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2-Nitrobenzaldehyde: a convenient UV-A and UV-B chemical actinometer for drug photostability testing

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Abstract

We report the development of a new 'photochemical titration' actinometric method for measurement of UV-B (290–320 nm) and UV-A (320–400 nm) light dose during drug photostability testing. It is based upon photolysis of aqueous 2-nitrobenzaldehyde solution, a well-characterized reaction that has been previously demonstrated to be useful as an accurate and reliable actinometric method. Our new method can be performed by a chemical technician using only common reagents. It has been developed for use with xenon arc lamp illumination chambers which are commonly employed for drug photostability testing, but can be readily modified for use with the other illumination sources.

Keywords: Drug photostability; Actinometer; 2-Nitrobenzaldehyde

1. Introduction

Photostability testing of drug substances and products is conducted in order to determine if and to what degree they are likely to undergo photodegradation under the illumination conditions encountered during their manufacture, storage, and handling. A number of important issues such as loss of efficacy and formation of toxic photoproducts are associated with drug photodegradation [1]. The results obtained from photostability testing are highly dependent upon such variables as choice of illumination source, sample placement, temperature, and humidity. In order to standardize such testing, a monograph describing the guidelines for photostability testing of drug substances and products has been published by the International Committee on Harmonization (ICH) [2].

Actinometric measurements are made during photostability testing in order to characterize the irradiance (light dose) to which samples are exposed. Several options exist for making actinometric measurements. Some laboratories employ an instrumental approach such as radiometry or spectroradiometry. In order to be used for measuring irradiance, instrumental methods must be calibrated by the use of standard illumination

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sources or by chemical actinometry. This calibration must be carried out on a regular basis.

Typically, a chemical actinometer is employed as a solution containing a chemical compound that undergoes a specific chemical reaction as a result of photon absorption. The rate at which this reaction takes place is related to the rate at which photons are absorbed by the actinometer. In the case of drug photostability studies, the actinometer solution is transferred to an appropriate container and illuminated under the same conditions as the drug samples. The actinometer reaction is followed as a function of illumination time and these data are used to compute the irradiance to which the drug samples are exposed in the illumination chamber used for photostability testing. Some means of measuring irradiance is absolutely essential in order for the results of drug stability tests to have any quantitative significance.

The use of chemical actinometry to measure light dose during drug photostability testing offers several distinct advantages over instrumental methods. Most importantly, it is an absolute method in that it allows the absolute number of photons impinging upon a sample in a given wavelength range over a given time interval to be determined. These data can readily be converted to more convenient units (eg., $W-h^{-1}\ m^{-2}$). In addition, the actinometer can sometimes be irradiated in the same vessel as the samples (i.e.,when samples are liquid solutions), thereby eliminating the need to correct for reflectance losses.

Many chemical actinometry methods have been previously reported [3]. Unfortunately, most of these methods require the dedication of expensive laboratory instruments or involve difficult, time-consuming procedures. The general principles of chemical actinometry are discussed in detail elsewhere [4,5]. The currently approved quinine HCl actinometric method for drug stability testing using quinine HCl solutions as outlined by the ICH monograph [2] is relatively simple [6] but has been demonstrated to have some serious disadvantages [7]. These disadvantages limit the usefulness of quinine actinometry. The use of *trans*-2-nitrocinnimaldehyde solutions as an actinometer for drug photostability testing has also recently been reported [8]. This actinometric method involves following the *trans-cis* isomerization of 2-nitrocinnimaldehyde as a function of illumination time by UV–vis spectroscopy. The purpose of our investigation was to develop a reliable chemical actinometer for use in both the UVB (290–320 nm) and UVA (320–400 nm) regions that is simple and inexpensive to use on a routine basis.

The photochemistry of 2-nitrobenzaldehyde (2-NB) was first described by Ciamician and Silber in 1900 [9]. Bowen et al. [10], Lucy and Leighton [11], and Pitts [12] established the quantum yield for 2-NB photolysis; 0.5 from 300 to 410 nm in solid, liquid, and vapor phases. This means that absorption of 1 einstein (i.e.,1 mole of photons) having wavelengths from 300 to 410 nm will cause 0.5 mole of 2-NB to be converted to product. The use of 2-NB was proposed by Pitts in 1965 [13] as a convenient actinometer for atmospheric photochemistry experiments. Pitts also found that the quantum yield for conversion of 2-NB to product is independent of wavelength and temperature.

