Tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence enhanced by silver nanoparticles

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Mixtures of silver(I) and citrate that are used to produce silver nanoparticles evoke intense chemiluminescence with tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV), which can be exploited for the determination of citrate ions and other analytes over a wide concentration range.

The use of metal ion catalysts and, more recently, nanoparticle materials in chemiluminescence reactions have provided new avenues to enhance the inherent sensitivity and expand the analytical applications of this mode of detection. The oxidation of luminol with hydrogen peroxide has traditionally been catalysed by peroxidases or transition metal ions such as cobalt(II) or copper(II), but the catalytic action of nanoparticles on this chemiluminescence reaction has been exploited to determine colloidal ferric hydroxide and a range of organic compounds that readily react with nanogold.[1] DNA hybridisation and functionalised gold- and silver-nanoparticle labels for immunoassay have been quantified at exceedingly low levels with luminol chemiluminescence, but the nanoparticles were dissolved to ions prior to detection.[2] Direct chemiluminescence reactions of gold nanoparticles with acidic permanganate, alkaline periodate and carbonate, or bis(2,4,6-trichlorophenyl)oxalate and hydrogen peroxide have also been examined,[3] but not yet applied to analysis.

Tris(2,2′-bipyridyl)ruthenium(II) is an extremely versatile and widely used chemiluminescence reagent, particularly for the detection of tertiary amines and carboxylic acids.[4] Several innovative approaches for the incorporation of this reagent into electrode surfaces involving nanoparticle materials have been reported[5,6] and it was recently found that the electro-chemiluminescence determination of pentoxyverine with tris(2,2′-bipyridyl)ruthenium(II) was considerably enhanced by the addition of colloidal gold to the detection cell.[7]

We have examined the chemiluminescence evoked by reactions between tris(2,2′-bipyridyl)ruthenium(II), cerium(IV) and nanoparticle materials, using flow-injection analysis.† Silver and gold nanoparticles prepared by adding sodium citrate to heated solutions of appropriate metal ions‡ produced an intense emission with these reagents and could be detected down to 0.038 and 0.13 mg of metal per litre, respectively. However, far less chemiluminescence was detected from the reaction with silver nanoparticles that were isolated from citrate with sodium carbonate[8] or synthesised by reducing silver ions with dimethylformamide instead of citrate.[9]

A comparison of the chemiluminescence response from silver nitrate and sodium citrate solutions and mixtures of the two components (both before and after heating to promote the formation of nanoparticles) revealed the strong enhancement by silver nitrate of the chemiluminescence reaction between citrate, tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) (Table 1). No enhancement was observed when silver nitrate was replaced by other nitrate salts. Silver acetate gave a similar enhancement to silver nitrate, but silver permanganate was much less effective and produced a brown precipitate on mixing with tris(2,2′-bipyridyl)ruthenium(II).

The chemiluminescence reaction between citrate, tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) (without silver nitrate) is relatively slow, but has previously been used to determine citrate by measuring the emission over an extended period of time in stopped-flow or batch reaction cells.[10,11] The mechanism of this reaction is thought to involve oxidation of both citrate and tris(2,2′-bipyridyl)ruthenium(II) with cerium(IV) to form an intermediate radical and tris(2,2′-bipyridyl)ruthenium(III), which react to produce the original ruthenium complex in an electronically excited state that emits light at 610 nm.[10] In support of this proposal, we observed that the direct reaction of citrate with tris(2,2′-bipyridyl)ruthenium(III) – prepared by oxidising tris(2,2′-bipyridyl)ruthenium(II) with solid lead dioxide and filtering off the excess oxidant – evoked an emission that was far less intense than that detected when cerium(IV) was used as the oxidant.

There is considerable interest in the development of new methods to determine citric acid (or citrate ion), due to the frequent use of this compound in food and pharmaceuticals, and its role in cellular oxidative metabolism.[13,14] To determine citrate using flow-injection analysis,† the length of tubing between the final confluence point and the detection coil was optimised (46 cm) to capture the maximum emission from the reacting solution. Under these conditions, the relative standard deviation for replicate injections of 1 × 10^{-6} M sodium citrate was 2.0% and the limit of detection (4 × 10^{-9} M) was superior to previously reported approaches for the determination of citrate based on tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence,[10–12] and all

Table 1 Chemiluminescence response from reaction with tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV)

Silver nitrate	0.08 V
Sodium citrate	0.9 V
Mixture of sodium citrate and silver nitrate	2.3 V
Mixture after heating and cooling	12.4 V

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flow-injection analysis methods discussed in a recent review on the determination of citric acid.[13]§

Two-fold dilution of the sodium citrate solutions with silver nitrate (5×10^{-3} M) prior to injection did not have a significant effect when a 46 cm mixing tube was used, but with shorter mixing lengths the intensity was enhanced, particularly for the more concentrated citrate solutions, which extended the linear range from around one order to over three orders of magnitude (Fig. 1). To demonstrate the analytical utility of this system, we quantified citrate in a commercially available urinary alkaliniser (Citravescent®, Rhône–Poulenc Rorer Australia) without the dilution steps required in methods that did not involve silver nitrate enhancement. Calculated values were within 2% of that stated on the product. As the addition of silver nitrate removed the need for prolonged reactions times[10,11] and sample dilution steps, the overall analysis time was reduced. Problems associated with the instability of tris(2,2′-bipyridyl)ruthenium(III)[15] were avoided by generating the reagent on-line through the oxidation of tris(2,2′-bipyridyl)ruthenium(II) with cerium(IV).

