

# FRACTIONAL EXTRACTION AND PROPERTIES OF HEMICELLULOSES

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After the excellent presentations which you have just read on the hemicelluloses, you may wonder with reason whether there is anything left to discuss. We should, therefore, like to state the basis, somewhat arbitrary at best, of the present review. The first limitation is the use of the restricted definition of hemicellulose, which excludes starch, pectins and water-extractable polysaccharides, except those freed for extraction by strong swelling agents, such as liquid ammonia or dimethyl sulphoxide. The second limitation is consideration only of experiments in which the hemicellulose mixtures were precipitated so that we know something of the yield, at least, of the product. While work on softwoods is emphasized, some pertinent papers dealing with other materials have been included.

The separation of polymers must, in its very nature, be a fractionation procedure and there are two main types of fractionation, precipitation and solution. In general, precipitation procedures are preferred, but there are some special reasons which make solution fractionation of particular interest to the chemist interested in hemicelluloses. Most important, perhaps, is the fact that separation of the hemicelluloses from the more inert and insoluble cellulosic component of the wood has to be done by solution anyway. Closely associated with this is the consideration that the avoidance of as many different manipulations in purifying as possible makes the material balance more accurate. If one is interested in determination of structure and high purity, this is less important, but if one is interested in the composition of the original wood, it becomes very important. Similarly, the simplification of purification procedures and elimination of steps makes it more likely that the hemicellulose has been isolated unchanged, a goal rarely if ever attained.

Extraction techniques are made especially difficult in this field by the nature and structure of wood. The morphology of the fibre, the varying accessibility of the components, and the strong sorptive forces exerted by cellulose, all combine to prevent as complete separation as one would wish. Nevertheless, the same series of scientific breakthroughs that have accelerated progress in the whole field of hemicelluloses have also given fresh impetus to the investigation of fractional extraction.

In 1946, Partridge<sup>1</sup> demonstrated the applicability of paper chromatography to the separation of sugar mixtures. This made possible investigations of polysaccharides that were previously out of the question; in particular, one could analyse extracted hemicellulose fractions readily and reasonably accurately. In 1947, Yan<sup>2</sup>, working with Purves, investigated

liquid ammonia as an extractant. This was the first use in fractional extraction of a nonaqueous solvent. In 1956, Hägglund, Lindberg, and McPherson<sup>3</sup> explored a second such solvent, dimethyl sulphoxide. This exhibited a certain specificity in its action, as will be discussed later. Still more specific was the use of alkali borate extractions, introduced the same year by Jones, Wise, and Jappe<sup>4</sup>. It was also this year that Anthis<sup>5</sup>, working with Wise, demonstrated unequivocally the existence of a chemical linkage between mannose and glucose in the cell-wall polysaccharides of spruce, which led to the establishment of glucomannans as a class. Finally, in 1957, Hamilton and Quimby<sup>6</sup> demonstrated the superiority of sodium hydroxide over potassium hydroxide for extracting hemicelluloses.

These developments changed the rate of progress tremendously, but they also changed the direction. Prior to this, successively extracted fractions showed a gradual change in properties. The structure of the hemicelluloses was not well understood and fractionation according to molecular size, as in the case of homologous polymers, might have been expected. Now, as we learned more of the complexity of the hemicellulose mixtures, it became clearer that fractionation according to structure was within our reach.

Naturally enough, everyone studies his own particular problems and uses extraction procedures chosen from his viewpoint. This variety of procedures has made it hard to compare results, but in this review we shall try to trace the development of fractional extraction from the start.

We shall not discuss the simplest case of fractional solution, repeated extraction with liquid of the same composition. The omission is not serious, however, for it is well known that the bulk of the extraction is done by the first charge. The composition of the extracted hemicellulose has been found to change with later repetitions, but the small amounts involved make this a rather trivial observation for most purposes.

The first real fractional extractions used the same liquid at different temperatures. Preece<sup>7</sup> extracted "free hemicellulose" from boxwood with 4 per cent sodium hydroxide at room temperature and then "combined hemicellulose" with boiling 4 per cent sodium hydroxide. We know now that the material extracted with hot alkali, and the residue as well, have been greatly altered by the treatment and the procedure is not satisfactory for most purposes, but Preece's work shows two important points, first, that fractional extraction of wood hemicelluloses is possible (his yields were 7.7 and 6.4 per cent respectively, of the dry wood) and, second, that it might be incomplete (there was still "xylan" in the residue). Of course, the principle of fractional extraction of the hemicelluloses was established much earlier when Cross, Bevan, and Smith<sup>8</sup> showed that alkali extracts only part of the furfuroids.

