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Effect of oxidant type on the chemiluminescence intensity from the reaction of tris(2,2′-bipyridyl)ruthenium(III) with various organic acids.

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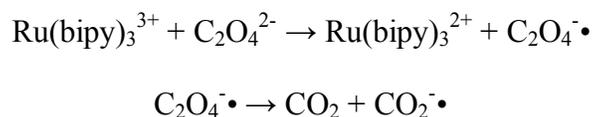
Abstract

An investigation into the chemiluminescence of fourteen organic acids and tris(2,2′-bipyridyl)ruthenium(II) was undertaken. Particular emphasis was placed upon the method of production of the reagent, tris(2,2′-bipyridyl)ruthenium(III), with cerium(IV) sulfate, potassium permanganate, lead dioxide and electrochemical generation. Analytically useful chemiluminescence was observed when Ce(IV) or potassium permanganate were employed as oxidants. The kinetics of analyte oxidation was related to the intensity of the chemiluminescence emission, which increased by three orders of magnitude for tartaric acid after forty hours of oxidation.
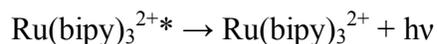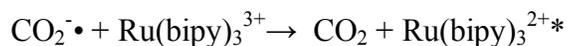
Keywords: tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence, organic acids, Ce(IV), electrochemiluminescence.

1. Introduction

Low-molecular mass organic acids are found in many natural and synthetic matrices.[1-4] They are often metabolites of biochemical pathways including the citric acid cycle, carbohydrate fermentation and ethanol oxidation.[5] Commonly, they are found in food products including juices[2], wine[2], honey[3] and also as vitamin supplements. Determination of organic acids in urine and other bodily fluids has facilitated the diagnosis of central nervous system diseases, neuroblastoma and nephrolithiasis.[5] Current analytical techniques include capillary electrophoresis[5, 6], HPLC[7, 8], gas chromatography[9] and enzymatic procedures.[10, 11] Various organic acids have been determined using tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence.[12-17] Due to the poor sensitivity and slow kinetics of these reactions this type of chemiluminescence has not been exploited or investigated with these analytes to the same extent as those containing amine moieties.[18, 19]

Electrochemical oxidation is by far the most common method used to generate tris(2,2′-bipyridyl)ruthenium(III). Seventy percent of papers published since 1998 on tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence have utilised this method of generation.[18] However, organic acid determinations have predominantly been achieved through the use of cerium(IV) sulfate as the oxidant.[13-15, 20] Rubinstein and Bard investigated the electrochemiluminescence of oxalate and some organic acids with tris(2,2′-bipyridyl)ruthenium(II)[21] and proposed the following reaction mechanism.

$$Ru(bipy)_3^{3+} + C_2O_4^{2-} \rightarrow Ru(bipy)_3^{2+} + C_2O_4^{-}\bullet$$

$$C_2O_4^{-}\bullet \rightarrow CO_2 + CO_2^{-}\bullet$$

$$CO_2^-\bullet + Ru(bipy)_3^{3+} \rightarrow CO_2 + Ru(bipy)_3^{2+}*$$

$$Ru(bipy)_3^{2+}* \rightarrow Ru(bipy)_3^{2+} + h\nu$$

Scheme 1: Electrochemiluminescence reaction mechanism for the reaction between oxalate and tris(2,2′-bipyridyl)ruthenium(III)[21]

These authors[21] also proposed that tris(2,2′-bipyridyl)ruthenium(III) did not have the oxidising strength to produce a radical intermediate from pyruvate and other organic acids.[21] However, upon the addition of the auxiliary oxidant Ce(IV) they postulated the following reactions:

$$Ce^{4+} + CH_3COCO_2^- \rightarrow Ce^{3+} + CH_3COCO_2\bullet$$

$$CH_3COCO_2\bullet \rightarrow CH_3CO\bullet + CO_2$$
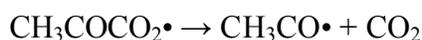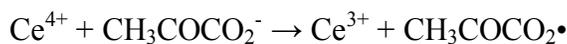
Scheme 2: Reaction of Cerium(IV) and pyruvate[21]

