PERKIN 2

Free-radical reactions of carbohydrate moieties in macromolecular structures. EPR evidence for the importance of steric and stereoelectronic effects and for the influence of inclusion in cyclodextrins

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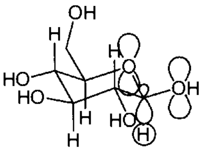
EPR experiments involving the attack of oxygen-centred free radicals on polysaccharides in aqueous solution reveal two sources of regioselectivity in C–H abstraction. For SO$_4^{-\bullet}$ (derived from one-electron reduction of S$_2$O$_8^{2-}$), hydrogen-atom abstraction is encouraged by SOMO–σ* interactions involving β-C–O bonds which eclipse the orbital of the unpaired electron at the developing radical centre. In addition, for a series of cyclodextrins, the reactivity observed with SO$_4^{-\bullet}$ points to the selective reaction of the latter within the cyclodextrin cavity; these findings are in contrast to those obtained with $^\bullet$OH (from H$_2$O$_2$).

Introduction

The considerable contemporary interest in the free-radical reactions of carbohydrates is a reflection of the importance of radical reactions involving sugar rings, for example, in free-radical damage to nucleic acids (induced, for instance, by ionizing radiation[1] or transition-metal activation of oxygen or peroxides[2]), the involvement of radicals in the enzymic degradation of polysaccharides and related macromolecular structures[3] and the incorporation of water-soluble oligosaccharides into copolymers *via* radical chemistry.[4] Whilst a variety of approaches has been employed in mechanistic and structural studies, involving spectroscopic and analytical techniques, product studies and molecular modelling, we believe that EPR spectroscopy can provide extremely important and detailed information to complement these approaches. For example, there have been extensive EPR studies of the species generated by X- or γ-irradiation of solid samples (of *e.g.* glucose, polysaccharides and nucleic acids; see ref. 5 and references therein). Although the spectra tend to be broad and therefore ill-defined, the main conclusions to be drawn parallel those reached on the basis of the highly resolved spectra obtained from continuous-flow studies of the reaction of $^\bullet$OH (from transition-metal/peroxide systems) with various carbohydrates.[6] It has been concluded, for example, that the highly-reactive hydroxyl radical is generally unselective in its attack *via* C–H abstraction, though we have drawn attention to the preference for abstraction of the C–H adjacent to the ring-oxygen in *furanose* compounds (*e.g.* ribofuranose, sucrose),[7] a finding attributed to a favourable stereoelectronic interaction *via* stabilization of the incipient radical by the lone pair of electrons on the ring oxygen in the transition state.

When considering the related electron-transfer and C–H abstraction reactions of SO$_4^{-\bullet}$ (an oxygen-centred radical which is believed to be somewhat less reactive than $^\bullet$OH) we have employed some simple carbohydrates as templates for attack in EPR experiments, so as to reveal the factors which govern the rate of reaction.[8] Our findings, for example that for α-D-glucose the selectivity of abstraction is in the order C2 ≃ C6 > C5 ≃ C1 > C3 ≃ C4, and that for β-D-glucose the order is C1 > C5 ≃ C6 > C2 > C3 > C4, provide more evidence of stereoelectronic control in free radical reactions. Thus the relative facility of attack at C1 in β-D-glucose reflects the importance of overlap between the developing radical centre on both neighbouring oxygen atoms as the axial C1–H is removed (see **1**), and the preference for C2 attack in α-D-glucose reflects the stabilization in the transition state offered by overlap involving the incipient SOMO and the σ* antibonding orbital of the axial C1–O bond (see structure **2**): the latter interaction is also believed to explain the "locked" structure preferred for α,β-dioxygen-substituted radicals, and the effect of a β-oxygen in retarding both the one-electron oxidation of such radicals[9] by Fe^{III} and the free-radical addition to methacrylic acid and related compounds.[10] A similar effect is believed to encourage formation (by SO$_4^{-\bullet}$) of the C5- and C6-derived radicals which exhibit geometries in which the overlap is maximised (*e.g.* **3**).[8] Studies of *myo*-inositol provide evidence to support this conclusion and also indicate that equatorial C–H abstraction is favoured over axial attack, in the absence of this effect.



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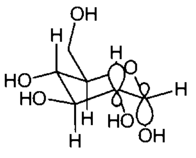

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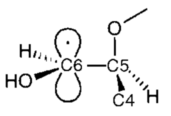

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In the work to be described here, we aimed firstly to explore the operation of steric, stereoelectronic and enthalpic effects in the reaction of some polysaccharides derived from glucose, not least in the context of the use of free-radical copolymerization reactions of starch and acrylates in the paint industry.[4] Secondly, we were particularly interested in the reactions of cyclodextrins as model compounds for studying free-radical attack at different surfaces of the toroidal molecules, and also because they offer possibilities for inclusion of one or other component of the redox couple to be employed (*e.g.* Ti^{III}, Fe^{II}, Cu^{I} with H$_2$O$_2$, S$_2$O$_8^{2-}$, HOOSO$_3^-$). We note that relatively little has been reported concerning radical attack on cyclodextrins. EPR

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spectroscopy has, for example, been successfully applied to observe the bimodal inclusion of stable aminoxyls within the hydrophobic cavity,[11] and to provide evidence for the related complexation of short-lived radicals.[12] However, there have been few successful attempts to identify first-formed radicals produced *via* hydrogen-atom abstraction from the cyclodextrin molecule itself (though ref. 13 describes some time-resolved EPR studies of reactions of excited-state ketones generated photolytically).

