Saccharification process of cellulose in 97% sulfuric acid monitored by sulfuric acid induced ultraviolet absorption behaviour

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Mercerized cellulose in concentrated sulfuric acid was found to give rise to a new ultraviolet absorption band assigned to 5-hydroxymethyl-2-furfural (HF). The time dependence of the HF absorption shows that the saccharification process of cellulose readily takes place in 97% sulfuric acid in spite of the very low water content. The dehydration reaction of the glucose unit appears to induce the decomposition of cellulose to HF with high efficiency.

(Keywords: cellulose; sulfuric acid; saccharification)

INTRODUCTION

Cellulose is one of the most typical natural polymers and has been studied intensively and extensively[1–3]. Many studies employing spectroscopic methods have been used to monitor molecular motion, microstructure, and the reaction of cellulose both in solution and the solid state, e.g. nuclear magnetic resonance (n.m.r.)[4–6], cross-polarization/magic angle spinning (CP/MAS) n.m.r.[7,8], dielectric relaxation[9], and electron paramagnetic resonance[10]. This paper describes the ultraviolet (u.v.) absorption behaviour of the new products that appear when cellulose is dissolved in sulfuric acid. The results give information on the saccharification process of cellulose in sulfuric acid.

EXPERIMENTAL

Cellulose powder (200–300 mesh pulverized filter paper, Toyo Roshi) was boiled in 0.5% NaOH for 20 min, washed with distilled water several times, and then dried. After this pretreatment, the cellulose was mercerized in 18% NaOH at 22°C for 2 h. U.v. absorption spectra were measured on a Shimadzu UV-2200. All the measurements were carried out in aerated sulfuric acid (Wako Co. Super Special Grade, 97%) or aqueous sulfuric acid diluted using non-fluorescent distilled water (Kanto Chemical). The solutions used for the u.v. measurements were about 2.5×10^{-5} M in the glucose unit. ^{1}H and ^{13}C n.m.r. spectra were recorded on a JEOL GSX-400 spectrometer, using deuterated chloroform as the solvent and tetramethylsilane (TMS) as the internal standard.

RESULTS AND DISCUSSION

Cellulose shows no important absorption bands in the 250–400 nm region just after being dissolved in 97% sulfuric acid. However, new absorption bands at 323 and 257 nm (error ±2 nm) appear gradually and their intensities increase very much with time at room temperature. The absorption behaviour of glucose in sulfuric acid is found to agree precisely with that of cellulose; namely, new peaks at 323 and 257 nm come to be quite strong with the lapse of time. *Figure 1* shows the u.v. absorption spectra of cellulose and glucose in 97% sulfuric acid 5 days after making the solutions. Since cellulose and its unit glucose in sulfuric acid show the same change in u.v. absorption, the new absorption bands around 320 and 260 nm are considered to originate from a chemical species resulting from the reaction of the glucose unit with sulfuric acid. Moreover, the thin-layer chromatography (t.l.c.) experiments clarified that the new product is a small molecule not attached to cellulose.

Some have already reported that 5-hydroxymethyl-2-furfural (HF) is produced from glucose by dehydration with sulfuric acid[11–14]. Since the t.l.c. results indicate that the new product agrees with HF, we measured the u.v. absorption spectrum of HF in several solvents. The spectrum shows little variation from one organic solvent to another, although increased polarity of the solvent causes a slight shift of all the bands to lower energies; the peak wavelength is 274 nm in cyclohexane, 279 nm in tetrahydrofuran, 283 nm in dimethyl sulfoxide and propylene glycol, and 284 nm in water. However, the u.v. absorption of HF suffers a dramatic red shift in 97% sulfuric acid and is identical with the absorption spectra observed for cellulose and glucose in 97% sulfuric acid, as shown in *Figure 1**. As a matter of course, the spectrum of HF does not change with time. This shift would be

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*The discrepancy in the intensity below 300 nm in *Figure 1* is due to the u.v. absorption of an inorganic compound that is gradually produced in sulfuric acid even in the absence of organic compounds

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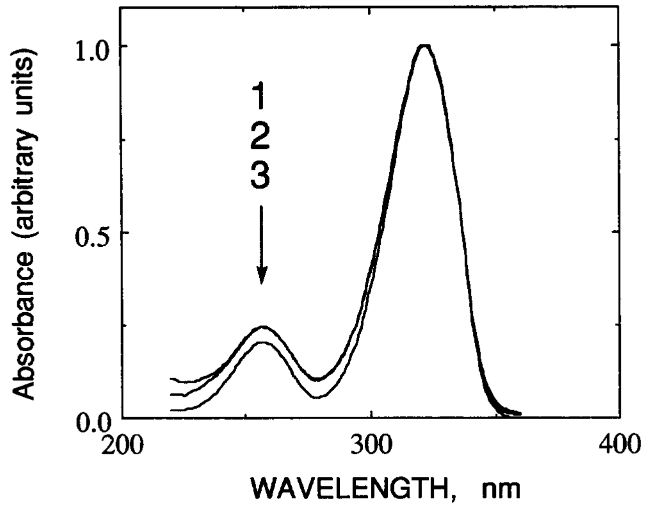

Figure 1 U.v. absorption spectra of the 97% sulfuric acid solutions of cellulose (1) and glucose (2) 5 days after making the solutions. The u.v. absorption spectrum of 5-hydroxymethyl-2-furfural (3) in 97% sulfuric acid is also shown