The final product in Scheme 2 ($CH_3CO\bullet$) is a strong reducing agent which reacts with tris(2,2′-bipyridyl)ruthenium(III) to produce the excited species in a similar fashion to the carbon dioxide anion radical in Scheme 1. Knight and co-workers[17] used Ce(IV) to determine pyruvate by electrochemiluminescence. Other groups employed Ce(IV) to oxidise tris(2,2′-bipyridyl)ruthenium(II) for the determination of oxalate[22], tartaric acid[15, 22], pyruvic acid[13, 15] and citrate[13] *via* simple chemiluminescence. All authors[13, 15, 22] reported a strong dependence upon sulfuric acid and cerium(IV) concentrations and noted that the reaction kinetics were different for each analyte.

To date, there has been no systematic investigation of the relationship between oxidant and analyte in tris(2,2′-bipyridyl)ruthenium(II) chemiluminescent reactions. In this work,

fourteen organic acids were subjected to oxidation both electrochemically and chemically in the presence of tris(2,2′-bipyridyl)ruthenium(II).

2. Experimental

2.1 Flow Injection Analysis

The FIA was constructed from a Gilson Minipuls^{TM} 3 Peristaltic pump (John Morris Scientific Pty. Ltd., Australia) and a six port injection valve (Valco Instruments Company, Model E60-220) using PVC pump tubing (1.02 mm i.d., Protech Group Pty. Ltd., Coolum Beach, QLD, Australia). A spiral PTFE tubing flow cell constructed in-house was positioned directly in front of a photomultiplier tube (Electron Tubes, UK) biased at 900 V (Thorn EMI power supply, Electron Tubes, UK). Signals were recorded on a strip chart recorder (Yokogawa Electric Works Ltd., Tokyo, Japan). The instrument was enclosed in a light-tight box made in-house.

Procedure 1

A simple two-line manifold was employed with a 70 μL injection loop. The organic acids (0.01 M) were injected into a running stream of tris(2,2′-bipyridyl)ruthenium(II) (0.1 mM), which merged with either cerium(IV) sulfate (1mM) or potassium permanganate (1 mM) at the point of detection.

Procedure 2

Cerium(IV) sulfate (10 mL of 1mM) was added to 20 mL of organic acid (0.01 M). Samples were taken at different time intervals and injected into a sulfuric acid (0.05 M) carrier stream. Tris(2,2′-bipyridyl)ruthenium(III) was prepared off line by the addition of cerium(IV) sulfate, before merging with the sample at the point of detection.

Procedure 3

Tris(2,2′-bipyridyl)ruthenium(III) (0.1 mM in 0.05 M sulfuric acid) was prepared off-line by the addition of solid lead dioxide (4g in 100 mL). The solution was filtered on line prior to merging with a sulfuric acid carrier (0.05 M) into which the sample (10mM in deionised water) was injected.

Procedure 4

All electrochemical experiments were carried out using a μ-Autolab Type II (Eco Chemie) potentiostat. 3mm diameter glassy carbon disk electrodes embedded in a 6mm diameter teflon body (CH instruments) were used as working electrodes with a 1 cm^2 platinum gauze and an Ag/AgCl (3M KCl) electrode as counter and reference electrodes respectively. Light emission during the electrochemiluminescence experiments was detected using a 9828SB photomultiplier tube (Electron Tubes, UK) positioned underneath the cell. A specially designed holder and quartz glass cell was used to position the working electrode opposite the PMT in a reproducible manner. The PMT was biased at +500 V using a high voltage supply (Electron Tubes, UK) and signal amplification was performed using an amplifier constructed in house. The entire electrode assembly was housed inside a light-tight box which also served as a Faraday

cage. Data acquisition and manipulation were performed using the auxiliary channel of the potentiostat and GPES software package (Eco Chemie, Netherlands). Organic acids (1 mM) were added to tris(2,2′-bipyridyl)ruthenium(II) (1mM in appropriate buffer solution) in a 2.5:1 molar ratio. The electrochemiluminescent reaction was initiated in each case by scanning the potential between 0 V and 1.35 V.

