

Determination of Absolute Rate Constants for the Reversible Hydrogen-atom Transfer between Thiyl Radicals and Alcohols or Ethers

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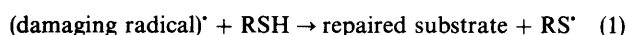
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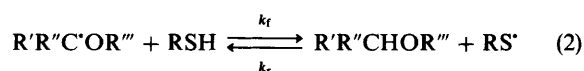
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Absolute rate constants have been determined for the reversible hydrogen-transfer process $R^{\bullet} + RSH \rightleftharpoons RH + RS^{\bullet}$ by pulse radiolysis, mainly through direct observation of the RS^{\bullet} radical formation kinetics in water–RH (1 : 1, v/v) mixtures. The thiols investigated were penicillamine and glutathione; the RH hydrogen donors were methanol, ethanol, propan-1-ol, propan-2-ol, ethylene glycol, tetrahydrofuran and 1,4-dioxane with the abstracted hydrogen being located α to the hydroxy or alkoxy function. Rate constants for the forward reaction of the above equilibrium (in radiation biology referred to as ‘repair’ reaction) were typically of the order of $10^7 - 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ while hydrogen abstraction from RH by thiyl radicals (reverse process) occurred with rate constants of the order of $10^3 - 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. This yields equilibrium constants of the order of 10^4 . Based on these data, standard reduction potentials could be evaluated for the $R^{\bullet}R''C^{\bullet}OH/H^+//R^{\bullet}R''CHOH$, $R^{\bullet}R''CO/H^+//R^{\bullet}R''C^{\bullet}(OH)$ and $R^{\bullet}R''CO//R^{\bullet}R''C^{\bullet}O^-$ couples from methanol, ethanol and propan-2-ol. Effective hydrogen-atom abstraction by RS^{\bullet} required activation by neighbouring groups of the C–H bond to be cleaved in RH. No such process was observed for the RS^{\bullet} reaction with $-CH_3$ groups, e.g. in 2-methylpropan-2-ol. Several halogenated hydrocarbons, including some anaesthetics (e.g. halothane) and $Fe(CN)_6^{3-}$ have been tested with respect to their ability to disturb the $(CH_3)_2C^{\bullet}OH + RSH \rightleftharpoons (CH_3)_2CHOH + RS^{\bullet}$ equilibrium through an irreversible electron-transfer reaction with the reducing α -hydroxyl radical, thereby drawing the equilibrium to the left-hand side. The respective efficiencies are found to be related to the electronegativities of the electron acceptors. The results are briefly discussed in terms of their biological relevance.

The mercapto group participates in many biological processes owing to its high affinity to transition metals, its strong nucleophilicity (as thiolate), and its reducing character.^{1–4} Thiyl free radicals are important chemical intermediates derived therefrom and have been identified in biological systems, for example, by EPR studies on the peroxidase catalysed reduction of peroxides in cellular systems.⁵ In radiation biology, thiols have attracted much attention since they were shown to exhibit a protective role against the deleterious action of ionizing radiation which is achieved by the free radical scavenging capacity of thiols based on their pronounced H-atom donor ability.² This reaction, in radiation biology known as ‘repair reaction’, is accompanied by the formation of thiyl radicals.

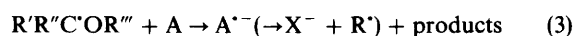


Studies with 2,5-dimethyltetrahydrofuran⁶ and propan-2-ol^{7–9} have shown that reaction (1) is, in fact, reversible and correctly expressed in terms of an equilibrium:



This is understandable considering the similarity of the bond dissociation energies for the thiol S–H (ca. 380 kJ mol^{-1}) and some C–H bonds activated by substituents such as α -hydroxy or α -alkoxy groups (ca. $380 - 395 \text{ kJ mol}^{-1}$).¹⁰ H-atom abstractions by thiyl radicals, in general, have also been documented in quite a number of cases.¹¹

Carbon-centred α -alkoxyl and α -hydroxyl radicals ($R''' = H$) are known to exhibit reducing properties and for their ability to transfer electrons to appropriate acceptors, A, according to the general reaction (3).¹²



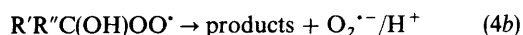
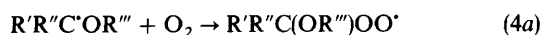
Irreversible removal of the $R^{\bullet}R''C^{\bullet}OR'''$ radicals causes, of course, a shift of equilibrium (2) to the left-hand side. This consequence has recently been used to determine the absolute rate constant $k_r = 1.4 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ for the reaction of penicillamine thiyl radicals, $PenS^{\bullet}$, with propan-2-ol.⁷ Similar rate constants have been evaluated for the thiyl radicals derived from glutathione, GS^{\bullet} , ($k_r = 1.2 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) and cysteine, $CysS^{\bullet}$, ($k_r = 2.0 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) with propan-2-ol.^{8,9}

Although the rate constants, k_r , are lower by several orders of magnitude compared with k_f (ca. $10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) for the corresponding repair reactions, equilibrium (2), nevertheless, opens a potential route to thiyl radical-inflicted biological damage. Since alcohols and ethers, owing to the presence of α -hydroxy/alkoxy-substituted C–H bonds, may serve as model compounds for DNA sugars, one of the implications would be a possible thiyl-induced damage to this most vital cellular constituent. The actual efficiency of this process would, of course, be determined by the extent to which a reaction analogous to eqn. (3) can take place. Besides xenobiotics like CCl_4 , many naturally abundant electron acceptors should be considered in this respect. Furthermore, carbon-centred radicals [as formed in equilibrium (2)] are known to react with molecular oxygen ($k \approx 2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$).¹³ In the first step this leads usually to a peroxy radical [eqn. (4a)] which, in the case of $R''' = H$, may subse-

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quently cleave superoxide in a pH-dependent process [eqn. (4b)].²



The reduced protection which is observed upon interaction of ionizing radiation with, for example, DNA in the presence of both thiol and oxygen (or Cu^{II})^{14,15} would thus find a rationale in the above-described mechanism. In essence, the existence of equilibrium (2) may even provide an additional possibility to explain the so-called 'oxygen effect' in radiation biology. Absolute determination of k_r , via the use of oxygen as scavenger for the C-centred alcohol or ether radicals is, unfortunately, not possible because the competing reaction of thiyl free radicals with oxygen is too fast.^{16,17}

