Oxidation of phenols employing polyoxometalates as biomimetic models of the activity of phenoloxidase enzymes

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A kinetic study of the oxidation of substituted phenols with either vanadium(V) polyoxotungstate, $[\alpha\text{-SiV}^{V}W_{11}O_{40}]^{5-}$ (*viz.* $SiW_{11}V$), or manganese(III) polyoxotungstate, $[\alpha\text{-SiMn}^{III}W_{11}(H_2O)O_{39}]^{5-}$ (*viz.* $SiW_{11}Mn$), has been carried out. Because the redox potentials of the polyoxometalate $SiW_{11}V$ and $SiW_{11}Mn$ compounds, *viz.* POMs, are 0.67 and 0.76 V/NHE, respectively, and those of the phenolic substrates are in the range of 0.4–0.9 V/NHE in water, the resulting oxidation reactions are exoergonic or slightly endoergonic. The reactivity of oxidation of substituted phenols by $SiW_{11}V$, at 50 °C in buffered (pH = 4) water solution, has been found to correlate with the σ^+ parameter of the substituents, yielding an Okamoto–Brown ρ value (*i.e.*, −3.1) consonant with the electronic requirements of a rate-determining electron-transfer route. The negligible value of the solvent kinetic isotope effect ($k_H/k_D = 1.06$) obtained for the oxidation of *p*-MeO-phenol with $SiW_{11}V$ in H_2O *vs.* D_2O solution was also in favour of a rate-determining one-electron abstraction from the substrate, followed by fast deprotonation of the intervening radical cation. Additional support to an outer-sphere oxidation with $SiW_{11}V$ was provided by a satisfactory correlation of the reactivity *vs.* redox potential of the phenols, and by a Marcus analysis of the experimental oxidation data. A sizeable value for the reorganisation energy (λ) was accordingly obtained. The slightly stronger oxidant $SiW_{11}Mn$ resulted to be more reactive than $SiW_{11}V$ by *ca.* two powers of magnitude. Our scrutiny of the reactive behaviour of the two POMs towards phenols may provide a model of interpretation for the phenoloxidase activity of the laccase enzymes, a class of multicopper oxidases endowed with redox potentials well comparable with those of the two POMs.

Introduction

Heteropolyacids, and particularly their conjugate polyoxometalate (POM) anions, represent an increasingly important class of environmentally friendly catalysts. POMs catalyse the oxidation of a number of organic substrates in the presence of either molecular oxygen or other oxygen donors,[1] under rather mild conditions. A full issue of *Chemical Reviews* in the late 1990s covered several facets of POMs chemistry.[1–4] Because the redox center in these anionic electron acceptors is deeply buried within the oxometalate Keggin cage (Fig. 1),[1,2] POMs are believed to provide unambiguous examples of outer-sphere oxidants.[3]

By changing their chemical composition, the redox properties of POMs can be varied within a significant range, to tailor catalytic proficiency finely. Whereas the synthetic value of POM-induced reactions is well appreciated,[4] kinetic and mechanistic features of the oxidation step are less investigated. Depending on the nature and redox properties of the substrate, electron-transfer,[3,5] O-transfer,[6] or radical pathways[5b] have been suggested to operate. Finally, POMs can be viewed as model systems of some metalloenzymes (*i.e.*, biomimesis) involved in oxidation processes of wide biochemical significance.

The aim of the present investigation is twofold. We describe our study with two *polyoxotungstates*, namely, $[\alpha\text{-SiV}^{V}W_{11}O_{40}]^{5-}$ (*viz.* $SiW_{11}V$) and $[\alpha\text{-SiMn}^{III}W_{11}(H_2O)O_{39}]^{5-}$ (*viz.* $SiW_{11}Mn$).[5b] The redox power of the V^V-containing $SiW_{11}V$ ($E^{1/2}$ = 0.67 V)[5c] and of the Mn^{III}-containing $SiW_{11}Mn$ ($E^{1/2}$ = 0.76 V)[5c] species (all redox data will be referred to the normal hydrogen electrode, NHE) is moderate, and thereby confined to the one-electron oxidation of substrates having low redox potential. The phenols (0.4–0.9 V, in water at pH = 4)[7] met this requirement and, in fact, the POMs have found use as catalysts in the oxidation of phenolic substrates relevant for lignocellulosic chemistry, or else for the removal of phenolic pollutants or dyes from industrial wastewaters.[5b,8] We investigate here the reactivity of the two POMs in the oxidation of a series of phenols, aiming at ascertaining and characterising a *bona-fide* electron-transfer route of oxidation. Moreover, because the redox potential of these two POMs compares well with that of a class of fungal enzymes, *i.e.*, the laccases, which is in the 0.6–0.8 V range,[9] we are addressing a biomimetic issue. Laccases are multicopper oxidases,[10] and catalyse the one-electron oxidation of appropriate

