AN IMPROVED HOLOCELLULOSE PROCEDURE

by

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INTRODUCTION

It has long been desirable to isolate the total polysaccharide fraction of extractive-free wood in a substantially unchanged state. Although earlier attempts had been made to separate this fraction from plants by chlorination and extraction to remove the lignin (11, p. 1140), the first successful attempt was made by Ritter and Kurth (6). Alternate chlorinations and basic alcohol extractions left a residue which contained virtually all of the carbohydrate material in extractive-free wood. They called this residue "holocellulose".

Their original method was applied to maple wood and the chlorinated wood meal was extracted with 15 per cent pyridine in 95 per cent ethanol. The sample was chlorinated in a chlorination chamber and then transferred to a Soxhlet extractor for extraction with the organic, basic solution. The alternate chlorinations and extractions were repeated until the sample was substantially free of lignin, at which time a hypochlorite bleach was applied to the sample. The total time required was about ten hours.

When this method was applied to spruce wood (5), it was found necessary to increase the pyridine concentration to 50 per cent and eliminate the hypochlorite bleach, since it caused excessive carbohydrate removal.

This method was later improved by Van Beckum and Ritter (9) by using a 3 per cent solution of monoethanolamine in 95 per cent ethanol. In subsequent work (10) they made improvements and the method was later accepted as Tappi Standard analytical method, T 9-m (8). A convenient chlorination and extraction device for this procedure was described by Kurth (4).

In the Tappi Standard method a two gram sample of extractive-free wood meal is weighed out into a fritted glass crucible, which is then mounted on a suction flask. The crucible is surrounded by an iced-water bath during the chlorination to minimize polysaccharide degradation and hydrolysis.

The sample is moistened, chlorinated for several minutes, washed with 95 per cent ethanol, extracted twice with a 3 per cent solution of monoethanolamine in 95 per cent ethanol at about 75° C., washed twice with 95 per cent ethanol at room temperature and twice with cold water. This procedure is repeated until the sample is white after chlorination or until no darkening occurs upon addition of the basic solution.

The residue is then washed with 95 per cent ethanol until the filtrate is neutral and then twice with ether. It is air dried and oven dried at 105° C. for 2 1/2 hours or <u>in vacuo</u> at 60° C. if further analysis of the

holocellulose is planned.

The object of this research was to improve the current method for the determination of holocellulose in wood in at least one of several ways. First, it was believed that a solvent other than 95 per cent ethanol might be more effective. Second, an organic base other than monoethanolamine might be more effective. Third, either the organic base or the solvent might make the end-point more readily observable. A method comparable to the Tappi Standard method would also be of value if the use of ethanol could be avoided, since it is difficult for many industrial laboratories to obtain non-denatured ethanol.

SAMPLE PREPARATION

The sample used in all of the following experiments, unless otherwise stated, was from an 80 year old Douglas-fir tree which was logged in Linn County, Oregon. The log was hand barked, chipped in a commercial-size Norman-type chipper and hand-picked to remove knots and dirt particles. The chips were then ground in a Wiley mill. The particles were classified by size with a Rotap shaker giving a coarse fraction which was retained on a 40 mesh screen, a fraction which passed a 40 mesh screen but was retained on a 60 mesh screen, a fraction which passed a 60 mesh screen but was retained on an 80 mesh screen and fines which passed an 80 mesh screen and were discarded. The coarse fraction was reground until the entire sample passed a 40 mesh screen.

The two wood meal fractions were then extracted in a large Soxhlet-type extractor with an azeotropic mixture of ethanol and benzene. The samples were then thoroughly extracted with water at 100° C. The extracted wood residues were air dried and stored in air tight glass bottles.

EXPERIMENTAL PROCEDURE

PRELIMINARY INVESTIGATION

Since the material which is removed from the wood by the Tappi Standard method is chlorinated lignin, or chlorolignin, it was decided to attempt an isolation of a sample of chlorolignin so that its solubility in various common solvents might be conveniently determined. A search of the literature revealed that many methods had been used in preparing and isolating chlorolignin. Since a sample similar to that which is removed in the Tappi Standard procedure was desirable, chlorinated wood was extracted with various organic solvents, some with and some without bases.

