Using the Agilent Cary Eclipse to measure the chemiluminescence from a ruthenium complex

Application Note

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Introduction

Chemiluminescence is emission resulting from a chemical reaction, as opposed to fluorescence or phosphorescence, both of which are emission resulting from the absorption of light. Since the emission is generated without light, no source is needed when measuring the signal. In a conventional fluorescence spectrophotometer the light source must be off during the measurement phase, so as not to interfere with the signal being measured. The chemiluminescence signal is also generally weak. Achieving good signal to noise and a low limit of detection depends on how well the sample is shielded from room light. It is absolutely essential that the instrument has excellent light-sealing capabilities. The Agilent Cary Eclipse fluorescence spectrophotometer addresses both these requirements which makes it an excellent analyser for chemi- or bio- luminescent applications. With a red-sensitive detector as standard and the excellent sensitivity of the gratings in the visible region, emissions well into the red can be measured with the Eclipse, without the expense of purchasing components which allow measurement in this region.

This application note demonstrates the Cary Eclipse chemiluminescence capabilities by measuring the emission from a ruthenium complex upon reaction with codeine. Figure 1 shows a photograph of the mixing of the two non-emitting samples to produce a chemiluminescence product.

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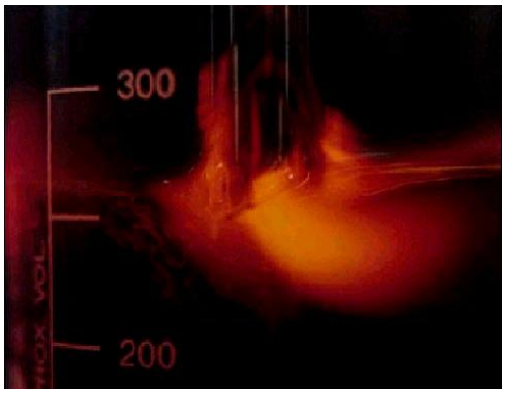

Figure 1: Photograph of tris(2,2' - bipyridyl)ruthenium(II) chemiluminescence via the reaction with codeine

Method

The ruthenium complex, tris(2,2'-bipyridyl)Ruthenium(II) (1 mM) in H_2SO_4 (0.05 M), was oxidised to the 3+ oxidation state with solid lead dioxide which is subsequently removed by filtration. The ruthenium 3+ species was then merged with a flowing stream of codeine (1 mM) which is oxidized by the ruthenium. The ruthenium is subsequently reduced to the 2+ oxidation state producing an excited state intermediate which then returns to the ground state by the emission of a photon centered around 610 nm. Figure 2 shows a schematic of the reaction process.

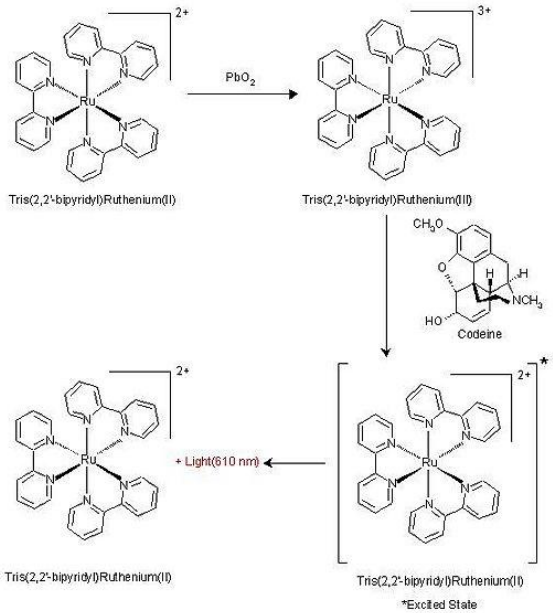

Figure 2: Schematic of the reaction between Ruthenium and codeine to generate chemiluminescence around 610 nm

Discussion

The chemiluminescence of the ruthenium complex was generated by passing the Ru(III) species and codeine through a specially designed flow cell of spiral geometry. This cell was positioned on the face of the emission window inside the sample compartment. Upon mixing of the 2 reagents, emission occurred which was measured as a function of wavelength using the Cary Eclipse Scan application. Since the instrument comes standard with a R928 red-sensitive detector, the chemiluminescence could measured with excellent S/N up to 800 nm, as shown in Figure 3.

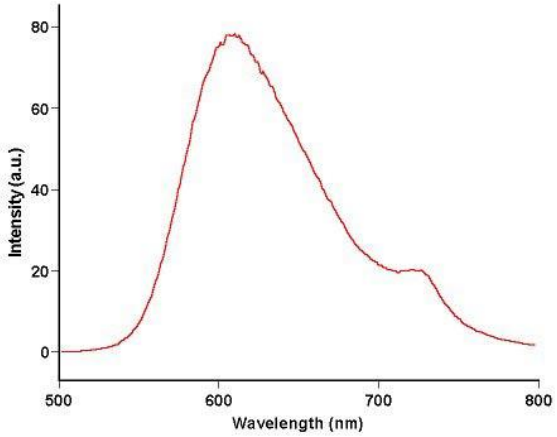

Figure 3: Chemiluminescence of Tris(2,2'-bipyridyl)Ruthenium(II) complex

Emission from the ruthenium(II) species can also be generated by exciting the complex with light. In this case, the emission is due to phosphorescence[1]. This emission has the same spectral characteristics as the emission generated through chemiluminescence, as the emission occurs from the same excited state. The only difference is that this excited state is produced via a chemical reaction, in the case of chemiluminescence, whereas in phosphorescence emission the absorption of energy in the form of light produces the excited state. Figure 4 shows both spectra overlaid.

Note : The phosphorescence spectrum was recored from a 1 mM solution of the tris(2,2' - bipyridyl)ruthenium(II) chloride salt dissolved in H_2SO_4 (0.05 M).

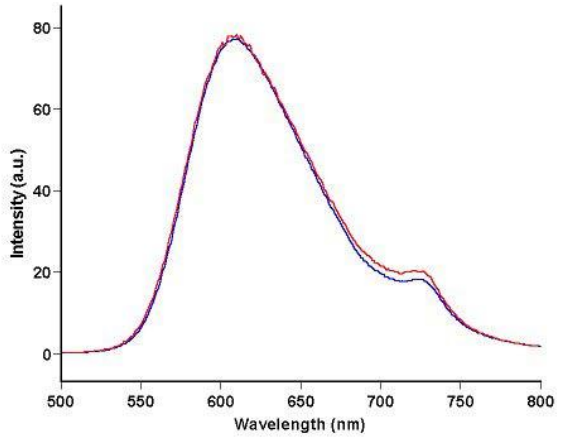

Figure 4: Chemiluminscence (Red) and Phosphorescence (Blue) spectra of Tris(2,2'-bypyridyl)Ruthenium(II) complex

References

1. Gerardi, R. D., Barnett, N.W. Lewis, S.W., Anal. Chim. Acta, 378, 1999, pg 1-41

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