

CHEMICAL AND PHYSICAL NATURE OF COLOR BODIES IN  
KRAFT MILL EFFLUENTS BEFORE AND AFTER  
LIME TREATMENT

by

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## ABSTRACT

Untreated and lime-treated wastes from the decker stage of a linerboard mill were acidified to give acid-insoluble and acid-soluble color bodies. These color bodies were then fractionated on Bio-Gel columns into many fractions. The fractionated color bodies were chemically and physically analyzed.

The study shows that color bodies having an apparent "weight average" molecular weight ( $M_w$ ) of less than 400 are not removed by lime treatment and those having  $M_w$  of 5000 and above are completely removed. The molecular weights of all acid-soluble color bodies and lime-treated acid-insoluble color bodies were of approximately the same magnitude.

Infrared spectroscopy data indicate that the acid-insoluble color bodies contain a high proportion of conjugated carbonyl groups where conjugation with an aromatic ring is probable. The acid-soluble fractions seem to contain nonconjugated carboxyl groups and may be associated with carbohydrate material. However, the aromatic nature of color bodies is quite evident. All color bodies possess a negative charge.

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## SECTION I

### CONCLUSIONS

1. Eight series of composite unbleached kraft mill waste samples shipped from Riceboro, Georgia to Appleton, Wisconsin during the investigation showed no appreciable changes in color, total solids, fixed solids, and volatiles during shipment.
2. The lime treatment process under study removed an average of about 86 percent of the color and 57 percent of the total organic carbon from the waste effluent during the period of approximately 15 months over which the samples were collected.
3. The absorbance of radiation by color bodies obeys the Beer-Lambert law in the visible wavelength region but it varies with pH and time of storage of the waste sample.
4. Carbonate content of color bodies was found to increase during processing, freeze-drying and conditioning and was due to absorption of carbon dioxide from the atmosphere.
5. Freeze-drying of the colored wastes will prevent significant changes in color bodies during storage. However, freeze-drying of the color bodies of lime-treated waste caused a significant decrease in their sedimentation coefficient indicating a decrease in molecular weight, or increase in hydration of the sedimenting molecules.
6. Upon redissolution the freeze-dried color bodies gave a precipitate which was mainly silica.

7. Freeze-dried color bodies can be separated into acid-insoluble and acid-soluble fractions by acidification to pH 1.0 with hydrochloric acid followed by filtration. These materials can be further fractionated into various molecular weight ranges on Bio-Gels.
8. In aqueous media color bodies were soluble as salts (especially sodium). Decationization by cation exchange resin resulted in precipitation of color bodies.
9. Infrared spectroscopy shows that the acid-insoluble color bodies contain a high proportion of carbonyl groups (carboxyl, ketone, or both) conjugated with an aromatic ring, whereas the acid-soluble fractions seem to contain nonconjugated carboxyl groups. The acid-soluble material seems to be associated with carbohydrate material.
10. Most of the color bodies are ligninlike in character and appear to consist of lignins which have been degraded to various degrees.
11. Color bodies having a weight average molecular weight less than 400 are not removed by lime treatment; those having molecular weights above 5000 are completely removed. Color bodies in the range of molecular weights 400 to 5000 are partially removed by lime treatment.
12. All color bodies possess a negative charge.



## SECTION II

### RECOMMENDATIONS

Analysis of the data obtained during the work period pinpoints the following recommendations and desirable future studies.

#### Recommendations

1. Because the absorbance of radiation by color bodies varies with pH and time, the spectrophotometric studies must be performed at a constant pH as soon as possible after sampling.
2. Freeze-dried color bodies must be conditioned in air to a constant weight before storage to minimize weight fluctuations caused by carbon dioxide absorption.

#### Suggestions for Future Studies

The following future work would be desirable for further understanding and improvement of the lime-treatment process.

1. Effect of carbohydrate content on the lime-treatment of mill effluents.
2. Effect of multivalent cations on lime treatment of mill effluents.
3. The study of the effect of different degrees of pulping on the molecular weight of color bodies in mill effluents and their subsequent lime treatment.

4. It would be useful to obtain additional UV- and IR-spectra of samples with considerably lower inorganic content. Tentative suggestions regarding possible differences in relative amounts of aromatic and aliphatic molecular units would be worthy of further investigation using nuclear magnetic resonance (NMR) spectroscopy.

### SECTION III

#### INTRODUCTION

Recently, the pulp and paper industry has given major attention to the effect of mill effluents on receiving water color and to the development of means for reducing the discharge of colored organic material to such waters. Major sources of color from the pulp mill are the caustic extraction stage in bleaching, and the unbleached screening and decker filtrates. It is believed that the colored material originates from lignins and lignin derivatives which are washed out of the cooked pulp. Since lignin is highly resistant to microbiological degradation, the color passes through the biological treatment processes. The colored effluents make the receiving waters brownish in color and reduce the light penetration in water. This reduction in light intensity affects aquatic plants by reducing photosynthesis and thereby adversely affects the dissolved oxygen content of water.

The lime-treatment process developed by the National Council of The Pulp and Paper Industry for Air and Stream Improvement (NCASI) is capable of removing about 90 percent of the color from both bleaching and pulping effluents<sup>1-5</sup>. This process has gone through the pilot-plant stage and at present is being used by several mills.

Although the technology of lime treatment is well developed, conflicting results have been reported with respect to the underlying chemistry of the process. However, recent studies by Dence, et al.<sup>6</sup> have shown that the removal of colored material from spent caustic extraction liquor with

lime is a chemical rather than a physical process and that color removal is dependent on (a) the presence of enolic and phenolic hydroxyl groups, and (b) on the molecular weight of solids contained in the liquor. No data on the molecular weight distribution were reported.

This report presents work done on the decker wastes from the Interstate Paper Corporation kraft linerboard mill at Riceboro, Georgia.

The general objective of the project was the isolation of the colored components of the dilute kraft waste liquors before and after lime treatment and their subsequent characterization. It is generally known that such brown-colored materials are complex mixtures of more-or-less acidic polymers which are chemically sensitive. Such materials when isolated frequently tend to condense further into intractable, amorphous solids. Although the colored fractions, herein described, appeared to be reasonably stable one must assume that each separation may have been accompanied by minor chemical changes, at least.

The project approach was divided into three major categories:

1. Initial Characterization and Handling of Colored Wastes.
2. Isolation and Fractionation of Color Bodies.
3. Characterization of Color Bodies.

## SECTION IV

### INITIAL CHARACTERIZATION AND HANDLING OF COLORED WASTES

#### Sampling and Handling

Twenty-four-hour composite samples of untreated decker waste (U-Series), lime-treated waste (LT-Series), and returned waste [supernatant from sludge holding ponds returned to the treatment process (R-Series)] were shipped by air from Riceboro, Georgia to Appleton, Wisconsin. The samples were shipped in five-gallon polyethylene jugs enclosed in especially designed wooden crates provided by The Institute of Paper Chemistry. The usual transit time was two to three days. Samples in transit longer than three days were discarded. Eight series of samples were received over a period of 15 months in this manner. The R-Series were used for purposes of comparison only.

#### Chemical Characterization

Upon receipt at the Institute, representative aliquots of the liquid wastes were chemically analyzed and the data are given in Tables 1, 2, and 3. A comparison of these data with that obtained before shipment from Riceboro, Georgia, indicated that no appreciable changes in color, total solids, fixed solids, and volatiles occurred during shipment of the samples from Riceboro to Appleton under the sampling and shipping conditions recommended by the Institute. Calculation from the data showed that on an average about 86 percent color and 57 percent total organic carbon are removed by the lime-treatment process under study.

TABLE 1

## ANALYTICAL DATA ON KRAFT LINERBOARD UNTREATED DECKER WASTE WATER

Sample Designation	U1	U2	U3	U4	U5	U6	U7	U8	Range	Avg
Month of Sampling	Jan.	March	May	Sept.	Oct.	Dec.	Jan.	March		
pH	10.9	11.6	9.6	10.1	10.3	10.3	11.2	10.2	9.6-11.6	10.5
Color Units at original pH at pH 7.6	905 705	-- 380	-- 560	1100 800	980 660	1100 820	2000 1200	440 300	440-2000 300-1200	1087 680
Sodium, mg/l	341	466	336	372	402	286	443	350	286-466	374.5
Calcium, mg/l	61	132	44	2.4	9.2	14	16	22	2.4-132	28.0
Organic Nitrogen, mg	--	--	1.7	2.4	2.8	--	--	--	1.7-2.8	2.3
Organic Carbon, mg/l	--	125	120	200	190	195	355	125	120-355	186
Total Solids, mg/l	1360	1526	1212	1540	1600	1340	2100	1450	1212-2100	1516
Fixed Solids (Ash), mg/l	920	1241	898	1070	1090	893	1320	1140	893-1320	1073
Volatiles, mg/l	440	285	314	470	510	447	780	310	310-780	443
Carbonate, mg/l	--	--	121	178.5	184	132.5	256	140.5	121-256	172

TABLE 2

## ANALYTICAL DATA ON KRAFT LINERBOARD LIME-TREATED DECKER WASTE WATER

Sample Designation	LT1	LT2	LT3	LT4	LT5	LT6	LT7	LT8	Range	Avg
pH	12.1	12.3	12.1	12.2	12.2	12.2	12.5	12.2	12.1-12.5	12.2
Color Units										
at original pH	250	--	--	360	280	280	400	150	150-400	287
at pH 7.6	105	90	50	160	120	120	170	60	50-170	109
Sodium, mg/l	306	405	310	414	411	264	434	396	264-434	367.5
Calcium, mg/l	488	505	440	335	340	378	407	456	335-505	419
Organic Nitrogen, mg/l	--	--	3.6	3.0	2.1	--	--	--	2.1-3.6	2.9
Organic Carbon, mg/l	--	60	45	110	90	100	120	45	45-120	80
Total Solids, mg/l	2060	2239	1818	2080	1920	1790	2220	2210	1790-2239	2042
Fixed Solids (Ash), mg/l	1630	1827	1420	1580	1560	1380	1730	1740	1380-1827	1608
Volatiles, mg/l	430	412	398	500	360	410	490	470	398-500	434
Carbonate, mg/l	--	--	171	150	82.5	96.5	217.5	250	82.5-250	161.2

TABLE 3

ANALYTICAL DATA ON RETURNED WASTE WATER  
FROM LIME-ORGANIC-SLUDGE HOLDING PONDS

Sample Designation	R1	R2	R4	R5	R7	R8	Range	Avg
pH	11.6	11.4	11.5	11.5	11.6	11.4	11.4-11.6	11.5
Color Units								
at original pH	855	--	520	860	860	900	520-900	779
at pH 7.6	660	580	320	480	500	520	320-660	501
Sodium, mg/l	--	500	368	417	520	578	368-578	475
Calcium, mg/l	62	34	0.8	2.0	4	< 2	1-62	17
Organic Nitrogen, mg/l	--	--	1.5	1.6	--	--	1.5-1.6	1.6
Organic Carbon, mg/l	--	156	103	178	220	196	103-220	171
Total Solids, mg/l	1490	1577	1380	1640	1900	2040	1380-2040	1671
Fixed Solids (Ash), mg/l	1060	1238	1090	1240	1470	1560	1060-1560	1276
Volatiles, mg/l	430	339	290	400	430	480	290-480	395
Carbonate, mg/l	--	--	255	292	344	390	255-390	320



## Spectrophotometric Examination of Liquid Wastes

The ultraviolet and visible spectra of the waste samples of Series One (U1, LT1, R1) and -Two (U2, LT2, R2) were found to be similar and representative of other series. The absorption characteristics of Series Two, discussed in the following sections, may be considered to apply to all samples.

The spectra were recorded with a Beckman Model DK-2 ratio recording spectrophotometer, at the original pH and pH 7.6. Distilled water was used as a reference for all of the samples. The following was observed.

### Visible Spectra

- a. All of the samples exhibited an increase in absorbance as the wavelength decreased (750-350 nm).
- b. None of the samples contained an absorption maximum in this region.
- c. The untreated waste (U2) and the sludge pond supernatant (R2) gave comparable absorbance values.
- d. The lime-treated waste (LT2) exhibited lower absorption than the other two samples through this region.
- e. After adjusting the waste samples at pH 7.6 a general decrease in absorbance was observed.

### Ultraviolet Spectra

- a. The samples exhibited increased absorbance as the wavelength decreased (350-230 nm).

- b. The absorption patterns of U2 and R2 samples were quite similar. R2 gave higher absorption values.
- c. LT2 samples gave the lowest absorption values.

The effects of dilution, pH, and lime on absorbance were also studied.

#### Effect of Dilution on Absorbance at pH 7.6

Visible spectra were recorded on a series of four dilutions of the U2 sample. The pH was adjusted to 7.6 in each case.

The decreases in absorbance values with dilution were found to be linear, demonstrating that the Beer-Lambert law was being observed in the visible region.

#### Effect of pH on Absorbance at Constant Dilution

The pH of the U2 sample was varied between 11.3 and 2.2 in six steps. The absorbance data illustrated that the lowest pH value gave the lowest absorbance value. As the pH increased, the absorbance values increased until a pH of approximately 5 was obtained. Further pH increases resulted in lowering of the absorbance values to a pH of approximately 6.7. Near this point, the absorbance values then increased with increasing pH values. A duplicate run after 3 days showed the same trend. The reason for the higher absorbance at pH 5.0 was not pursued as it falls out of the scope of this project.

The rate of absorbance change per unit of pH change was found to be greater at the lower wavelengths.

### Effect of Time on Absorbance

Absorption spectra of the U2 sample at pH 7.6 were run after 0, 3, 6, and 24 hours. All samples exhibited an increase in absorbance as the wavelength decreased (700 to 230 nm). Absorbance at three wavelengths (420, 280, and 254 nm) registered a decrease in absorption with increase in storage time from 0 to 24 days. Absorbance values measured at all wavelengths after three days were higher than those measured at zero days, and the absorbance value at 254 nm after 24 days was higher than that of six days. The exact reason for such behavior is not known.

The above study suggests that in order to be able to compare different wastes, the spectrophotometric study should be performed at a constant pH and that in case the liquid waste cannot be analyzed immediately upon receipt, it should be processed under mild conditions and stored in a state in which minimum possible changes occur (see following).

### Waste Storage

Reductions in color were observed during the storage of wastes in the liquid form over longer periods, even at 5°C. Freeze drying of the colored wastes was found to prevent appreciable changes in color bodies. Freeze-dried material was readily soluble in water.

It was thought that any appreciable physical change occurring in color bodies during freeze drying could be detected by observing changes in the sedimentation coefficient values of the color bodies before and after freeze drying.

The sedimentation coefficient, defined as the velocity of sedimenting molecule per unit field, is a function of the anhydrous molecular weight of the sedimenting substance and the partial specific volume of the solute. It decreases with decrease in molecular weight, and increase in hydration of the sedimenting molecule.

The sedimentation coefficients were determined according to the method described by Schachman<sup>6</sup> with the ultracentrifuge. The results indicated that, although some variation occurred, the U and R samples showed little average decrease in sedimentation values whereas the LT samples showed consistently an average decrease of over 45 percent. Obviously, either the molecular weight of the substance is decreasing or the size and hydration of the molecule is increasing. However, the reason for the decrease in sedimentation coefficients was not further investigated.

It should be pointed out here that upon redissolution in water the freeze-dried material gave an opaque colorless sediment which upon emission spectrographic analysis was found to be mainly silica. Microscopic investigation under polarized light also showed the presence of starch. It is possible that the loss of these materials from the colored solutions resulted in lower sedimentation coefficients of the freeze-dried color bodies.

The handling and freeze drying of the wastes is explained in the experimental part of this report. Chemical analysis of the freeze-dried color bodies of all samples (U-, LT-, and R-Series) are given in Tables 4, 5, and 6. The sludges obtained by centrifuging the carbonated colored

TABLE 4

ANALYTICAL DATA ON FREEZE-DRIED COLOR BODIES  
FROM UNTREATED WASTE WATER

Sample Designation	U2-1C	U3-1C	U4-1C	U5-1C	U6-1C	U7-1C	U8-1C	Range	Avg
Sodium, percent	24.8	23.4	24.4	25.4	24.0	23.8	28.8	23.4-28.8	24.9
Calcium, percent	0.3	0.5	0.1	0.2	0.1	0.1	0.2	0.1-0.5	0.2
Nitrogen, percent	0.05	0.07	0.1	0.06	--	--	--	0.05-0.1	0.07
Chloride, percent	--	--	0.5	0.7	0.82	0.6	0.96	0.5-0.96	0.71
Organic arbon, percent	10.4	10.9	16.6	17.4	18.1	19.6	9.6	10.4-19.6	14.7
Total Solids, g/100 ml	0.12	0.09	0.11	0.11	0.09	0.17	0.12	0.09-0.17	0.00
Fixed Solids (Ash), percent	84.0	78.3	74.3	78.3	73.7	69.1	85.5	69.1-85.5	77.6
Volatiles, percent	16	21.7	25.7	21.7	26.3	30.9	14.5	16-30.9	22.4
Carbonate, percent									
in Total Solids	18.6	6.4	16.4	16.6	11.4	12.5	7.6	6.4-18.6	12.8
in Fixed Solids	--	9.4	25.1	23.1	17.2	24.6	10.0	9.4-25.1	18.2

All values calculated on the basis of od total solids taken as 100 percent.