Preparation of Chlorolignin

Twenty-five grams of the previously extracted 40 to 60 mesh wood meal were placed in a large fritted glass funnel. The wood was moistened and chlorine gas was drawn through the sample with slight suction for five minutes. The sample was then treated with four 50 milliliter portions of the solvent at room temperature. The extracts were evaporated to about 50 milliliters, poured into 10 volumes of cold water and acidified if basic. The suspensions were centrifuged, the supernatant liquid was decanted and the precipitates were dried *in vacuo* at 55° C.

The supernatant aqueous solution in each case evidently contained a substantial amount of chlorolignin since its color was a very dark orange. No attempt was made to recover this water-soluble chlorolignin. Shorygina and Kolotova (7) found that chlorolignin was quite easily hydrolyzed in water or aqueous alkali solutions with a marked increase in hydroxyl groups and a corresponding increased solubility in water.

Table I. Yield and Appearance of Various Chlorolignin Preparations.

Solvent	Yield	Description
3% monoethanolamine in 95% ethanol	1.0 gram	Light orange powder
1,4-dioxane	1.0 gram	" " "
95% ethanol	1.0 gram	" " "
Methanol	1.0 gram	" " "
2% KOH in 95% ethanol	0.5 gram	Dark brown powder

A similar sample of the wood meal chlorinated by the above method, air dried and extracted with methanol in a Soxhlet extractor gave an increased yield of 1.1 grams, but the physical appearance of the product was the same.

Twenty-five grams of the moist, chlorinated wood meal were suspended in 250 milliliters of 3 per cent (v/v) monoethanolamine in 95 per cent ethanol at 75° C. and agitated

for 10 minutes. The wood meal was filtered and washed with one hundred milliliters of 95 per cent ethanol. The monoethanolamine-ethanol extraction and ethanol wash were repeated once. The total extracts and washes were combined and evaporated *in vacuo* to about 50 milliliters and poured into 10 volumes of cold water. The precipitate was collected by centrifugation and dried at 55° C. *in vacuo*. The yield consisted of 0.3 grams of a brown, amorphous powder. Identical results were obtained when a saturated solution of ammonia in 95 per cent ethanol at the same temperature was used. The low yields were evidently due to the hydrolysis of the chlorolignin in the moist, hot, basic solutions to water soluble products.

An attempt was made to obtain chlorolignin from 72 per cent sulfuric acid lignin by a similar procedure, but the isolatable yield was impractically low.

Solubilities of the Chlorolignins

Accurately weighed 0.100 gram samples of chlorolignin were placed in small test tubes. Exactly three milliliters of the various solvents at 25° C. were added, and the solutions were thoroughly mixed. The relative solubilities were determined after centrifugation by a visual comparison of the color of the solution and the amount of residue remaining.

Table II. Solubilities of the Chlorolignin Preparations in Various Solvents

Method of Chlorolignin Preparation	Order of decreasing solubility → 1	2	3	4	5	6
3% monoethanolamine in 95% ethanol @ 25° C.	D/M	D	E	M	I	A
1,4-dioxane @ 25°C.	D	D/M	M	E	I	A
95% ethanol @ 25°C.	Completely soluble in all.					
Methanol, Soxhlet	M	D	D/M	E	I	A
3% monoethanolamine in 95% ethanol @ 75°C.	E	D/M	D	M	I	A
95% ethanol saturated with NH_3 @ 75°C.	D	D/M	D	M	I	A

D-1,4-dioxane; M-methanol; E-95% ethanol; I-isopropanol; A-acetone; D/M-50% methanol in 1,4-dioxane.

Although the results of these experiments appeared somewhat indefinite, it was noted that dioxane and 50 per cent methanol in dioxane were, in almost all cases, among the best solvents.

When the above procedure was used to determine the effectiveness of several organic bases in dioxane it was found that dioxane alone proved to be at least as effective as the basic solutions. Therefore, it was decided that the various bases would have to be evaluated by their use in a holocellulose procedure.

HOLOCELLULOSE DETERMINATIONS

An attempt was made to determine the value of the iced-water jacket prescribed in the standard method. It was found that without the water jacket the results were somewhat lower and often varied by more than one per cent with the same number of chlorinations. This should be expected since the chlorination of wood is a highly exothermic reaction. It was decided to use the iced-water jacket in all subsequent chlorinations in this research.