The only other application of hot alkali extraction to the fractional solution of wood hemicelluloses is the work of Mitchell, Rogers, and Ritter<sup>9,10</sup> on maple holocellulose. It has been used, however, on such materials as corn cobs and cottonseed hull bran<sup>11</sup>.

Sands and Nutter<sup>12</sup> used a different fractional extraction to separate "free" and "combined" hemicelluloses from mesquite wood. The wood was extracted with 10 per cent sodium hydroxide, then delignified, and, finally, a second fraction was extracted with 10 per cent sodium hydroxide.

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Anderson and his co-workers used this technique on locust, lemon, birch<sup>13</sup>, cottonwood<sup>14</sup>, and white pine<sup>15</sup>. Rogers, Mitchell, and Ritter<sup>16</sup> and Clermont<sup>17</sup> used modified variants on maple sawdust and on spruce holo-cellulose, respectively. Rogers, *et al.*<sup>16</sup> used an interesting procedure—steeping the material first in 20 per cent sodium hydroxide and then diluting to 3 per cent before filtering. In none of the above cases is there much difference in the nature of the extracts, only a slight increase in mannose in the second extraction for softwoods and a correspondingly slight decrease in xylose and uronic anhydrides. Opinions are divided as to the effect of delignification. Some believe that a chemical bond is actually broken, others that lignin simply inhibits swelling to the extent that complete removal of the hemicelluloses is hindered, but there is no conclusive evidence. In any case, delignification is helpful in any extraction, indeed it is quite essential for softwoods, as Nelson and Schuerch have shown<sup>18</sup>.

Swelling is also a factor in achieving fractionation by extraction sequences with increasing alkali concentration where the swelling increases correspondingly. It should be remembered, however, that Schuerch<sup>18</sup> has shown, in extracting hemicelluloses from hardwoods, that there is an optimum swelling concentration above which the swelling of the cellulose inhibits the removal of the pentosans.

Successive extractions with alkali of increasing concentration were introduced very early. In 1931, Anderson<sup>11</sup> extracted corn cobs with 4 per cent sodium hydroxide, followed by a second extraction with 17 per cent sodium hydroxide. Many groups of chemists have used some type of concentration sequence (*Nables 1 and 2*).

Table 1. Sequences of varying concentration: KOH

Alkali concentration (%)	Investigators
5-16	Wise, <i>et al.</i> <sup>20-22</sup>
5-16	Brasch and Jones <sup>23</sup>
5-24	Wise, <i>et al.</i> <sup>19, 24</sup>
5-24	Runkel and Lüthgens <sup>25</sup>
5-24	Karnik <sup>26</sup>
5-16-24	Wise and Ratliff <sup>27</sup>
1-4-7-16	J. O. Thompson, Swanson, and Wise <sup>22</sup>
1-4-7-16	Most <sup>28</sup>

Table 2. Sequences of varying concentration: NaOH

Alkali concentration (%)	Investigators
1-5	Millett and Stamm <sup>29</sup>
1-2-3-4-6-12	Nelson and Schuerch <sup>18</sup>
0.5-1-2-12	Roudier <sup>30</sup>
1-4-10	Aspinall and McKay <sup>31</sup>
5-10-18	Painter and Purves <sup>32</sup>
4-17.5	Laidlaw, <i>et al.</i> <sup>33</sup>
4-10	Laidlaw, <i>et al.</i> <sup>33</sup>

Some fractionation occurs in these extractions as seen in *Table 3*, but it is not very great; the glucuronoxyylan group of hemicelluloses is, in general, more easily extracted than the glucomannan group and fractionation is, therefore, more apparent in the softwoods where there is more of the latter. Really efficient separation only came when specific action was attained by the use of different compounds for extraction.

