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Effect of Deuteration, Oxidation, and Hydrogen-Bonding on the Infrared Spectrum of Cellulose

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Regenerated cellulose films were immersed in heavy water (99.58% D_2O) at temperatures up to 52° C for as long as 100 hours in an attempt to effect a substitution of D for the H in the OH groups of the cellulose. The intensity of the OH vibration in the 3-micron region of the spectrum of the films decreased very little. A weak OD vibration appeared in the 4-micron region. The results indicate that only a small fraction of the OH groups of the cellulose participated in an exchange under the conditions of the experiments.

The infrared spectra from 2 to 16 microns of cellulose nitrate, nitrogen dioxide oxidized cellulose, alginic acid, and sodium alginate are recorded. A comparison of the spectra of these polysaccharides indicates that the strong band at 5.75 microns in the spectrum of the oxidized cellulose is due to the C=O vibration of the carboxyl group, and that the bands at 6.07 and 7.01 microns are due to nitrogen-oxygen vibrations.

The intense bands in the 3-micron region of the spectra of a number of cellulose derivatives, attributed to hydroxyl groups, were examined with the aid of the high-dispersion lithium fluoride prism. The minima of the bands were exactly at or close to 2.86 microns (3,500 cm^{-1}). The possibility of the existence of a common type of hydrogen bond in the cellulose derivatives is discussed.

I. Introduction

A continuation of work previously reported [1] [1] has led to further observations on the structure of cellulose. The infrared spectrophotometric measurements reported here pertain to three separate aspects of cellulose chemistry. The experiments and spectral measurements represent an attempt to answer questions relating to (a) the exchange reaction between regenerated cellulose and deuterium oxide, (b) the spectrum of cellulose containing carboxyl groups, and (c) the nature of hydrogen bonding in cellulose and its derivatives. The experiments bearing on the exchange reaction will be discussed first. The work on the exchange reaction should be considered as a progress report. It is presented in such detail because the results appear to be in somewhat surprising contrast to earlier findings.

[1] Figures in brackets indicate the literature references at the end of this paper.

II. Deuteration of Cellulose

Champetier and Viallard [2] reported that native cellulose undergoes the heterogeneous exchange reaction represented as follows:

$$C_6H_7O_2(OH)_3+3D_2O\rightarrow C_6H_7O_2(OD)_3+3H_2O,$$

and that the reaction goes to completion in 36 hr at 30° C. This implies that all of the hydroxyl groups are accessible to the D_2O molecules and hence available for the exchange. On the other hand, Frilette, Hanle, and Mark [3] studied the reaction, employing both native and regenerated cellulose and concluded that the hydroxyls were not equally accessible. Only 64 percent of the hydroxyl groups of the native cellulose and 66 to 89 percent of the hydroxyls in viscose rayon were found to participate in the exchange in their experiments in 168 hr at 25° C.

Whether all or only a part of the hydroxyls in cellulose are available for the exchange reaction

has an important bearing upon the present-day concept of the structure of this natural polymer. Cellulose is generally believed to consist of a network of long chain molecules arranged in more highly ordered (crystalline) regions and less well-ordered (amorphous) regions. Hydroxyl groups buried inside the crystalline regions would not be expected to be as accessible, and hence as free, to participate in the exchange with deuterium oxide as hydroxyl groups elsewhere in the cellulose.

The extent of the exchange reaction in the studies referred to above was deduced from the change in density of the heavy water. However, in this work infrared spectrophotometry was used in detecting the replacement of hydroxyl hydrogen by deuterium. This method has been applied successfully to the evaluation of the exchange between D_2O and β-methyl glucoside, β-methyl xyloside, α-methyl mannoside, L-rhamnose, and L-sorbose.[2] It was found using the spectroscopic method that the hydroxyl hydrogens of these water soluble compounds are completely exchanged for deuterium in a few hours at 25° C. The exchange is noted in the infrared spectra by the appearance of intense absorption at about 4 μ with simultaneous disappearance of the intense absorption at 3 μ. The absorption at 3 μ is known to be characteristic of hydroxyl hydrogens bonded through a hydrogen bridge to another oxygen atom, and that at 4 μ to hydroxyl deuterium similarly bonded.

