On-line generation of a colloidal manganese(IV) reagent for chemiluminescence detection

Zoe M. Smith, Jessica M. Terry, Christopher M. Hindson, Neil W. Barnett, Paul S. Francis *

Centre for Chemistry and Biotechnology, Faculty of Science and Technology, Deakin University, Locked Bag 20000, Geelong, Victoria 3220, Australia

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ABSTRACT

Chemiluminescence was observed from reactions with a Mn(IV) colloid generated by reduction of KMnO$_4$ with Na$_2$S$_2$O$_3$. Due to the limited stability of the colloid under acidic conditions and the poor reproducibility of the chemiluminescence signal when the reagent was prepared by published methods, an *in situ* (on-line) method of preparation utilising flow injection analysis methodology was developed. This approach provided superior emission intensities to the conventional Mn(IV) reagent, although less enhancement was obtained from the addition of formaldehyde. The selectivity of the reagent was 'tuned' towards specific classes of analyte through convenient modification of reaction conditions. Contrary to some previous reports, we attribute this chemiluminescence to the formation of an electronically excited Mn(II) species, based on direct comparison of the spectral distribution with that of other chemiluminescence reactions.

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1. Introduction

The chemiluminescence of 'soluble' Mn(IV) was published in 2001 by researchers in our laboratory [1,2]. These studies showed that the characteristic red emission observed upon reaction of this Mn(IV) colloid with a wide range of analytes emanated from an electronically excited Mn(II) species; the same emitter as that generated in reactions with acidic KMnO$_4$ [1–4]. Despite this common emitter, the selectivity of the two reagents is markedly different, as Mn(IV) generates an intense emission with a far broader range of analytes [1,5]. Since these initial investigations, the reagent has been used for the determination of many classes of compounds, such as alkaloids, antioxidants, pharmaceuticals and biologically significant thiols and disulfides [5–9].

The preparation of this Mn(IV) reagent is based on the method of Jáky et al. [10,11], involving the slow dissolution of freshly precipitated manganese dioxide (formed by the reduction of KMnO$_4$ with excess sodium formate) in 3 M orthophosphoric acid to yield a transparent brown colloidal suspension. The process is lengthy, requiring numerous steps (including filtration, sonication, heating, cooling, titration and dilution) which makes same-day analysis impractical. In addition, the reagent generally requires a formaldehyde enhancer in order to obtain analytically useful chemiluminescence signals [1,5]. This enhancer is undesirable due to its classification as a carcinogen, but no other compounds have been found to provide the same increase in sensitivity for most analytes [1,12].

An alternative method of preparing colloidal Mn(IV) was developed by Perez-Benito et al. [13], in which KMnO$_4$ is simply reduced by sodium thiosulfate (Na$_2$S$_2$O$_3$) under neutral conditions according to Eq. (1):

$$8MnO_4^- + 3S_2O_3^{2-} + 2H^+ \rightarrow 8MnO_2 + 6SO_4^{2-} + H_2O \qquad (1)$$
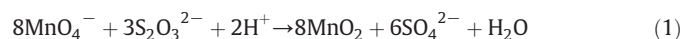
Mn(IV) sols prepared in this manner have been used to investigate the kinetics of oxidation reactions with various organic compounds (such as formic acid, lactic acid, oxalic acid and L-tryptophan) [14–18]. In a doctoral thesis on the application of manganese-based chemiluminescence reagents to detect food components, Agater demonstrated that the reaction of glucose or fructose with this Mn(IV) sol produced sufficient light to detect the sugars over the range of 5×10^{-4} M to 0.1 M [19]. Flow injection analysis methodology was used to insert the analyte into an acidic carrier solution that merged with the Mn(IV) colloid. The emission from these reactions was found to have the same spectral distribution as those of related chemiluminescence reactions with permanganate [19].

In similar, more recent studies, Du and Wang applied this Mn(IV) sol to the chemiluminescence determination of ascorbic acid [20], perphenazine and chlorpromazine [21], using flow injection analysis methodology to acidify the colloid and then merge it with analyte and enhancer solutions. In spite of the substantial evidence for a Mn(II) emitter formed in many closely related reactions with KMnO$_4$ [4,22] and the conventional Mn(IV) colloidal reagent [2,5], these authors ascribed the emission to the generation of singlet molecular oxygen [20,21]. Herein we describe the on-line generation of colloidal Mn(IV) for chemiluminescence detection in flow injection analysis and high-performance liquid chromatography. This approach

enables the convenient manipulation of reaction conditions, which we found to significantly influence the selectivity of the reagent. We also present spectroscopic evidence regarding the nature of the emitting species.

2. Experimental

2.1. Chemicals and reagents

Deionised water (Continental Water Systems, Victoria, Australia) and analytical grade reagents were used unless otherwise stated. Chemicals were obtained from the following sources: epinephrine hydrochloride, gallic acid, ofloxacin, papaverine hydrochloride, sodium formate, sodium polyphosphate (+80 mesh), synephrine, thiosemicarbazide, trifluoroacetic acid, L-tryptophan and L-tyrosine from Sigma-Aldrich (New South Wales, Australia); formaldehyde (37% w/w), orthophosphoric acid (85% w/w) and $KMnO_4$ from Chem-Supply (South Australia, Australia); cocaine hydrochloride, codeine, morphine, noscapine, oripavine and thebaine from GlaxoSmithKline (Victoria, Australia); aqueous soluble starch, manganese(II) sulfate monohydrate and vanillin from Ajax Finechem (New South Wales, Australia); ascorbic acid and caffeine from BDH (Poole, England); methanol and sulfuric acid from Merck (Victoria, Australia) and salbutamol hemisulfate from Fluka (New South Wales, Australia). Stock solutions of analytes (1×10^{-3} M) were prepared in deionised water. Sulfuric acid (~10 drops in 250 mL) was added to the solutions of codeine, epinephrine, morphine, noscapine, oripavine and thebaine to aid dissolution. The stock solutions were diluted daily with deionised water to obtain working concentrations.

