628. *Infrared Spectra of Periodate-oxidised Cellulose.*

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The intensities of the hydroxyl band in films of cellulose and periodate-oxidised cellulose, and of the aldehydic-carbonyl band in the oxidised films have been measured. Together with intensity changes that occur when the oxidised films are dried and heated these indicate that the aldehyde groups of the oxidised material exist in various forms, but mainly as hemialdal groups. The number of free aldehyde groups increases on drying and heating at the expense of the hydrated and the hemialdal groups respectively.

ROWEN, FORZIATI, and REEVES [1] showed that in the infrared spectrum of periodate-oxidised cotton cellulose there was little or no absorption attributable to free aldehyde groups. They suggested this was due to hydration of all the aldehyde groups or hydration

[1] Rowen, Forziati, and Reeves, *J. Amer. Chem. Soc.*, 1951, **73**, 4484.

of some and hemiacetal formation by the rest. Similar absence of the carbonyl band was reported for spectra of the hydrates of the periodate-oxidation products of methyl 4,6-*O*-benzylidene-α-D-glucoside [1] (infrared) and of methyl α-D-glucoside [2] (ultraviolet). It is the rule rather than the exception that there is little or no carbonyl absorption in the spectra of periodate-oxidised carbohydrates. The aldehyde groups in periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside are not hydrated but are present in a hemialdal structure,[3] and only on sublimation *in vacuo* does the normal form of the substance change into the anhydrous dialdehyde containing free aldehyde groups.[4]

In periodate-oxidised cellulose many structures have been suggested to account for the absence of free aldehyde groups,[5] and the problem is more complicated than for sugar derivatives because of the possibility of the formation of cross-links between chains. Zhbankov [6] reported a linear relation between the intensity of an infrared band at 910 cm.$^{-1}$ and the aldehyde content determined chemically. He concluded that this band was due to hydrated aldehyde groups or to hemiacetal groups. Zhbankov favoured the latter, but he did not consider any other possibilities. He stated that this band represents the only possibility of analysis for aldehyde groups in periodate-oxidised cellulose. This is not so, as Higgins and McKenzie [7] have shown. They observed the dependence of carbonyl intensity on the moisture content of oxidised cellulose fibres. It appeared to them that the aldehyde groups are readily hydrated and that some of the primary alcohol groups are either oxidised or involved in hemiacetal groups, but that there was no clear evidence for the presence of the last-named groups.

Evidence for the structure of periodate-oxidised cellulose from changes in the 1500—800 cm.$^{-1}$ region alone is unsatisfactory because of the numerous overlapping bands that cannot be assigned definitely to particular vibrations. Further, periodate and periodic acid oxidation render cellulose more amorphous [8,9] and this physical modification itself results in spectral changes.

Although numerous structures for periodate-oxidised cellulose can be written, involving linkages between different units of one chain or between different chains, the forms in which the aldehyde groups can be present are limited to the following: (1) free aldehyde, ·CHO; (2) hydrated aldehyde, ·CH(OH)$_2$; (3) hemialdal, ·CH(OH)·O·CH(OH)·; and (4) hemiacetal, ·CH(OH)·O·CH$_2$·. The formation of the last three from the first involves respectively addition of one molecule of water per aldehyde group, addition of one molecule of water per two aldehyde groups, and rearrangement between an aldehyde group and one of the remaining alcohol groups without addition of water. A study of the conditions under which the intensity of the carbonyl band changes should indicate the form(s) in which the aldehyde groups are present, and a knowledge of the hydroxyl content of the samples would supplement this. This approach has the advantage that the bands to be measured can be assigned with certainty.

EXPERIMENTAL

The experiments have been carried out with films of viscose and " Cuprophane " (cuprammonium cellulose). Very thin films were prepared by regeneration from viscose solution that had been squeezed between warmed glass plates whose surfaces had been finely ground to avoid interference fringes in the spectra.[10] The plates were slid apart and immersed for about

[2] Hurd, Baker, Holysz, and Saunders, *J. Org. Chem.*, 1953, **18**, 186.
[3] Guthrie and Honeyman, *J.*, 1959, 2441, and references therein.
[4] Goldstein, Lewis, and Smith, *Chem. and Ind.*, 1958, 595.
[5] Head, *J. Textile Inst.*, 1952, **43**, T1, and references therein.
[6] Zhbankov, *Optika i Spektroskopiya*, 1958, **4**, 318.
[7] Higgins and McKenzie, *Austral. J. Appl. Sci.*, 1958, **9**, 167.
[8] Nevell, *J. Textile Inst.*, 1956, **47**, T287.
[9] Davidson, *J. Textile Inst.*, 1941, **32**, T109.
[10] Brown, Holliday, and Trotter, *J.*, 1951, 1532.