The present study has been undertaken in order to obtain further quantitative data on equilibrium (2) and the associated kinetics by direct time-resolved measurements using pulse radiolysis. The reactions of interest have been investigated for a variety of aliphatic alcohols and ethers and two different thiols, penicillamine and glutathione. In order to disturb equilibrium (2) deliberately, various electron scavengers have been employed, namely, halogenated aliphatic compounds and $Fe(CN)_6^{3-}$ (as a model compound for Fe^{III} -containing biomolecules). The halogenated substrates are of particular interest, since their reduction [in analogy to eqn. (3)] would lead to radicals (particularly peroxy radicals formed in an oxygen-containing environment) which are known to be the potential starting point for lipid peroxidation or the degradation of natural cellular protectors such as Vit E.^{18,19}

Finally, from the absolute rate constants determined for equilibrium (2), and on the basis of the known reduction potentials of RS^{\cdot} radicals,^{20,21} it became possible to calculate the standard reduction potential for some α -hydroxyl radicals for which some conflicting data have been reported.²²

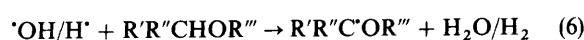
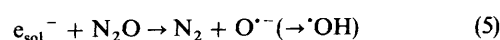
Experimental

All chemicals were of the purest grade and were used as received. The water was Millipore filtered. Solutions were deaerated by bubbling with N_2 for ca. 1 h per l of sample and subsequently saturated with N_2O . The pH was generally adjusted to ca. 5 by use of $HClO_4$ (mainly to prevent deprotonation of the thiols). Experiments were all conducted at room temperature.

The studies were performed by pulse radiolysis.²³ Radicals were generated by applying short pulses of high-energy electrons, from a 1.55 MeV Van de Graaff accelerator, to the solution. Dosimetry was based on the oxidation of thiocyanate by $\cdot OH$ radicals in N_2O -saturated aqueous solution to yield $(SCN)_2^{\cdot-}$ and by using $\epsilon(SCN)_2^{\cdot-} = 7200 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 500 nm. The radiation chemical yield of this marker process amounts to $G \approx 6$ in aqueous solutions (G denotes the yield of species, generated or destroyed, per 100 eV absorbed energy; $G = 1$ corresponds to ca. $0.1 \mu\text{mol J}^{-1}$). The absorbed doses in the investigated mixed-solvent systems were adjusted for the respective electron densities by applying relative density approximations. Generally, pulses of ca. 0.3 μs duration were applied with doses of the order of 2–8 Gy per pulse ($1 \text{ Gy} = 1 \text{ J kg}^{-1}$).

The primary radicals produced by radiolysis of water–alcohol or water–ether mixtures are solvated electrons, H^{\cdot} , $\cdot OH$, and different organic radicals (R^{\cdot}). Under the experimental conditions used all the primary reactive species, $e_{\text{sol}}^{\cdot-}$, H^{\cdot} and $\cdot OH$, were eventually converted into alcohol- or ether-derived radicals, mostly α -hydroxyl or α -alkoxyl rad-

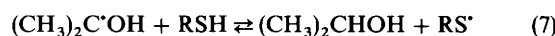
icals [as outlined in eqn. (5) and (6)]. These processes were usually completed within the duration of the pulse ($\leq 1 \mu\text{s}$).



Results and Discussion

Direct Determination of Rate Constants for Equilibrium (2)

The results published previously⁷ on the absolute rate constants of the forward and back reactions of equilibrium (2) for a singular system, namely, (PenSH)–propan-2-ol were based on indirect competitive methods. Direct measurements, e.g. by following the formation kinetics of the thiyl radical, however, also appear feasible and would certainly lend further convincing support for the reversible H-atom transfer mechanism. The generation of thiyl radicals is achieved by the forward reaction of equilibrium (7)

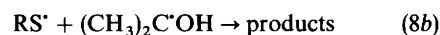


At higher thiol concentrations (e.g. $10^{-2} \text{ mol dm}^{-3}$) the forward reaction goes to completion within the pulse duration ($\leq 1 \mu\text{s}$). Optical absorption measurements conducted at 330 nm (λ_{max} for PenS $^{\cdot}$, $\epsilon = 1200 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)²⁴ immediately after the pulse gave $G(\text{PenS}^{\cdot}) \approx 6.0$ for aqueous solutions, i.e. a yield which corresponds to the yield of primary radicals ($e_{\text{sol}}^{\cdot-}$, H^{\cdot} , and $\cdot OH$) available in such systems.

Kinetically, the RS^{\cdot} radical formation is described by expression (I), formulated for the general case of alcohols, ethers and thiols, and their respective radicals:

$$d[RS^{\cdot}]/dt = k_r[R'R''C'OR'''] [RSH] - (k_t[RS^{\cdot}][R'R''CHOR'''] + 2k_g[\text{radicals}]^2) \quad (I)$$

The rate constant k_r , would, accordingly, be represented by the slope of a $d[RS^{\cdot}]/dt$ vs. $[RSH]$ graph while the RS^{\cdot} -induced H-atom abstraction, k_r , would show up in the intercept. The latter includes also the rate constant $2k_g$ for the possible termination reactions (8) of all radicals present in the system.



Actual measurements, as they have now been carried out, were restricted to those thiyl radicals which show a sufficiently intensive optical absorption for an unambiguous direct detection. This is the case for PenS $^{\cdot}$ and GS $^{\cdot}$ (the thiyl radicals from penicillamine and glutathione) which exhibit molar absorption coefficients of $\epsilon = 1200$ and $800 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$, respectively.²⁴

The rates of formation of RS^{\cdot} radicals, generated upon pulse irradiation of N_2O -saturated alcohol or ether–water mixtures (1 : 1, v/v) of different thiol concentrations (pH 5) via reactions (5)–(7) were monitored at 330 nm for propan-2-ol, propan-1-ol, ethanol, methanol, 2-methylpropan-2-ol, ethylene glycol, tetrahydrofuran, and 1,4-dioxane as non-aqueous component. k'_{obs} values have been calculated from the observed half-life of the thiyl radical formation signals and plotted vs. thiol concentration.

As expected, straight lines were obtained for all systems investigated. The absolute rate constants for the so-called repair reactions, i.e. the H-atom abstraction from GSH and PenSH by $R'R''C'OR'''$ radicals, k_r , were calculated from the respective slopes. These values are listed in Table 1, column (a).

