Application Note 70:

Fast Stern Volmer analysis in flow

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Abstract

This application note illustrates how a Vapourtec E-Series can be configured with a spectrophotometer for Stern-Volmer fluorescence quenching studies.

This set up was developed for undergraduate teaching laboratory sessions. The results obtained in both batch and flow highlight the benefits of the flow system over manual data collection for determining Stern-Volmer quenching rates.

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Background

Flow chemistry makes 'accelerating chemistry' easy. By integrating auto-samplers and in-line detectors, flow chemistry systems can automate high-throughput experimentation and reaction optimization [1–3], while significantly reducing manual labor [4,5].

In-line monitoring is a standard procedure in many industrial flow chemical processes, enabling real-time quality assessment of the process. This can improve safety and prevent chemical waste in the event of out-of-specification process conditions[6–9]. Despite these benefits, in-line reaction monitoring is not yet fully implemented in academic research settings, requiring further off-line analysis of the synthesized products.

Advanced spectrometers, such as benchtop NMR, are becoming increasingly available with accessories that enable continuous flow compatibility[10,11]. However, these are relatively large instruments and at an inaccessible price for many academic research groups.

Optical spectroscopies such as UV-Vis, Raman or IR are popular tools for in-line monitoring, and can be easily integrated with the Vapourtec R-Series systems. Reaction kinetics can be evaluated by monitoring UV, IR or Raman spectral output of the reaction. In-line data can also assist to easily screen several reactions before further analysis.

These in-line analytical capabilities are not only used to evaluate traditional chemical reactions (A+B→C). Optical spectroscopies can also be used to evaluate photocatalysts and elucidate the primary event in a photocatalytic reaction [12].

Fluorescence is a type of intramolecular deactivation, in which an electronically excited molecule relaxes to its ground state by emitting a photon of light. The presence of other chemical species can inhibit this radiative decay, and the Stern–Volmer relationship allows to study the kinetics of these processes [12].

The principle is that a fluorophore's emission will be quenched in the presence of a species which provides a non-radiative decay pathway of the fluorophores excited state. This is described by the Stern-Volmer relationship:

$$\frac{I_f^0}{I_f} = 1 + k_q \tau_0 [Q]$$

The ratio of initial fluorescence intensity (I0f) to the quenched fluorescence intensity (If) is related to the concentration of the quenching species ([Q]). A plot of I0f/If vs. [Q] should yield a linear relationship with a gradient equal to the product of the fluorescence lifetime (τ0) and the quenching rate coefficient (kq). The fluorescence lifetime is a physical constant of a molecule at a specified solvent and temperature.

The quenching rate coefficient is also a constant, which indicates the efficiency of the quenching process between the photocatalyst and the quenching molecule. This allows the relative efficiency of energy- or electron-transfer between a photocatalyst and quenching species to be determined for a given solvent and temperature. Alternatively, if fluorescence is not quenched in the presence of a given molecule, it indicates that molecule is not able to mediate the primary

electron (or energy) transfer step of a photocatalytic mechanism by itself. It may be an incompatible substrate or redox mediator for that photocatalytic process.

The importance of this technique was highlighted by MacMillan and co-workers, when they reported the dependency of an acetate base for the photochemical activation of a thiol [13].

Stern-Volmer fluorescence quenching analysis is notoriously labor intensive and needs to be done with care due to a few factors: (i) multiple solutions of varying concentrations need to be prepared accurately in order to obtain a straight line, (ii) the cuvette must be carefully cleaned and dried between measurements, as traces of certain solvents, especially acetone, are efficient quenching species, and (iii) oxygen is also potent quenching species, which can be difficult to exclude during transfer to a spectrophotometer [9].

For these reasons, Noël and co-workers developed a continuous flow platform for fully automated Stern-Volmer analysis 5. Their system, based on a high resolution spectrophotometer, was proven to be extremely effective and convenient. However, the cost of these spectrophotometers are prohibitive to many teaching labs.

The remainder of this application note describes a continuous flow experimental set up to run Stern-

Volmer analysis, the experiments were conducted by Christopher G. Thomson, Arno Kraft and Filipe Vilela of Heriot-Watt University.

PASCO Scientific Incorporated recently released the PS-2600 wireless spectrophotometer, which is a small device capable of measuring light between 380-950 nm (Figure 1). The device can be used for absorption spectroscopy measurements with a

broad-band white LED source, as well as fluorescent emission measurements with either a 405 nm or 500 nm LED excitation source.

