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Determination of Chemical Composition of Wood Pulp Hydrolyzates by Paper Chromatography

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DETERMINATION OF CHEMICAL COMPOSITION OF WOOD PULP HYDROLYZATES BY PAPER CHROMATOGRAPHY

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Abstract

The available literature on paper chromatography was surveyed. Special attention was given to those techniques dealing with the analysis of wood pulp hydrolyzates. Five hardwood pulp hydrolyzates were analyzed by paper chromatographic means. All hardwood pulp hydrolyzates analyzed tended to fall in a relatively narrow range of chemical composition. Glucose and xylose were found to be the main constituents with traces of mannose and galactose present in some pulp hydrolyzates.

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INTRODUCTION

Recently the need for an accurate means of separating and identifying simple sugars obtained by the hydrolysis of wood pulp has become apparent. By the use of paper chromatography such monosaccharides as galactose, mannose, xylose and glucose have been successfully separated on a quantitative level. Not only does paper chromatography provide the necessary accuracy and sensitivity for such separations, but it is relatively simple and inexpensive in operation.

This technique has found many applications in the study of various phases of the paper industry. It has been used extensively in the analysis of the polysaccharide portion of cellulosic fibers, in determining the hemicellulose composition of pulp (13) and as an aid in the continued search for the structure of lignin. Paper chromatography has also been used to trace the fate of carbohydrates through various processes in the paper industry and has found use in the study of pulping inhibitors, analysis of tall oil and in tannin research. A more detailed summary of the uses of paper chromatography in the paper industry has been offered by E. F. Dickey (10, 9).

OBJECT

The object of this report is to survey pertinent literature on paper chromatography, to obtain information on the results of past analyses and to select the method of paper chromatography best suited for the planned experimental work on a comparison of the chemical composition of wood pulp hydrolyzates.

MECHANISM OF PAPER CHROMATOGRAPHY

Although there is still some discussion as to the

actual mechanism of chromatographic separations, the following definition is given by Williams (29).

"By chromatography is meant those processes which allow the resolution of mixtures by effecting separation of some or all their components in concentrated zones on or in phases different from those in which they are originally present, irrespective of the nature of the force or forces causing the substances to remove from one phase to another."

Paper chromatography can be defined generally as the partition of a solute between a mobile solvent phase and a stationary phase consisting of a cellulose-water complex (18). A simplified view is that of continuous extraction of a solute between two solvents, one stationary and one moving. A more detailed discussion of the theory involved as well as other forces present can be found in a variety of sources (1, 31, 3, 5, 6, 27, 26).

TECHNIQUE

Although many variations in technique are employed in paper chromatography, some basic steps apply to all. These steps include:

1) Preparation of a solution containing the substances to be identified.
2) Chromatographic separation of the solution into its individual components.
3) Drying of the chromatogram and its identification with selected reagents.
4) Quantitative measurement of the components.

Details on the various methods and equipment used can be found in the many laboratory manuals on the subject (1, 6, 5, 3, 14).

Preparation of Solution

In the analysis of pulp hydrolyzates, the preparation of the solution to be analyzed is of special importance, since care must be taken or excessive decomposition of the

sugars will occur (25). A large excess of glucose which will hinder detection of small amounts of galactose, mannose and rhamnose can also be avoided at this point by only partial hydrolysis of the sample (19).

Hydrolysis of the pulp sample is usually accomplished with 72% sulfuric acid (25, 19, 18, 20). Saeman and his co-workers (25) reported that a one-hour primary hydrolysis with 72% sulfuric acid at 30°C followed by a secondary hydrolysis with dilute acid for one hour under a pressure of fifteen p.s.i. gave acceptable results. Block (3) also suggested a two-stage hydrolysis for wood pulp. A one gram pulp sample was digested at room temperature with 72% sulfuric acid for four hours, after which water was added and the mixture refluxed for six hours. Das (8) reported better results using formic acid in the analysis of cellulose, although Mazumdar (20) has disputed this claim. Fellegi (12) reported that the use of milder acids such as phosphoric or acetic prevents decomposition in the hydrolysis of beta and gamma cellulose. Studies of the effects of varying concentrations of strong acids have been made by both La Diega (19) and Wittwer (30).