*Table 3.* Fractionation by varying alkali concentration slash: pine holocellulose\*

KOH concentration (%)	Percentage of total	Mannose units (%)	Pentose units (%)	D.P.	Uronic acid (%)
1	42	5.4	49.5	100	28.0
4	17	10.2	56.1	105	20.4
7	19	15.2	48.9	120	16.8
16	22	22.6	27.8	130	12.8

\* Data from Thompson, Swanson and Wise<sup>22</sup> except last column from Most<sup>28</sup>.

The first experiments describing the use of different materials did not exhibit any very specific action, nor, indeed, were they used for this purpose. For instance, Voss, Bauer, and Pfirschke<sup>34</sup> extracted beechwood with 0.2 per cent sodium hydroxide, followed by extraction with a solution containing 5 per cent sodium hydroxide and 3 per cent sodium chloride. Actually, this is an extension of studies begun much earlier by Schmidt. The first paper by Schmidt on cellulose and its incrustants was published in 1921<sup>35</sup>, before the work already discussed, but he never seems to have isolated his very soluble xylan and less soluble xylan from a single source. In all his work on beechwood, which was summarized in 1937<sup>36</sup>, he drew his conclusions from weight losses and properties of the residues. Both Schmidt<sup>37</sup>, and later Voss<sup>34</sup>, added salt to the alkali to hinder solubility of  $\beta$ -cellulose, not to increase the solubility of the non-celluloses.

Other early experiments in different extractants were aimed merely at altering the pH of the alkali used. Such a sequence was Bennett's extraction of cornstalk holocellulose<sup>38</sup>, which used 2 per cent sodium carbonate, 4 per cent sodium hydroxide and 10 per cent sodium hydroxide. This resembles Mitchell, Rogers, and Ritter's earlier sequence used on maple<sup>9,10</sup>, except that Bennett did not mention the use of hot alkali. Separations achieved in this way are not very startling and, as the yields in Thomas' study of aspen show<sup>39</sup>, most of the extraction is done by the stronger alkalis (*Table 4*).

It might be argued that the use of liquid ammonia is another example of this type, but this is only superficially so. Ammonia is a weak base, but liquid ammonia is a very strong swelling agent and facilitates subsequent extraction. It was first used by Yan and Purves<sup>2, 40</sup> as an extractant for lignin and, later, carbohydrates and it was shortly after used by Bishop and Adams<sup>41</sup> in a fractionation of wheat straw holocellulose. This does not strictly belong in a discussion of wood chemistry, but it is the first use of liquid ammonia in a fractionation sequence and also the first use of paper partition chromatography for evaluating hemicelluloses fractionally extracted. Both techniques were applied to wood the same year by Björkqvist

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and Jörgensen<sup>42</sup>. The fractionating capacity is probably best shown by the recent paper of Milford, Watts, and Purves<sup>43</sup> in which spruce holocellulose was extracted first with liquid ammonia at 20°, then with water at 75°, and finally with 10 per cent sodium hydroxide at 5°. The ammonia deacetylates the acetylated derivatives and removes lignin and a little carbohydrate. The warm water removes what is mainly a glucuronoarabinoxylan. There is glucose and galactose in the mixture, but no mannose.

Table 4. Extraction of aspen holocellulose

<i>Extractant</i>	<i>Yield, on holocellulose</i>
Water at 20°	3.9
Water at 100°	9.1
5% KHCO <sub>3</sub>	0.6
5% K <sub>2</sub> CO <sub>3</sub>	1.6
1.4% KOH	1.3
5.6% KOH	10.8
13.9% KOH	6.3
23.8% KOH	1.1
Residue	65.0
	Total 99.7

The sodium hydroxide removes a mixture of which over half is glucomannan. The extracted pulp still contains 9 per cent of mannose residues. This was reduced to 3 per cent by extraction with 18 per cent sodium hydroxide, but the extract was not worked up.

