A new type of manganese-Schiff base complex, catalysts for the disproportionation of hydrogen peroxide as peroxidase mimics

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New manganese(II) and manganese(III) complexes of substituted *N*,*N*′-bis(salicylidene)-1,2-diimino-2-methylethane have been prepared and characterized. Elemental analysis, IR and EPR spectroscopies, mass spectrometry, magnetic measurements and the study of their redox properties have confirmed their respective formulae as $Mn^{II}L(H_2O)_2$ and $Mn^{III}L(H_2O)_n(ClO_4)$. Electron-withdrawing substituents on the phenyl rings of the ligand stabilize the oxidation state (II) for manganese, but the electron-donating substituents on the Schiff bases are those that lead to Mn(III) complexes, which behave as efficient peroxidase mimics in the presence of the water-soluble trap ABTS. The rate of peroxidase activity of the present complexes is significantly higher than that of other series of Mn-Schiff base compounds, probably due to their versatility in adopting in solution a structure that allows the coordination of the hydrogen peroxide substrate molecule to the manganese.

Introduction

In recent years the coordination chemistry of manganese has been intensively studied[1–3] due to its occurrence in the active sites of several enzymes that participate in the chemistry of reactive oxygen species, such as Mn catalase,[4] Mn superoxidase dismutase,[5] Mn ribonucleotide reductase,[6] the oxygen-evolving complex (OEC),[7,8] or, in particular, the Mn peroxidase that protects cells against hydrogen peroxide induced oxidative stress.[9] Oxidative stress is implicated in the pathogenesis of neurodegenerative disorders and brain ischemia, and hydrogen peroxide plays a central role in this stress.[10]

Spectroscopic data taken from these biological enzymes show a manganese centre surrounded by O- or N-donor ligands. This is the case of the interpretation of the EXAFS experiments by Klein and co-workers[11] for the OEC or the crystal structure at 2.06 Å resolution[12] of the manganese peroxidase from the white rot basidiomycete *Phanerocaete chrysosporium* (which utilizes this enzyme to degrade lignin).

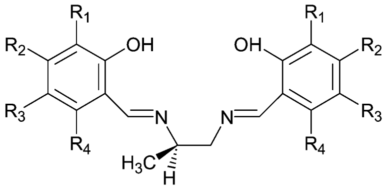
Manganese complexes involving tetradentate ONNO Schiff base derivatives of *N*,*N*′-bis(salicylidene)ethylenediamine (known as salen) are one of the most versatile and interesting synthetic systems, the result of the development of the research in Mn coordination chemistry. Thus, some of these types of complexes were found to be artificial mimics of the biological enzymes cited above,[2,13] and also to act as catalysts for important industrial redox processes.[14]

Our research experience with manganese-Schiff base complexes has led us in the past to prepare different synthetic models that reproduce in part the reactivity of some of these enzymes.[15–18] We have found that some minor changes in the design of the Schiff base have an important incidence on the catalytic activity of the final complexes. Steric effects determine the optimal aproximation of a substrate susceptible of oxidation,[15] but also electronic properties (electron-donor or -withdrawing character) of the substituents in the phenyl rings of the salen moiety induce the stabilization of a particular state of oxidation for Mn and/or a variation in the redox reversibility character of the Mn complex.[16] The arrangement of the coordination positions of the ligand would involve polymeric structures, which also arise from the use of other polydentate bridging anions.[19] Besides, it is worthy to note that some catalytic activities need the previous coordination of the substrate molecule to the manganese centre, so the potentially active complexes should have a vacancy in the coordination sphere or a labile ligand.[18]

Herein we report a new series of Mn-Schiff base complexes, named in the text as Me-salen or L (see Scheme 1), and we compare their peroxidase activity *versus* other compounds formed from different Schiff bases. Thus, we might understand the structural motifs that play a key role in the mechanism of the processes catalyzed by the biological enzymes.

Results and discussion

The tetradentate Schiff bases, H_2L, used in this study readily react with manganese(II) perchlorate, in the presence of sodium



	R_1	R_2	R_3	R_4
H_2L^1	H	H	Br	H
H_2L^2	H	H	Cl	H
H_2L^3	H	H	NO_2	H
H_2L^4	OEt	H	H	H
H_2L^5	Br	H	Br	H
H_2L^6	Cl	H	Cl	H
H_2L^7	Br	H	NO_2	H
H_2L^8	OMe	H	Br	H
H_2L^9	H	OMe	H	OMe

Scheme 1

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hydroxide and air, to give two types of compounds owing to the different nature of the Schiff bases. Thus, in spite of the oxidant conditions, ligands with electron-withdrawing substituents in their phenyl rings (*e.g.*, NO_2) stabilize compounds formulated as $[MnL(H_2O)_2]$ with manganese in oxidation state II. This conduct has been previously found with this kind of substituent.[16,20,21] Such behaviour can be explained as a charge balance that starts when electron-withdrawing substituents provoke deficitary-charge rings, which subsequently accept charge from the phenoxy and imine groups. As result of this balance, the electronegativity of these last groups, which should coordinate manganese, decreases. This determines the low capacity of this type of ligand to stabilize the trivalent manganese.[16,17] On the other hand, Mn(III) complexes have been easily stabilized with the rest of the Schiff base ligands, and formulated as $MnL(H_2O)_n(ClO_4)$. Analytical and spectroscopic data are in accordance with these empirical formulae.