Since inorganic salts interfer with the separation of sugars by paper chromatographic means, they must be removed from the hydrolyzate before chromatographic separation. Although the use of barium carbonate to precipitate the sulfate has been reported (3, 18, 12), most workers favor the use of various ion exchange resins such as Amberlite IR4B to remove the interfering ions (25, 3, 2, 12).

After the pH of the salt-free solution has been adjusted to a pH 4-5, the hydrolyzate is concentrated by vacuum evaporation using standard laboratory equipment. An efficient means of concentrating a large number of samples in a minimum of time has been described by Saeman and co-workers (25).

Chromatographic Separation

The specific equipment used in chromatographic separations varies with the experimenter, although certain general

features are found in all publications (1, 6, 5, 3). Cabinets and accessories for chromatographic separations are available commercially; however, additional useful features can be incorporated with an original design (25).

Although Whatman #1 filter paper is the usual type of paper used, other varieties have been employed for specific separations where faster or slower moving solvent fronts were desired (1, 6, 5). Most workers with wood pulp hydrolyzates have found Whatman #1 paper to be suitable for all separations.

Many solvents have been tried by various investigators, but only a few have been reported to give acceptable results. Systems involving mixtures of ethyl acetate, pyridine and water have been found suitable for separation of xylose, arabinose, mannose and glucose (3), but two-dimensional chromatography using systems of butanol, pyridine and water, and ethyl acetate, acetic acid and water have been used more extensively (2, 24, 23, 4, 25, 18). Although the pyridine system fails to separate mannose and arabinose, while the acid system fails to separate glucose and galactose, a combination of the two yields separation of all sugars commonly found in wood pulp hydrolyzates (18, 25).

Actual separation of hydrolyzate sugars is accomplished in a suitable cabinet which has been previously saturated with solvent vapors. Saturation usually requires at least 24 hours after a container of the solvent has been placed in the cabinet (3). Small volumes of hydrolyzates are then applied at regular intervals across the sheet of filter paper by means of micro pipets and dried by a hot air stream such as from a hair dryer (6, 3). These spots must be exact and accurate, for according to Block (3), spotting is the most important single factor for a successful paper chromatograph. To avoid "tailing" of the separated sugars a maximum application of sixty gammas is recommended by Cramer (6). For quantitative separation known concentrations of known sugars are spotted alternately with the unknown spots. This not

only allows easy identification of the unknown sugars, but also tends to cancel out outside influences, since both known and unknown samples are separated and developed under the same conditions.

Although some work has been done using circular paper chromatography (14, 12), the majority of the work with pulp hydrolyzates has been done using the descending method. The spotted paper is suspended from a trough in the sealed cabinet and the solvent solution is poured into the troughs. Separation of sugars varied in time depending on the solvent system used, the temperature and the type of filter paper. Using the butanol-pyridine-water system with Whatman #1 filter paper at room temperature, Lea (18) reported times of from sixteen to twenty hours. To obtain excellent separation Quick (24) used the following procedure. After developing with an ethyl acetate-acetic acid-water (1:2:2) solvent for twelve hours, the chromatogram was dried and developed again with the same solvent for twenty-four hours. After another drying, the chromatogram was then developed with a butanol-pyridine-water (10:3:3) solution for twelve hours, dried, and received a final development of twenty-four hours with the butanol-pyridine-water system.

The time of developing as well as the solvent system used is determined by the degree of separation of the various components. Between stages and before application of the desired indicator, the developed sheet must be dried to remove residual solvent. The length and temperature of drying depends on the solvent used, varying from air drying at room temperature to oven drying at 105°C.

Identification of Sugars

Once the chromatograph has been developed and dried, the positions of the various sugars must be established. Two basic means have been used for this purpose; illumination with ultraviolet light and application of selected chemical indicator. Bernardin and Piper (2) suggested a combination of these two by spraying first with an indicator

solution, followed by illumination with ultraviolet light.