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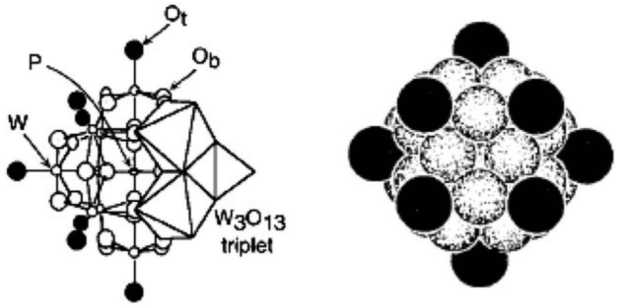

Fig. 1 Primary structure of the polyoxotungstate anion $PW_{12}O_{40}^{3-}$. Reprinted (in part) with permission from: N. Mizuno and M. Misono, *Chem. Rev.*, 1998, **98**, 199–217 (ref. 1). Copyright 1998, American Chemical Society.

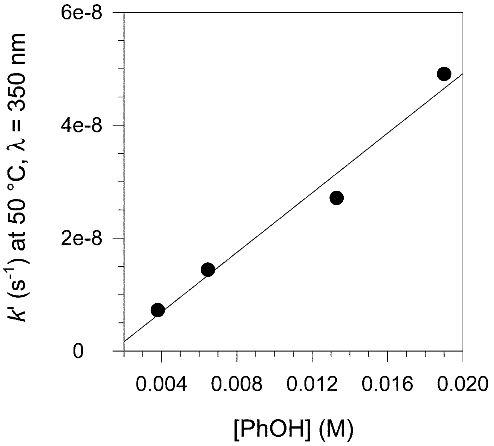

Fig. 2 Graphic determination of the second-order rate constant for the oxidation of PhOH with $SiW_{11}V$ at 50 °C.

substrates with the concomitant reduction of dioxygen to water. Phenols, or phenolic residues in lignin, are natural substrates of laccases owing to their low redox potential (phenoloxidase activity), and this matches the feature delineated above for the substrates that are accessible to POMs. Structure–reactivity studies aimed at ascertaining mechanistic requirements of the oxidation by laccases are a hot topic,[11] and POMs are certainly easier to investigate than the enzymes. We hope to provide sufficient information here that, besides serving well to understand mechanistic details of the oxidation by POMs, may also throw additional light on the monoelectronic reactivity of laccases, in view of the existing analogy of redox features.

Results

Kinetic investigation

Kinetic data. The two POMs were accessible to us from a previous investigation where their full characterisation is reported.[5b,c] They are able to oxidise phenols into quinones whenever a good match of redox potential between oxidant and substrate exists. In aqueous solution they behave as sufficiently strong electrolytes so as to allow neglect of their counterions (*i.e.*, K^+ or Na^+) under our operating conditions.[12] We have verified that the spectrophotometric features of $SiW_{11}V$ are particularly convenient to perform a kinetic study of the oxidation process. In fact the UV-Vis spectrum of the yellow $SiW_{11}V^V$ species (λ_{max} = 350 nm, ε = 2700 M^{-1} cm^{-1})[5c] changes appreciably into the purple $SiW_{11}V^{IV}$ species (λ_{max} = 500 nm, ε = 630 M^{-1} cm^{-1})[5c] upon mixing with a solution of phenol, according to the process outlined in eqn (1).

$$PhOH + SiW_{11}V^V \rightarrow PhO^\bullet + H^+ + SiW_{11}V^{IV} \qquad (1)$$

At 25 °C the reaction is not fast, and is more conveniently followed at 50 °C in the thermostated cell-holder of the spectrophotometer. Under pseudo first-order conditions with respect to POM (*i.e.*, excess of substrate), namely, $[SiW_{11}V]$ = 4 × 10^{-4} M, [PhOH] in the range 4 × 10^{-3} to 0.019 M, in aqueous solution at pH = 4 (acetate buffer, 0.1 M), the drop of absorbance of $SiW_{11}V^V$ at 350 nm is monitored. Due to the gradual appearance of spurious absorption bands, the acquired OD *vs.* time data points are less ambiguously handled by extrapolating the initial rates, in order to obtain pseudo-first-order k' data. The reaction turns out to be first order in both POM and PhOH. The rate constants k', determined at 50 °C from at least duplicated experiments at three-to-four different concentrations of PhOH, are converted into the second-order rate constant by determining the slope of a k' *vs.* $[PhOH]_o$ plot, as shown in Fig. 2. All the second-order rate constants for the oxidation of the substituted phenols reported in Table 1 have been obtained in the same way. Typical uncertainty of the kinetic determinations ranges from 3 to 8%.