The 2-NB photoreaction (Fig. 1) proceeds via an intramolecular rearrangement involving transfer of an $-NO_2$ oxygen atom to the aldehyde functionality yielding the nitrosobenzoic acid product [9]. The photoreaction is thus not O_2 dependent. This is important since chemical actinometry is carried out in a closed vessel and depletion of O_2 would cause the rate of the 2-NB photochemical rearrangement to decrease if the reaction was O_2 dependent.

Like many other actinometers, the aqueous 2-NB solution actinometer can be employed in either high optical density or low optical density modes. The high optical density mode is preferred because of its relative simplicity. A high optical density actinometer has an absorbance >2 across

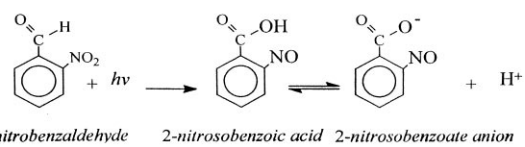

Fig. 1. Photochemical rearrangement of 2-NB to 2-nitrosobenzoic acid and dissociation of the product yielding H^+.

the wavelength range of interest so that essentially all incident photons are absorbed. This experimental approach results in the conversion of 2-NB exhibiting zero-order (linear loss of 2-NB as a function of illumination time) kinetic behavior. The low optical density mode involves a dependence of the rate constant for actinometer consumption upon the molar absorptivity which is a function of wavelength. This complicates matters significantly when polychromatic sources such as those used in drug photostability testing are used. The two modes, high optical density and low optical density, are differentiated primarily by the concentration of the actinometer solution.

Pitts [13] developed a very simple high optical density 2-NB actinometry method in which the increase in $[H^+]$ derived from dissociation of the 2-nitrosobenzoic acid photoproduct (Fig. 1) was followed as a function of illumination time. Pitts found that the increase in $[H^+]$ was linear with illumination time (zero-order) and the slope of the resulting plot was used to calculate photon flux, which can be easily related to irradiance. According to Pitts the rate of hydrogen ion formation (rate of 2-NB consumption) is given by:

$$\frac{\mathrm{d}\,[H^+]}{\mathrm{d}t} = \frac{nAt}{2NV} \quad (1)$$

where $\mathrm{d}[H^+]/\mathrm{d}t$ is obtained from the slope of the calibration plot, n is the photon flux (photons $cm^{-1}\ s^{-1}$), A is the cross sectional area of the irradiated solution (cm^2), t is irradiation times, N is Avogadro's number, and V is the actinometer solution volume(L). Thus, Eq. 1 is simply rearranged and solved for the photon flux (n).

We have expanded upon the work of Pitts by adding a specific quantity of base (NaOH) to the aqueous 2-NB actinometer solution. The desired photon dose was calculated from the 200 $W\text{-}h^{-1}\ m^{-2}$ UV exposure as specified in the ICH monograph [2] and the corresponding amount of base required to neutralize the 2-nitrosobenzoic acid formed by photolysis of 2-NB was readily calculated. Thus, the new method essentially takes the form of a photochemical acid-base titration. The solution is initially basic due to the addition of NaOH. When the desired dose of UV photons has been absorbed by the actinometer solution, enough acid is formed so that the added NaOH is neutralized and the pH drops very rapidly.

The resulting actinometry technique involves the introduction of an appropriately constructed vessel containing the actinometer solution into the illumination system and allowing the exposure to continue until the added base is neutralized. The actinometer solution pH is monitored as a function of illumination time and the data used to construct a titration curve. Alternatively, phenolphthalein indicator can be added to the 2-NB and NaOH actinometer solution so that a visible color change (from pink to yellow) can be used to determine the end point.

2. Experimental

2-Nitrobenzaldehyde (98%) and ethanol (95%) were obtained from Aldrich. Sodium hydroxide (ACS) and pH indicators (bromcresol green, bromthymol blue, phenol red, neutral red, thymol blue, and phenolphthalein) were obtained from Fisher. Purified water used for preparing aqueous photolysis solutions was generated by the use of in-house deionized water that was further purified using a Barnstead E-Pure laboratory water purification system with an activated carbon cartridge providing water of >18 M resistivity.