Many reagents have been used to identify the separated sugars. O-anino biphenyl was found to give excellent results by both Bernardin and Piper (2) and Timell and co-workers (10). The relative stability of the reagent, as well as that of the colors produced, was found to be superior to that of other substances used. Aniline hydrogen phthalate has also been found to give excellent results (12, 18, 24, 30), but the stability of the reagent as well as that of the color produced was not good. Various other reagent, such as p-anisidine hydrochloride (23), aniline oxalate (16), Somogy sugar reagent (25) and the modified Seliwanoff's reagent (3), have been used by workers in the field.

Once the sugars have been located it is a simple matter to identify them qualitatively either by comparison with the known sugars on the same sheet or by calculation of their R_f values; i.e...the ratio of the distance traveled by the sugar to that traveled by the solvent front.

Quantitative Determination

Two methods are commonly used for quantitative determination of the separated sugars. Visual comparison of unknown spot area and intensity with known spots was reported by some workers, but this did not give the needed accuracy in most cases. Greater accuracy can be obtained using a Photovolt reflectance unit or a Densitometer as suggested by McCready and McComb (21) to measure the color and the intensity of both known and unknown spots. Both Fellegi (12) and La Dieu (17) reported excellent results using this method.

Elution of the individual sugars from the chromatogram for quantitative identification either by chemical or spectophotographic means is used by many workers. Lea (18) and Quick (24) both used the periodate chemical method of determining the actual amounts of sugars after the sugars had been eluted from the chromatogram. If the eluted sugar

solution is reacted with a color reagent such as previously mentioned, the absorbence of the resulting solution can be measured using a spectrophotometer such as a Beckman DU instrument, and can be compared with the absorbencies of known concentrations. Excellent results using this method have been reported (23, 28, 25). Bernardin and Piper (2) found a recovery of ± 0.1% of known glucose samples with this method.

RESULTS

Many authors have reported results obtained using paper chromatography. Gustafeson (13) obtained information concerning the hemicellulose present in wood which would not have been obtainable without the use of paper chromatography. Das (7, 8) showed the presence of glucose, xylose, arabinose and small amounts of rhamnose in raw cotton and also ran tests on chlorine dioxide, Cross and Bevan, Norman and Jenkins and Chlorite alpha-cellulose.

Fellegi (12), who did extensive work with beta and gamma-cellulose, found that glucose composed the largest percentage of beta-cellulose hydrolyzate, while gamma-cellulose hydrolyzate contained largely xylose and mannose. Xylose was found to be the much larger constituent in deciduous gamma-cellulose hydrolyzate (Table I).

Pridham (23), using 72% sulfuric acid to hydrolyze spruce pulp samples, found the average concentration of sugar in the hydrolyzate to be 27.0 mg/ml. A break-down of the sugars found as well as the amounts present is presented in Table II.

Lea (18), as a part of a study on neutral sulfite semichemical pulping, used paper chromatography in the analysis of aspen wood, holocellulose and NSSC pulp. All hydrolyzates contained a major percentage of glucose and xylose with traces of galactose, mannose, arabinose and rhamnose (Table III). Hemicellulose, aspenwood and NSSC pulp were also analyzed for pentose content, the hemicellulose showing the largest percentage (Table IV).

PRECISION

Results obtained by Kritchevsky and Anderson (16) show the precision with which sugars can be identified in pulp hydrolyzates using paper chromatography. R_f values obtained with reference sugars and those obtained with aqueous extracts agree to two significant figures, showing excellent precision (Table V).

The precision of the absorption spectrophotometric method of quantitative analysis was shown by Bernardin and Piper (2). Known amounts of glucose were recovered with a percent error of 0.7% (Table VI). This method was also studied by Quick (24) who ran samples of galactose, glucose, mannose, arabinose, xylose and rhamnose. Over 96% recovery was reported with all but galactose and arabinose (Table VII).