Extension of the study to phenols substituted with stronger electron-withdrawing groups than *p*-Cl is prevented by the fact

Table 1 Second-order rate constants for the oxidation of substituted phenols by the two POMs, $SiW_{11}V$ and $SiW_{11}Mn$, in buffered (pH = 4) aqueous solution (*cf.* eqn (1))

ArOH	$E^{1/2}$/V *vs.* NHE[a]	$k(SiW_{11}V)/M^{-1}\ s^{-1}$ (50 °C)	$k(SiW_{11}V)/M^{-1}\ s^{-1}$ (25 °C)	$k(SiW_{11}Mn)/M^{-1}\ s^{-1}$ (25 °C)
p-Br-phenol	0.85	6.7 × 10^{-3}	—	—
Phenol	0.88	7.2 × 10^{-3}	5.3 × 10^{-4}	—
m-MeO-phenol	0.87	0.012	—	—
p-Cl-phenol	0.90	0.013	—	—
p-Ph-phenol	0.78	0.020	—	—
p-Me-phenol	0.79	0.052	1.3 × 10^{-2}	74
p-MeO-phenol	0.66	9.0	7	700
2-MeO-4-Me-phenol	0.62	7.0	2	590
2,6-diMeO-phenol	0.57	2.6	0.74	—
p-OH-phenol	0.48	54	32	9500

[a] From ref. 7.

that the redox potential rises above 1 V and, consequently, the ΔE between $SiW_{11}V$ ($E^\circ = 0.67$ V) and the substrate becomes significantly negative, slowing the endoergonic process so much that no decrease or increase in the absorption bands of the POM is clearly perceptible. On the other extreme, with a subset of electron-rich phenols the kinetic study could be performed at 25 °C (Table 1).

The $SiW_{11}Mn$ analogue, being a stronger oxidant (*i.e.*, $E^{1/2} = 0.76$ V)[5c] than $SiW_{11}V^V$, allows to follow the oxidation of the substituted phenols at 25 °C (Table 1) easily, by monitoring the disappearance of the $\lambda = 490$ nm band ($\varepsilon = 290$ M^{-1} cm^{-1})[5c] of the coordinated Mn^{III}. However, experimental problems (low ε value of POM, lack of stability of the absorption) precluded to investigate phenols substituted with electron-withdrawing groups. On the other extreme, *p*-OH-phenol (*viz.* hydroquinone) represents the fastest reacting phenol we could kinetically follow with our stopped-flow spectrophotometric device. It can be observed that, for equal substrate and temperature, the stronger oxidant $SiW_{11}Mn$ is more reactive than $SiW_{11}V$ by *ca.* two powers of magnitude.

Hammett-like correlation. The wider range of X-substituted phenols investigated at 50 °C with $SiW_{11}V$ allows attempting a correlation of the rate constants with the σ^+ parameter of the substituents, according to the Okamoto–Brown relationship (eqn (2)):

$$\log(k_X/k_H) = \rho\sigma^+ \qquad (2)$$

Within the experimental errors, a fairly linear plot is obtained ($r^2 = 0.90$) having $\rho = -3.1$ (Fig. 3).

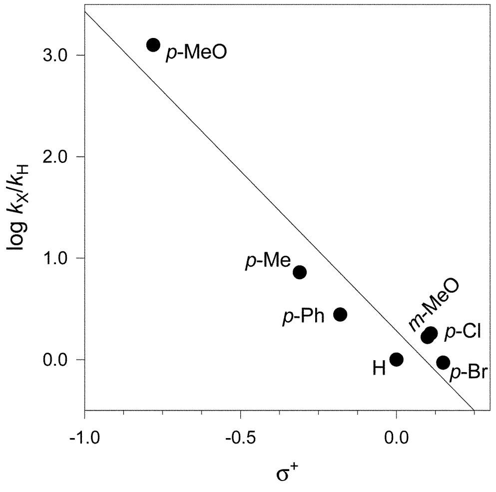

Fig. 3 Okamoto–Brown correlation of the kinetic data for the oxidation of substituted phenols by $SiW_{11}V$, in buffered water (pH = 4) at 50 °C.

The relevance of the substituent effects upon this oxidation process is noticeable, and the reactivity spans over three powers of magnitude: it is speeded up by electron-donor substituents, whereas electron-withdrawing ones retard it. The trend is in agreement with a rate-determining electron transfer from the substrate to the POM oxidant, giving rise to the aryl oxide radical ($ArO^\bullet$) through the fleeting intermediacy of the $ArOH^{\bullet+}$ species. The more limited number of substrates that could be investigated with $SiW_{11}Mn$ prevents us from attempting an analogous structure–reactivity correlation.

