 and 5 (Figs. 4 and 5)\* were different from those made on runs no. 1 and 2 (Figs. 2 and 3). The basis for the extrapolations made on the first two runs was the experience of students at The Institute of Paper Chemistry, who fitted a line through points taken over a period of from 100 to 200 hours. The basis for the second series of extrapolations was the prevailing practice in the literature, which advocates a much closer watch of the osmotic head and the extrapolation of those points plotted during the first three to ten hours following the maximum point of the curve.

The second factor affecting the observed osmotic heights was that of temperature. During the days that observations were made, the bath was controlled to within  $\pm .005^{\circ}\text{C}$ . However, during a period of extreme fluctuations in room temperature between day and night, the bath would drop or rise in temperature up to a maximum of  $\pm 2$  degrees. A more common variation was about  $\pm .5$  to  $\pm 1$  degree. On the days following re-establishment of the bath temperature, the osmotic head was observed to have a thermal lag of about eight to ten hours, depending on the degree of variation on the previous night. The effects of this thermal lag can be seen quite well on the chart of osmotic pressure run no. 1 (Fig. 2). Note the periods from 20 to 30 hours and from 46 to 52 hours on the plots of osmometers no. 2 and 4.

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\* See Appendix D

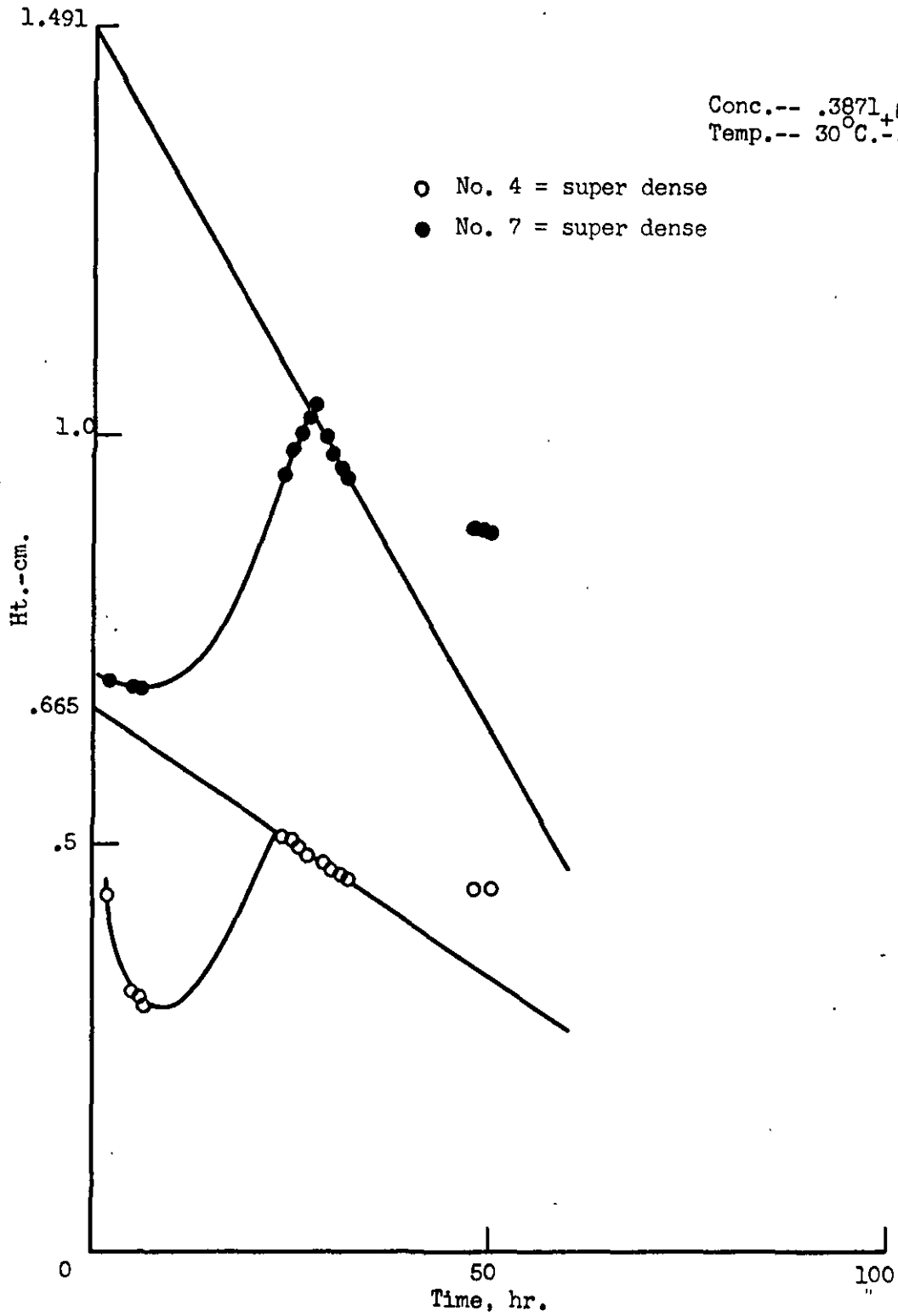


Fig. 4. Osmotic Pressure Determinations, Spruce Milled Wood Lignin

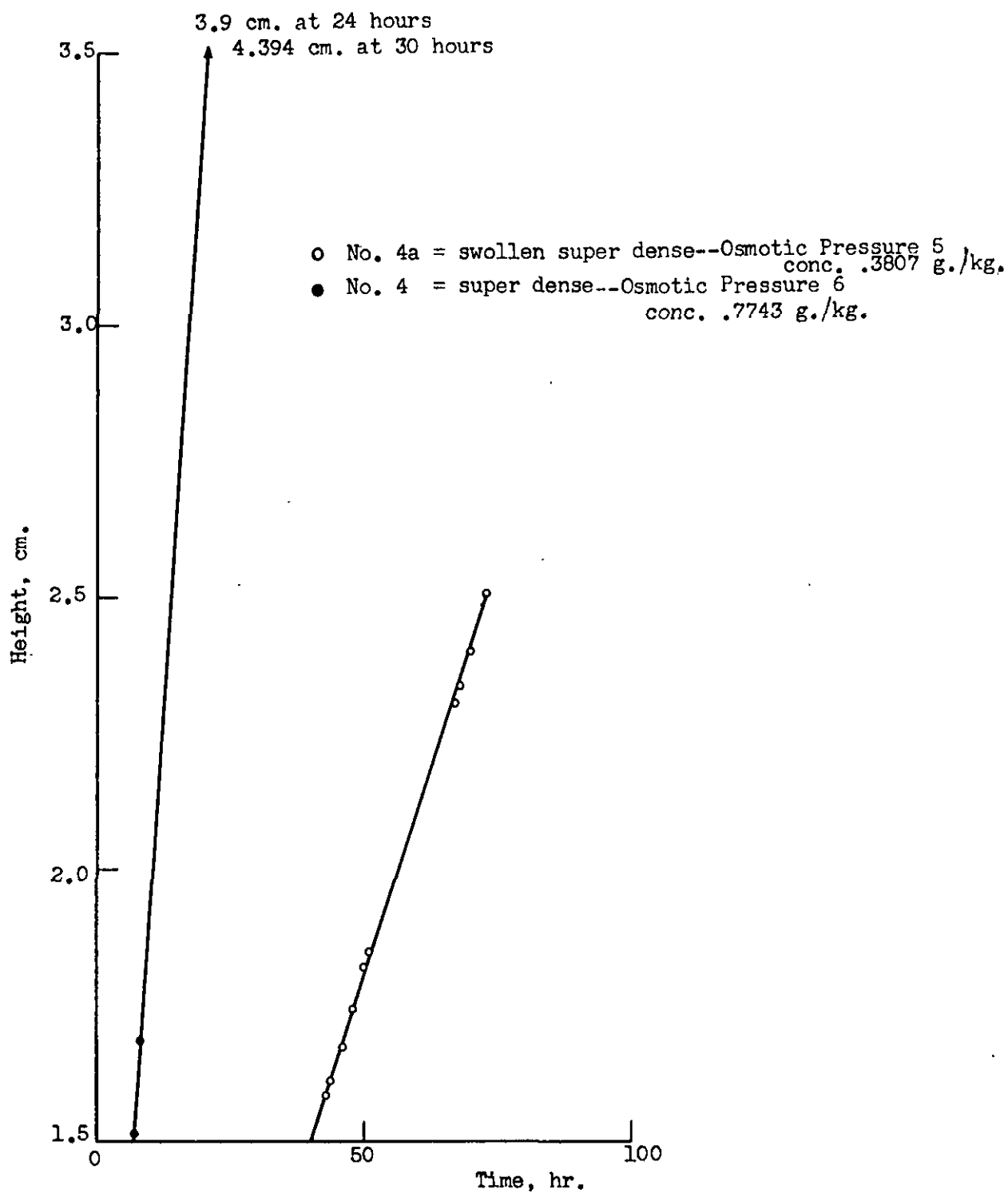


Fig. 5. Osmotic Pressure Determinations, Spruce Milled Wood Lignin

Another thermal lag was that of the solution coming to thermal equilibrium after it was placed in the osmometer and the unit returned to the bath. This effect is in evidence on the charts of osmotic pressure runs no. 4 and 5 (Figs. 4 and 5). Note that it, too, covers an initial period of about ten hours.

#### MOLECULAR WEIGHTS

At the conclusion of the experimentation described herein, the molecular weight of lignin could not be determined with great precision. Error was inherent in the diffusion effects, the membrane constants, and the temperature effects. The range of molecular weights determined was from approximately 9000 to 16,000 which agrees well with Bjorkman's value of 11,000 (see Fig. 6). It is hoped that greater precision can be obtained in the continuation of this project.