Two types of regenerated cellulose films were used in the work to be reported. There was no essential difference in results. The first was prepared by deacetylation of cellulose acetate [1], and the second was commercial cellophane sheeting obtained from the Sylvania Division of the American Viscose Co. The films were 3 to 13 μ thick and were mounted in a stainless steel frame to facilitate handling. The mounted films were dried in an oven for 16 hr at 105° C in a special glass chamber (capacity 10 ml) with its ground glass cover ajar. Liquid heavy water, 99.56-percent D_2O, was run into the chamber from a sealed storage bottle, completely filling it, and the cover was put in place and sealed with a thin film of silicone grease. The cellulose was kept in contact with the D_2O for periods of time ranging from 25 to 100 hr at temperatures from 25° to 52° C. The film was removed and either dried or immediately placed in the beam of the infrared spectrophotometer [4] in room air at 40-percent relative humidity. Thus the transmission of the film in the 2- to 4-μ region could be measured within a few minutes with little opportunity for moisture from the air to enter the film and reverse the reaction.

The absorption at 3 μ of the film prepared according to the procedure just described would represent OH groups in the cellulose that had not exchanged with D_2O and OH in water in the cellulose that had not been eliminated by the drying or by diffusion into the D_2O. The contribution of this ordinary water to the absorption at 3 μ would be small, at least at the time the film was removed from the D_2O.

In order to find out whether there was a reversal of the deuteration reaction and removal of the deuterium from the cellulose during the measurements, deuterium-treated films were dried under two conditions, in an oven at 105° C and over anhydrous calcium sulfate at room temperature. The films were removed periodically, and the transmittance at 4 μ relative to that of the undeuterated cellulose was computed. As may be seen from the lower curve in figure 1, the rate of loss of the deuterium, as evidenced by decrease in the 4-μ band, was so slow at room temperature as to be insignificant for the first several hours.

In the first experiment designed to observe the extent of reaction, the regenerated cellulose film

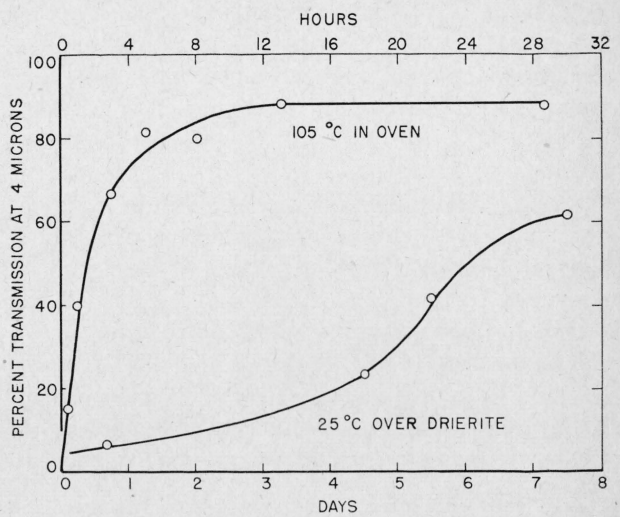


FIGURE 1. *Comparative rates of loss of deuterium from cellulose under two conditions of drying.*

[2] Unpublished experiments carried out in collaboration with Lester Kuhn of the Ballistics Research Laboratory, Aberdeen Proving Ground, Aberdeen, Md.

was exposed to 99.56-percent D_2O as outlined above for 72 hr at 25° C. Following the treatment the film was dried over anhydrous calcium sulfate at 25° C for 48 hr, and its spectrum was then recorded with the Perkin Elmer [5] infrared spectrometer. The spectrum of the treated film had an extremely weak band at approximately 4 μ with no apparent change in intensity of the band at 3 μ.