Results and discussion

EPR experiments: continuous flow and *in-situ* photolysis

Our approach, details of which have been described earlier,[6–8] has mainly involved use of a continuous-flow system to study, by EPR, radicals formed when reagents are mixed *in situ*. The redox couples used to generate $^{\bullet}OH$ and $SO_4^{-\bullet}$ are shown in reactions (1)–(7) (for details, see Experimental section). Note

$$Ti^{III} + H_2O_2 \longrightarrow Ti^{IV} + HO^{\bullet} + OH^- \qquad (1)$$

$$Fe^{II}\text{–}EDTA + H_2O_2 \longrightarrow Fe^{III}\text{–}EDTA + HO^{\bullet} + OH^- \qquad (2)$$

$$Ti^{III} \text{ (or } Fe^{II}\text{–}EDTA) + S_2O_8^{2-} \longrightarrow Ti^{IV} \text{ (or } Fe^{III}\text{–}EDTA) + SO_4^{-\bullet} + SO_4^{2-} \qquad (3)$$

$$Ti^{III} + Cu^{II} \longrightarrow Ti^{IV} + Cu^{I} \qquad (4)$$

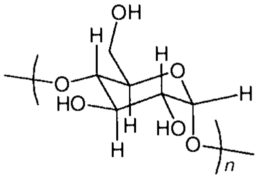
$$Cu^{I} + S_2O_8^{2-} \longrightarrow Cu^{II} + SO_4^{-\bullet} + SO_4^{2-} \qquad (5)$$

$$Cu^{I} + HOOSO_3^- \longrightarrow Cu^{II} + HO^{\bullet} + SO_4^{2-} \qquad (6)$$

$$Ti^{III} \text{ (or } Fe^{II}\text{–}EDTA) + HOOSO_3^- \longrightarrow Ti^{IV} \text{ (or } Fe^{III}\text{–}EDTA) + HO^- + SO_4^{-\bullet} \qquad (7)$$

that $^{\bullet}OH$ can be very efficiently formed from the reaction of either Ti^{III} or Fe^{II}–EDTA with H_2O_2 [reactions (1) and (2)];[6,14] the $Cu^{I}/HOOSO_3^-$ couple [reaction (6)] is a less effective source.[15,16] Though $SO_4^{-\bullet}$ can be generated from Ti^{III} or Fe^{II}–EDTA and $S_2O_8^{2-}$ [reaction (3)], the most effective method (leading to highest steady-state radical concentrations) involves Cu^{I} (from Ti^{III} and Cu^{II}) and peroxydisulfate [reactions (4) and (5)].[17] We have also employed direct photolysis (with a 1 kW Hg–Xe lamp) of $S_2O_8^{2-}$ in a slowly-flowing solution to generate $SO_4^{-\bullet}$ *in situ*.[18]

(i) Reactions of $^{\bullet}OH$ and $SO_4^{-\bullet}$ with starch. Starch (**4**) consists of glucose units joined by α-(1,4)-glucoside links; in its native form it is not water soluble on account of the size of the molecule and intramolecular hydrogen-bonding between glucose units. For the experiments to be described here the starch was degraded to a water-soluble form, with *ca.* eight glucose units per molecule (see Experimental section).



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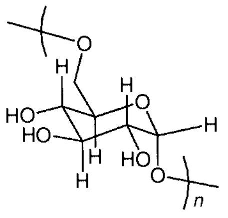

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Reactions of $^{\bullet}OH$. The EPR spectrum obtained from $^{\bullet}OH$ (from Ti^{III}/H_2O_2, Fig. 1*a*) is similar to that observed with α-D-glucose[6] though less well-resolved; this broadening reflects either (or both) the polymeric nature of the molecule, resulting in a slower rate of tumbling of the radicals, giving broader (more anisotropic) signals, or the result of the detection of overlapping signals from the formation of similar (but not identical) radicals from different glucose moieties in the polymer chain.

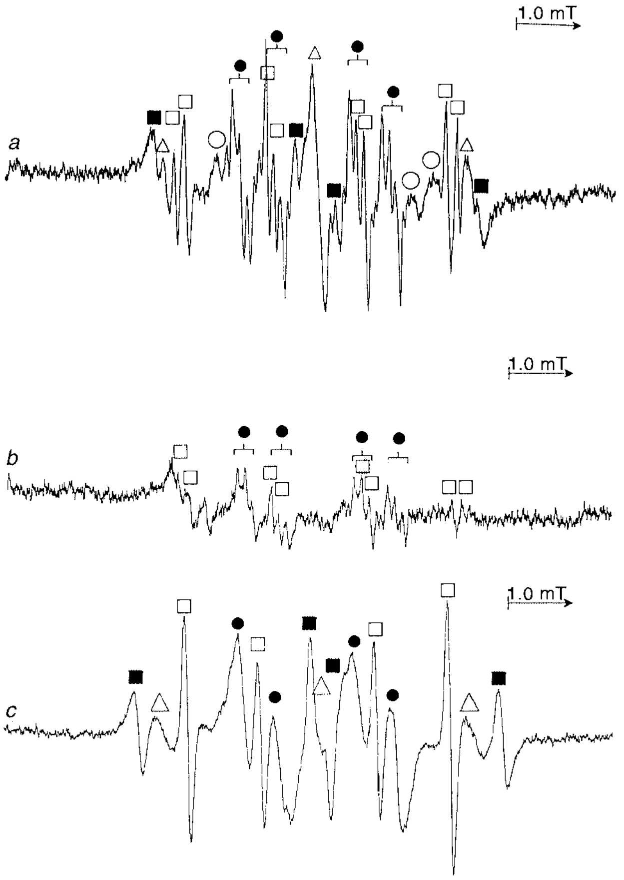


Fig. 1 EPR spectra obtained from the reaction of solutions of glucose-based polysaccharides with $HO^{\bullet}$ or $SO_4^{-\bullet}$: *a*) enzyme-degraded starch (24 g dm^{-3}) and $HO^{\bullet}$ (from Ti^{III}/H_2O_2) at pH 4: □ C_2, ■ C_3, △ C_4, ○ C_5, ● C_6; *b*) enzyme-degraded starch (24 g dm^{-3}) and $SO_4^{-\bullet}$ (from $Cu^{I}/S_2O_8^{2-}$) at pH 2.5: □ C_2, ● C_6; *c*) dextran (average M_r 10 500, 10 g dm^{-3}) and $HO^{\bullet}$ (from Ti^{III}/H_2O_2) at pH 4: □ C_2, ■ C_3, △ C_4, ● C_6.

