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LED-array photocalorimetry: Instrument design and application to photostability of nifedipine

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ABSTRACT

A photocalorimeter was designed to analyse quantitatively the photostability of pharmaceuticals. Its application is demonstrated with reference to two solution phase test systems; the photodegradations of 2-nitrobenzaldehyde (2-NB) and nifedipine. Light emitting diode (LED) arrays were used to illuminate the sample and reference channels of the calorimeter. Five LEDs were used to create an array from 360 to 700 nm. A power supply system was constructed that zeroed the instrument by supplying a preset voltage to the sample side LEDs and varying that supplied to the reference LEDs until a zero calorimetric signal was obtained. The photodegradation of 2-NB was zero-order and varied in proportion to the input voltage supplied to the LED array. Analysis of the data (the rate of reaction was determined to be equal 1.04×10^{-6} mol dm^{-3} s^{-1} by pH titration) determined a reaction enthalpy of 5.0 ± 0.6 kJ mol^{-1}. In the case of nifedipine, the LEDs in the array were operated individually in order to determine the causative wavelength of degradation. This was found to be 360 nm, in agreement with the literature.

1. Introduction

Photochemical degradation affects an increasing number of pharmaceuticals; quantification of photostability is thus essential to ensure appropriate measures are taken to ensure stability and product performance over an acceptable time period. Despite this, there are no 'standard' photostability assays or conditions. The International Conference on Harmonization (ICH) has issued photostability testing guidelines (document Q1B) [1], but this test does not fully specify the wavelength range or distribution of the irradiating light source. It also requires only a pass/fail decision, rather than quantification of photodegradation.

With most photodegradation assays the sample is irradiated prior to isolation and quantification of the analyte (or any degradants). In other words, photodegradation and analysis are not concomitant. This arises in part as a consequence of the complex nature of typical pharmaceutical samples and in part because of a lack of apparatus dedicated to photostability testing. A further problem is that any physical change upon irradiation (such as a change in polymorph) is not quantified because isolation of an analyte usually involves some form of solvent extraction. These factors act to limit current photostability testing regimes and mean there is a gap in current analytical science.

Photocalorimetry (the measurement of the heat change when a sample is irradiated) offers an alternative method which addresses all of the issues noted above. Principally, this is because the data recorded (power as a function of time) are acquired in real-time, in a non-invasive manner and are easily interpreted. At present this is usually in a qualitative form although quantitative interpretation is sometimes achievable. Further, by using an array of light emitting diodes (LEDs) as the light source it is possible to design (and hence define) the spectral distribution of light irradiating the sample. This means that standard photostability conditions can be developed which, in principle, should be both reproducible and repeatable. The purpose of this work therefore is to report the development of an LED-array photocalorimeter and to demonstrate its applicability to pharmaceutical photostability testing by studying both a test and reference reaction (the photodegradation of 2-nitrobenzaldehyde) and the degradation of a model drug (nifedipine).

2. Materials and methods

2.1. Instrument design

The principle of photocalorimetry is not new, with instrument designs consistent with modern apparatus being recorded in the

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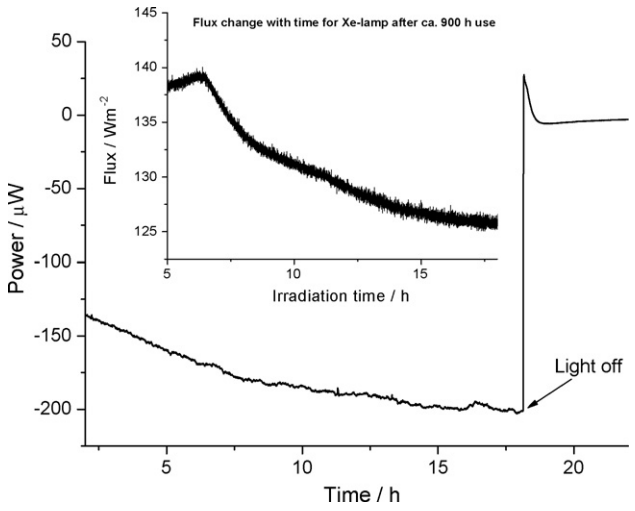