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## APPENDIX A

### TECHNIQUES USED

Certain techniques were developed and adopted for use in the work described herein. The points made in the following paragraphs are small details which made the work easier or the results more reliable.

#### FILLING THE OSMOMETER

When the osmometer was filled, the operations were done in a 400-ml. beaker with a height to diameter ratio of approximately two to one. This was filled with solvent so that the cell of the osmometer was completely immersed.

In filling the osmometer, enough solution (or solvent) was used to fill both the capillary and the filling tube after the hypodermic needle was withdrawn. The stainless steel rod was then inserted nearly to the bottom of the filling tube. After sealing the osmometer with mercury, the tip of the capillary was wiped off with filter paper which was then held there as the meniscus was lowered by raising the stainless steel rod. If the osmometer was being filled with solution, the outside was rinsed off with clean solvent before it was returned to the jacket. These precautions were not taken during conditioning of the osmometer to a given solution.

After the osmometer was returned to the bath in the jacket, the head (for an actual osmotic pressure run) was set one or two cm. below the expected equilibrium head. After about one-half hour, the head was reset to a few mm. below the expected equilibrium point. Readings were begun at any time after that.

ASSEMBLING THE OSMOMETER

One of the brass plates in each set has the screws soldered to it. This plate was placed in a Bakelite jar cap about one cm. in depth which was filled with enough solvent to cover the plate. The gasket and membrane were then inserted and the osmometer set over them. The other membrane and gasket were set on the osmometer and the second plate set over the screws. During this operation and the subsequent tightening of the plates, the second membrane was kept wet by pouring a few drops of solvent on it from a small (50 ml.) beaker.