TABLE 5

ANALYTICAL DATA ON FREEZE-DRIED COLOR BODIES  
FROM LIME-TREATED WASTE WATER

Sample Designation	LT2-1C	LT3-1C	LT4-1C	LT5-1C	LT6-1C	LT7-1C	LT8-1C	Range	Avg
Sodium, percent	28.5	26.6	27.2	28.2	26.4	29.1	28.6	26.4-29.1	27.8
Calcium, percent	0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1-0.2	<0.1
Nitrogen, percent	0.04	0.04	0.4	0.04	--	--	--	0.04-0.4	0.13
Chloride, percent	--	--	0.6	0.79	0.98	0.6	0.78	0.6-0.98	0.73
Organic Carbon, percent	5.5	6.00	11.2	10.3	11.3	11.1	6.3	5.5-11.3	9.0
Total Solids, g/100 ml.	0.11	0.08	0.1	0.09	0.095	0.124	0.11	0.08-0.124	0.1
Fixed Solids (Ash), percent	90.6	52.2	81.4	84.4	82.5	83.4	90.2	52.2-90.6	80.7
Volatile, percent	9.4	47.8	18.6	15.6	27.8	16.6	9.8	9.8-47.8	19.3
Carbonate, percent									
In Total Solids	18.6	6.4	16.4	16.6	11.4	17.1	9.8	6.4-18.6	13.8
In Fixed Solids	--	9.4	25.1	23.1	17.2	24.4	11.0	9.4-25.1	15.0

TABLE 6

ANALYTICAL DATA ON FREEZE-DRIED COLOR BODIES  
FROM RETURNED WASTE WATER

Sample Designation	R2-1C	R4-1C	R5-1C	R7-1C	R8-1C	Range	Avg
Sodium, percent	29.4	27.5	27.4	27.3	29.2	27.3-29.4	28.2
Calcium, percent	0.3	<0.1	<0.1	0.1	<0.1	<0.1-0.3	0.1
Nitrogen, percent	0.08	0.06	0.08	--	--	0.06-0.08	0.07
Chloride, percent	--	0.6	0.9	0.5	0.52	0.5-0.9	0.63
Organic Carbon, percent	12.0	10.3	13.7	13.1	11.5	10.3-13.7	12.1
Total Solids, g/100 ml	0.13	0.1	0.13	0.168	0.17	0.1-0.168	0.12
Fixed Solids (Ash), percent	80.5	83.5	81.1	79.8	82.5	79.8-83.5	81.5
Volatile, percent	19.5	16.5	18.9	20.2	17.5	16.5-20.2	18.5
Carbonate, percent							
In Total Solids	26.0	20.6	18.0	20.2	19.7	18.0-26.0	20.9
In Fixed Solids	--	24.8	25.7	26.5	24.4	24.4-26.5	25.3

wastes were also freeze-dried and analyzed (data not given). These sludges were found to contain mainly fibers, calcium carbonate, and very low amounts of sodium.

Analytical data on the freeze-dried color bodies did not show any particular trend. Fluctuations in the data were found to be due to carbon dioxide absorption during processing, freeze drying, and conditioning of freeze-dried material. Data calculated on a carbon dioxide-free basis showed lesser variations but still no particular trend was evident.

Solutions of freeze-dried color bodies were tested for color, absorbance, and total organic carbon (TOC). The plots of color versus organic carbon, color versus absorbance (254 nm) and absorbance (254 nm) versus organic carbon gave linear relationships. Because no color losses were noticed upon freeze drying nor upon longer storage periods in the dry state, all color bodies were freeze-dried and stored until used for further study.



## SECTION V

### ISOLATION AND FRACTIONATION OF COLOR BODIES

The freeze-dried color bodies contained a large amount of ash. They seem to occur naturally as sodium salts. For characterization of color bodies, it was desirable to isolate them as free color bodies and to separate them from the inorganic constituents. Ion exchange resins, dialysis, sorption on carbon and on synthetic resin, and gel permeation chromatography was tested as means for accomplishing the desired goal.

#### Ion Exchange Resin

Ion exchange resins are often used for removal of mineral constituents from water. When the original wastes or waste fractions from a Bio-Gel column were passed through a column of Amberlite IR-120 (hydrogen form), most of the cations were removed from solution and most of the color bodies remained in solution. Sometimes, insoluble materials separated from the aqueous solution. This could be prevented by addition of up to one volume of 95 percent ethanol per volume of aqueous solution. Sulfates and chlorides in the wastes were converted to sulfuric and hydrochloric acids by the resin and remained with the color bodies in the eluates of a cation exchange column. Although Amberlite IR-120 seemed to be satisfactory for removal of cations, a subsequent treatment to remove mineral acids was needed.

Mixed bed resins were used to remove both cations and anions in a single treatment. When solutions of the original wastes were passed through a column of the mixed bed resin, Amberlite MB3, 15 to 20 percent of the color was retained by the column and could not be recovered. In addition, the solids

passing through the column still contained 3-5 percent ash. This was mainly silica, but its presence in the isolated color bodies was undesirable.

Amberlite MB3 is a mixture of strong exchange resins, Amberlite IR-120 and IRA-140, and thus it is possible that some of the color bodies were sorbed on the strong anion exchange resin. However, when the waste was treated with Amberlite IR-120 and then with the weak anion exchange resin, namely IR-45, 15 to 20 percent of the color was still retained by the anion exchange resin. But in this case, at least a part of the sorbed color could be removed by elution with ammonium hydroxide giving a solution containing ammonium salts with excess of ammonium hydroxide. Presumably, the ammonia could have been removed by evaporation followed by treatment with Amberlite IR-120 to remove ammonium ions but this was not done. A remaining difficulty in any case would still be the presence of silica in the demineralized solutions.

#### Dialysis

Dialysis was tested as a means of isolating the color bodies. Cellulose acetate tubing having an average pore size of 40 A. was used for dialysis at pH 10.5, 7.2, and 2.9. About 50 percent of the color passed through the tube with the mineral constituents.

When a solution of the untreated waste was first treated with Amberlite IR-120 to remove cations, and then was dialyzed against distilled water using a similar tube, about 80 percent of the original color remained in the dialysis bag and presumably was free of at least the main part of the inorganic constituents. Since we were searching for a procedure which would recover all of the color bodies, work on dialysis was discontinued.

### Sorption on Carbon

Carbon is often suggested for removal of color from waste waters especially the small amounts of color remaining after other treatments. Attempts to use Darco Grade 60 (Atlas Chemical Industries, Wilmington, Delaware) for isolation of color bodies from the original wastes were not successful.

When untreated waste was treated with this carbon and filtered, the aqueous filtrate and washings had very light tan color and contained 85 percent of the starting material by weight. The carbon containing color bodies was washed first with 50 percent aqueous ethanol and then with 50 percent pyridine. The aqueous ethanol eluate contained 8.7 percent and aqueous pyridine eluate 5.6 percent of the starting material. The color fractions, however, were contaminated with a small undetermined amount of colloidal carbon which could not be removed by filtration. In addition, some color was irreversibly sorbed on the carbon.

### Sorption on Synthetic Resin

Information supplied by the Rohm & Haas Company representatives indicated that the Amberlite XAD-2 resin is capable of removing color from waste waters. When an aqueous solution of the original waste was passed through a XAD-2 column, no appreciable color was removed. But when the solution was first decationized by IR-120 resin and then passed through a bed of Amberlite XAD-2 resin about one-third of the color was retained and could be removed from the column by elution with 50 percent ethanol.

### Gel-Permeation Chromatography (GPC)

GPC was attempted as a means for obtaining ash-free color bodies from both untreated and lime-treated decker wastes. Bio-Gel P-2 column having an

exclusion limit of molecular weight 2600, a total bed volume of 426 ml and an approximate void volume of 162 ml was used for this purpose.

Fifteen ml of 10 percent solutions of color bodies from the untreated (U5-1C) and lime treated (LT5-1C) were separately fractionated in the above column at flow rates of 0.2 to 0.3 ml/min. Distilled water was used as eluent. The eluate was monitored by a UV source (280 nm) and the fractions were collected at ten-minute intervals. Seventy-seven to ninety fractions were collected in this way and analyzed for color, total solids, fixed solids, volatiles (by difference), absorbance at 280 nm (data not plotted here), and pH.

The data obtained for each fraction were calculated as a percentage of the respective parent sample (untreated or lime treated) and then divided by the volume of that fraction to obtain the percentage of color per milliliter in each fraction. The total solids, fixed solids, and volatiles were all expressed as percentages of the total solids of the parent sample. Figures 1 and 2 are plots of these parameters and of pH against elution volume.

A general comparison of these figures shows that:

- a. Curves of U5-1C and LT5-1C display two major peaks.
- b. The total- and fixed-solids of U5-1C and LT5-1C elute at approximately the same elution volume.
- c. The maxima of the LT5-1C volatile curve at higher elution volumes does not coincide with that of the color curve as is the case with U5-1C.

Figure 3 shows plots of color-to-volatile ratio of U5-1C and LT5-1C fractions versus their respective elution volumes. A general decrease in these ratios

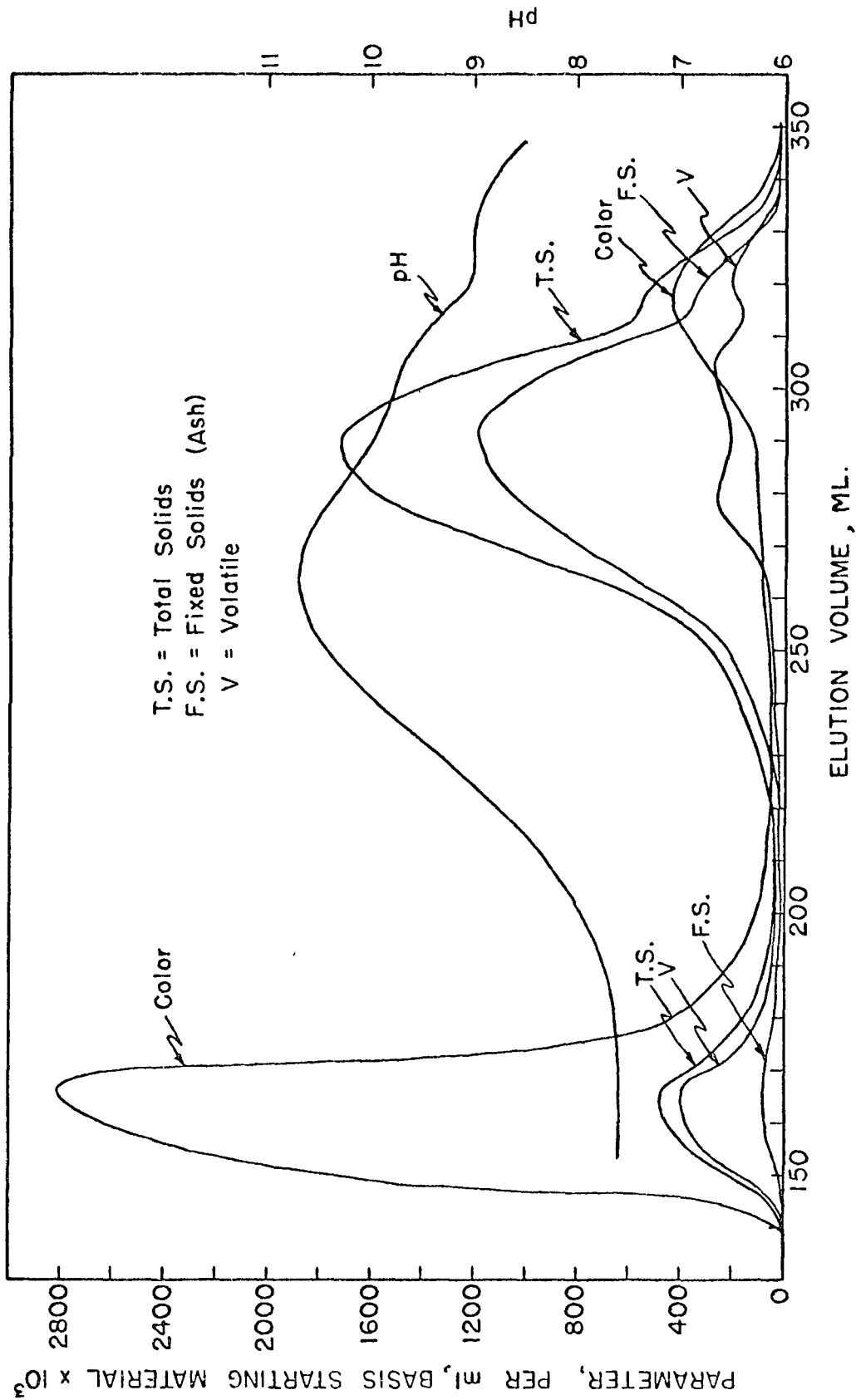


Figure 1. Gel Permeation Chromatography of Untreated Dilute Kraft Mill Decker Waste. Parameter Calculated as Percent of Untreated Waste Present in One ml of the Collected Fraction

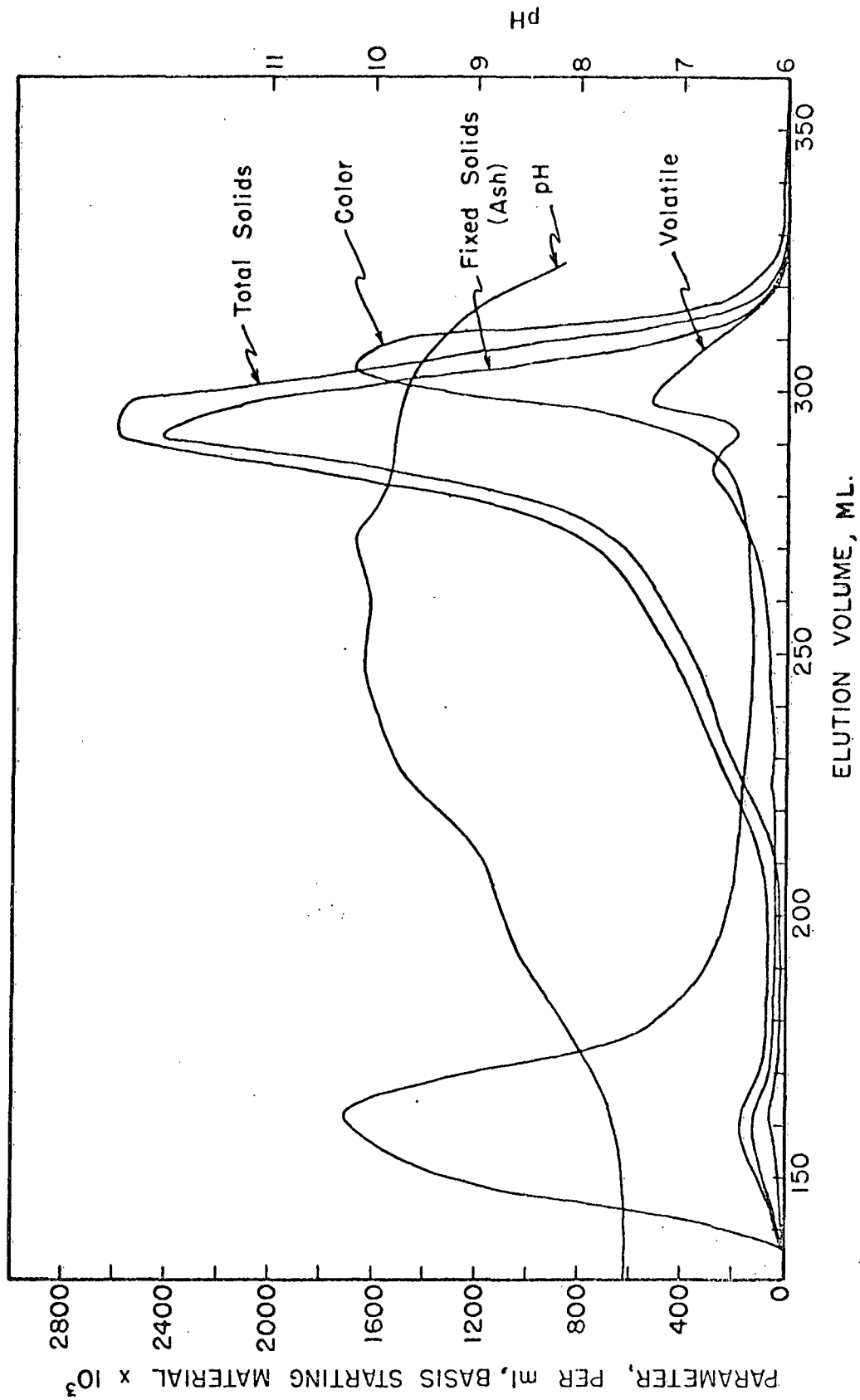


Figure 2. Gel Permeation Chromatography of Lime-Treated Dilute Kraft Mill Decker Waste. Parameter Calculated as Percent of Lime-Treated Waste Present in One ml of the Collected Fraction