Table 1 Absolute rate constants for forward and reverse reactions of equilibrium (2)

alcohol/ether	RSH	$k_f/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$		$k_r/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$				K_2^a
		(a)	(b)	(a)	(b)	(c)	(d)	
propan-2-ol	PenSH	1.2×10^8 ^b		1.0×10^4		1.4×10^4	1.1×10^4	1.0×10^4
	GSH	1.6×10^8		n.d.		n.d.	n.d.	n.d.
propan-1-ol	PenSH	8.0×10^7		8.0×10^3		7.0×10^3	5.0×10^3	1.2×10^4
	GSH	9.1×10^7		2.5×10^3		2.4×10^3	2.2×10^3	3.8×10^4
ethanol	PenSH	1.3×10^8	1.1×10^8	1.4×10^3	3.3×10^3	n.d.	n.d.	5.1×10^4
	GSH	3.6×10^7		n.d.		1.8×10^3	0.7×10^3	2.9×10^4
methanol	PenSH	6.5×10^7	5.0×10^7	1.5×10^3	3.9×10^3	n.d.	n.d.	2.1×10^4
	GSH	3.0×10^7	3.0×10^7	$\leq 10^3$		n.d.	n.d.	$\geq 3 \times 10^4$
2-methylpropan-2-ol	PenSH	3.9×10^7		1.3×10^3		1.5×10^3	0.8×10^3	3.2×10^4
	GSH	1.0×10^8		n.d.		1.2×10^4	0.3×10^4	1.3×10^4
ethylene glycol	PenSH	0.9×10^8	0.8×10^8	9.0×10^3	7.5×10^3	n.d.	n.d.	1.3×10^4
	GSH	6.7×10^7		n.d.		$\leq 3 \times 10^3$	n.d.	$\leq 2.2 \times 10^4$
tetrahydrofuran	PenSH	7.8×10^7	7.5×10^7	3.0×10^3	2.5×10^3	n.d.	n.d.	2.8×10^4
	GSH							

(a) Determined *via* measurement of RS^\bullet radical formation [eqn. (I)], k_{obs} calculated from the first half-life determination and (b) *via* computer simulation (see text). (c) Determined from slope and (d) from intercept of plots according to eqn. (II).

^a Calculated from mean values for k_f and k_r ($K_2 = k_f/k_r$). ^b From ref. 7. n.d.: not determined.

Error limits: $\pm 10\%$ for k_f ; $\pm 50\%$ for k_r and K_2 .

They are all of the order of 10^7 – $10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ as is characteristic for the reaction of C-centred radicals with thiols.^{25,26}

Calculation of the rate constants for the reverse reaction, *i.e.* the H-atom abstraction from alcohol or ether molecules by RS^\bullet , k_r , is possible from the intercepts. It must be recognized, however, that the intercepts include also the radicals recombination processes [eqn. (8)]. Numerical values for k_r were thus obtained by correcting for this on the basis of $2k_8 = 1.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ as measured for the reaction (8a), *i.e.* thiyl radical recombination.⁷ For the termination reactions (8b) and (8c) the same values were assumed. (A constant dose of *ca.* 8 Gy has been applied to ensure comparable conditions for all individual experiments). In the case of 2-methylpropan-2-ol this correction factor amounted, in fact, to exactly the observed intercept indicating that there is no direct reaction of thiyl radicals with this alcohol ($k < 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). As mentioned already above, 2-methylpropan-2-ol has no α -hydroxy substituent and consequently no activated C–H bond.

For all the other alcohols and ethers net intercepts were observed after correction for $2k_8$. Dividing these figures by the corresponding alcohol or ether concentrations, absolute second-order rate constants, k_r , were obtained. The values are listed in Table 1, column (a). (The alcohol concentrations for the 1 : 1 v/v solutions were evaluated from *Handbook of Chemistry* tables: methanol 12.8 mol dm^{-3} ; ethanol 8.9 mol dm^{-3} ; propan-1-ol 6.7 mol dm^{-3} ; propan-2-ol 6.7 mol dm^{-3} ; 2-methylpropan-2-ol 5.3 mol dm^{-3} ; ethylene glycol 9.0 mol dm^{-3} ; tetrahydrofuran 6.2 mol dm^{-3} ; 1,4-dioxane 5.9 mol dm^{-3} ; all ± 0.3 to allow for uncertainties due to non-linear density changes).

It is recognized that the above kinetic treatment is not absolutely correct as it includes certain approximations. Thus an exact evaluation of k_{obs} from the RS^\bullet formation kinetics would have to consider also all the time-dependent changes in overall radical concentrations caused by the radical-radical reactions (8). Justification for our simplified approach is, however, achieved by a computer simulation. In this case, the pseudo-first-order rate constants for the GS^\bullet radical formation, k_{obs} , were obtained by a best-fit procedure for the experimental signals taking into account the decay kinetics of radicals *via* reactions (8), *i.e.* $2k_8 = 1.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ for (8a), (8b) and (8c), and starting concentrations of $[\text{GS}^\bullet] = 0$ and $[\text{R}^\bullet\text{R}^\bullet\text{C}^\bullet\text{OR}^\bullet] = [\text{all primary radicals}]$, the latter cal-

culated from dosimetry measurements. The respective k'_{obs} , obtained by this procedure, were plotted again as a function of $[\text{GSH}]$ and shown on Fig. 1(a) for methanol, ethanol, 1,4-dioxane and 2-methylpropan-2-ol as co-solvent.

