



INSTITUTE OF
PAPER CHEMISTRY
Appleton, Wisconsin

MOLECULAR STRUCTURE OF LIGNOSULFONATES

Project 2421

Report Six

A Progress Report

To

PULP MANUFACTURERS RESEARCH LEAGUE

February 1, 1967

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MOLECULAR STRUCTURE OF LIGNOSULFONATES

SUMMARY

This report covers recent experimental work that has been done under Project 2421 and also gives a review of developments in the project.

Sedimentation velocity ultracentrifuge molecular weight determinations were carried out on two sets of Sephadex fractionated calcium lignosulfonates supplied by Dr. Forss. Progressive changes from 5,000 to 170,000 were found. These results are interpreted in light of other information on molecular weight properties of the lignosulfonates. Supporting evidence for the validity of the Benko diffusion method for molecular weight determination was secured.

A group of electrodialed and heat-treated spent sulfite liquor samples were fractionated by solvent precipitation and Sephadex methods. Ultracentrifuge molecular weights were determined. The molecular weight values secured were surprisingly low, and little evidence of polymerization due to the heat treatment was found.

An interpretative review of the work carried out under this project is given, and the significance of the findings is discussed.

INTRODUCTION

The work covered by this report was concerned with two main phases of activity. Ultracentrifuge sedimentation velocity molecular weights were determined for two series of Sephadex fractions of calcium lignosulfonates supplied by Dr. Kai Forss. Fractionation experiments and molecular weight determinations on the fractions were carried out on a group of heat-treated and nonheat-treated electrodialyzed sulfite liquors intended for use as plywood adhesives.

The study of the Sephadex fractionated calcium lignosulfonates made possible a testing of the ultracentrifuge techniques for molecular weight determinations of these materials and an evaluation of the molecular weight properties from such fractionations.

The fractionations and molecular weight evaluations of the plywood adhesive liquors provide basic physical chemical data on the properties of these materials.

MOLECULAR WEIGHT DETERMINATIONS FOR FRACTIONATED CALCIUM LIGNOSULFONATES

EXPERIMENTAL

Dr. Forss supplied two series of fractions of calcium lignosulfonates, one fractionated on Sephadex G-50 and the other fractionated on Sephadex G-75. For the ultracentrifuge determinations, the fractions were dried in vacuo over P_2O_5 and were dissolved at approximately 0.5% in $1/3M$ calcium chloride. The partial specific volume of three fractions was determined pycnometrically. Because of the close agreement of these determinations, the average value of 0.611 was used for all the fractions.

A Beckman Model E ultracentrifuge was used for both sedimentation velocity and diffusion coefficient determinations. The sedimentation runs were made by centrifuging the sample 30 minutes at 56,100 r.p.m., using schlieren optics. The diffusion runs were made by centrifuging the sample 30 minutes at 2,095 r.p.m., using Rayleigh optics. Sedimentation coefficients and diffusion coefficients were calculated by use of an IBM Model 1620 computer. Molecular weight values were secured from these data by means of the Svedberg equation.

RESULTS

The ultracentrifuge data secured on the Sephadex G-50 series of fractions are shown in Table I. The regular progression of changes in diffusion coefficients and sedimentation coefficients is evident, and the resulting spread of molecular weights from 80,600 to 3,300 covers quite a wide range.

In the Sephadex G-75 separation, only five fractions were available. Of these, the first was insoluble in the $1/3M$ calcium chloride solvent. The

ultracentrifuge molecular weights of the remaining four are shown in Table II, in comparison to the values obtained for the Sephadex G-50 separation.