This attempt to deuterate the cellulose was followed by seven other experiments in which the time of exposure was varied from 25 to 100 hr and the temperature from 25° to 52° C. In no case was more than a barely perceptible diminution of intensity of the 3-μ band observed. This negative result is in sharp contrast to the complete disappearance of the 3-μ band in the spectra of the carbohydrates mentioned above.

In the final experiment, the dried film of regenerated cellulose was exposed for 100 hr at 52° C to the D_2O. The recording of the spectrum by the Baird spectrophotometer was started under room conditions of 40-percent relative humidity 1 min after the film was removed from the reaction vessel, and the recording was completed in 4 min. The spectrum of this treated film was recorded at 20-min intervals during the first 3 hr of its approach to equilibrium with the 40-percent relative humidity of the room. The spectrum of the dried, undeuterated, regenerated cellulose, figure 2, A, is compared with that of the same specimen 60 min after removal from the liquid D_2O, figure 2, B, and 100 minutes after removal, figure 2, C. The spectrum of the treated film immediately after removal from the D_2O is not shown because of the partial overlapping of the 4-μ band into the 3-μ band.

The absorption at 3.47 μ, attributable to the C—H stretching vibration, serves as an internal standard to which the variations in intensity of the other bands may be referred. A comparison of the intensity of the 3-μ band before and after exposure to the D_2O reveals little change in the intensity of this band. Assuming the Lambert-Beer law to be applicable to this system and further assuming the contribution of any sorbed H_2O to be low, one would have expected a 50-percent deuterium exchange to increase the percentage transmittance from the observed value of 17 percent (fig. 2, A) to about 40 percent.