Since the apparatus used in the standard method is somewhat cumbersome an attempt was made to improve it. The illustrated apparatus (Figure 1) proved quite satisfactory. It was easily made and the jacket could be removed to facilitate working with the sample when it was not being chlorinated. No suction was necessary for chlorination as the pressure of the chlorine gas caused it to pass through the sample. Adjustment of the flow of chlorine was not as critical as with the standard apparatus in which too small a flow can cause the drawing of iced-water into the sample, and too great a flow can cause the chlorine to bubble through the iced-water and into the hood.

Preliminary analyses were performed on the extracted wood meal which passed a 40 mesh screen and was retained on a 60 mesh screen. It was found that fourteen chlorinations and extractions were required to produce a

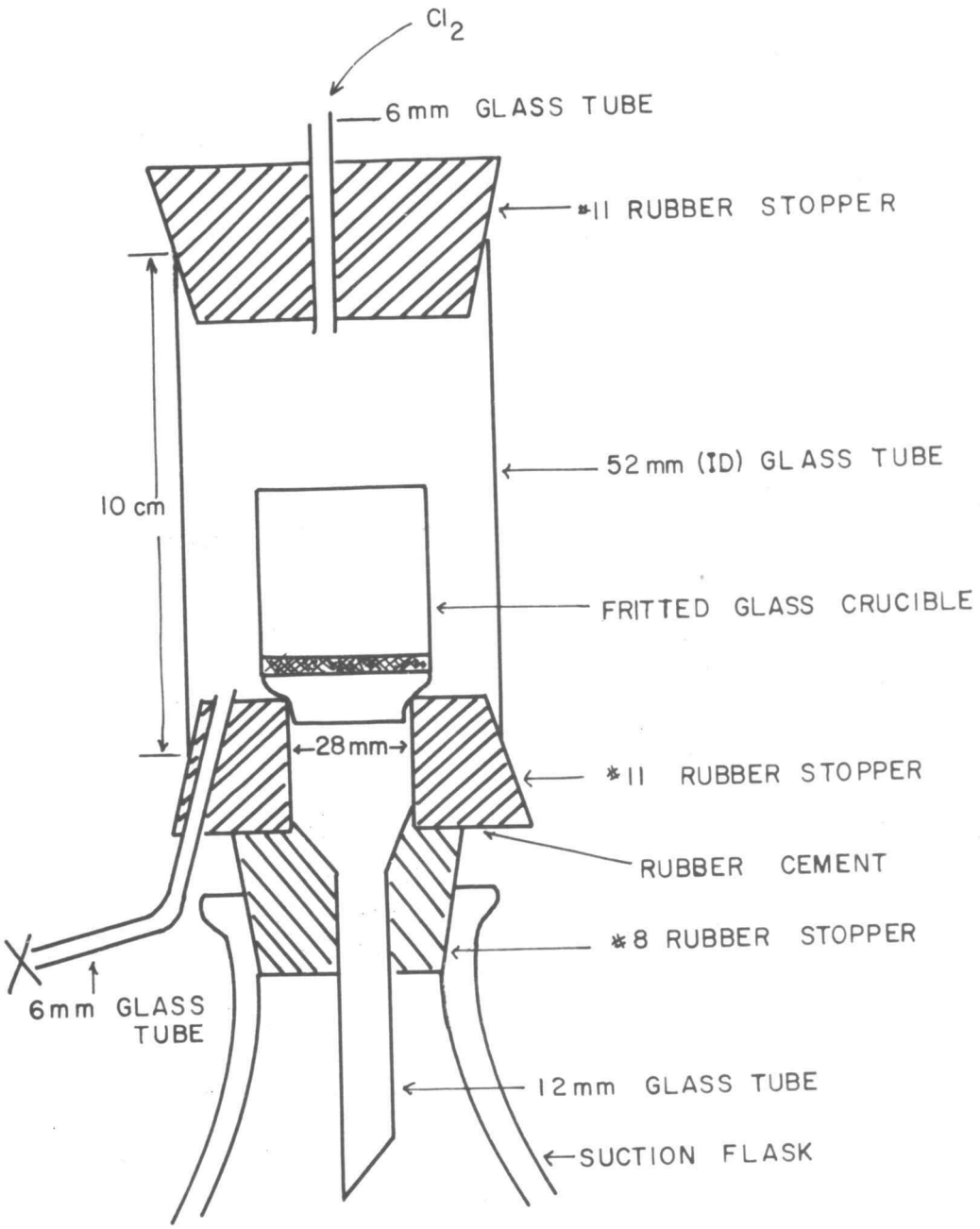

FIGURE 1. HOLOCELLULOSE APPARTUS

substantially lignin-free product by the standard procedure. Atchison (1) found that holocellulose prepared from 40 to 60 mesh wood and 60 to 80 mesh wood were essentially identical, but the use of 60 to 80 mesh wood decreased the amount of time necessary for preparation. Therefore, it was decided to use 60 to 80 mesh wood meal for the analyses.