2.2 Reagents

All chemicals were supplied by Sigma-Aldrich unless otherwise noted. Tris(2,2′-bipyridyl)ruthenium(II) (0.1mM), cerium(IV) sulfate (1mM) and potassium permanganate were prepared in 0.05 M sulfuric acid. All organic acids (See Figure 1) were dissolved in deionised water at a concentration of 0.01 M. Buffer solutions used for pH range experiments were glycine/hydrochloric acid (pH 2-3.5), sodium acetate/acetic acid (pH 4-5.5) and potassium dihydrogen phosphate/ sodium hydrogen phosphate (pH 6-8) at 0.1 M.

3. Results and Discussion

3.1   Cerium(IV) sulfate

The use of Ce(IV) in tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence has been restricted due to its low solubility at greater than pH 3 and its oxidising strength.[23] The latter restriction is a benefit for the determination of organic acids as oxidation of the analyte is necessary to produce a species that reacts with tris(2,2′-bipyridyl)ruthenium(III) to emit light. Table 1 presents the emission intensities of fourteen organic acids upon reaction with tris(2,2′-bipyridyl)ruthenium(II)/cerium(IV) (utilising procedure 1).

The choice of aqueous medium was vital to the generation of chemiluminescence; for example perchloric acid quenched the emission observed with ascorbic acid. Conversely, tartaric and malic acid exhibited an increase in emission intensity in the presence of perchloric acid. Four of the six organic acids that produced light upon reaction with tris(2,2′-bipyridyl)ruthenium(III) in the presence of Ce(IV) contained a hydroxyl moiety alpha to a carboxylic acid.

Much has been published on the oxidation of organic acids using Ce(IV) in sulfuric acid.[24-29] Singh *et al.* stated that the maleic acid/cerium(IV) oxidation was sluggish by nature but resulted in the production of glyoxylic acid.[24] The oxidation of fumaric acid by cerium(IV) perchlorate was described also as being relatively slow.[28] All papers published reported the transient formation of a cerium(IV)/organic acid complex. The kinetics of the reaction between tartaric acid and cerium(IV) have been investigated by Sengupta[25] and Ali *et al.*[27] It was shown that the final products of oxidation are formic acid and carbon dioxide. Initially, the formation of a red tartrate-Ce(IV) complex was observed.[25] In the current study, cerium(IV) sulfate was added to each acid in a 5:1 molar ratio and the results were monitored both visually and with UV-visible spectrometry. In the case of tartaric acid the aforementioned red complex[25]was observed, and some of the other acids also exhibited a colour change upon mixing. Following initial reaction most of the solutions became colourless albeit at different rates. This is postulated[25] to coincide with the decomposition of the red complex to form an intermediate radical followed by reaction with more Ce(IV) to afford, in the case of

tartaric acid, glyoxylic acid.[25]   Hydration and oxidation then occurs resulting in formic

acid formation.[25]

The complex formation of tartaric acid was followed using UV-Visible spectrometry at

430 nm after the method of Drake and co-worker[26] and was found to be complete within

five minutes.   The chemiluminescence was observed to increase in intensity over the

same five-minute period.   The emission intensities for all acids were then monitored after

addition of cerium(IV) sulfate over a period of one hundred and forty hours utilising

procedure 2 (see figure 2).   The chemiluminescence of tartaric acid increased

dramatically in the first 25 hours.   The experiment was repeated for all acids resulting in

similar trends.   The control showed no fluctuation in intensity over the time period

studied. Temperature remained constant throughout the experiment.

