Anal. Chem. **2010,** *82,* 4174–4180

Mechanism of Permanganate Chemiluminescence

Christopher M. Hindson,[†] Paul S. Francis,[†,‡] Graeme R. Hanson,[§] Jacqui L. Adcock,[†] and Neil W. Barnett*[,†]

School of Life and Environmental Sciences and Institute for Technology Research and Innovation, Deakin University, Geelong, Victoria 3217, Australia, and Centre for Advanced Imaging, University of Queensland, St. Lucia, Queensland 4072, Australia

Spectroscopic and synthetic methods have been exploited to deduce the mechanism for acidic potassium permanganate chemiluminescence. We have employed electron paramagnetic resonance (EPR) spectroscopy with a continuous flow assembly to monitor the formation of radical intermediates in real time generated from substrate oxidation by manganese(VII). These transient species react with manganese(III) in solution to produce the previously characterized manganese(II)* emission source. Using UV−vis, EPR, attenuated total reflection (ATR)-FT-IR, and chemiluminescence spectroscopies, we have established that there are two distinct enhancement mechanisms that in combination afford a 50-fold increase in emission intensity when the reaction is conducted in the presence of phosphate oligomers. In addition to preventing disproportionation of the manganese(III) precursor, the phosphate oligomers form protective "cage-like" structures around the manganese(II)* emitter, thus preventing nonradiative relaxation pathways.

Since its first documented use by Harvey in 1917,[1] the utility of acidic potassium permanganate chemiluminescence in the field of analytical science has grown considerably and the number of scientific papers detailing its applications is well into the hundreds.[2,3] Because of its importance as an analytical reagent, significant attention has also been given to elucidating the mechanism of the reaction with organic or inorganic substrates that, under the right conditions, affords quantitative light emission.[2] The inherent complexity of the mechanism of permanganate oxidations stems from the number of valence states that may be exhibited by manganese and their markedly different chemistries.[4] In fact, even the reaction of permanganate with the relatively simple oxalate molecule in acidic aqueous media has been the subject of vigorous academic discussion for more than 60 years.[5−13]

In order to formulate a unified scheme for acidic permanganate chemiluminescence, we must first consider the features of this reaction that have already been proven. Two earlier papers from our laboratory[14,15] have unequivocally shown that the characteristic red emission (λ_{max} = 734 ± 5 nm) of permanganate chemiluminescence emanates not from the once postulated singlet oxygen but rather from relaxation of the Mn(II) $^4T_1$ excited state to the $^6A_1$ ground state. The earlier of these two papers[14] also demonstrated that this same chemically induced phosphorescence occurred regardless of the analyte used or the initial oxidation state of the manganese reagent [Mn(III), Mn(IV), or Mn(VII)]. Despite producing indistinguishable corrected emission spectra, each of these three reagents exhibit significantly different degrees of sensitivity and selectivity, and demand vastly different reaction conditions to produce measurable amounts of light.[2,3,16] This suggests that the same excitation scheme is operating for the tri-, tetra-, and heptavalent oxidants, but their different chemistries (with respect to stability and solubility) require specific conditions to efficiently access the excited state. For this reason, the characterization of intermediates participating within the light-producing pathway of permanganate chemiluminescence is required to accurately devise a generalized excitation scheme.

Another feature of permanganate chemiluminescence that is not yet understood is the mechanism by which it can be greatly enhanced when conducted in the presence of certain species. For example, it is well-known that when the reaction proceeds in the presence of phosphate oligomers (polyphosphates) such as sodium hexametaphosphate (Graham's salt), an approximate 50-fold enhancement of the emission intensity can be realized and the λ_{max} is blue-shifted to 689 ± 5 nm.[2,3] While the name "hexametaphosphate" implies a 12-membered ring of alternating phosphorus and oxygen atoms, it should be noted that commercial

* To whom correspondence should be addressed. E-mail: neil.barnett@deakin.edu.au.

[†] School of Life and Environmental Sciences, Deakin University.

[‡] Institute for Technology Research and Innovation, Deakin University.

[§] Centre for Advanced Imaging, University of Queensland.

(1) Harvey, E. N. *J. Biol. Chem.* **1917**, *31*, 311–336.
(2) Adcock, J. L.; Francis, P. S.; Barnett, N. W. *Anal. Chim. Acta* **2007**, *601*, 36–67.
(3) Hindson, B. J.; Barnett, N. W. *Anal. Chim. Acta* **2001**, *445*, 1–19.
(4) Dash, S.; Patel, S.; Mishra, B. K. *Tetrahedron* **2009**, *65*, 707–739.
(5) Adler, S. J.; Noyes, R. M. *J. Am. Chem. Soc.* **1955**, *77*, 2036–2042.
(6) Launer, H. F.; Yost, D. M. *J. Am. Chem. Soc.* **1934**, *56*, 2571–2577.
(7) Taube, H. *J. Am. Chem. Soc.* **1948**, *70*, 1216–1220.
(8) Pimienta, V.; Lavabre, D.; Levy, G.; Micheau, J. C. *J. Phys. Chem.* **1994**, *98*, 13294–13299.
(9) Pimienta, V.; Lavabre, D.; Levy, G.; Micheau, J. C. *J. Phys. Chem.* **1995**, *99*, 14365–14371.
(10) Kovacs, K.; Vizvari, B.; Riedel, M.; Toth, J. *Phys. Chem. Chem. Phys.* **2004**, *6*, 1236–1242.
(11) Kovacs, K. A.; Grof, P.; Burai, L.; Riedel, M. *J. Phys. Chem. A* **2004**, *108*, 11026–11031.
(12) Reckley, J. S.; Showalter, K. *J. Am. Chem. Soc.* **1981**, *103*, 7012–7013.
(13) Malcolm, J. M.; Noyes, R. M. *J. Am. Chem. Soc.* **1952**, *74*, 2769–2775.
(14) Barnett, N. W.; Hindson, B. J.; Jones, P.; Smith, T. A. *Anal. Chim. Acta* **2002**, *451*, 181–188.
(15) Adcock, J. L.; Francis, P. S.; Smith, T. A.; Barnett, N. W. *Analyst* **2008**, *133*, 49–51.
(16) Brown, A. J.; Francis, P. S.; Adcock, J. L.; Lim, K. F.; Barnett, N. W. *Anal. Chim. Acta* **2008**, *624*, 175–183.