A solar simulator used for illumination of samples in the experiments reported here incorporating a 1 kW Xe arc lamp, optical bench, and sample illumination chamber was obtained from Spectral Energy Corporation. The lamp output was filtered through a water filter with quartz windows to remove most of the IR radiation and optical filters to remove wavelengths below 290 nm. The output of the illumination system was focused onto the face of a 1 cm quartz cuvette that was thermally equilibrated with a constant temperature water bath at 25°C. A magnetic stirrer was mounted under the cuvette so that the samples could be stirred while being illuminated. An electric shutter was controlled by a darkroom timer to provide precise control of illumination times. This solar simulator was constructed to provide illumination that closely matches terrestrial sunlight [8].

The measurement of pH was carried out for all samples using a VWR model 8005 pH meter with an Orion model 9103 pH electrode. Buffer solutions at pH 6, 7, 9, and 10 were used to calibrate the pH measurements. A three point calibration was performed using three of the above buffers whenever a pH measurement was made.

2.1. 2-NB photolysis

In order to repeat the work as described earlier by Pitts [13], a solution of 2-NB at a concentration of 0.1 M was prepared in 50% ethanol per 50% H_2O. The initial pH was determined and an aliquot of this solution was transferred to a 1-cm quartz cuvette. The cuvette containing the 2-NB solution was then placed in the sample chamber of the solar simulator. The solution was illuminated for several seconds and then an aliquot was removed and the pH again determined. This process was repeated until the pH was determined for several illumination time intervals. The data thus obtained allowed the change in $[H^+]$ to be followed as a function of illumination time.

2.2. 2-Nitrobenzaldehyde photochemical titration

A target irradiance for drug photostability testing is specified in the ICH guidelines [2] as 200 W-h^{-1} m^{-2}. The target light dose of 200 W-h^{-1} m^{-2} was first converted to units of J m^{-2} in the following manner:

$$(200\ \text{W-h m}^{-2})\ (3600\ \text{J W}^{-1}\ \text{h}^{-1}) = 7.2 \times 10^5\ \text{J m}^{-2} \quad (2)$$

This value can then be expressed in units of J cm^{-2}:

$$(7.2 \times 10^5\ \text{J m}^{-2})\ (0.01\ \text{m cm}^{-1})^2 = 72\ \text{J cm}^{-2} \quad (3)$$

The 0.1 M 2-NB actinometer solution absorbs all photons between 290 and 400 nm when placed in a 1 cm cell. If a vessel having an opening on its face of 1 cm^2 is employed, 72.0 J of energy will be absorbed by the solution when the target light dose of 200 W-h^{-1} m^{-2} is reached.

It must be kept in mind that all the photons having wavelengths in the 290–400 nm range convert the 2-NB to 2-nitrosobenzoic acid with equivalent efficiency. However, a polychromatic illumination source (e.g. Xe-arc or fluorescent tube) emits a different number of photons during a given time interval at a each wavelength. If one considers the total energy flux in the 290–400 nm range provided by several illumination systems (e.g., Xe arc, fluorescent, and metal halide), each system will provide a greater or lesser fraction of that total energy over specific bandwidths (e.g., 300–302 nm). An illumination system that produces a relatively large number of photons at shorter wavelengths (high energy photons) when compared with another illumination system will deliver the desired energy dose to a sample using fewer photons than an illumination source that produces fewer photons at shorter wavelengths. It is thus necessary to estimate the weighted-average photon energy incident upon and absorbed by the actinometer. This estimation is necessary with all the chemical actinometer systems when a polychromatic light source is used, and is strongly dependent upon the light source used for photostability testing.

The weighted-average photon energy incident upon the actinometer solution (and samples) can be estimated by an examination of the energy distribution data for the light source of interest. These data are available from a number of sources [9] and from specific illumination device manufacturers. For example, the manufacturers of commonly employed illumination chambers for drug photostability studies frequently provide tables of spectroradiometric data. These can be used to estimate the weighted-average photon energy for that specific illumination source.