Spectroscopic and magnetic characterization of the complexes

IR spectra. The most important IR features are collected in Table 1. These data suggest the coordination of the Schiff base to the metal through both phenol oxygen and imine nitrogen atoms, and show the tetradentate character of the ligands in the complexes. Complexes **1**, **2**, **4**, **5**, **6**, **8** and **9** show broad unsplit bands at *ca.* 1120 cm^{-1}, together with bands at 630 cm^{-1}, that are indicative of the presence of a non-coordinated perchlorate counterion. These stretching frequencies were not observed for complexes **3** and **7**.

FAB mass spectrometry. All compounds exhibit peaks in the FAB mass spectra relating to fragments $[MnL]^+$, which are indicative of the Schiff base coordination to the metal (Table 1). Other minor signals could be attributed to $[MnL]_2^+$ units, suggesting a possible μ-phenoxy-bridge dimeric structure with oxo bridges sensitive to the FAB technique.[16]

Magnetic studies. The complexes formulated as $MnL(H_2O)_n(ClO_4)$ exhibit room temperature magnetic moments in the range 4.7–5.0 B.M. (see Table 2), close to the spin-only value of 4.9 B.M. expected for a magnetically dilute high-spin d^4 manganese(III) ion. In contrast the complexes from 5-NO_2-substituted ligands show markedly elevated magnetic moments close to the spin-only value of 5.9 B.M. expected for the high-spin d^5 magnetically dilute manganese(II) ion.

Electrochemical studies

The electron-donor or -withdrawing character of the substituents on the phenyl rings of the ligands has a decisive influence on the electrochemical behaviour of the complexes. The quasi-reversible one-electron reduction-oxidation wave observable by cyclic voltammetry was generally situated at more negative potentials ($E_{1/2}$) as the electron-donor character of the cited substituents increased. Contrary to this, as the electron-withdrawing character of the substituents was higher, the potentials ($E_{1/2}$) became more positive.

Although the shape of the cyclic voltammograms for all the complexes is quite similar (Fig. 1), with a peak-to-peak separation from 0.080 to 0.312, further studies using normal pulse voltammetry confirmed the involvement of two different redox systems. Cathodic or anodic currents were observed by normal pulse voltammetry with reference to the blank current (without electroactive compounds). This fact shows the reversible nature of these systems but also indicates the presence of two different redox systems associated to the different Mn oxidation

Table 1 IR spectroscopic and FAB spectrometric data for the complexes **1**–**9**

Complex	$\nu(C{=}N)^a$/ cm^{-1}	$\nu(C–O)^a$/ cm^{-1}	$\nu_3(ClO_4^-)$/ cm^{-1}	FAB m/z^b
1	1623 (1632)	1286 (1281)	1115	494
2	1617 (1632)	1298 (1274)	1121	405
3	1633 (1637)	1335 (1338)	–	426
4	1617 (1630)	1297 (1272)	1118	424
5	1620 (1628)	1288 (1280)	1120	651
6	1632 (1637)	1293 (1284)	1120	474
7	1636 (1645)	1330 (1325)	–	584
8	1615 (1625)	1295 (1285)	1120	553
9	1598 (1618)	1256 (1245)	1122	456

[a] Ligand band values in parentheses. [b] Corresponding to the fragment $[MnL]^+$.

Table 2 Magnetic moment at room temperature and electrochemical data for the complexes **1**–**9**

Complex	μ/B.M.	E_{ox}/V	E_{red}/V	$E_{1/2}$/V	ΔE_{ox-red}/V
1	4.7	0.037	−0.055	−0.009	0.092
2	4.8	0.094	−0.009	0.042	0.103
3	5.6	0.391	0.202	0.297	0.189
4	5.0	−0.116	−0.256	−0.186	0.140
5	4.7	0.094	−0.130	−0.018	0.224
6	4.7	0.156	−0.156	0.000	0.312
7	5.8	0.491	0.240	0.366	0.251
8	4.7	−0.034	−0.114	−0.074	0.080
9	5.0	−0.151	−0.312	−0.232	0.161