10.1021/ac100363s
Published on Web 04/07/2010

hexametaphosphate is a complex mixture of ~90% linear and ~10% cyclic phosphate oligomers.[17] It is also pertinent to note that typical analytical conditions demand that polyphosphates be present in large excess (~1% w/v) with respect to the oxidant concentration (~0.5 mM).[2,3] Owing to their innocuous nature and the superior detection limits afforded in their presence, polyphosphates are the most commonly utilized enhancer of permanganate chemiluminescence. For this reason, we have also investigated the chemistry governing this enhancement mechanism to gain a more complete understanding of the processes that ultimately lead to the analytical utility of this important light-producing reaction.

Herein, we report the use of attenuated total reflection (ATR)-FT-IR, UV−vis, and electron paramagnetic resonance (EPR) spectroscopies together with synthetic chemistry in conjunction with existing experimental evidence to deduce the mechanism of acidic potassium permanganate chemiluminescence and its enhancement in the presence of polyphosphates.

MATERIALS AND METHODS

Reagents. Potassium permanganate, manganese(II) sulfate monohydrate, and sodium dihydrogen orthophosphate were from Ajax (Auburn, NSW, Australia). Ascorbic acid, sodium hexametaphosphate (96%), sodium borohydride, and sulfuric acid were from BDH (Poole, England). Morphine was from GlaxoSmithKline (Port Fairy, Vic., Australia). Dilutions were performed with deionized water (Millipore, Milli-Q Water System, Bedford, MA).

Preparation of Solutions. The permanganate chemiluminescence reagent (0.5 mM) was prepared by dissolving $KMnO_4$ in deionized water with 1% w/v sodium hexametaphosphate and adjusting the solution to pH 2.0 with 2 M H_2SO_4. Manganese(III) (0.5 mM) was prepared by dissolving manganese(II) sulfate monohydrate in an aqueous solution of sodium hexametaphosphate (1% w/v) and adjusting the pH to 2.0 with H_2SO_4 prior to and again after the addition of sufficient $KMnO_4$ to form the characteristic pink color of manganese(III). The concentration was determined by titration, and the solution was diluted as required.

UV−vis and ATR-FT-IR Spectroscopy. UV−vis spectra were acquired using a Cary 300 UV−vis absorption spectrophotometer (Varian) at a scan rate of 200 nm/min using 1 nm band widths and quartz cuvettes (1 cm path length). IR spectra were collected with a Perkin-Elmer System 2000 Fourier Transform Infrared (FT-IR) spectrometer equipped with a SPECAC model 11900 variable angle attenuated total reflection accessory and a Germanium Internal Reflective Element set at a 45° angle of incidence. Spectra were collected at a resolution of 4 cm^{-1} and averaged over 256 scans.

Electron Paramagnetic Resonance (EPR) Spectroscopy. EPR spectra were collected with a Bruker Elexsys E580 continuous wave spectrometer fitted with an X-band Super HighQ cavity. The continuous flow manifold consisted of two lines, one containing the reagent solution and the other containing the substrate. Both solutions were pumped using a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, Balwyn, Vic., Australia) with PVC pump tubing (DKSH, Caboolture, Qld, Australia) into PTFE tubing at a flow rate of 4.3 mL/min (per line) before merging and entering an AquaX cell placed in the microwave cavity. A Bruker ER036 Teslameter and Bruker microwave frequency counter were used for calibration of the magnetic field and microwave frequency, respectively. Low temperatures (1.8 K) were obtained using an Oxford ESR910 flow through cryostat in conjunction with an Oxford instruments ITC-503 temperature controller. Bruker's Xepr software was used for spectrometer tuning, signal averaging, and plotting. Computer simulation of the EPR spectra and generation of energy level diagrams and transition roadmaps were carried out with Molecular Sophe[18] (MoSophe, v2.1.4) running on a personal computer with the Mandriva 2009.1 linux operating system.