TABLE I
MOLECULAR WEIGHTS OF CALCIUM LIGNOSULFONATES
FRACTIONATED ON SEPHADEX G-50

Fraction No.	Diffusion Coefficient $\times 10^7$	Sedimentation Coefficient $\times 10^{13}$	Molecular Weight
1	5.3	6.31	80,600
2	8.7	4.01	30,900
3	9.2	3.21	23,600
4	10.7	2.64	16,600
5	12.1	1.97	11,000
6	18.5	1.54	5,600
7	21.2	1.03	3,300

TABLE II
MOLECULAR WEIGHTS OF CALCIUM LIGNOSULFONATES
SEPHADEX SEPARATIONS

Fraction No.	Molecular Weights	
	G-50	G-75
1	80,600	--
2	30,900	168,280
3	23,600	71,100
4	16,600	37,350
5	11,000	7,600
6	5,600	--
7	3,300	--

It is evident that Sephadex G-75 provides a more complete separation on the basis of molecular weight than Sephadex G-50. Sephadex G-75 Fraction One is presumably of higher molecular weight than the value of 168,000 obtained for Fraction Two, and the values decrease progressively to a low of 7,600.

To the best of our knowledge, this is the first time that numerical molecular weight values have been obtained for calcium lignosulfonates fractionated

on Sephadex. The logical results lend credence to the ultracentrifuge sedimentation velocity method for application to these materials. The regular progression of values from 170,000 to a low of 5,000 raises doubts as to the hypothesis of association in the lignosulfonates.

Having these data makes it possible for us to answer a question that has been with us throughout this project. That is, what is the reliability of the diffusion coefficient method of determining molecular weights developed by Dr. Benko.

We are justified in taking the diffusion coefficients determined by the ultracentrifuge as equivalent to those determined by the porous plate method of Dr. Benko. From the diffusion coefficient data secured for the Sephadex G-50 series of fractions, we have calculated molecular weights as inversely proportional to the square of the ratio of diffusion coefficients, relative to that of potassium chloride, as done by Dr. Benko. The results are shown in Table III, along with the ultracentrifuge sedimentation velocity values. It is evident that the results are in the same order and of the same order of magnitude. This lends support to the Benko method for approximating molecular weights of lignosulfonates.

TABLE III
DIFFUSION MOLECULAR WEIGHTS
OF CALCIUM LIGNOSULFONATES

Fraction No.	Diffusion Coefficient x 10 ⁷	Diffusion Molecular Weight	Sedimentation Molecular Weight
1	5.3	93,000	80,600
2	8.7	34,300	30,900
3	9.2	29,500	23,600
4	10.7	22,700	16,600
5	12.1	17,000	11,000
6	18.5	7,600	5,620
7	21.2	5,700	3,300

FRACTIONATION OF ELECTRODIALYZED SPENT SULFITE LIQUORS

The use of electrodialyzed and heat-treated spent sulfite liquor as a component of plywood adhesives is a promising outlet for these materials. It was judged to be worthwhile in this project to characterize these substances as to molecular weight and other physical chemical properties as an aid in their development.

EXPERIMENTAL

Three samples of treated Interlake spent sulfite liquor were secured from the League staff. One of these, 65-8R198, had been electrodialyzed only. The other two, 65-24R44 and 65-24R45, had been electrodialyzed and heat treated.

The first process applied was fractional precipitations of the solids from these solutions. Acetone precipitation from water solution was used. After some preliminary experimentation on proportions, the following procedure was applied:

A quantity of the liquor containing three grams of solids was diluted to 50 ml. with water. With vigorous agitation, 250 ml. of acetone were added. The suspension was centrifuged at 2,500 r.p.m., and the precipitate was washed with acetone and dried. Two additional fractions were secured by sequential addition of 90 and 200 ml. of acetone. It was found that further addition of acetone would not produce a precipitate.

Ultracentrifuge sedimentation velocity molecular weights were secured on the fractions obtained.

A second fractionation applied was separation of the solid on Sephadex columns. Sephadex G-25, G-75, and G-100 were used. Twenty-gram samples of the absorbents were formed into columns in 2-cm. glass tubes. Samples of the treated spent liquors containing 3 g. of solids were introduced to the columns and eluted with water. Fifty-milliliter fractions were taken. These were freeze dried and ultracentrifuge sedimentation velocity molecular weights secured for the fractions.