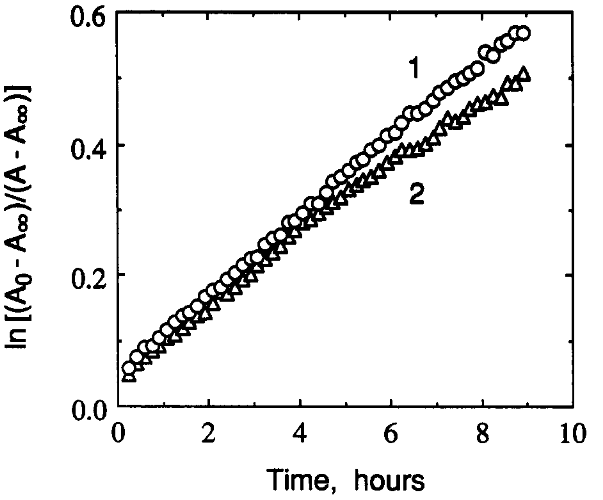

Figure 2 First-order plots for the reactions from cellulose to HF (1) and from glucose to HF (2) in 97% sulfuric acid at 25°C

induced by the change from CHO to $CHOH^+$ [15]. Actually, a bathochromic shift is observed for the u.v. absorptions of both furfural and HF with increasing sulfuric acid content in aqueous solution; the 277 nm peak of furfural in water shifts to 287 nm (61% sulfuric acid), 291 nm (69%), 314 nm (83%), and reaches as high as 317 nm (90%), while the peak intensity increases very much at concentrations over 83%. The shifts are confirmed to be reversible by repeated additions of concentrated sulfuric acid and water into the same solution. A similar phenomenon is reported to be observed in the case of α,β-unsaturated ketones[16,17] and α,β-unsaturated alicyclic ketones[18] in sulfuric acid solutions. The phenomenon occurs as a result of protonation. The new product was ascertained to show the same behaviour when the concentration of sulfuric acid was changed.

In order to prove the production of HF, we tried to isolate HF and identify it by n.m.r. We mixed cellulose (0.1 g) into 97% sulfuric acid (50 ml), left the solution for a week at room temperature, and then extracted the product with chloroform after adding water (500 ml) to the sulfuric acid solution at low temperature. The ^1H and ^{13}C n.m.r. spectra of the product clearly show that HF is formed in the reaction: ^1H n.m.r. δ (ppm) 9.6 (CHO), 7.2 and 6.5 (ring), 4.7 (CH_2); ^{13}C n.m.r. δ (ppm) 178 (CHO), 161, 152, 123, and 110 (ring), 57.6 (CH_2).

Thus it is concluded that: (i) cellulose is decomposed into glucose units by 97% sulfuric acid; (ii) HF is produced by the dehydration of glucose with sulfuric acid; and (iii) the intensities of the u.v. absorption peaks of HF at 323 and 257 nm increase with time. Although 71.2 to 72% sulfuric acid can be a good solvent for cellulose[19], the above conditions (low concentration of cellulose in 97% sulfuric acid) are found to accelerate the degradation process.

The above results demonstrate that the degradation reaction of cellulose with sulfuric acid can be monitored by measuring the u.v. absorbance A of HF at 320 nm over a period of time. We observed the saccharification process of cellulose with 97% sulfuric acid in an optical cell without stirring at 25°C and compared it with the conversion of glucose to HF. *Figure 2* shows the first-order plots for the reactions from cellulose to HF through glucose and from glucose to HF in 97% sulfuric acid at room temperature; A_0, the initial absorbance of HF at time = 0, is zero and A_∞ is the value measured more than 4 days later. The plots give essentially straight lines and very similar values for the rate constants ($1.46 \times 10^{-5}\,s^{-1}$ for glucose and $1.66 \times 10^{-5}\,s^{-1}$ for cellulose), even though the reaction is mainly due to slow self-diffusion of molecules in viscous sulfuric acid. The final conversion ratios for cellulose to HF and glucose to HF can be determined using the extinction coefficient of HF in 97% sulfuric acid ($\varepsilon_{322} = 2.62 \times 10^4$). For example, when cellulose with a concentration of 2.72×10^{-5} M in the glucose unit was dissolved in 97% sulfuric acid with stirring at 25°C, the final concentration of HF was found to be 2.54×10^{-5} M, meaning that the conversion ratio was 93%. In the case where the solution was not stirred, the ratio was no more than 77%. As for glucose, the conversion ratios were found to be almost 100% both with and without stirring.

The good agreement between the rate constants for the reactions whose plots are shown in *Figure 2* means that the degradation process of cellulose to small molecules takes place efficiently and it is faster than the formation of HF, even though the concentration of water is very low in the reaction solutions. Thus the dehydration reaction of the glucose unit appears to induce the decomposition of cellulose with high efficiency. We will in the future try to clarify the change of cellulose molecular weight during the reaction in order to determine whether the decomposition takes place randomly or stepwise from both ends, like a peeling reaction.

In summary, the present paper demonstrates, as a preliminary work, that: (i) the saccharification reaction of cellulose to HF can be monitored using the absorption intensity of HF; and (ii) the degradation of cellulose readily occurs in 97% sulfuric acid at room temperature and the conversion ratio is high under the above conditions.

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