Energies expressed in units of W m^{-2} over 2 nm bandwidths in the 290–400 nm range obtained from manufacturer-supplied spectroradiometeric data [14] for a 1 kW Xe arc illumination system (Table 1) were used to compute a weighted-average photon energy value in the following manner:

$$\frac{\sum_{290\ \text{nm}}^{400\ \text{nm}} (\lambda_{\text{nm}})(\text{Irradiance}_{\text{W m}^{-2}})}{\sum_{290\ \text{nm}}^{400\ \text{nm}} \text{Irradiance}_{\text{W m}^{-2}}} \quad (4)$$

Table 1
Spectroradiometric data for 1 kW Xe are solar simulator illumination system

Wavelength (nm)	Irradiance (W m^{-2})	Wavelength (nm)	Irradiance (W m^{-2})
290	0.011	348	0.774
292	0.018	350	0.769
294	0.026	352	0.800
296	0.035	354	0.826
298	0.057	356	0.863
300	0.068	358	0.833
302	0.088	360	0.850
304	0.108	362	0.906
306	0.146	364	0.899
308	0.173	366	0.920
310	0.190	368	1.023
312	0.220	370	1.062
314	0.264	372	0.966
316	0.302	374	1.007
318	0.331	376	1.041
320	0.352	378	1.088
322	0.416	380	1.212
324	0.443	382	1.250
326	0.464	384	1.227
328	0.504	386	1.254
330	0.543	388	1.295
332	0.565	390	1.399
334	0.598	392	1.418
336	0.623	394	1.545
338	0.661	396	2.067
340	0.691	398	1.954
342	0.711	400	1.565
344	0.713		
346	0.740		

The product of wavelength and irradiance over each bandwidth is summed over the wavelength range of interest (290–400 nm) and divided by the irradiance over each bandwidth summed over the range of interest. The weighted-average value for the photon wavelength obtained via the calculation described above using the data from Table 1 was 374 nm. These weighted-average values in wavelength units can easily be converted to energy units (see below).

The 374 nm weighted-average wavelength obtained above can first be expressed in meters:

$$(374\ \text{nm})\left(\frac{1\ \text{m}}{1\times 10^{9}\ \text{nm}}\right) = 3.74\times 10^{-7}\ \text{m} \qquad (5)$$

The energy of a 374 nm photon is computed readily by:

$$E = h\nu = \frac{hc}{\lambda} \qquad (6)$$

where h is Planck's constant (6.63×10^{-34} J s^{-1}) and c is light velocity (2.99×10^{8} m s^{-1}).

Thus:

$$E = \frac{hc}{\lambda} = \left(\frac{(6.63\times 10^{-34}\text{J s})(2.99\times 10^{8}\ \text{m s}^{-1})}{3.74\times 10^{-7}\ \text{m}}\right) = 5.30\times 10^{-19}\ \text{J} \qquad (7)$$

If on average, each photon delivers 5.30×10^{-19} J of energy to the actinometer solution, then:

$$72.0\ \text{J}\left(\frac{1\ \text{photon}}{5.30\times 10^{-19}\ \text{J}}\right) = 1.36\times 10^{20}\ \text{photons} = 2.26\times 10^{-4}\ \text{einstein} \qquad (8)$$

Recall (Fig. 1) that the photochemical reaction upon which this technique is based is represented by the following equation:

$$\text{2-nitrobenzaldehyde} + h\nu \rightarrow \text{2-nitrosobenzoic acid} \quad (9)$$

then:

$$\text{2-nirosobenzoic acid} \rightarrow H^+ + \text{2-nitrosobenzoate anion} \quad (10)$$

The number of moles of $[H^+]$ formed is equivalent to the number of moles of HA formed. The 2-NB actinometer has a quantum yield = 0.5 across the 290–400 nm range. This will require:

$$(2.26 \times 10^{-4}\ \text{einstein})(\Phi) = 1.13 \times 10^{-4}\ \text{einstein} \quad (11)$$

Thus, 1.13×10^{-4} moles of $[H^+]$ produced corresponds to 2.26×10^{-4} einstein of absorbed photons and will neutralize 1.13×10^{-4} moles of added base. If a different light dose for photostability testing is desired, the amount of added NaOH can be easily adjusted. For example, if a light dose of 100 W-h^{-1} m^{-2} is desired using the same illumination system, the amount of NaOH added is simply reduced by half to 5.65×10^{-5} moles.

Several other options with regard to illumination sources are available according to ICH guidelines [2]. We have thus carried out several calculations including weighted-average wavelength, weighted-average photon energy, and the amount of added NaOH required for performing the 2-NB photochemical titration with four other illumination sources based upon spectral irradiance data from 300 to 400 nm (Table 2).