1½ hr. at room temperature in a coagulating solution containing 110 g. of sulphuric acid and 500 g. of sodium sulphate decahydrate per l. The films, quite transparent, were washed three times in distilled water: the last two wash-waters were free from sulphate and acid. The films were removed from the water on the glass plates, dried, and then lifted off easily with compressed air. The " Cuprophane " film was obtained commercially. It was washed with water to remove any softening agent present.

The oxidations were performed in the dark on loose film immersed in 0·1M-sodium meta-periodate, the consumption of which was determined by Müller and Friedberger's method.[11] The degree of oxidation is expressed in this paper as a percentage, a consumption of one molecule of periodate per glucose unit corresponding to 100%. The oxidised viscose films were very difficult to handle. The best way of transferring them to distilled water for washing, and of removing them for drying, was to stroke a glass plate along the underside of a film until one end of it adhered to the plate and then withdraw the latter in such a way that the surface tension fastened the rest of the film to the plate as it broke the surface. A sheet of polypropylene was used once as the dry film was more easily removed from this than from glass.

Films were mounted on a frame and placed in the sample compartment (which could be evacuated) of the spectrometer for measurement of the spectra. Thirty minutes' drying *in vacuo* over phosphoric anhydride was necessary to remove the adsorbed water band at *ca.* 1640 cm.$^{-1}$ from films ~5 μ thick. This is much longer than Brown, Holliday, and Trotter found,[10] but they measured the change in the hydroxyl stretching band where the decrease on drying is small compared with the intensity of the cellulosic hydroxyl band. In practice the films were dried even longer before the measurements were recorded. The dried films adsorbed water very quickly when re-exposed to the air, and one minute's exposure was sufficient to produce a distinct band at *ca.* 1640 cm.$^{-1}$.

The films were heated in an oven at 105° unless stated otherwise. The hot films were transferred to the sample compartment which was evacuated within one minute of their leaving the oven. This procedure gave reproducible results; and any uptake of water vapour must have been insignificant, as measurements at *ca.* 1640 cm.$^{-1}$ and the constancy of the carbonyl intensity confirmed.

When necessary, film thicknesses were measured with a " Magna " gauge at five or more points within the area covered by the beam. To compare the hydroxyl intensity in films before and after oxidation, the C–H band intensity (optical density) was used as a measure of effective sample thickness. This is because of possible density changes during the oxidation and non-uniformity of film thickness. After oxidation, comparison of values of the optical density of the hydroxyl band itself (O.D.$_{O-H}$) for any one film is also permissible. The same applies to the carbonyl band (O.D.$_{C=O}$).

The spectra were measured with a Unicam SP. 100 spectrometer fitted with a rock-salt prism. The measured half-band width of the carbonyl band was 29 cm.$^{-1}$ both before and after heating, and the effective slit width at 1734 cm.$^{-1}$ was calculated as *ca.* 7 cm.$^{-1}$. Reducing this to *ca.* 5 cm.$^{-1}$ did not increase the peak height of the carbonyl band. The value for the extinction coefficient of the aldehyde group used here in the calculations was measured by Cross and Rolfe[12] with an effective slit width of 6 cm.$^{-1}$, but the half-band width was not stated. Optical densities were measured by a base-line technique. The choice of base-line was straightforward for the carbonyl band, rather less so for the adsorbed water and the 3 μ hydroxyl bands, and difficult for the C–H band which is overlapped by the hydroxyl absorption. Various methods of drawing the C–H base-line were tried. The one that gave the most consistent results for O.D.$_{C-H}$ in the same film before and after drying (when the extent of overlap changes considerably) was to draw a smooth curve from the absorption minimum at *ca.* 3050 cm.$^{-1}$ to join the transmission curve at 2600 cm.$^{-1}$. Further, by this method, the ratio O.D.$_{O-H}$: O.D.$_{C-H}$ for four separate dry viscose films varied by ±3% only. Because of the almost constant half-band width (Δν$_{\frac{1}{2}}$) of the hydroxyl band (in dry samples) its intensity has been measured by optical density instead of band area, since determination of the latter does not seem warranted in the present circumstances.