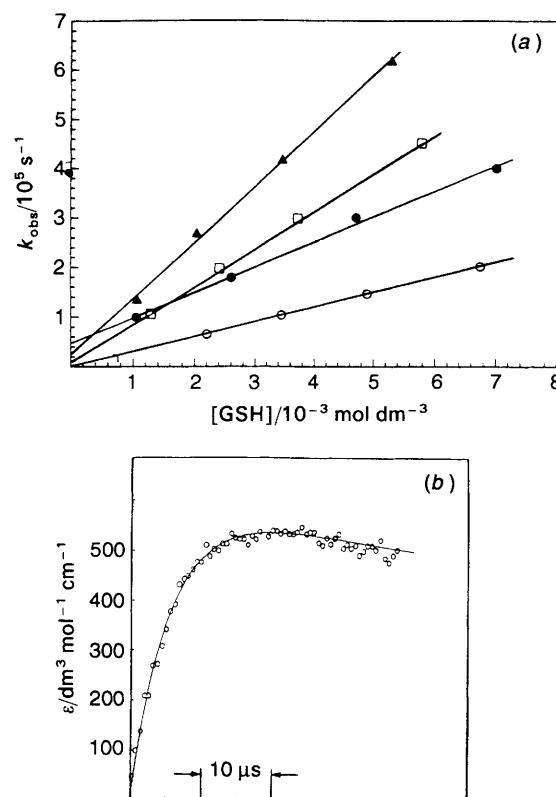


Fig. 1 (a) Pseudo-first-order rate constant (k_{obs}) for GS^\bullet formation, measured at 330 nm, *vs.* $[\text{GSH}]$ concentration. N_2O -saturated, pH 5, 1 : 1 (v/v) water-alcohol or water-ether mixtures; (●) methanol, (▲) ethanol, (□) 1,4-dioxane, (○) 2-methylpropan-2-ol. (Error limits of individual data points: $\pm 10\%$). (b) Formation kinetic of GS^\bullet radical taken at 330 nm in methanol-water system (conditions as above) containing $2.6 \times 10^{-3} \text{ mol dm}^{-3}$ GSH ; full-line calculated by using eqn. (I) and $k_f = 5.0 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k_r = 3.9 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k_{(8a)} = k_{(8b)} = k_{(8c)} = 1.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, initial yield of $\cdot\text{CH}_2\text{OH} = 3.8 \times 10^{-6} \text{ mol dm}^{-3}$ and $\epsilon_{\text{GS}^\bullet} = 850 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. (A significant deviation from the fit is observed already for a 20% variation in the rate constant for the radical-radical decay).

In the case of 2-methylpropan-2-ol the straight line obtained now passes through the origin, as expected. For all other alcohols and ethers net intercepts were observed reflecting the back reaction k_r . The absolute rate constants of the forward and back reactions, k_f and k_r , were calculated from the slopes and intercepts of the corresponding straight lines, respectively, and listed under columns (b) in Table 1.

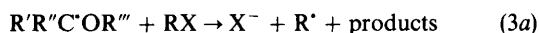
The k_{obs} values derived from the computer simulation treatment differed at most by a factor of two from those evaluated from the simple half-life determination with deviations in both directions. As can be appreciated from Fig. 1(a), a relatively high error limit must be allowed for k_r , since small changes in the slope of the straight lines will exert a significant influence on the intercept. We consider an error limit of at least $\pm 50\%$ which, in turn, must allow almost a factor of two in accuracy for k_r and K_2 without, however, affecting their orders of magnitude.

The general validity of the reaction mechanism represented by reactions (2) and (8) and the absolute rate constants obtained in this work was demonstrated by yet another computer simulation which included also the back reaction of eqn. (2). The experimental signals were fitted by using differential eqn. (I) and k_f , k_r as listed in Table 1 columns (b), $2k_8 = 1.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $G_{\text{primary radicals}} = 6$, and the molar absorption coefficient for GS^{\bullet} radical, $\epsilon_{330} = 850 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. The kinetic fits were very good throughout the entire set of GSH concentrations used. This is exemplified in Fig. 1(b) for the methanol-water system containing $2.6 \times 10^{-3} \text{ mol dm}^{-3}$ GSH.

Table 1 includes values for k_r obtained *via* an indirect method using electron acceptors (see next section). It includes also the equilibrium constants $K_2 = k_f/k_r$ ($=K_7$ for the specific case of propan-2-ol-containing systems), derived from all available kinetic data. The equilibrium constants amount to *ca.* 10^4 for all investigated systems. This means that the reversible hydrogen-abstraction equilibrium is located far to the right-hand (RS^{\bullet}) side under equimolar conditions.

Determination of Rate Constants for H-atom Abstractions by Thiol Radicals (k_r) *via* an Indirect Method

In order to confirm the results for the absolute rate constants obtained in the previous section, additional experiments were performed in which k_r values were determined *via* an indirect method. The latter employs electron acceptors, *e.g.* halogenated compounds RX, in order to disturb equilibrium (2) deliberately *via* the irreversible, general electron-transfer reaction



Full details have been given in earlier publications.⁷⁻⁹ In short: depending on the $k_f[\text{RSH}]/k_{3a}[\text{RX}]$ ratio the $\text{R}'\text{R}''\text{C}'\text{O}\text{R}'''$ radicals will react competitively with both the thiol and the electron scavenger. Any RS^{\bullet} radical generated *via* the forward reaction of equilibrium (2) will, however, subsequently equilibrate again *via* the back reaction of this equilibrium and engage in another cycle of the competitive scheme, and so on. Eventually, all RS^{\bullet} radicals will thus suffer irreversible removal from the equilibrium.

In an experimental example PenS^{\bullet} radicals generated upon pulse irradiation of an N_2O -saturated propan-2-ol-water mixture (1 : 1, v/v) pH 5, containing $10^{-2} \text{ mol dm}^{-3}$ PenSH had been shown to decay *via* pure second-order kinetics according to reaction (8).^{8,9} Addition of various amounts of CCl_4 , an efficient electron scavenger, lead to a change in decay kinetics to pseudo-first order with respect to the CCl_4 concentration. However, in the absence of the alcohol the kinetics remained at second order even in the presence of

CCl_4 . This clearly demonstrated that the thiol radical did not directly react with CCl_4 but that the CCl_4 -dependent decay of the PenS^{\bullet} had to be rationalized in terms of a PenS^{\bullet} -mediated generation of the reducing $(\text{CH}_3)_2\text{C}'\text{OH}$ radical, followed by the irreversible removal of the latter from equilibrium (2) *via* reaction (3a). In the present investigation these findings have now been generalized by employing a series of halogenated aliphatics, RX, as electron scavengers. Some of them are, in fact, used as anaesthetics.

Equilibrium (2) and its disturbance by reaction (3a) (or reaction (3), in general) are mathematically described through expression (II), relating in particular the variables k_{obs} and $[\text{RX}]$:

$$[\text{R}'\text{R}''\text{C}'\text{O}\text{R}'''] / k_{\text{obs}} = 1/k_f + [k_r/(k_f k_{3a})][\text{RSH}]/[\text{RX}] \quad (\text{II})$$

Since RS^{\bullet} and alcohol radicals are quantitatively removed in such systems by equilibration [eqn. (2)] and the rather fast process (3a), it is not necessary to include the bimolecular termination reactions [eqn. (8)].