Since the clarity of the end-points obtainable with the various solvents and organic bases was not known, it was decided to stop at a point several per cent above the actual holocellulose value with the standard solvent and base and then repeat the procedure with the new bases and solvents with exactly the same number of chlorinations and extractions and under identical conditions. It was found that nine chlorinations and extractions were satisfactory in this respect.

The solvents were used in lieu of ethanol and the bases were used in lieu of monoethanolamine, with the same volume to volume concentration (3 per cent). Otherwise, the procedure was exactly as described in Tappi Standard method T 9-m (8).

The lignin content of the extracted wood meal was found to be 27.84 per cent by the Tappi Standard 72 per cent sulfuric acid method T 13-m (8). Therefore, the correct holocellulose content should be approximately 72.16 per

cent. In Table III it is seen that the Tappi Standard method left approximately 4.5 per cent lignin in the wood meal after nine chlorinations and extractions.

Table III. Conditions and Yields of Holocellulose Preparations

Solvent	Base	Temperature[a]	Apparent % Holocellulose
95% ethanol	Monoethanolamine	75° C.	76.72[b]
"	Morpholine	"	84.43
"	Ethylenediamine	"	82.36
"	Butylamine	"	78.98
"	Triethylamine	"	98.50
"	Triethanolamine	"	-[d]
Methanol	Monoethanolamine	65° C.[c]	83.57
Methanol-dioxane (1:1)	Monoethanolamine	"	78.80
Dioxane	Monoethanolamine	75° C.	69.74
"	Morpholine	"	80.93

a-basic solution; b-standard method; c-B.P. of methanol; d-filtration impractically slow.

When one of the designated solvents was substituted for ethanol in the basic solution, but not in the wash solution, the results were almost identical to those obtained when ethanol was used exclusively. Similar results were obtained when a solvent other than ethanol was used only for the wash solution.

It is obvious from the above table that a 3 per cent solution of monoethanolamine in dioxane is superior to any of the other combinations tried. Therefore, it was decided to determine the effect of the concentration and the temperature of the basic solution using dioxane and monoethanolamine. The procedure followed was the same as described above.

First, the temperature of the basic solution was varied from 25° C. to 95° C. keeping the monoethanolamine concentration constant at 3 per cent. Following this the temperature of the basic solution was held constant at 50° C., while the concentration of the monoethanolamine was varied from one to seven per cent. Nine chlorinations and extractions were performed in each case.

The results of these experiments (Figures 2 and 3) showed that the concentration of the monoethanolamine should be at least 3 per cent, since below that value a marked decrease in the solvent ability of the basic solution occurred. Since no marked decrease in solvent ability occurred at lower temperatures, it appeared that the optimum temperature would be a temperature high enough so that the chlorolignin would be removed within a reasonable time, but low enough so that excessive carbohydrate removal would not occur. The temperature selected for further work was 50° C., while the concentration of the monoethanolamine