The effect of two different solvent systems on accuracy was studied by Timell and co-workers (28). The pyridine-butyl-water system showed excellent results when used on a glucose, mannose and xylose mixture, while poorer but still acceptable results were obtained with an ethyl-acetate-acetic acid-water system (Table VIII).

Pridham (23) by evaporation of the hydrolyzate found the total sugars present in a sample to be 27.3 mg/ml as compared with 27.0 mg/ml determined by paper chromatography or a 99% recovery. Excellent results can be expected, most authors reporting errors of from ± 4% (23) to ± 1-2% (25).

Table I

Sugars Found in Beta and Gamma Cellulose Hydrolyzates (12)

	% Glucose	% Xylose	% Mannose
Beta-cellulose			
coniferous	85 - 90	1	5 - 10
deciduous	75	20	---
Gamma-cellulose			
coniferous	8 - 12	45	45
deciduous	---	80 - 85	---

Table II

Hydrolysis of Spruce Pulp (23)

Sugar	Volume of hydrolyzate applied to chromatogram(λ)	Determined weight of sugar (γ)	Calc. sugar concentration in hydrolyzate (mg/ml)
Glucose	5	12.2	24.40
	10	24.9	24.90
Mannose	20	28.3	1.30
	30	38.2	1.40
Xylose	40	39.0	0.97
	60	55.0	0.92

Table III

Sugars Found by Chromatography in Hydrolyzates of Aspenwood Shavings, Pulp and Holocelluloses (13)

	% Glucose(b)	% Galactose(b)	% Mannose(b)	% Xylose (b)	% Arabinose(b)	% Rhamnose(b)
Aspen shaving	68.5	trace	1.3 (c)	29.0	1.3 (c)	trace
Holocellulose	70.0	1.2	1.4 (c)	25.9	1.4 (c)	sl. trace
Pulp (NSSC)	76.0	sl. trace	trace	24.0	sl. trace	sl. trace

b) Percentage as a pentose or hexosan of the total pentosans and hexosans present.

c) Mannans and arabans were determined together and each was arbitrarily assumed to constitute half of the total.

Table IV

Quantitative Sugar Determinations (18)

Material Hydrolyzed	Type of Hydrolysis	Reference Sugar	Pentosans % (a)	Pentosans % (b)
Hemicellulose	Lignin filtrate	Rhamnose	69.5	77.5
Aspenwood shaving	"	"	16.2	17.3
NSSC pulp	"	"	17.0	17.7
	"	Ribose	19.8	17.7

Table V

R_f Values Obtained with Each of Four Chromatograms (16)

Solvent Used	R_f Values			
	Galactose	Glucose	Arabinose	Xylose
Ethyl acetate-pyridine-water (2:1:2)	0.24	0.29	0.34	0.38 ref.
	0.24	0.29	0.34	0.38 ag. extr.
sym-Collidine satd. with water	.35	.40	.44	.53 ref.
	.35	.40	.44	.52 ag. extr.
n-Butyl alcohol-acetic acid-water (4:1:5)	.16	.18	.22	.28 ref.
	.16	.18	.22	.28 ag. extr.

Table VI

Recovery of Glucose by Chromatographic Analysis (2)

Amount of Glucose Applied (γ)	Amount of Glucose Recovered (γ)	Per Cent Error
200	200.7	0.4
200	199.1	0.5
200	200.0	0.0
200	200.0	0.0
200	203.0	1.5
200	203.0	1.5
200	198.2	0.9
Average	200.6	0.7

Table VII

Quantitative Chromatographic Sugar Study (24)

(Strips eluted by the complete elution method)

Sugar	Recovered, Mg	Known, Mg	Recovered, %	Standard Dev., Mg
D-galactose	24.2	26.7	90.6	1.5
D-glucose	19.7	20.3	97.0	1.2
D-mannose	13.7	12.8	107.0	1.2
L-arabinose	23.5	25.1	93.6	0.7
D-xylose	414.1	430.4	96.2	17.1
L-rhamnose	9.5	10.3	96.2	0.2