Signals can be unambiguously assigned to the C2-, C3-, C4-, C5- and C6-derived radicals (see Table 1) by comparison with spectra from α-D-glucose. The signals from the C2-derived radicals are of particular interest: the presence of a small doublet splitting (0.16 mT) assigned to the C1–hydroxy group (as in the case of α-D-glucose) suggests that a substantial portion of the abstraction occurs from the terminal glucose unit. The signals assigned to the C4-derived radical closely resemble those observed in the α-D-glucose and cyclodextrin spectra (see below), *i.e.* a triplet with hyperfine splitting of 2.51 mT. It was not possible to determine accurately the hyperfine splitting constants for the C5-derived radicals from this spectrum as they are rather broad and weak and partially overlap the signals assigned to the easily characterized signals from C2- and C6-derived radicals. The signal due to the C6-derived radical consists of a doublet of doublets, with a remarkably low β-proton splitting of 0.53 mT confirming that the radical is in a locked conformation[8] (there are also several smaller splittings although these are not as well resolved as in the α-D-glucose spectra). Peaks at the extremities of the spectrum, together with matching peaks indicated near the centre of the spectrum, are assigned to the C3-derived radical (with two slightly different axial β-proton splittings of 2.25 and 2.90 mT); signals due to the C1-derived radical may well be obscured by signals assigned to the C6-derived radical(s).

We conclude that, largely as expected on the basis of the reaction of $^{\bullet}OH$ with α-D-glucose, the reaction of $^{\bullet}OH$ with starch appears to be relatively non-selective.

Table 1 EPR parameters for the radicals observed from the reaction of $^{\bullet}$OH with some glucose-based polysaccharides in aqueous solution

	Hyperfine splittings/mT[b]			
Radical[a]	a(α-H)	a(β-H)	a(other)	g-value[c]
Starch				
C2		1.29 (1)[d] 2.85 (1)	0.16 (1)	2.0031
C3		2.90 (1) 2.25 (1)		2.0031
C4		2.51 (2)		2.0031
C5[e]		3.4 (1) 0.9 (1) 0.6 (1)		2.0031
C6	1.84 (1)	0.53 (1)	[f]	2.0032
Dextrans				
C2		1.16 (1) 3.01 (1)		2.0031
C3		2.90 (1) 2.70 (1)		2.0031
C4		2.53 (2)		2.0031
C6	1.85 (1)	0.64 (1)	[f]	2.0031

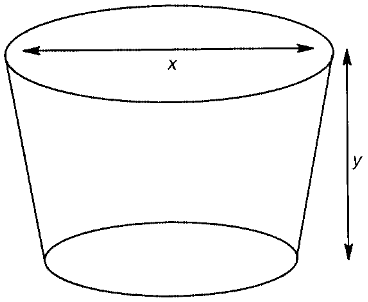
[a] Denotes site of abstraction. [b] ±0.01 mT. [c] ±0.0001. [d] Values in parentheses indicate the number of hydrogens. [e] Hyperfine splittings could not be determined as accurately for this species. [f] Further splittings are present but are not sufficiently well resolved to make a precise measurement.

Reactions of $SO_4^{-\bullet}$. The reaction of $SO_4^{-\bullet}$ with an aqueous solution of partially degraded starch at pH *ca.* 2.5 gave a relatively weak EPR spectrum (see Fig. 1*b*). The broad nature of the signals makes precise assignment difficult but it is clear that the dominant radicals formed are C2- and C6-derived. This result is entirely consistent with that expected from the results of the reaction of $SO_4^{-\bullet}$ with α-D-glucose, as described earlier,[8] which gave an EPR spectrum showing predominant abstraction at these positions. This selectivity is believed to reflect the favourable overlap in the resulting radicals (and in the transition state for their formation) between the singly-occupied orbital and the (eclipsing) β-C–O bond.

(ii) Reactions of $^{\bullet}$OH and $SO_4^{-\bullet}$ with dextrans. We next investigated the related reactions of aqueous solutions of dextran [poly-1,6-linked-α-D-glucose (**5**)]. Two polymer samples were studied with average molecular weights (M_r) 10 500 and 188 000.

Reactions of $^{\bullet}$*OH*. The spectra obtained with $^{\bullet}$OH at pH 4 (see, for example, Fig. 1*c*) were very similar in appearance (the higher molecular weight polymer sample showing slightly broader lines, as might be expected) and also resembled those obtained from the starch oligomers. Signals can be assigned to radicals formed by hydrogen-abstraction from positions C2, C3, C4 and C6 (see Table 1); there are no signals assignable to attack at C1 or C5 though, of course, these may be obscured. It is interesting to note that the pattern of reactivity broadly reflects that detected for α-D-glucose and starch, for which the C5 radical tends to be of relatively low intensity: steric reasons may be even more important in this case. The EPR parameters in the various α-glucose-derived radicals are strikingly similar, indicating that the chair geometry is retained in the polymers. For both sets of oligomers/polymers, the narrowness of the lines ($\Delta H \sim 0.2$ mT) compared with solid-state/immobilized spectra is presumably an indication of rapid internal molecular motions of the carbohydrate chains.

Reactions of $SO_4^{-\bullet}$. Reactions of $SO_4^{-\bullet}$ with each sample of dextran under similar conditions led to weaker spectra, clearly related to that shown in Fig. 1*c*, but dominated by signals from the C2 and C6-derived radicals as noted above. This, again, is believed to reflect σ*–SOMO interactions.



