The measurement of singlet oxygen lifetime sensitised using Rose Bengal

The study of singlet oxygen (1^1 \text{O}_2) is of interest, principally, as it is a highly reactive species. It can be produced by photosensitisation, usually of a molecule such as a dye or porphyrin. Thus, by the appropriate selection of sensitiser, the presence of oxygen and light, 1^1 \text{O}_2 can be selectively generated. From a biological aspect it has the ability to damage and destroy cells, which has lead to interest in its use as an anticancer agent in photodynamic therapy (PDT).

Sensitised production of 1^1 \text{O}_2

There are several types of molecules that can be used as sensitisers to generate 1^1 \text{O}_2. Common ones employed in PDT include porphyrins, although dyes such as Methylene Blue and Rose Bengal have been frequently used. The photosensitisation reaction that can occur when oxygen collides with a photosensitising agent (PA), sometimes referred to as a type II reaction, can be summarised below.

\[
PA(S_0) \xrightarrow{h
u} PA(S_1) \xrightarrow{k_{Sc}} PA(T_1)
\]

\[
PA(T_1) + \text{3}^1 \text{O}_2 \xrightarrow{k_{ST}} PA(S_0) + 1^1 \text{O}_2
\]

Where \(S_0\) and \(S_1\) are the singlet ground and excited states, \(T_1\) the first excited triplet state, \(k_{Sc}\) and \(k_{ST}\) the rate constants for intersystem crossing and energy transfer. 3^1 \text{O}_2 is the ground state of oxygen.

In practical terms this means that light is used to excite the photosensitiser (usually in the visible) and the emission from 1^1 \text{O}_2 monitored (in the near infrared, NIR). The decay time of 1^1 \text{O}_2 is usually in the microsecond time range and depends on the solvent used.

Measurement of the 1^1 \text{O}_2 lifetime

The emission of 1^1 \text{O}_2 occurs close to 1275nm, in the near infrared part of the spectrum. This means that a NIR sensitive detector is required and instrumentation which can measure decay times on the microsecond time scale.

The photosensitising agent, Rose Bengal, requires an excitation wavelength around 530nm. The HORIBA Scientific TemPro-01, equipped with a S-535 excitation source (SpectraLED emitting at 535 nm) operating on the phosphorescence timescale and a H10330-45 detector, is well suited for such a measurement. This equipment is shown in Fig. 1. The SpectraLED pulse rate is automatically adjusted to suit the time range and its duration can be altered which allows further control over the source intensity. These parameters are set by the DataStation control software. The apparatus phosphorescence time ranges, with a resolution from 83ns / channel is well suited for measuring lifetimes from a microsecond onward. It is also capable of coping with the high level of “dark counts” or noise associated with the use of NIR sensitive detectors for lifetime measurements.
Time-resolved decay of Rose Bengal sensitised $^1$O₂ emission in different solvents.

To demonstrate the measurement of the sensitised decay of $^1$O₂ two solvents were chosen for this work; ethanol, where a time-resolution of 83ns/chnl was employed and acetone, where 167ns/chnl was selected. Making use of a lower resolution can be useful if a shorter data acquisition time is desired. In the following results it can clearly be seen that both time ranges yield a good quality of data, with HORIBA Scientific DAS6 analysis software used to fit the data to a single exponential decay model.

The time-resolved decay of Rose Bengal sensitised $^1$O₂ emission in ethanol is shown in Fig. 2, along with the fit and weighted residuals.

Fig. 2. Decay (red) of $^1$O₂ in ethanol, monitored at 1275nm with excitation of Rose Bengal made using a SpectraLED-535. The fit and weighted residuals (green) are shown and the lifetime obtained was 16μs.

Fig. 2 clearly shows a good fit to the single exponential decay model. The decay shown in Fig. 3 was measured in the same manner, but using acetone as a solvent. In this case it was expected that a longer-lived decay would be obtained and this proved to be correct. A lifetime of 50μs was recovered from the DAS6 analysis. Again a good fit was obtained using a single exponential decay model.

Fig. 3. Decay (red) of $^1$O₂ in acetone, monitored at 1275nm with excitation of Rose Bengal made using a SpectraLED-535. The fit and weighted residuals (green) are shown and the lifetime obtained was 50μs.

Summary

The time-resolved emission of $^1$O₂ monitored at 1275nm after excitation of a photosensitiser, Rose Bengal, using a SpectraLED source could be measured using the TemPro system equipped with a NIR detector. Good fits were made to a single exponential decay model and the influence of solvent on the time-resolved kinetics was clearly evident.