Table VIII

Influence of Two Different Solvent Systems on Accuracy (28)

Solvent system	Glucose γ/ml.		Mannose γ/ml.		Xylose /ml.	
	applied	found	applied	found	applied	found
Pyridine-butyl alcohol-water (10:3:3)	100.0	100.5	100.0	100.0	100.0	101.0
Ethyl acetate-acetic acid-water (9:2:2)	112.0	111.0	96.2	97.0	70.0	70.3

SOURCES OF ERROR

As preciously mentioned, the major error occurs during the hydrolysis of the pulp sample, due either to incomplete hydrolysis or degradation of the components (18). Some masking of sugars present in small amounts also occurs if glucose is present in large percentages. Minor errors may occur due to variation in conditions, although these errors may be all but eliminated if reference samples are run concurrently.

CONCLUSION

After surveying the available information, it was decided to use a two-stage hydrolysis with sulfuric acid (25) followed by separation involving the n-butyl alcohol-pyridine-water or the etayl acetate-acetic acid-water solvent system. Absorption spectrophotometry using o-amino biphenyl as a color reagent was selected as the means of quantitative analysis.

LITERATURE CITED

1. Balson, J. N., and Talbot, B. E., "A Guide to Filter Paper and Cellulose Powder Chromatography," London, H. Reeve Angel & Co. Ltd., and Maidstone, W. R. Balston Ltd., 1952.

2. Bernardin, L. J. and Piper, C. V., Tappi 41, no. 1: 16 - 18 (Jan., 1958).

3. Block, Richard J., Durrum, Emmett L. and Zweig, Gunter, "A Manual of Paper Chromatography and Paper Electrophoresis," Second Edition, Academic Press Inc. Publishers, New York, 1958.

4. Boggs, L. A., Anal. Chem. 24, no. 10: 1673-5 (Oct., 1952).

5. Brimley, R. C. and Barrett, "Practical Chromatography" London: Chapman & Hall Ltd., Reinhold Publishing Corporation, New York, 1953.

6. Cramer, Friedrich, "Paper Chromatography," London, Macmillan & Co. Ltd., New York, St. Martin's Press, 1954.

7. Das, D. B., Mitra, M. K., and Wareham, J. F., Nature 174, no. 4422: 228-9 (July 31, 1954).

8. Das, D. B., Mitra, M. K., and Wareham, J. F., Nature 174, no. 4440: 1058-9 (Dec. 4, 1954).

9. Dickey, E. E., Tappi 38, no. 3: 138-9A.

10. Dickey, E. E., Tappi 39, no. 10: 735-6 (Oct., 1956).

11. Dimler, R. J., Schaefer, W. C., Wise, C. S. and Rist, C. E., Anal. Chem. 24, no. 9: 1411-14 (Sept., 1952).

12. Fellegi, Jan, Zellstoff u. Papier 7, no. 3: 65-9 (March, 1958

13. Gustafeson, Charley, Paper ja Puu, no. 9: 383-90 (1956).

14. Henze, Joachim, Naturwissenschaften 44, no. 16: 442-3 (1957).

15. Kalkwarf, Donald R., and Frost, Arthur A., Anal. Chem. 26, no. 1: 191-5 (Jan., 1954).

16. Kritchevsky, Gene, and Anderson, Arthur B., J. Am. Soc. 77, no. 12: 3391 (June 20, 1955).

17. La Diega, Guido Noto, Annali di Chimica (Rome) 46, no. 12: 1208-16 (1956).

18. Lea, David C., Tappi 37, no. 9: 393-9 (Sept., 1954).

19. Macmillan, W. G., Sengupta, A. B., and Dutt, A. S., Nature 178, no. 4546: 1346-7 (Dec. 15, 1956).

20. Mazumdar, A. K., and Sarkar, P. B., Nature 172, no. 4388: 1047-8 (Dec. 5, 1953).

21. McCready, R. M., and McComb, E. A., Anal. Chem. 26, no. 10: 1645-7 (Oct., 1954).

22. "Paper Chromatography," Encyclopedia of Chemical Technology, The Interscience Encyclopedia Inc., New York, Vol. 3: 929.