The chemiluminescence was possibly due to the reaction of the products of oxidation,

formic or glyoxylic acid, with tris(2,2′-bipyridyl)ruthenium(III).   These postulated

intermediates were then subjected to Procedure 1 and while no light was observed from

formic acid, the glyoxylic acid produced an intense response (*eg.* 1mM resulted in 160 V

signal) that could be seen with the naked eye. The responses from formic acid and

glyoxylic acid did not change over time (140 hours).

Mehrotra *et al*. disagreed with the proposal by Sengupta[25] that glyoxylic acid was an

intermediate in this reaction and proposed a different intermediate species; HOOC-

CH(OH)-CH(OH)•.[30] This species may also produce chemiluminescence with tris(2,2′-

bipyridyl)ruthenium(III) but is not likely to be long-lived, therefore precluding the observation of the intense emission seen from the tartaric acid/cerium(IV) solution after twenty hours of reaction. Hence, the formation of glyoxylic acid from the Ce(IV) oxidation of tartaric acid may be a likely source of the increase in chemiluminescence. Neumann and co-workers attempted to identify the radical species produced by the oxidation of glyoxylic acid with Ce(IV) using rapid-flow EPR measurements.[31] They postulated a structure for the radical species based on evidence of Ce(III) complexation (see Figure 3). Vijai and co-workers proposed the existence of a radical in the mechanism of the reaction between tris(2,2′-bipyridyl)ruthenium(III) and glyoxylic acid with peroxodisulfate as the oxidant, however no evidence was provided to support this postulation.[32] Sengupta showed that the rate constant of the reaction increased with an increase in temperature.[25] Consequently, the reaction mixtures were heated at 70 °C for 1 hour and as expected, this substantially increased the emission intensities (see Table 2).

Three calibrations of tartaric acid were performed at different concentrations of Ce(IV) over a period of 90 hours. The molar ratios of Ce(IV) to analyte and chemiluminescence intensities were calculated and are shown in Table 3. The results obtained for low concentrations of tartaric acid ($1 \times 10^{-7} - 1 \times 10^{-5}$ M) followed a different trend and therefore possibly a different mechanism to those of 1×10^{-4} M to 1×10^{-3} M. Low concentrations of analyte exhibited minimal intensities, increases were observed over time and with increases in molar ratios for the first two calibration sets. High concentrations of tartaric acid exhibited significant increases in emission intensity, possibly a consequence of the oxidation of tartaric acid to glyoxylic acid followed by the

formation of a radical species. The signal intensities of the high concentrations improved as the number of moles of Ce(IV) increased relative to tartaric acid. The responses recorded were reproducible at constant temperature, with no variation in intensity observed in the controls.

3.2 Potassium Permanganate

Acidic potassium permanganate has been shown to elicit chemiluminescence upon reaction with some organic acids.[33, 34] It has also been utilised as the oxidising agent in tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence.[35, 36] Emission intensities of the fourteen acids upon reaction with potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II)/potassium permanganate are presented in Table 1 (Procedure 1).

Of particular interest were the results for two geometric isomers (see Figure 1) maleic and fumaric acids. Initially, fumaric acid produced light upon reaction with tris(2,2′-bipyridyl)ruthenium(II) in the presence of potassium permanganate, whereas the majority of the maleic acid emission from the tris(2,2′-bipyridyl)ruthenium(II)/potassium permanganate system was actually resulting from potassium permanganate (emission intensity with and without tris(2,2′-bipyridyl)ruthenium(II) was 0.35 V). This result demonstrated, for the first time, a degree of selectivity between two stereoisomers possibly due to the differing kinetics of the respective initial analyte oxidations.