The spectrum in figure 2, C was recorded 40

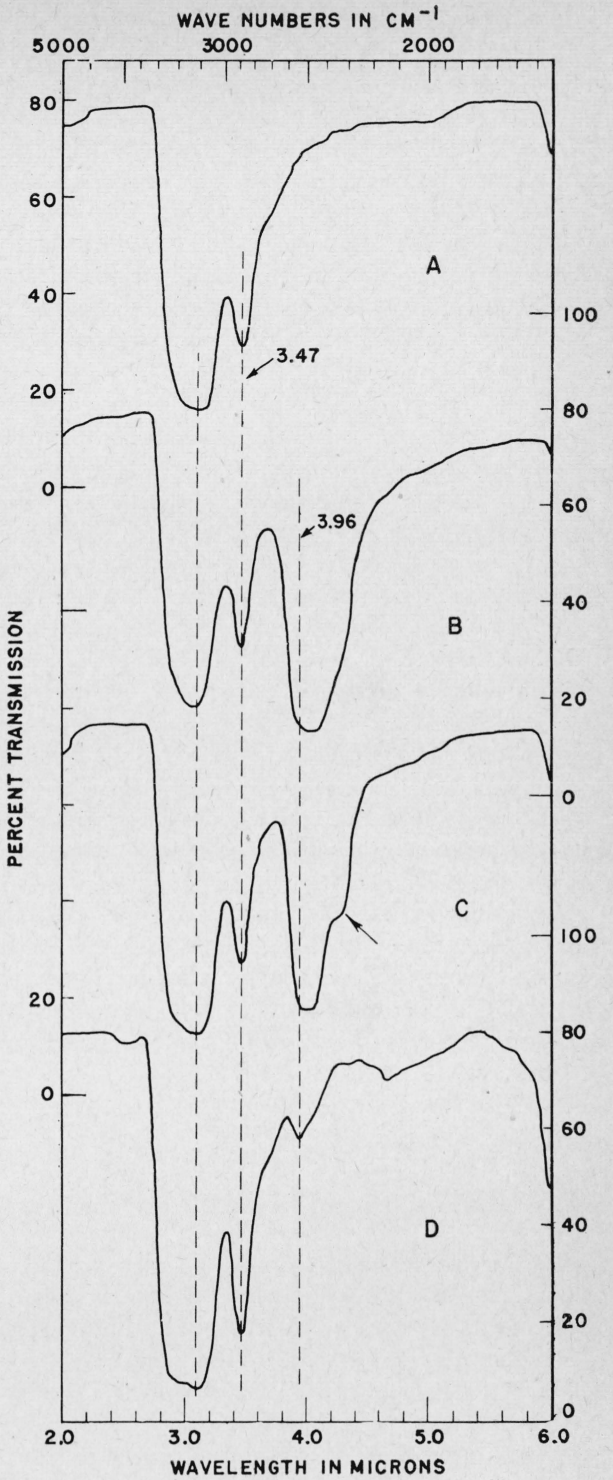


FIGURE 2. *Effect of deuteration on the infrared absorption spectrum of a film of regenerated cellulose.*

A, Cellulose before exposure to heavy water; *B*, cellulose 60 min after exposure to heavy water for 100 hr at 52° C; *C*, same as *B*, but 40 min later; *D*, same as *C* after attempts to remove O—D band by drying for 5 days at 25° C over anhydrous calcium sulfate, drying in oven at 105° C, immersion in water at 25° C for 6 hr, and drying.

min after obtaining that in B. During this time the OD band at 4 μ had decreased in intensity and showed the shoulder or secondary peak indicated by the arrow. This vibration at 4.3 μ was present in the spectra for most of the eight experiments and suggests that the OD band may be made up of at least two bands representing two vibrational frequencies. The absorption in the region of 4 μ for the deuterated carbohydrates mentioned above was usually found to consist of two or three separate and distinct vibrations. An attempt was made to eliminate the OD absorption from the film giving the spectrum shown in figure 2, C by drying it over anhydrous calcium sulfate in a desiccator at 25° C for 18 hr. A fairly strong OD band was still present. The film was then immersed in ordinary water at 25° C for 6 hr and dried. The spectrum of the film, figure 2, D, still showed a small but significant absorption band at 4 μ. Evidently the removal of the last trace of deuterium from the film is difficult.

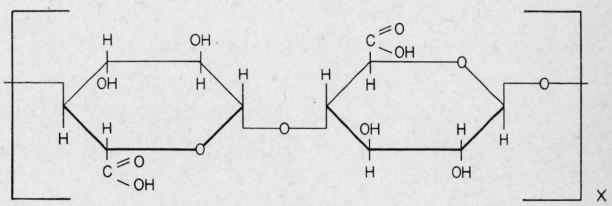
The relative decrease in intensity of the band at 3 μ in figure 2, B and the residual absorption at 4 μ in figure 2, D indicate that some deuteration has taken place. However, it appears that the extent of reaction was either small, or that the HOH formed as a result of the reaction remained in the cellulose film and did not migrate into the surrounding D_2O in the time and at the temperature of these experiments. Since such extremely slow diffusion of normal H_2O from the film into the D_2O seems unlikely, it must be concluded that the extent of deuteration of the regenerated cellulose film under the above conditions was much less than 50 percent, contrary to the findings of previous workers.

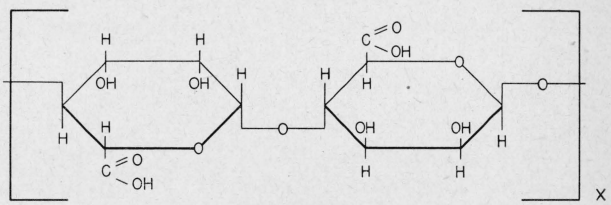
III. Carboxyl Group in Oxidized Cellulose

Earlier work showed that cellulose oxidized with nitrogen dioxide had three main new absorption bands [1] located at 5.75, 6.07, and 7.81 μ. Nitrogen dioxide is said to be specific and to oxidize the primary alcohol group on the number six carbon atom to a carboxyl group [7, 8].