Fig. 1. Power–time data showing the drift in the instrument response with an old (ca. 900 h) Xe-arc lamp. Note that the signal returns to zero and remains horizontal once the lamp is turned off. Inset graph: corresponding light flux data for the same lamp over an equivalent time period showing equivalent drift.

literature as long ago as 1939 [2]. In basic terms, the sample is irradiated with light (which is directed into the sample ampoule from an external light source) and any heat change that occurs is recorded with the calorimeter. The major drawback with nearly all the photocalorimetry apparatus recorded in the literature, including our own [3], is that the light source is usually extremely powerful (typically a 300 W Xe-arc lamp) and hence a large light power is introduced into an extremely sensitive instrument. This can make it extremely difficult to distinguish what may be quite small power signals originating from the sample under investigation. In addition to producing too much light, Xe-arc lamps have many drawbacks in this particular application; these include a relatively short lifetime (ca. 1000 h), significant heat generation and a gradual degradation in light output intensity over time. Furthermore, as the lamp ages the light flux produced by the lamp drifts over relatively short time periods; this change in flux results in a concomitant drift in the response of the calorimeter. The data in Fig. 1 serve to illustrate this point. Because nearly all modern calorimeters are differential instruments (that is, the data from the sample are compared with those recorded simultaneously from an inert reference) if a single light source is used a beam-splitting arrangement is required. This immediately makes it difficult to zero the instrument prior to calibration and data capture. The most problematic drawback however, from the perspective of recording repeatable data, is that the arc strikes in a different location every time the lamp is fired. This means that the focussing and shuttering arrangements must be adjusted with each use, a time-consuming effort that still does not result in consistency of instrument performance. Such variability precludes the use of the instrument for studying samples unless a large power output from the sample is expected.

To overcome these issues, we replaced the Xe-arc lamp light source of our photocalorimeter with an LED array. The immediate benefits of LED technology are low cost, long life times (greater than 10,000 h), low power consumption and heat generation and consistency of light output.

The basic design of the photocalorimeter did not change from that we reported previously [3] and the reader is referred to that paper for full design details. Here we report only the design and use of LED arrays. Five LEDs were mounted into an aluminium holder angled at 15° from vertical (this angle giving the best compromise between the need to mount several LEDs and the fact that optimal transmission of the light from the LEDs to the light guide occurs when the angle from vertical is zero). The light guide was mounted into the bottom of the aluminium holder and carried light into the ampoule. Separate LED arrays were mounted on the sample and reference sides. A small printed circuit board was designed to operate the array; this accommodated both the in-line resistor needed for each LED (to ensure the current input to each LED did not exceed its operational maximum) and a switch to allow irradiation of the sample by individual LEDs. Because the sample and reference ampoules had dedicated LED arrays there was no need for any form of beam-splitting arrangement.

Further, because the spectral power distribution (SPD) from each LED array was proportional to its input voltage, there was no need for any shuttering or focussing assemblies; any adjustments in the light powers on the two sides required to achieve a zero baseline could be made electronically. The SPD on the sample side can be set to a predetermined level (in this case, by supplying a specific voltage to the LED array) while the light intensity on the reference side is adjusted until the calorimeter output power is zero. Hence, the calorimeter itself is used as a null-adjust balance to ensure parity between the sample and reference sides. Note that this does not mean that the light intensities irradiating the sample and reference sides are equal; it means that the power outputs recorded by the calorimeter on the sample and reference sides are equal, a situation that accounts for variability in the apparatus as well as light intensity.

To ease further the operation of the instrument, we designed a power supply system capable of increasing the power to the reference LEDs to blank the instrument automatically. Initially the system supplies a preset voltage to the sample side LED array (say, 15 V) while a lower power is supplied to the reference side LED array. The initial value of the reference side voltage is equal to the sample side voltage $-0.5\times$(maximum output voltage of the D/A converter). The raw output signal from the calorimeter (which, for the TAM system is the voltage signal generated by the thermopiles) is monitored and after a preset time (7 min, to allow the system to reach equilibrium) the power supply acts to drive the calorimeter output signal to zero (assuming it is not already so) by increasing the reference side power. This cycle repeats every 7 min until the calorimeter output signal falls to 0 ± 0.1 mV, up to a value of sample side voltage $+0.5\times$(maximum output voltage of the D/A converter). Details of the electronic arrangement of the power supply are available on request.