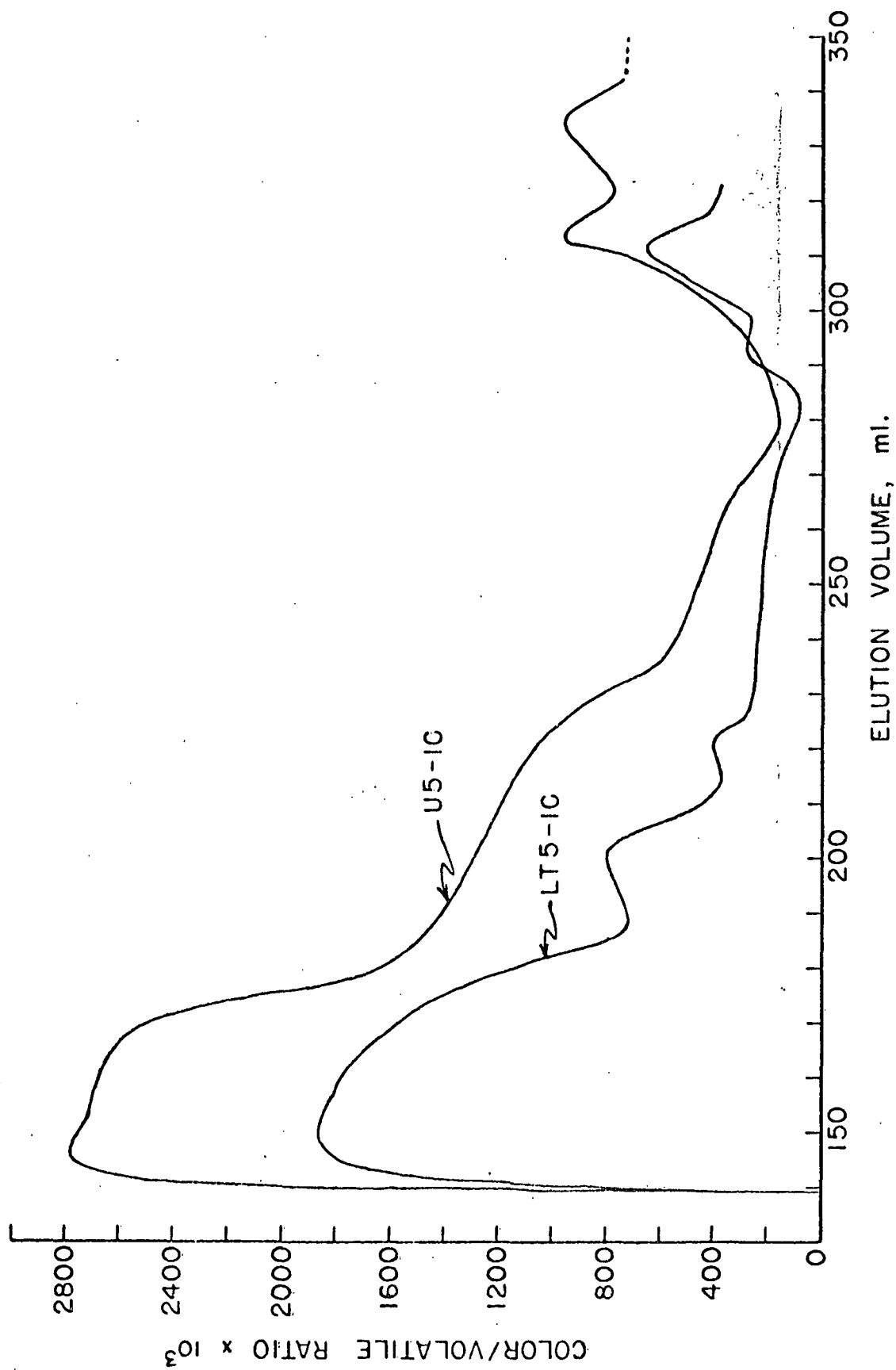


Figure 3. Gel Permeation Chromatography of Untreated (U5-1C) and Lime-Treated (LT5-1C) Dilute Kraft Mill Wastes. Ratio of Color Units to Volatiles x 10<sup>3</sup> Versus Elution Volume

is observed as the elution volume increases. The ratios of LT5-1C fractions as expected, are lower than those of U5-1C.

In order to further evaluate the degree of removal, ratios of the maximum values of the two major peaks of U5-1C and LT5-1C (Figures 2 and 3) were calculated for color, volatile- and fixed-solids. The results are given in Table 7. A significant decrease in these ratios after lime treatment indicates that comparatively higher amounts of color bodies eluting at lower elution volumes (higher molecular weights) are removed during lime treatment.

TABLE 7  
EFFECT OF LIME TREATMENT ON THE FRACTIONS  
OF U5-1C AND LT5-1C WASTES

	U5-1C			LT5-1C		
	Maximum Value at Elution			Maximum Value at Elution		
	Volume of			Volume of		
	167 ml (A)	320 ml (B)	Ratio (A)/(B)	162 ml (A)	305 ml (B)	Ratio (A)/(B)
Color Units	24,000	3,600	6.7	3,000	3,000	1.0
Volatile, percent	2.65	1.39	2.8	67	49.3	1.35
Fixed Solids, percent	0.46	2.16	0.21	0.36	6.8	0.05

As the main aim of GPC, at this point, was to obtain ash-free color bodies, the fractionated U5-1C and LT5-1C color bodies were combined according to the following code to give three large fractions in each case.

Combined Fractions	Approximate Elution Volume, ml
U5-1C (A)	Between 123 to 195
U5-1C (B)	Between 195 to 293
U5-1C (C)	Between 293 to end of colored fraction
LT5-1C (A)	Between 120 to 193
LT5-1C (B)	Between 193 to 283
LT5-1C (C)	Between 283 to end of colored fraction



These combined fractions were then analyzed for ash, volatiles, sodium, and calcium. The weight average molecular weights ( $M_w$ ) were also determined by the ultracentrifuge and results are given in Table 8. The molecular weights of the middle fractions (B) are the lowest in both, U5-1C and LT5-1C, cases. It should be noted that in extremely complex and heterogeneous mixtures, such as these color bodies, the  $M_w$  values should not be taken as absolute values.

TABLE 8  
ANALYSIS OF FRACTIONATED COLOR BODIES

Combined Fraction Designation	Fixed Solids (Ash), percent	Volatile, percent	Sodium, <sup>a</sup> percent	Calcium, <sup>a</sup> percent	$M_w$
U5-1C (A)	26.8	73.2	9.1	0.2	11,400
U5-1C (B)	89.0	11.0	28.3	0.1	74
U5-1C (C)	71.7	28.3	21.5	<0.1	124
LT5-1C (A)	48.7	51.3	13.3	0.27	760
LT5-1C (B)	92.9	7.1	32.9	<0.1	70
LT5-1C (C)	79.9	20.1	26.2	0.1	85 <sup>a</sup>

<sup>a</sup>Analyzed in ash and calculated on respective combined fraction.

The data further indicate that, although fractions containing color bodies of different molecular weights and sizes could be obtained, GPC was not very effective in giving ash free color bodies under these conditions.

Paper chromatography was also tried. The chromatograms produced light tan-colored bands and zones which were separated and eluted to yield seven fractions. Although some fractions were more highly colored than others, all fractions carried tan to brown coloration. Furthermore, the inorganic materials appeared to be spread over several fractions. For this reason this method was also abandoned.

In order to further deash the color bodies, a combination method of acidification and GPC was developed.

### Acidification and Gel Permeation Chromatography

The effluent color was pH dependent. A decrease in pH decreased the color and also precipitated some color bodies.

Although nearly all of the color remained in solution when a dilute solution of the waste was acidified, up to 80 percent of the color bodies in the untreated wastes were precipitated when hydrochloric acid was added to a solution of the original waste which contained 15-20 percent solids. In addition, a large portion of the color remaining in the acid solution could be isolated by sorbing it on Amberlite XAD-2 resin and desorbing with aqueous ethanol. These techniques were used for the isolation of acid-insoluble and acid-soluble color bodies from the untreated (U7-1C) and lime-treated (LT7-1C) freeze-dried solids.

The acid-insoluble (U and L) and acid-soluble (UX and LX) color bodies of both U7-1C and LT7-1C wastes were fractionated into nine to twelve (A to M) fractions using the Bio-Gel P-2 column (200 cm long and 2.5 cm in diameter) having an exclusion limit of molecular weight 2600. The first fraction, "A," from each run was further fractionated into three to seven (A1 to A7) fractions using the Bio-Gel P-60 column (100 cm long, 2.5 cm diameter) having an exclusion limit of molecular weight 60,000. Details are given in the experimental part of this report.

All fractions obtained in this manner were analyzed for color, total organic carbon (TOC) and absorbance at 280 nm. The data were used for calculating percentage yields and removal (by lime) of color and TOC. Results are given in Table 9. All values are calculated on the basis of untreated, unbleached decker waste, U7-1C, taken as 100 percent. Values in parentheses show percentage of color and brackets percentage of TOC.

TABLE 9  
FRACTIONATION OF COLOR BODIES  
Material Balance<sup>a</sup>

Untreated, Unbleached Decker Waste, (U7-1C)			Lime Treatment (-86) [-57.2]			Lime-treated Waste (LT7-1C)		
(100) [100]						(14) [42.8]		
Acid Treatment			Acid Treatment			Acid Treatment		
Acid-insoluble, U	Acid-soluble, UX	Loss	Acid-insoluble, L	Acid-soluble, LX	Loss	Acid-insoluble, L	Acid-soluble, LX	Loss
(63) [59]	(30) [18]	(7) [23]	(3.7) [3.6]	(7.7) [10.2]	(2.6) [2.9]	(3.7) [3.6]	(7.7) [10.2]	(2.6) [2.9]
Fractn. P-2 Gel	P-2 Gel		Fractn. P-2 Gel	P-2 Gel		Fractn. P-2 Gel	P-2 Gel	
UA (49.8) [32.8]	UXA (9.9) [4.2]		LA (1.2) [0.68]	LXA (1.9) [2.0]		LA (1.2) [0.68]	LXA (1.9) [2.0]	
UB (7.6) [6.5]	UXB (7.5) [5.0]		LB (0.3) [0.24]	LXB (0.7) [1.1]		LB (0.3) [0.24]	LXB (0.7) [1.1]	
UC (1.9) [1.1]	UXC (3.4) [2.8]		LC (0.18) [0.22]	LXC (1.5) [2.5]		LC (0.18) [0.22]	LXC (1.5) [2.5]	
UDE (1.8) [1.5]	UXD (3.7) [1.8]		LDE (0.11) [0.35]	LXD (0.7) [1.6]		LDE (0.11) [0.35]	LXD (0.7) [1.6]	
UF (2.5) [5.0]	UXE (3.2) [2.0]		LF (0.5) [0.74]	LXE (1.6) [1.9]		LF (0.5) [0.74]	LXE (1.6) [1.9]	
UG (0.6) [1.5]	UXF (1.6) [1.0]		LG (0.14) [0.11]	LXF (0.2) [0.37]		LG (0.14) [0.11]	LXF (0.2) [0.37]	
UHJ (1.0) [2.3]	UXG (1.8) [1.0]		LHJ (0.21) [0.27]	LXG (0.6) [0.36]		LHJ (0.21) [0.27]	LXG (0.6) [0.36]	
UK (1.5) [4.7]	UXH (0.5) [0.2]		LK (0.4) [0.54]	LXH (0.1) [0.19]		LK (0.4) [0.54]	LXH (0.1) [0.19]	
UL (0.4) [0.2]	UXJ (0.2) [0.07]		LL (0.07) [0.05]	LXJ (0.06) [0.13]		LL (0.07) [0.05]	LXJ (0.06) [0.13]	
UM (1.7) [0.6]			LM (0.17) [0.15]	LXK (0.3) [0.08]		LM (0.17) [0.15]	LXK (0.3) [0.08]	
				LXL (0.04) [0.037]			LXL (0.04) [0.037]	
				LXM (0.02) [0.04]			LXM (0.02) [0.04]	
P-60	P-60		P-60	P-60		P-60	P-60	
UA1 (11.7) [6.8]	UXA1 (0.8) [0.6]		LA1 (0.67) [0.22]	LXA1 (0.07) [0.06]		LA1 (0.67) [0.22]	LXA1 (0.07) [0.06]	
UA2 (19.9) [9.7]	UXA2 (1.2) [1.0]		LA2 (0.38) [0.13]	LXA2 (0.13) [0.25]		LA2 (0.38) [0.13]	LXA2 (0.13) [0.25]	
UA3 (9.8) [3.0]	UXA3 (1.7) [0.87]		LA3 (0.26) [0.26]	LXA3 (0.36) [0.32]		LA3 (0.26) [0.26]	LXA3 (0.36) [0.32]	
UA4 (3.8) [1.8]	UXA4 (2.6) [0.86]			LXA4 (0.42) [0.31]			LXA4 (0.42) [0.31]	
UA5 (2.1) [1.0]	UXA5 (1.8) [0.96]			LXA5 (0.48) [0.14]			LXA5 (0.48) [0.14]	
	UXA6 (0.4) [0.10]			LXA6 (0.07) [0.03]			LXA6 (0.07) [0.03]	
	UXA7 (0.11) [0.17]			LXA7 (0.02) [0.01]			LXA7 (0.02) [0.01]	

<sup>a</sup>Calculated on the basis of untreated, unbleached decker waste, U7-1C; parentheses show color and brackets the TOC values.

Total amounts of material input and output during fractionation fluctuated and some losses were observed in the mass balance. These calculations were based on cumulative values and so the errors per fraction would be a lot smaller. Maximum loss was observed in TOC values when fraction UA was further fractionated through Bio-Gel P-60. In this case 32.8 percent TOC was put on the column and only 22.3 percent (total of UA1 to UA5) was recovered. It is believed that some lower molecular weight material was retained in the column and did not elute out at the collected elution volumes.

Table 9 shows that 86 percent of total color and about 57 percent of total organic carbon (TOC) are removed by the lime treatment process, indicating that the remaining 43 percent TOC contributes to only 14 percent of the total color. It is possible that part of this TOC is in the form of carbohydrate degradation products, resin acids, etc.

In the case of untreated acid-insoluble color bodies (U), 79 percent color ( $49.8 \times 100/63$ ) and 44.6 percent TOC ( $32.8 \times 100/59$ ) were present in the first fraction, UA, whereas in the case of lime-treated acid-insoluble color bodies the first fraction, LA, contained only 32.4 percent color and 18.9 percent TOC.

Acid-soluble fractions, on the other hand, give even lower color values (in fact 33 percent or less) in the first fraction, indicating that in this case more color bodies are of lower molecular weights.

Our experience has shown that GPC runs could not be quantitatively duplicated. However, they showed somewhat similar trends. In order to minimize the experimental error, especially because there was no clear-cut demarcation line between the two adjacent fractions, the data in Table 9 were divided by their respective fraction volumes and the values per ml thus obtained were plotted against eluate

fractions in Figures 4 to 7. (Acid-insolubles and acid-solubles are coded as [ins.] and [sol.], respectively.)

The dotted area between the untreated and lime-treated curves corresponds to the amounts of color, and TOC removed by the lime-treatment process. Absorbance at 280 nm for each fraction was also measured and when plotted as above, gave a pattern similar to that of color. The TOC pattern for the untreated acid-insoluble and lime-treated acid-soluble fractions were not similar to that of their respective color, indicating the presence of some "noncolored" organic carbon in these fractions.