2.3. Experimental procedure

The procedure used to perform photochemical titration actinometry experiments involved preparation of 0.1 M 2-NB in 0.03 M NaOH using 50% ethanol per 50% H_2O solvent. Exactly 4.0 ml of the 2-NB solution was transferred into a 1 cm path length quartz UV–vis cuvette (NSG Precision Cells). This provides enough base to slightly exceed (5%) the desired light dose of 200 W h^{-1} m^{-2} from a filtered Xe-arc lamp based upon the data from Table 1. In order to measure the desired light dose, the cuvette must be masked so that only 1 cm^2 of one the faces is exposed to light. Experiments were conducted in which 2-NB actinometer solutions with added NaOH were illuminated using the Xe-arc solar simulator in masked 1 cm quartz cells. The actinometer solution pH was monitored as a function of illumination time.

Acid-base indicators are frequently employed in acid-base titration experiments in order to visualize the end point by color change rather than by monitoring pH with a meter. In addition to the obvious requirement that the indicator must not absorb strongly in actinometer range (290–400 nm), it also must not undergo photoreaction and it must produce an observable color change in 2-NB actinometer solution. Several acid-base indicators were evaluated for use with the 2-NB photochemical titration method; bromcresol green,

Table 2
Weighted-average wavelength photon energy data for several UV fluorescent illumination sources[a]

Illumination source	Weighted-average wavelength (nm)	Photon energy (J)	Added base required (moles)
Japan UV[b]	356.7	5.56×10^{-19}	1.07×10^{-4}
USA UV-A[c]	356.2	5.56×10^{-19}	1.07×10^{-4}
Vita-lite[d]	385.6	5.14×10^{-19}	2.33×10^{-4}
Cool white[e]	394.0	5.03×10^{-19}	2.38×10^{-4}

[a] Data from ref. [16].
[b] Toshiba FL40BL.
[c] Sylvania F20T12/BLB.
[d] Vita-Lite Duro-test.
[e] Sylvnia F20T12/CW.

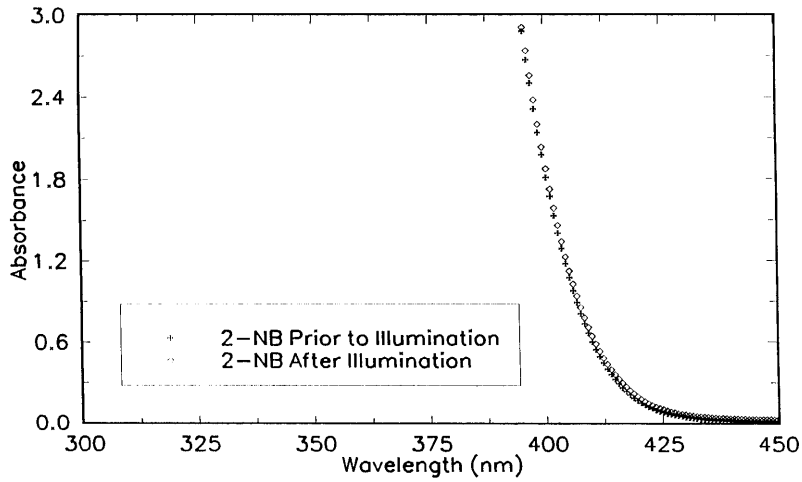

Fig. 2. UV-vis absorption spectrum of a 0.1 M 2-NB and 0.03 M NaOH solution prepared in 50% H_2O per 50% ethanol both before and after illumination in the solar simulator for 60 min.

bromthymol blue, phenol red, neutral red, thymol blue, and phenolphthalein. In each case, a working solution of the indicator was prepared and an aliquot was added to the 2-NB actinometer solution. For example, 20 μl of a 0.1% phenolphthalein indicator solution was added to the 2-NB and NaOH actinometer solutions prior to illumination. In order to determine if phenolphthalein directly photolyzes, a solution containing 20 μl of the phenolphthalein indicator solution diluted to 4.0 ml with 50% ethanol per 50% H_2O was illuminated in the solar simulator for 1 h in a 1 cm quartz cuvette.