APPENDIX B  
LEAK TEST DATA  
MEMBRANE CORRECTIONS

Figs. 6 through 14

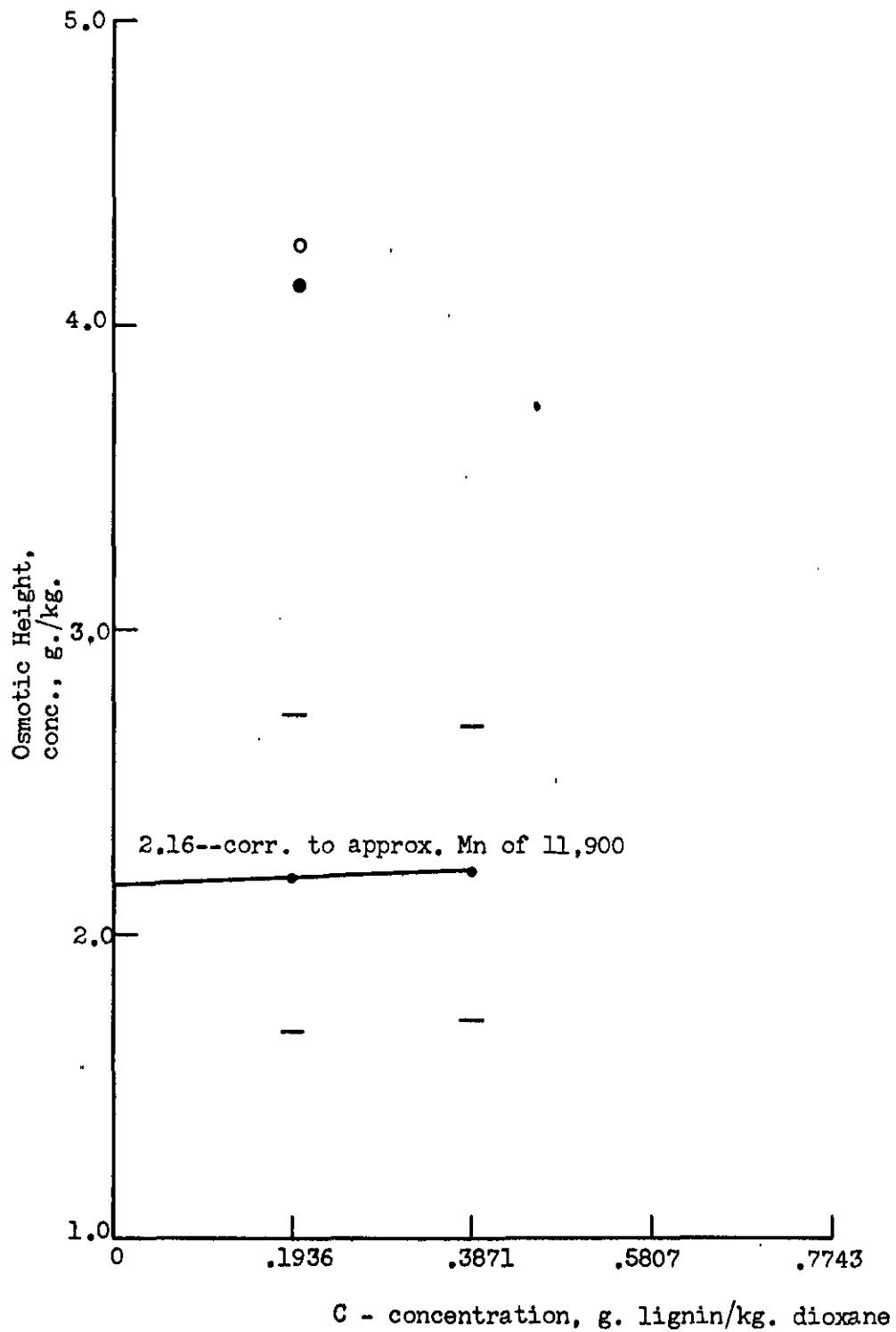


Fig. 6. Infinite Dilution Osmotic Pressure

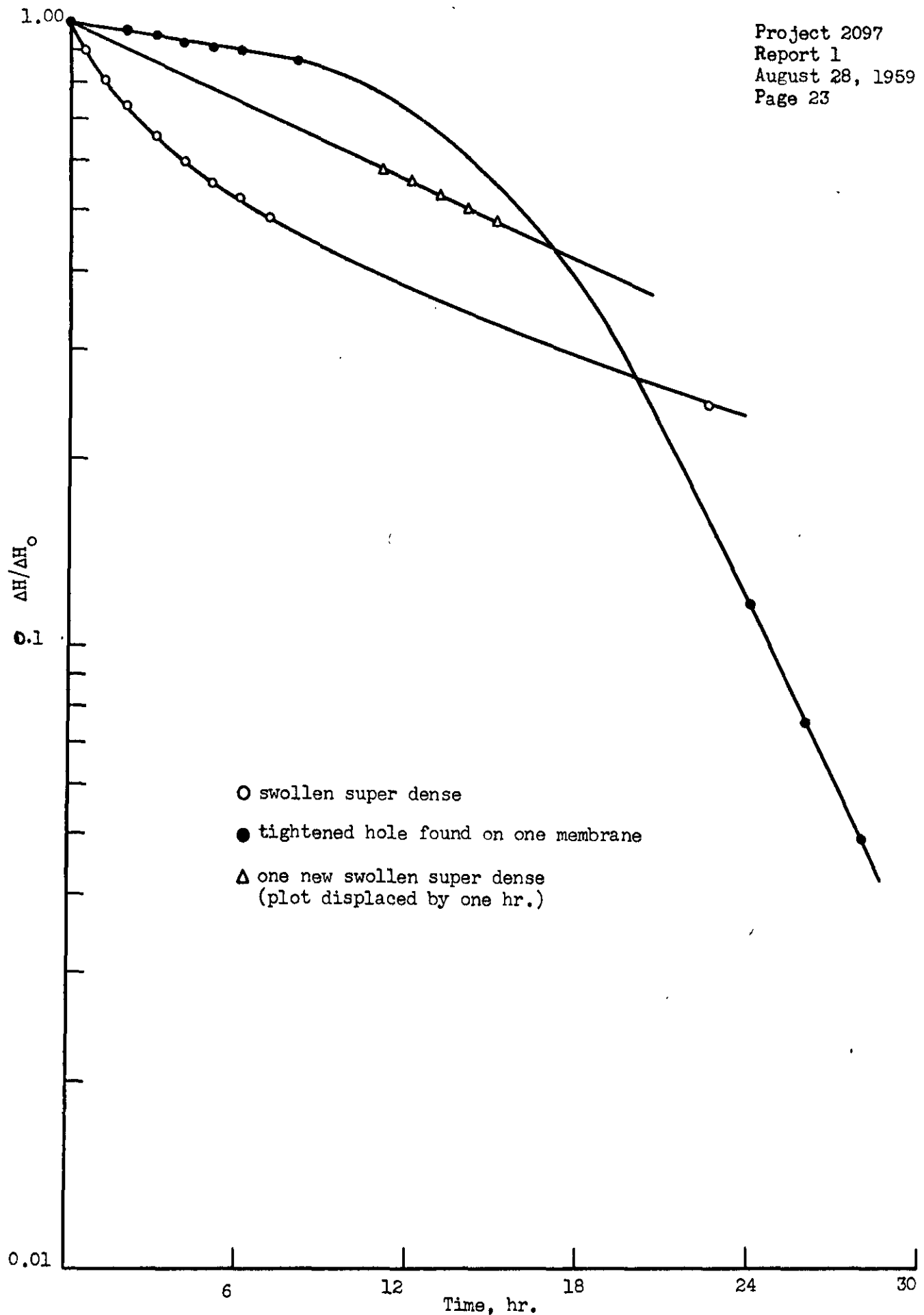