2.2. Materials

2-Nitrobenzaldehyde (2-NB, >98%) and nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl 1-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate, $C_{17}H_{18}N_2O_6$, >98%) were purchased from Sigma (UK). Ethanol (>99.5%) was purchased from Hayman Ltd. Deionised water was used throughout. All materials were used as received.

LEDs were purchased from Roithner Laser Technik GmbH (Vienna, Austria) with output maxima of 360, 370, 380 and 395 nm. White light LEDs (400–700 nm) were also purchased from Roithner Laser Technik GmbH. All LEDs were 5 mm in diameter and had epoxy resin caps.

The controlling power supply unit was designed and built in-house using components from RS Ltd. (UK).

2.3. Preparation of solutions

2-NB (1.5211 g) was accurately weighed and crushed in a pestle and mortar. The solid was dissolved in a 1:1 (v/v) water:ethanol mixture by stirring for 1 h. The solution was then made up to 100 mL

using the same solvent. The final concentration of 2-NB was thus 0.1 M.

Nifedipine (500 mg) was dissolved in ethanol (50 mL) and stirred with a magnetic stirrer until the solid had dissolved (30 min). Solutions were prepared in a dark room under red light to minimise photodegradation during preparation. The final concentration of nifedipine was 1% (w/v).

2.4. Photocalorimetry

Calorimetric data were recorded with a 2277 Thermal Activity Monitor (TAM, TA Instruments, USA). The TAM works on a heat-conduction principle; the sample is surrounded by a heat-sink which is maintained at a constant temperature. Any heat released (or absorbed) by the sample is quantitatively exchanged with the heat-sink via a series of thermocouples (a thermopile). The thermopile produces a voltage signal which is amplified and converted to a power signal. Each channel comprises a sample and a reference side; the sides are connected in opposition and hence the output data are the differential response of the sample and reference thermopiles. The instrument was fitted with 20 mL calorimetric channels into which the photocalorimeter was designed to fit. Stainless steel cells were used throughout. The sample cell was charged with solution (2-NB or nifedipine, 4 mL). The reference cell was charged with the appropriate blank solvent (4 mL). The apparatus was allowed to equilibrate in the TAM before the commencement of sample irradiation. Experiments were conducted at 25 °C (2-NB) and 37 °C (nifedipine).

The LED array was mounted externally from the calorimeter and the output light was directed to the sample and reference cells via liquid light guides (model 77554 LOT-Oriel Ltd., UK). Data were recorded with the dedicated software package Digitam 4.1. Three replicates of each measurement were taken and data are recorded throughout as the mean ± S.D. The instrument was calibrated periodically using the electrical substitution method and was set to an amplifier range of 100 μW.

2.5. pH titration

Experiments were performed at 25 °C with the photocalorimeter apparatus (i.e. in the 20 mL stainless steel ampoule with light input via a liquid light guide) but instead of being sited in a TAM the unit was housed in a heating block (as no calorimetric data were required and this allowed easy access for pH measurement). Samples (4 mL, prepared as above) were irradiated for various periods of time (10 min increments to a total irradiation time of 3 h). The pH of the solution was determined after each period of irradiation with a pH meter (Hamilton glass electrode).

3. Results and discussion

3.1. Chemical actinometry

The benefits of chemical actinometry and our reasons for selecting 2-NB as a model system have been discussed previously [3–4]. Earlier experiments with the Xe-arc lamp photocalorimeter suggested the photodegradation of 2-NB proceeded with zero-order kinetics [3], although determining an accurate deflection from the baseline was difficult because of the irreproducibility of the Xe-arc lamp. Before use the SPDs of both the sample and reference LED arrays were measured with a spectroradiometer (AvaSpec-2048, Avantes Ltd., UK), Fig. 2. It can be seen that the integrated areas of the two arrays are dissimilar. This effect manifests itself during a photocalorimetry experiment as a non-zero baseline, and is in part the reason we designed the power supply system discussed earlier.