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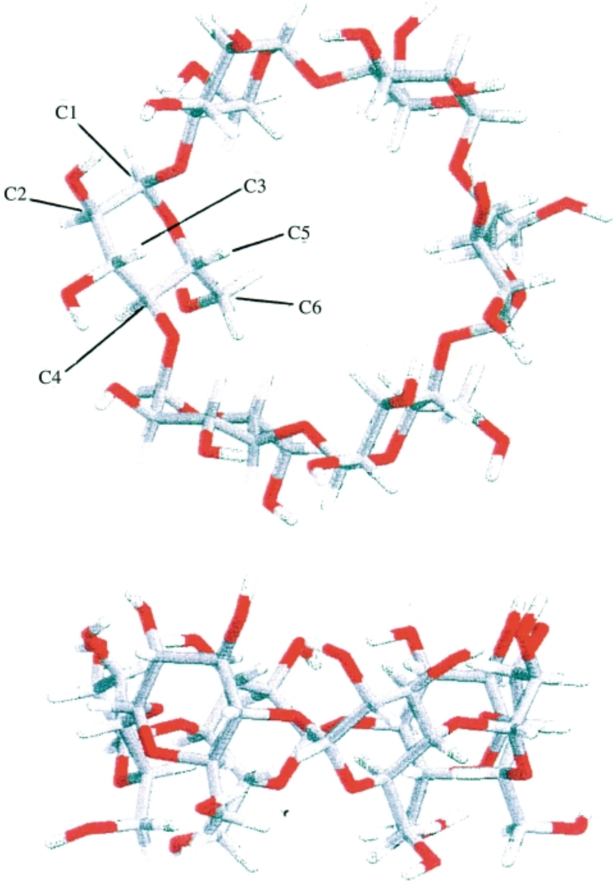
	x^a/nm	y/nm	Cavity volume/Å^3
α	0.57	0.78	174
β	0.78	0.78	262

[a] X = internal diameter

Fig. 2 Physical properties of cyclodextrins (taken from ref. 21).

(iii) Reactions of $^{\bullet}$OH and $SO_4^{-\bullet}$ with cyclodextrins. α-, β-, and γ-Cyclodextrins are cyclic species which contain six, seven and eight glucose units per molecule, respectively, joined by α-(1,4)-glycosidic bonds. They exist as toroidal structures (see *e.g.* **6**) in which the exocyclic C6-units occupy the narrower end; they are generally believed to have hydrophilic exteriors and hydrophobic interiors.[19] The C3–H and C5–H bonds are directed inside the cavity and the C1, C2 and C4 C–H bonds outside the ring[19] (see *e.g.* structure **7** for α-cyclodextrin which is based upon X-ray crystal-structure coordinates[20] and generated using RASMOL[21]).



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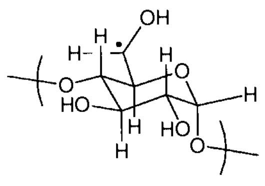
Reaction of $^{\bullet}$*OH with α-cyclodextrin*. The EPR spectrum obtained from $^{\bullet}$OH (Ti^{III}/H_2O_2) and an aqueous solution of α-cyclodextrin (**7**) at pH *ca.* 2.5 (see Fig. 3*a* and Table 2) is



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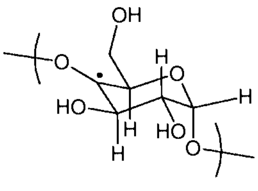

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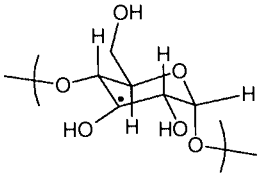

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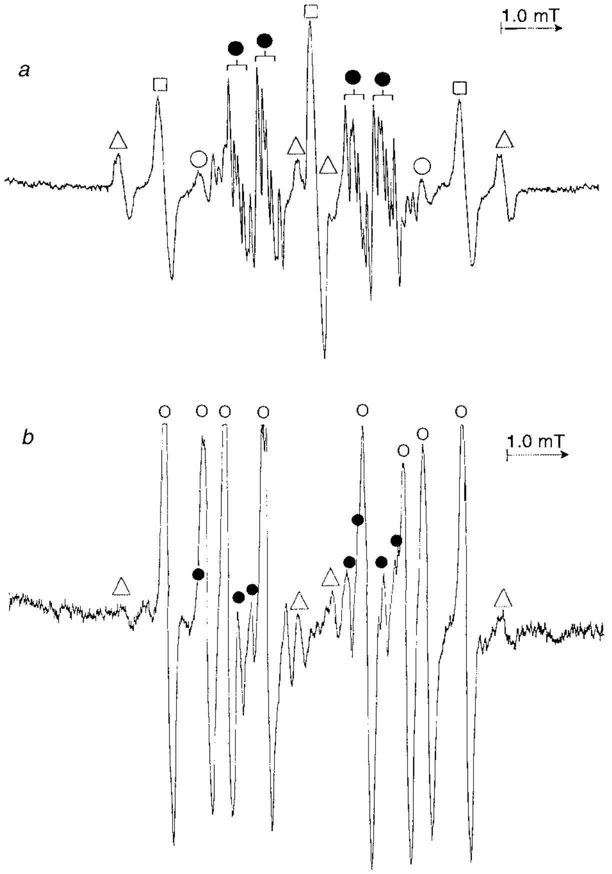


Fig. 3 EPR spectra obtained from the reaction of a solution of α-cyclodextrin (1.7×10^{-3} mol dm^{-3}) with *a*) $HO^{\bullet}$ (from Ti^{III}/H_2O_2) at pH 2.5: △ C_3, □ C_4, ○ C_5, ● C_6; *b*) $SO_4^{-\bullet}$ (from $Cu^I/S_2O_8^{2-}$) at pH 2.5: △ C_3, ○ C_5, ● C_6.

very similar to that observed from the reaction of α-D-glucose and the related oligomers. The high intensity is notable and suggests the radical termination rate is somewhat reduced compared to comparable open-chain species (the cyclic structure might be expected to lead to a reduction in $2k_t$). Dominant features include a strong spectrum with a doublet of doublets, 1.89 and 0.47 mT (and several well-resolved smaller splittings) assigned to the C6-derived radical (**8**); note that the very low β-proton splitting (0.47 mT) is characteristic of a locked conformation (*cf.* structure **3** above). Other clear features include a relatively intense triplet (2.45 mT) assigned to the C4-derived radical (**9**), which as expected has splittings from two axial β-protons, and a doublet of doublets, 3.28 and 2.99 mT, assigned to the C3-derived radical (**10**) (with splittings from the axial C2 and C4 protons). A trace of the C5-derived radical (for assignment, see below) is also detectable.