Silver(I) ions have been shown to catalyse the oxidation of various carboxylic acids with cerium(IV), *via* the formation of 1 : 1 complexes between silver(I) and substrate.[16] The enhancement of the chemiluminescence from reactions between citrate, tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) could therefore be attributed to the catalytic effect of silver(I) ions on the oxidation of citrate with cerium(IV). The spectral distribution of the chemiluminescence from the enhanced reaction matched that of the reaction without silver nitrate. An examination of the chemiluminescence intensity–time profiles for this reaction in the presence and absence of silver nitrate, using stopped-flow instrumentation, confirmed that the enhancer increased the rate of light production.

However, silver nitrate also enhanced the direct reaction between citrate and tris(2,2′-bipyridyl)ruthenium(III) in the absence of cerium(IV), when the reagent was prepared by off-line oxidation with lead dioxide. Silver(I) ions may also catalyse the oxidation of citrate by tris(2,2′-bipyridyl)ruthenium(II) *via* a silver citrate complex, but an examination of solutions containing silver(I) and citrate using dynamic light scattering revealed the initial formation of nanoparticles with an average size of 19 nm, even immediately after mixing at room temperature.¶ The mechanism of silver nitrate enhancement on chemiluminescence reactions between citrate, tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) thus appears to involve a combination of (a) complexes formed between silver(I) and citrate, which increase the rate of oxidation by cerium(IV), and (b) the initiation of citrate-capped silver nanoparticles, which provide a large catalytic surface. Electrostatic interactions between the negatively charged citrate-capped nanoparticles and the positively charged reagent also may decrease the ligand π–π* energy gap and raise the quantum efficiency of the reaction.[6,7] In relatively concentrated solutions, far greater enhancement was observed when the mixtures of sodium citrate and silver nitrate were heated to promote the formation of nanoparticles (Table 1), but heating was detrimental to the chemiluminescence response for most of the citrate calibration standards mixed with silver nitrate.

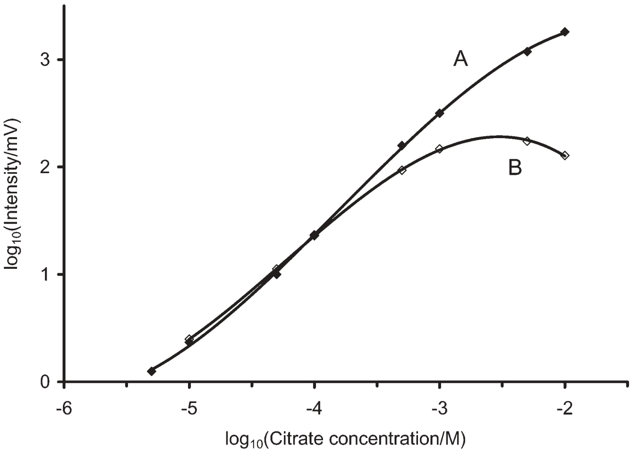

Fig. 1 Log–log calibration for citrate with flow-injection analysis using 1 cm mixing length: (A) enhanced by silver nitrate; (B) no enhancement.

Preliminary investigations with selected amino acids and organic acids that slowly react with tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) suggest that silver ions could be used to increase the sensitivity of detection and/or the analytical range of numerous other analytes. Solutions of glycine, proline and tartaric acid (5×10^{-5} M) that contained silver nitrate (2.5×10^{-3} M) elicited chemiluminescence with tris(2,2′-bipyridyl)ruthenium(II) and cerium(IV) that was approximately two orders of magnitude more intense than analyte solutions without silver nitrate. The presence of silver nitrate in a 5×10^{-5} M solution of ascorbic acid did not significantly enhance the chemiluminescence intensity, but – as with sodium citrate – enhancement was observed at higher analyte concentrations, which extended the analytical range for ascorbic acid by over an order of magnitude. This chemistry could also be exploited to apply tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence to new analytes, such as compounds that readily interact with citrate-capped nanoparticles.

Notes and references

† The flow-injection analysis manifold was constructed as previously described,[17] except that a coil of PTFE tubing (1.02 mm i.d.) was used as the detection cell. Samples were injected into a carrier line containing 1 mM $Ru(bipy)_3^{2+}$ in 50 mM H_2SO_4, which merged with 1 mM cerium(IV) sulfate in 50 mM H_2SO_4. All manifold tubing was 0.8 mm i.d. PTFE.

‡ Gold nanoparticles were synthesised by heating hydrogen tetrachloroaurate (0.3 mM, 50 mL) to reflux, adding sodium citrate (34 mM, 1.5 mL) and vigorously stirring for 15 min (until the solution became a burgundy red colour). Silver nanoparticles were prepared by heating silver nitrate (2 mM, 125 mL) to reflux and adding sodium citrate (1% w/v, 5 mL). Heating was continued until a yellow colloid was observed. The nanoparticles were used immediately after cooling.

§ In the review,[13] one method is erroneously cited as having a superior detection limit of '10^{-8} mM'. The limit of detection in the original paper was 10 pmol, which in the 10 μL injection volume corresponds to a concentration of 1×10^{-6} M.

¶ Dynamic light scattering was measured with a Malvern 4700 apparatus with 10 mW Ar^+ ion laser at 488 nm (90° angle; 25 °C). Multiple scattering and particle–particle interactions were negligible due to the dilute particle concentration in the samples. The time autocorrelation functions were analysed by an inverse Laplace transform algorithm, CONTIN, to obtain a distribution of diffusion constants. The diffusion coefficients were converted to hydrodynamic radii *via* the Stokes–Einstein equation using the viscosity of water as 0.89 mPa s at 25 °C.

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