The thesis of Schoettler<sup>44</sup> (The Institute of Paper Chemistry, 1952) has not been published as such. It dealt with the resistant xylan of kraft pulp and included some data of interest here. When aspen kraft pulp was treated with 17.5 per cent sodium hydroxide, a complex mixture of hemicelluloses is extracted, of course, but Schoettler found that with 24 per cent potassium hydroxide, he could get a yield of 5.7 per cent of a pure xylan from the residual  $\alpha$ -cellulose (90.5 per cent xylose residues, 8.9 per cent ash). Glucuronic acid residues were probably removed during the kraft cook. This preferential action of the two alkalis in the case of aspen is remarkable, since it has since been shown, in the case of softwoods, that sodium hydroxide is the better extractant. In 1957, Hamilton and Quimby<sup>6</sup> studied the action of the two alkalis at various concentrations on Western hemlock. In the same year, Aspinall, Laidlaw, and Rashbrook<sup>45</sup> used an extraction sequence on partially delignified Sitka spruces of 4 per cent sodium hydroxide—24 per cent potassium hydroxide—delignification—10 per cent sodium hydroxide. The last fraction was largely glucomannan. Later work by Hamilton and N. S. Thompson<sup>46, 47</sup>, Dutton and Hunt<sup>48</sup>, and Murray, Morak, and Ward<sup>49-51</sup> has adequately demonstrated the value of extracting softwood hemicelluloses with potassium hydroxide and then sodium hydroxide. It has also emphasized again the importance of swelling. In order to remove everything soluble in a given extractant, the structure must be as available as possible to the extractant, which can only be achieved at high states of swelling. Even then, sorption phenomena will

prevent absolutely complete removal. The difference between the behaviour of aspen and of other woods is still unexplained.

Jones, Wise, and Jappe<sup>4</sup> introduced the use of alkali borate in 1956 and Jones, Merler, and Wise<sup>52</sup> used it in fractionally extracting aspen wood the next year. The sequence (water, 1 per cent potassium hydroxide, 10 per cent potassium hydroxide, 20 per cent potassium hydroxide, and 20 per cent potassium hydroxide + 4 per cent boric acid) gave mainly xylan polymers in the first four and mainly glucomannans in the final fraction. The value of this reagent was immediately recognized and it has been used by many groups since. The same is true of the use of dimethyl sulphoxide, also introduced in 1956 by Hägglund, Lindberg, and McPherson<sup>3</sup> as a solvent for hemicelluloses. The list of those who have made use of extraction sequences containing one or both of these solvents includes Jones, Merler, and Wise<sup>52</sup>, Lindberg and Meier<sup>53, 54</sup>, Croon<sup>55</sup>, Mian and Timell<sup>56-62</sup>, Nelson<sup>63</sup>, Leopold<sup>64</sup>, Murray, Morak, and Ward<sup>49-51</sup>. Koshijima<sup>65</sup> described an interesting variant last year; he extracted pine holocellulose with dimethyl sulphoxide acidified with acetic acid, then dimethyl sulphoxide acidified with boric acid, then water, and finally a more acid dimethyl sulphoxide mixture with boric acid. The pH values of his dimethyl sulphoxide extractants were 6.8, 5.2, and 4.3 respectively. Decreasing amounts of pentose and increasing amounts of glucomannan were evident in his fractions.

To sum up the present status of fractional extraction of softwood hemicelluloses, the problem is to effect as good a separation as feasible of the xylan-containing components and of the glucomannans and related compounds during extraction. We can come nearer this with some of the recent techniques described, but we are not near enough. We can remove almost all the xylan-containing components, but some of the galactoglucomannan group are so readily available that they are also removed in the extract. By removing the xylan, however, it is possible in a later extraction to get a nearly pure glucomannan. It will not represent the entire mannose content of the tree, however. *Table 5* shows the composition of this fraction by different methods. In this table, Lindberg and Meier<sup>53</sup> used a sequence of dimethyl sulphoxide—hot water—14 per cent potassium hydroxide—14 per cent potassium hydroxide + 3 per cent boric acid—24 per cent potassium hydroxide + 3 per cent boric acid. This represents

*Table 5.* Crude glucomannan fractions

<i>Source</i>	<i>Yield</i>	<i>Composition</i>					<i>Investigator</i>
		<i>Gluc.</i>	<i>Gal.</i>	<i>Man.</i>	<i>Arab.</i>	<i>Xyl.</i>	
Spruce holo.	7.6*	[17.7]		81.0	—	1.3	Lindberg and Meier
Pine holo.	2.9	[24.4]		72.6	1.2	1.8	Meier
Pine holo.	2.3	[24.7]		70.3	2.3	2.7	Meier
Slash pine holo.	2.0	[24.5]		70.2	Minor	5.3	Nelson
Spruce holo.	9.6	36.3	1.2	60.3	0.6	1.6	Morak and Ward
Spruce holo.	5.1	21.6	1.9	75.9	—	0.6	Morak and Ward

\* Total amount extracted.