Many organic acids containing carboxyl groups have absorption bands at approximately 5.75, 6.07, and 7.81 μ [9, 10]. It was, therefore, tentatively concluded at that time that the three absorption bands at 5.75, 6.07, and 7.81 μ were in some way connected with the carboxyl groups. However, our attention was called to the fact that the two intense bands at 6.07 and 7.81 μ were also present in the spectrum of cellulose nitrate. Although the amounts of cellulose nitrate formed in the reaction with nitrogen dioxide are said to be relatively small [7, 8], it is possible that under the conditions of our experiments appreciable amounts of cellulose nitrate were formed. The cellulose nitrate and adsorbed nitrogen dioxide might possibly give rise to the strong bands at 6.07 and 7.81 μ. Any adsorbed water in the film would make a small contribution to the 6.07-μ band, as possibly would carbonyl groups. In view of the above it was, therefore, desirable that the spectrum of the NO^2 oxidized cellulose be compared with the spectrum of cellulose nitrate and with a closely related polysaccharide molecule containing carboxyl groups.

The polysaccharide alginic acid is a polymer of D-mannopyrano-uronic acid. The stereoisomeric difference between NO_2 oxidized cellulose (celluronic acid) and alginic acid may be seen from the following structural formulas. The units in alginic acid are linked through the number one and number four carbon atoms, and like NO_2 oxidized cellulose they have carboxyl groups in the number six position. The alginic acid films used in this work were prepared from sodium alginate. One-half gram of the latter, obtained from the Algin Corporation of America, was dissolved in 30 ml of distilled water con-





taining 0.3 ml of a 5-percent stock solution of Aerosol O. T. (The Aerosol O. T. facilitated stripping of the film and had no effect on the spectrum of the polymer). The solution was cast on a chromium-plated surface using a blade with a 0.04-in. clearance. This yielded a film 5.5 μ thick. The film so obtained was then converted into alginic acid by treatment with 2 N hydrochloric acid. The resulting alginic acid film was found to be gelatinous and quite strong after washing in cold water. The films were dried in air for 24 hr and then over anhydrous calcium sulfate for 48 hr before recording the spectrum. The cellulose nitrate was prepared by the method of Davidson [6]. One-half gram of dried nitrated cotton was dissolved in 100 ml of warm ethyl acetate. A film 6.9 μ thick was then prepared with the aid of a 0.04-in. Bird film applicator. The NO_2 oxidized cellulose film was prepared from regenerated cellulose obtained by deacetylation of cellulose acetate [1]. The cellulose film so obtained was suspended at room temperature in a glass chamber that was evacuated. Dry nitrogen dioxide was then passed into the chamber. The films were left in the nitrogen dioxide atmosphere for 2 hr and then soaked for another 2 hr in distilled water with three changes of water. The spectrum of the three polysaccharides prepared in the above manner are shown in figure 3.

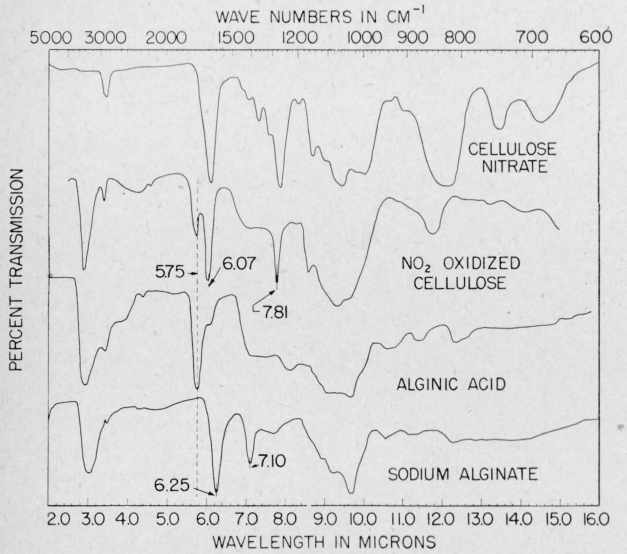


FIGURE 3. *Comparison of spectra of cellulose nitrate, oxidized cellulose, and alginic acid.*