There was no evidence for either the C1- or C2-derived radicals. On the basis of the results for α-D-glucose, it would be expected that they would be formed although it is possible that signals due to the C1-derived radicals are obscured by the signals assigned to the C6-derived radical.

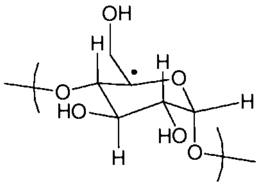
The results are clearly in contrast to those for the reaction of $^{\bullet}OH$ with α-D-glucose[6] for which hydrogen-atom abstraction is non-selective. In this case, the dominance of the signals assigned to the C4- and C6-derived radicals suggests that abstraction by $^{\bullet}OH$ is occurring predominantly from the C–H bonds on the outside of the torus. The possible explanation for this behaviour is discussed below.

Table 2 EPR parameters for the radicals observed from the reaction of $^{\bullet}OH$ with α-cyclodextrin

	Hyperfine splittings/mT[b]			
Radical[a]	*a*(α-H)	*a*(β-H)	*a*(other)	*g*-value[c]
C3		3.28 (1) 2.91 (1)		2.0031
C4		2.45 (2)		2.0031
C5[d]		3.23 (1) 0.99 (1) 0.64 (1)		2.0031
C6	1.89 (1)	0.47 (1)	0.14 (1) 0.13 (1) 0.08 (1)	2.0032

[a] Denotes site of abstraction. [b] ±0.01 mT. [c] ±0.0001. [d] Hyperfine splittings determined from the spectrum of this radical obtained in higher concentration for reaction with $SO_4^{-\bullet}$.

Reaction of $SO_4^{-\bullet}$ with α-cyclodextrin. The EPR spectrum observed from the reaction of $SO_4^{-\bullet}$ with α-cyclodextrin at pH *ca.* 2.5 (Fig. 3*b*) is dramatically different from that observed with $^{\bullet}OH$. The spectrum is dominated by an eight-line pattern, with proton hyperfine splittings of 3.23, 0.99, and 0.64 mT, assigned by comparison with the results from α-D-glucose,[6] to the C5-derived radical (**11**). The largest splitting in this radical is evidently from the axial C4-proton while the smaller splittings are from the diastereotopic C6-protons (this radical is also expected to exist in a locked conformation). Other rather weak signals include a doublet of doublets, assigned to the C3-derived radical. We note that signals assigned to the C5 and C3 radicals, as well as C1-derived radicals, have been observed in a time-resolved EPR study of the *in-situ* photolysis of acetone and related carbonyl compounds in aqueous solutions containing cyclodextrins (see below).[13] The hyperfine splittings are similar to those reported here.



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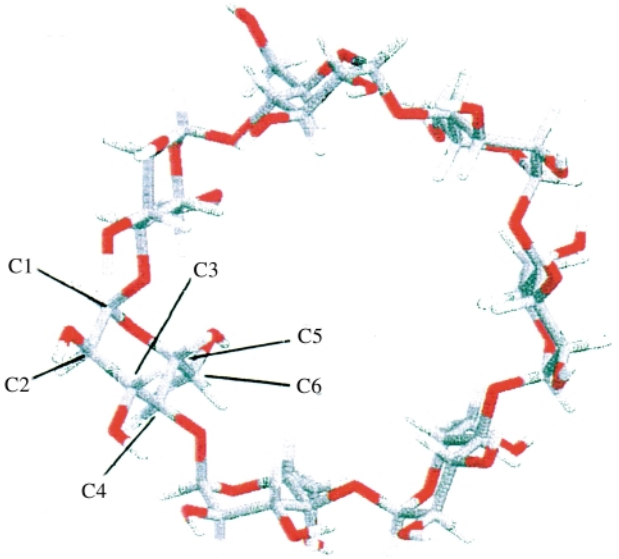
The dominance of the signal assigned to the C5-derived radical and the presence of the C3-derived radical provide clear evidence that abstraction by $SO_4^{-\bullet}$ is occurring exclusively from the C–H bonds *inside* the cyclodextrin cavity (and similarly for the excited state ketones[13]).

Reaction of $^{\bullet}OH$ with β-cyclodextrin. β-Cyclodextrin (**12**)[22] consists of seven glucose units per molecule; as with α-cyclodextrin the C3 and C5 C–H bonds are inside the cavity while the C1, C2 and C4 C–H bonds are positioned on the outside surface.[19]

Table 3 EPR parameters for the radicals observed from the reaction of $^{\bullet}$OH with β-cyclodextrin

Radical[a]	Hyperfine splittings/mT[b]			g-value[c]
	a(α-H)	a(β-H)	a(other)	
C3[d]		3.39 (1) 2.87 (1)		2.0031
C4		2.69 (2) 3.31 (1)		2.0031
C5[d]		0.97 (1) 0.64 (1)		2.0031
C6	1.87 (1)	0.56 (1)	0.14 (1) 0.12 (1) 0.08 (1)	2.0032

[a] Denotes site of abstraction. [b] ±0.01 mT. [c] ±0.0001. [d] Hyperfine splittings determined from the spectrum of this radical obtained in higher concentration for reaction with $SO_4^{-\bullet}$.