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the last but one fraction. Meier<sup>54</sup> used the same sequence. The results shown are for the water extraction and for the 24 per cent potassium borate. Nelson<sup>63</sup> used a series of sodium hydroxide extracts of varying strength, followed by a sodium borate hemicellulose which is that given. In the case of the two examples from Morak and Ward<sup>50</sup>, the xylans were first removed with potassium hydroxide. The final extractant was then sodium hydroxide in the first case and potassium borate in the second.

Let us discuss briefly some of the properties of these unpurified extracted hemicelluloses. The reasons for my interest in the unpurified fractions have been indicated; let me review a few of the results.

The sequence studied most thoroughly is essentially in three stages—potassium hydroxide, potassium borate, and sodium hydroxide—although several successive extractions were actually made with each to get exhaustive extraction with each reagent. The analyses in *Table 6* are for the alcohol-insoluble precipitate from the first extract with each reagent.

*Table 6.* Comparison of fractions from spruce holocellulose

Extractant	Yield	Composition of hydrolysate				
		Galactose	Glucose	Mannose	Arabinose	Xylose
KOH	21.02	14.0	9.9	21.7	7.9	46.5
KBO <sub>2</sub>	5.26	3.6	19.8	75.4	—	1.2
NaOH	4.90	2.0	54.5	41.3	—	2.2

The first fraction is the usual mixture obtained with potassium hydroxide. The second is a practically pure galactoglucomannan. We are not sure yet of the sodium hydroxide fraction. It is a mixture and probably contains the same glucomannan as the preceding along with some degraded glucans from cellulose, but this has not yet been proved.

To get optical rotations (*Table 7*) we dissolved each fraction in the extractant used (incidentally, this is not always easy), but we cannot draw any important conclusion other than that there are differences between the hemicelluloses from holocelluloses, sulphite pulp and kraft pulp, which we already knew from the chromatograms. A study of the rotations of the borate complexes would be needed to evaluate this series thoroughly.

*Table 7.* Optical rotations

Source	KOH hemi.	KBO <sub>2</sub> hemi.	NaOH hemi.
	$[\alpha]_D^{25}$	$[\alpha]_D^{25}$	$[\alpha]_D^{25}$
Chlorite holocellulose	-40.7	-65.8	-78.4
Chlorited sulphite pulp A	-77.4	-72.7	-21.3
Chlorited sulphite pulp B	-78.9	-74.4	-22.6
Chlorited kraft pulp	-108.2	-79.6	-46.4

X-ray diagrams were obtained of all the precipitated hemicelluloses and of all the residues at each stage. I am repeating here (*Figure 1*) the diagrams

of the three hemicellulose fractions extracted from spruce sulphite pulp combined for convenient comparison. Kraft pulp and holocellulose furnish similar sets of diffractograms<sup>51</sup>. There is no doubt that the fractions are crystalline, in the sense that polymers are crystalline. There is no doubt that they are different from each other, but the difference between the borate and the sodium hydroxide hemicelluloses is not great. The peaks at about 12.5 and about 20.5 are similar in both. They are also similar to Cellulose II. The patterns of the potassium hydroxide hemicelluloses correspond roughly to those of purified glucuronoxylans, but the main peak is at an intermediate value between the strong reflection for xylan and xylan hydrate of Marchessault and Timell<sup>66</sup>. The pattern of the borate fraction strongly resembles that of the hydrolysed glucomannan described by Lindberg and Meier<sup>53</sup>. However, we shall at this time heed the warning of Björkqvist, Jørgensen, and Wallmark<sup>67</sup> and draw no conclusions as to identity.