A representative plot of $[\text{CH}_3\text{CH}_2\text{OH}]/k_{\text{obs}}$ vs. $1/[\text{CCl}_4]$ for a CCl_4 -containing solvent mixture of ethanol and water, containing also PenSH , is displayed in Fig. 2. It shows a reasonably good straight line which intercepts the y axis at *ca.* $5 \times 10^4 \text{ mol dm}^{-3} \text{ s}$.

With the determination of k_{3a} (see next section) and k_f (Table 1) it was then possible to evaluate the absolute rate constants, k_r , for the PenS^{\bullet} -induced H-atom abstraction from various alcohols and ethers, not only from the intercepts but also, as confirmation, from the slopes of the respective $[\text{R}'\text{R}''\text{C}'\text{O}\text{R}'''] / k_{\text{obs}}$ vs. $[\text{RSH}]/[\text{CCl}_4]$ plots. The actual values derived from the respective intercepts and slopes are listed in Table 1, columns (c) and (d), respectively (in one case they are based on data obtained with $\text{CCl}_3\text{COOCH}_3$ instead of CCl_4). As can be seen, they agree very well with those rate constants determined *via* the direct measurements.

The above analysis also involves one simplifying assumption, namely, it does not consider a possible $\text{CCl}_3 + \text{RSH} \rightarrow \text{CHCl}_3 + \text{RS}^{\bullet}$ reaction, the rate constant of which is not known. If it occurred fast enough to generate additional RS^{\bullet} radicals this would result in a slowing of the RS^{\bullet} decay process and consequently in too-low rate constants. However, our previous study⁷ did not reveal any difference between a CCl_4 -propan-2-ol-RSH and a *p*-nitroacetophenone(PNAP)-propan-2-ol-RSH system. Since there is no

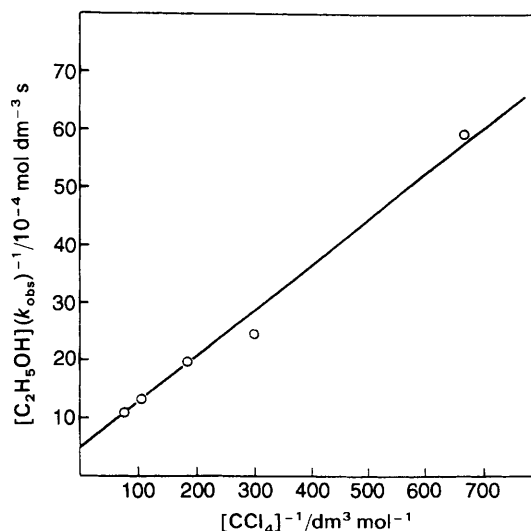
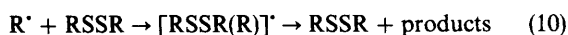
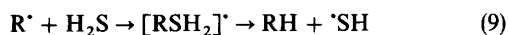


Fig. 2 $[\text{C}_2\text{H}_5\text{OH}]/k_{\text{obs}}$ vs. $1/[\text{CCl}_4]$; k_{obs} denotes first-order rate constant for PenS^{\bullet} decay measured at 330 nm. N_2O -saturated, pH 5, 1 : 1 (v/v) ethanol-water mixture, $1 \times 10^{-2} \text{ mol dm}^{-3}$ PenSH

indication of a correspondingly disturbing reaction $\text{PNAP}^{\cdot-} + \text{RSH}$, we conclude that the above $\cdot\text{CCl}_3 + \text{RSH}$ reaction is not of deciding significance.

The good agreement between the rate constants obtained via the different methods allows, incidentally, yet another conclusion. Namely, that the observed results do not indicate formation of any stable intermediate complex between α -hydroxyl- or α -alkoxyl-alkyl radicals and thiols, as has been suggested for the reducing C-centred radicals with H_2S^{27} or disulfides.²⁸



The existence of such a complex, with any considerable stability, in our systems would have led to higher apparent k_r values from the direct method in comparison with the indirect determination in the presence of electron acceptors.⁷

Determination of k_{3a}

In order to calculate k_r from eqn. (II) the rate constants k_{3a} for the reduction of RX by the various organic radicals had to be determined separately for each particular solvent system used. This was achieved by means of a competition method with PNAP as reference compound, as described previously.⁷

Since the reduction of PNAP constitutes an inner-sphere electron transfer,^{25,29} it was necessary to separate the two processes involved, namely, the initial adduct formation [eqn. (11)] and the subsequent, base-catalysed decay of the adduct to $\text{PNAP}^{\cdot-}$ [eqn. (12)]. (In the case of alcohols $\text{R}'' = \text{H}$.)



Mechanistically, addition of $\text{R}'\text{R}''\text{C}'\text{O}\text{R}'''$ takes place at the nitro-group of PNAP, and the characteristic features of the resulting adduct radical resemble several transient adducts of $\text{R}'\text{R}''\text{C}'\text{O}\text{R}'''$ to tetranitromethane in water and other solvents.³⁰⁻³²

Reasonably good time-resolved separations of both processes could be achieved for the weaker of the reducing organic radicals, particularly for $\cdot\text{CH}_2\text{OH}$ radicals. In this case the adduct is kinetically stable over the entire timescale of the investigation, *i.e.* no decay into $\text{PNAP}^{\cdot-}$ could be detected (at pH 5). For the other extreme, namely, the $(\text{CH}_3)_2\text{C}'\text{OH}$ -induced process, the adduct decayed so fast that reaction (11) became the rate-determining step for the $\text{PNAP}^{\cdot-}$ formation.

The adduct radicals, $(\text{PNAP}-\text{R}'\text{R}''\text{C}'\text{O}\text{R}''')^{\cdot}$, are character-

ized by optical absorption bands with λ_{max} around 330 nm.²⁹ The evaluation of k_{11} was, therefore, generally based on the analysis of the formation kinetics at this wavelength, *i.e.* from the slope of $k_{\text{obs}} = \ln 2/t_{1/2}$ vs. $[\text{PNAP}]$ plots. The data are summarized in the fourth column of Table 2.