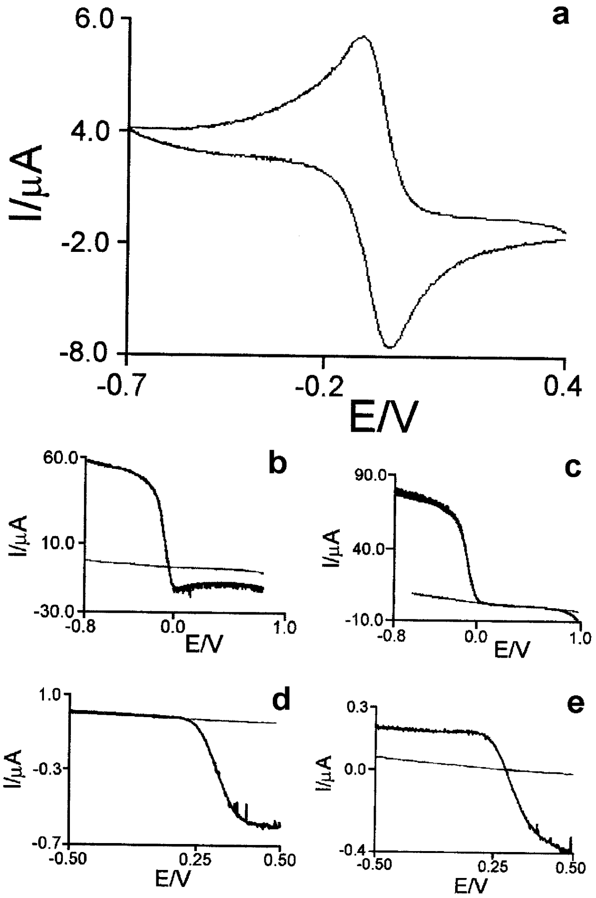


Fig. 1 (a) Cyclic voltammogram for **8** at scan rate of 0.02 V s^{-1}; (b) normal pulse voltammogram (NPV) with anodic scan of **8**; (c) NPV with cathodic scan of **8**; (d) NPV with anodic scan of **7**; (e) NPV with cathodic scan of **7**.

states. Complexes **1**, **2**, **4**, **5**, **6**, **8** and **9** (Mn^{III} complexes) exhibit anodic and cathodic currents when an anodic potential scan is performed, but when a cathodic potential scan was used, only cathodic currents were observed. This behaviour suggests that only the oxidized forms of the implied redox systems Mn^{II}–Mn^{III} are present in solution. However, complexes **3** and **7** (Mn^{II} with electron-withdrawing substituents on the phenyl rings of the ligand) show an opposite behaviour: when the initial potentials were more positive than the wave range, both anodic and cathodic currents were observed, meanwhile only an anodic current was recorded when the initial potentials were more negative than the wave voltage range. The presence of the redox systems Mn^{II}–Mn^{III} in reduced forms is in accordance with these voltammograms.

EPR spectroscopy

The oxidation state and the geometry of the complexes can be elucidated by EPR spectroscopy. Mn(II) complexes **3** and **7** were active in X-band perpendicular-mode EPR. Their spectra in glassy-like solution at 9 K showed the usual Mn(II) ($S = 5/2$) six-line hyperfine pattern (assigned to the $\Delta M_I = 0$ spin-allowed transitions) at $g = 2$. Such a strong signal corresponds to the magnetically dilute mononuclear high-spin Mn(II).

Parallel-mode EPR (microwave field H_1 is parallel to the static field H_0) has proven to be a powerful tool to investigate integer-spin paramagnetic species. Using this technique, an electron $\Delta M_S = 0$ transitions can be detected, which are hardly observable with a conventional EPR method ($H_1 \perp H_0$, perpendicular mode). Mn(III) has an integer electron spin, $S = 2$, and has been shown for powdered samples to develop a characteristic sextet pattern in parallel-mode EPR.[22] This sextet is centred at around $g_{\text{eff}} = 8$ and split by the hyperfine interaction $A = 43$–56 G due to the Mn nucleus ($I = 5/2$). A similar sextet is also detectable with perpendicular-mode EPR but signal intensity is significantly weaker and peak resolution worse.[22,23]

The spectra of Mn(III) were interpreted taking into account that zero-field splitting (ZFS) dominates the spin Hamiltonian of Mn(III). ZFS was reported to be about $D \sim 3.4$–5.0 cm^{-1} for Mn(III) in tetragonal complexes,[24–26] while the electron Zeeman interaction at X-band EPR magnetic fields is an order of magnitude smaller, 0.2–0.3 cm^{-1}, while the Mn hyperfine term is $\sim$0.1 cm^{-1}.

Fig. 2 shows the parallel-mode EPR spectra for a series of Mn(III)-Schiff base complexes. Only the low-field portion of the spectra is shown, since no additional lines were detected at higher fields. The centre position of the sextet ($g_{\text{eff}} = 8.09$) and hyperfine splitting ($A_{\parallel} = 43$ G) are identical among the present complexes and closely resemble those previously reported for similar compounds.[23] The signal at $g_{\text{eff}} = 8.09$ should be assigned to $g_{\text{eff}(2)}$.[27] Assuming typical $D \sim 3.4$–5.0 cm^{-1} and $g_{\parallel} = 1.99$,[24–26] one estimates $E = 0.25$–0.31 cm^{-1} ($h\nu_0 = 0.31$ cm^{-1} corresponds to $\nu_0 = 9.37$ GHz in our experiments). Thus, significant rhombic splitting within the $M_S = \pm 1$ NK (non-Kramers) doublet ($6E = 1.5$–1.8 cm^{-1}) explains the absence of the second $g_{\text{eff}(1)}$ transition in the spectra. The slight ZFS rhombicity, $E/D = 0.05$–0.09, assumes that Mn(III)-Schiff base accepts a highly symmetric coordination geometry in frozen solutions. A slight rhombicity has also been observed for substitutional Mn(III) in rutile single crystal ($E/D = 0.04$)[24] and Mn(III)(dibenzoylmethane)$_3$ in a polycrystalline powder ($E/D = 0.06$).[25] In both these cases, Mn(III) is found in an approximately tetragonal coordination, with two axial O ligands slightly more distant than four equatorial O ligands. Tetragonal geometry is also expected for Mn(III)-Schiff base complexes, with two O and two N from the tetradentate Schiff base ligand forming an equatorial coordination to the ion. The tetragonal field terms are also those that are found in the five-coordinate square-pyramidal geometry. Thus, parallel-EPR data is consistent with either a $^5B_{1g}$ symmetry ground state of an octahedral Mn(III) geometry or a $^5B_1$ symmetry ground state of a five-coordinate square-pyramidal Mn(III) geometry.[28]