Chemiluminescence Spectra. Spectra were measured on a Cary Eclipse spectrofluorimeter (Varian, Mulgrave, Vic., Australia) fitted with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan). A two-line continuous flow assembly was employed (flow rate 3 mL/min for each line), whereby an integrated glass Y-piece and spiral flow cell (0.5 mm i.d., 90 μL volume, Embell Scientific, Murwillumbah, NSW, Australia) was positioned in front of the emission window of the instrument. Spectra were smoothed using a Savitzky-Golay function (10 points). Emission spectra were corrected for the wavelength dependence of the detector response and monochromator transmission by multiplication with a correction factor that was established with a quartz-halogen tungsten coiled lamp (45 W) of standard spectral irradiance (OL245, Optronics Laboratories, Orlando, FL). The lamp was operated at 6.5 A DC, which was supplied by a programmable constant current source (OL65A, Optronics Laboratories).

RESULTS AND DISCUSSION

Nature of the Excitation Source. Reaction of Mn(III) with a suitably powerful reductant ($NaBH_4$) has been shown to produce the same corrected chemiluminescence emission spectra as the tetra- and heptavalent manganese species.[19] Thus, it seems plausible that a one electron reduction of Mn(III) produces the Mn(II)* excited state. A candidate electron source could be a radical substrate oxidation product, and to test this postulate, electron paramagnetic resonance (EPR) spectroscopy was employed to monitor the reaction conducted under analytical conditions in real time. A benchmark analyte in terms of the amount of light produced upon reaction with permanganate is the opiate alkaloid morphine.[20] It can routinely be detected at subnanomolar levels, and so, when present at millimolar concentrations, we thought it reasonable that its radical oxidation product would be present at high enough concentrations to be detected and characterized by EPR (if indeed radical species are functioning as an excitation source). We have employed continuous flow EPR spectroscopy in conjunction with a manifold to mix the reactant solutions immediately prior to entering the AquaX cell in the microwave cavity. An aqueous solution of potassium permanganate (0.5 mM, pH 2) was mixed

(17) McCullough, J. F.; Vanwazer, J. R.; Griffith, E. J. *J. Am. Chem. Soc.* **1956**, *78*, 4528–4533.

(18) Hanson, G. R.; Noble, C. J.; Benson, S. Biological Magnetic Resonance. In *High Resolution EPR: Applications to Metalloenzymes and Metals in Medicine*; Hanson, G. R., Berliner, L. J., Eds.; Springer: New York, 2009; Vol. 28, pp 105−173.

(19) Anastos, N.; Barnett, N. W.; Hindson, B. J.; Lenehan, C. E.; Lewis, S. W. *Talanta* **2004**, *64*, 130–134.

(20) Abbott, R. W.; Townshend, A.; Gill, R. *Analyst* **1986**, *111*, 635–640.

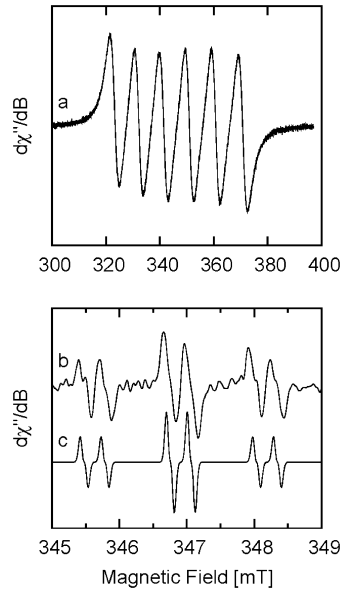


Figure 1. EPR spectra of a solution containing MnO_4^- (0.5 mM), sodium hexametaphosphate (1% w/v), and morphine (1 mM). The morphine was mixed with the other components before entering the microwave cavity in a continuous flow assembly. Details of the continuous flow assembly are given in the Materials and Methods section. (a) Experimental spectrum recorded using a modulation amplitude of 0.4 mT, microwave power of 16 mW, $\nu = 9.7557$ GHz. (b) Experimental spectrum recorded using a modulation amplitude of 0.08 mT, microwave power of 16 mW, $\nu = 9.7557$ GHz, and (c) computer simulation of (b) using the spin Hamiltonian parameters given in the text.

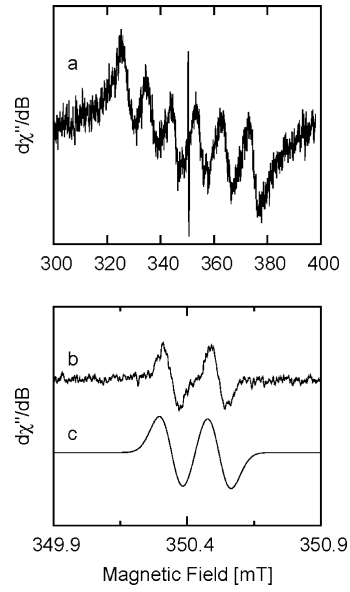
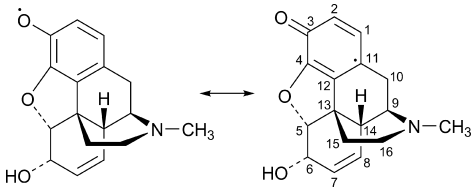


Figure 2. EPR spectra of a solution containing MnO_4^- (0.5 mM), sodium hexametaphosphate (1% w/v), and ascorbic acid (1 mM). The ascorbic acid was mixed with the other components before entering the microwave cavity in a continuous flow assembly. Details of the continuous flow assembly are given in the Materials and Methods section. (a) Experimental spectrum recorded using a modulation amplitude of 0.4 mT, microwave power of 20 mW, $\nu = 9.8345$ GHz. (b) Experimental spectrum recorded using a modulation amplitude of 0.07 mT, microwave power of 39 mW, $\nu = 9.8345$ GHz, and (c) computer simulation of (b) using the spin Hamiltonian parameters given in the text.

