strap molasses. The effluent was collected in eleven fractions. Data on these fractions are listed in Table I. Fractions two and three of Table I had a strong, unpleasant odor characteristic of beet molasses; all of the fractions were nearly colorless. A total of 21.7 g. of sucrose (93.3% of that present) of excellent purity was obtained on concentration of the effluent. The residual sirups obtained on complete solvent removal were unpalatable.

TABLE I

EFFLUENT FRACTIONS FROM THE CHROMATOGRAM OF MOLASSES ON CLAY

Type of molasses	Fraction	Volume of fraction, ml.	Solids in fraction, g.	Sucrose, First crop, g.	Sucrose, First crop, M. p., °C.	Sucrose, First crop, $[\alpha]^{28}\text{D}$, c 5, water	Sucrose, Second crop, g.	Sucrose, Second crop, $[\alpha]^{28}\text{D}$, c 5, water
Cane blackstrap	1	450	0.3	...			..	
	2	260	.3	...			..	
	3	130	.6	...			..	
	4	290	5.9	2.74			0.11	
	5	310	6.7	2.91	180–182	+66.8°	.17	
	6	400	6.5	2.80			.16	
	7	370	3.7	1.00	179–181		.45	
	8	180	1.0	0.07			.37	
	9	1280	2.4	1.09	180–182		..	
	Total	3670	27.4	10.61	180–182[a]	+65.9°	1.26	+65.0°
Beet	1	270	0.2	...			..	
	2	190	.2	0.05			..	
	3	200	.2	.15	180–182		0.05	
	4	210	1.4	.30			.44	
	5	230	2.8	1.44	180–182		.68	
	6	390	4.9	2.92	180–182	+66.7°	.90	
	7	470	5.5	2.70	180–182		2.46	
	8	360	5.3	1.89			1.87	
	9	350	3.9	1.00	180–182		1.97	
	10	350	2.6	1.70			..	
	11	880	1.9	1.21	180–182		..	
	Total	3900	28.9	13.36	180–182[a]	+66.5°	8.37	+64.0°

[a] Mixed melting point with sucrose (m. p. 180–182°) of composite samples: from cane blackstrap molasses, 180–182°; from beet molasses, 180–182°.

Acknowledgment.—We are pleased to acknowledge the assistance in this work of Mr. John Kolbas.

Summary

When either cane blackstrap molasses or beet molasses is adsorbed on a suitable clay and the chromatogram so obtained is developed with dilute alcohol, a high purity, crystalline sucrose is recoverable from the effluents in a yield of 74% of the sucrose present for cane blackstrap molasses and 93% for beet molasses.

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A Diffusion Study of Lignin Sulfonic Acids in Sulfite Waste Liquor

BY DERROL PENNINGTON AND D. M. RITTER

Available data support the conclusion that lignin sulfonic acids from the sulfite wood pulping process not only exhibit chemical inhomogeneity but are heterogeneous in respect to their molecular weights, which are thought to be high. Unfortunately, however, most values given in the literature offer only qualitative evidence of the molecular size. Their variations from 400 to 6,000 suggest the cryoscopic method by which they were determined to be unsuitable for use with lignin sulfonic acids.[1] This is not surprising since cryoscopic data are expressive of thermodynamic activities and their interpretation in terms of the molecular dimensions for polyvalent strong electrolytes such as lignin sulfonic acids is complicated by lack of knowledge of activity coefficients and degrees of dissociation. Interpretation of osmotic pressure data is subject to the same difficulties and is further complicated by the inevitable presence of Donnan equilibria. Reproducible molecular weights of 400 to 20,000

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(1) P. Klason, "Beitrag zur chemischen Zusammensetzung Fichtholzes," Berlin, 191[illegible], pp. 18, 37; *Arkiv. Kemi Mineral. Geol.*, **6**, 13 (1917); *Chem. Zentr.*, **90**, I, 62 (1919); K. Melander, *Kemi Och Bergvetenskop*, 10–12 (1918); *Medd. fra Pappersmk.*, **29**, 1921; M. Samec and I. Ribaric, *Kolloidchem. Beihefte*, **24**, 157 (1927).

have been estimated by Schwabe and Hasner[2] from data on dialysis of lignin sulfonic acid preparations through "Cella filters." These values lack authenticity since they were obtained by using as membranes ultrafilters which those authors and others[3] recognize to lack sufficient porosity for study of particles with molecular weights exceeding 5,000.