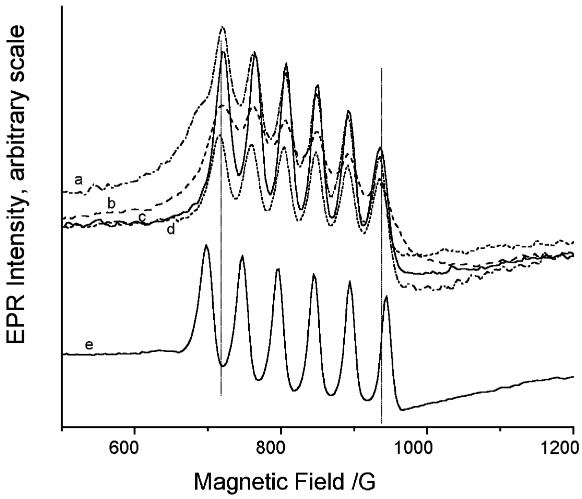


Fig. 2 Parallel-mode EPR (microwave field H_1 is parallel to the static field H_0) of **2** (a), **5** (b), **6** (c) and **8** (d) in toluene:dmf:EtOH (2:1:drop) solution. Spectrum (e) corresponds to [Mn(3,5-Br-*salpn*)$(H_2O)_2$]ClO_4, taken from ref. 18. Conditions: frequency 9.37 GHz, $T = 9$ K, 20 mW microwave power.

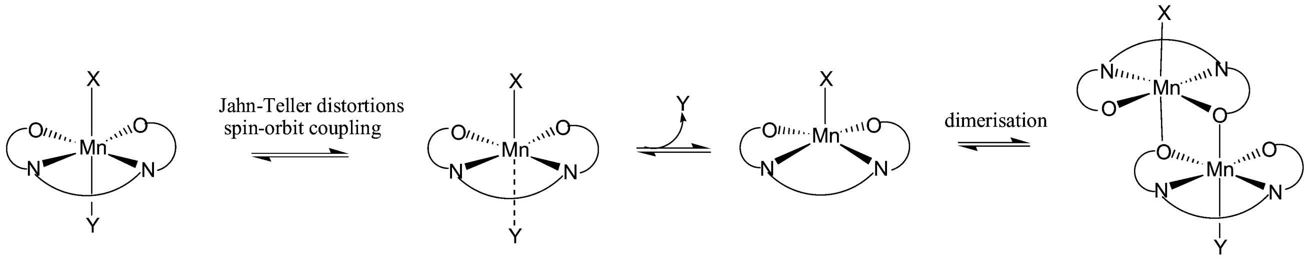
In conclusion, the overall structural study of the manganese-Schiff base complexes $Mn^{II}L(OH_2)_2$ suggest an octahedral geometry around the manganese with the tetradentate Schiff base occupying the equatorial plane with coordinated water molecules filling the axial positions. An unequivocal structure in solution for the $Mn^{III}L(OH_2)_n(ClO_4)$ complexes cannot be established. All the data limit the geometries of the likely candidates to a five-coordinate site with square-pyramidal geometry (**III**) or a six-coordinate site with octahedral geometry (see Scheme 2). But the latter include two possible structural motifs: (1) monomer complexes **I** where the manganese atom is surrounded by the Schiff base in the equatorial plane with two water molecules occupying the apical positions or (2) a μ-phenoxy dimer structure **IV**, suggested by some mass spectrometry data and the known tendency of this type of complex to dimerize in solution through μ-phenoxy-bridges.[16,29,30]

These three possible structures share the stable Mn-Schiff base unit where the tetradentate ligand is situated in a square plane. The extreme high lability of the two axial positions for Mn-Schiff base complexes (see, for example, Lee *et al.*'s[31] detailed kinetic study of the evaporation of water from hydrated Mn-salen complexes) justifies the behaviour in solution for these systems with the easy displacement of a coordinated water (or any other solvent) molecule.

This equilibrium leads to square-pyramidal geometries from octahedral units through tetragonally elongated octahedral geometries (**II**) due to the usual Jahn–Teller distortions for octahedral Mn(III). But the donor character of the phenoxy oxygens of the Schiff base is enough to establish coordination to the manganese atom of the neighbouring Mn-Schiff base unit, forming μ_2-phenoxy-bridged dimers. The labiliness of the axial ligands and the coexistence of forms **I**, **III** and **IV** (refer to Scheme 2) in solution will influence to the reactivity showed by these complexes in some catalytic processes.

