

INTERACTIONS BETWEEN LIGNIN AND CELLULOSE IN ORGANIC LIQUID AND SALINE DENSITY GRADIENT COLUMNS¹

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ABSTRACT

Despite significant differences in their densities, lignin and carbohydrates associate so strongly, even in physical mixtures, that no separation takes place in liquid density gradient columns. Ball-milled cellulose, lignin, and wood exhibit much higher densities in salt gradients than in organic liquid gradients indicating penetration of the saline solutions into voids or "free volume" in the polymers that are inaccessible to organic liquids.

Additional keywords: *Populus tremuloides*, *Picea sitchensis*, ball-milled wood, enzyme lignin, cotton, Avicell.

INTRODUCTION

Wood is a composite material made up of the structural polysaccharides cellulose and hemicellulose and the amorphous phenylpropanoid polymer lignin. These polymers are so closely interrelated in wood that none of them can be completely extracted, even if powerful solvents or reagents are used that dissolve the isolated substances. It is not known whether this behavior merely reflects an intimate physical intermingling of the polymers in wood (incrustation theory) or whether they are interlinked by occasional covalent chemical bonds to form a true graft polymer in the so-called lignin-carbohydrate complex (for a recent review, see Grushnikov and Shorygina 1970).

Little or no lignin can be extracted with organic solvents from sawdust or Wiley-milled wood. However, when wood is subjected to vibratory ball-milling, some lignin of low carbohydrate content (milled wood

lignin, MWL) can be extracted, for example with dioxane-water mixtures (Björkman 1956). The amount of lignin extractable from aspen (*Populus tremuloides* Michx.) and the carbohydrate content of the extract increase with milling time (see Table 1).

The distribution of lignin across wood cell walls is not uniform; the narrow compound middle lamella between cells and the interstices at cell corners contain much higher concentrations of lignin than the broad secondary cell-wall thickenings (Fergus and Goring 1970). Vibratory ball-milling produces particles smaller than wood cell-wall cross sections (Björkman 1956). If lignin and the polysaccharides are merely physically intermixed in wood, it can be conjectured that MWL of low carbohydrate content is derived from the lignin-rich fragments of middle lamellae that have become exposed to solvent by extensive mechanical breakdown of the cell wall during milling. The carbohydrate-rich fractions are derived from secondary cell-wall particles (Sarkanen and Ludwig 1971).

Published values for the density of lignin—about 1.34 g/cc (Stamm 1969)—are much lower than those for cellulose. It therefore seems that physical separation of middle lamella lignin from lignin-carbohydrate complexes of secondary cell-wall origin should occur in liquid density gradient columns (Oster and Yamamoto 1963)

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TABLE I. Amounts and carbohydrate contents of lignin^a from "bone-dry" ball-milled aspen

Milling time ^b Hr	Total lignin extracted	Klason lignin content of extract	Carbohydrate content of extract
	Pct	Pct	Pct
4	27.8	69.1	8.4
6	48.2	57.2	17.4
8	44.7	55.4	18.5
16	63.0	42.8	27.8
42	80.2	45.7	23.9

^aExtractable with dioxane-water (96:4 v/v).^bMilled in air.

and perhaps lead to isolation of purer lignin fractions.

RESULTS AND DISCUSSION

Organic liquid density gradients

A suspension of vibratory ball-milled aspen was dispersed ultrasonically in a mixture of dried, degassed toluene/carbon tetrachloride ($d = 1.300$) and added cautiously to a density gradient column made from the same liquids (density range 1.300–1.590). A sharp band formed at density 1.440; no particles floated at the levels for lignin or cellulose densities.

An enzyme lignin was isolated from Sitka spruce (*Picea sitchensis*) by the method of Pew and Weyna (1962); this had a residual carbohydrate content of 6%. A dispersion of this lignin formed a narrow band at $d = 1.340$ on a toluene/ CCl_4 column. A dispersed sample of ball-milled cotton linters was added to the same column. As the cellulose particles sedimented through the lignin layer, they picked up varying amounts of lignin, so that a distribution of agglomerates resulted between the density of the lignin (1.340) and that of pure cellulose (1.510). Similarly, when milled cellulose sedimented through a layer of milled aspen wood in another toluene/ CCl_4 column, a distribution of agglomerates was obtained between $d = 1.440$ and 1.510. This indicates a very pronounced physical attraction between the cellulose and lignin particles.

This lignin-carbohydrate interaction was further demonstrated: Homogeneous mixtures of 1 g of spruce lignin ($d = 1.340$) with either 4 g of sorbitol ($d = 1.467$ in toluene/ CCl_4 columns) or 4 g of milled linters ($d = 1.510$) were made by prolonged spatulation in toluene/ CCl_4 or by milling for 5 to 30 min, both followed by ultrasonic dispersion. When these mixtures were added to columns, only single sharp bands were formed at $d = 1.432$ (sorbitol/lignin) and 1.465 (cellulose/lignin). The sorbitol could be extracted quantitatively from the initial mixture with water, and the MWL fraction from the cellulose/lignin mixture with dioxane-water (9:1 v/v).