The Table shows examples of the results.

[11] Müller and Friedberger, *Ber.*, 1902, **35**, 2652.
[12] Cross and Rolfe, *Trans. Faraday Soc.*, 1951, **47**, 354.

Measurements on periodate-oxidised viscose films.

	$O.D._{H_2O}$	$O.D._{C=O}$	$O.D._{O-H}$	$(\Delta\nu_{\frac{1}{2}})_{O-H}$ (cm.$^{-1}$)	$O.D._{C-H}$	$\dfrac{O.D._{O-H}}{O.D._{C-H}}$	$\dfrac{O.D._{C=O}}{O.D._{C-H}}$
Film 1. 97% *oxidised.*							
Air-dry: unheated	0·069	0·031	0·507	360	0·150	3·38	0·207
Dry: unheated	0·011	0·065	0·473	260	0·167	2·83	0·392
Dry: 4 hr. at 105°	0·008	0·146	0·429	245	0·161	2·67	0·907
Dry: 22½ hr. at 105°	0·008	0·175	0·403	255	0·161	2·50	1·09
Dry: 45 hr. at 105°	0·009	0·200	0·399	250	0·160	2·50	1·25
Dry: 68½ hr. at 105°	0·009	0·203	0·389	250	0·159	2·45	1·28
68½ hr. at 105°; left 72 hr. in air	0·049	0·114	0·466	305	0·150	3·11	0·76
Re-dried at 30°	0·013	0·125	0·427	250	0·164	2·61	0·76
Film 2. *Unoxidised.*							
Dry: unheated	0·002	—	0·564	260	0·158	3·57	—
ca. 100% *oxidised.*							
Dry: unheated	0·006	0·066	0·638	265	0·210	3·04	0·314
Dry: 26 hr. at 105°	0·016	0·270	0·521	255	0·205	2·54	1·32
Film 3. *ca.* 80% *oxidised.*							
Dried *in vacuo* over P_2O_5 45 min.	0·009	0·047					
Dried *in vacuo* over P_2O_5 140 min.	0·009	0·054					
1 hr. at 105°	0·005	0·112					
1 hr. at 105°; left 7 days in air ...	0·063	0·055					

RESULTS AND DISCUSSION

900 *cm.*$^{-1}$ *Region.*—In the spectra of unoxidised viscose sheet and " Cuprophane " there was a band at 897 cm.$^{-1}$. On oxidation of the sample, this band increased in width and moved to 891 cm.$^{-1}$. (Since this peak is the only one near 900 cm.$^{-1}$ it must be the same as that measured by Zhbankov [6] at 910 cm.$^{-1}$). The transmission at this frequency was less in the oxidised samples, but it was not clear if this involved an increase in band intensity because the background transmission also decreased considerably throughout a wide region. After reduction of oxidised " Cuprophane " with excess of potassium borohydride the band was definitely weaker. Forziati and Rowen [13] noted that the intensity of a band at 893 cm.$^{-1}$ was greater in amorphous cellulose (prepared by grinding) and in regenerated cellulose than in bacterial cellulose and residues from the hydrolysis of cotton linters. Zhbankov [6] considered that the increase on grinding was due to oxidation but this does not explain the increase in regenerated cellulose. On the other hand X-ray measurements on cellulose oxidised by periodate [8] and periodic acid [9] showed that oxidation rendered the sample more amorphous, and partial decrystallisation of cotton by means of ethylamine, where no oxidation occurs, slightly increased the intensity of the band under discussion.[14] Present work confirms the latter observation. It appears that the increase in absorption near 900 cm.$^{-1}$ is not due solely to the formation of a new chemical grouping as Zhbankov thought, and in fact other measurements (see below) show that the aldehyde groups in periodate-oxidised cellulose are present in more than one form.