In order to evaluate k_{3a} standard competition kinetics, expressed in terms of eqn. (III), were applied.

$$(G\epsilon)_0/(G\epsilon) = 1 + k_{3a}/k_{11} \times [\text{RX}]/[\text{PNAP}] \quad (III)$$

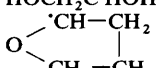
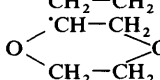
$(G\epsilon)_0$ and $(G\epsilon)$ denote the respective products of radiation chemical yield and molar absorption coefficient of the adduct radicals in the absence and presence of scavenger RX. The rate constants k_{3a} , derived from the slopes of $(G\epsilon)_0/(G\epsilon)$ vs. $[\text{RX}]/[\text{PNAP}]$ plots, are collected in the last column of Table 2. Although a direct comparison is not feasible because the values refer to different solvents, the observed decrease by a factor of *ca.* 100, on going from the tetrahydrofuran- to the methanol-derived radical reflects, at least qualitatively, a corresponding trend for the reduction potentials of the respective radicals.²²

In addition to these kinetic data the results presented above allow one to estimate molar absorption coefficients for some of the PNAP-radical adducts. They can be derived from the $(G\epsilon)_0$ values recorded at 330 nm and are listed in the third column of Table 2. In the case of N_2O -saturated methanol, ethylene glycol and 1,4-dioxane solutions, only one type of organic radical is available for reaction with PNAP, namely, the α -hydroxyl/alkoxyl type species. In the case of methanol, this takes into account that the small fraction of $\text{CH}_3\text{O}^{\cdot}$ radicals (7%), formed in the reaction of $\cdot\text{OH}$ with this alcohol besides $\cdot\text{CH}_2\text{OH}$,³³ is rapidly converted into α -hydroxyl radicals according to eqn. (13) with $t_{1/2} \approx 0.2 \mu\text{s}$.³⁴



In the case of ethylene glycol it considers that extrusion of a water molecule from the α -hydroxyl radical, $\text{CH}_2\text{OHC}'\text{HOH}$, a process occurring efficiently at $3 > \text{pH} > 7$,³⁵ can be excluded at the experimental pH 5. The radiation chemical yield of the PNAP-radical adducts of interest can thus be taken as $G \approx 6$. For ethanol, propan-1-ol and THF, α -hydroxyl/ α -alkoxyl radicals are formed with efficiencies of only 85% ($G = 5.1$),³³ 53% ($G = 3.2$)³³ and 92% ($G = 5.5$),³¹ respectively. Based on these G values the molar absorption coefficients are calculated to be 2.08×10^4 , 2.09×10^4 , 2.04×10^4 , 1.79×10^4 , 1.53×10^4 and $1.38 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ for the PNAP adducts with the α -hydroxyl/ α -alkoxyl radicals from methanol, ethanol, ethylene glycol, dioxane, propan-1-ol and THF, respectively. The first three are practically identical, while lower ϵ values are indicated for the cyclic ether- and propan-1-ol-derived species. The latter may

Table 2 Yield (in terms of $G\epsilon$) and formation kinetics (k_{11}) of PNAP-radical adducts, and rate constant for reduction of CCl_4 by α -hydroxyl and α -alkoxyl radicals (k_{3a})^a

alcohol/ether	radical	$(G\epsilon)_{330}$	$k_{11}/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	$k_{3a}^b/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$
propan-1-ol	$\text{CH}_3\text{CH}_2\text{C}'\text{HOH}$	57 300	6.7×10^8	3.3×10^7
ethanol	$\text{CH}_3\text{C}'\text{HOH}$	106 700	6.7×10^8	2.3×10^7
methanol	$\cdot\text{CH}_2\text{OH}$	125 000	4.9×10^7	7.9×10^5
ethylene glycol	$\text{HOCH}_2\text{C}'\text{HOH}$	122 600	9.4×10^7	2.0×10^6 ^c
tetrahydrofuran		75 700	6.2×10^8	9.1×10^7
1,4-dioxane		92 000	2.4×10^7	1.6×10^6

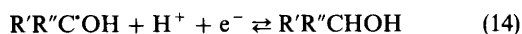
^a N_2O -saturated water-alcohol or water-ether (1 : 1 v/v) mixtures, pH 5, containing $\leq 10^{-3} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ PNAP. ^b Systems as above with CCl_4 added. ^c As above with $\text{CCl}_3\text{COOCH}_3$ instead of CCl_4 , *i.e.* rate constant refers to reduction of trichloroacetic acid methyl ester. Error limits for all three data sets: $\pm 10\%$.

not necessarily reflect an inherent electronic property of the adduct radicals but just a, possibly structure related (steric hindrance), lower kinetic stability. In other words, some of the radical adducts may have already decayed into the final product at the time of measurement. The actual yields contributing to the observed $(G\epsilon)_0$ values would thus be lower than the total yields of the initial free hydroxyl/alkoxyl radicals and this, in turn, would require a corresponding correction of the low molar absorption coefficients to higher values.

Reduction Potentials

Based on the equilibrium constants for the reversible hydrogen transfer [eqn. (2)] (Table 1) it is possible to evaluate the one-electron reduction potentials for some of the alcohol-derived α -hydroxyl radicals, for which some conflicting data have been reported.^{22,36–39}

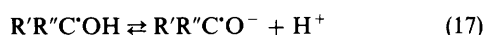
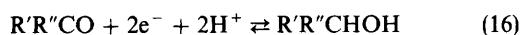
Equilibrium (2), formulated for alcohols, can be viewed as a combination of reactions (14) and (15):



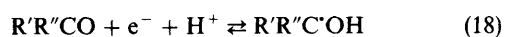
With $E_{15}^\circ = 1.34$ V reported for the reduction potential of the PenS* radical,^{20,21} E_{14}° can be obtained from expression (IV) ($T = 298$ K).

$$E_{14}^\circ = E_{15}^\circ + (RT/nF)\ln K_2 \quad (IV)$$

Data on the two-electron redox potential of alcohols [eqn. (16)][†] as well as on the pK values of the corresponding α -hydroxyl radicals [eqn. (17)]⁴⁰ are available in the literature for propan-2-ol, ethanol and methanol.



They were employed to calculate E_{18}° and E_{19}° , the one-electron reduction potentials of α -hydroxyl radicals in their neutral and anionic form, through expressions (V) and (VI).



$$E_{18}^\circ = 2E_{16}^\circ - E_{14}^\circ \quad (V)$$

$$E_{19}^\circ = E_{18}^\circ - 0.0592pK_{17} \quad (VI)$$

All the data related to propan-2-ol, ethanol and methanol are compiled in Table 3. It is noted that our present E_{18}° and E_{19}° values add credit to the majority of the data published previously (also listed in Table 3) and obtained *via* different methods and reference compounds.^{22,36–39} The good agree-

ment is, in turn, taken as general support for the overall reaction mechanism and kinetic determinations in our work on the reversible hydrogen transfer between thiol and carbon-centred radicals. Note, however, that our measurements refer to solutions of high alcohol content, which may well contribute to the small differences between our and the probably most reliable of the other (ref. 36 and 38) values.