Thus, although the lignin and carbohydrates were not chemically bonded and physical incrustation was not involved, no separation according to density occurred.

Salt density gradients

The interaction of the lignin and carbohydrates to yield particles of homogeneous consistency might be ascribed to hydrogen bonding or to electrostatic charges acquired during milling. Although such forces were not overcome in the nonpolar organic solvents, they ought to be dissipated in strong electrolyte solutions.

Columns were therefore prepared from saturated potassium iodide and water (density range 1.300–1.720) and saturated cesium chloride and water (density range 1.250–1.850). In the KI columns, ball-milled wood again formed only a single narrow band. However, this band occurred at a much higher density, viz. 1.675. Milled cotton cellulose sank to the bottom of KI columns, indicating a density of over 1.720. Enzyme lignin showed a density of 1.450. A 4:1 mixture of milled cellulose and enzyme lignin gave only a single band at $d = 1.680$.

Similar results were observed in CsCl columns. The densities of enzyme lignin, milled wood, and milled cotton cellulose in CsCl columns were 1.449, 1.673, and 1.724. A 4:1 cellulose/lignin mixture gave only a single sharp band at 1.680.

Thus, the physical forces between lignin

TABLE 2. *Densities^a (g/cm³) of wood, lignin, and cellulose in gradient columns*

Column	Enzyme lignin	Wood	Ball-milled cotton	Avicell
Toluene/carbon tetrachloride	1.340	1.440	1.510	1.549
Potassium iodide	1.450	1.675	>1.720	1.650
Cesium chloride	1.449	1.673	1.724	1.648

^aDensity values are expressed in terms of the density of the liquid at the height of the column where the particles come to rest.

and cellulose are too strong to be neutralized even in concentrated salt solutions. Even though vibratory ball-milling may liberate some carbohydrate-free (i.e., chemically unbonded) lignin from wood—as indicated by its solubility in dioxane-water (Table 1)—the physical association of this lignin with the wood polysaccharides precludes its separation from lignin-carbohydrate complex by flotation in density gradients.

Anomalies in densities

The values for the densities of lignin, cellulose, and wood observed in saline columns (Table 2) were much higher than those found in organic liquid columns. A sample of microcrystalline Cellulose I (Avicell) had a density of 1.648 in a CsCl column and 1.650 in a KI column. Ball-milling destroys the crystallinity of cellulose, but upon treatment with water it partially recrystallizes as Cellulose II (Caulfield and Steffes 1969). However, X-ray diffraction showed that material treated with KI or CsCl solution did not recrystallize. It therefore appears that amorphous cellulose has a higher density (ca. 1.724)

than crystalline cellulose (ca. 1.650) in saline systems.

The density values for cellulose in saline columns were higher than any in the literature (Table 3). The density of cellulose in organic liquid columns has been related to its degree of crystallinity (Caulfield and Steffes 1969); higher densities are associated with greater crystallinity. Because the density of Avicell measured in the salt gradients is slightly higher than the maximum density calculated from X-ray data, some residual content of amorphous cellulose in this preparation seems indicated.

An antiparallel relation between the density of cellulose in organic liquids and its higher "apparent" density in water as a buoyancy medium (Table 3) has been discussed by Hermans (1949). The density of a solid measured by liquid displacement is unique only when there is no interaction to change the internal structure and dimensions of the solid or its components. Water penetrates into portions of the cellulose matrix inaccessible to nonswelling organic liquids. Depending on the extent of swelling, an infinite number of displaced volumes are possible and computed density

TABLE 3. *Densities (g/cm³) of cellulose measured by various methods (Ruck 1967)*

	Method			
	X-ray diffraction	Water displacement	Helium displacement	Organic liquid displacement
Cellulose I	1.591-1.630	1.604-1.612	1.567-1.588	1.516-1.553
Cellulose II	1.580-1.660	1.601-1.615	1.531-1.550	1.512-1.536

values have less meaning. The high densities of lignin, cellulose, and wood now found in saline solutions (Table 2) are ascribable to even better swelling and liquid penetration into the free volume (Tobolsky 1960) in the polymers. This enhanced swelling may be due to the large amount of unstructured water present in solutions of high ionic strength (Dobbins 1970). Anomalous high density values would also be obtained using conventional pycnometric procedures (Kellogg and Wangaard 1969), as deeper penetration and even solubilization would result in a reduced displacement volume.

SUMMARY

Attempts to separate physical mixtures of cellulose and lignin in organic liquid and saline solution density gradient columns failed. In spite of appreciable differences in density, such mixtures showed little tendency to fractionate according to their densities, suggesting a strong physical attraction between the two polymers. Densities of the individual components measured in columns of nonswelling liquids are normal. Densities measured in columns capable of swelling the components are anomalously high because of the penetration of the liquid into portions of the free volume of the polymers.

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