23. Pridham, John B., Anal. Chem. 28, no. 12: 1967-8 (Dec., 1956).

24. Quick, Robert H., Anal. Chem. 29, no. 10: 1439-41 (Oct., 1957).

25. Saeman, Jerome F., Moore, Wayne E., Mitchell, Raymond L., and Millett, Merrill A., Tappi 37, no. 8: 336-43 (Aug., 1954).

26. Strain, Harold H., Sato, T. R., and Engelke, Hohn, Anal. Chem. 26, no. 1: 90-100 (Jan., 1954).

27. Thoma, John A., and French, Dexter, Anal. Chem. 29, no. 11: 1645-8 (Nov., 1957).

28. Timell, T. E., Glaudemans, C. P. J., and Currie, A. L., Anal. Chem. 28, no. 12: 1916-20 (Dec., 1956).

29. Williams, T. I., and Weil, H., Nature 170: 503 (1952).

30. Wittwer, Rudolf, Faserforschung und Textilechnik 6, no. 2: 58-62 (Feb., 1955).

31. Wood, Scott E., and Strain, Harold H., Anal. Chem. 26, no. 2: 260-4 (Feb., 1954).

EXPERIMENTAL DESIGN

Using the procedure selected under "Conclusion" in the literature survey, it was decided to analyze quantitatively, the hydrolyzates of three NSSC pulps of different yields. Unbleached NSSC pulps of approximately 80% and 70% yield and a bleached pulp made from the 70% yield NSSC pulp were the three pulps which were chosen to be evaluated. If time permits, additional pulp hydrolyzates will be analyzed.

EXPERIMENTAL SECTION

Objective

The object of the experiment was to analyze quantitatively for simple sugars the pulp hydrolyzates from unbleached NSSC pulps of 80% and 70% yields and from a bleached pulp made from the 70% yield NSSC pulp.

Methods and Equipment Used

A pulp sample equivalent to 0.3 g of cellulose was reacted with 3 ml of 72% sulfuric acid at 30°C for one hour. The mixture was stirred as required to put the hollocellulose and some of the extractives into solution. The solution was then diluted with 84 ml of water and heated for one hour in a steam autoclave at 15 p.s.i.

After the strongly acid solution from the autoclave had cooled, approximately 30 g of Amberlite IR45 ion exchange resin was added and the mixture was agitated by means of a magnetic stirrer till a pH of about 4.0 was reached. This took on the average about 45 minutes. The pH value was determined with pH paper. The mixture was then filtered through ordinary filter paper to remove both ion exchange resin and undissolved lignin.

The dilute sugar solution was concentrated to about 20 ml by use of a rotating flask evaporator at a temperature of about 50°C and a pressure of about 20 mm of mercury. The concentrated solution was stored in a refrigerator until it was analyzed by paper chromatographic means. At that time it was allowed to reach room temperature.

All chromatograms were developed in a standard chromatographic cabinet available commercially. The stainless steel interior of the cabinet had inside dimensions of 27½" length x 26" height x 19½" width; it was large enough to accomodate eight chromatographic sheets simultaneously (figure 1). Whatman #1 filter paper was used in all separations, adjusted

so that the solvent flowed cross-direction or perpendicular to the machine direction. The cabinet was located in a constant temperature (72°F) room during all separations.

Exact volumes of the concentrated pulp hydrolyzate, varying from one to thirty microliters each, were applied at three centimeter lateral intervals, four centimeters from the upper edge of the chromatographic sheet. The size of the spot was kept to a minimum by use of a stream of hot air to dry the solution as it was applied. Known reference sugars of known concentrations were also applied in a similar manner on the same sheet.