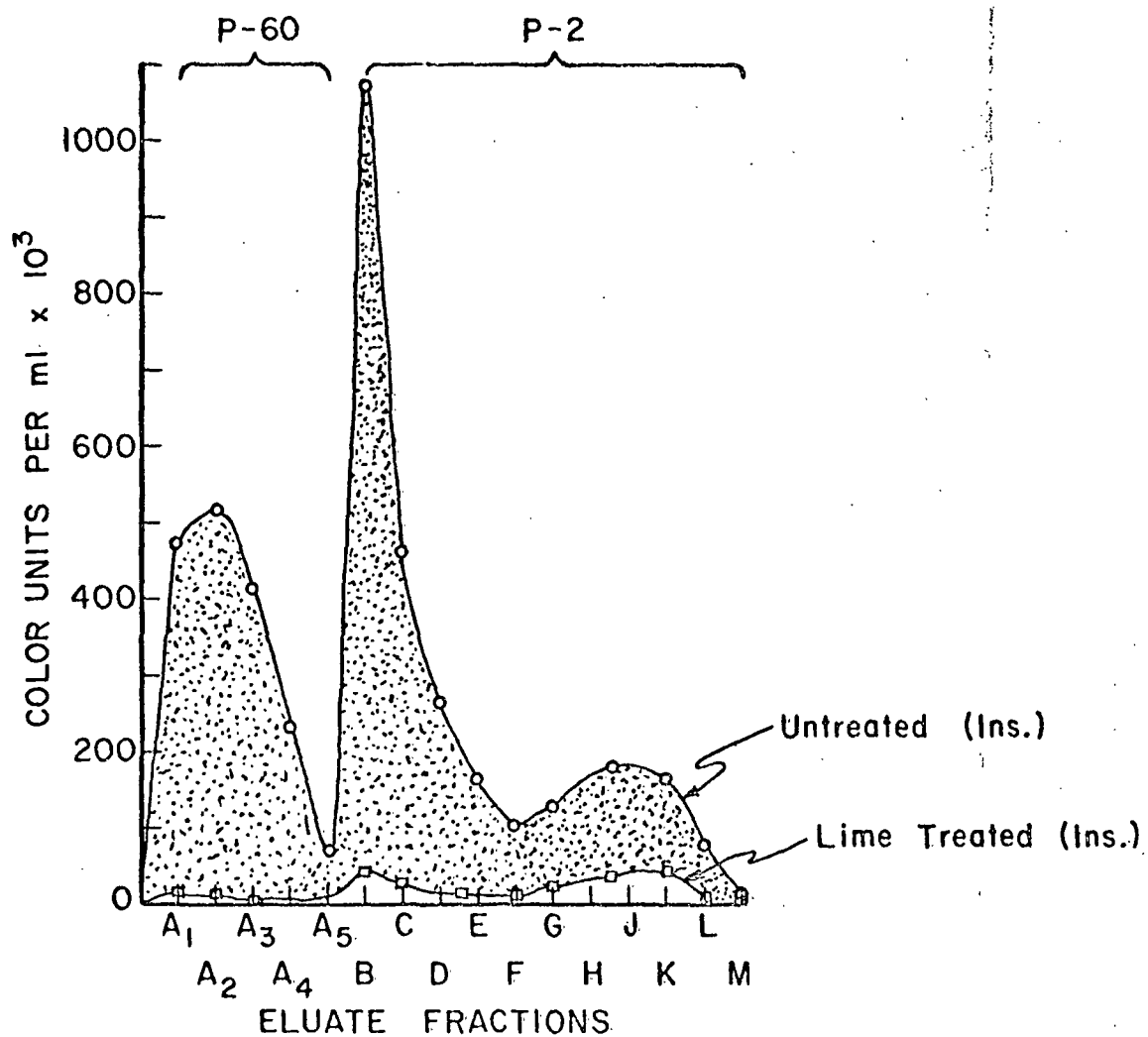


Figure 4. Gel Permeation Chromatography of Acid-Insoluble Color Bodies. Color Units Calculated as Percent of Untreated Waste Present in One ml of the Collected Fraction

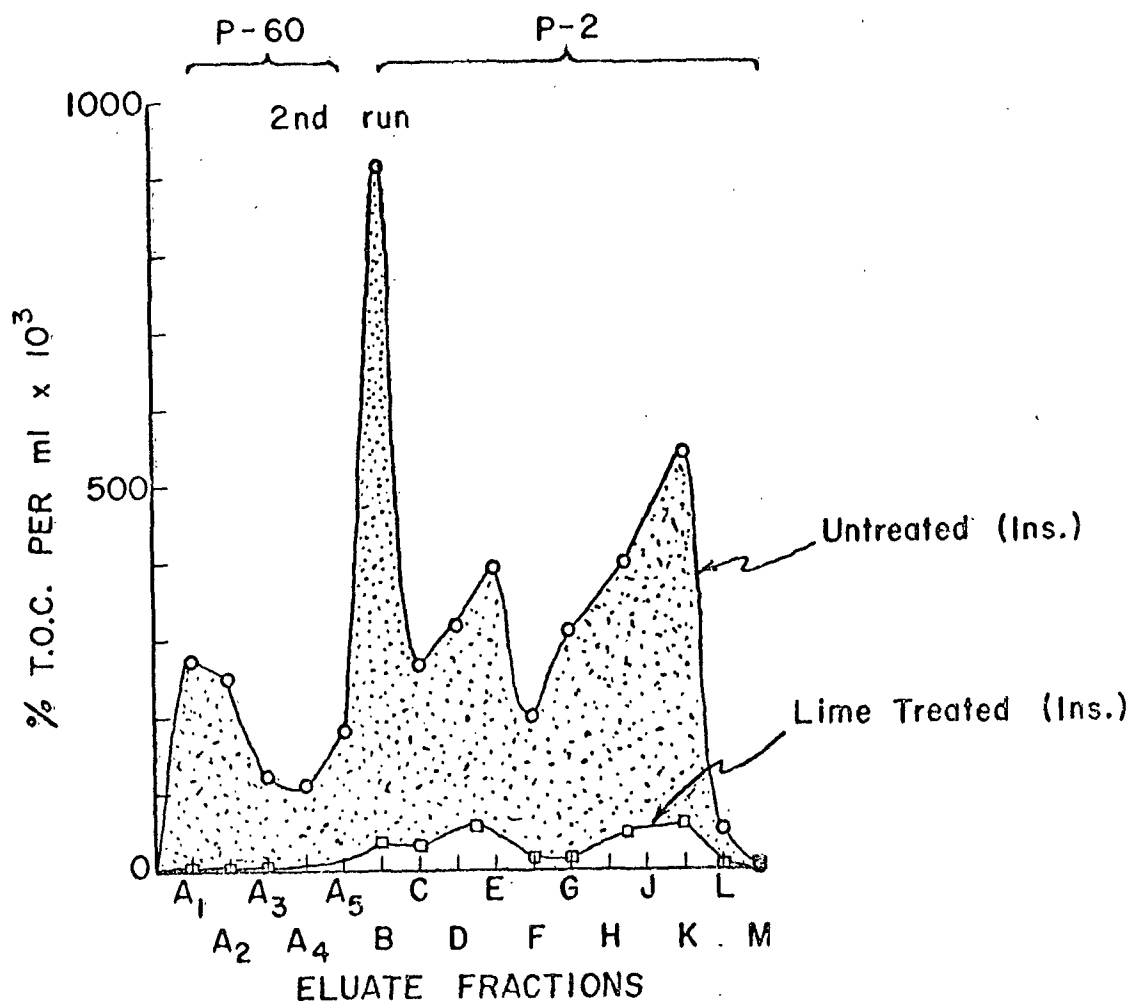


Figure 5. Gel Permeation Chromatography of Acid-Insoluble Color Bodies. TOC Calculated as Percent of Untreated Waste Present in One ml of the Collected Fraction

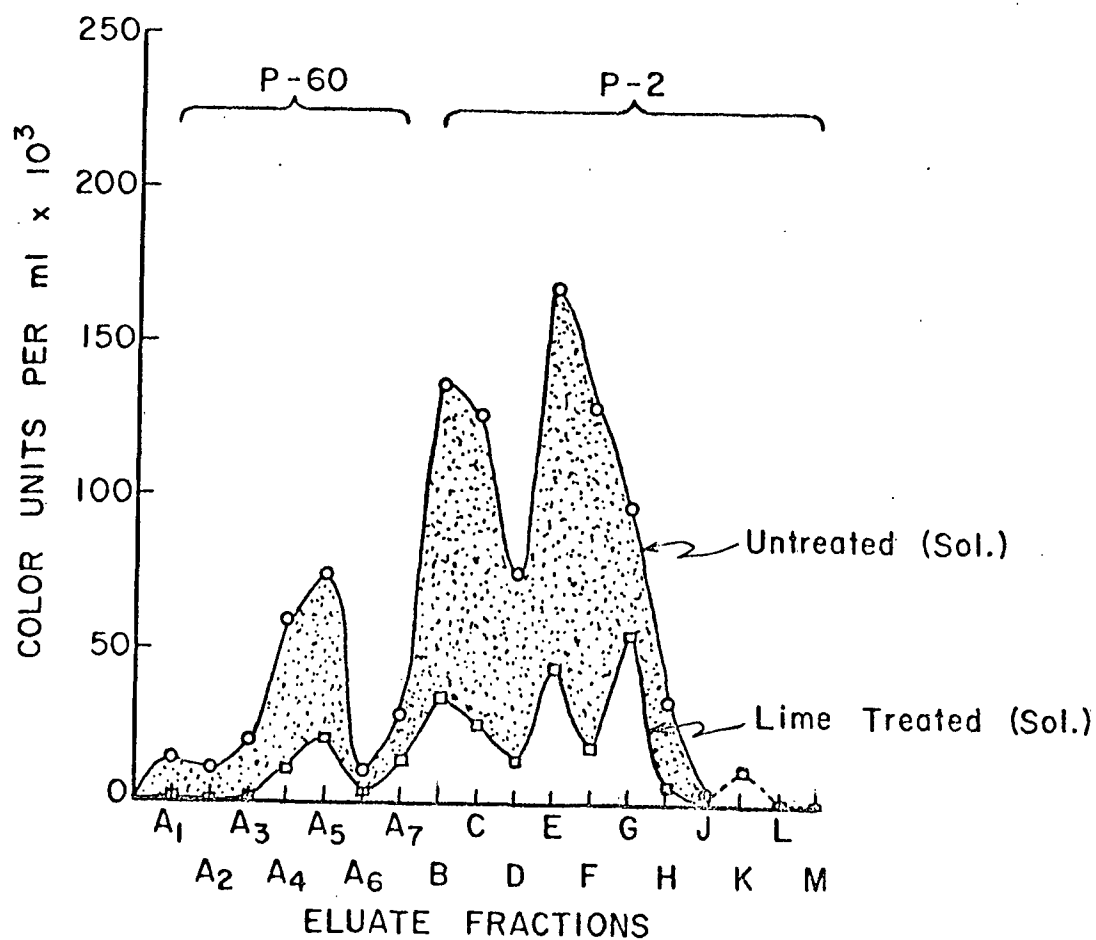


Figure 6. Gel Permeation Chromatography of Acid-Soluble Color Bodies. Color Units Calculated as Percent of Untreated Waste Present in One ml of the Collected Fraction



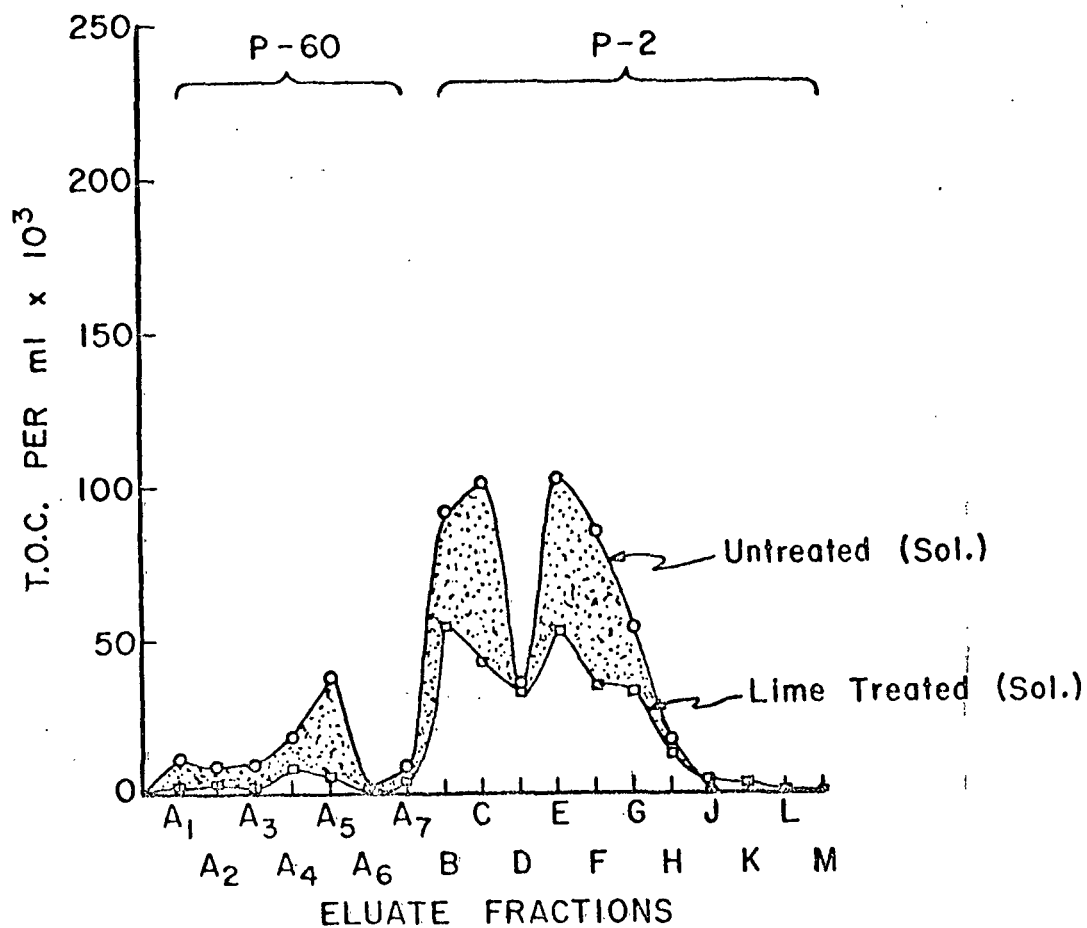


Figure 7. Gel Permeation Chromatography of Acid-Soluble Color Bodies  
 TOC Calculated as Percent of Untreated Waste Present in  
 One ml of the Collected Fraction

SECTION VI  
CHARACTERIZATION OF COLOR BODIES

Elemental Analysis

Acid-insoluble and acid-soluble fractions were isolated from three untreated and two lime-treated wastes. Color of each fraction was determined by the American Public Health Association method (Pt Co), and the recovery of the original color was calculated. Five fractions were analyzed for carbon, hydrogen, methoxyl, nitrogen, and ash. Absorptivities (absorbance/solids in g/l) were determined at 420 nm ( $a_{420}$ ) as a measure of original color, and at 280 nm ( $a_{280}$ ) and the maximum near 200 nm ( $a_{\max}$ ) as a measure of the lignin content. The results are summarized in Table 10. Absorptivities for Indulin 'C' and analytical data for an alkali lignin from pinewood are included in the table. Analytical data were not obtained for Indulin C, but the values for the alkali lignin are believed to be good approximations of the composition of Indulin.

The data in Table 10 include three values which may be used as approximate measures of lignin content, namely: methoxyl, absorptivity at 280 nm and absorptivity at the maximum. Calculated ratios of absorptivity at 420 nm (as a measure of color) to each of these are included in the table.

The data suggest that at least most of the color bodies are ligninlike and that they consist of lignins which have been degraded to varying degrees. The lignin in all of the wastes appear to have lost somewhat more methoxyl than Indulin C, with those in the more soluble fractions having lost the greatest amount of methoxyl.

The ratios of the absorptivity at 420 nm to the absorptivity at 280 nm and at the 'maximum' suggest that, except for some greater loss of methoxyl, the lignins in the acid-insoluble fractions from the untreated wastes are very similar to the

TABLE 10

ACID-INSOLUBLE AND ACID-SOLUBLE FRACTIONS  
(Color Recovery, Analytical Data, and Absorptivity)

Original Waste Fraction	Lignin <sup>a</sup>	U4-1C		U7-1C		U8-1C		LT7-1C		LT8-1C	
		Acid-Ins.	Acid-Sol.	Acid-Ins.	Acid-Sol.	Acid-Ins.	Acid-Sol.	Acid-Ins.	Acid-Sol.	Acid-Ins.	Acid-Sol.
Color recovery, percent	--	84	14	63	30	46	42	26	55	36	54
Carbon, percent	63.4	55.34	--	56.89	47.92	--	--	51.82	48.30	--	--
Hydrogen, percent	5.6	5.25	--	5.30	4.87	--	--	5.55	5.47	--	--
Nitrogen, percent	--	0.36	--	0.33	0.68	--	--	0.25	0.42	--	--
Ash, percent	--	9.78	--	7.81	6.04	--	--	17.18	4.56	--	--
"Oxygen" by Difference, percent	31.0	29.3	--	29.7	40.5	--	--	25.2	41.2	--	--
Methoxyl, percent	14.2	11.23	--	8.51	5.20	--	--	4.09	4.37	--	--
<sup>a</sup> <sub>420</sub>	1.54	1.38	0.74	1.41	1.25	1.09	0.95	0.72	1.04	0.64	0.92
<sup>a</sup> <sub>280</sub>	19.0	16.7	10.5	16.9	12.9	13.2	14.1	10.5	12.1	10.5	12.1
<sup>a</sup> <sub>max</sub>	83.2	77.5	51.6	78.0	51.9	73.1	60.6	56.8	50.0	68.4	63.4
<sup>a</sup> <sub>420</sub> /MeO	0.11	0.12	--	0.17	0.24	--	--	0.18	0.24	--	--
<sup>a</sup> <sub>420</sub> / <sup>a</sup> <sub>280</sub>	0.081	0.083	0.070	0.083	0.097	0.083	0.067	0.069	0.086	0.061	0.076
<sup>a</sup> <sub>420</sub> / <sup>a</sup> <sub>max</sub>	0.018	0.018	0.014	0.018	0.024	0.015	0.016	0.013	0.021	0.009	0.014

<sup>a</sup>Analytical data for an alkali lignin from pinewood, absorptivities for Indulin C.

lignins in Indulin C. The lignins in the acid-soluble fractions and those from the lime-treated wastes appear to have been more degraded.

### Ultraviolet and Visible Spectra

Ultraviolet and visible spectra were determined of Indulin 'A' and acid-insoluble and acid-soluble fractions isolated from the untreated and two lime-treated wastes. All spectra were determined at a pH of about 7.5.

Absorptivity values were calculated from these spectra at definite wavelengths and the results of only U7-1C and LT7-1C series are plotted in Figures 8 and 9.

Figure 8 shows that in the visible range all samples gave simple absorption curves and exhibited an increase in absorptivity as the wavelength decreased. Comparatively, the lime-treated color bodies showed lower absorptivity values than the untreated color bodies. However, the lime-treated acid-insoluble color bodies showed the lowest absorptivity values, even lower than acid-soluble color bodies. This is not surprising as the LT7-1C (ins.) contained only 3.7 percent of the total color and LT7-1C (sol.) contained 7.7 percent (see Table 9).

In the ultraviolet range (Figure 9) one reaches the same conclusions as in the visible range except that here the characteristic absorption bands at 205 nm and 208 nm are obtained.