For further logical development of methods for separation and fractionation of the lignin sulfonic acids more knowledge is required concerning the molecular sizes involved, and a diagnostic method is needed for assessing the results of reactions which may cause cleavage of molecules. In this study such information has been obtained by measurement of average integral diffusion coefficients. Average molecular weights are not always sensitive to changes in size distribution but in this work measurements over successive intervals have furnished a sequence of values which express the inhomogeneity of the substances under investigation. For this purpose free diffusion has been measured in sintered Pyrex glass diaphragm cells of the type used so successfully by Northrop and Anson,[4] and other investigators,[5] for similar measurements on protein macromolecules.

Experimental

Preparation of Sulfite Waste Liquor.—A material was used in the diffusion runs which represented the bulk of sulfite waste liquor and yet was as free as possible from such non-ligneous substances as simple sugars and inorganic salts. A sulfite waste liquor from western hemlock was neutralized with calcium oxide and fermented with yeast until all fermentation had ceased. The fermented liquor was filtered and de-ashed by means of a cation-exchange resin and the strongly acid solution was neutralized with ammonium hydroxide. The solution of ammonium salts was extracted with ether and evaporated to dryness. This material had an ash content of 1.12%, and a methoxyl content of 8.50%. The equivalent weight determined by ammonia removal was 210.

Diffusion Methods.—Diffusion cells of the type described by Northrop and Anson[4] were constructed from 6 cm. Corning Pyrex glass funnels with sintered glass disks of medium porosity. The cells fitted closely into 400-ml. beakers which contained the solution into which diffusion took place. To prevent evaporation during the long diffusion periods the beakers were covered with rubber membranes pierced by a hole through which passed the neck of the diffusion cell. In operation cells and beakers were mounted in a constant temperature air-bath maintained at 25.00 ± 0.05°.

Five per cent. ammonium carbonate solution was employed as the diffusion medium to reduce the influence of the charge carried by the lignin sulfonate anions.[6] At the beginning of each experiment sufficient of this buffer solution was prepared and bottled to last through the successive intervals as a solvent of constant composition. Each diffusion period was of the minimum duration required to obtain sufficient diffusate for analysis. After each interval the beaker was removed, the outside of the cell and membrane was washed and the washings were combined with the diffusate solution. Another beaker containing fresh solution was placed in contact with the cell and the diffusion was continued. Other procedures and precautions observed while using the diffusion cells were those described by previous investigators.[4,5]

Since the material being studied was a mixture, the only means of determining the weight of solids diffusing was to evaporate an aliquot of the solution to dryness and to weigh the residue. Usually it was necessary to evaporate 50 ml. of solution and vaporize 2.5 g. of ammonium carbonate in order to weigh samples of the order of 20 mg. Nevertheless, it was found possible to duplicate the weights to within 2%, which enabled diffusion coefficients to be calculated with a reproducibility of 5%. Methoxyl and sulfur analyses were carried out by standard micro methods. The determination of the periodic acid oxidation is to be described in a forthcoming communication.

Results

The diffusion coefficients were calculated from the integrated form of Fick's law

$$D = \frac{2.303\,V}{2K\,\Delta t}\log\frac{C_0}{C_0 - 2C_2}$$

It is based on the condition that the volumes on each side of the sintered glass membrane be the same. V is the volume of the diffusion cell, and K is the cell constant determined by diffusing 0.1 N potassium chloride for which the diffusion coefficient at 25° was assumed equal to 1.631 as determined by McBain and Liu.[5] C_2 equals the concentration achieved in the lower volume at the end of the interval, and C_0 the concentration in the cell at the beginning of the interval.

Diffusion coefficients based on the rate at which the total solids diffused are given in Table I. Data concerning the diffusion of methoxyl-bearing components of the liquor are given in Table II. In Table III are given data based on the rate of diffusion of periodic acid-oxidizable material. Diffusion of sulfur-containing substances is shown in Table IV.[7]

Discussion

Diffusion data have not often been applied to examination of mixtures. Interpretation of results in the case of a mixture such as sulfite waste

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(2) K. Schwabe and L Hasner, *Cellulosechem.*, **20**, 61 (1942).