After all the spots were thoroughly dry, the chromatographic sheet was suspended from a glass trough (figure 2) in the chromatographic cabinet. Small Petri dishes were placed in the bottom of the cabinet and filled with the solvent to be used. The cabinet was then sealed and allowed to come to equilibrium for at least twenty-four hours.

When this saturation period was over, 150 ml of solvent (n-butanol : pyridine : water - 10:3:3 by volume) were added to the trough. The cabinet was sealed again to allow separation to occur. Developing time varied from sixteen to seventeen hours and was not critical as long as the descending solvent front did not reach the bottom edge of the chromatographic sheet.

After separation of the sugars, the sheet was removed from the cabinet, air dried for fifteen minutes at room temperature and, then, oven dried at 105°C for five minutes. The sheet was next lightly sprayed with the color reagent (0.4 g o-amino biphenyl, 100 ml glacial acetic acid, and 20 ml of water), allowed to stand at room temperature for fifteen minutes and finally held at 105°C for five minutes. After this treatment the separated sugars were plainly visible under ultraviolet light.

Qualitative identification of the unknown sugars was

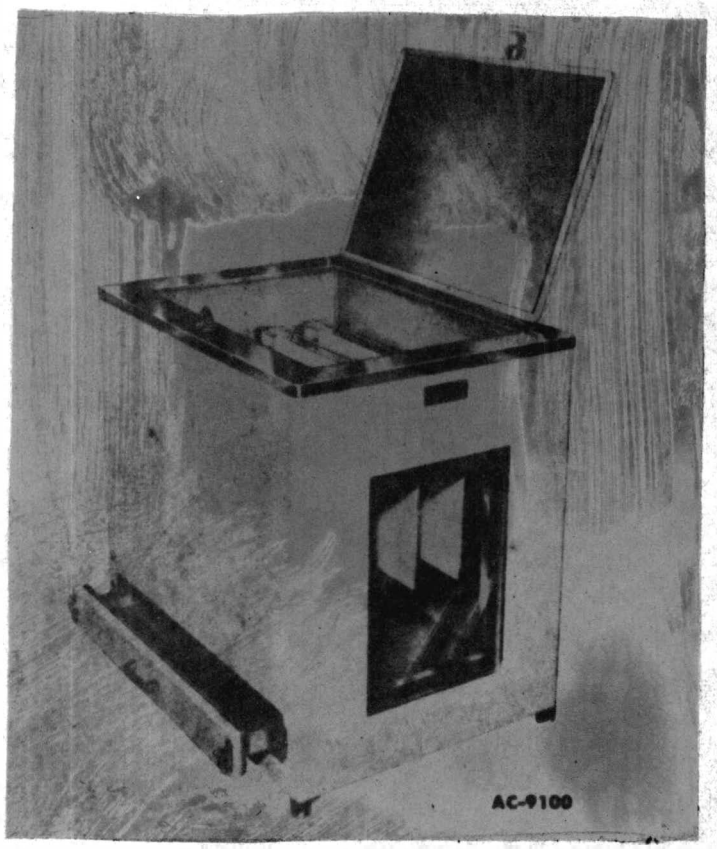

Figure 1: Chromatographic Cabinet

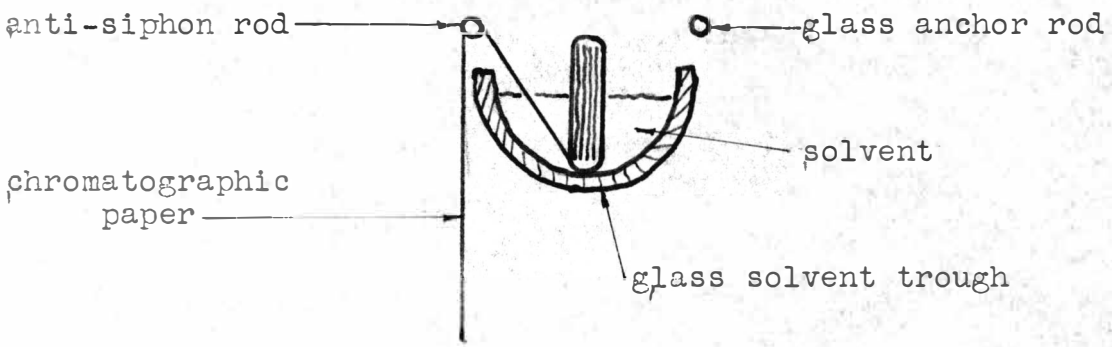

Figure 2: Solvent Trough Assembly

made by comparison with known sugars on the sheet and by calculation of the R_f values of each sugar. For quantitative evaluation of the unknown sugars, the spots were cut from the sheet in small circles of about two centimeters in diameter and each placed in separate test tubes. After the addition of 6 ml of the color reagent, the test tubes were stoppered with corks wrapped in aluminum foil and placed on a mechanical shaker for twenty minutes. This effectively removed the sugars from the chromatographic paper and permitted the use of absorption spectrophotometry for quantitative determinations.

After elution of the sugars, the solutions were filtered through glass wool to remove traces of filter paper and brought to a volume of twelve milliliters with the color reagent. The solutions were heated in boiling water for forty-five minutes to obtain full color development and cooled to room temperature for final analyzing.

The absorbencies of the various solutions were determined at a wave length of 380 millimicrons using a Beckman B spectophotometer. From the absorbencies of known concentrations of the reference sugars, a graph of absorbency versus concentration was plotted. The concentration of the unknown sugars was then simply determined from their absorbency values.