It is noted in figure 3 that the two questionable bands (6.07 and 7.81 μ, measured to the second decimal place in order to compare their positions in the two spectra) are absent in alginic acid, but present in cellulose nitrate. The intense absorption at 5.75 μ in the spectrum of alginic acid is undoubtedly due to the C=O vibration of the carboxyl group. The shift in band from 5.75 to 6.25 μ, which occurs in the neutralization of the alginic acid, is noted in figure 3. This behavior is apparently characteristic of the carboxyl group when it is converted to the ionized carboxylate group. It has been observed [10, 11] when the acid group is converted to the sodium salt. It then appears, at this point, that although the band at 5.75 μ in the NO_2 oxidized cellulose is certainly due to a C=O vibration in the chain molecules, it is highly probable that the other two bands (the one at 6.07 and 7.81 μ) are related to nitrogen-oxygen vibrations [12].

IV. Hydrogen Bonding in Cellulose

In the earlier work [1] the regeneration of the cellulose from cellulose acetate was accompanied by a shift in the minimum of the OH band to slightly longer wavelengths. The minimum appeared at approximately 3,500 cm^{-1} (2.86 μ) in cellulose acetate and at approximately 3,400 cm^{-1} (2.94 μ) in regenerated cellulose. This fact suggested that the hydrogen bridges between the unacetylated OH groups in cellulose acetate were relatively weaker [13] than the hydrogen bridges between the OH groups in regenerated cellulose. The apparent difference in positions of the minima is shown in figure 4, in which the spectra of three different cellulose acetates and one sample of methyl cellulose may be seen. The cellulose triacetate used in obtaining the spectrum in figure 4 was obtained from the Eastman Kodak Co. It was insoluble in acetone and, therefore, cast from a 10-percent chloroform solution. In preparing the 10-percent solution, five parts of alcohol were added to 100 parts of the chloroform to facilitate dispersion of the cellulose triacetate. The film made with a 0.04-in. Bird film applicator was 7.6 μ in thickness. The cellulose acetate had an acetyl content of approximately 54 percent (as acetic acid); it was prepared and partially deacetylated as previously described [1]. The

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methyl cellulose (4,000 centipoises) used in this work was obtained from the Dow Chemical Co. A 2½-percent water solution was spread with a blade having a clearance of 0.006 in. The resulting film (4.6 μ) was then removed from the glass plate with the aid of a razor blade and a pair of tweezers.

It is noted in figure 4 that the commercial cellulose triacetate and methyl cellulose have OH bands (due to a few unreacted hydroxyl groups) whose minima are at approximately 2.87 μ. On the other hand, the partially deacetylated acetates had OH bands whose minima are at 3.0 μ.