To investigate the affect the reaction of potassium permanganate with the organic acids had on the chemiluminescence, oxidant and acid were mixed prior to detection. Only four of the acids exhibited an increase in response over time (see Figure 4). Citric acid

which had previously exhibited no light emission in the presence of potassium permanganate was found to emit light with high sensitivity after approximately five hours. The responses for all four acids increased dramatically in the first five hours, with tartaric acid as an exception each acid then proceeded to decrease in intensity with time.

Unlike cerium(IV), potassium permanganate is soluble at most pH values. Therefore, the chemiluminescence of organic acids was investigated from pH 1.5 to pH 8.5. Above pH 8.5 the blank response, from the reaction of tris(2,2′-bipyridyl)ruthenium(III) with hydroxide ions, was more intense than the analyte signal.[37] Increasing the pH afforded chemiluminescence that had not been previously observed, for example; succinic acid and aspartic acid, albeit at low intensities. The pH profile for succinic acid showed increases in intensity at pH 4 and pH 6, which corresponds to the pK_a values of 4.16 and 5.61. A relationship between emission intensities and pK_a values did not exist for all of the analytes studied.

3.3 Lead Dioxide

Lead dioxide has been used as an oxidant to generate tris(2,2′-bipyridyl)ruthenium(III) for the sensitive determination of various analytes including codeine, thebaine, proline, glyphosate, and ranitidine.[38-41] The reagent is filtered prior to injection to prevent valve damage, however this also precludes analyte oxidation due to the presence of residual lead dioxide.[42] Not surprisingly, the responses obtained from all fourteen analytes upon reaction with the lead dioxide-generated tris(2,2′-bipyridyl)ruthenium(III), were significantly poorer than those obtained when either Ce(IV) or potassium permanganate

were employed as oxidants (Procedure 3). For example, the intensity of the citric acid signal was six orders of magnitude less than that obtained with Ce(IV). The results illustrate the necessity for the pre-oxidation of these particular analytes in order to achieve analytically useful chemiluminescence upon reaction with tris(2,2′-bipyridyl)ruthenium(III).

3.4 Electrochemiluminescence

In situ electrochemiluminescence facilitates the oxidation of both analyte and reagent in close proximity to the detector.[18] Consequently, this approach potentially offers advantages similar to those seen above with either Ce(IV) or potassium permanganate. Specifically the oxidative modification of certain analytes to afford species that will elicit chemiluminescence from their reaction with tris(2,2′-bipyridyl)ruthenium(III). Table 1 shows the results achieved using electrochemical oxidation, which proved to be less sensitive and selective towards the analytes than either Ce(IV) or potassium permanganate. The change in selectivity most probably resulted from a greater variety of oxidation pathways that exist for the acids in electrochemiluminescence than those of the chemical oxidants.[43] Oxidant strength may have been a contributing factor in relation to the measured chemiluminescence intensities given that Ce(IV) (E^0 = 1.72 V)[44] and potassium permanganate (E^0=1.5 V)[44] are stronger oxidants than tris(2,2′-bipyridyl)ruthenium(III).[19] Notably the electrochemiluminescence maximum was observed in each case close to 1.2 V, which is close to the oxidation potential for the reagent. It should be noted that the electrochemiluminescence results were obtained in quiescent solutions using a potential scan lasting only a few seconds for each measurement (see Procedure 4). As a consequence only a very small quantity of tris(2,2′-

bipyridyl)ruthenium(III) reagent is generated relative to the chemiluminescence experiment and the reactions leading to light emission are diffusion limited.

4. Conclusion

The choice of oxidant in the chemiluminescence of tris(2,2′-bipyridyl)ruthenium(II) is vital to selectivity and sensitivity. The variation in response could be a result of oxidant strength, products of oxidation, and the stability of an intermediate radical species. For example, the isomeric species fumaric and maleic acids showed different reactivity's with the tris(2,2′-bipyridyl)ruthenium(II)/potassium permanganate system. The reaction of some organic acids with oxidant prior to reaction with tris(2,2′-bipyridyl)ruthenium(III) increased signal intensity and, in the case of tartaric acid, by approximately three orders of magnitude. The dependence of the oxidation and chemiluminescence, upon the molar ratios of Ce(IV) to analyte and the identification of the species present in solution over time, are currently being investigated to assist in applying the increases in signal intensity to real samples.