Kinetic isotope effect determination. The rate constant of oxidation of *p*-MeO-phenol by $SiW_{11}V$ has been determined at 25 °C in H_2O or in D_2O in separate experiments. Rapid exchange of D for H within the phenolic substrate in D_2O solution is ensured, and the effect of this exchange upon the oxidation of ArOD by the POM has been kinetically measured and compared with the non-deuterated case. The k_H/k_D ratio comes out as 1.06, and this negligible 'solvent kinetic isotope effect' is in line with a fast deprotonation (or dedeuteration) of $ArOH^{\bullet+}$ (or of $ArOD^{\bullet+}$) to yield $ArO^\bullet$, whereas the generation of $ArOH^{\bullet+}$ (or $ArOD^{\bullet+}$) by electron transfer to the POM is rate-determining.

Activation parameters. By using two of the most reactive phenols from Table 1, the second-order rate constants of oxidation by $SiW_{11}V$ could be measured at different temperatures (Table 2). By plotting the rate constants according to the Eyring equation ($\log k_H/T = \log k_B/h - \Delta H^{\neq}/RT + \Delta S^{\neq}/R$), we could calculate the activation parameters of the oxidation process. While the enthalpy of activation of *p*-MeO-phenol is expected and found to be lower than that of *p*-Me-phenol, keeping in mind the lower redox potential of the former substrate and its faster kinetic process, the entropy of activation comes out as slightly more negative.

Driving force of the oxidation step. The $\log k$ values (at 50 °C, from Table 1) for the oxidation by $SiW_{11}V$ correlate to the available half-wave redox potentials ($E^{1/2}$, in V *vs.* NHE)[7] of the substituted phenols, giving a fairly linear plot (Fig. 4; $r^2 = 0.92$). Hence, compelling evidence stems in favour of a rate-determining electron transfer step of oxidation, because the reactivity trend across the series is dominated by the difference in redox potential between the POM oxidant and the reducing phenolic substrate. Additional evidence that this ΔE is the driving force of the oxidation process of interest (eqn (1)) has been sought within the Marcus theory framework.[13]

Marcus correlation. The Marcus equation (eqn (3)) provides a quantitative relationship between the kinetic data of an electron transfer reaction ($\Delta G^{\neq}$) and the thermodynamic

Table 2 Rate constants for the oxidation of two phenols with $SiW_{11}V$ in buffered aqueous solution (pH = 4) at different temperatures

	k/M^{-1} s^{-1}					
ArOH ($E^{1/2}$)	50 °C	37 °C	25 °C	13 °C	$\Delta H^{\neq}$/kcal mol^{-1}	$\Delta S^{\neq}$/cal K^{-1} mol^{-1}
p-Me-phenol (0.79)	5.2×10^{-2}	2.9×10^{-2}	1.3×10^{-2}	—	10.4	−32.3
p-MeO-phenol (0.66)	11.8	10.8	7.1	3.3	5.7	−35.9

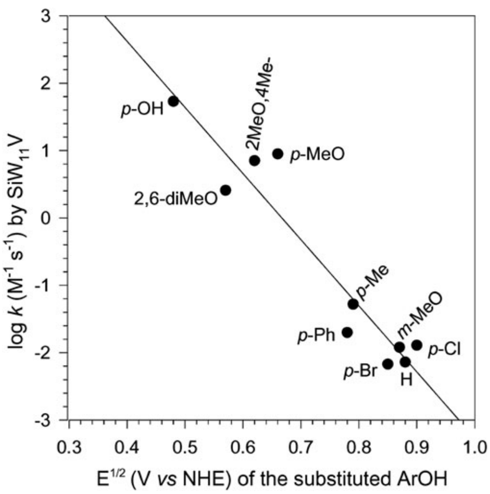

Fig. 4 Correlation of the rate constants of oxidation by $SiW_{11}V$ (at 50 °C) *vs.* the redox potential of the substituted phenols.

driving force ($\Delta G^{\circ\prime}$) for the transfer of electron between a donor substrate and an acceptor species.[13]

$$\Delta G^{\neq} = (\lambda/4)(1 + \Delta G^{\circ\prime}/\lambda)^2 \qquad (3)$$

In order to obtain the $\Delta G^{\circ\prime}$ data, several parameters need to be known (see eqn (4)) besides the variation of the electrochemical driving force (ΔE, in V) that is obtained as $\Delta E = E_{POM} - E_{ArOH}$ for the phenolic substrates of our series, and duly converted into ΔG° data ($\Delta G^{\circ} = -nF\Delta E$).

$$\Delta G^{\circ\prime} = \Delta G^{\circ} + (z_{ox} - z_{red} - 1)e^2 f/\varepsilon r_{12} \qquad (4)$$