Fig. 7. Leak Test Determinations, Osmometer No. 1

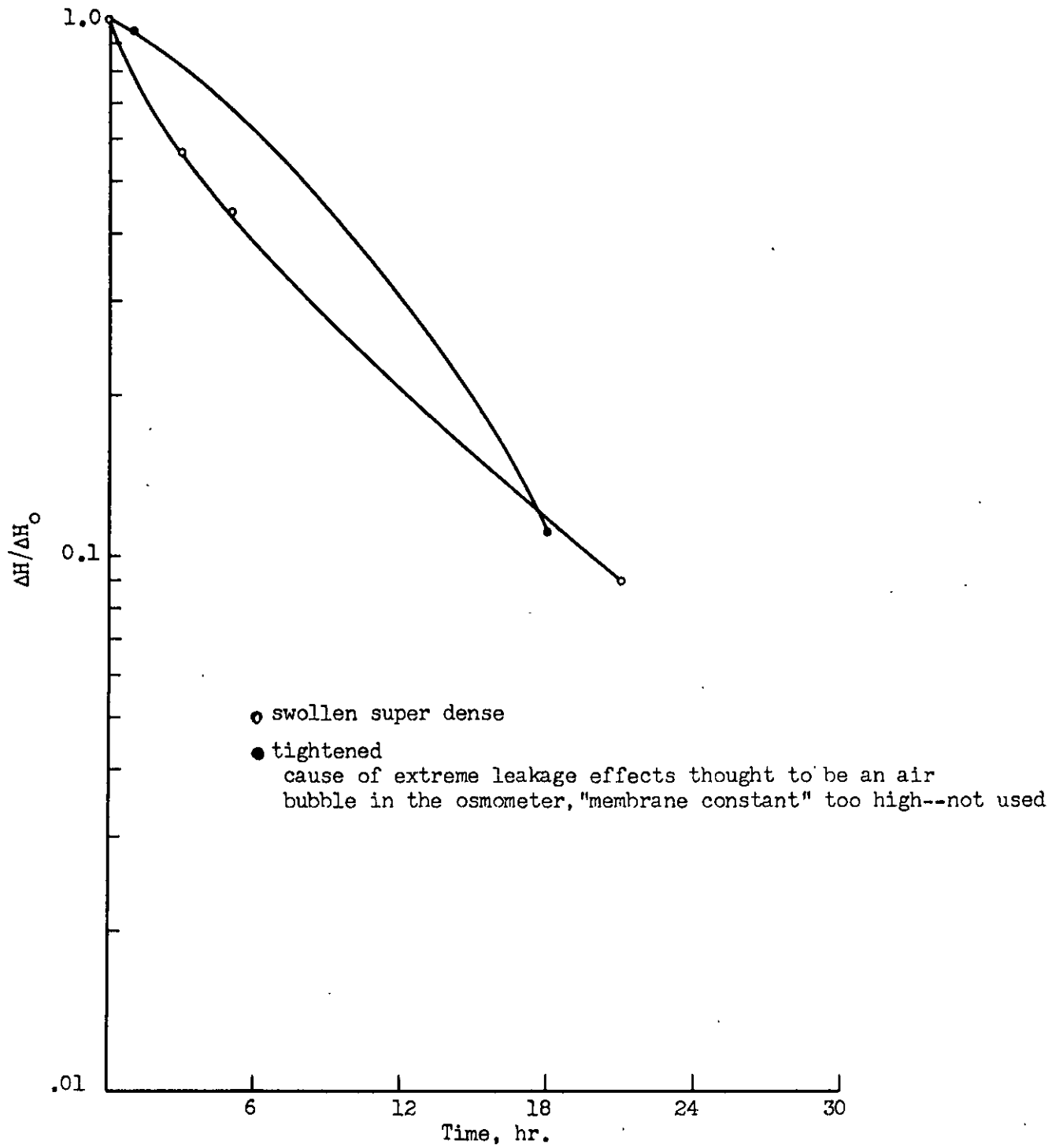


Fig. 8. Leak Test Determinations, Osmometer No. 1

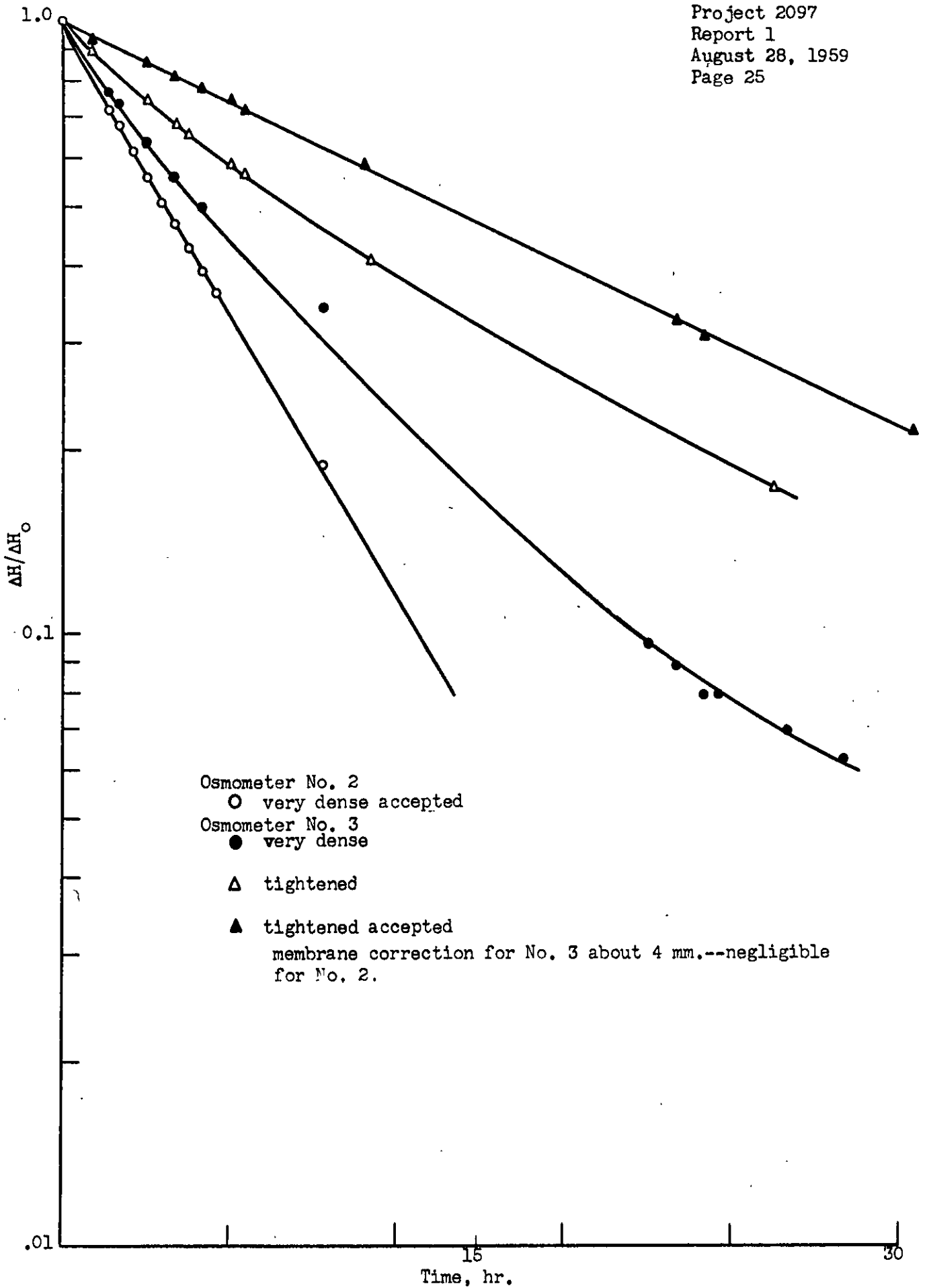


Fig. 9. Leak Test Determinations, Osmometers No. 2 and 3.