It can be said that all fractions contain ligninlike color bodies. The differences in absorptivity values can be due to differences in ash contents and different levels of degradation of color bodies.

### Infrared Spectra

Infrared (IR) spectra were determined for acid-insoluble and acid-soluble components of both untreated and lime-treated wastes (Figure-10). The IR spectra were analyzed to see whether or not important functional group differences were associated with

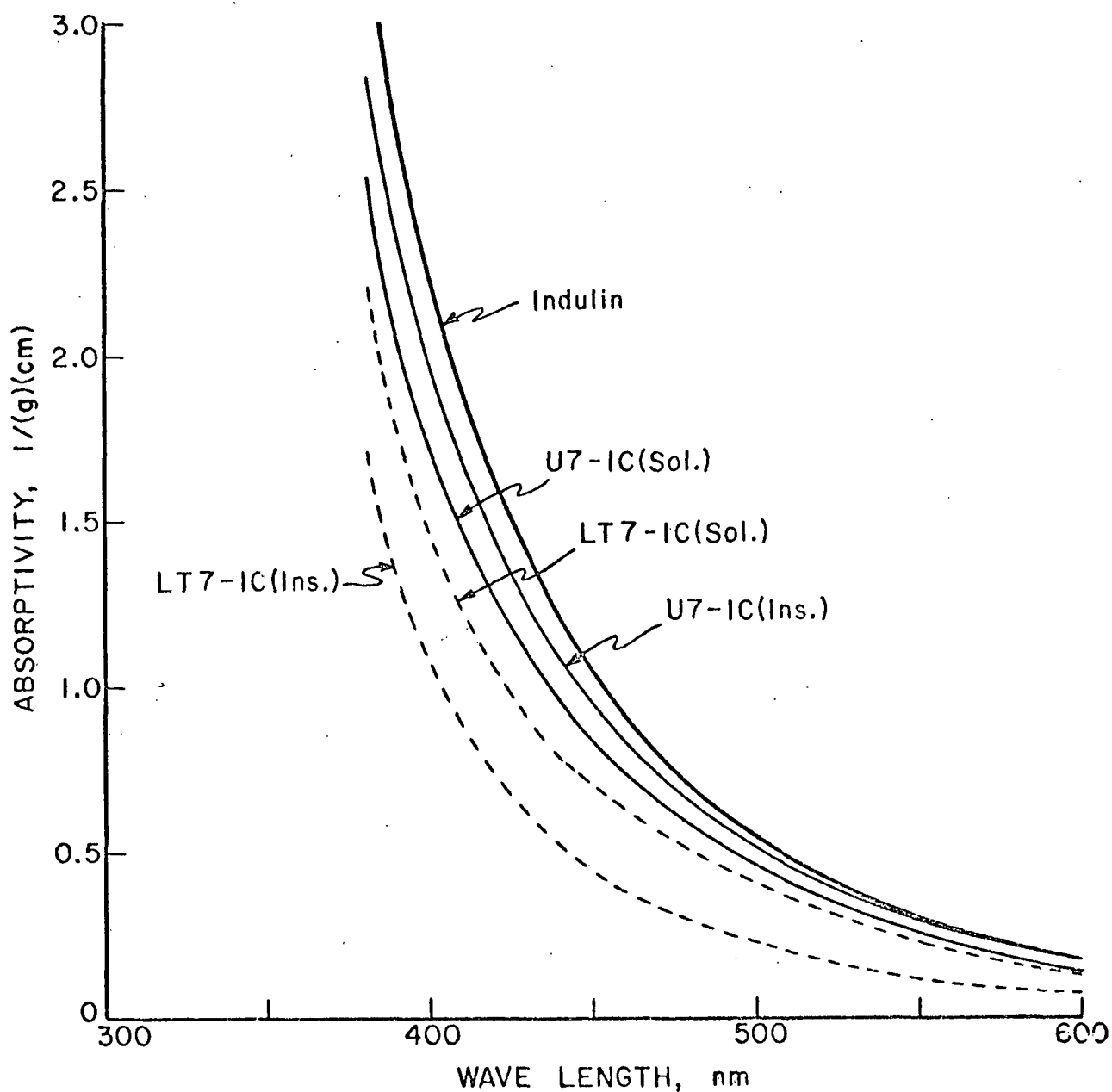


Figure 8. Absorptivity Versus Wavelength (Visible Range) of Indulin "A," Acid-Insoluble and Acid-Soluble Color Bodies of Untreated and Lime-Treated Kraft Mill Decker Wastes

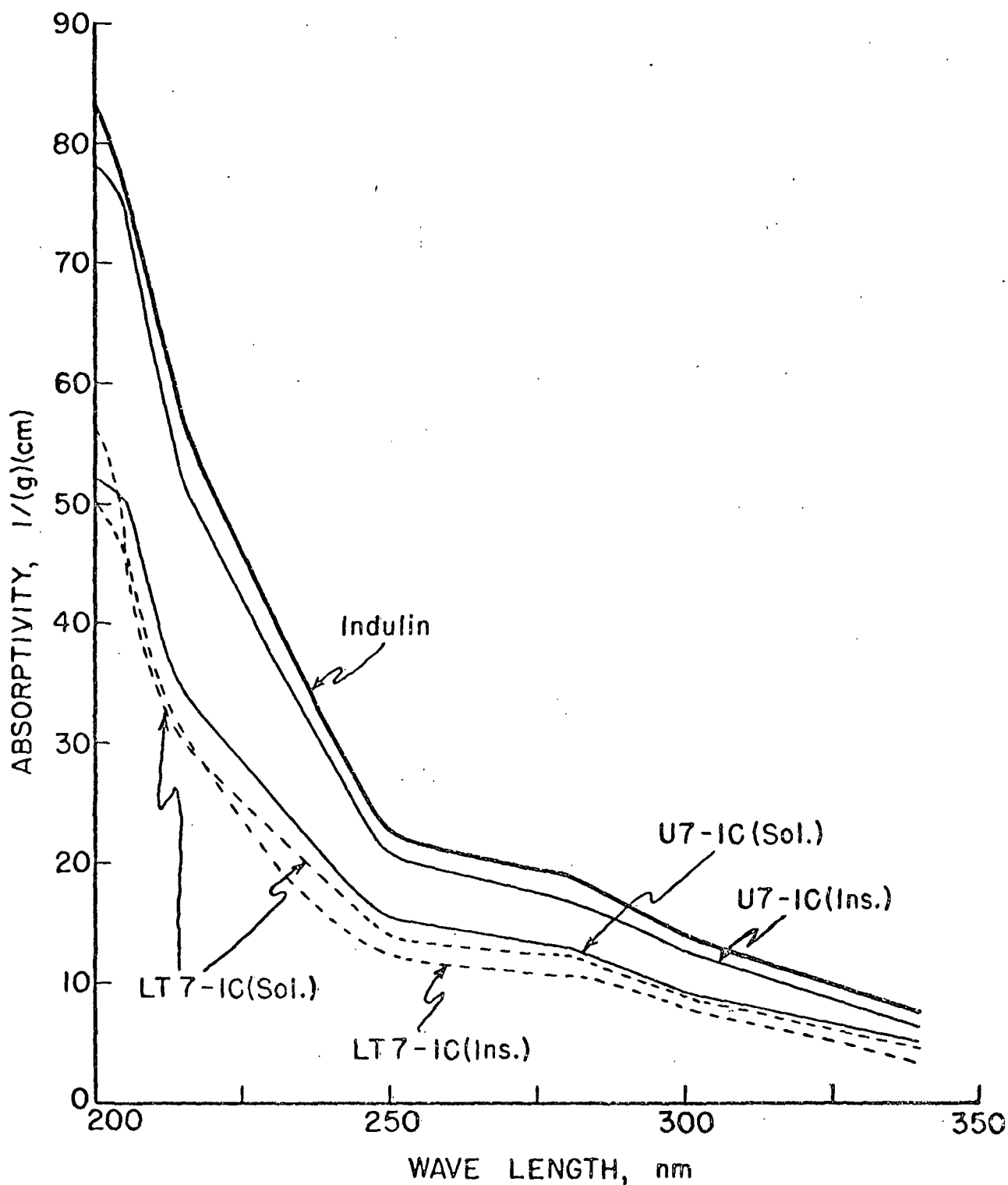


Figure 9. Absorptivity Versus Wavelength (Ultraviolet Range) of Indulin "A," Acid-Insoluble and Acid-Soluble Color Bodies of Untreated and Lime-Treated Kraft Mill Decker Wastes

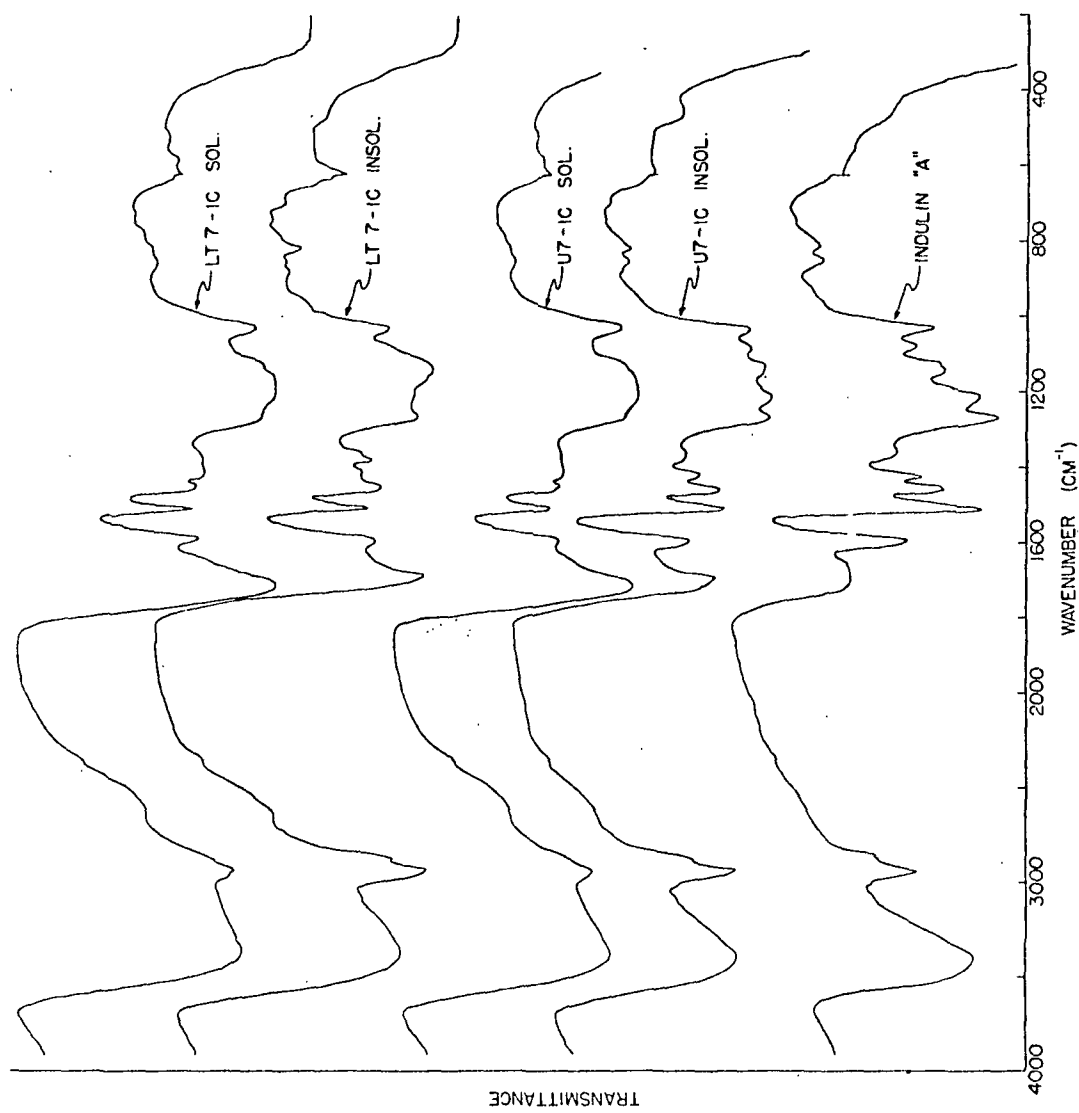


Figure 10. Infrared Spectra of Kraft Mill Decker Effluent Color Bodies

the differences in the treatment prior to isolation. It should be realized that detailed interpretation of many of the absorption bands is not possible because of the complexity of the molecular system, uncertainties always present in comparison of solid state spectra, and the interference by impurities (elemental analyses revealed ash contents from 4.56 to 17.18 percent).

All fractions, as well as Indulin, gave absorption bands at 1460, 1500, and 1600  $\text{cm}^{-1}$ , indicating the presence of aromatic structures. Differences in the intensity of the bands suggested a lower ratio of aromatic to aliphatic groups in the acid-insoluble fractions than in Indulin.

The main differences in the IR spectra were in the absorption bands due to carbonyl groups. Indulin 'A' gave a relatively weak carbonyl band at 1690  $\text{cm}^{-1}$ , whereas the acid-insoluble fractions gave a strong band at about 1695  $\text{cm}^{-1}$  and the acid-soluble fractions gave a strong band at approximately 1715  $\text{cm}^{-1}$ . Different carbonyl stretching frequencies distinguish the acid-soluble from the acid-insoluble samples, but lime treatment itself does not influence these frequencies. The acid-insoluble fractions seem to contain a high proportion of carbonyl groups (carboxyl, ketone, or both) conjugated with an aromatic ring, whereas the acid-soluble fractions seem to contain nonconjugated carboxyl groups.

The intensities of bands normally associated with the methoxyl group correlate well with the experimental methoxyl contents. Comparison of certain bands related to aromatic structures and aliphatic structures suggests possibly marked differences between the acid-soluble and acid-insoluble materials.

The spectra of acid-soluble samples reveal very few, if any, significant differences as a result of lime treatment. The samples appear to be very similar in structure. The acid-insoluble samples show greater differences as a result of lime treatment, but these differences may be entirely due to differences in ash content and methoxyl content.



The broad, intense absorption band near  $1200\text{ cm}^{-1}$  in the spectra of acid-soluble materials may be due, in part, to associated carbohydrate material.

### Molecular Weight Distribution

Molecular weights of some selected fractions of acid-insoluble and acid-soluble components of the untreated and lime-treated color bodies were determined by the sedimentation equilibrium method using the analytical ultracentrifuge<sup>7</sup>. The maximum and minimum values, in other words the molecular weight ranges of the above-mentioned color bodies, are plotted in Figures 11, 12, 13, and 14. The dotted area represents the molecular weight ranges and also indicates the heterogeneity of each fraction. To simplify matters, the apparent "weight average" molecular weights ( $M_w$ ) for each fraction were calculated from the range data and are plotted in Figures 15 and 16.

Figure 15 shows that  $M_w$  of untreated acid-insoluble components are much higher than that of the lime-treated acid-insoluble component. The  $M_w$  values drop sharply, level off, and then rise slightly near fractions "J" and "K." Theoretically, the gel chromatography should yield samples with regularly decreasing molecular weights. The reason for this shift in property of the gel was not pursued.

Acid-soluble color bodies, on the other hand, seem to behave differently (Figure 16). In this case, the A1 and A5 fractions of the lime-treated color bodies show higher  $M_w$  values than that of the untreated color bodies. It seems that some of the higher molecular weight material in the lime-treated color bodies did not precipitate out during the acid treatment, indicating a slightly different character. It should be noted here also that comparatively more color remained in solution when the lime-treated material was acidified (see also Table 9).