Scheme 1. Proposed Phenoxyl Radical and Carbon Centered Resonance Form Generated upon Reaction of Morphine with Permanganate



in the cavity with an aqueous solution of morphine (1 mM), and initially, we examined a wide field range (modulation amplitude 0.4 mT) to see whether we could observe resonances from Mn(III). However, the only resonances observed were those from the |±1/2> Kramers doublet arising from high spin Mn(II) (Figure 1a). Recording the spectrum around $g = 2$, with a smaller modulation amplitude (0.08 mT), enabled the resolution of smaller superhyperfine splittings and, in conjunction with substantial signal averaging (63 scans), produced the spectrum of the oxidized morphine radical (Figure 1b).

Computer simulation of the spectrum using the Molecular Sophe computer simulation software suite[18] and the spin Hamiltonian parameters (presented below) yields the spectrum shown in Figure 1c characteristic of a carbon centered resonance form of the phenoxyl radical outlined in Scheme 1. This triplet of doublets is centered at $g = 2.00912$ with the splitting pattern arising from hyperfine coupling to two diastereotopic C10 protons ($a^H = 12.0 \times 10^{-4}$ cm^{-1}) which appear magnetically equivalent in the EPR spectrum and further coupling to the C1 proton ($a^H = 2.9 \times 10^{-4}$ cm^{-1}) and C2/C9 protons ($a^H = 0.57 \times 10^{-4}$ cm^{-1}). Given that, to the best of our knowledge, this is the first reported EPR spectrum of a morphine radical, a comparison of the coupling constants could not be made.

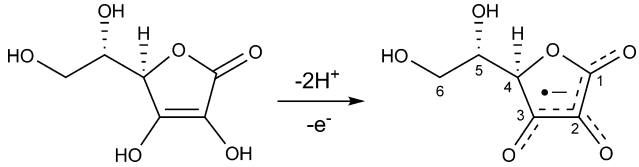
Although the chemiluminescent oxidation of phenols (such as morphine) by permanganate is well-known,[2] this reagent also elicits an analytically useful response upon reaction with certain substrates that do not bear this functionality. If our earlier postulation that a one electron reduction of the manganese(III) precursor generates the emitting species was correct, a nonphenolic radical analyte oxidation product should also be detectable by EPR spectroscopy. L-Ascorbic acid was ideally suited to test this because it does not contain a phenol, it can be routinely determined by permanganate chemiluminescence well below micromolar concentration levels,[19] and its EPR spectrum has been known for 50 years[21] and is comprehensively characterized.[22] The only resonances observed over a large field range were those from the |±1/2> Kramers doublet arising from high spin Mn(II) (Figure 2a) and the ascorbyl radical (Figure 2b) generated under identical conditions to those used to produce the morphine radical.

The observed doublet,[18,21] centered at $g = 2.00512$ with $a^H = 1.66 \times 10^{-4}$ cm^{-1}, is characteristic of the ascorbyl radical anion[21,22] where the electron is delocalized within the 1,2,3-tricarbonyl system and the splitting pattern is due to coupling to the C4 proton (Scheme 2).

By reacting ascorbate with dissolved oxygen, Lagercrantz[23] was the first to resolve the ascorbyl radical anion proton hyperfine structure which split each line of the doublet into sets of triplets

(21) Yamazaki, I.; Mason, H. S.; Piette, L. *J. Biol. Chem.* **1960**, *235*, 2444–2449.
(22) Laroff, G. P.; Fessende, Rw; Schuler, R. H. *J. Am. Chem. Soc.* **1972**, *94*, 9062–9073.
(23) Lagercrantz, C. *Acta Chem. Scand.* **1964**, *18*, 56.

Scheme 2. Oxidation of Ascorbic Acid to Generate the Ascorbyl Radical Anion



arising from coupling to the C5 proton. The hyperfine structure was not resolved here which is common for chemical generation of this transient species under aerobic conditions at ambient temperatures.[24] In an important paper, and of consequence to this study, Laroff et al.[22] demonstrated that this radical anion persisted even at pH 0 which supports our assignment at pH 2 (Scheme 2).