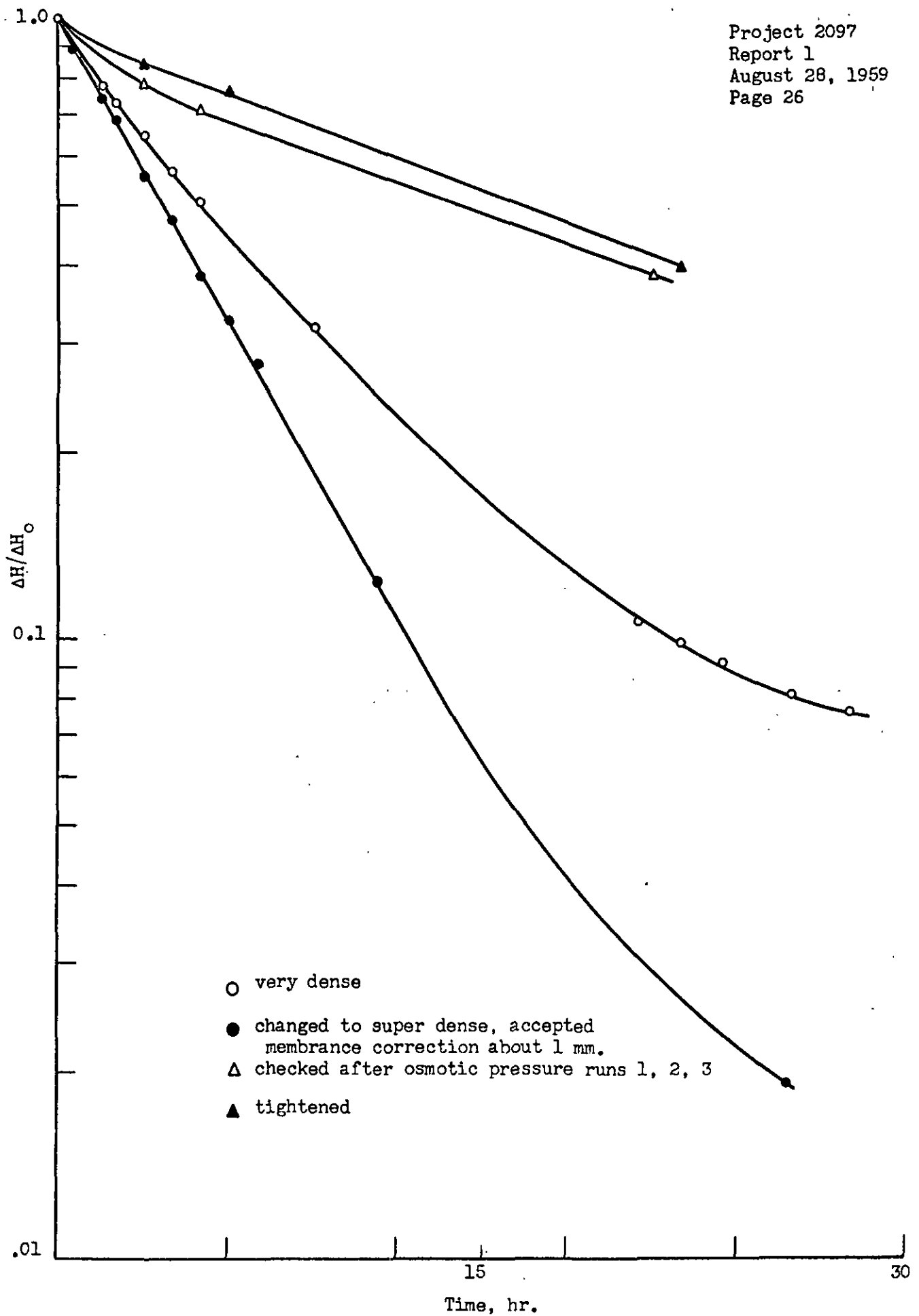


Fig. 10. Leak Test Determinations, Osmometer No. 4



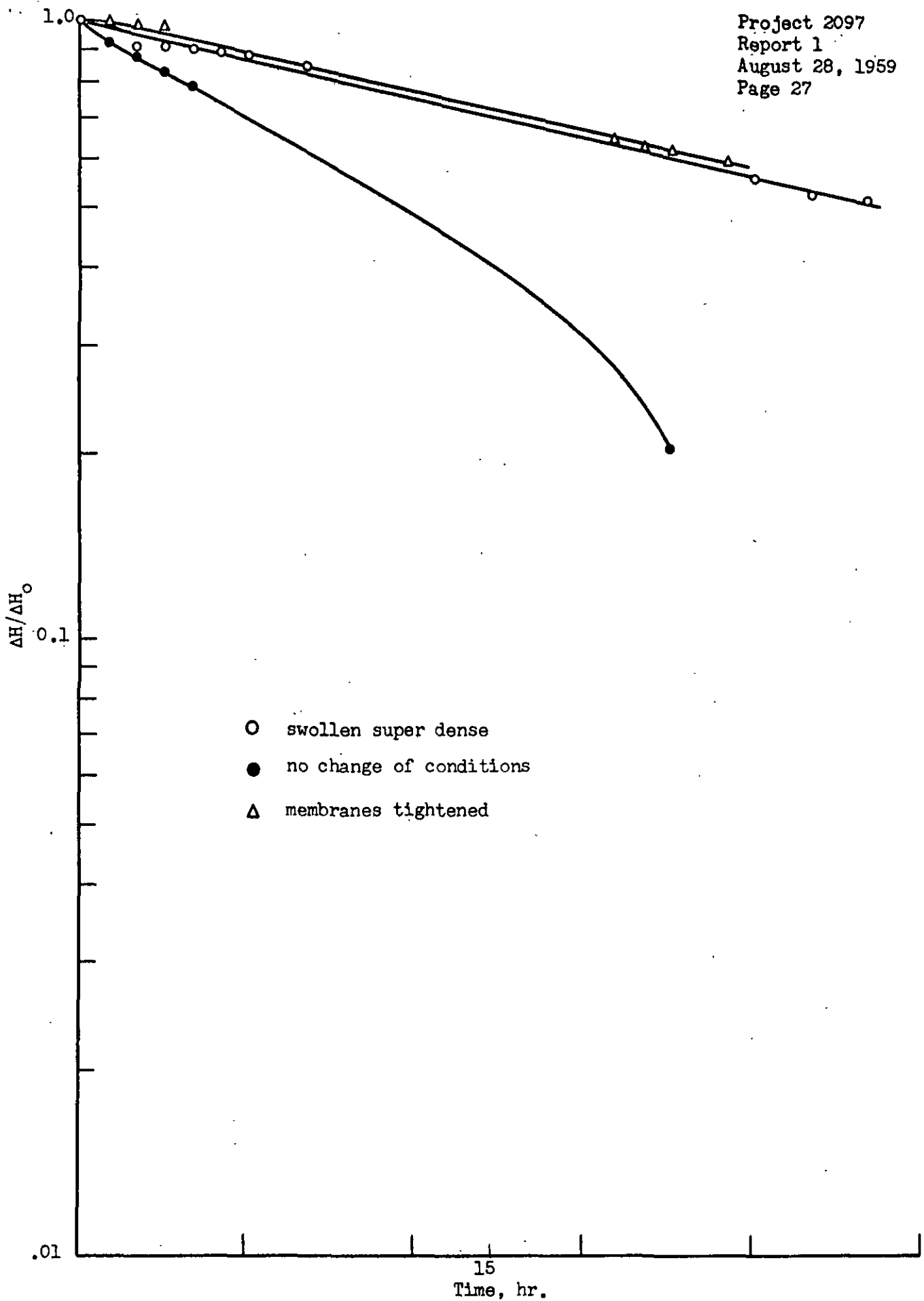


Fig. 11. Leak Test Determinations, Osmometer No. 4a

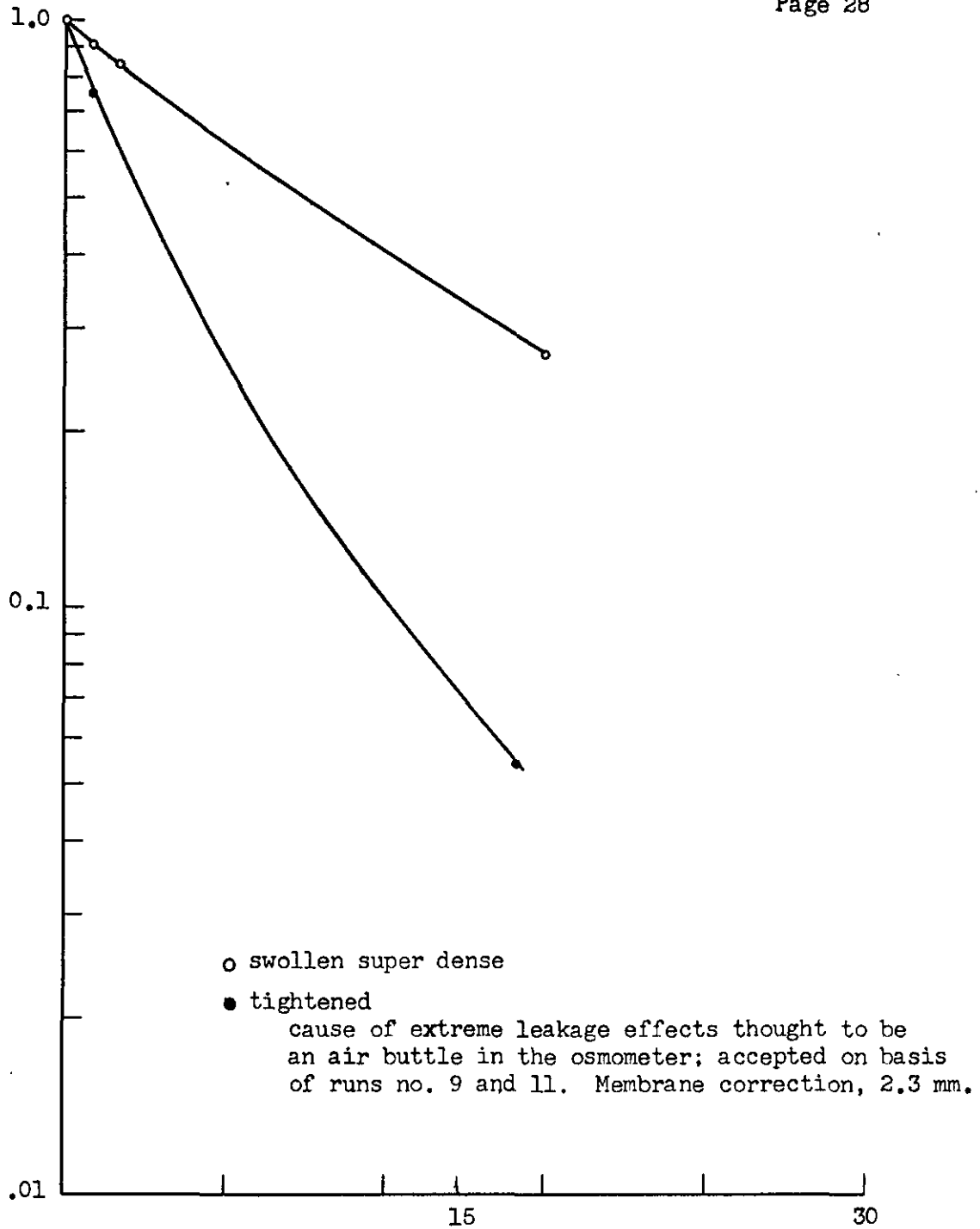


Fig. 12. Leak Test Determinations, Osmometer No. 4a

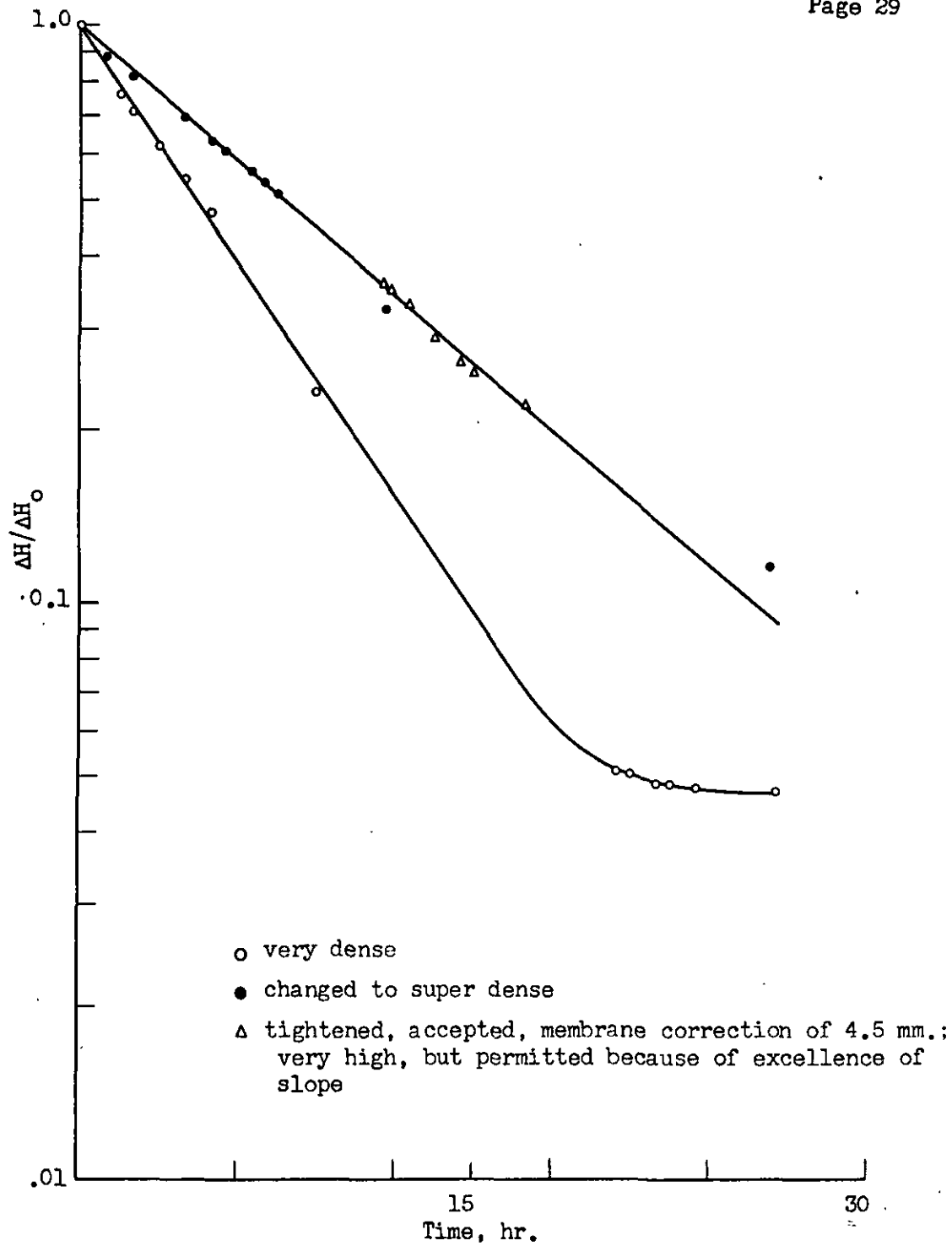


Fig. 13. Leak Test Determinations, Osmometer No. 7

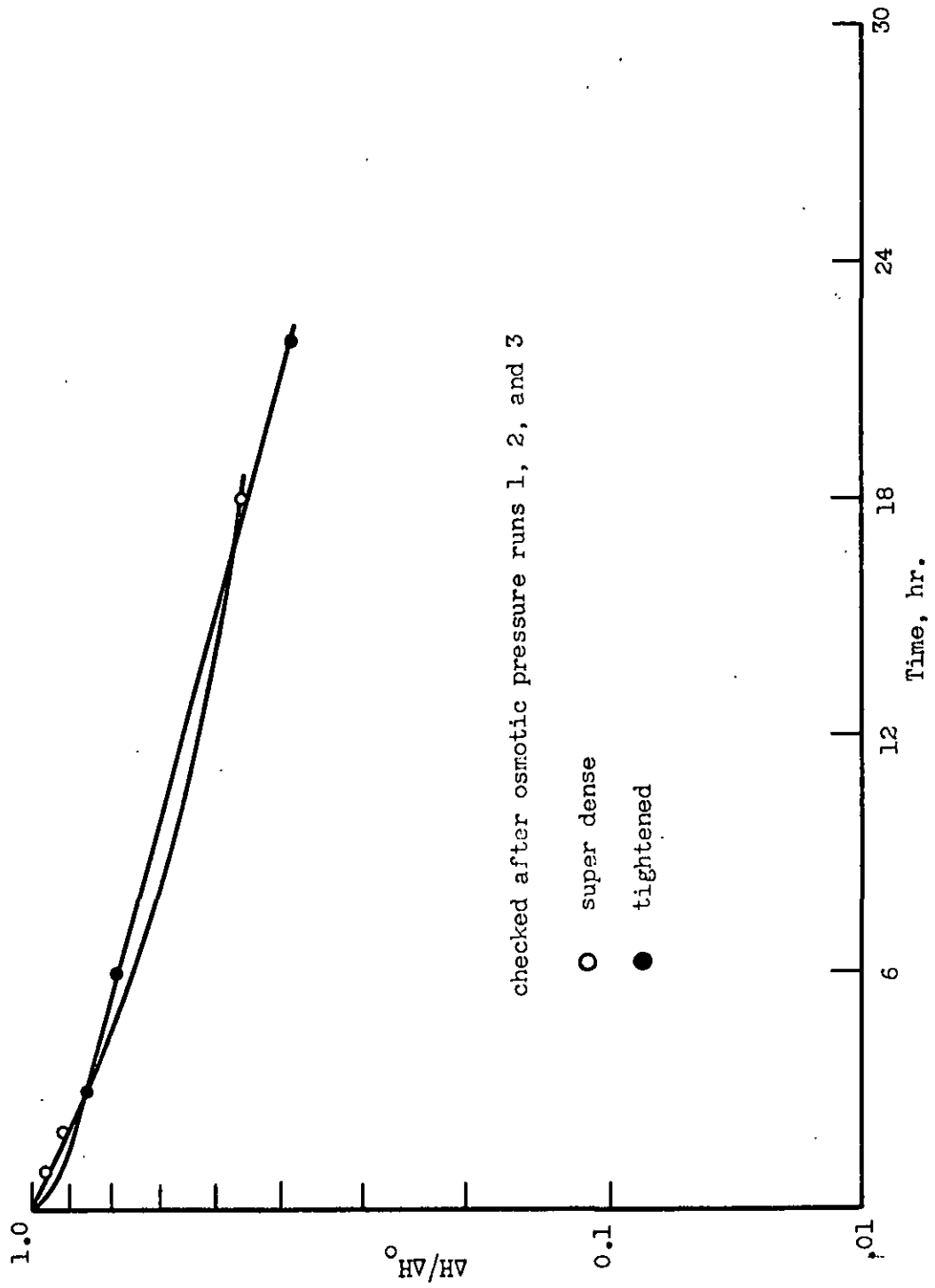


Fig. 14. Leak Test Determinations, Osmometer No. 7

APPENDIX C

PREPARATION OF LIGNIN SOLUTIONS

Two stock solutions of lignin in dioxane were prepared, one in a 50-ml. volumetric flask and the other in a 100-ml. flask. Aliquots were pipetted into other 100-ml. volumetric flasks and diluted to 100 ml. with pure dioxane.