(3) Jander and Spandau, *Z. physik. Chem.*, **A187**, 24 (1940); H. Brintzinger, *ibid.*, **A187**. 317–334 (1940); Spandau and Gross, *Ber.*, **74B**, 362 (1941); Vollmar, *Oesterr. Chem. Ztg.*, **44**, 13–15 (1941).

(4) Northrop and Anson, *J. Gen. Physiol.*, **12**, 543 (1929); Anson and Northrop, *ibid.*, **20**, 575 (1937).

(5) McBain and Liu, THIS JOURNAL, **53**, 59 (1931); McBain, Dawson and Barker, *ibid.*, **56**, 1021 (1934); Friedman and Klemm, *ibid.*, **61**, 1747 (1939); Friedman and Ray, *J. Phys. Chem.*, **46**, 1140 (1942); Fletcher, Gulland, Jordan and Dibben, *J. Chem. Soc.*, 30 (1944).

(6) Hartley and Robinson, *Proc. Roy. Soc.* (London), **A134**, 20 (1931); Valko, *Trans. Faraday Soc.*, **31**, 230 (1935); Dean, THIS JOURNAL, **67**, 31 (1945).

(7) A few secondary results can be calculated from the direct observations with the aid of an assumption regarding the composition of the ligneous substances. An unpublished method developed by the authors for the purification of lignin sufonic acids by quinoline extraction yields a product representing the major portion of the methoxyl-containing material with a methoxyl content of about 12% and an equivalent weight for periodic acid reduction of about 525. If the ligneous substances are assumed to have that composition, a residue of methoxyl-free solids will be diffusing at each interval. The diffusion coefficients for residual material calculated on that basis are nearly the same for all intervals. The values correspond closely to $D = 0.4 \pm 0.06_{\text{av.}}$ with remarkable agreement considering the fact that the calculations are based upon small differences between analytical values varying ±2%. Values based on the periodate oxidation data can be judged to approximate $D = 0.4$ though the agreement is poor. The periodate equivalent weight of this low molecular weight fraction appears to be about 110–115.

TABLE I

DIFFUSION COEFFICIENTS BASED ON TOTAL SOLIDS DIFFUSING

Cell 1. Volume 185 ml., $K = 0.742$ cm.

Time, hours	Cumulative % solids diffused	C_0 mg./ml.	C_2 mg./ml.	D cm.²/day
0- 120	11	6.278	0.720	0.27
120- 317	24	5.558	.810	.22
317- 531	35	4.748	.681	.20
531-1034	50	4.067	.962	.16
1034-1494	62	3.105	.715	.17

Cell 2. Volume 174 ml., $K = 0.600$ cm.

Time, hours	Cumulative % solids diffused	C_0 mg./ml.	C_2 mg./ml.	D cm.²/day
0- 120	9.5	6.211	0.590	0.26
120- 317	21	5.621	.745	.23
317- 531	32	4.876	.676	.22
531-1034	48	4.200	.957	.16
1034-1494	59	3.243	.681	.17

TABLE II

DIFFUSION COEFFICIENTS OF METHOXYL-BEARING COMPONENTS

Cell 1. Volume 185 ml., $K = 0.742$ cm.

Time, hours	% Methoxyl in diffusate	Cumulative % methoxyl diffused	C_0 mg. MeO/ml.	C_2 mg. MeO/ml.	D_2 cm.²/day
0- 120	4.96	7.3	0.500	0.036	0.16
120- 317	6.20	17	.464	.050	.16
317- 531	6.49	27	.414	.049	.16
531-1034	7.50	41	.365	.072	.12
1034-1494	7.31	52	.293	.052	.12

(Methoxyl content of material remaining in cell = 10.10%.)

Cell 2. Volume 174 ml., $K = 0.600$ cm.

Time, hours	% Methoxyl in diffusate	Cumulative % methoxyl diffused	C_0 mg. MeO/ml.	C_2 mg. MeO/ml.	D_2 cm.²/day
0- 120	5.81	6.8	0.499	0.034	0.18
120- 317	6.47	16	.465	.048	.17
317- 531	6.42	25	.417	.043	.16
531-1034	7.12	39	.374	.068	.13
1034-1494	7.18	48	.306	.049	.12

(Methoxyl content of material remaining in cell = 10.04%.)