Mechanism of Phosphate Mediated Enhancement of Chemiluminescence. *Stabilization of Reaction Intermediates.* Under certain conditions, the reduction of permanganate to bivalent manganese is known to proceed via hexa-, penta-, tetra-, and trivalent intermediates.[4] Although manganese(V) and manganese(VI) species have been directly detected spectrophotometrically during the alkaline oxidation of sulfite by permanganate,[25] they are known to be highly unstable in acidic media[26] and so their level of participation in the chemiluminescence light producing pathway (~pH 2) was judged to be negligible. To compliment the findings outlined above, we have investigated the manganese(III) intermediate as an immediate precursor to the characterized manganese(II) emission source while exploring the mechanism of phosphate mediated enhancement. We note here that, under mildly acidic conditions, trivalent manganese shows a propensity to disproportionate to more stable oxidation states[27] according to eq 1:

$$2\mathrm{Mn(III)}_{(aq)} \rightleftharpoons \mathrm{Mn(IV)}_{(aq)} + \mathrm{Mn(II)}_{(aq)} \qquad (1)$$

However, manganese(III) can be stabilized by a number of ways including: (i) increased acidity; (ii) increased manganese(II) concentrations; and (iii) the presence of complexing agents.[27] A large molar excess of pyrophosphate over manganese(III) has been shown on numerous occasions[26,28−30] to effectively "trap" this species and prevent its disproportionation during the permanganate reduction pathway.

Figure 3 shows the UV−vis absorption spectra of the products from the oxidation of manganese(II) sulfate (3.3 mmol) by potassium permanganate (0.158 mmol) in acidic solution (0.25 L, pH 2, with (Figure 3A) and without (Figure 3B) sodium hexametaphosphate (1% w/v). The spectrum collected when the reaction was performed in the presence of hexametaphosphate shows a prominent absorption band centered at 510 nm which is in

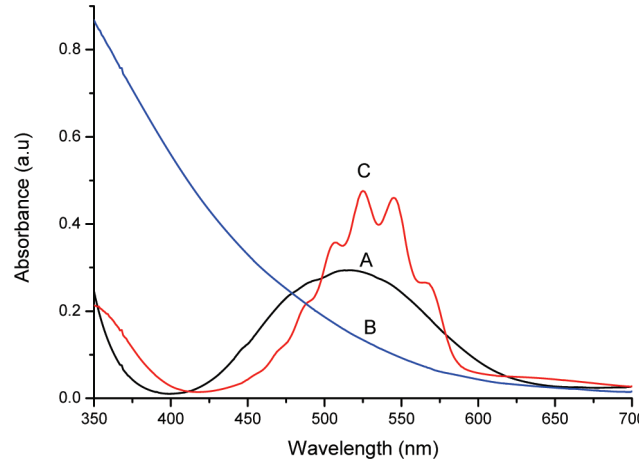


Figure 3. UV−vis absorption spectra of the manganese chemiluminescence reagents prepared (A) with and (B) without 1% (w/v) sodium hexametaphosphate. Spectrum (A) is typical of the formation of manganese(III) while (B) indicates the preferred formation of manganese(IV). Spectrum (C) is the absorption spectrum of the manganese(VII) reagent (after ~5-fold dilution). Comparison of (A) and (C) shows that the absorbance due to unreacted permanganate is negligible in spectrum (A). For more details see text.

agreement with the published spectra of the pyrophosphonatomanganese(III) complex.[31] Careful comparison of this spectrum (Figure 3A) with that of the permanganate chemiluminescence reagent (Figure 3C) shows that the contribution due to unreacted manganese(VII) was negligible. In the absence of polyphosphate enhancers, hydrated manganese(IV) oxide species are formed and the solution has the characteristic murky yellow-brown color of colloidal MnO_2.[8] The UV−vis spectrum of this solution (Figure 3B) is typical of colloidal manganese(IV), with an absorbance maximum at around 300 nm (results not shown) that gradually decreases with increasing wavelength.[8] This spectrum results from a combination of the absorbance of MnO_2 at the surface of the aggregates together with Rayleigh scattering contributions of the floc where the magnitude of the latter term would depend on the size and concentration of these species. These absorption spectra also agree with those previously published for the manganese(VII)/(IV)/(III) chemiluminescence reagents.[14]

By inhibiting disproportionation of manganese(III), the polyphosphates are effectively facilitating an increased concentration of the species which, upon reaction with the radical analyte oxidation intermediate, generates the Mn(II)* emitter. This postulate is supported by a recent paper from our laboratory[32] where the addition of 0.6 mM manganese(II) to the 1 mM permanganate chemiluminescence reagent (with 1% w/v sodium hexametaphosphate) 24 h prior to reaction afforded up to a 70-fold increase in emission intensity. Of course, the forerunner of a trivalent species would be manganese(IV), and given its tendency to form insoluble oxides (Figure 3B), polyphosphates appear to be preventing flocculation, thus facilitating their further reduction. When the two aforementioned solutions were reacted with $NaBH_4$, only the trivalent manganese reagent produced intense chemiluminescence (manganese(IV) afforded a relative emission intensity of 0.05%). This result indicates that when the formation

(24) Zhang, L. B.; Lay, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12624–12637.
(25) Simandi, L. I.; Jaky, M.; Schelly, Z. A. *J. Am. Chem. Soc.* **1984**, *106*, 6866–6867.
(26) Simandi, L. I.; Jaky, M. *J. Am. Chem. Soc.* **1976**, *98*, 1995–1997.
(27) Davies, G. *Coord. Chem. Rev.* **1969**, *4*, 199–224.
(28) Jaky, M.; Simandi, L. I.; Maros, L.; Molnarpe, I. *J. Chem. Soc.-Perkin Trans. 2* **1973**, 1565–1569.
(29) Simandi, L. I.; Jaky, M. *J. Chem. Soc.-Perkin Trans. 2* **1973**, 1856–1860.
(30) Jaky, M.; Simandi, L. I. *J. Chem. Soc.-Perkin Trans. 2* **1972**, 1481–1487.
(31) Diebler, H.; Sutin, N. *J. Phys. Chem.* **1964**, *68*, 174–180.
(32) Slezak, T.; Terry, J. M.; Francis, P. S.; Hindson, C. M.; Olson, D. C.; Wolcott, D. K.; Barnett, N. W. *Anal. Chem.* **2010**, *82*, 2580–2584.