All of the flasks were cleaned by first allowing them to stand for a day with potassium dichromate cleaning solution in them. This was followed by four rinsings with tap water, four with distilled water, and four with reagent grade acetone. They were then allowed to air dry.

STOCK SOLUTIONS

The lignin used in preparing the stock solutions was taken from the blended portion coded 2097-1074-124-U (Notebook 1704, p. 124), the lignin extracted from the meal not subjected to benzene-alcohol refluxing.

0.1998 g. lignin/100 ml. dioxane      0.0998 g. lignin/50 ml. dioxane

Based on coefficient of expansion of dioxane of  $0.00108/^{\circ}\text{C}$ . and density of dioxane  $1.0353-20^{\circ}/4^{\circ}$ , solutions by weight are:

0.1998 g./103.217 g. ( $22.8^{\circ}\text{C}$ .)      0.0998 g./51.609 g. ( $22.8^{\circ}\text{C}$ .)

DILUTE SOLUTIONS

Prepared at  $22.8^{\circ}\text{C}$ ., concentrations by weight are:

0.1936 g. lignin/kg. dioxane	10 ml. dil. to 100 ml.
0.3871 g. lignin/kg. dioxane	20 ml. dil. to 100 ml.
0.5807 g. lignin/kg. dioxane	30 ml. dil. to 100 ml.
0.7743 g. lignin/kg. dioxane	40 ml. dil. to 100 ml.
0.9669 g. lignin/kg. dioxane	50 ml. dil. to 100 ml.

APPENDIX D

Footnote to Page 14

It can be seen that the plot of osmotic pressure run no. 5 (and also no. 6) cannot be extrapolated as indicated in the body of the report. The statements on page 14 were made before conclusion of these runs, in the expectation that the results would be similar to those of run no. 4.

A number of hypotheses can be proposed to explain the steady rise of the osmotic head in these runs. Among these are:

1. Absorption of water in the system
2. Degrading of the lignin
3. Degrading of the dioxane to a corresponding peroxide
4. An air bubble discovered in the system

Repeats of these runs must be made, making sure that no air bubbles exist in the systems, and analytical studies can be made from that point if the same phenomenon occurs.