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The EPR spectrum observed from the reaction of $^{\bullet}$OH (from Ti^{III}/H_2O_2) with β-cyclodextrin at pH *ca.* 2.5, (Fig. 4*a*) is similar to that for α-cyclodextrin, the most notable difference being the very high intensity of the central line (associated with C4). Signals attributable to the C6-derived radicals are clear and there are relatively strong signals present which are assigned to the C5-derived radical; signals from C3-derived radicals can also be detected. As with α-cyclodextrin, it was not possible to detect unambiguously the presence of C1- or C2-derived radicals. Broadly similar results (Fig. 4*b*) were obtained with Fe^{II}–EDTA/H_2O_2: the C4-radical spectrum was again found to be prominent. The C6-derived radical is reduced in intensity and the C3-derived radical is no longer detectable. (This is believed to reflect selective oxidation of these radicals by Fe^{III} as observed previously.[9]) As with α-cyclodextrin, hydrogen-abstraction reactions of $^{\bullet}$OH with α-cyclodextrin appear to occur predominantly from the outer surface of the torus, although in this case there appears to be a greater amount of abstraction from the C5-position.

Reaction of $SO_4^{-\bullet}$ with β-cyclodextrin. The spectrum from $SO_4^{-\bullet}$ and β-cyclodextrin at pH *ca.* 2.5, (Fig. 4*c*) is similar to that observed for α-cyclodextrin. It is dominated by an eight-line pattern, with splittings of 3.31, 0.97 and 0.64 mT, assigned to the C5-derived radical. The C3-derived radical is also present, but at a higher concentration with respect to the signals due to the C5-derived radical than for the α-cyclodextrin. Signals due to the C6-derived radical can also be clearly distinguished. There is no evidence for the presence of any other radical species in significant concentrations. This result is consistent with the reaction of $SO_4^{-\bullet}$ occurring predominantly inside the cyclodextrin cavity, as suggested for α-cyclodextrin and as may also occur for triplet-state ketones.[13]

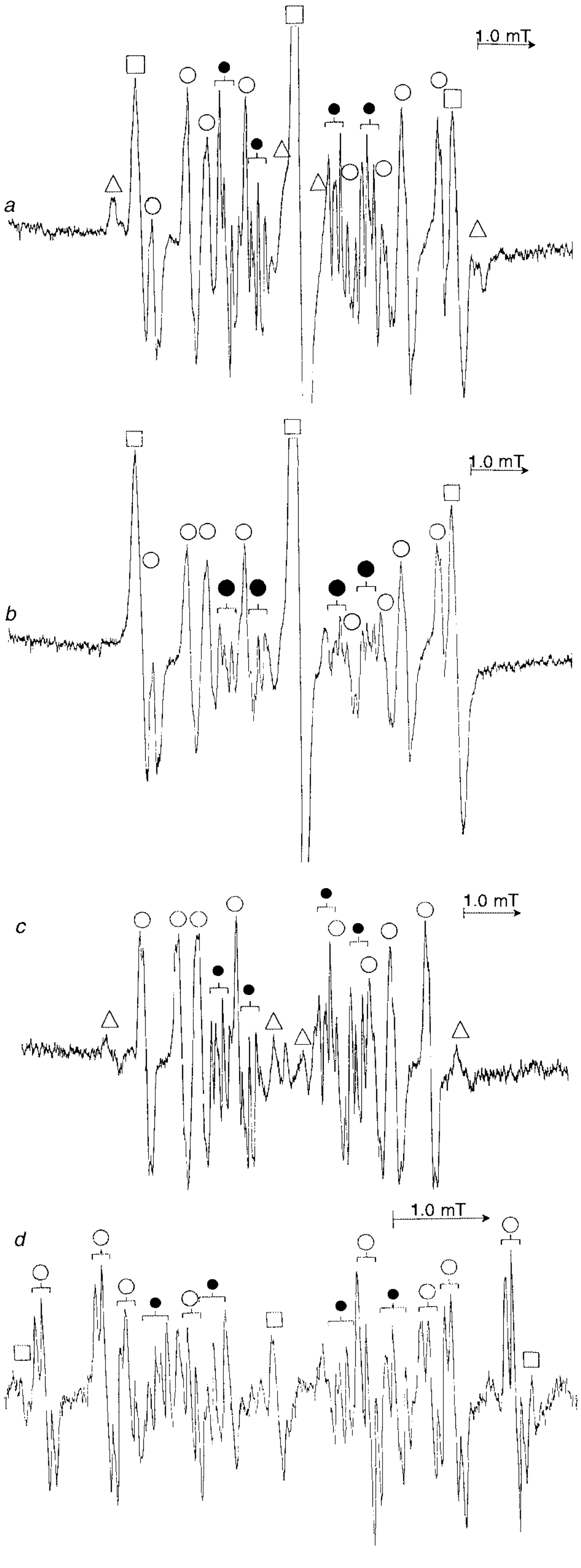
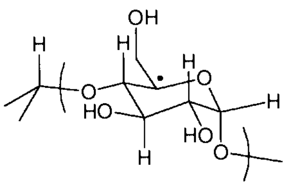


Fig. 4 EPR spectra obtained from the reaction of a solution of β-cyclodextrin (5×10^{-3} mol dm^{-3}) with *a*) HO$^{\bullet}$ (from Ti^{III}/H_2O_2) at pH 3.5: △ C_3, □ C_4, ○ C_5, ● C_6; *b*) HO$^{\bullet}$ (from Fe^{II}/H_2O_2) at pH 3.5: □ C_4, ○ C_5, ● C_6; *c*) $SO_4^{-\bullet}$ (from $Cu^{I}/S_2O_8^{2-}$) at pH 2.5: △ C_3, ○ C_5, ● C_6; *d*) $SO_4^{-\bullet}$ (from $S_2O_8^{2-}$ photolysis): □ C_4, ○ C_5, ● C_6, (NB expanded spectrum).