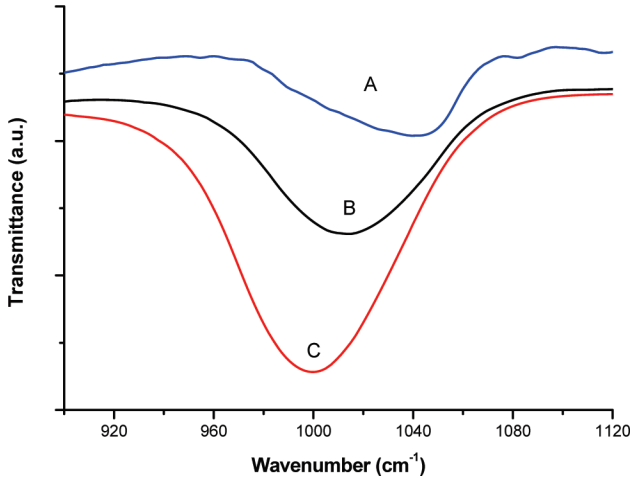


Figure 4. ATR-FT-IR spectra of (A) 1% w/v sodium hexametaphosphate adjusted to pH 2.0 using H_2SO_4, with (B) 0.5 mM manganese(II) or (C) 0.5 mM manganese(III).

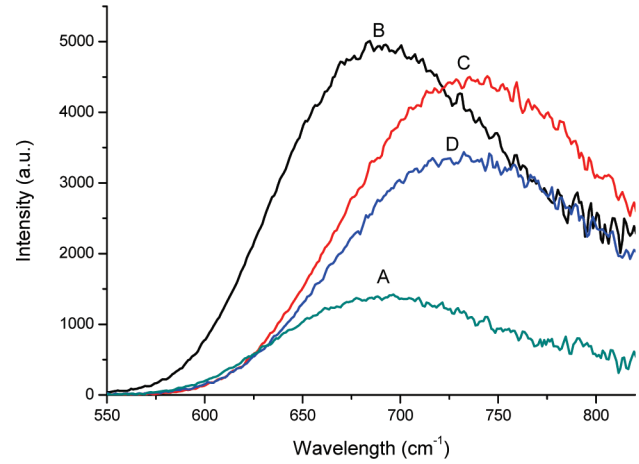


Figure 5. Corrected chemiluminescence emission spectra from the reaction between (A) redissolved manganese(III)−polyphosphate coacervate and 1 mM $NaBH_4$; (B−D) 0.5 mM $KMnO_4$ and 1 mM morphine with (B) 1% w/v sodium hexametaphosphate, (C) 3 M sodium pyrophosphate, or (D) 3 M sodium dihydrogen orthophosphate.

of insoluble manganese(IV) species is favored, i.e., in the absence of polyphosphates, even powerful reductants are not capable of efficiently producing the excited state, presumably because only the MnO_2 at the surface of the aggregate is available for further reduction. This reasoning is consistent with the requirement of 3 M H_3PO_4 to solubilize manganese(IV) when it is prepared as a chemiluminescence reagent.[16] Scattering contributions of the floc could also see a diminished amount of light reaching the detector. It has previously been noted that, in analytical applications of permanganate chemiluminescence without the use of polyphosphate enhancers, more acidic conditions are required to obtain the greatest emission intensity.[2] On the basis of the established chemistry of manganese in solution,[16,27] this can be attributed to stabilization of manganese(III) and solubilization of manganese(IV).

Manganese−Polyphosphate Coacervates. To assess the possibility of a 2-fold phosphate mediated chemiluminescence enhancement mechanism, the formation of protective structures around the manganese(II)* excited state was investigated. Figure 4A shows the ATR-FT-IR spectrum of the aqueous sodium hexametaphosphate solution (1% w/v, pH 2) without manganese. The stretching mode of nonbridging −PO groups of the linear phosphates[33,34] (Figure 4A: $\upsilon_s = 1092$ cm^{-1}) is shifted by the addition of bivalent manganese (0.5 mM, Figure 4B) and becomes more prominent when manganese(III) is present (0.5 mM, Figure 4C). This spectral change is consistent with the "cagelike" coordination sites observed for alkali earth metal polyphosphate coacervates at relatively low metal concentration.[35]