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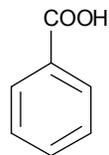44. D. R. Lide, ed., *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, 2002-2003.

Figure(s)

Figure 1: Structures of fourteen organic acids investigated in this study.
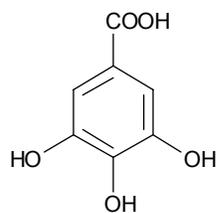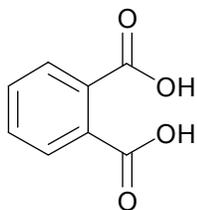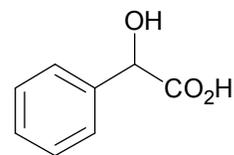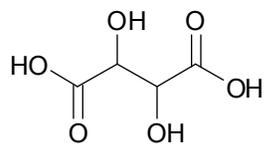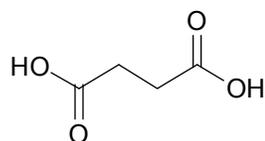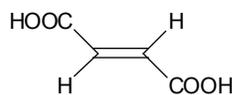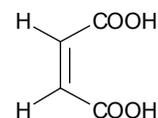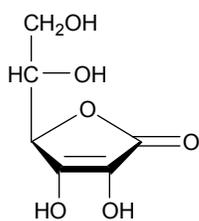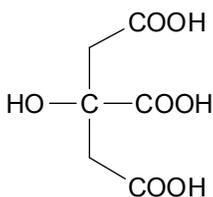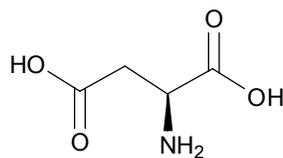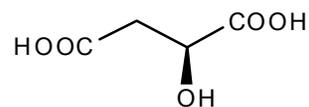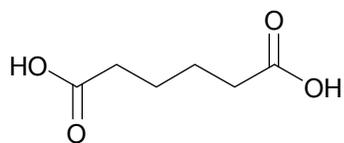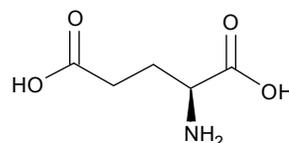