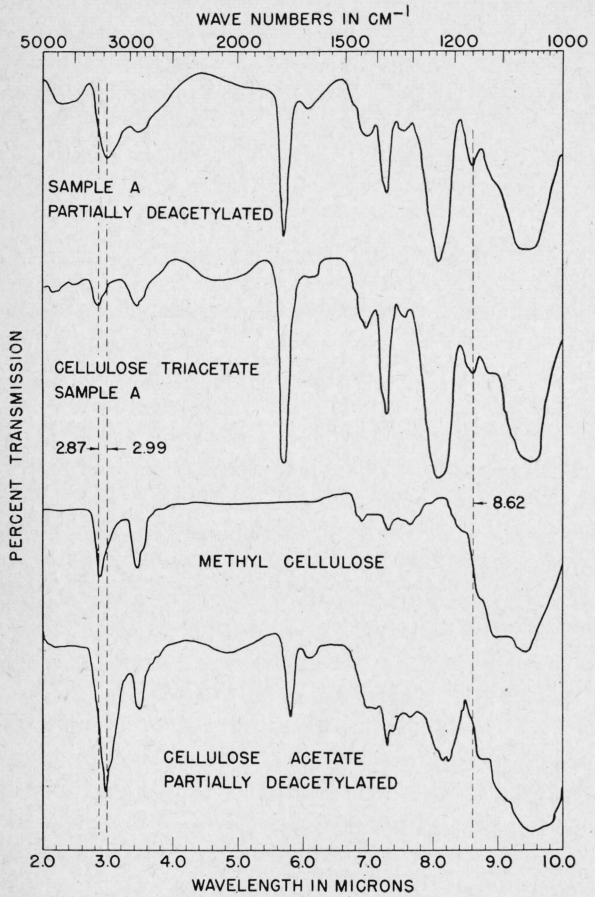


FIGURE 4. *Comparison of the infrared absorption spectra of some cellulose derivatives.*

In view of the above difference in the position of the minimum of the OH band, it was of interest to examine the band with the aid of the wide dispersion lithium fluoride prism in an additional experiment designed to detect, in a qualitative manner, the effect of water molecules and the band intensities upon the position of the mini-

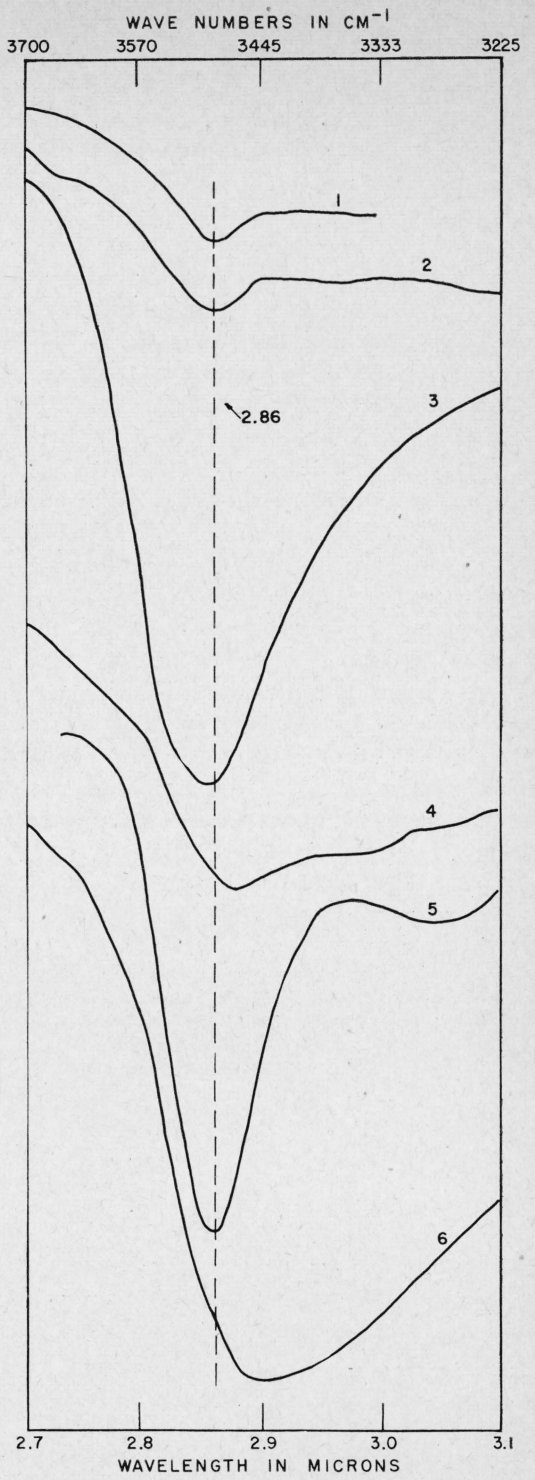


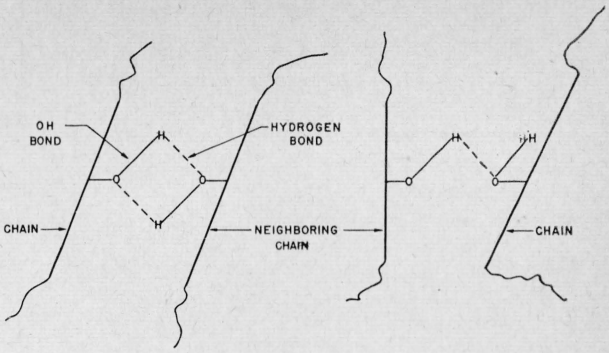
FIGURE 5. *Infrared absorption spectra in the hydroxyl region for several cellulose derivatives.*