Disturbance of Equilibrium (2) by Halocarbons as Electron Scavengers

In a thiol-containing environment the effective turnover of thiol radicals into C-centred radicals within equilibrium (2) is strongly dependent on the presence of an electron acceptor being able to remove these C-centred radicals irreversibly from the equilibrium. PNAP and CCl₄ have already been shown to be operative in this respect (see above, and ref. 7). In the present study several more haloalkanes were employed to test the mechanism and to extract independent values for k_r , the rate constant for the abstraction of H atoms from propan-2-ol by thiol radicals. In addition, absolute rate constants were measured for the reduction of these halogenated substrates by (CH₃)₂C'OH radicals.

The general system studied was propan-2-ol–water (1 : 1, v/v) containing 1×10^{-2} mol dm⁻³ PenSH at pH 5. The decay kinetics of the PenS* radicals, which in these systems are formed immediately after the *ca.* 0.5 μ s pulse and monitored at 330 nm, was accelerated upon addition of the electron-accepting halocarbon, namely, CCl₃CO₂CH₃, CCl₃CCl₃, CF₃CCl₃, CCl₃CHCl₂ and the anaesthetic halothane CF₃CHBrCl.

The experimental results, plotted according to expression (II), for the three latter substrates are shown in Fig. 3. The linearity obtained confirms the conclusions made and discussed already in connection with the CCl₄ system (Fig. 2). The graphs provide, in particular, numerical information on k_r (intercept: $1/k_r$) and k_f (slope: $k_f/k_r k_{3a}$).

The absolute rate constants, k_{3a} , for the reduction of the electron acceptors by (CH₃)₂C'OH radicals were determined separately in the same solvent mixture but in the absence of thiol. Again, the competition method with PNAP as reference compound was employed (see above). The respective k_{3a} values obtained *via* application of expression (III) are listed in Table 4. They identify CCl₄ as the most efficient electron acceptor while CCl₃CHCl₂ is the least effective in this series. No reaction at all was indicated with CHCl₃, CH₂Cl₂, and the anaesthetics isoflurane (CHF₂–O–CHCl–CF₃), enflurane (CHF₂–O–CF₂–CHClF) and methoxyflurane (CH₃–O–CF₂–CHCl₂), even at $\geq 10^{-2}$ mol dm⁻³ of these substrates. All these compounds possess too negative reduction potentials (< -1.4 V) to be reduced by (CH₃)₂C'OH radicals.^{22,41,42}

Introducing these k_{3a} values into expression (II) and taking $k_f = 1.2 \times 10^8$ dm³ mol⁻¹ s⁻¹ gave the k_r values for the PenS*-induced H-atom abstraction from propan-2-ol which are listed in Table 4. As expected, they are practically the

Table 3 Redox potentials (referring to 1 : 1 v/v water–alcohol mixtures)

alcohol	K_2	pK ₁₇ ^a	E_{16}°/V^b	E_{14}°/V	E_{18}°/V	E_{19}°/V
propan-2-ol	1.0×10^4	12.03	0.25	1.57	-1.32 (-1.39; ^c -1.80; ^d -1.50 ^e)	-2.03 (-2.10; ^{c,e} -2.50 ^d)
ethanol	3.8×10^4	11.51	0.47	1.61	-1.14 (-1.25; ^c -1.10 ^{d,e})	-1.82 (-1.93; ^c -2.50; ^d -1.80 ^e)
methanol	2.9×10^4	10.71	0.55	1.60	-1.05 (-1.18; ^c -0.90; ^d -0.97 ^e)	-1.68 (-1.81; ^c -2.50; ^d -1.60 ^e)

^a Ref. 40; ^b see footnote; ^c Ref. 36; ^d Ref. 37; ^e Ref. 38; ^{c-e} see also Ref. 22. Error limit ± 0.10 V, based on high error limit of k_r and K_2 .

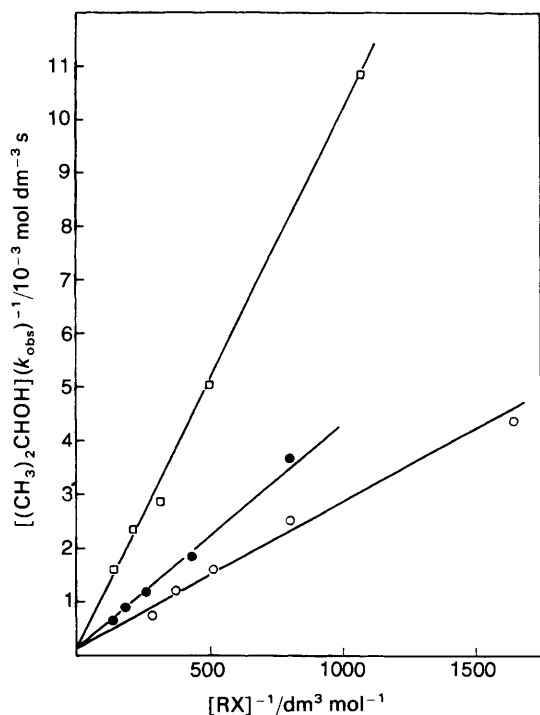


Fig. 3 $[(\text{CH}_3)_2\text{CHOH}]/k_{\text{obs}}$ vs. $1/[\text{RX}]$; k_{obs} denotes first-order rate constant for PenS' decay measured at 330 nm. N_2O -saturated, pH 5, 1:1 (v/v) propan-2-ol-water mixture, $1 \times 10^{-2} \text{ mol dm}^{-3}$ PenSH. RX: (\square) $\text{CCl}_3\text{CHCl}_2$; (\bullet) CF_3CCl_3 , (\circ) CF_3CHClBr (halothane).

same with a mean value of $(1.2 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, irrespective of the nature of the halocarbon.

$\text{Fe}(\text{CN})_6^{3-}$ as Electron Scavenger and RS^\cdot Formation from Disulfides

Finally, the following experiments demonstrate the limitations one may encounter in the reversible H-atom transfer systems. For example, a particularly potent electron acceptor, better than any of the above halocarbons, is $\text{Fe}(\text{CN})_6^{3-}$ ($E^\circ = +0.36 \text{ V}$) and it may, accordingly, be considered a suitable substrate to disturb equilibrium (2). However, it cannot be applied in the thiol-containing model system, since free mercapto groups react thermally with hexacyanoferrate (III).