Figure 2: Intensity vs time profiles, utilizing procedure 2, for A) tartaric acid B) ascorbic acid C) citric acid D) mandelic acid E) gallic acid. Cerium(IV) sulfate was added in a 1:2 volume ratio oxidant to acid. Temperature remained constant throughout the experiment
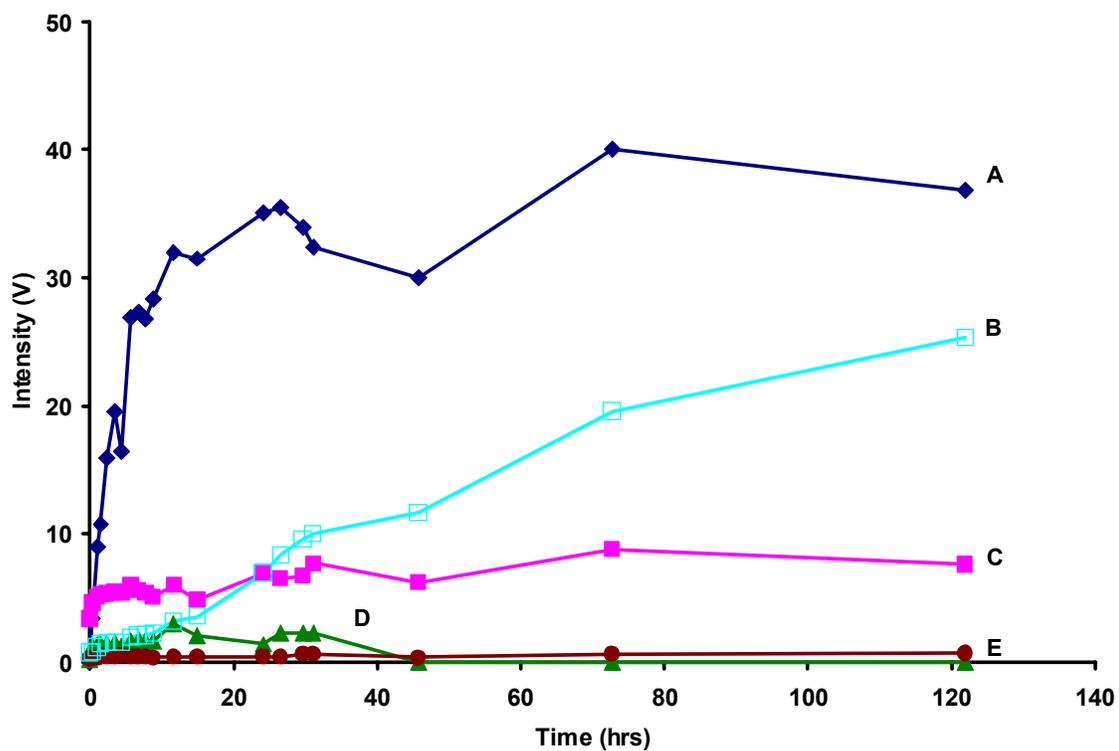
Figure 3: Proposed structure of the radical species produced upon the oxidation of glyoxylic acid with Ce(IV)[31]
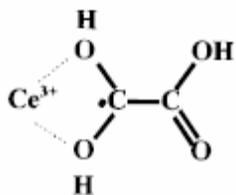
Figure 4: Intensity vs time profiles for A) fumaric, B) tartaric, C) maleic, and D) citric acids in the presence of potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II).
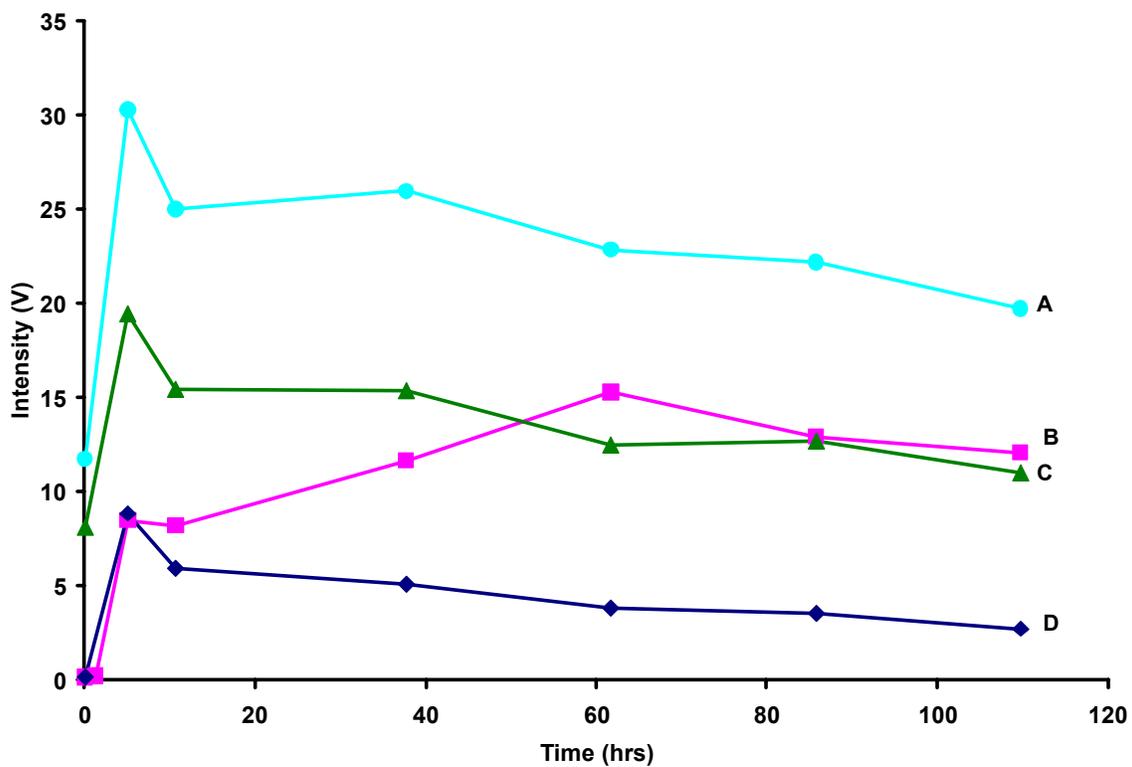
Table 1: Emission intensities of 14 organic acids upon reaction with tris(2,2′-bipyridyl)ruthenium(II) oxidised by Ce(IV), KMnO$_4$ and electrochemistry. (all intensities are displayed in V).