If such a species is forming during permanganate chemiluminescence, it should be possible to isolate the manganese(III)−polyphosphate coacervate that would be a precursor for the phosphate protected manganese(II)* emission source. Some 40 years ago, Griffith and Buxton[36] used ethanol to extract their newly synthesized 12-membered ring cyclohexaphosphate from the aqueous phase, and a similar protocol was applied here to a solution of the manganese(III) hexametaphosphate chemiluminescence reagent. The collected products were bright pink solids with no definitive structure after three recrystallizations. The amorphous nature of this species is not surprising given that commercial hexametaphosphate is a complex mixture of linear and cyclic phosphate oligomers[17] of which any number could be participating in the coacervation process. The UV−vis and ATR-FT-IR spectra of the redissolved product were indistinguishable from those recorded for the manganese(III) chemiluminescence reagent (Figures 3A and 4C, respectively), indicating that the species removed from the solution was indeed a manganese(III)−polyphosphate coacervate. To further confirm this, the redissolved solid was reacted with $NaBH_4$ (1 mM) which produced the characteristic blue-shifted corrected emission spectra of polyphosphate enhanced manganese chemiluminescence (Figure 5A, $\lambda_{max} = 689 \pm 5$ nm). Figure 5B−D shows the corrected emission spectra of the reaction between permanganate and morphine when mediated by an excess of phosphate species of differing chain lengths. As expected, the spectrum of the reagent solution containing hexametaphosphate (Figure 5B) was identical to that observed for the redissolved manganese(III)−polyphosphate compound, but the emission spectra of reactions in the presence of pyro- and orthophosphate (Figure 5C,D, respectively) were not blue-shifted ($\lambda_{max} = 734 \pm 5$ nm).

This finding is of particular importance when considering the operation of a 2-fold polyphosphate enhancement mechanism. As mentioned earlier, pyrophosphate prevents disproportionation of manganese(III)[26,28−30] and has been shown to increase the emission intensity of permanganate chemiluminescence,[20] albeit to a far lesser degree than hexametaphosphate. Preventing disproportionation effectively facilitates an increased concentration of the precursor to the emission source which we would expect to lead to an eventual increase in the amount of light produced by the reaction. However, given that the emission is not blue-shifted, we can assume that pyrophosphate is not interacting with the emitter in the same manner as the long chain phosphates; i.e., they cannot form protective structures and are, therefore, not

(33) Michelmore, A.; Gong, W. Q.; Jenkins, P.; Ralston, J. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2985–2992.
(34) Chapman, A. C.; Thirlwell, L. E. *Spectrochim. Acta* **1964**, *20*, 937–947.
(35) Dias, F. A.; Carlos, L. D.; Messadeq, Y.; Ribeiro, S. J. L. *Langmuir* **2005**, *21*, 1776–1783.
(36) Griffith, E. J.; Buxton, R. L. *Inorg. Chem.* **1965**, *4*, 549–551.

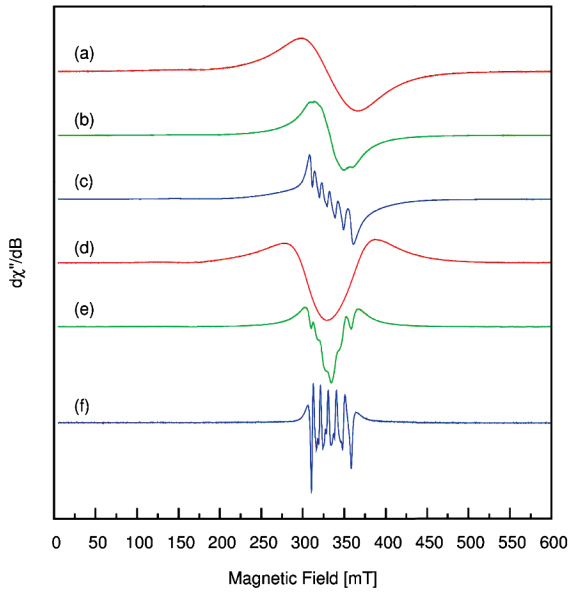


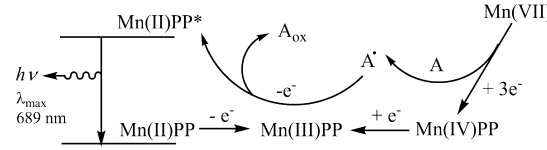
Figure 6. Frozen solution (1.8 K) EPR spectra of solutions of Mn(II) (0.5 mM) and hexametaphosphate. First derivative spectra of (a) aqueous Mn(II), $\nu = 9.3744$ GHz, (b) aqueous Mn(II) and 0.1% (w/v) sodium hexametaphosphate, $\nu = 9.3776$ GHz, and (c) aqueous Mn(II) and 1% (w/v) sodium hexametaphosphate, $\nu = 9.7639$ GHz. Second derivative spectra of these solutions are shown in (d−f), respectively.

as effective at enhancing the reaction. This suggestion is supported by the results of a previous investigation[14] that compared the level of enhancement afforded by different strong complexing agents including EDTA, methylenediphosphonic acid, nitrilotri(methylenephosphonic acid), iminodi(methylenephosphonic acid), and pyridine-2,6-dicarboxylic acid. Each resulted in relative emission intensities that were comparable to orthophosphate with a further 50-fold increase in emission intensity being unique to hexametaphosphate.