We therefore switched the model system by introducing disulfides instead of thiols, where thiyl radicals can be generated *via* a reductive pathway:

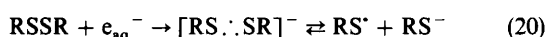


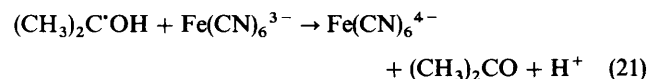
Table 4 Rate constants for the reduction of RX by $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ radicals (k_{3a}), and for the H-atom abstraction from propan-2-ol by PenS' radicals (k_r)

RX	$k_{3a}/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	$k_r/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$
CCl_4^a	1.2×10^8	1.4×10^4
$\text{CCl}_3\text{COOCH}_3$	1.2×10^8	1.3×10^4
CCl_3CCl_3	8.7×10^7	1.0×10^4
CF_3CCl_3	3.7×10^7	1.1×10^4
CF_3CHClBr (halothane)	5.0×10^7	1.3×10^4
$\text{CCl}_3\text{CHCl}_2$	1.2×10^7	1.1×10^4
CHCl_3	no reaction	
CH_2Cl_2	no reaction	
$\text{CHF}_2\text{OCHClCF}_3$ (isoflurane)	no reaction	
$\text{CHF}_2\text{OCF}_2\text{CHClF}$ (enflurane)	no reaction	
$\text{CH}_3\text{OCF}_2\text{CHCl}_2$ (methoxyflurane)	no reaction	

^a From ref. 7. Error limits for both data sets: $\pm 10\%$.

This process proceeds *via* a $2\sigma/1\sigma^*$ three-electron-bonded radical anion intermediate which, in the absence of any excess thiolate, typically suffers complete equilibration to the thiyl radical within less than a few μs (except for backbone-linked species such as reduced lipolate for which much longer lifetimes of the radical anion prevail).⁴³

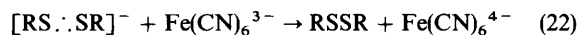
A suitable system to study this reductive pathway to thiyl radicals is an N_2 -saturated water propan-2-ol (1:1, v/v) solution, pH 4.5, containing also the disulfide of interest, *e.g.* $(2-5) \times 10^{-3} \text{ mol dm}^{-3}$ glutathione-, dimethyl-, or diethyl-disulfide. The reactive species available under these conditions at the end of a 1 μs pulse are mainly solvated electrons and $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ radicals (the latter result from reaction of $\cdot\text{OH}$ and H^\cdot with propan-2-ol and, to some extent, from direct ionization of propan-2-ol molecules). Among these only the electrons, but not the $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ radicals,^{44,45} are able to reduce the above disulfides. The formation of RS^\cdot radicals is indicated by their, generally weak, optical absorptions at around 330 nm. If we now add small concentrations of $\text{Fe}(\text{CN})_6^{3-}$, *e.g.* $(0.6-3.3) \times 10^{-4} \text{ mol dm}^{-3}$, the electrons still react with the disulfide. The $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ radicals, however, instead of suffering bimolecular inactivation (mainly disproportionation) will now react with the hexacyanoferrate(III) in an electron-transfer process.



Direct observation of this reaction is possible by monitoring the bleaching of the $\text{Fe}(\text{CN})_6^{3-}$ absorption at 410 nm, and at $3.3 \times 10^{-4} \text{ mol dm}^{-3}$ $\text{Fe}(\text{CN})_6^{3-}$ this occurs with $t_{1/2} \approx 1 \mu\text{s}$ ($k = 2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$).⁴⁵

In view of the possible formation of additional $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ radicals through reaction of RS^\cdot with propan-2-ol [equilibrium (2)] it would be expected that two distinct stages of the bleaching process could be observed at higher $\text{Fe}(\text{CN})_6^{3-}$ concentrations. The rate-determining step for the slower of these stages would be the H-atom abstraction from propan-2-ol by RS^\cdot which, on the basis of the measured k_r value, occurs with a half-life of *ca.* 8 μs . However, no such observation was made in all our disulfide systems. This does not invalidate the concept of the reversible H-atom transfer involving thiyl and alcohol radicals but, rather, it reflects an inherent property of the disulfide system. As formulated in eqn. (20) the reduction of disulfides proceeds *via* a radical anion intermediate which itself is still a strong reductant ($E^\circ \approx -1.6 \text{ V}^{21}$). For example, it readily transfers an electron to molecular oxygen with $k \approx 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ²¹ and will do the same and most probably even faster to the even better

electron acceptor hexacyanoferrate(III):



At higher $\text{Fe}(\text{CN})_6^{3-}$ concentrations [necessary to distinguish between the two above-mentioned $\text{Fe}(\text{CN})_6^{3-}$ bleaching stages] reaction (22) is fast enough to compete successfully against the dissociation of $[\text{RS}\cdot\text{SR}]^-$ into $\text{RS}\cdot$ and RS^- . Therefore, whenever it would be possible to identify an $\text{RS}\cdot$ -mediated formation of $(\text{CH}_3)_2\text{C}\cdot\text{OH}$ radicals in a disulfide system the $\text{RS}\cdot$ radicals are, unfortunately, not produced any more.

In essence, the disulfide-hexacyanoferrate(III) system is not suitable for the study of a thiyl radical-induced H-atom abstraction reactions.

Conclusion

The reversible H-atom transfer between thiols and activated C—H bonds, involving $\text{RS}\cdot$ and C-centred radicals, has been substantiated and quantified for a number of further examples. In case of ethers and alcohols the general $>\text{C}\cdot + \text{RSH} \rightleftharpoons \text{RS}\cdot + >\text{CH}\cdot$ equilibrium lies on the right-hand side (K of the order of 10^4). Nevertheless, as shown in this study it may effectively be disturbed and forced to the left by scavenging the reducing α -hydroxyl or α -alkoxyl radicals, formed as $>\text{C}\cdot$ in these systems, by suitable electron acceptors. Since thiyl radicals may well be generated enzymatically,⁵ the above equilibrium, in association with the irreversible electron transfer from C-centred radicals to the electron acceptor, thus provides a possible scenario for thiyl radical-induced cellular damage.

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