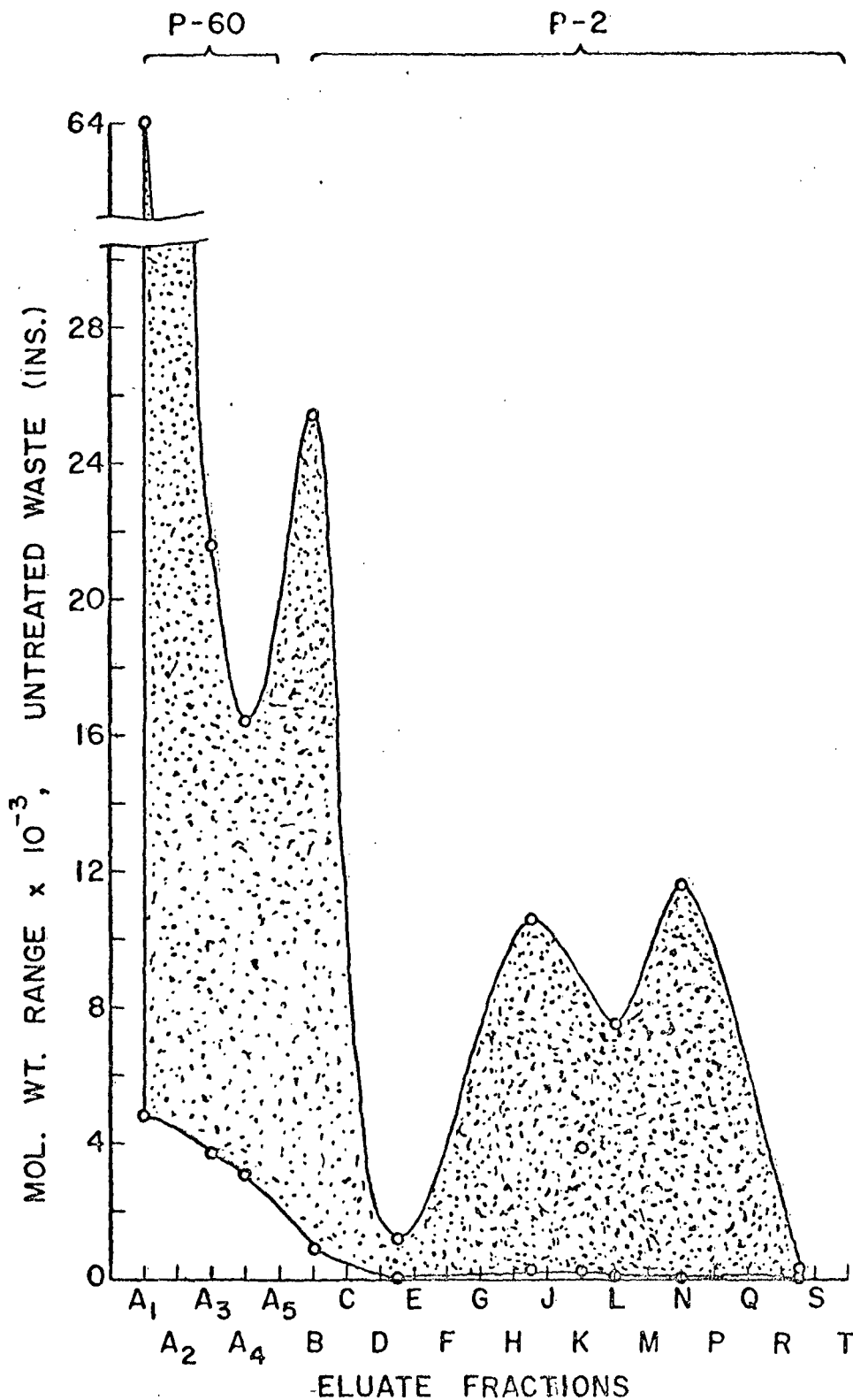


Figure 11. Gel Permeation Chromatography of Untreated Acid-Insoluble Color Bodies of Dilute Kraft Mill Decker Wastes. Molecular Weight Ranges and Distribution

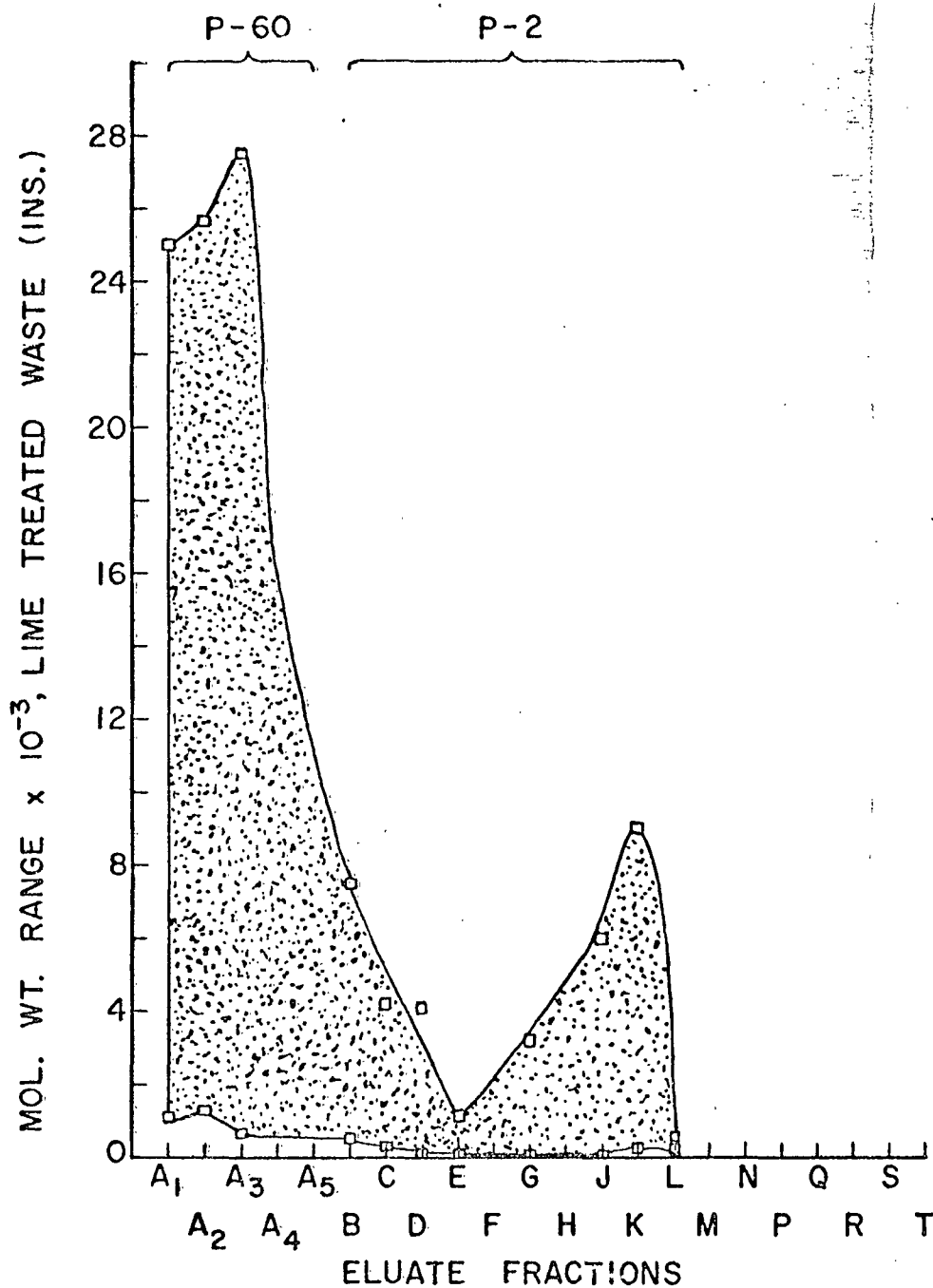


Figure 12. Gel Permeation Chromatography of Lime-Treated Acid-Insoluble Color Bodies of Dilute Kraft Mill Decker Wastes. Molecular Weight Ranges and Distribution

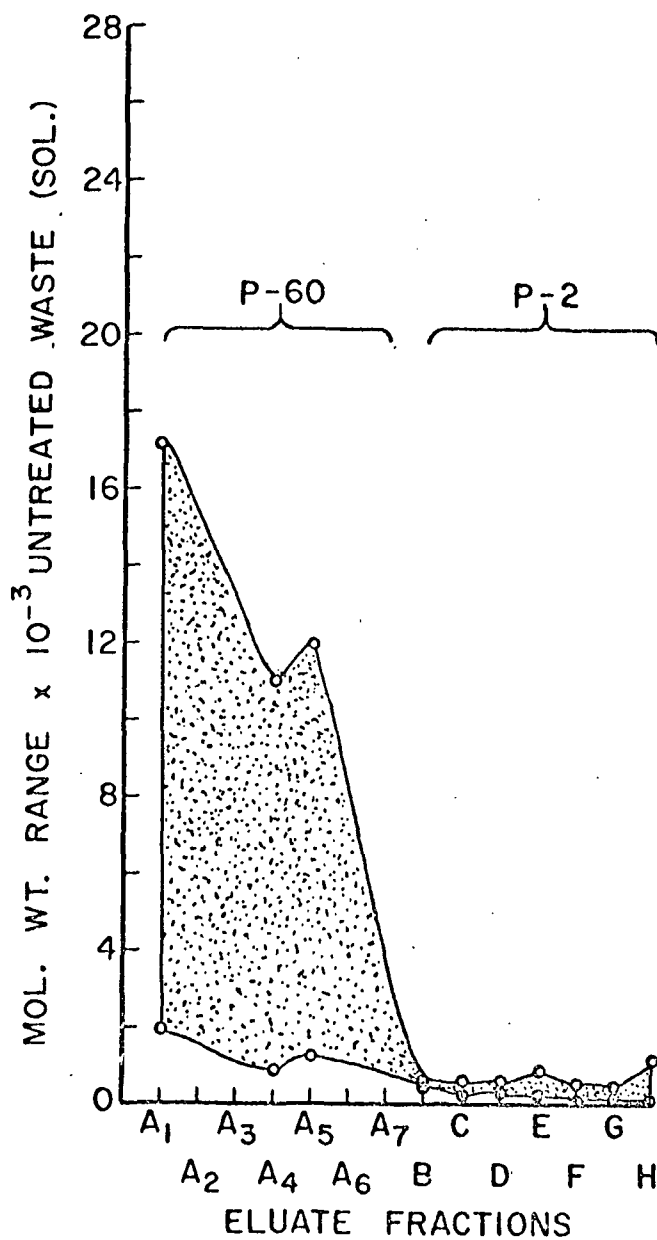


Figure 13. Gel Permeation Chromatography of Untreated Acid-Soluble Color Bodies of Dilute Kraft Mill Decker Wastes. Molecular Weight Ranges and Distribution

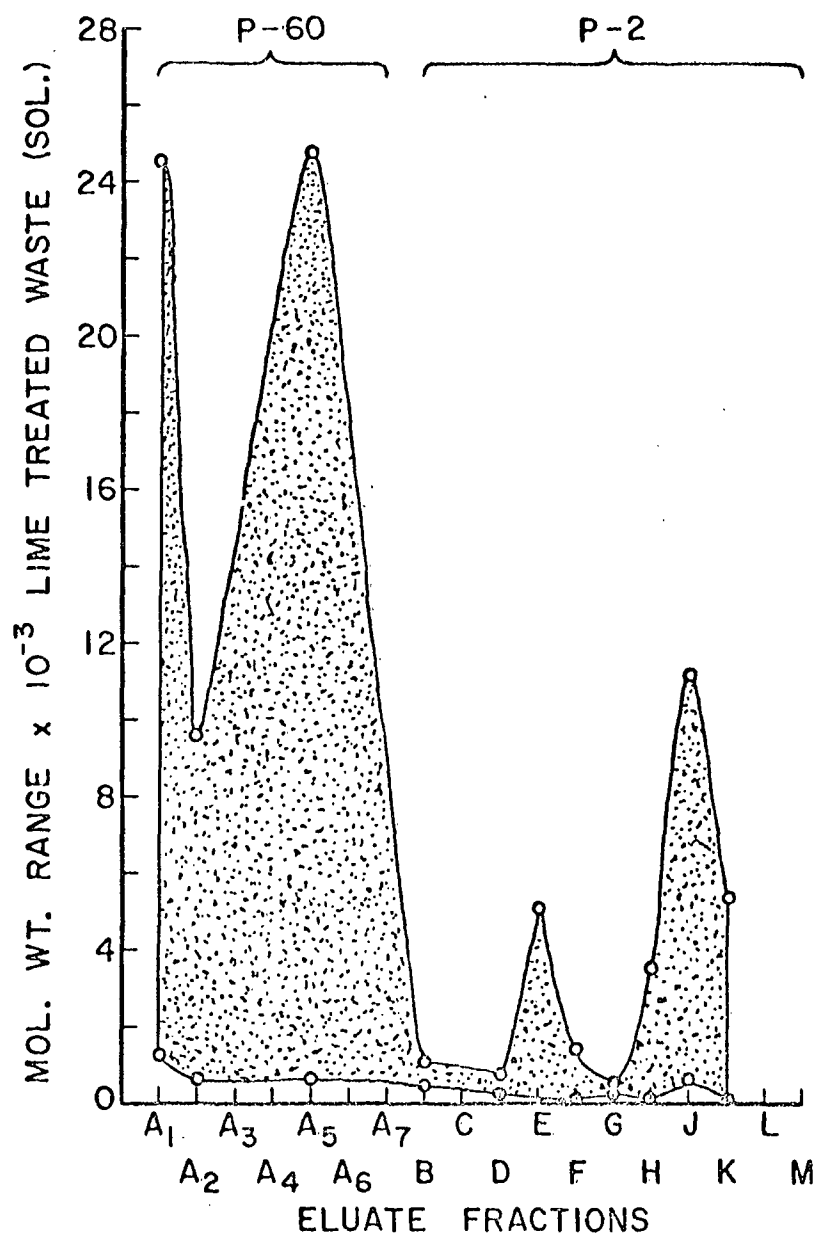


Figure 14. Gel Permeation Chromatography of Lime-Treated Acid-Soluble Color Bodies of Dilute Kraft Mill Decker Wastes. Molecular Weight Ranges and Distribution

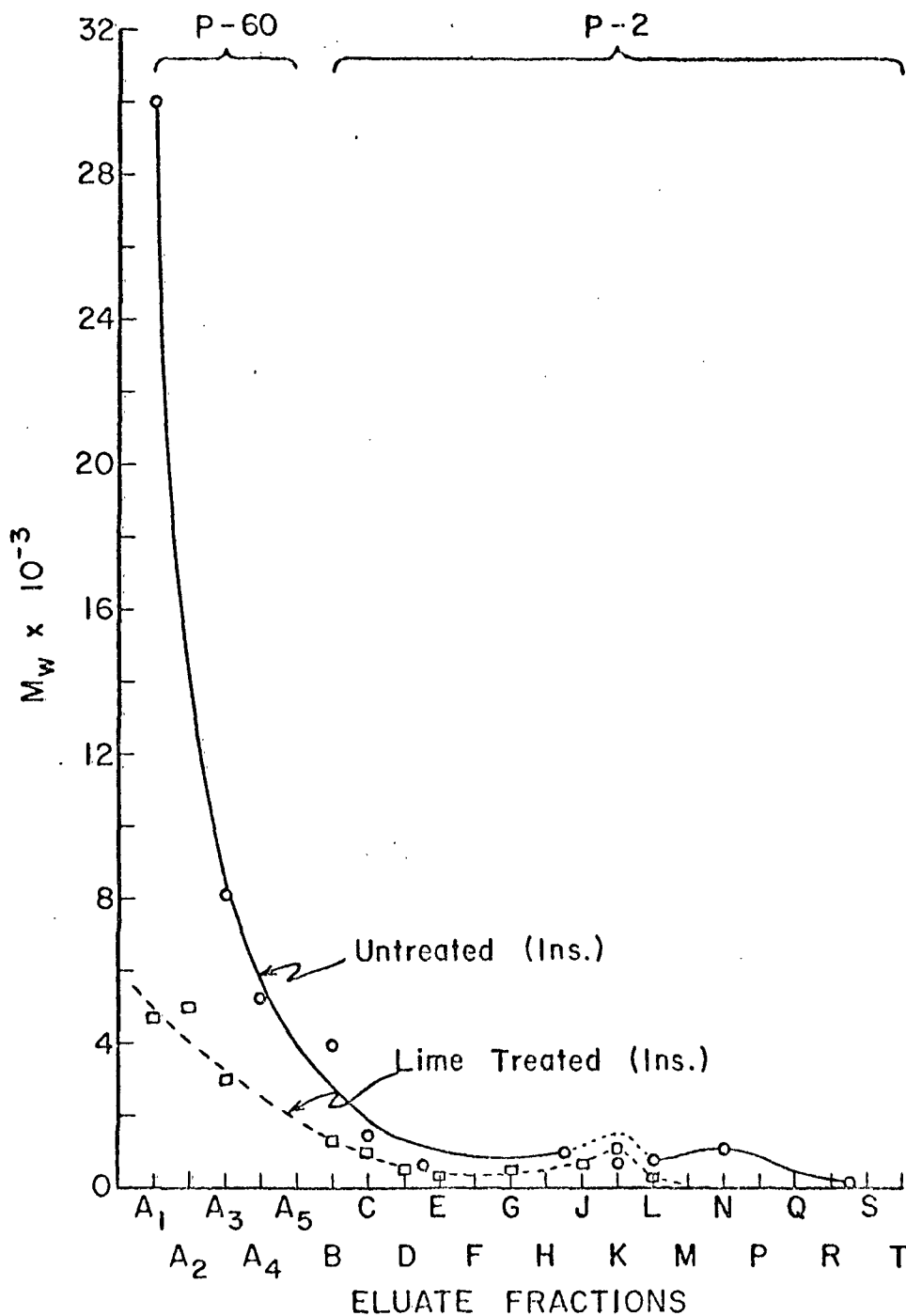


Figure 15. Gel Permeation Chromatography of Acid-Insoluble Color Bodies of Untreated and Lime-Treated Dilute Kraft Mill Decker Wastes. Weight Average Molecular Weight ( $M_w$ ) Distribution

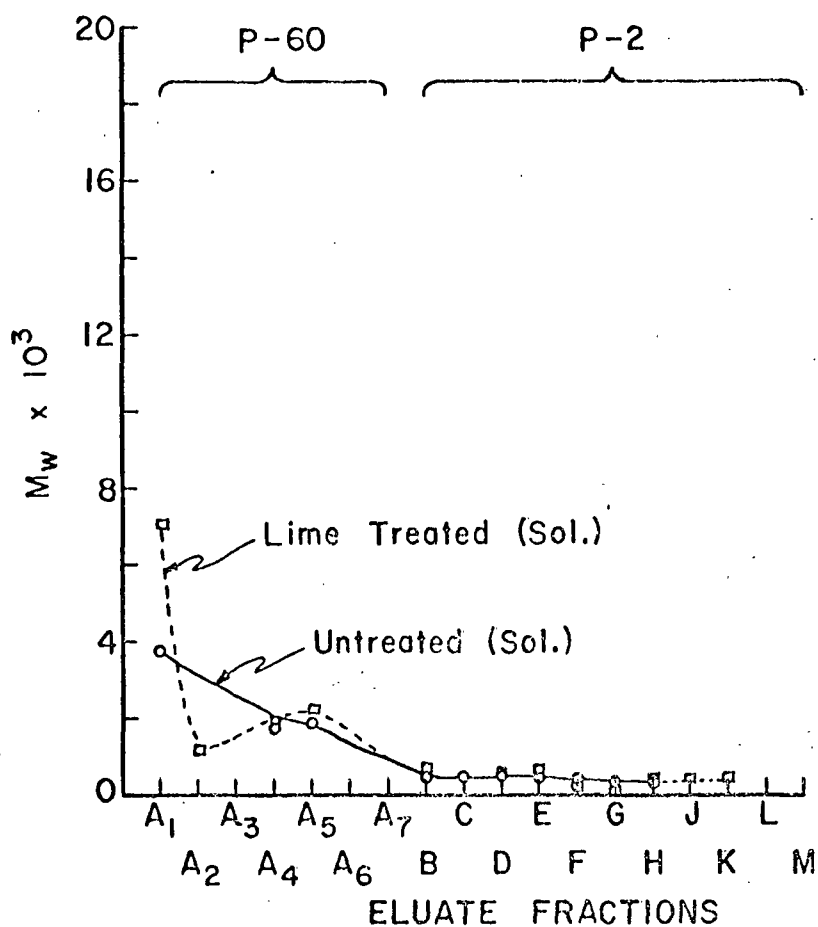


Figure 16. Gel Permeation Chromatography of Acid-Soluble Color Bodies of Untreated and Lime-Treated Dilute Kraft Mill Decker Wastes. Weight Average Molecular Weight ( $M_w$ ) Distribution

The percentage removal of color was calculated from the analytical data for both acid-insoluble and acid-soluble components and is plotted against their respective molecular "weight averages" in Figures 17 and 18.