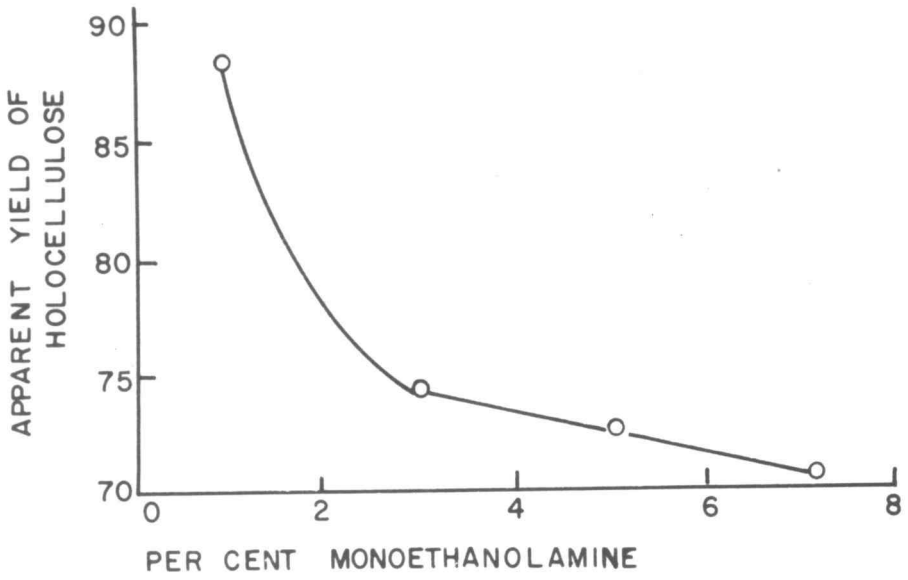

FIGURE 2. EFFECT OF CONCENTRATION OF THE BASIC SOLUTION

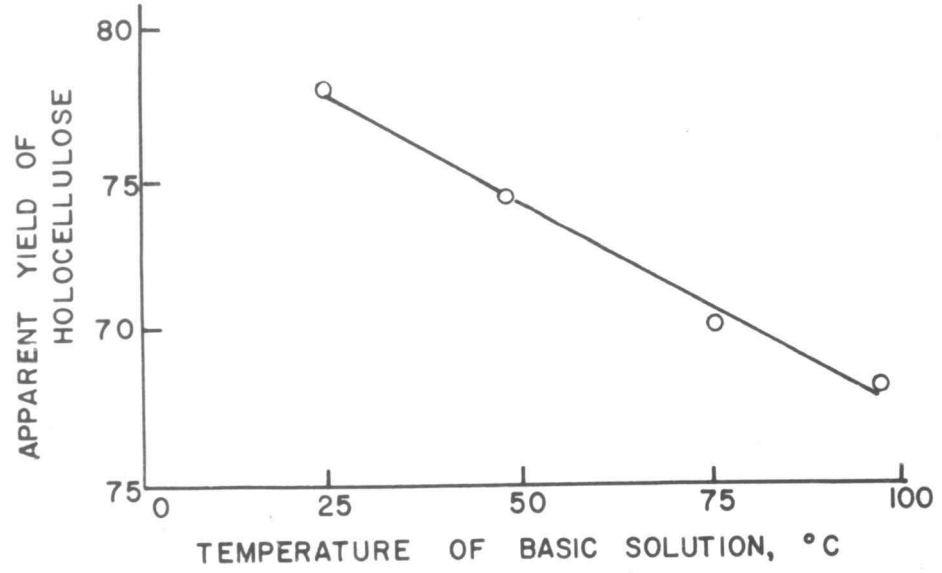

FIGURE 3. EFFECT OF TEMPERATURE OF THE BASIC SOLUTION

solution was fixed at 5 per cent.

In order that more representative results might be obtained a sample of a hard wood, white oak, was used in addition to the Douglas-fir previously described. The white oak was ground in a Wiley mill, and the 60 to 80 mesh portion was used. The wood meal was exhaustively extracted with an ethanol-benzene azeotrope followed by water at 100° C. The lignin content of this sample was found to be 19.22 per cent by the 72 per cent sulfuric acid method.

The procedure adopted for the holocellulose determinations was as follows: The sample consisted of air-dried, extracted, 60 to 80 mesh wood meal whose moisture content was known accurately. Two grams of the sample were weighed out accurately into a fritted glass crucible. The crucible was mounted in the apparatus shown in Figure 1 and surrounded by iced-water. About twenty milliliters of cold water were added to the sample, and after stirring the excess water was removed by moderate suction.

The upper stopper was placed firmly on the apparatus, and chlorine was passed through the sample for three minutes, the sample was stirred and chlorinated for two more minutes. The sample was covered with dioxane, stirred, dried by suction after one minute and the iced-water jacket was removed.

Two successive portions of 5 per cent (v/v) monoethanolamine in dioxane at 50° C. were added and stirred

thoroughly. Each portion was allowed to stand for two minutes before removal by suction. The sample was next washed twice with dioxane at room temperature and twice with cold water.

The above procedure was repeated limiting the chlorinations to one uninterrupted three minute chlorination for all following treatments. When the sample appeared white after chlorination, or when very little color change occurred upon addition of the basic solution, the sample was assumed to be substantially lignin free.