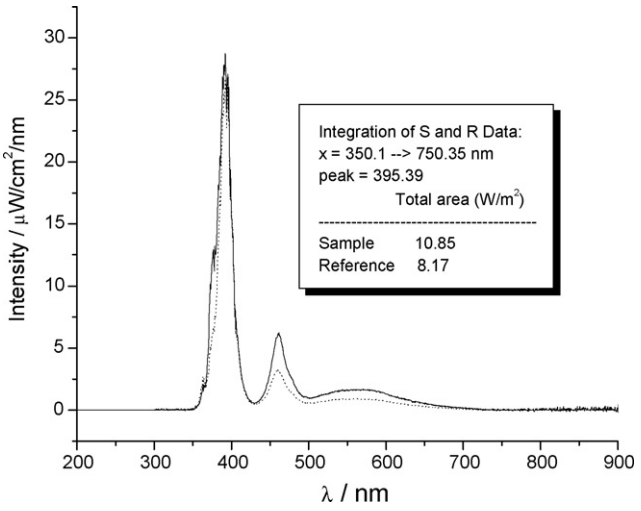

Fig. 2. Spectral power distribution (SPD) for the sample (solid line) and reference (dashed line) LED arrays.

The power supply adjusted the light intensity produced by the reference array so that a zero baseline was achieved prior to recording 2-NB data.

To record photostability data the input voltage to the sample LED array was set to 15 V. Typical data for the photodegradation of 2-NB are given in Fig. 3. It is immediately apparent that the data are representative of zero-order kinetics and that excellent stability is observed over several hours.

The power deflection for a zero-order reaction is given by;

$$\Phi = k\Delta HV \quad (1)$$

where Φ is the power output (μW) from the calorimeter, k is the zero-order rate constant, ΔH is the reaction enthalpy and V is the volume of sample. To effect a quantitative analysis requires knowledge of either the rate of degradation or the enthalpy of reaction. Since neither value was available in the literature, we determined the value of the rate constant by pH titration. The resulting rate constant value was 1.04×10^{-6} mol dm^{-3} s^{-1}. Given that the average power deflection recorded for the photodegradation of 2-NB was 21.0 ± 2.6 μW, the enthalpy of reaction can be calculated to be 5.0 ± 0.6 kJ mol^{-1}.

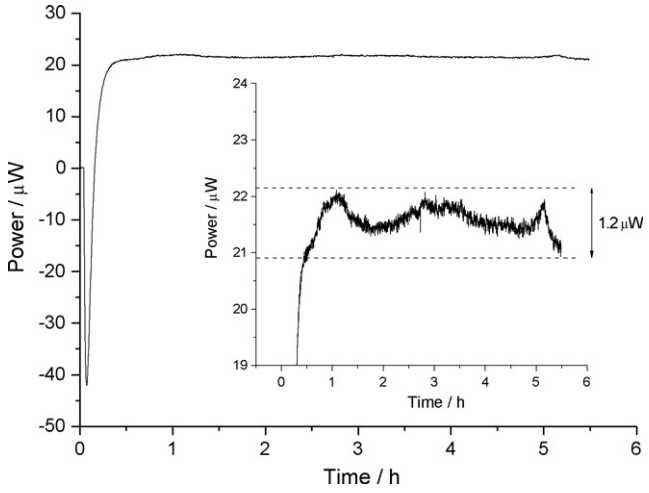

Fig. 3. Power–time data for the photodegradation of 2-NB with the LED array operating at 15 V. Inset graph: A close-up plot of the constant deflection region showing stability to ±0.6 μW.