TABLE III

DIFFUSION COEFFICIENTS OF PERIODATE OXIDIZABLE COMPONENTS

Cell 2. Volume 174 ml., $K = 0.600$ cm.

Time, hours	C_0 mmoles periodate reduced/ml.	C_2 mmoles periodate reduced/ml.	D cm.²/day
0- 120	0.0257	0.0033	0.36
120- 317	.0224	.0038	.31
317- 531	.0186	.0030	.26
531-1034	.0156	.0042	.22
1034-1494	.0114	.0030	.24

liquor is of increased difficulty because the components are not only heterodispersed, but also chemically inhomogeneous. However, the results of only partially successful efforts at physical and chemical fractionation of the lignin sulfonic acids indicate a chemical similarity sufficiently close that to a first approximation the ligneous portion of sulfite waste liquor can be treated as a single substance,[7]

TABLE IV

DIFFUSION COEFFICIENTS OF SULFUR-BEARING COMPONENTS

Cell 1. Volume 185 ml., $K = 0.742$ cm.

Time, hours	% Sulfur in diffusate	Cumulative % of sulfur diffused	C_0 mg. S/ml.	C_2 mg. S/ml.	D cm.²/day
0- 120	8.49	13	0.4697	0.0612	0.31
120- 317	8.50	28	.4085	.0688	.26
317- 531	8.11	40	.3397	.0552	.23
531-1034	7.51	56	.2845	.0721	.18
1034-1494	7.24	67	.2124	.0515	.18

(S content of material remaining in cell = 6.48%.)

Cell 2. Volume 174 ml., $K = 0.600$ cm.

Time, hours	% Sulfur in diffusate	Cumulative % of sulfur diffused	C_0 mg. S/ml.	C_2 mg. S/ml.	D cm.²/day
0- 120	9.01	12	0.4560	0.0532	0.32
120- 317	8.11	25	.4038	.0604	.26
317- 531	7.73	36	.3434	.0522	.24
531-1034	7.98	53	.2904	.0766	.22
1034-1494	6.86	63	.2138	.0470	.18

(S content of material remaining in cell = 6.51%.)

The values of diffusion coefficients obtained in this work differ from those ordinarily expressed in that the quantities determined are average integral diffusion coefficients. Examination of the Sutherland–Einstein equation[8] $D = (1/6\pi\eta r) \times (iRT/N)$ shows the diffusion coefficient to be the product of two factors. The contribution from Stokes' law is related to the weight of the particle through its radius, and the osmotic factor is dependent upon the number of particles. The type of average is therefore composite and the values will lie between those calculable from weight-average and from number-average molecular weights. In spite of the difficulty of interpreting diffusion data for complex mixtures on a sound theoretical basis certain general conclusions can be drawn from an examination of the diffusion coefficients given in the Tables I through IV:

1. The heterodisperse character of the principle components of sulfite waste liquor is obvious from examination of diffusion coefficients calculated on whatever basis is chosen, be it weight, methoxyl or sulfur transfer or diffusion of periodate-reducing power.
2. Over each interval methoxyl-bearing material, assumed to be the ligneous fraction, diffuses more slowly than the balance of the substances. The lignin sulfonic acids therefore appear to be the largest molecules present. Deductions described in footnote 7 suggest the non-methoxyl material to be for the most part rather small in molecular size.
3. Sulfur transfer occurs more rapidly than methoxyl migration. Since an important fraction of the sulfur is associated with the methoxyl-bearing material and must therefore diffuse at the same rate, another portion of the sulfur must be associated with non-methoxyl-bearing organic

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[8] Sutherland, *Phil. Mag.*, [6] **9**, 781 (1905); Einstein, *Z. Elektrochem.*, **14**, 235 (1908).

components smaller in size than the ligneous material.[9]

4. While the average integral diffusion coefficients of substances oxidized by periodic acid are higher than corresponding values determined on any other basis, it is obvious that a part of the ability to reduce periodic acid is associated with a macro-molecule. That is particularly apparent during the fourth and fifth intervals. This suggests that the lignin sulfonic acids contain structural features oxidizable by periodic acid. The deductions in footnote 7 indicate that the non-ligneous material which reacts with periodic acid is made up of relatively small molecules of low equivalent weight. These are doubtless carbohydrate materials resistant to fermentation and to the action of sulfite pulping chemicals.