A number of continuous-flow experiments were next performed to establish whether or not certain cyclodextrin-derived radicals might be particularly susceptible to further oxidation (by Cu^{II}, $SO_4^{-\bullet}$ or $S_2O_8^{-}$) or reduction (by Cu^{I}), as a possible explanation for the apparent selectivity observed: Cu^{I} and Cu^{II} are known to remove organic radicals rapidly, by reduction and oxidation, respectively (see *e.g.* ref. 17). In order to investigate any potential effects from copper ions on the spectrum observed from the reaction of $SO_4^{-\bullet}$ with cyclodextrins, the reaction of $SO_4^{-\bullet}$ with β-cyclodextrin was monitored at various initial Cu^{II} concentrations (using the continuous-flow EPR technique) and, see later, in the absence of Cu^{II}, by utilizing a photolytic approach. The spectra observed from the reactions of $SO_4^{-\bullet}$ with β-cyclodextrin at initial concentrations of Cu^{II} between 1.75×10^{-5} and 7×10^{-5} mol dm^{-3} showed no significant differences in relative radical intensities, which appears to rule out such an explanation.

The final set of continuous-flow experiments involved following the reaction of $SO_4^{-\bullet}$ at various initial concentrations of $S_2O_8^{-}$ to investigate the possibility of radical oxidation by the peroxydisulfate. The initial concentration of sodium peroxydisulfate was varied between 0.004 and 0.016 mol dm^{-3}: no significant differences in the spectra were observed, which appears to rule out the occurrence of selective radical oxidation by $S_2O_8^{-}$.

(iv) Photolysis of β-cyclodextrin–sodium peroxydisulfate solutions. Photolysis experiments involved slowly flowing an aqueous solution of β-cyclodextrin (0.014 mol dm^{-3}), sodium peroxydisulfate (0.1 mol dm^{-3}) and acetone (5% v/v) through the EPR cavity (at a flow rate of approximately 1–2 cm^{3} min^{-1}, which avoids the effects of substrate depletion) while it was exposed to an intense beam of uv light ($\lambda > 250$ nm). The EPR spectrum observed is dominated by the signals due to the C5-derived radical (Fig. 4*d*) and allows a further triplet splitting of 0.06 mT in this spectrum to be resolved. This extra splitting is evidently due to long-range coupling from two separate protons, possibly the axial γ-proton at the C3-position and either the equatorial γ-C1-proton or the δ-proton at the C1-position of the adjacent glucose unit (**13**). There are also signals due to the C3- and C6-derived radicals and a small central signal. These results strongly suggest that the similar selectivity observed in the metal-mediated $SO_4^{-\bullet}$ reaction reflects the selective H-abstraction of $SO_4^{-\bullet}$ rather than a dependence upon further reaction (oxidation/reduction) of metal ions.



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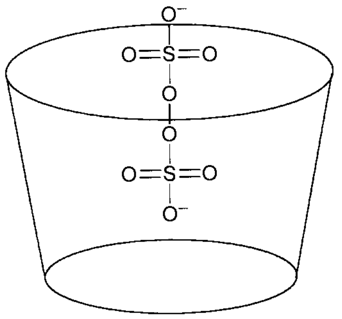
(v) Comparison of the reactions of $^{\bullet}OH$ and $SO_4^{-\bullet}$ with α- and β-cyclodextrins. $^{\bullet}OH$ and $SO_4^{-\bullet}$ appear to behave very differently in their hydrogen-atom abstraction reactions with cyclodextrins. $^{\bullet}OH$ reacts with α-cyclodextrin predominantly at the C4- and C6-positions of the individual glucose units whereas the reaction of $SO_4^{-\bullet}$ occurs almost exclusively at C5 (control experiments suggest that the differences are due to selective reaction of $SO_4^{-\bullet}$). When the size of the cyclodextrin is increased (in experiments with β-cyclodextrin) the reaction of $SO_4^{-\bullet}$ becomes less selective, with evidence for more abstraction from the C3- and C6-positions. In both cases, $SO_4^{-\bullet}$ is only observed to abstract hydrogen atoms from inside the cyclodextrin cavity. The preferential reaction of $SO_4^{-\bullet}$ at the C5-position of both forms of the cyclodextrin is particularly unexpected given that $SO_4^{-\bullet}$ primarily abstracts the C2- and C6-hydrogen atoms from α-D-glucose and starch. The cyclic nature of the polysaccharide clearly influences its reaction.

Given that abstraction is apparently occurring inside the cavity of the cyclodextrin molecule then it is not unreasonable to assume that formation of $SO_4^{-\bullet}$ also occurs there. If this is the case then it may be a result of either the transition-metal ion involved or the peroxide being complexed inside the cyclodextrin cavity. Since the slow-flow photolysis experiment in the absence of any transition-metal ions, also showed predominant reaction inside the cyclodextrin cavity, we conclude that the selectivity is not a result of complexation of either the Ti^{III} or Cu^{II} employed in the $Ti^{III}/Cu^{I}/S_2O_8^{2-}$ redox couple (previous studies involving benzoyl peroxide led to a suggestion that formation of a radical from a peroxide complexed in a cyclodextrin molecule might result in hydrogen-atom abstraction from the C3-, C5- and C6-positions).[23] We suggest that the peroxydisulfate itself is either complexed within the cyclodextrin cavity (**14**) or across one end (**15**), possibly *via* hydrogen bonding.

Computer modelling (using Cerius 2 software) suggests that the distance between the two negatively charged oxygen atoms of a peroxydisulfate molecule is approximately 0.57 nm. The internal diameters of the wider end of the α- and β-cyclodextrin cavities are 0.57 and 0.78 nm, respectively, while both are 0.78 nm deep.[19] These dimensions suggest that both **14** and **15** are viable structures. We note, however, that inclusion within the cyclodextrins evidently does not substantially alter the rapidity of the electron-transfer reaction with the transition metal (Cu^{I}).