RESULTS

The weight yield and the ultracentrifuge sedimentation velocity molecular weight data for the first two fractions from the fractional precipitation of the electrodialyzed and heat-treated spent sulfite liquors (EP) are shown in Table IV. P_1 and P_2 represent the first and second fractions, respectively. R44 and R45 were electrodialyzed and heat treated. R198 was electrodialyzed only. Other fractions were taken but were too small for molecular weight determinations.

It should be noted that the precipitation yields were only a small part of the total liquor solids. The molecular weight values are surprisingly low for calcium lignosulfonates. There is a suggestion that the molecular weight values for the heat-treated materials are slightly higher than the untreated, but the difference is slight.

The results for the fractions obtained from Sephadex G-25 are shown in Table V. The third fractions were too small for evaluation. The molecular weights are low and there is no evidence of difference due to heat treatment.

The yield and molecular weight for fractions obtained from Sephadex G-75 are shown in Table VI. In this case three fractions were obtained, but the small first fraction was insoluble in the $1/3M$ calcium chloride used as a solvent.

The molecular weight values are quite low and there is no appreciable evidence of effect of heat treatment.

TABLE IV

MOLECULAR WEIGHTS OF EP LIQUOR
FRACTIONAL PRECIPITATION SAMPLES

Sample No.	Yield, %	Molecular Weight
R44P ₁	17.5	17,250
R44P ₂	12.0	10,840
R45P ₁	11.5	12,690
R45P ₂	3.4	2,030
R198P ₁	9.5	10,450
R198P ₂	5.7	4,170

TABLE V

MOLECULAR WEIGHTS OF EP LIQUOR
G-25 FRACTIONATED SAMPLES

Sample No.	Yield, %	Molecular Weight
R44(1)	69	9,900
R44(2)	30	2,000
R45(1)	59	10,280
R45(2)	35	2,040
R198(1)	49	15,150
R198(2)	49	1,220

TABLE VI
MOLECULAR WEIGHTS OF EP LIQUOR
G-75 FRACTIONATED SAMPLES

Sample No.	Yield, %	Molecular Weight
R44(1)	2.5	--
R44(2)	80	12,900
R44(3)	17	550
R45(1)	2.5	--
R45(2)	56	5,230
R45(3)	22	370
R198(1)	2.5	--
R198(2)	80	3,600
R198(3)	--	--

The results of fractionation on Sephadex G-100 are shown in Table VII. Again, the small first fractions are insoluble. There is some indication of higher molecular weights for the heat-treated materials, even though the ranges are quite low.

TABLE VII
MOLECULAR WEIGHTS OF EP LIQUOR
G-100 FRACTIONATED SAMPLES

Sample No.	Yield, %	Molecular Weight
R44(1)	1.6	--
R44(2)	61	9,010
R44(3)	12	760
R45(1)	1.7	--
R45(2)	78	5,090
R45(3)	0.1	560
R198(1)	0.7	--
R198(2)	75	2,650
R198(3)	1.5	530

In an effort to secure more complete separation of these materials, a more complete fractionation was carried out on Sephadex G-100 and G-75. In this series five fractions were obtained in each case.

The results of the Sephadex G-100 fractionation are shown in Tables VIII, IX, and X. In this procedure, the first two small fractions were found to be insoluble. Although there is no significant difference in molecular weights in the series, the lower yield for the first three fractions of electrodialed only R-198 suggest some polymerization due to heat treatment.