Carbonyl (1734 *cm.*$^{-1}$) *and Adsorbed-water* (ca. 1640 *cm.*$^{-1}$) *Bands.*—Air-dry samples of 15, 26, 44, 76, and 100% oxidised films all showed absorption at 1734 cm.$^{-1}$ which increased from a weak shoulder to a distinct peak with increasing oxidation. The carbonyl band in all these samples increased when they were heated, while the adsorbed water band at *ca.* 1640 cm.$^{-1}$ (broad) decreased. One 100% oxidised film was heated for equal times at 80°, 60°, and 105°. The intensity of the carbonyl band increased, while that of the adsorbed water band decreased, with rise in temperature. An unoxidised sample was heated in air at 105° for as long as 65 hours without absorption appearing in the carbonyl region.

It was found that intense drying of the samples over phosphoric anhydride *in vacuo* increased the carbonyl intensity, but that once the adsorbed water was removed any further

[13] Forziati and Rowen, *J. Res. Nat. Bur. Stand.*, 1951, **46**, 38.
[14] O'Connor, DuPré, and Mitcham, *Textile Res. J.*, 1958, **28**, 382.

increase was extremely small and not even definite. The adsorbed-water band is the only other one markedly different in the air-dry and the chemically dried samples. Heating a dried sample either in air or *in vacuo* produced a rapid increase in the carbonyl-band intensity which, within 5 hours, reached three-quarters of the value attained after 3 days (see Table). The intensity in a 100% oxidised film continued to increase slowly even after 6 days and did not reach a limit in a practicable length of time. One therefore cannot construct a calibration curve of optical density of the carbonyl band against % oxidation from which to determine the latter accurately in any other sample. It seems certain that the large increase in the first 5 hours is due to the liberation of free aldehyde groups and not to formation of simple aldehydes by degradation. The largely reversible nature of the change is slight additional evidence in favour of this idea. Thus, when highly oxidised samples that had been heated were exposed to the atmosphere the carbonyl intensity decreased considerably, though it was still greater even after a few days than in the unheated samples dried over phosphoric anhydride (see Table).

If a heated sample was kept dry while cooling, the carbonyl intensity did not diminish at all, even after 3 hours by which time the sample was quite cool, whereas exposure of the cooled film to the atmosphere produced an immediate decrease. Water is therefore essential for the reverse process.

The two-stage increase in the carbonyl intensity strongly suggests that the aldehyde groups concerned were originally combined in two different forms in oxycellulose. It is very likely that drying over phosphoric anhydride dehydrates only groups of type (2), since the hemialdal group is known to be difficult to dehydrate.[4,15] The further increase on heating must be due to dehydration of hemialdal groups rather than conversion of hemiacetal groups into free aldehyde groups, because water is required to reverse the change.

The decrease which occurred after the film had been re-exposed to the atmosphere for a few days was much larger than the initial increase on drying of an air-dry film at 30°. Rehydration of the aldehyde groups freed at 30° could only account, therefore, for a small part of this decrease. The remainder of it was due to re-formation of hemialdal groups and not formation of more hydrated groups. This is shown by the small increase only in carbonyl intensity on drying of the exposed film again at 30° (Table).

Determination of the number of free aldehyde groups per chain unit from the optical density of the carbonyl band in these samples is complicated by four factors: values have to be assumed for the molecular weight of the chain unit and the extinction coefficient of the carbonyl band; the film density must be known or assumed since the thickness is not uniform; and direct measurement of the effective thickness is difficult in these thin, non-uniform films. The second and the fourth factor are likely to introduce most error. With values of 140 mole^{-1} l. cm.$^{-1}$ for ε, taken from measurements on two saturated aldehydes,[12] of 178 for the molecular weight, and of 1·5 for the density,* such a calculation yielded values of *ca.* 0·3 and 0·5 free carbonyl group per oxidised unit corresponding to the optical densities of 0·175 and 0·270 respectively in films 1 and 2 after *ca.* 24 hours' heating (see Table). This means that only 15% and 25%, respectively, of the total aldehyde groups are free, the remainder presumably being in the form of hemiacetal groups plus any remaining hemialdal groups.