One final set of experiments involved the reaction of *peroxymonosulfate* with Ti^{III} in the presence of β-cyclodextrin. We have shown that reduction of peroxymonosulfate by Ti^{III} induces decomposition to form $SO_4^{-\bullet}$ [reaction (7)] while in the presence of Cu^{II}, $^{\bullet}OH$ is produced [reactions (4) and (6)].[15,16] This striking difference in behaviour has been established by EPR spectroscopy, by monitoring the reaction in the presence



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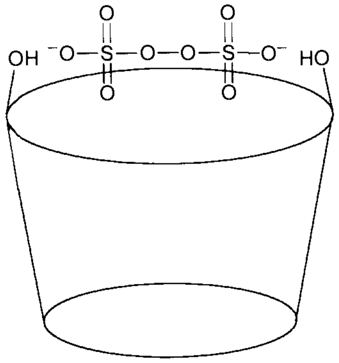

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of alkenes such as methacrylic acid as the hyperfine splittings of an $^{\bullet}$OH adduct radical are different to those for an $SO_4^{-\bullet}$ adduct. We aimed to monitor the reactions of $^{\bullet}$OH and $SO_4^{-\bullet}$, formed from peroxymonosulfate, with β-cyclodextrin, since it has previously been reported[24] that the peroxymonosulfate anion is not complexed by cyclodextrins and thus it might be expected that both the $^{\bullet}$OH and $SO_4^{-\bullet}$ spectra would be dominated by signals due to abstraction from C–H bonds outside the cavity.

The EPR spectrum observed from the reaction of $Ti^{III}/Cu^{II}/HOOSO_3^-$ with α-cyclodextrin was found to be similar to that observed for the reaction with $Ti^{III}/Cu^{II}/H_2O_2$: it is dominated by signals from C6-derived radicals and shows weaker signals due to C3-, C4- and C5-derived radicals (presumably from reaction with $^{\bullet}$OH); this result suggests, as expected,[24] that the peroxymonosulfate anion is not complexed inside the cyclodextrin cavity. Attempts to monitor the reaction of $Ti^{III}/HOOSO_3^-$ in the absence of Cu^{II} (to generate $SO_4^{-\bullet}$) were unsuccessful; no EPR spectrum was observed. This is presumably due either to reaction (7) being too slow for effective use on the continuous-flow time-scale or to rapid oxidation of any radicals produced by residual peroxymonosulfate.[16] This result is in marked contrast to the intense signals produced by the reaction of Cu^I with $S_2O_8^{2-}$, which in itself may reflect acceleration of electron-transfer *via* inclusion of either or both reagents.

Finally, we return to the behaviour of $^{\bullet}$OH (from either Ti^{III} or Fe^{II}–EDTA with H_2O_2) in its reaction with the cyclodextrins (it is also worth noting that the very close similarity of the behaviour of these two metal ions provides further very striking evidence that the Fe^{II}–EDTA/H_2O_2 redox couple generates $^{\bullet}$OH rather than a ferryl species). The observation that attack of $^{\bullet}$OH occurs predominantly on hydrogens directed *outside* the cavities suggests either that the transition metal cannot be effectively accommodated within the cavity, or, perhaps more likely, that the rate constants of $^{\bullet}$OH formation by reaction of H_2O_2 with Ti^{III} or Fe^{II}–EDTA bound within the cavity are significantly reduced.

Experimental

All chemicals were purchased from the Sigma-Aldrich Company and were used without further purification except for starch (see below). EPR Spectra were recorded on a Bruker ESP-300 spectrometer equipped with an X-band klystron and 100 kHz modulation. Deionised water was used for all experiments.

The continuous flow apparatus employed a three-way mixing system. The flow rate, typically 40 $cm^3\ s^{-1}$ was maintained with a Watson-Marlow 502s peristaltic pump, positioned on the inlet tubes. The delay time between mixing and passage through the EPR cavity was *ca.* 30 ms. All solutions were deoxygenated both prior and during use by purging with oxygen-free nitrogen. Final adjustment of pH was made by addition of conc. ammonia or conc. sulfuric acid solutions to the metal-ion stream. For $^{\bullet}$OH studies at pH ~4, typical concentrations after mixing were: stream 1, Ti^{III} 1.6×10^{-3} mol dm^{-3} or Fe^{II} 1.0×10^{-3} mol dm^{-3} with 1.1 equivalents of EDTA (added as the disodium salt); stream 2, H_2O_2 8.3×10^{-3} mol dm^{-3}; stream 3, substrate 1–25 g dm^{-3}; for $SO_4^{-\bullet}$ studies at pH ~2: stream 1, Ti^{III} 1.6×10^{-3} mol dm^{-3}, stream 2, $Na_2S_2O_8$ 8.5×10^{-3} mol dm^{-3}; stream 3, substrate 1–25 g dm^{-3} and $CuSO_4$ 2.9×10^{-5} mol dm^{-3}. Hyperfine splittings were taken directly from the field scan, itself calibrated by reference to Fremy's salt. *g*-Values were obtained by comparison with spectra from $^{\bullet}$CHMeOH (*g* 2.0031) recorded under identical conditions.

For the photolysis studies, a single stream was flowed through a flat quartz EPR cell positioned in the EPR cavity. Photolysis was carried out using the unfiltered radiation from a Hanovia 977B-1 1 kW mercury-xenon arc. The flow was maintained at 1–2 ml min^{-1} using a Watson Marlow 101U peristaltic pump. Solutions typically contained 5% acetone (v/v), $Na_2S_2O_8$ 0.1 mol dm^{-3} and substrate 5×10^{-3} mol dm^{-3}. All solutions were deoxygenated both prior and during use by purging with oxygen-free nitrogen.

In order to prepare solutions for EPR study, starch was typically added to water at 40 °C, with vigorous stirring, the temperature was increased to 60 °C and an α-amylase enzyme added. The temperature was raised to 70 °C (the temperature at which the enzyme is active) for 30 minutes and a clear solution was formed. The temperature was then raised to 90 °C for 30 minutes to denature the enzyme and the system allowed to cool to give a cloudy white aqueous suspension of oligomeric sugars which was used without further purification.

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