TABLE VIII

FRACTIONATION ON SEPHADEX G-100
SAMPLE R-45

Fraction	Yield, %	Molecular Weight
1	3.90	--
2	4.04	--
3	56.5	14,600
4	28.7	3,600
5	7.85	630

TABLE IX

FRACTIONATION ON SEPHADEX G-100
SAMPLE R-44

Fraction	Yield, %	Molecular Weight
1	3.50	--
2	2.39	--
3	35.9	16,520
4	49.8	9,630
5	8.25	340

TABLE X
FRACTIONATION ON SEPHADEX G-100
SAMPLE R-198

Fraction	Yield, %	Molecular Weight
1	1.49	--
2	1.09	--
3	12.6	16,800
4	74.5	6,240
5	10.4	350

The corresponding data for fractionation on Sephadex G-75 are shown in Tables XI, XII, and XIII. Here, there is found no evidence to support the idea of polymerization due to heat treatment.

TABLE XI
FRACTIONATION ON SEPHADEX G-75
SAMPLE R-45

Fraction	Yield, %	Molecular Weight
1	1.20	--
2	2.45	--
3	0.74	26,500
4	59.5	6,740
5	36.2	3,920

TABLE XII
FRACTIONATION ON SEPHADEX G-75
SAMPLE R-44

Fraction	Yield, %	Molecular Weight
1	0.70	--
2	0.03	--
3	5.40	--
4	91.8	6,830
5	1.92	300

TABLE XIII
FRACTIONATION ON SEPHADEX G-75
SAMPLE R-198

Fraction	Yield, %	Molecular Weight
1	0.78	--
2	2.20	--
3	14.5	17,360
4	71.8	6,390
5	10.7	680

There are several conclusions that can be drawn from the fractionation and molecular weight studies that have been conducted. One is that the molecular weights of these materials are low for adhesive use. The average of only several thousand is below the high polymer range that is normally associated with good adhesion. A second conclusion is that the appreciable quantities of material having molecular weight of only a few hundred is certainly not contributing to the effectiveness of the adhesive. It might be worthwhile to experimentally cut this fraction out and to evaluate the refined product.

The combination of the finding of little significant gain in molecular weight on heat treatment with the increase in viscosity known to result from this treatment leaves a puzzling question of what action is taking place. One possibility is that solvation is involved. This should be investigated by running viscosities on dilute solutions to find whether the difference in viscosity disappears under these conditions.

REVIEW OF PROJECT

This is an appropriate time to review the work that has been done under Project 2421 as a basis for deciding on the future course of action. In the following paragraphs the various phases of the study are summarized.

MOLECULAR WEIGHT

Ultracentrifugation

The theoretical background and exploratory experiments on the application of sedimentation velocity ultracentrifugation to lignosulfonic acids and their salts were given in Report One. It was shown that use of the ultracentrifuge with solutions of free lignosulfonic acids in water solutions was of doubtful validity, but work in potassium chloride solutions gave reasonable results. Molecular weight values in the order of 10,000 were obtained in potassium chloride ranging from 0.01 to 0.3M concentration. Other lignosulfonic acid preparations secured from Dr. Benko gave molecular weight values as high as 20,000.

In the present report, application of the ultracentrifuge to two series of Sephadex fractionated calcium lignosulfonates supplied by Dr. Forss is presented. Smoothly progressing molecular weight changes from 5,000 to 170,000 are observed. The application of the ultracentrifuge to the determination of molecular weights of lignosulfonates has been firmly established. The only restrictions are the limited availability of the instrument and the considerable time involved in its operation.

A test of the diffusion rate method of Dr. Benko against ultracentrifuge molecular weight determinations gave encouraging agreement (this report).

Light Scattering

A considerable amount of work was done on the application of light scattering to the determination of molecular weights of lignosulfonates (Report Three). Difficulties were encountered with strong fluorescence and with nonlinearity of the data plots. The best of the data obtained gave molecular weights of the order of 300,000. This compares to 20,000 for the same material from the ultracentrifuge.

At the present time, the light-scattering method is not considered reliable for lignosulfonates. Since the method has been applied with more apparent success by other workers, it seems possible that our difficulties have been involved with the particular samples used.