1, Cellulose acetate butyrate (0.03 equivalent) film thickness 13 μ; 2, cellulose acetate butyrate (0.52 equivalent) film thickness 14 μ; 3, cellulose acetate butyrate (0.94 equivalent) film thickness 12 μ; 4, benzyl cellulose, film thickness 3 μ; 5, ethyl cellulose, film thickness 12 μ; 6, regenerated cellulose, film thickness 2 μ.

mum. For this preliminary experiment, the intensity of the band could be varied by changing the hydroxyl content. The change in intensity of the band was made possible by means of a series of cellulose acetate butyrate esters available at the Bureau. It would have been more desirable to use a cellulose acetate series to study the effect of varying hydroxyl content for this experiment; however, such a series was not immediately available. For the study, three samples of the mixed ester having three different hydroxyl contents, 0.03 equivalent, 0.52, and 0.94 were chosen. (One equivalent of OH here means that there is one hydroxyl group in each anhydroglucose unit). In addition, the following three materials were also examined at the same time: ethyl cellulose, benzyl cellulose, and regenerated cellulose. In order to minimize any possible effect of water on the positions of the OH bands, the above six samples were compared after drying over anhydrous calcium sulfate for 24 hr in a specially constructed cell having rock-salt windows. It was thus possible to obtain the spectra of these dry films with the aid of this cell and a lithium fluoride prism.

The spectra of the six derivatives of cellulose are shown in figure 5. It is noted that the first, second, third, and fifth spectra all have minima at 3,500 cm^{-1} (2.86 μ). The minima of the benzyl (spectrum 4) and regenerated cellulose (spectrum 6) appear to be shifted to slightly longer wavelengths. It is apparent that the position of the minimum in the dried mixed ester (spectra 1, 2, and 3) is at 2.86 μ and that the difference, 0.12 μ, previously observed in figure 4, is an effect that is most likely due to additional hydrogen bonding in the deacetylated cellulose acetate.

An absorption band in the region of 3,500 cm^{-1} (2.86 μ) has been observed in dilute carbon tetrachloride solutions of alcohols. The position of this band has been attributed [14, 15] to the type of hydrogen bonding between the two alcohol molecules, which are associated in some manner, which leads to the formation of a dimer alcohol molecule. The presence of this vibration in different dried cellulose derivatives suggests that the mechanism by which the cellulose chains are bound together may possibly be of the "dimer" type and common to many cellulose derivatives. This association may be imagined as follows:



Although these bridges may exist in ordered (crystalline) regions of cellulose, they would not be present in large numbers in the disordered (amorphous) regions.

In the disordered regions many of the OH groups on neighboring chains are not close enough to each other to enter into a hydrogen bonding arrangement. This inability of some of the OH groups in cellulose to undergo hydrogen bonding in the amorphous regions should give rise to frequencies characteristic of free OH vibrations. The frequencies reported for free OH groups in alcohols in solutions [16, 17, 18] are found to be in the region of 2.72 to 2.75 μ. Small inflections in the region of the spectra of cellulose acetate butyrate (0.52 equiv.—spectrum 2 in fig. 5) and in regenerated cellulose (spectrum 6) suggest the presence of a few free OH groups in these derivatives. However, additional work will have to be done on thicker films before the presence of free OH groups in cellulose and its derivatives is definitely established.

The preliminary work described here suggests the advisability of examining the short wavelength region, under high dispersion, of samples of dried and undried cellulose and its derivatives, both deuterated and nondeuterated, having varying tensile strengths and different degrees of crystallinity.

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