Figure 1 – Image of the PASCO Scientific Inc. PS-2600 Wireless Spectrophotometer & Fluorometer

We have incorporated the PS-2600 with the Vapourtec easy-Photochem flow chemistry system for a variety of potential teaching applications, including:

i. Kinetic analysis of aqueous dye degradation by a heterogeneous photocatalyst within the immobilized photocatalyst reactor and
ii. Assessing the photostability of homogeneous organophotocatalysts irradiated by the photochemical reactor UV-150.

We chose to study the fluorescence quenching of aqueous fluorescein using DABCO. This is an advanced physical chemistry undergraduate laboratory experiment at Heriot-Watt University. This provided us with a large sample of unbiased experimental data for comparison.

In the undergraduate experiment, students are required to make six aqueous solutions of fluorescein with a range of DABCO concentrations (0–0.5 M). This is achieved by carefully adding the correct mass of fluorescein and DABCO and diluting to the correct volume to achieve an accurate concentration. This is time consuming and prone to human error. With our flow system adaptation, only two solutions with accurate concentration need to be prepared. As shown in Figure 2, the two solutions and solvent converge at a four-way cross-mixing junction with varying flow rates, achieving a constant concentration of fluorescein whilst varying the concentration of quencher. This eliminates the need to prepare multiple solutions, reducing waste, manual labor and human error.

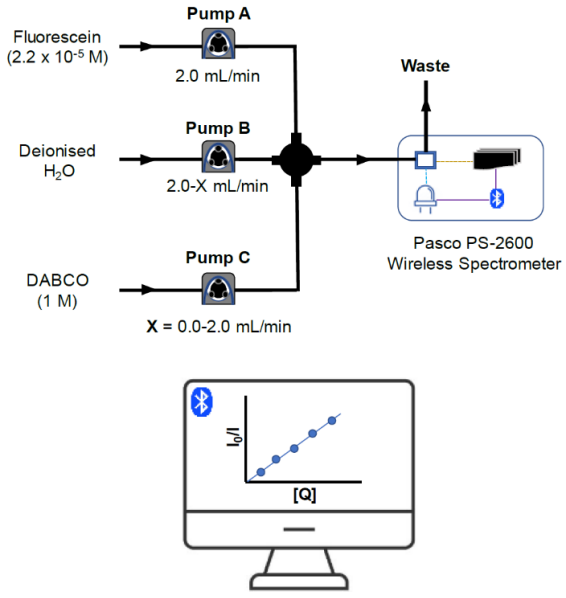

Figure 2 – Flow system schematic used for semi-automated Stern-Volmer fluorescence quenching analysis

Setup

All the experiments were performed using a Vapourtec E-Series flow chemistry system, equipped with three V-3 peristaltic pumps. The PASCO PS-2600 spectrophotometer was connected to the system using a flow-through quartz cuvette (Hellma GmbH & Co. KG; 176-761-15-40). The total cuvette chamber volume was 140 µl. Three separate solutions were pumped to a four-way cross junction mixer. The resultant flow mixture then flows to the cuvette within the PS-2600 for analysis before being collected in a waste bottle. A schematic flow diagram is displayed in Figure 2.

Reagents

All reagents were commercially available and used without further purification. Fluorescein disodium salt and 1,4-diazabicyclo[2.2.2]octane (DABCO) were purchased from Alfa Aesar. Deionized water is produced on site at the Heriot-Watt University teaching laboratories.

System Parameters

System solvent: deionized water
Solution A: Aq. fluorescein solution (2.2 x 10−5 M)
Flow rate A: 2 ml/min
Solution B: deionized water
Flow rate B: 2.0 - X ml/min
Solution C: Aqueous DABCO) solution (1 M)
Flow rate C: 0.0 – 2.0 ml/min = X

Procedure

The three pumped solutions mix in four-way cross-junction mixer before entering the spectrophotometer for analysis. The outflow was discarded to waste collection. The system was initially primed with deionized water.

The experiment started by flowing water and fluorescein solution at 2 ml/min each, to take a blank fluorescence spectrum and measure the initial fluorescence intensity (I0f, excitation at 405 nm). The final concentration of the fluorescein solution after the mixing junction was 1.1 x 10−5 M. The peak emission intensity wavelength of fluorescein in water was identified at 517 nm, as Figure 3 shows.