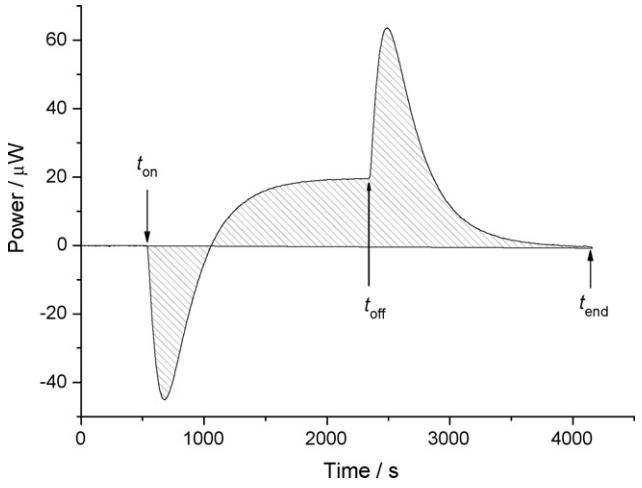

Fig. 4. Power–time data for the photodegradation of 2-NB with the LED array turned on for 30 min at 15 V and the integration to determine the total area.

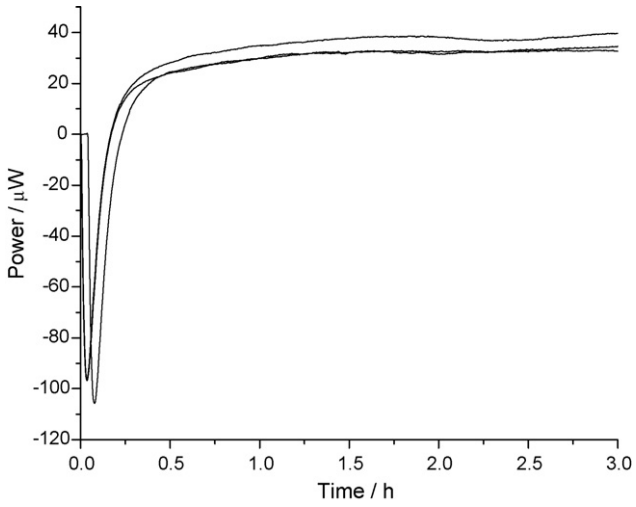

Fig. 5. Power–time data for the photodegradation of nifedipine (three experiments) with the LED array operating at 15 V.

Although the deflection seen when using the LED array was much more stable than that seen for the Xe-arc lamp used previously, the use of a power deflection can introduce errors because the choice of value is somewhat subjective. An alternative strategy is to irradiate the sample for a defined period of time (in this case 30 min) and measure the total heat change. This includes the power as the light is switched on and off and the power from photodegradation. The disturbances caused by switching the light on and off should be equal and opposite and thus are removed automatically from the data when the area is calculated. The benefit of this approach is that the power value used in the calculation is not subjective (the choice of time points to integrate between is fixed because the times at which the light was switched on and off are known absolutely). Typical data recorded in this way are given in Fig. 4. The area determined was 20.79 ± 2.5 μJ, which corresponds to an enthalpy of reaction of 5.0 ± 0.6 kJ mol^{-1}. Although in this instance the two approaches are comparable, the latter method may give more precise data when the apparatus does not produce such repeatable deflections and we include it here for completeness.