The sample was then washed with dioxane until neutral and twice with diethyl ether. After air drying the residue was dried at 105° C. for 2 1/2 hours before weighing. If analyses were to be performed on the holocellulose it was either dried *in vacuo* or air dried with a small portion used for a moisture determination.

The lignin content of the holocellulose samples thus prepared was determined by the standard 72 per cent sulfuric acid method. Since the carbohydrate fraction most easily removed from wood is the hemicellulose fraction, which contains a relatively large percentage of pentosans, the pentosan content of the holocellulose was determined and compared with that of the original wood. The procedure followed was the bromate-bromide titration method as described in Tappi Standard method T 223-m (8).

Since acetyl groups are generally the functional groups most easily removed by hydrolysis from carbohydrate material in wood, the acetyl content of the holocellulose was determined and compared to that of the original wood. The acetyl content was determined by the semi-micro method of Clark (3).

The ash content of the holocellulose was found to be negligible. The average results of several analyses are tabulated on table IV.

Table IV. Results of Analysis of Extractive-free Wood and of the Holocellulose

(Per cent composition based on weight of oven-dry, extractive-free wood)

Material	Yield	Lignin	Pentosan	Acetyl
Douglas-fir wood	-	27.84	6.2	0.84
Douglas-fir holocellulose, air-dried	72.41	0.02	6.0	0.77
White oak wood	-	19.22	24.1	2.39
White oak holocellulose, dried *in vacuo* @ 60°C.	80.17	0.14	22.0	1.80

THE BASIC SOLUTION

It was noted that the monoethanolamine-dioxane solution was quite cloudy when prepared, but after standing overnight a yellow, oily, water-soluble substance precipitated leaving a clear, colorless supernatant solution.

Commercial grade 1,4-dioxane without purification was used in this investigation. By the addition of an acidified potassium iodide-starch solution, it was found to contain a large amount of peroxides. It was believed that the formation of the yellow, oily precipitate with monoethanolamine might be caused by the presence of these peroxides.

For example, when twenty-five milliliters of a one per cent aqueous solution of monoethanolamine were treated with five milliliters of three per cent hydrogen peroxide, after twenty-four hours a definite yellow tinge developed which was similar in appearance to an aqueous solution of the precipitate from the dioxane solution. What appears to be the same color develops in monoethanolamine after standing for several months.

It was thought desirable to determine the amount of monoethanolamine lost due to this reaction with the dioxane. Solutions of monoethanolamine in dioxane ranging from one to five per cent were prepared and allowed to stand for twenty-four hours. At the end of this period the clear supernatant solution was titrated with 0.1 normal sulfuric acid to determine the amount of monoethanolamine present. It was found that the loss was about 0.9 milliliters for each one hundred milliliters of dioxane, which represents a decrease of about 0.9 per

cent in the concentration of the basic solution.

Table V. Results of Analysis of Supernatant Layer of Basic Solution after 24 Hours.

Per cent monoethanolamine in dioxane	
Added	Found
1.00	0.22
2.00	0.93
3.00	1.98
4.00	3.01
5.00	4.21

Standing for several months did not appear to increase the amount of separation appreciably, but the clear supernatant solution became dark yellow. The use of this solution did not appear to have any effect on the holocellulose procedure, but due to its color it was felt that a fresh solution was desirable.

The 168 to 171° C. fraction of Eastman white label monoethanolamine was used in this investigation. Distillation was necessary to remove the yellow color which had developed upon standing.

DISCUSSION

The suggested apparatus (Figure 1) was found to be much more convenient to use than the apparatus previously described in the literature (4). It was quite easily constructed with no expensive materials.

The total number of chlorinations and extractions necessary to prepare holocellulose by the new procedure was nine for 60-80 mesh Douglas-fir wood meal and five for white oak, whereas nine chlorinations and extractions using the Tappi Standard procedure on Douglas-fir left approximately 4.5 per cent lignin in the product as determined by the standard 72 per cent sulfuric acid method. Experience has shown that the last few per cent of the lignin are removed relatively slowly. Therefore, it can be assumed that the proposed procedure decreased by at least two or three the number of chlorinations and extractions necessary.