While the preceding experiments confirm the formation of a manganese(III)−polyphosphate coacervate under identical conditions to those used for permanganate chemiluminescence, they do not indicate that their formation facilitates the enhancement of light emission. Because the final products of permanganate chemiluminescence reactions are the oxidized analyte and ground state manganese(II), it was considered desirable to ascertain if a manganese(II)−polyphosphate coacervate was readily formed under typical analytical conditions. This would establish that the immediate precursor and successor of the emitting species were capable of forming these molecular encapsulated entities, thus providing convincing evidence for the existence of a similarly protected manganese(II) excited state. Although attempted, the same experimental procedure used above to demonstrate the formation of manganese(III) coacervates was not conclusive. The redissolved solids could not be analyzed by UV−vis while maintaining the same metal to phosphate ratio used for chemiluminescence because bivalent manganese does not absorb strongly at these wavelengths and must, therefore, be present at much higher concentrations than deemed appropriate here.

With this in mind, 0.5 mM manganese(II) solutions prepared at pH 2.0 were frozen and analyzed using EPR spectroscopy. The first (Figure 6a−c) and second derivative (Figure 6d−f) low

Scheme 3. Postulated Mechanism for Permanganate Chemiluminescence in the Presence of Polyphosphates



temperature (1.8 K) X-band EPR spectra show the effect of increasing hexametaphosphate concentration. The manganese(II) spectrum (Figure 6a) measured in the absence of polyphosphates reveals a broad featureless resonance centered at $g = 2.033$ with a peak to peak line width of 66.4 mT. The lack of resolved manganese hyperfine coupling ($I = 5/2$) results from dipolar broadening due to solute aggregation within the frozen aqueous solution.[37] Previous studies[37,38] have shown that this effect can be overcome with the introduction of experimentally inert solutes. Bearing this in mind, we would expect the resolution of manganese hyperfine coupling upon the addition of polyphosphates, stemming from the formation of manganese−polyphosphate complexes or clathrates. Increasing the concentration of sodium polyphosphates from 0% to 0.1% and finally to 1% (w/v) (Figures 6a,b,c and 6d,e,f) show increasing resolution of the manganese hyperfine coupling. At a sodium polyphosphate concentration of 1% (w/v), the level used for routine chemiluminescence analysis, the manganese hyperfine coupling is almost completely resolved, with only minimal manganese−manganese dipolar broadening[37] present. The six hyperfine resonances arise from allowed transitions within the |±1/2> Kramers doublet of the high spin ($S = 5/2$) Mn(II) species. The second derivative spectra (Figure 6f) reveals additional resonances of weaker intensity which have been attributed to the forbidden $\Delta M_I = \pm 1,2,3$ transitions which, at low temperatures, are known to become more prominent with increased additive concentrations.[38] A weak resonance around $g_{eff} = 4.3$ is also apparent in the EPR spectra (results not shown) of aqueous Mn(II) and Mn(II) polyphosphate complexes measured at pH 2 and presumably arises either from a formally forbidden $\Delta M_S = \pm 2$ transition within the |±1> Kramers doublet or from an interdoublet transition.

These findings compliment two recent studies[35,39] which have shown that when the polyphosphate−metal ratio is greater than 6, as it is in Figure 6c,f, the metal centers are strongly coordinated to the oxygen atoms of the linear phosphate polyhedra which promotes the formation of "cagelike" structures. Altering this ratio by increasing the metal ion concentration causes saturation of the cage meaning the metal centers then also occupy external sites which in turn promotes cross-linking of the phosphate chains and complete coacervation. With respect to permanganate chemiluminescence, we suggest that the reason optimized reaction conditions demand such a large excess of polyphosphate over manganese is to facilitate the formation of these protective cages and maintain their structural integrity throughout the reaction.

CONCLUSIONS

In light of the findings outlined above, the mechanism for acidic potassium permanganate chemiluminescence is depicted

(37) Ross, R. T. *J. Chem. Phys.* **1965**, *42*, 3919–3922.
(38) Allen, B. T.; Nebert, D. W. *J. Chem. Phys.* **1964**, *41*, 1983–1985.
(39) Silva, M. A. P.; Franco, D. F.; de Oliveira, L. F. C. *J. Phys. Chem. A* **2008**, *112*, 5385–5389.

in Scheme 3. For simplicity, we have shown the mechanism for the starting oxidant, heptavalent manganese.

Initial reaction of the substrate (A) with manganese(VII) generates a radical intermediate ($A^{\bullet}$), as shown in the EPR spectra of the oxidized analyte (Figures 1 and 2). UV−vis (Figure 3A) and ATR-FT-IR (Figure 4C) spectra show that reduction of Mn(VII) generates the polyphosphate stabilized[26,28−30] trivalent manganese species Mn(III)PP. The absence of MnO_2 floc indicates that Mn(IV) is also present as a coacervate. Further reduction of Mn(III)PP by $A^{\bullet}$ generates the protected manganese(II) $^4T_1$ excited state Mn(II)PP* (Figure 5) and the oxidized substrate (A_{ox}). Relaxation to the $^6A_1$ ground state Mn(II)PP (Figures 1a, 2a, 4a, and 6) is accompanied by light emission centered at 689 ± 5 nm (Figure 5A,C). We are currently investigating the coordination sphere of the manganese polyphosphate centers of different valencies as permanganate reduction proceeds toward excitation and light emission, and the results of these experiments will be reported in due course.

ACKNOWLEDGMENT

The authors thank the Australian Research Council for funding this research.

Received for review February 9, 2010. Accepted March 29, 2010.

AC100363S