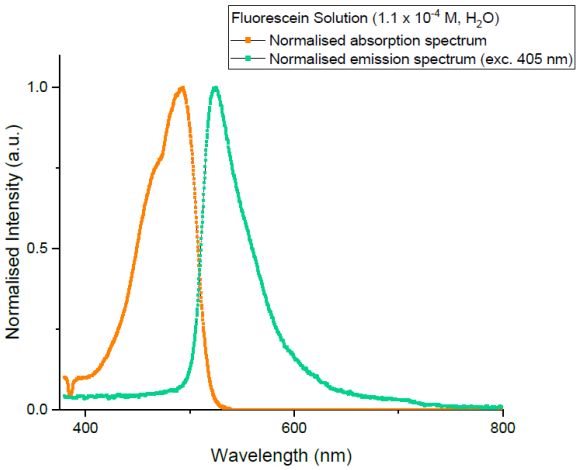

Figure 3 - Normalized absorption and emission spectrum of an aqueous fluorescein sodium salt solution used in this study

The PS-2600 was programmed to collect intensity data at 517 nm every second with a bandwidth of 4 nm. The analysis began by flowing only water through cuvette from pump B (4 ml/min). This established the residual baseline signal (Ibaseline), which must be subtracted from I0f and IXf. The value of I0f was then obtained by flowing fluorescein solution and water (pumps A and B at 2 ml/min each). After achieving a steady state of fluorescence intensity for approximately 100 seconds, the flow rates of pumps B and C were synchronously altered, such that their net-flow rate was maintained to 4 ml/min. This maintained a net-concentration of fluorescein solution, whilst varying the concentration of DABCO solution (0–0.5 M).

The conditions were maintained to achieve 100 seconds of steady state intensity. This was repeated six times in total, changing the flow rate of pumps B and C in steps of 400 µl/min. After completion, the system was flushed with fresh deionized water (50 ml) and then ethanol (50 ml) to clean the cuvette and system. The pumps were then left to pump air through the system to facilitate drying of the tubing and cuvette.

Results and Discussion

The mixed solution was irradiated with 405 nm to induce fluorescence, and its intensity was monitored for at least 100 seconds of steady state conditions. The flow rates of pumps B and C were varied to find values of I0 f and Ix f for five different concentrations of DABCO, consistent with the undergraduate experiment. An example of the raw data trace obtained from the PS-2600 spectrophotometer is presented in Figure 4.

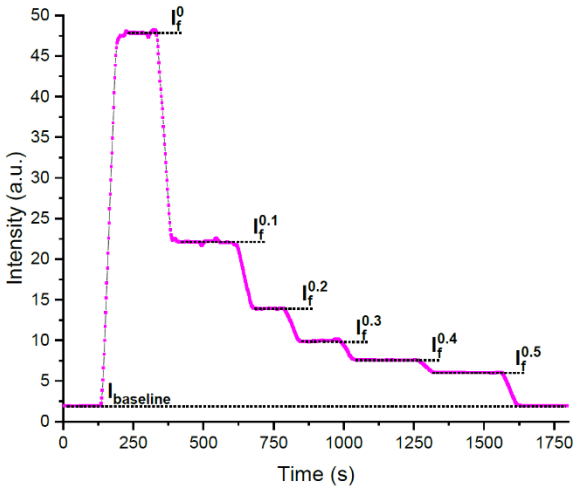

Figure 4 - Change in fluorescence emission intensity ($I_f{}^X$, where X is the concentration of DABCO (M)), recorded at 517 nm by the PS-2600

The data was exported and analyzed using OriginLab software [14]. The average intensity of each steady state was calculated before plotting those values against the concentration of DABCO.

The processed results were compared with data from undergraduate students, acquired with a commercial fluorimeter (Jenway). The results for four independent experiments acquired in flow and three acquired manually by undergraduates, are displayed in Figure 5.