Quite often the last few treatments with the Tappi Standard procedure are prolonged by exceedingly slow filtration. This is due to carbohydrate hydrolysis by the excessive number of chlorinations. No slow filtrations were experienced with the proposed method.

The total amount of time required for a determination on Douglas-fir averaged slightly less than two hours by the proposed method, excluding the time necessary for

sample preparation and drying of the holocellulose. The time required by the Tappi Standard procedure on the same sample averaged slightly over three hours. The time saved by the new method is quite important since continuous observations and manipulations are required throughout.

The end-point color change of the proposed procedure did not appear to be greatly improved over that of the standard procedure. It is possible that the addition of another substance to either the basic solution or to one of the wash solutions might improve this feature, since lignin exhibits color reactions with numerous organic and inorganic compounds (11, p. 414-424).

The holocellulose prepared by the proposed procedure compares quite favorably with that prepared by the standard method. Douglas-fir holocellulose showed a loss of 0.2 per cent pentosans and 0.07 per cent acetyl groups, while white oak showed a loss of 2.1 per cent pentosans and 0.59 per cent acetyl groups, all based on the weight of the oven-dry extractive-free wood. It has been assumed that the removal of the lignin causes the carbohydrates with very low degrees of polymerization to be readily dissolved (11, p. 1143).

Kurth and Ritter (5) stated that the apparent loss of pentosans was due to the formation of furfural from the lignin in the wood. This would account for a part of

the pentosans which appear to be lost. However, the acetyl group loss may be due to the loss of carbohydrate material since isolated lignin does not contain volatile acid groups.

The cost of commercial grade dioxane, including handling, is approximately $2.75 per gallon at Oregon State College, while 95 per cent ethanol (tax-free) costs about $1.25 per gallon. Since dioxane decreases the total number of chlorinations and extractions and also eliminates slow filtration, this increase in cost is felt to be of little disadvantage, especially since many laboratories can not obtain tax-free ethanol.

SUMMARY

The amount of chlorolignin removed from chlorinated wood by one extraction was found to be approximately the same with several common solvents. These chlorolignins were isolated and their relative solubilities in various solvents were determined. Of the solvents used dioxane and 50 per cent methanol in dioxane appeared to be the most effective.

When various solvents and bases were substituted for 95 per cent ethanol and monoethanolamine in the Tappi Standard procedure a marked difference in effectiveness was found. Only one combination, however, proved to be more effective than 95 per cent ethanol and monoethanolamine. This was dioxane and monoethanolamine.

When the concentration of the monoethanolamine in the dioxane was below 3 per cent the solution exhibited a marked decrease in its solvent ability. Also, it was found that the solvent ability of the monoethanolamine solution increased approximately proportionally to the temperature of the solution.

Holocellulose determinations were performed on two different species of wood using 5 per cent monoethanolamine in dioxane at 50° C. as the basic solution, and dioxane at room temperature as the organic wash solvent.

The yield of the resulting holocellulose products agreed consistently within one per cent of the value predicted by lignin determinations. An analysis of the holocellulose revealed that only a small loss of pentosans and acetyl groups had evidently occurred.

The procedure recommended for the holocellulose determination is as follows: The sample shall consist of extractive-free, 60 to 80 mesh wood meal whose moisture content is accurately known. Two grams of the sample are weighed into a tared fritted glass crucible (C or M porosity).

The crucible is mounted on the apparatus shown in Figure 1 and surrounded by iced water. After moistening the sample, the top stopper is placed firmly into the apparatus and chlorine gas is passed through the sample for three minutes. The sample is then stirred and chlorinated for 2 more minutes.

Dioxane at room temperature is now added to cover the sample and the mixture is stirred. Suction is applied to remove the dioxane. Then the iced-water jacket is drained and removed. Five per cent monoethanolamine in dioxane at 50° C. is added and stirred. After two minutes the basic solution is removed by suction and the treatment is repeated.

The sample is then washed twice with dioxane at room

temperature and twice with cold water. The above procedure is repeated limiting all subsequent chlorinations to one uninterrupted 3 minute treatment with the iced-water jacket in place. When the product appears white after chlorination, or when very little color change occurs upon addition of the basic solution, essentially all of the lignin is removed.

After washing with dioxane until neutral the sample is washed twice with ether and then air dried. The holocellulose is either dried for 2 1/2 hours at 105° C. or at 55 to 60° *in vacuo* before weighing.

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