In our case, the charge of $SiW_{11}V$ is $z_{ox} = -5$, whereas for the ArOH substrates $z_{red} = 0$. The dielectric constant ε in water at 50 °C is 70 D, $f = 0.6$ and e^2 is $= 331$.[14] An evaluation of the size (r_1) for POMs of this type is available as 12.5 Å,[8d] whereas for a typical phenol, $r_2 = 3.2$ Å;[15] these values combine to give $r_{12} = r_1 + r_2 = 15.7$ Å. The reckoned $\Delta G^{\circ\prime}$ data (Table 3) are finally used to solve the Marcus equation (eqn (3)) on the basis of an appropriate value for the reorganisation barrier (λ) of the reaction, to be obtained from combining the two self-exchange λ values (eqn (5)):

$$\lambda_{calc} = (\lambda_{POM} + \lambda_{ArOH})/2 \qquad (5)$$

A self-exchange λ_{ArOH} value of 55 kcal mol^{-1} has been reported recently,[16] whereas literature values for λ_{POM} cluster in the 30–50 kcal mol^{-1} range.[3,17] This leads to a λ_{calc} value of 53 kcal mol^{-1} as the reorganisation energy required in our electron-transfer oxidation (eqn (1)).

The experimental rate constants (k at 50 °C, from Table 1), suitably converted into $\Delta G^{\neq}$ data (given in Table 3) according to the relationship $\ln k = \ln(k_B T/h) - \Delta G^{\neq}/RT$, are represented as black points in a $-\Delta G^{\neq}$ *vs.* $\Delta G^{\circ\prime}$ plot (Fig. 5). These points are not interpolated by the theoretical $\Delta G^{\neq}$ curve calculated from eqn (3) according to the $\Delta G^{\circ\prime}$ data (Table 3) if the above $\lambda = 53$ kcal mol^{-1} barrier is used, whereas they are interpolated when a λ value of 79 kcal mol^{-1} is forced into eqn (3). Even when using the more limited set of kinetic data determined at 25 °C with this POM, a similar and high value of the reorganisation energy, namely $\lambda = 72$ kcal mol^{-1}, is required for fitting the calculated $\Delta G^{\neq}$ curve to the experimental data (plot not shown).

Likewise, the few kinetic data acquired for the oxidation of substituted phenols with $SiW_{11}Mn$ (Table 1), once converted into $\Delta G^{\neq}$ data and plotted in the $-\Delta G^{\neq}$ *vs.* $\Delta G^{\circ\prime}$ space, are interpolated by a calculated $\Delta G^{\neq}$ curve (eqn (3)) whenever a $\lambda = 61$ kcal mol^{-1} energy is imposed (Fig. 6).

Discussion

A spectrophotometric kinetic study of the oxidation of substituted phenols, the redox potential of which spans over *ca.* 0.4 V across the series, has been performed with two POM compounds in buffered (pH = 4) aqueous solution. Most of the kinetic results have been gathered with the V^V-containing $[\alpha\text{-}SiV^VW_{11}O_{40}]^{5-}$ POM (*viz.* $SiW_{11}V$), which is a weaker oxidant ($E^{1/2} = 0.67$ V) and accordingly enabled the kinetic study to be performed at 50 °C. A Hammett $\rho = -3.1$ value has been determined from the oxidation of the substituted phenols, in addition to a $k_H/k_D = 1.06$ value for the solvent kinetic isotope effect. The Mn^{III}-containing $[\alpha\text{-}SiMn^{III}W_{11}(H_2O)O_{39}]^{5-}$ POM (*viz.* $SiW_{11}Mn$), a stronger oxidant ($E^{1/2} = 0.76$ V), was more reactive than $SiW_{11}V$ by *ca.* two powers of magnitude; in fact, the kinetic study with $SiW_{11}Mn$ could be run at 25 °C but, due to experimental problems, is more limited.

The redox potential difference (ΔE) between the POM and the reducing phenols drives the reactivity of the oxidation process, as indicated by fitting the kinetic data of both $SiW_{11}V$ and $SiW_{11}Mn$ *vs.* ΔG° within the Marcus equation. From this parabolic fitting, the strong relevance of the λ parameter

Table 3 Experimental rate constants (k, from Table 1) and $\Delta G^{\neq}$ data for the oxidation of substituted phenols by $SiW_{11}V$ ($E^{1/2} = 0.67$ V) in buffered aqueous (pH = 4) solution at 50 °C; the calculated $\Delta G^{\circ\prime}$ data (from eqn (4)) are also given

ArOH	$E^{1/2}$/V *vs.* NHE	ΔG°/kcal mol^{-1} [a]	$k/M^{-1}\ s^{-1}$	$\Delta G^{\neq}$/kcal mol^{-1} [b]	$\Delta G^{\circ\prime}$/kcal mol^{-1} [c]
p-Br-phenol	0.85	4.15	6.7×10^{-3}	22.10	3.06
Phenol	0.88	4.84	7.2×10^{-3}	22.06	3.75
m-MeO-phenol	0.87	4.61	0.012	21.73	3.52
p-Cl-phenol	0.90	5.30	0.013	21.68	4.21
p-Ph-phenol	0.78	2.54	0.020	21.40	1.45
p-Me-phenol	0.79	2.77	0.052	20.79	1.68
p-MeO-phenol	0.66	−0.23	9.0	17.49	−1.32
p-OH-phenol	0.48	−4.38	54	16.35	−5.47

[a] From $\Delta E = E_{POM} - E_{ArOH}$, and converted into kcal mol^{-1} (1 V = 23.06 kcal mol^{-1}). [b] From $k = 6.7 \times 10^{12} \exp(-\Delta G^{\neq}/RT)$ at 50 °C. [c] From eqn (4).