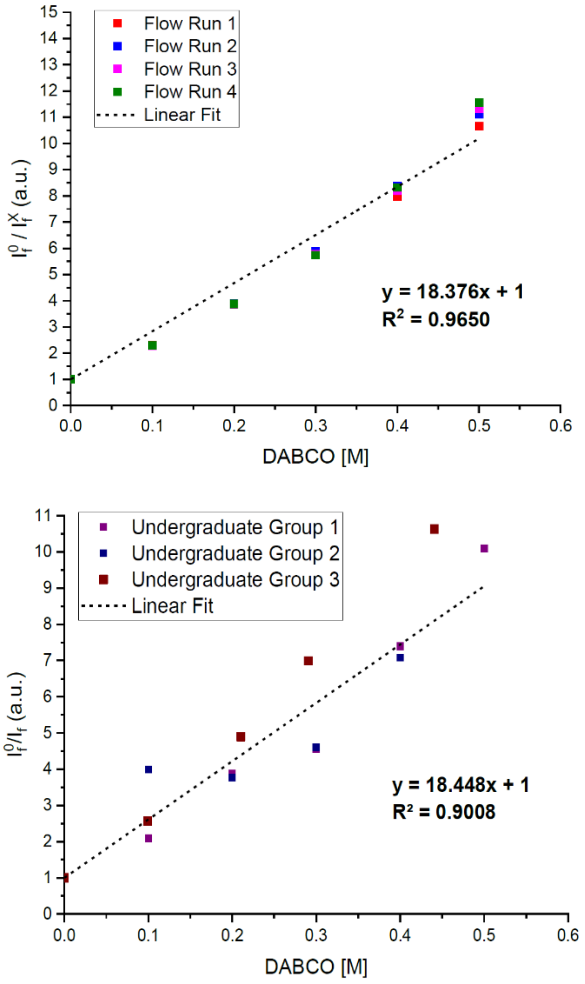

Figure 5 - Results of the Stern-Volmer fluorescence quenching analysis (A) performed using the PS-2600 and flow system (B) Performed by three, independent groups of undergraduate students using a commercial fluorimeter

The value of k_q was determined using τ_0 (4x10−9 s, H_2O, 298 K)[24]. The values of k_q and R^2 for the individual runs and the combined data sets from each method are presented in Table 1.

The results obtained with the flow system are far more consistent than those obtained manually. Additionally, the R^2 values for the individual flow experiments were generally higher, although one group of undergraduates achieved an R^2 value of 0.99, demonstrating that manual analysis can provide good linear relationships if done carefully.

Table 1 – Comparison of k_q and R^2 values obtained

Method	Entry	k_q ($x10^9$, l mol^{-1} s^{-1})	R^2 Value
E-Series + PS-2600	1	4.434	0.98
	2	4.627	0.97
	3	4.635	0.97
	4	4.712	0.96
Total Data Set Average		***4.594***	***0.97***
Manual + Commercial Fluorimeter	1	5.410	0.99
	2	4.399	0.86
	3	4.027	0.96
Total Data Set Average		***4.612***	***0.90***

Undergraduate students are given four hours to complete the analysis, which is in part due to their inexperience, but it also reflects that this is a time-consuming technique if performed manually. The flow system has several significant benefits: a single experiment's data collection can be completed within 20 minutes; time is saved through producing fewer sample solutions; there is no longer need for cleaning and drying cuvettes between measurements; and the results obtained are far more reproducible.

It is important to recognize that the analysis performed in flow should always give good linear relationships, as the error in the x-axis is only defined by the preparation of the two initial solutions as all other human error is removed. However, this does not mean that the result obtained is accurate if the concentrations of the initial stock solutions are inaccurate. The analysis should be repeated until consistent results are obtained in triplicate, which would require a minimum of six stock solutions for the flow system, compared with eighteen for manual analysis. The four flow system experiments were achieved within 3 hours, including familiarizing with the E-Series, setup and sample preparation. This is an hour less than the time period given to undergraduate students to complete a single manual analysis.

Conclusions

We have performed Stern-Volmer fluorescence quenching analysis in continuous flow by using a Vapourtec E-Series combined with a PS-2600 spectrophotometer. The flow system provided high quality data, as well as significantly reducing manual labor and the overall time required for the analysis. This provides an accelerated method to assess the relative efficiencies of quenching by photocatalytic substrates, as well as helping to elucidate photocatalytic mechanisms.

This would be an ideal set-up for undergraduate teaching laboratories, which would simultaneously reduce the tedium of the traditional analysis, as well as introduce undergraduates to principles of flow chemistry, which are not present in many degree programs. This system configuration is an affordable method for automated reaction monitoring of continuous flow processes that feature chromatic or fluorescent chemical species, and especially photocatalysis.

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