Peroxidase essays

The peroxidase-like activity of the complexes was followed by the oxidation of the diammonium salt of 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) at pH 6.8. ABTS is colourless and it reacts readily with hydrogen peroxide in presence



I
octahedral geometry

II
tetragonally elongated
octahedral geometry

III
square pyramidal
geometry

IV
μ-phenoxy-bridged
dimer

Scheme 2

of a peroxidase catalyst to yield a stable green coloured radical cation (see Scheme 3),[32] which absorbs light at 415, 650, 735 and 815 nm.[33] In most cases, the strongest absorption at 415 nm is used for detection purposes. The oxidation potential of ABTS, as well as the comproportionation constant for the $2e^-$ oxidized and the fully reduced species to provide ABTS$^{\bullet+}$, is invariable over a wide range of pH. In the absence of the complex, a solution of ABTS and H_2O_2 is stable for several hours without showing any formation of ABTS$^{\bullet+}$. The reaction of ABTS with H_2O_2 in the presence of **1**–**9** generates ABTS$^{\bullet+}$ and the characteristic absorption bands of this species could be established (Table 3, Fig. 3). Further oxidation to the corresponding dication was not observed.

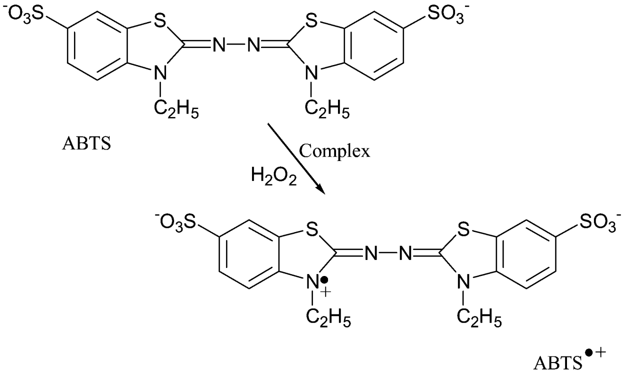
Fig. 3 shows the UV spectrum of the ABTS radical cation obtained after hydrogen peroxide decomposition by **8** (a), and it is compared with the activity of another compound of a Mn(salpn) series previously reported by us (b).[17] The comparision reveals that the present complexes of the type Me-(salen) surprisingly present a *ca.* 30-fold greater peroxidase activity than Mn(salpn), using the same substituents on the phenyl rings of the Schiff base.

An explanation for this behaviour may arise from the different structural features found by EPR in solution for these two series of complexes, manifested in their respective hyperfine splitting constants.[17,18] Fig. 2 illustrates such differences, showing the EPR spectra for the present Mn(Me-salen) complexes (top) compared to a typical Mn(salpn) parallel-EPR spectrum (bottom). We interpret these spectroscopic results as an indication of their different tendency to stabilize the octahedral geometries **I** (see Scheme 2). The flexible three-membered alkyl chain between the imine groups in the Mn(salpn) compounds favours a better stabilization of the octahedron. But the asymmetric Schiff base ligand Me(salen) has a shorter two-carbon chain between imine groups that constricts the chelate ring once nitrogens coordinate to the metal. In addition, a steric effect arises from the methyl group attached to this chain. The conjuction of both factors affects to the stability of a high-symmetry octahedral geometry for Mn(Me-salen) complexes. This hypothesis is also in accordance with the $A_{\parallel}$ values of about 56 G reported[18,22,25] for octahedral Mn(III) complexes with a known structure solved by X-ray crystallography, meanwhile the smaller ^{55}Mn hyperfine coupling of 43 G found in the present work is, in the literature, assigned to square-pyramidal or tetragonally elongated octahedral geometries.[28,34]

As result of these structural considerations we propose that Mn(Me-salen) complexes have either a vacancy in the coordination sphere (square-pyramidal geometry) or a labile ligand (tetragonally elongated octahedral geometry). Therefore, these complexes are easily able to coordinate a substrate molecule such as hydrogen peroxide.

This behaviour might be explained by the solution equilibria phenomena represented in Scheme 2. Thus, Mn(Me-salen) would have a greater capacity to coordinate the H_2O_2 substrate molecule and also would evolve to the μ-phenoxo-bridged dimer **IV**. As the oxidation of a bound hydroperoxide to generate O_2 would involve an intramolecular two-electron transfer reaction, this would be possible by reduction of two manganese atoms in **IV** from (III,III) to (II,II).

On other hand, the peroxidase activity is also influenced by the redox potentials of the complexes of the series Mn(Me-salen). Table 3 collects the intensity of the bands for the ABTS$^{\bullet+}$ formed by catalysis of each complex. Despite the fact that all of them catalyse the disproportionation of hydrogen peroxide with significant rates, a greater catalytic behaviour is observed for those with negative $E_{1/2}$ *versus* SCE and better reversible electrochemical behaviour. Nevertheless, comparisons to Mn(salpn) complexes[17] reveal that the redox potentials are not always the determining factor in the peroxidase rate of these complexes.