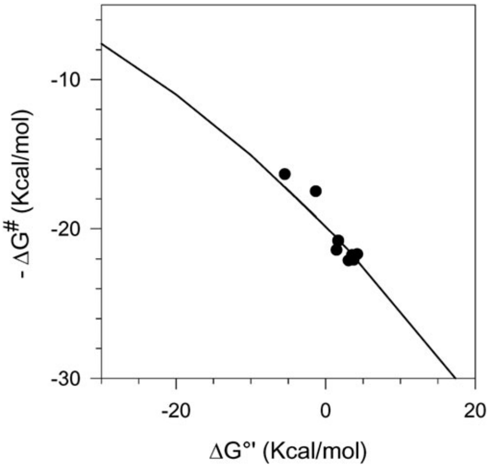

Fig. 5 Marcus plot for the oxidation of substituted phenols with $SiW_{11}V$, at 50 °C. The calculated curve (from eqn (3)) interpolates the experimental kinetic data for $\lambda = 79$ kcal mol^{-1}.

emerges. The high reorganisation energy appraised for this electron-transfer reactions, which is larger for the weaker oxidant $SiW_{11}V$ than for the stronger oxidant $SiW_{11}Mn$, is indeed larger than expected from the self-exchange energies available for both POM and ArOH.[3,6a,16,17] Because the electron abstraction takes place in a polar aqueous medium, it is likely that an extensive restructuring of the Keggin-like cage[1] of the highly charged POMs is needed. This inference complies with the strongly negative value of the entropy of activation determined here and elsewhere,[6a] and also with the strong solvation energy of a highly charged POM recently evaluated for an epoxidation reaction investigated in a polar solvent.[18] Our study, therefore, gives a novel assessment of the reorganisation energy of polyoxotungstates in electron transfer reactions in water, providing a higher λ value that approaches the 70–80 kcal mol^{-1} range.

In addition, one can also suggest that even the self-reorganisation energy of the phenols is perhaps larger (*i.e.*, 60–65 kcal mol^{-1}) than reported in the literature.[16] This could have some bearing with the current debate about the mechanism of oxidation of phenols, for which various mechanistic options are possible.[16,19] In fact, the oxidation process delineated in eqn (1) requires the extraction of one electron plus one proton from the phenolic substrate, to produce the phenoxyl radical: a different timing could be envisioned for the two extraction events, or else they could occur in one single step. First of all, our results suggest that a 'deprotonation-first' mechanism (eqn (6))[19a] is highly unlikely, because the kinetic study is performed at pH = 4 in water, and the phenolic substrates have $pK_a \gg pH$, so that a (rate determining) deprotonation can be ruled out.

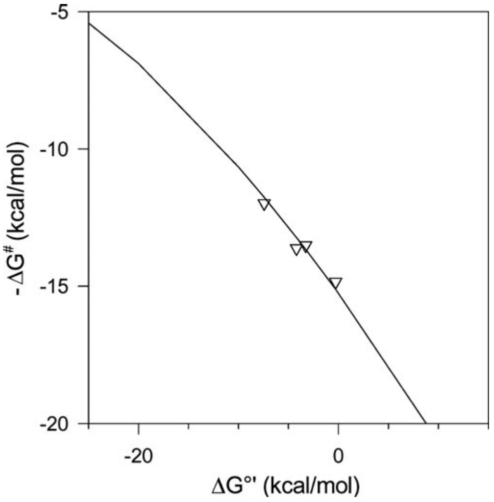

Fig. 6 Marcus plot for the oxidation of substituted phenols with $SiW_{11}Mn$, at 25 °C. The calculated curve (from eqn (3)) interpolates the experimental kinetic data for $\lambda = 61$ kcal mol^{-1}.

$$\mathrm{ArOH} \overset{K_a}{\rightleftharpoons} \mathrm{ArO^-} + \mathrm{H^+} \overset{K_{ET}}{\rightleftharpoons} \mathrm{ArO^\bullet} \qquad (6)$$

Direct extraction of an H-atom (a HAT route, eqn (7)), that is, removal of a proton and an electron combined together,[19f,i] can also be excluded in view of the sizeable value of the ρ parameter measured here. Our evidence points to a substantial effect of the substituents over a polar transition state, which contrasts to the outcome of radical HAT processes where low ρ values are typically obtained.[20] Additionally, the nature of the abstracting $R^\bullet$ species in eqn (7) would be uncertain.