By means of the Sutherland–Einstein equation[8] the average integral diffusion coefficients can be translated into average molecular weights. Thus determined[10] the sizes of the methoxyl-bearing components vary from an average molecular weight of 3,100 in the first interval to an average molecular weight of 10,000 in the last two intervals. It is apparent that on this basis well over 60% of the methoxyl-bearing material has an average molecular weight equal to 10,000 or more. If the assumptions made in footnote 7 are accepted, the molecular weights of the non-ligneous substances average about 250.

Diffusion analysis is being applied to the study of lignin sulfonic acid preparations, and to the examination of the products of their reactions.

Summary

The heterodisperse character of the lignin sulfonic acids in sulfite waste liquor has been demonstrated by determination of a sequence of average-integral diffusion coefficients observed over successive intervals of diffusion.

Calculations made by application of the Sutherland–Einstein equation to the experimentally determined diffusion coefficients show the lignin sulfonic acids to vary in molecular weight from 3,000–10,000 or more.

The non-ligneous substances in sulfite waste liquor appear to be of low molecular weight, leaving the lignin sulfonic acids as probably the only macromolecules.

[9] A higher average integral coefficient for the diffusion of sulfur could arise from the presence of rapidly diffusing salts such as ammonium sulfate or ammonium sulfite. Since with cells of the dimensions used in this work only 10% of any ammonium sulfate originally present would remain after the third interval, while 60% of the total sulfur would not have migrated, inorganic salt transfer can account for only a small portion of the increased rate of diffusion.

[10] The equation used for the calculation is $M = 18.3/D^3$, where M = the average molecular weight, D = the average integral diffusion coefficient, and the constant 18.3 contains a density value = 1.35 g./cm.^{3} obtained on the material described in footnote 7 and a viscosity value for five per cent. aqueous ammonium carbonate solution of $\eta = 1.072 \times 10^{-2}$ poise. The remainder of the constants in the Sutherland–Einstein equation were obtained from "International Critical Tables."

Although the Sutherland–Einstein equation was derived from assumptions valid only in infinitely dilute solutions, it has been applied with success by Northrop and Anson, and by McBain to egg albumin at 1% concentration and by Friedman to several sugars [see Friedman and Carpenter, This Journal, **61**, 1745 (1939), also Dean and Loring, *J. Biol. Chem.*, **157**, 717 (1945)]. In solutions as dilute as those used for this work (0.5%) the error introduced in the calculated molecular weights is certainly not greater than that corresponding to the precision claimed in the measurements.

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The Pigments of Cottonseed. II. Gossycaerulin, a Blue Quinonoid Gossypol Derivative Occurring in Cooked Cottonseed

By Charlotte H. Boatner, Carolyn S. Samuels, Catherine M. Hall and Maizie C. Curet

In the first recorded investigation of the pigments of cottonseed, Kuhlmann[1] reported the formation of a brilliant blue pigment when acidified cottonseed foots were subjected to steam distillation for the recovery of fatty acids. Following Kuhlmann's original partial characterization of "cottonseed blue" nothing further has been reported relative to this pigment.

During isolation of gossypol from ethereal extracts of non-defatted cottonseed by a modification[2] of a procedure recommended by Carruth,[3] a brilliant green precipitate was obtained upon heating the crude gossypol acetic acid suspended in a mixture of diethyl ether and acetic acid. Prolonged heating (three to four days) of a suspension of pure gossypol in concentrated hydrochloric acid produced a similar green product. The absorption spectra of chloroform solutions of the green products exhibited a slight maximum at 610 mμ in addition to that at 365 mμ which is characteristic of gossypol.

A blue compound has now been prepared free of gossypol by heating pure gossypol in methanol acidified with sulfuric acid. It has been named gossycaerulin because of its color and its occurrence in cooked cotton seed. This blue pigment was probably a component of Kuhlmann's "cottonseed blue."

The reaction of gossypol in methyl alcohol solu-

[1] Kuhlmann, *Compt. rend.*, **53**, 444–452 (1861).

[2] Boatner, Caravella and Samuels, This Journal, **66**, 838 (1944).

[3] Carruth, *ibid.*, **40**, 647–663 (1918).