Figure 17 shows that color bodies, in the acid-insoluble components, having a  $M_w$  of less than 400 are not removed by the lime treatment and those above  $M_w$  5000 are completely removed. The intermediate range ( $M_w$  400 to 5000) apparently undergoes partial removal. However, over 80 percent removal occurs above a  $M_w$  of 700 to 1000.

In the case of acid-soluble component, percent removal levels off at 85.

The results indicated that any treatment which will increase the molecular size and weight of color bodies will favor the complete removal of color bodies.

#### Electrokinetic Properties

As the amounts of individual fractions of acid-insoluble and acid-soluble color bodies of untreated and lime-treated components at hand at this stage were extremely small, GPC fractions having approximately the same color-to-TOC ratios were combined to get somewhat larger amounts for these experiments. The following combining schedule was followed:

U7-1C (ins.)	U7-1C (sol.)	LT7-1C (ins.)	LT7-1C (sol.)
UA1+2	UXA1 thru 3	LA1+2	LXA1 thru 7
UA3	UXA <sup>4</sup>		
UA4+5	UXA5+6	LA3 + LB thru LM	LXB thru F
UB+C	UXB thru J		(LXG thru LXM were not combined)
UD thru K			
UL			
UM thru T			



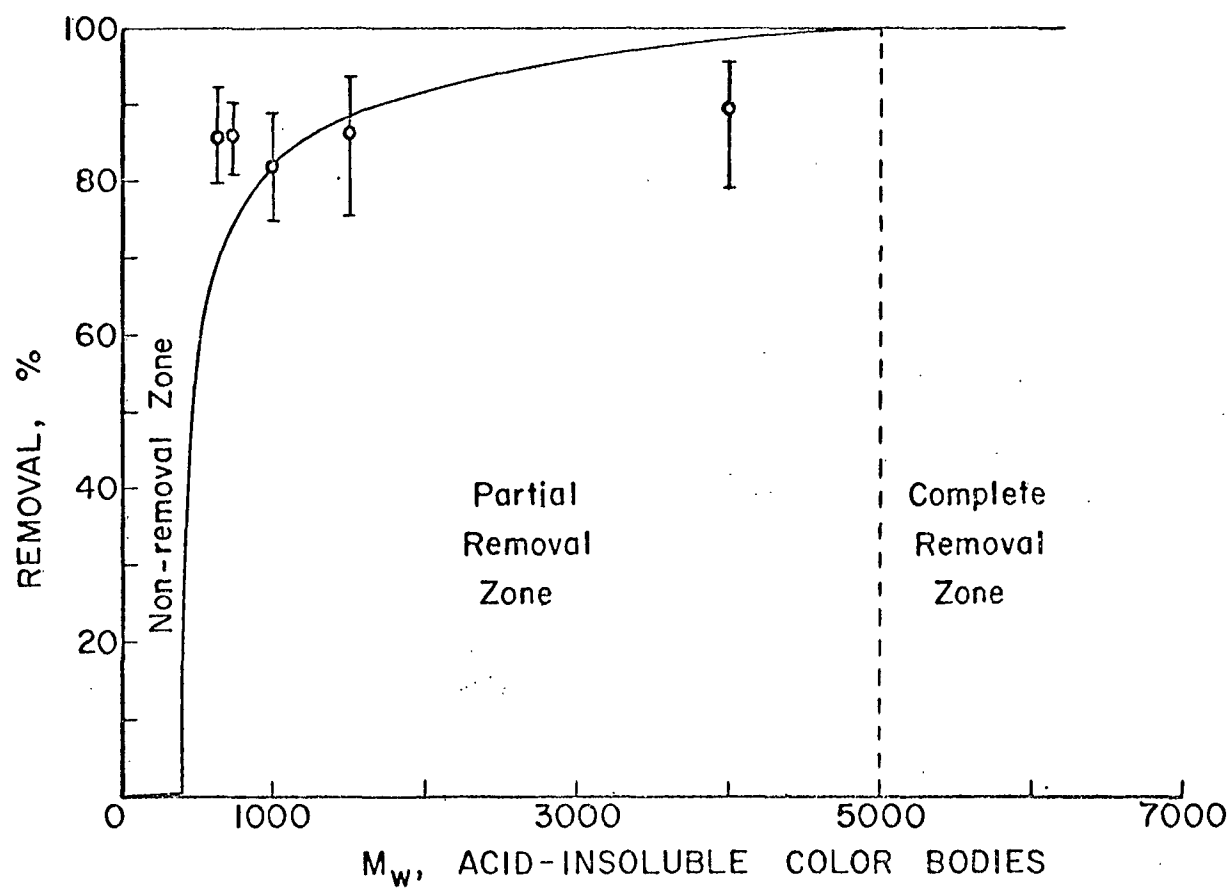


Figure 17. Weight Average Molecular Weight ( $M_w$ ) of Acid-Insoluble Color Bodies Versus the Degree of Removal by Lime Treatment

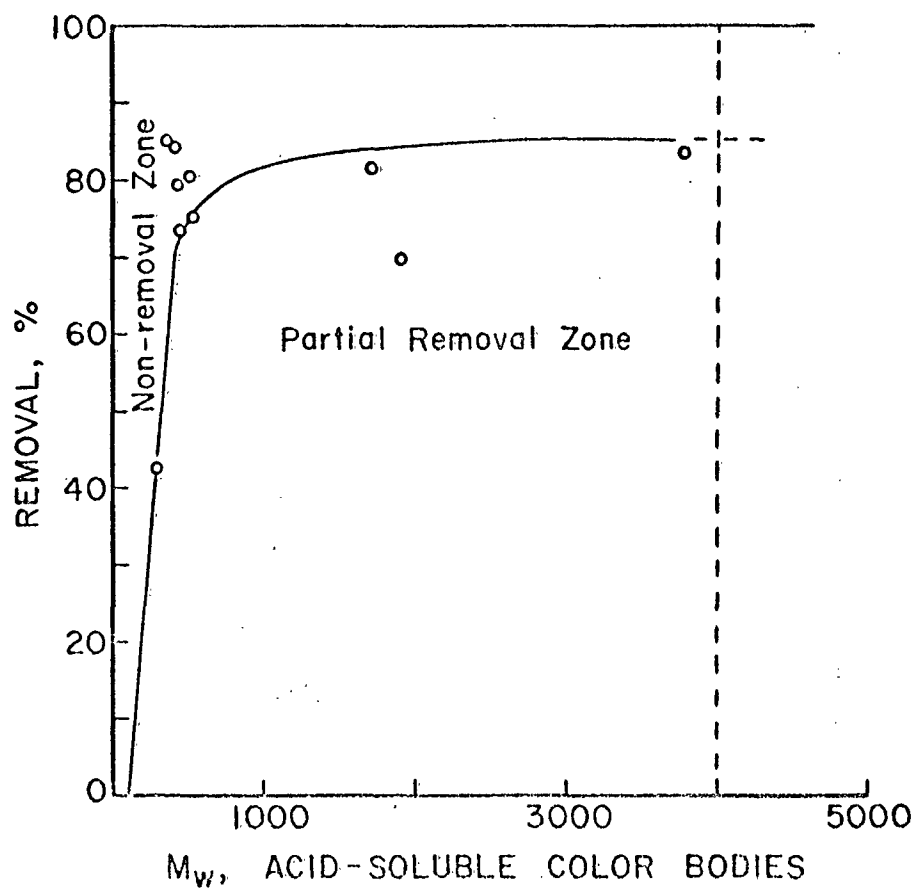


Figure 18. Weight Average Molecular Weight ( $M_w$ ) of Acid-Soluble Color Bodies Versus the Degree of Removal by Lime Treatment

Only U7-1C (ins.) were chemically analyzed. The results are reported in Table 11.

TABLE 11  
ANALYTICAL DATA ON COMBINED FRACTIONS  
U7-1C (INS.) COLOR BODIES

Combined Fraction Code	Carbon, percent	Hydrogen, percent	Methoxyl, percent	Ash, percent
UA1-2	53.46	5.53	9.60	8.88
UA4-5	48.52	5.29	7.67	13.60
UB-C	39.53	4.65	5.16	32.27
UD-K	48.35	5.88	2.08	8.54

The data show that the ash is highest in the middle fractions, UA4-5 and UB-C. This is similar to what was observed before when U5-1C and LT5-1C were fractionated by GPC (Figures 1 and 2). The methoxyl contents of these combined fractions decrease from UA1-2 to UD-K.

Gel electrophoresis<sup>8</sup> was used to achieve migration of color bodies under an electric field. All color bodies migrated toward the anode, indicating that they were negatively charged.

Electrophoretic mobilities were calculated according to Ornstein<sup>9</sup> and relative mobilities were obtained by dividing these values by the Indulin C values. Results are given in Table 12.

Except for one untreated acid-insoluble fraction, UA1-2, and two lime-treated acid-soluble fractions, LXH and LXJ, all color bodies showed higher mobilities than Indulin, indicating a higher density of negative charge per molecule.

TABLE 12

RELATIVE MOBILITIES OF COLOR BODIES BY  
GEL ELECTROPHORESIS

Sample	Relative Mobility <sup>a</sup>	Charge on Color Bodies
UA1-2	0.969	Negative
UA4-5	1.051	"
UB-C	1.010	"
UD-K	1.092	"
UL	1.054	"
UM-T	1.033	"
UXA1-3	1.030	"
UXA4	1.010	"
UXA5-6	1.071	"
UXB-J	1.071	"
LA1-2	1.071	"
LA3, B-M	1.071	"
LXA1-7	1.010	"
LXB-F	1.054	"
LXG	1.033	"
LXH	0.989	"
LXJ	0.989	"
LXK	1.033	"
LXL	1.076	"

---

<sup>a</sup> Mobility of color bodies divided by that of Indulin  
'C.'

## Pyrolysis Gas Chromatography of Color Bodies

In order to learn as much as possible from the small amounts of color bodies available, pyrolysis gas chromatography was carried out. A comparison of the complex chromatograms of selected color bodies to those obtained from a kraft liquor (Indulin C) qualitatively supported the other data which relate these fractions to kraft lignin. It might be expected that the more highly degraded smaller molecules found in the acid-soluble fractions would at least resemble lignin in chemical behavior. By inspection of Figure 19, the chromatograms of the several pyrolyzed fractions appear to be consistent with these hypotheses:

- (1) the color bodies are degraded lignin fragments.
- (2) the lower the solubility in acid and the higher the molecular weight the more nearly do the fractions resemble isolated kraft lignin.

Although such chromatograms are relatively complex, it may be possible to utilize the technique in future studies to elucidate further the chemical nature of such materials.

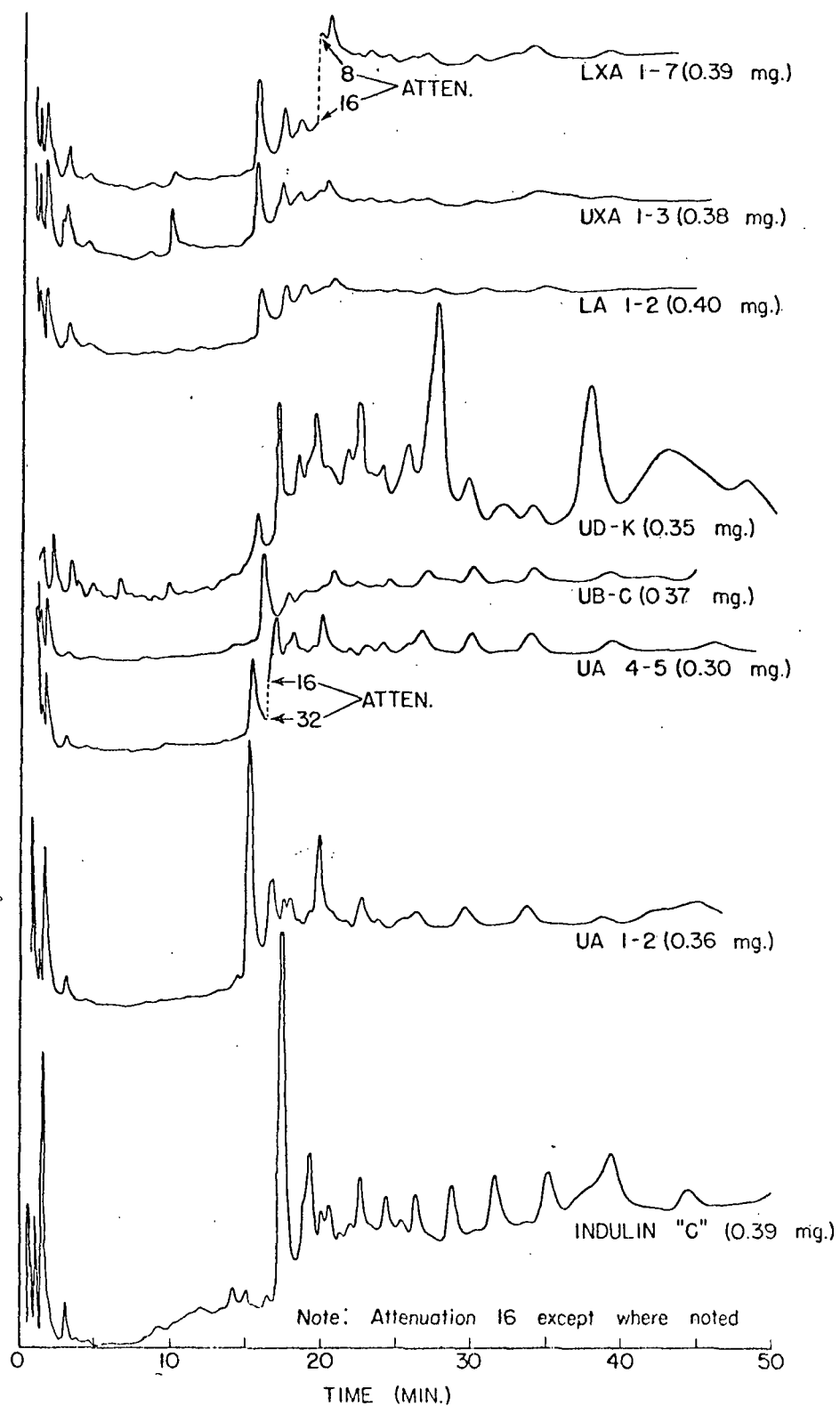


Figure 19. Pyrolysis Gas Chromatograms of Fractionated Color Bodies from the Kraft Mill Decker Effluents

## SECTION VII

### EXPERIMENTAL

#### Processing and Freeze-Drying of Wastes

Untreated and lime-treated colored samples were concentrated under reduced pressure to one-tenth of their original volume. (Untreated samples, if concentrated further, were very difficult to freeze-dry.) Most of the calcium, especially in the lime-treated samples, was precipitated by carbonating the samples to a pH of 10.2. The carbonated samples were then centrifuged in the Beta-centrifuge at 9000 rpm for 15 minutes. This speed and time was sufficient to give clear solutions. If, in some cases, slight turbidity was still present, these samples were filtered through a Millipore filter paper. The pH of the solution was checked at every stage. The colored but clear solutions were frozen in strong glass containers (centrifuge bottles) and dried under high vacuum. This freeze-dried material formed a low density powder and was readily soluble in water. The dried samples could be kept in airtight bottles for longer periods without any significant change.