$$\mathrm{ArOH} \overset{R^\bullet}{\rightleftharpoons} \mathrm{ArO^\bullet} + \mathrm{HR} \qquad (7)$$

The satisfactory correlation of the rate constants of oxidation by $SiW_{11}V$ *vs.* the redox potential of the phenols at 50 °C (Fig. 4), in combination with the negligible value ($k_H/k_D = 1.06$) of the kinetic isotope effect experiment, are consonant with a rate-determining electron-transfer (k_{ET}, in eqn (8)) from the reducing substrates to the oxidising POM, followed by fast deprotonation of the intervening radical cation of the phenol.

$$\mathrm{ArOH} \overset{k_{ET}}{\rightleftharpoons} \mathrm{ArOH^{\bullet +}} \rightarrow \mathrm{ArO^\bullet} + \mathrm{H^+} \qquad (8)$$

Electron-donor substituents decrease the redox potential of the aromatic substrate and, by stabilising the polar radical-ion intermediate, make the oxidation faster, while electron-withdrawing ones slow it down, as indicated by the negative sign of the ρ parameter, besides its sizeable value. Finally, for a given substrate, the oxidation reaction is faster with the stronger oxidising POM (*i.e.*, $SiW_{11}Mn$), as it is reasonable for a rate-determining electron transfer. The possibility of a proton-coupled electron transfer (PCET) route,[16,19] as a subtle alternative to the rate-determining electron transfer, has been considered. In the PCET route the phenolic proton and the electron move in a single kinetic step towards two different acceptor locations. However, the negligible value of the kinetic isotope effect obtained here argues against this hypothesis, for which k_H/k_D values in the range 2–4 are instead reported.[16,19f,g,21] Unfortunately, the lack of stability of these POMs in basic media prevents us from extending the investigation to higher pH values,[8c] to better ascertain any effect that a more extensive deprotonation of the substrate might have on the perspective of a PCET route. As far as the Marcus λ parameter is concerned, a high reorganisation energy appears required, and λ is larger the weaker the oxidant. The entropy of activation measured for *p*-MeO- and *p*-Me-phenol is rather negative, and slightly larger (a little beyond the experimental errors) with the more reactive out of the two substrates. This is in keeping with a profound reorganisation that both the ArOH and the POM must undergo upon electron transfer, as the large λ values confirm.

Conclusions

Our experimental data support the occurrence of an outer-sphere oxidation reaction of substituted phenols by two POM compounds in aqueous solution, and characterise this mechanistic route with ρ and k_H/k_D values, in addition to the correlation trends obtained, which can make a useful paradigm in future studies. As anticipated in the Introduction, in fact, our data with POMs may help to better appreciate reactivity features in one-electron oxidation processes with metalloenzymes. For example, the copper-containing laccases are endowed with redox potential values comparable[9,10] to those of the present vanadium- or manganese-containing POMs, and laccase enzymes are indeed involved in the oxidation of natural phenolic compounds. For these reasons, the two POMs here investigated would stand as good biomimetic models of the reactivity of fungal laccases. In contrast, the reorganisation energy (λ) of biological copper-containing electron-transfer sites is known to have a lower value than that of synthetic copper complexes.[22] This represents an advantage for the enzymes, enabling them to perform a fast oxidation of natural substrates even in the absence of a strong electrochemical driving force.[23] The reorganisation energy of our POMs is certainly much higher than that reported for a few copper-enzymes,[22b] in spite of similar redox features. We are therefore interested in studying the difference in reorganisation requirements between biomimetic and biological electron-transfer sites more deeply. Studies on the reactivity of oxidation by *Trametes villosa* laccase towards substituted phenols are under way for this purpose.

Experimental

General remarks

Phenolic substrates were high purity commercial samples (Aldrich) and used without further purification. A HI-TECH SFA-12 stopped flow instrument interfaced to a HP 8453 diode array spectrophotometer was employed in the kinetic measurements; a conventional UV-Vis spectrophotometer (PerkinElmer Lambda 18) was alternatively used. Quartz cells had a 1 cm optical path. Ultrapure water obtained with a MilliQ apparatus was used to prepare the buffer.