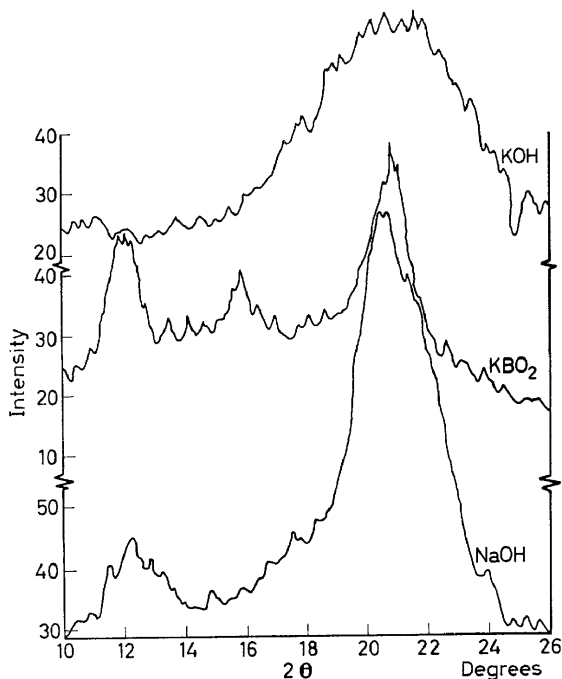


Figure 1. X-ray diffraction of hemicellulose of sulphite pulp ( $S_3$ )

The crystallinity was also investigated by means of the electron microscope. The general similarity of the crystals from the potassium hydroxide hemicellulose to the xylan crystals of Yundt<sup>68, 69</sup> and of Marchessault<sup>70</sup> is striking (Figure 2). This is, we believe, ample evidence that such crystals are *not* artefacts produced during the purification procedures.

Finally, we should like to discuss the molecular size of these fractions. We examined the potassium hydroxide extract of the holocellulose in detail.





*Figure 2.* Sprucewood hemicellulose extracted from chlorite holocellulose with 3.9 N KOH; chromium shadowed

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Light-scattering measurements gave a molecular weight of 45,000 and ultra-centrifuge measurements 42,000. The intrinsic viscosity in cupric ethylene-diamine was 0.52. When we used the calculated monomer weight of 145.8, this gave us a ratio of  $P_w$  (light scattering)/ $[\eta]_{CED}$  of 592. This is in reasonable agreement with similarly calculated ratios for 4-O-methylglucuronoxylans from the data of Goring and Timell<sup>71</sup>: 561 and 562. This is, of course, mostly fortuitous, for both they and we have shown that this ratio varies with the source of the hemicellulose.

In spite of all the uncertainties involved, we have used the value of 592 in calculating the D.P.'s in *Table 8*. We recognize that there is error in going from one hemicellulose to another and that the relation of D.P. to intrinsic viscosity may not be so nearly linear for hemicelluloses as for cellulose, but we wanted some approximation and we prefer to use a weight-average value rather than a number-average and a value derived on hemicelluloses rather than one derived from celluloses. In spite of the fact that these hemicelluloses were obtained from chlorited materials, the molecular weights are higher than most of those in the literature, except those of Goring and Timell<sup>71</sup> who were careful to avoid degradation. Some preliminary work at The Institute of Paper Chemistry indicates that our calculations for the borate fractions may be much too low.

*Table 8.* D.P. of extracted hemicelluloses

<i>Chlorited holocellulose from</i>	KOH <i>hemi.</i>	KBO <sub>2</sub> <i>hemi.</i>	NaOH <i>hemi.</i>
Sprucewood	308	254	355
Sulphite pulp <i>A</i>	201	148	361
Sulphite pulp <i>B</i>	201	148	319
Kraft pulp	396	201	373

Summing up briefly, it is now possible, for softwoods at least, to make a preliminary fractionation of hemicelluloses during the extraction process and to obtain the galactoglucomannan series in practically pure form. In the sequence used by us, this is the second of three fractions. It should be stressed that while we can reduce the number of steps in the sequences discussed, we are not obtaining our hemicelluloses unchanged so long as we use alkaline extractions. Acetyl groups and, perhaps, galactose units have been removed. It may be possible to devise a sequence which will separate the xylose group from the mannose group in the first extraction. Dimethyl sulphoxide looks promising but we have not been able to obtain the separations reported by Meier<sup>54</sup>. We are still investigating the material in our final fraction.

Let me close by giving credit to my co-workers at the Institute, Mr Swenson for the light scattering, Dr Kurath for the ultra-centrifuge, Dr Williams for the X-ray diagrams, Miss Smith and Mr Dearth for the electron micrographs, and Dr Wise, Dr N. S. Thompson, and Mr Dickey for valuable advice and occasionally comfort and condolence.

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