Scheme 3

Table 3 Values of the intensity of the absorption at different wavelengths in the UV/vis spectrum corresponding to the ABTS$^{\bullet+}$ formed in the peroxidase experiments

	Wavelength λ/nm			
Complex	415	650	735	815
1	1.545	0.578	0.684	0.604
2	0.826	0.387	0.502	0.495
3	0.982	0.333	0.396	0.343
4	2.070	0.757	0.894	0.779
5	1.548	0.597	0.723	0.668
6	1.341	0.503	0.596	0.463
7	0.882	0.392	0.543	0.521
8	2.852	1.012	1.114	1.002
9	2.671	0.988	1.165	1.021

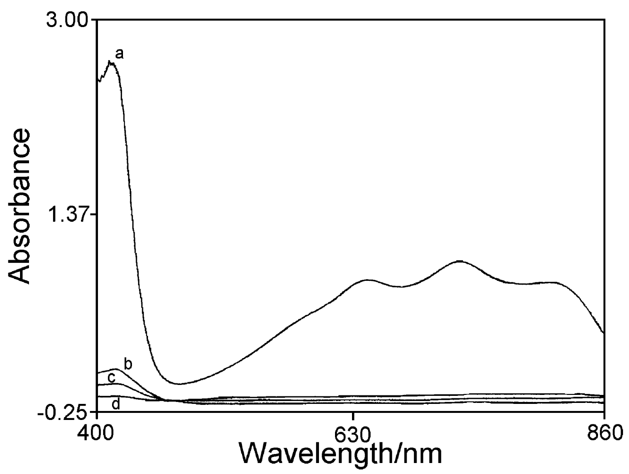


Fig. 3 UV absorption spectra of: (a) ABTS + H_2O_2 + **8**; (b) ABTS + H_2O_2 + [Mn(3-OMe-5-Br-salpn)$(H_2O)_2$]ClO_4, cited in ref. 17; (c) ABTS + H_2O_2, blank 1; (d) **8** + ABTS, blank 2, each recorded 10 min after mixing the solutions.

In the case of the present complexes we can establish a very interesting correlation between a higher reactivity and the existence of electron-donating substituents in the phenyl rings of the Schiff bases. The effects of these substituents: stabilizing Mn(III) *vs.* Mn(II) complexes, decreasing the redox potentials and increasing the reversibility of the electrochemical processes, may determine the peroxidase activity of their final complexes.

Conclusion

Mn(Me-salen) complexes appear to be efficient models for peroxidase activity. In this series of complexes we found that the presence of electron-donating substituents in the phenyl rings of the Schiff bases enhances the catalytic behaviour, although comparison with reported Mn-Schiff base complexes indicates that a more important factor might be a versatile solution structure, which allows coordination of the hydrogen peroxide substrate molecule. A detailed kinetic study of this catalytic process merits further investigation in order to propose a reaction mechanism for these peroxidase mimics.

Experimental

Materials and methods

Salicylaldehydes (Maybridge), 1,2-diaminopropane (Aldrich), manganese(II) perchlorate hexahydrate (Fluka) and ABTS (Aldrich) were used as received without further purification.

Physical measurements. Elemental analyses were performed on a Carlo Erba model 1108 CHNS-O elemental analyser. The IR spectra were recorded as KBr discs on a Nicolet PC5 FT-IR spectrophotometer and UV-Visible spectra on a Hewlett–Packard 8452A instrument. ^1H NMR spectra were recorded on a Bruker WH 300 MHz spectrometer using $CDCl_3$ as solvent and $SiMe_4$ as an internal reference. FAB mass spectra were recorded on a Kratos MS50 TC spectrometer connected to a DS90 data system, using metanitrobenzyl alcohol as a matrix. Room-temperature magnetic susceptibilities were measured using a Digital Measurement system 1660 vibrating magnetometer operating at 5000 G. $HgCo(NCS)_4$ was employed as a susceptibility standard. EPR measurements were carried out on a Bruker ESP300E X-band spectrometer equipped with an ER4116 DM dual-mode cavity and an Oxford 900 continuous flow cryostat. The typical temperature for EPR measurements was 9 K, and other experimental conditions are shown in the figure caption.

Electrochemical measurements. All measurements were performed using an EG&G PAR model 273 potentiostat, controlled by EG&G PAR model 270 software. A Metrohm model 6.1204.000 graphite disc coupled to a Metrohm model 628-10 rotating electrolyte device was used as a working electrode. A saturated calomel electrode was used as a reference and a platinum wire as an auxiliary electrode.

All measurements were made with *ca.* 10^{-3} mol dm^{-3} solutions of the complexes in dimethylformamide using 0.2 mol dm^{-3} NBu_4PF_6 as a supporting electrode. Cyclic voltammetry measurements were performed with a static graphite electrode, whilst direct-current and pulse voltammograms were recorded with the graphite disc rotating at 2000 revolutions per minute.

Preparation of the ligands

All the asymmetrical Schiff bases (Me-salen) have been prepared in an analogous manner by the reaction of the appropriately substituted salicylaldehyde with diamine, and is typified by the following preparation.