The acidic colloidal Mn(IV) reagent was prepared as previously described [23], based on the method of Jáky and co-workers [10,11]. Freshly precipitated MnO_2, obtained *via* the reduction of $KMnO_4$ with excess sodium formate was collected by vacuum filtration and washed with deionised water. Subsequently, 0.6 g of the wet material was added to 500 mL of orthophosphoric acid (3 M) and sonicated for 30 min. The colloid was heated at 80 °C for 1 h, cooled to room temperature and the concentration determined by iodometric titration. The stock Mn(IV) reagent was diluted daily to the required concentration (5×10^{-4} M) using orthophosphoric acid (3 M).

In initial experiments, the alternative colloidal Mn(IV) reagent (5×10^{-4} M) was prepared as described by Perez-Benito et al. [15]. Solutions of $KMnO_4$ (0.1 M, 1.25 mL) and $Na_2S_2O_3$ (1.88×10^{-2} M, 2.5 mL) were added to deionised water with stirring, and the mixture diluted to 250 mL.

For comparison of reagent selectivity, the acidic $KMnO_4$ reagent was prepared by dissolving the oxidant (1×10^{-3} M) in a solution of 1% (m/v) sodium polyphosphate before adjusting to pH 2.5 by dropwise addition of H_2SO_4.

2.2. Instrumentation

The flow injection analysis manifolds (Fig. 1) were constructed from a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, NSW, Australia) with bridged PVC pump tubing (white/white, 1.02 mm i.d., DKSH, Queensland, Australia), PTFE manifold tubing (0.8 mm i.d., Cole-Parmer Instrument Company, Illinois, USA) and a six-port injection valve (Vici 04W-0192L Valco Instruments, Texas, USA) equipped with a 70 μL sample loop. A custom built flow-cell (a tight coil of 0.8 mm i.d. PTFE tubing) was mounted flush against an extended range photomultiplier tube (Electron Tubes model 9828SB, ETP, NSW, Australia) and encased in a light-tight housing. The output signal from the detector was obtained using an e-corder 410 data acquisition system (eDAQ, NSW, Australia). Following analysis each day, the lines were flushed with a solution of manganese(II) sulfate in 3 M orthophosphoric acid and then with deionised water to remove any residual Mn(IV) from the manifold.

Chromatographic separations were carried out on an Agilent Technologies 1200 series liquid chromatography system, equipped with a quaternary pump, solvent degasser system and autosampler (Agilent Technologies, Victoria, Australia), using a reversed phase
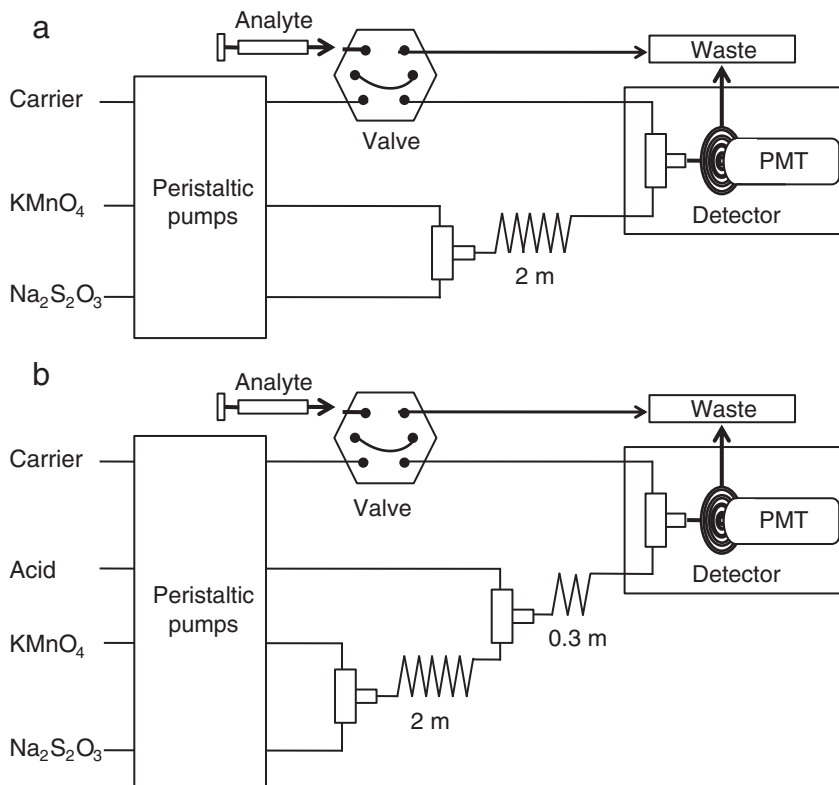

Fig. 1. Flow injection analysis manifolds.