Hydroxyl Stretching Band.—Since the four forms in which the aldehyde groups may be present differ in their hydroxyl content, information about this would supplement the above data. To determine the relative number of hydroxyl groups from measurements of the hydroxyl band intensity, it must be assumed that the intensity per group remains the same irrespective of changes in the chemical and physical environment, which occur, for example, during oxidation. There is experimental evidence that the intensity is sufficiently

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* The value of 1·5, estimated from measurements on oxidised filter paper,[16] is possibly too low. If so, the free carbonyl concentrations would be even lower than 15% and 25%.

[15] González, *Adv. Carbohydrate Chem.*, 1956, **11**, 97, and references therein.

constant in the samples concerned to permit a choice between groups which vary considerably in their hydroxyl content. Thus the hydroxyl intensity for a hydrated aldehyde group, where one hydroxyl group was free and the other intramolecularly hydrogen-bonded, was found to be very nearly twice that of a similarly bonded hydroxyl in a hemiacetal group.[17] There is little similar information on hydroxyl groups, of different chemical types, *intermolecularly* hydrogen-bonded to one another. The hydroxyl intensity for alcohols is increased by hydrogen-bonding,[18] while the band is shifted to a lower frequency and broadened, but the extent of such bonding in the substances examined here does not change much, as judged by the almost constant half-band width (Table) and frequency (*ca.* 3450 cm.$^{-1}$).

Oxidation always decreased the ratio $O.D._{O-H} : O.D._{C-H}$, measured in dry samples, and sometimes the ratio in *dry* unoxidised cellulose was even slightly greater than in an *air-dry* oxidised sample where adsorbed water would increase the hydroxyl intensity. This is evidence against the existence of all the aldehydes as hydrated groups, for this would involve a large increase (from 3 to 5 per chain unit) in the hydroxyl groups present and very probably an increase in the hydroxyl absorption as well. The presence of a large proportion of hydrated groups should also produce a much smaller hydroxyl, and a much greater carbonyl, absorption intensity on drying than are actually observed. On the other hand, the presence of a large proportion of hemiacetal groupings should cause the hydroxyl intensity in (dry) oxidised cellulose to be much smaller than observed, since there would be only one hydroxyl group where before oxidation there were three. (A large number of " extended " hemiacetal linkages such as $\cdot CH_2 \cdot O \cdot CH \cdot O \cdot CH \cdot OH$ would be required to account for the few free aldehyde groups in dry, completely oxidised samples where there are insufficient hydroxyl groups to react with the aldehyde groups on a 1 : 1 basis.)

If this interpretation is correct there must be a considerable proportion of hemialdal groups in periodate-oxidised cellulose. On the assumptions that the hydroxyl extinction coefficient is approximately constant and that all the groups originally hydrated would be dehydrated, the number of hemialdal groups in dry samples can be estimated semi-quantitatively from the results in the Table. This amounts to *ca.* 75% of the total aldehyde groups at 30°, falling to *ca.* 60% after about 24 hours' heating at 105°, the other 25% (approx.) (40% after heating) being composed of free aldehydes and hemiacetal groups. Consequently, in the heated samples the amount of free aldehydes does not exceed 40% and may be much smaller, depending on the number of hemiacetal groups present. From the relative $O.D._{C=O}$ values, it is less than *ca.* 15% in the dry, unheated state. The value of $\not> 40\%$ is not inconsistent with the two values of 15% and 25% calculated previously without invoking any results of measurements on the hydroxyl band.

The free aldehydes liberated on heating come from the hemialdal groups, and this could account for the decrease in hydroxyl intensity that occurs at the same time.

The main conclusions may be summarised as follows: (1) there are few free aldehyde groups in air-dry periodate-oxidised cellulose; (2) their number increases slightly when the sample is dried over phosphoric anhydride at 30° and considerably when it is heated at 105°; (3) the aldehyde groups liberated in the two stages in (2) are originally bound in two different ways by water, the hemialdal groups being more numerous than the hydrated groups; and (4) most of the aldehyde groups in 100% oxidised cellulose are in the form of hemialdal groups, the rest being free, hydrated, or in hemiacetal groups.

[16] Davidson, *J. Textile Inst.,* 1941, **32,** T109.
[17] Isbell, Smith, Creitz, Frush, Moyer, and Stewart, *J. Res. Nat. Bur. Stand.,* 1957, **59,** 41.
[18] Francis, *J. Chem. Phys.,* 1951, **19,** 505.