H_2L^1. To an ethanolic solution (100 mL) of 1,2-diaminopropane (0.75 mL, 8.71 mmol) was added 5Br-salicylaldehyde (3.50 g, 17.42 mmol). This mixture was refluxed in a round bottom flask fitted with a Dean–Stark trap to remove the water produced during the reaction. After refluxing for 3 h, the solution was concentrated to yield a yellow solid. The product was collected by filtration, washed with diethyl ether and dried in air. Yields were almost quantitative. M. p. 118 °C. Anal. found: C, 46.1; H, 3.6; N, 6.3%; calcd. for $C_{17}H_{16}Br_2N_2O_2$: C, 46.3; H, 3.6; N, 6.3%; ^1H NMR δ 1.40 (d, 3H), 3.71 (d, 2H), 3.85 (m, 1H), 6.8–7.4 (m, 6H), 8.20 (s, 1H), 8.29 (s, 1H), 13.20 (s, 2H); IR ν(C=N) 1632, ν(C–O) 1281 cm^{-1}.

H_2L^2. M. p. 164 °C. Anal. found: C, 58.0; H, 4.6; N, 8.0%; calcd. for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.1; H, 4.6; N, 8.0%; ^1H NMR δ 1.36 (d, 3H), 3.65 (d, 2H), 3.85 (m, 1H), 6.8–7.2 (m, 6H), 8.20 (s, 1H), 8.25 (s, 1H); IR ν(C=N) 1632, ν(C–O) 1274 cm^{-1}.

H_2L^3. M. p. 247 °C. Anal. found: C, 54.3; H, 4.4; N, 15.3%; calcd. for $C_{17}H_{16}N_4O_6$: C, 54.8; H, 4.3; N, 15.0%; ^1H NMR δ 1.46 (d, 3H), 3.80 (d, 2H), 3.92 (m, 1H), 6.9–7.2 (m, 6H), 8.35 (s, 1H), 8.37 (s, 1H); IR ν(C=N) 1637, ν(C–O) 1338 cm^{-1}.

H_2L^4. M. p. 82 °C. Anal. found: C, 68.0; H, 7.0; N, 7.5%; calcd. for $C_{21}H_{26}N_2O_4$: C, 68.1; H, 7.0; N, 7.6%; ^1H NMR δ 1.37 (d, 3H), 1.43 (t, 6H), 3.65 (d, 2H), 3.87 (m, 1H), 4.05 (q, 4H), 6.6–6.9 (m, 6H), 8.23 (s, 1H), 8.27 (s, 1H); IR ν(C=N) 1630, ν(C–O) 1272 cm^{-1}.

H_2L^5. M. p. 182 °C. Anal. found: C, 34.3; H, 2.3; N, 4.8%; calcd. for $C_{17}H_{14}Br_4N_2O_2$: C, 34.3, H, 2.3, N, 4.8%; ^1H NMR δ 1.42 (d, 3H), 3.75 (d, 2H), 3.94 (m, 1H), 7.3–7.7 (m, 4H), 8.18 (s, 1H), 8.22 (s, 1H), 14.15 (s, 1H), 14.27 (s, 1H); IR ν(C=N) 1628, ν(C–O) 1280 cm^{-1}.

H_2L^6. M. p. 144 °C. Anal. found: C, 48.5; H, 3.3; N, 6.6%; calcd. for $C_{17}H_{14}Cl_4N_2O_2$: C, 48.6; H, 3.3; N, 6.6%; ^1H NMR δ 1.40 (d, 3H), 3.75 (d, 2H), 3.90 (m, 1H), 7.1–7.4 (m, 4H), 8.19 (s, 1H), 8.22 (s, 1H), 13.90 (s, 1H), 14.10 (s, 1H); IR ν(C=N) 1637, ν(C–O) 1285 cm^{-1}.

H_2L^7. M. p. 249 °C. Anal. found: C, 38.2; H, 2.8; N, 10.4%; calcd. for $C_{17}H_{14}Br_2N_4O_6$: C, 38.5; H, 2.7; N, 10.5%; ^1H

NMR δ 1.44 (d, 3H), 4.03 (d, 2H), 4.28 (m, 1H), 8.2–8.5 (m, 4H), 8.70 (s, 1H), 8.82 (s, 1H); IR ν(C=N) 1645, ν(C–O) 1325 cm^{-1};

H_2L^8. M. p. 167 °C. Anal. found: C, 46.0; H, 4.0; N, 5.8%; calcd. for $C_{19}H_{20}Br_2N_2O_2$: C, 45.6; H, 4.0; N, 5.6%; ^{1}H NMR δ 1.41 (d, 3H), 3.71 (d, 2H), 3.88 (s, 6H), 3.92 (m, 1H), 6.9–7.3 (m, 4H), 8.22 (s, 2H), 13.62 (s, 2H); IR ν(C=N) 1625, ν(C–O) 1285 cm^{-1};

H_2L^9. M. p. 108 °C. Anal. found: C, 61.8; H, 6.5; N, 6.9%; calcd. for $C_{21}H_{26}N_2O_6$: C, 62.6; H, 6.5; N, 6.9%; ^{1}H NMR δ 1.34 (d, 3H), 3.58 (d, 2H), 3.68 (s, 6H), 3.76 (s, 6H), 3.73 (m, 1H), 5.6–6.0 (m, 4H), 8.31 (s, 1H), 8.39 (s, 1H); IR ν(C=N) 1618, ν(C–O) 1245 cm^{-1};

Complex preparation

All the complexes were synthesized by air oxidation of the solution made up from $Mn(ClO_4)_2 \cdot 6H_2O$ and the Schiff base. A typical preparation is outlined below.