#### Isolation of Acid-Insoluble Color Bodies from Freeze-Dried Solids

Freeze-dried solids of the untreated waste (14.3 g od basis) were dissolved under mechanical stirring in 60 ml of water and approximately 17 g of clean cellulose powder (Whatman standard grade) was suspended in the solution. The stirring was continued and the pH was adjusted to 1.0 with strong hydrochloric acid (1 vol concentrated acid to 2 vol of distilled water). The acidified mixture was filtered through a precoat of about 4 g of cellulose powder on a Buchner funnel and the filter cake was washed with a total of 50 ml of water in small portions. When the filtrate was just acid to Congo Red paper, some of the precipitate peptized

and formed a cloudy filtrate. The cloudy filtrate was mixed with about 3 g of acid-washed Fibra-Flo 11C (Johns-Manville filter aid), the mixture was filtered on a thin precoat of Fibra-Flo on a small Buchner funnel, and the filter cake was washed with water.

Both the cellulose powder and the Fibra-Flo filter cakes were separately extracted with 50 percent aqueous ethanol. The alcohol was evaporated from the combined solution reduced pressure whereupon a finely divided precipitate formed; the slurry containing this precipitate was subsequently freeze-dried, and designated as "acid-insolubles." The aqueous filtrate contained the "acid-soluble" material.

#### Isolation of Acid-Soluble Color Bodies

Both Amberlite MB3 and the combination Amberlite IR-120 followed by Amberlite IR-45 were tested for removal of the hydrochloric acid and other inorganics in the solution of acid-soluble color bodies. In both cases, large amounts of the color were retained by the resin.

All of the acid-soluble color was removed from the strongly acid solution with carbon (Draco), and there was some difficulty in filtering the carbon. Moreover, the sorbed color could not be removed completely from the carbon. Thus, neither ion exchange nor carbon seemed promising for separation of color bodies from the inorganic constituents.

Finally, it was found that a large portion of the acid-soluble color bodies could be sorbed on Amberlite XAD-2 (Rohm & Haas Co.) and could be removed by eluting the resin with 50 percent ethanol. Most of the color was sorbed at the top of the column from the strong acid solution, but it moved slowly down the column as the excess acid was washed from the column. The acid passed through the column faster than the color, and most of the latter remained on the column when essentially



all of the acid had been washed out. Additional color bodies were recovered by concentrating the aqueous solution and the washings which passed through the column, and then adding the concentrate to a smaller column of XAD-2. The sorbed color was readily removed by eluting the columns with 50 percent ethanol.

#### Paper Chromatography

Following preliminary experiments, 0.202 g of U<sup>4</sup>-1C was dissolved in 3.2 ml water and the solution was applied as evenly as possible to 8 strips of Whatman 3MM filter paper (23 cm x 28 cm). Thus, each strip carried approximately 0.0252 g of material. The strips were air dried overnight and were developed in an apparatus for descending chromatography. Chromatograms were developed in a mixed solvent designated herein as BWA, butane - water - acetic acid, 100:33:15. In about 2 hours the solvent had traveled 19 cm from the starting line. There was considerable streaking, but four more-or-less distinct brown-colored bands were present with additional brown-colored material at the solvent front and at the starting line. The papers were dried and were sectioned into seven bands or zones for subsequent elution.

The corresponding zones from 6 of the 8 original strips, equivalent to 0.152 g of the total, 0.202 g, were eluted with 50 percent aqueous ethyl alcohol.

The above procedure was repeated with a sample of U<sup>4</sup>-1C which had been treated with an ion exchange resin, Amberlite IR-120, to remove the cations. The chromatograms streaked or channeled badly, and no further work was done with this method of separation.

#### Color Measurement

Color was measured according to the platinum cobalt standard method of the American Public Health Association (APHA)<sup>10</sup>. The only modification of the method was

the use of a noncarbonate buffer for pH adjustment to 7.6. It was necessary to have color values at a constant pH of 7.6, because color was found to be pH dependent. Most natural waters have a pH range close to 7.6.

It should be noted that the color unit is a measure of color intensity. When it has been necessary to refer to the total amount of colored material in a solution this has been called total color and is the product of the color units and the volume of the solution.

#### Absorbance Measurement at 280 and 420 nm

The samples used for color measurement were also used for absorbance on the Beckman DU Spectrophotometer. The values obtained were multiplied by the dilution factor to give absorbance of the concentrated solutions. Absorptivity was calculated by dividing the absorbance values by concentration in grams per liter.

#### Determination of Solids

Total solids were determined by evaporation of a measured volume of waste at 105°C overnight. The resultant weight of solids was expressed in milligrams per liter (mg/l) of waste.

Fixed and volatile solids were determined by igniting the total solids at 600°C in an electric muffle furnace to constant weight, usually requiring one hour. The loss on ignition is reported as mg/l volatile and the residue as mg/l fixed solids.

#### Total Organic Carbon (TOC)

The Process Carbonaceous Analyser (Beckman & Co.) was used for this purpose. Because this instrument gives only total carbon values, TOC was determined by a modified direct method. The matters were further complicated by very small quantities of the

fractions. To conserve color bodies, the majority of the specimens tested for TOC had been examined for color which necessitated dilution within the color range of the platinum cobalt reference Nessler tubes.

For manual injections into the carbonaceous analyzer, an aliquot was prepared as follows:

A 5-ml aliquot was transferred to a 30-ml glass beaker from a 5-ml volumetric flask. The pH of the specimen was adjusted to 2.9 to 3.0 with a 0.1N HCl solution (normally two milliliters were necessary). The acidified sample was rinsed with distilled water into a 2.5 x 15 cm test tube, having a 2-ml graduation mark, and the volume was reduced to 2.0 ml by boiling over a gas flame. The boiled sample was then transferred back to the 5-ml volumetric flask and the test tube was rinsed with 3 ml of pH 10 buffer (borax and sodium hydroxide) and added to the volumetric flask to give a combined 5-ml pH 10.0 specimen. Prior to injection into the carbon analyzer, the specimens were cooled to room temperature under the cold water tap.

Twenty-three microliters of the specimens were injected at 3 to 5-minute intervals and an average reading was thus obtained from which the "blank" reading was subtracted to give the TOC value in mg/l.

The "blank" consisted of all the ingredients listed above minus the sample and was handled in the same manner as the sample.

The analyzer was operated at 950°C according to instructions in the instrument manual. In addition to the precautions listed in the operating manual, the following precautions are recommended for accurate results.

- a. The injection syringe should be checked often for burrs, cracks, etc., which cause rubber particles to drop into the combustion tube thus giving high readings.

- b. A constant slow needle insertion and retraction is essential to prevent "popping" of the combustion chamber rubber cap.
- c. Combustion chamber rubber cap should be replaced often.
- d. Tygon tubing close to the condenser, filters, and glass combustion chamber dome should be cleaned often.

#### Fractionation of Color Bodies

Two types of Bio-Gels (Bio Rad Co.) were used for this purpose. Bio-Gel P-2 (exclusion limit 2600) and P-60 (exclusion limit 60,000) were hydrated in distilled water and separately packed in 2.5 x 200 cm and 2.5 x 100 cm glass columns, respectively. The complete apparatus used for fractionation is shown in Figure 20.

The volume of the solutions used for fractionation was less than 3 percent of the void volume of the column (void volume,  $V_0$ , = total bed volume x 0.38). A measured quantity of the solution was added to the top of the column. A glass fiber filter was used on the gel so that upon addition of the solution, the gel surface is not disturbed. The eluate was allowed to flow into an automatic collecting device and the collector timer and the UV-cord recorder were started. When the solution dropped to just below the surface of the gel, one milliliter of distilled water was added to the column and elution continued. When the level was again slightly below the gel, more distilled water was added and the column was then connected to the constant head water reservoir through a filter and a flowmeter. The elution rate (0.2-0.3 ml/min) was controlled by a teflon stopcock with a needle adjust. Fractions were collected every 30 minutes. At the end of fractionation, which took three to four days, the collected fractions were combined according to the number of peaks on the UV recorded chart. The combined fractions were freeze-dried and used for study. (Aliquots of the fractions were taken for color, TOC, and absorbance before freeze drying.)

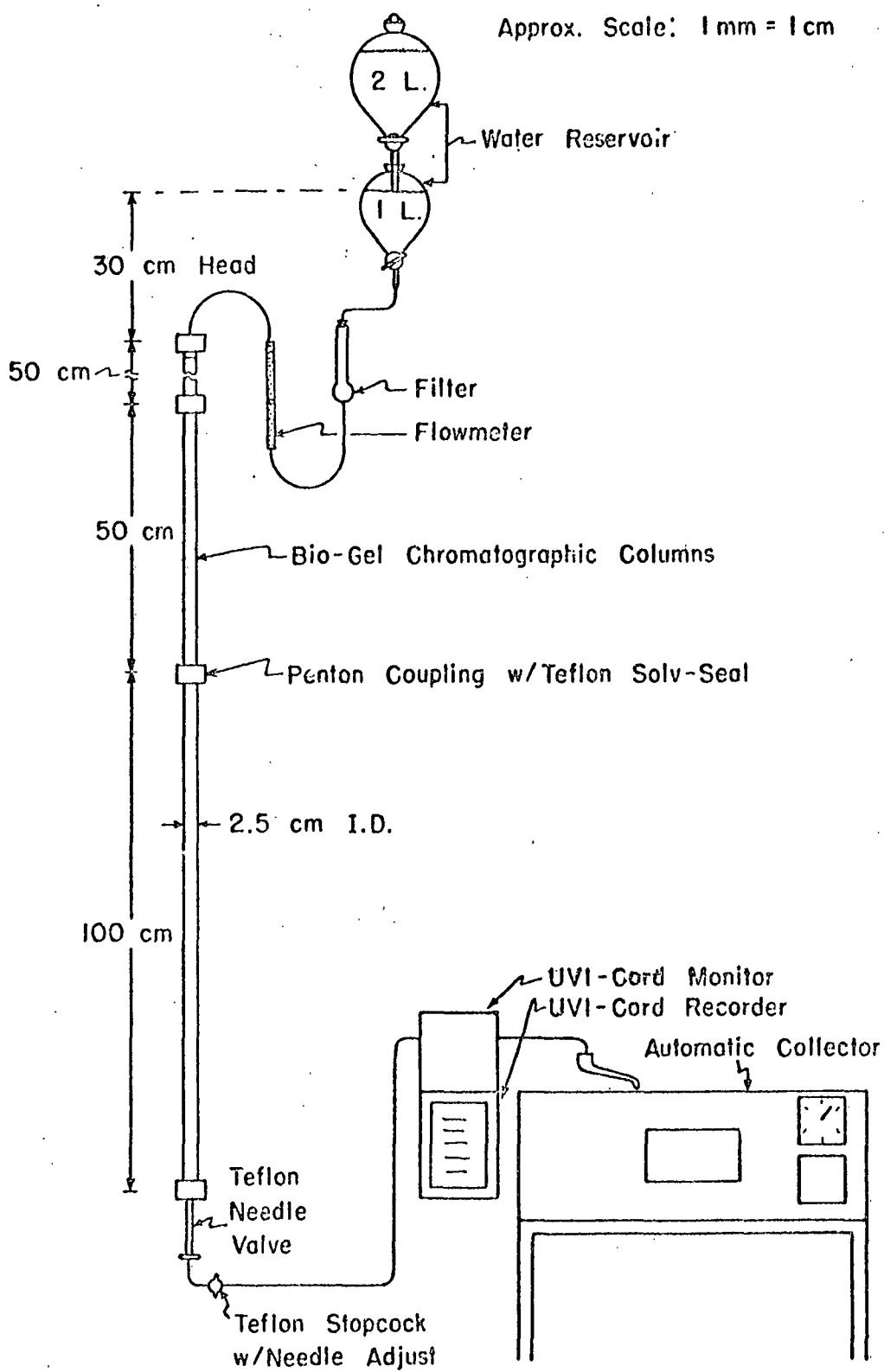


Figure 20. Diagram of Gel Permeation Chromatography Apparatus

## Determination of Molecular Weights

Molecular weights were measured by the sedimentation equilibrium method<sup>7</sup>. In this procedure the centrifuge is run at an appropriate motor speed for a period of time necessary to achieve an equilibrium condition (rate of transport in one direction due to sedimentation, balanced by the rate of transport due to diffusion in the other direction). Having achieved this condition, the technique requires the accurate measurement of the solute concentration distribution throughout the cell. The equilibrium condition is a practical one in which the concentration distribution does not change with time within the measuring experimental errors. Interference optics provides an accurate photographic record of concentration change from one position in the cell to another. A separate synthetic boundary run determines the initial concentration in terms of a total interference fringe shift.

Other data needed to compute molecular weights are rotor speed, temperature, partial specific volume of the solute, and density of the solution.

The densities of the solutions were measured according to the method described by Bauer<sup>11</sup> and plotted against their respective concentrations in g/ml. A straight-line plot was developed and the partial specific volume,  $\bar{V}$ , was calculated from the straight-line relationship using Equation (1).

$$\bar{V} = \frac{1}{d_0} - \left[ \left( \frac{1}{x} \right) \left( \frac{d - d_0}{d_0} \right) \right] \quad (1)$$

where

$x$  = concentration, g/ml

$d_0$  = density of solvent

$d$  = density of solution

A computer program (MOLWT) [a modification of the program by Teller<sup>12</sup>] was used for calculating the weight average molecular weights.

## Disk Electrophoresis

The techniques and apparatus used were as described by Davis<sup>8</sup> with slight modifications.

The apparatus consisted of two Plexiglas (3/8-inch) buffer vessels (16.5 cm diameter and 9.0 cm deep) each with a centrally located electrode. The bottom of the upper vessel had 12 equally spaced holes along a bottom circumference. Bored out serum stoppers in these holes accept 5 x 100 mm glass tubes (gel columns). This buffer vessel was supported above the other so that the lower ends of the gel columns extended about 1/4 inch below the surface of the buffer in the lower vessel. Any bubbles formed on the gel column ends were removed. The power supply was a RECO Model E 800-2, 750 volts, 200 ma maximum output.

Glass tubes (5 x 100 mm) were mounted with rubber caps in a rack and 1.4-ml small pore gel (separation gel) solution added to each followed by a water layer to insure a flat gel surface. After polymerization (30 minutes) the water was removed and the gel surface was washed with large pore spacer or stacking gel. Spacer gel (0.1 ml) was added, overlayed with water and photopolymerized for 30-45 minutes. After removal of the water layer, 0.1-ml colored sample (0.5 mg in 40 percent sucrose) was added. Buffer was then layered carefully to fill the tubes completely. The tubes were mounted in the grommets of the upper vessel, buffer was then added to the vessels and electrophoresis started. Length of the small pore separation gel was 7.2 cm.

Two preliminary runs were made. Sample UA 1-2 was run at 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg per tube. Sample UA 1-2 was also run at 0.5 mg per tube with gel concentrations of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 percent. At the higher monomer concentrations there was a general "smear" of material trailing the main single

band; however, no other discrete bands were visible. In subsequent runs, based on these results, a "normal" 7.5 percent gel was used with 0.5 mg sample per tube. Also, all samples were run at 400 volts, 34 ma for 60 minutes at room temperature. Following electrophoresis, the distance from the top of the separation gel in the middle of the colored band was measured and electrophoretic mobilities were calculated according to Ornstein<sup>9</sup>.

#### Pyrolysis-Gas Chromatography

Selected samples of color bodies from dilute waste liquor were subjected to pyrolysis-gas chromatography. The conditions were as follows:

Column: 20 percent Carbowax; 5 ft x 1/8 inch

Initial temp 75°; final temp 225°

Rate 10°/min

Detector: Hydrogen flame ionization

Temp 265°

Injector: Temp 225°

Carrier gas: Helium at 30 ml/min

Pyrolysis: 9.5 amp for 12 sec to produce a maximum temp of 650°.

Sample	Amount Hydrolyzed, mg
Indulin C	0.39
UA 1-2	0.36
UA 4-5	0.30
UB-C	0.37
UD-K	0.35
LA 1-2	0.40
UXA 1-3	0.38
LXA 1-7	0.39



Particles of suitable size and density for proper loading of the pyrolysis apparatus were prepared from the fluffy freeze-dried solids. A small droplet of water was placed on a microscope slide, the fluffy solid was carefully added to the droplet until a heavy paste was formed. The paste was dried in the air to a brittle solid from which suitable pieces were selected for the pyrolysis-GLC. The chromatograms are shown in Figure 19.

## SECTION VIII

### ACKNOWLEDGMENTS

The support of the President of The Institute of Paper Chemistry, Mr. John G. Strange, is acknowledged with sincere thanks. Mr. John W. Swanson, Director of the Division of Natural Materials and Systems of the Institute, was Project Director. Dr. Hardev S. Dugal, Research Associate of the Institute, was Project Leader and carried out much of the work himself and coordinated the work of others on this project. Dr. Marion A. Buchanan and Mr. Edgar Dickey were responsible for certain phases of isolation and characterization work. Portions of the experimental work were conducted by Messrs. Norman Colson, John Carlson, and Lowell Sell each of whom contributed to the work in the area of his specialty. Dr. Robert M. Leekley, Dr. Donald C. Johnson, and Mr. Carl Piper contributed through valuable suggestions and consultation.

Mr. Charles L. Davis, Jr., Pollution Control Director of Interstate Paper Corporation, and his staff periodically collected the samples of colored waste which were studied and contributed useful information about their lime treatment process.

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## SECTION IX

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