Osmotic Pressure

The Mechrolab high-speed membrane osmometer was applied to selected lignosulfonic acid preparations (Report Two). The data obtained in water solution and in $1/3M$ potassium chloride were unrealistic. However, when the solutions in the salt were neutralized, very satisfactory data were obtained. A number average molecular weight of 1,900 was calculated, as compared to the ultracentrifuge weight average of 20,000. A determination of the osmotic molecular weight of the methylated lignosulfonic acid in dimethylformamide gave a value of 2,420 (Report Five).

The osmotic pressure method for determination of number average molecular weight deserves more attention, particularly for carefully fractionated materials.

Association of Lignosulfonates

It has been suggested by other workers and some of the results of this program imply that lignosulfonate molecules associate in solution. Some of the evidence for this is:

- (1) The discontinuity of the equivalent conductivity and specific conductivity curves.
- (2) The negative slopes of the light-scattering curves.
- (3) The extreme difference between the light-scattering molecular weight, 100,000 up, and that determined by the method of sedimentation velocity, 12,000-20,000.
- (4) The inverse slope of the osmotic pressure curves in the absence of supporting electrolyte.
- (5) The high light-scattering molecular weight.
- (6) The difference between the osmotic pressure determinations in the presence of and in the absence of a Donnan equilibrium.

The most direct evidence for association was obtained from the conductivity determinations carried out in the project (Report Four). Distinct minima were found in the specific conductivity curves at a concentration of about 5×10^{-5} g./ml.

The finding of the conductivity minima, suggesting association, is difficult to reconcile with the sharp fractionations by molecular weight secured on Sephadex (this report). The subject should be further investigated, possibly by carrying out conductivity determinations on such sharp fractions as are obtained by the Sephadex separation.

Differential Thermal Analysis

A set of exploratory differential thermal analysis curves were run on a group of lignin preparations, including lignosulfonic acids (Report Two). Evidence for the strong hydrophilic character of these materials was obtained, and a number of interesting variations in endothermic and exothermic reactions were secured. This technique deserves further investigation.

Further studies of this differential thermal analysis behavior, coupled with chemical investigations could yield evidence of crystalline or second-order transitions, fusion, vaporization, sublimation, desolvation, decomposition, or solid-state reactions.

Disk Electrophoresis of Lignosulfonic Acids

A number of zone electrophoresis experiments were run on selected lignosulfonic acids (Report Five). This technique makes use of a combination of gel porosity and ionic mobility to effect a sharp separation of ionic species. Formation of striations of six to eight distinct bands was secured with the materials studied. Further work on identification of these bands has not been done.

The separation by disk electrophoresis involves a combination of ionic effects and molecular size. Further application of the method would require the development of microanalytical techniques to separate the bands formed and characterize their basic differences.

Fractionation of Plywood Adhesive Lignosulfonates

Electrodialyzed and heat-treated spent sulfite liquors have been fractionated, primarily on various grade of Sephadex, and characterized by ultracentrifuge

molecular weight determinations (this report). The molecular weight values secured were surprisingly low, and little evidence of polymerization due to the heat treatment was secured. These materials are being further investigated.

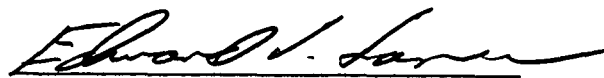
In summary, methods for molecular weight determinations of lignosulfonates have been extensively studied and a number of other physical chemical techniques have been investigated for application to these materials.

Ultracentrifugation has been established as a reliable tool for securing weight average molecular weights. Less satisfactory results were secured with light scattering. Encouraging results were secured with high-speed osmometry for number average molecular weights.

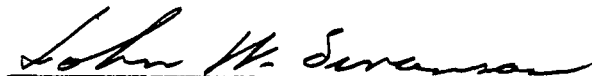
Differential thermal analysis, electrical conductivity, and gel electrophoresis were investigated for applicability to the lignosulfonate system.

Sephadex fractionation and sedimentation velocity ultracentrifugation were applied to electrodialyzed and heat-treated spent sulfite liquors with significant results being secured.

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