$MnL^1(H_2O)_2(ClO_4)$, 1. H_2L^1 (1.36 mmol, 0.60 g) was dissolved in 100 mL of a 1:1 methanol–ethanol mixture and 1.36 mmol (0.49 g) of $Mn(ClO_4)_2 \cdot 6H_2O$ was added to the initial yellow solution, which changed colour to green. (**CAUTION!:** Although no problems have been encountered in this work, perchlorates are potentially explosive and should be handled in only small quantities and with care!). After stirring for 10 min, 2.86 mmol (0.11 g) of NaOH, dissolved in a small quantity of water, was added and the mixture became dark. The progress of the reaction was followed by TLC for 3 days and the mixture was then filtered. The complex was obtained from the filtrate as a brown solid after crystallization. It was isolated by filtration and washed with diethyl ether and dried in air, yield: 0.61 g, 75%. Anal. found: C, 32.9; H, 2.9; N, 4.4%; calcd. for $C_{17}H_{18}Br_2ClMnN_2O_8$: C, 32.5; H, 2.9; N, 4.5%.

$MnL^2(H_2O)_2(ClO_4)$, 2. H_2L^2 (1.42 mmol, 0.50 g); 1.42 mmol (0.51 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 2.86 mmol (0.11 g) of NaOH; yield: 0.63 g, 85%. Anal. found: C, 38.3; H, 3.3; N, 5.3%; calcd. for $C_{17}H_{18}Cl_3MnN_2O_8$: C, 37.8; H, 3.3; N, 5.2%.

$MnL^3(H_2O)_2$, 3. H_2L^3 (1.34 mmol, 0.50 g); 1.34 mmol (0.49 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 2.86 mmol (0.11 g) of NaOH; yield: 0.43 g, 70%. Anal. found: C, 42.6; H, 3.5; N, 11.5%; calcd. for $C_{17}H_{18}MnN_4O_8$: C, 44.2; H, 3.9; N, 12.1%.

$MnL^4(H_2O)_{4.5}(ClO_4)$, 4. H_2L^4 (0.81 mmol, 0.30 g); 9.81 mmol (0.29 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 1.62 mmol (0.06 g) of NaOH; yield: 0.30 g, 60%. Anal. found: C, 41.6; H, 5.0; N, 4.6%; calcd. for $C_{21}H_{33}ClMnN_2O_{12.5}$: C, 41.7; H, 5.1; N, 4.6%.

$MnL^5(H_2O)_2(ClO_4)$, 5. H_2L^5 (1.67 mmol, 1.00 g); 1.67 mmol (0.61 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 3.34 mmol (0.13 g) of NaOH; yield: 0.63 g, 50%. Anal. found: C, 26.2; H, 1.8; N, 3.7%; calcd. for $C_{17}H_{16}Br_4ClMnN_2O_8$: C, 26.0; H, 2.0; N, 3.6%.

$MnL^6(H_2O)_2(ClO_4)$, 6. H_2L^6 (1.43 mmol, 0.60 g); 1.43 mmol (0.52 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 2.86 mmol (0.11 g) of NaOH; yield: 0.45 g, 55%. Anal. found: C, 34.0; H, 2.2; N, 4.3%; calcd. for $C_{17}H_{16}Cl_5MnN_2O_8$: C, 33.5; H, 2.6; N, 4.6%.

$MnL^7(H_2O)_2$, 7. H_2L^7 (1.87 mmol, 1.00 g); 1.87 mmol (0.68 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 3.74 mmol (0.15 g) of NaOH; yield: 1.06 g, 80%. Anal. found: C, 33.3; H, 2.5; N, 8.8%; calcd. for $C_{17}H_{16}Br_2MnN_4O_8$: C, 33.0; H, 2.6; N, 9.0%.

$MnL^8(H_2O)_2(ClO_4)$, 8. H_2L^8 (1.99 mmol, 1.00 g); 1.99 mmol (0.72 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 3.98 mmol (0.16 g) of NaOH; yield: 0.82 g, 60%. Anal. found: C, 34.0; H, 3.4; N, 4.0%; calcd. for $C_{19}H_{22}Br_2ClMnN_2O_8$: C, 34.7; H, 3.4; N, 4.3%.

$MnL^9(H_2O)_3(ClO_4)$, 9. H_2L^9 (0.75 mmol, 0.30 g); 0.76 mmol (0.27 g) of $Mn(ClO_4)_2 \cdot 6H_2O$; 1.62 mmol (0.06 g) of NaOH; yield: 0.39 g, 90%. Anal. found: C, 41.0; H, 4.4; N, 4.5%; calcd. for $C_{21}H_{30}ClMnN_2O_{13}$: C, 41.4; H, 4.6; N, 4.6%.

Peroxidase probes

Oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)sulfonic (ABTS) acid with H_2O_2 in the presence of the complexes was tested in the following manner. An aqueous solution of ABTS (50 μL; 0.009 M; 4.5×10^{-7} mol) and a methanolic solution of the complex (10 μL; 10^{-3} M; 10^{-8} mol) were added to water (3 mL). The oxidation of ABTS starts immediately after addition of an aqueous solution of H_2O_2 (50 μL; 10 M; 5×10^{-4} mol).

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