[a] **Sulfuric acid (0.05 M), perchloric acid (0.5 M).**

[b] **Due to the use of a different instrument, intensities are not directly comparable to chemiluminescence emission intensities.**

Organic Acid	Intensity (Ce(IV) in sulfuric acid[a])	Intensity (Ce(IV) in perchloric acid)	Intensity (KMnO$_4$ as oxidant)	Intensity (ECL[b])
Mandelic	3	2	0	0.7
Aspartic	0	0	0	0
Malic	0	0.4	0	0.3
Tartaric	0	0.2	0	0.5
Maleic	0	0	0.35	0.4
Fumaric	0	0	2	0
Gallic	1.2	1.4	0.5	0
Ascorbic	5	0.1	4.5	0.2
Citric	22.5	11	0	1.1
Benzoic	0	0	0	0.3
Glutamic	0	0	0	0.3
Adipic	0	0	0	0.3
Succinic	0	0	0	0.2
Phthalic	0	0	0	0.7

Table 2: Effect of heating upon the emission intensities of organic acids mixed with cerium(IV) sulfate prior to detection. Prior to heating: the analytes were mixed with Ce(IV) and then reacted with tris(2,2′-bipyridyl)ruthenium(II), After heating: the same solutions were then heated for one hour at 70°C.

Organic Acid	Intensity prior to heating (V)	Intensity after heating (V)
Citric	1.2	5.3
Ascorbic	5.2	6.5
Malic	0	1.3
Tartaric	0	6.8

Table 3: Increases in tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence observed for two different tartaric acid concentrations upon reaction with Ce(IV) over time.

Molar ratio	Time = 0 hrs	Time = 20 hrs	Time = 44 hrs	Time = 92 hrs
1 × 10⁻⁷ M tartaric acid				
5.6:1	8	10	10	0
56:1	0	10	0	0
560:1	0	0	0	0
1 × 10⁻⁶ M tartaric acid				
0.56:1	8	13	13	0
5.6:1	8	17	12	12
56:1	0	0	0	0
1 × 10⁻⁵ M tartaric acid				
0.056:1	78	20	20	7.5
0.56:1	27	133	115	112
5.6:1	5	0	0	0
1 × 10⁻⁴ M tartaric acid				
0.0056:1	13	37	33	28
0.056:1	38	212	178	165
0.56:1	463	1940	1990	PMT overloaded
1 × 10⁻³ M tartaric acid				
0.00056:1	35	87	85	93
0.0056:1	84	445	408	397
0.056:1	668	1880	2070	PMT overloaded

[a] The molar ratio was calculated as the number of moles of Ce(IV) present divided by the number of moles of analyte present.