Presentation of Results

The results obtained in the analyses of various pulp hydrolyzates by paper chromatographic means follow:

Table IX

Sugars Found in Various Pulp Hydrolyzates

Type Wood	Type Pulp	% Glucose	% Xylose	% Galactose	% Mannose
Aspen	Unbl. NSSC 80% yield (USA)	82.3	17.3	--	--
Aspen	Unbl. NSSC 70% yield (USA)	79.4	20.6	trace	sl trace

Table IX Cont.

Type Wood	Type Pulp	% Glucose	% Xylose	% Galactose	% Mannose
Aspen	Bl. NSSC 60% yield (USA)	83.1	16.9	trace	sl trace
Birch	Bl. Kraft (Sweden)	80.6	19.4	--	--
Birch	Bl. Soda (Canada)	80.7	19.3	--	--

All results reported above are the mean of at least two analyses.

Interpretation of Results

The increase in the percent glucose found for the 80% yield unbleached NSSC pulp as compared with the 70% yield unbleached NSSC pulp was not expected since it was thought that the more drastic treatment employed to obtain the lower yield pulp should attack the pentose fraction harder than the hexose fraction. However, it must be born in mind that the samples used were commercial pulps from different sections of the country and it is entirely possible that the pulps used were not 100% aspen as was assumed.

The decrease in the pentose content found between the unbleached NSSC pulp and the bleached NSSC pulp was as expected since chlorination under acid conditions followed by alkali neutralization should attack the xylose polymers to a greater extent than the glucose polymers.

It is of interest to note that both the kraft and soda bleached birch pulps closely approached the values found for 70% yield unbleached NSSC aspen pulp in hydrolyzate composition.

CONCLUSION

All hardwood pulp hydrolyzates analyzed tended to fall in a relatively narrow range of chemical composition. Glucose and xylose were found to be the main constituents with traces of mannose and galactose present in some pulp hydrolyzates.

The kraft and soda pulping processes, known to give lower yields than the NSSC pulping process, caused greater removal of the xylose polymers than the NSSC cooking process. The birch hydrolyzate dropped from about 38% xylose in the native wood (13) to approximately 19% in the bleached kraft and soda pulp hydrolyzates, while the aspen hydrolyzate dropped from 29% xylose in the native wood (18) to 17% in the bleached aspen NSSC pulp hydrolyzate. All three processes tended to remove all but traces of the mannose and galactose polymers.

In conclusion it might be noted that the techniques described in this paper lend themselves exceedingly well to relatively quick and accurate determinations of the chemical composition of pulp hydrolyzates.

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