Synthesis of the POMs

$SiW_{11}V$. $K_6[SiW_{11}V^{IV}O_{40}] \cdot 7H_2O$ was prepared by following literature procedures.[24,25] In short: 2.5 g (9.8 mmol) of $VOSO_4 \cdot 5H_2O$ was dissolved in 30 ml of aqueous 1 M sodium acetate buffer at pH 4.7, and 24.0 g (7.45 mmol) of $K_8[SiW_{11}O_{39}] \cdot 13H_2O$ added under vigorous stirring at room temperature.[26] The solution was heated to 60 °C, and 7.5 g of KCl added slowly during 3 min. A black–purple solid formed after keeping the reaction solution for one night at 5 °C, and was separated by filtration. The solid was recrystallised by dissolving in the minimum amount of water at 80–85 °C (about 10 g per 12 ml). Insoluble dark-green solid contaminants were separated by fast filtration of the hot solution. The filtrate (purple solution) was put in an ice bath for 2 h, and the formed solid was isolated by filtration and dried under vacuum; 70% yield. The purity of $SiW_{11}V$ was confirmed by UV-Vis spectroscopy and elemental analysis.[24,25]

A $[SiW_{11}V^VO_{40}]^{5-}$ stock solution was prepared by dissolving 7.0 g (2.25 mmol) of $K_6[SiW_{11}V^{IV}O_{40}] \cdot 7H_2O$ in 50 ml of water.[24,25] At room temperature, 60 μl (1.2 mmol) of Br_2 was added rapidly to this solution under stirring. The solution colour changed from purple to yellow during *ca.* 1 h. Traces of any formed solid were filtered off and the volume adjusted to 100 ml, to yield a solution with a polyoxometalate concentration of 22.5 mmol l^{-1}. The purity of the stock solution was confirmed by ^{51}V NMR.[25]

$SiW_{11}Mn$. $K_5[SiW_{11}Mn^{III}(H_2O)O_{39}] \cdot 9H_2O$ was prepared as follows.[27] Solution A was prepared by dissolving 20.0 g (6.2 mmol) of $K_8[SiW_{11}O_{39}] \cdot 13H_2O$ in 40 ml of water by heating to 90–95 °C while stirring. Solution B was prepared by dissolving 0.196 g (1.24 mmol) of $KMnO_4$ in 20 ml of water along with 1.8 ml of a 6.0 mol l^{-1} solution of HCl. Solution C was prepared by dissolving 1.22 g (4.96 mmol) of $Mn(CH_3COO)_2 \cdot 4H_2O$ in 20 ml of water. Solutions B and C were added dropwise and simultaneously to solution A. The resultant solution was kept at 90–95 °C for 30 min. After cooling to room temperature, an insoluble brown solid (MnO_2) was separated by filtration. The filtrate was concentrated to 10–12 ml and put in an ice bath for 3 h, giving a pink solid, which was filtered off. Recrystallization of this solid was performed by dissolving in the minimum amount of water at 70 °C (about 17 g per 10 ml). Insoluble brown solids were eliminated by filtration. The purple solution was put again in an ice-bath for 2 h, and $SiW_{11}Mn$ isolated by filtration and dried under vacuum; 60% yield. The purity of the compound was confirmed by UV-Vis spectroscopy and elemental analysis.[27]

A $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$ stock solution was prepared by dissolving 3.0 g (0.96 mmol) of $K_5[SiW_{11}Mn^{III}(H_2O)O_{39}] \cdot 9H_2O$ in distilled water, and adjusting the final volume to 100 ml, to give a polyoxometalate concentration of 9.6 mmol l^{-1}.

Kinetic measurements

Either a stopped flow or a conventional UV-Vis spectrophotometer were employed in the measurements. The thermostated cell-holder of the spectrophotometric instruments allowed to follow the kinetics at appropriate temperatures, in the 13–50 °C range. These were run in aqueous solution at pH = 4 (acetate buffer, 0.1 M) under pseudo-first-order conditions, at initial concentrations of [POM] = 4×10^{-4} and [ArOH] in the range 4×10^{-3} to 0.019 M. The drop of absorbance of $SiW_{11}V^V$ at 350 nm, or of $SiW_{11}Mn^{III}$ at 490 nm, was followed during the oxidation processes. In some cases, interference from absorption bands of the phenolic substrate prevented from reading the drop of absorbance of oxidised form of the POM clearly. This problem could be circumvented in the case of $SiW_{11}V^V$, by alternatively following the increase of absorption of the reduced $SiW_{11}V^{IV}$ form in the 500–520 nm range. A similar approach was not attainable in the case of $SiW_{11}Mn^{III}$, because the $SiW_{11}Mn^{II}$ form absorbs below 360 nm,[5c] in a region covered by absorption bands of the substrate. The contributions from the absorption of $SiW_{11}V^{IV}$ at 350 nm (ε = 295 M^{-1} cm^{-1}), from the absorption of $SiW_{11}V^{V}$ at 500 nm (ε = 3 M^{-1} cm^{-1}), and from the absorption of $SiW_{11}Mn^{II}$ at 490 nm (ε = 20 M^{-1} cm^{-1}) have been considered during experimental data treatments. For the kinetic isotope effect determination with $SiW_{11}V$, H_2O was substituted with D_2O, and *p*-MeO-phenol was used as the substrate at 25 °C, under otherwise identical experimental conditions as reported above.

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