3.2. Nifedipine

Nifedipine is a well-known photolabile substance and its photodegradation has been studied in solution where it decomposes into two major products, nitro-and nitroso-derivatives, under UV light [5–7]. Nifedipine has also found use as a test drug for photostability testing in the development of new photon sources for irradiation lamps [8], making it an excellent test candidate for the photocalorimeter. Fig. 5 shows the power–time data obtained for three repeat experiments irradiating nifedipine solutions with the LED array operating at 15 V. Again, pseudo zero-order kinetic behaviour is seen, the mean deflection being 35.8 ± 2.2 μW. Knowing that these data reflect a composite signal comprising two processes, we did not attempt to calculate any reaction enthalpies by determining photodegradation rate constants in a separate experiment. Previous work on nifedipine with photocalorimetry resulted in similar data to ours [9], although the authors report a higher causative wavelength of 455 nm. Part of the reason for this apparent discrepancy may lie in the fact that although the authors measured light intensities at the different wavelengths, they note that these are not comparable. They also note that quantitative interpretation of the nifedipine data is difficult. Rather, we were concerned with the ability of the instrument to detect degradation of the drug at specific wavelengths, and hence to determine causative wavelengths of degradation. Each LED in the array was turned on individually, at 15 V, and the power–time data recorded, Fig. 6. It is important to note that the instrument was zeroed before each experiment with each LED. It is apparent that each LED caused some response from the sample, and that in general zero-order behaviour is seen. In order to determine which LED caused most degradation, it is necessary to know the light intensities produced by each LED. This was determined with spectroradiometry. Integration of the SPD data gives the power produced by each LED (μW cm^{-2}). These data are given in Table 1, with the concomitant power deflections recorded with the TAM for the nifedipine sample. Since it can be assumed that the area of sample irradiated is constant, these light powers can be normalised (in this instance to 1000 μW cm^{-2}) and the corresponding power deflections from the nifedipine sample can be calculated. These data are also given in Table 1. It is apparent that while nifedipine undergoes some photodegradation at all wavelengths, the greatest effect is seen at 360 nm, where the normalised power deflection is ca. 10× greater than at any other wavelength. This observation agrees well with data from a kinetic evaluation of the degradation of nifedipine in methanol, where absorbance maxima at 238 and 361 nm were seen [10].

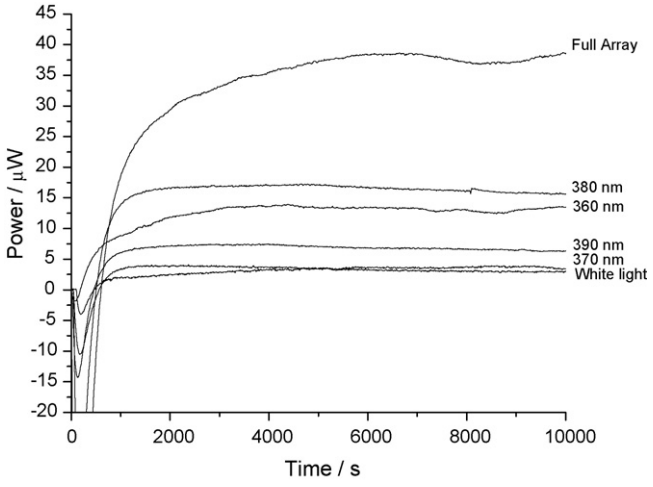

Fig. 6. Power–time data for the photodegradation of nifedipine at specific wavelengths with each LED operating individually at 15 V.

Table 1
Light intensities introduced to the sample and the concomitant power deflections recorded by the calorimeter, and the same values for normalised light powers

LED (nm)	Light power ($\mu W/cm^2$)	Average deflection (μW)	Normalised light power ($\mu W/cm^2$)	Normalised deflection (μW)
360	56.1	13.2	1000	235.3
370	299.2	3.9	1000	13.0
380	1166.5	16.8	1000	14.4
390	270.0	7.0	1000	26.0
White light	370.1	2.7	1000	7.3

In terms of instrument design, a future ideal would be to be able to adjust the SPD of each LED in the array to the same value. It would then be possible to identify the causative wavelength (or at least the wavelength region) of photodegradation simply from the power–time data. Even in its present form, however, the instrument is capable of quantitatively identifying photodegradation over narrow wavelength regions and thus has broad application to the pharmaceutical industry.

4. Summary

The use of LEDs as a light source in photocalorimetry has been successfully demonstrated to offer many benefits over the use of conventional Xe-arc lamps. Principally, LEDs offer more consistent performance with time, which is mirrored in more repeatable calorimetric data. In addition they are cheaper, last longer and are available in narrow wavelength ranges. The latter benefit permits the construction of LED arrays and, consequently, the ability to create custom spectral distributions for specific applications. Application of the instrument has been demonstrated with two model test systems; the photodegradations of 2-NB and nifedipine. The degradation of both systems followed zero-order kinetics, the magnitude of the calorimeter signal being seen to vary quantitatively with the input voltage supplied to (and hence SPD of) the LEDs. In the case of nifedipine, irradiating the sample with individual LEDs resulted in determination of the causative wavelength of photodegradation.

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