3. Results and discussion

The UV–vis absorption spectrum for a 0.1 M 2-NB and 0.03 M NaOH solution prepared in 50% ethanol per 50% H_2O is presented in Fig. 2. This figure confirms the complete absorbance of 290–400 nm range photons by the actinometer solution. At 400 nm, the wavelength within the 290–400 nm range at which the molar absorptivity reaches a minimum, the absorbance is approximately 2.4. This figure also demonstrates that complete absorbance across the 290–400 nm range is maintained after illumination of the actinometer solution in the solar simulator for 60 min. This confirms that complete absorption (i.e. $A > 2$) conditions are maintained during all of the experiments reported herein.

3.1. 2-NB photolysis

The original method of Pitts [13] was repeated without alteration. All the experiments with 2-NB exhibited the expected zero-order kinetic behavior (Fig. 3). As can be observed by an inspection of Fig. 3, the change in pH was very rapid. The actinometer solution exhibited a distinct yellow color after illumination, a result of 2-nitrosobenzoic acid formation.

A total of 14 measurements of photon flux were performed using the 2-NB method with the solar simulator in a temperature range from 25 to 40°C. The photon flux incident upon samples in the solar simulator used for our experiments was calculated from experimental data using equation 1. The photon flux over the range from 290 to 400 nm was found to be $3.70 \pm 0.07 \times 10^{-8}$ einstein s^{-1}. The uncertainty ($< 2\%$) reflects both the reproducibility of the method and fluctuations in the illumination source output and verifies that the rate of the 2-NB photolysis (and probably the quantum yield) is independent of temperature over the range examined. In order for the photon flux data to be useful in drug photostability stud-

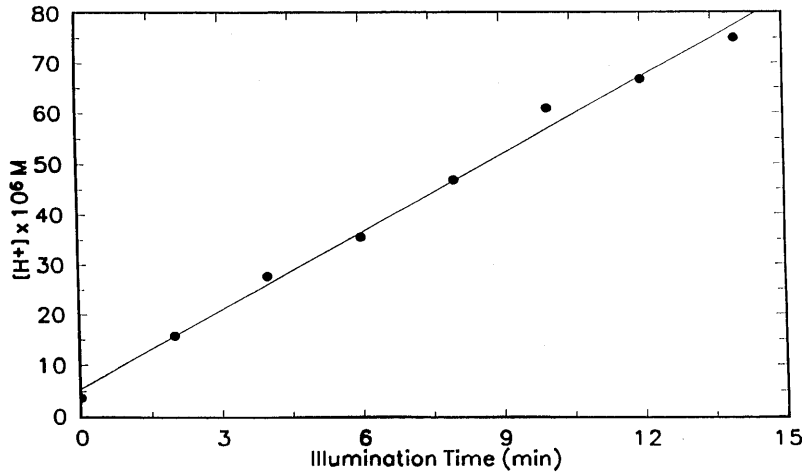

Fig. 3. First-order kinetic behavior of 0.1 M 2-NB 50% H_2O per 50% ethanol solution photolysis yields a linear increase in $[H^+]$ as a function of illumination time in the solar simulator.

ies, it must be converted to irradiance units as discussed above. The results obtained for irradiance using other illumination systems are likely to be significantly different than those reported here.

The results obtained indicate that the original actinometric method as described by Pitts [13] might be acceptable for drug photostability testing as long as the reaction is not allowed to proceed beyond the point that complete absorbance is maintained. These caveats are necessary primarily because the reaction occurred very rapidly in our experiments. It would be necessary to perform an actinometry experiment more than once during the course of a photostability test due to rapid 2-NB consumption; possibly at the beginning and then at the end of a test. This will allow an assessment of fluctuations in lamp output during the course of the experiments. Finally, measurement of the actinometer solution pH is somewhat tedious (aggravating) because it is initially fairly close to neutral pH which results in dissolution of CO_2 from the surrounding air causing drift. For all of the reasons listed above, we sought a simpler chemical actinometry method viz. the 2-NB photochemical titration method discussed herein.

3.2. 2-Nitrobenzaldehyde photochemical titration

The data presented in Table 3 and in Fig. 4 were obtained by performing a photochemical titration using the 2-NB actinometer with added NaOH as described herein. The data in Table 3

Table 3
Photochemical titration of a 0.1 M 2-NB and 0.03 M NaOH actinometer solution prepared with phenolphtalein indicator prepared using 50% ethanol per 50% H_2O illuminated in the solar simulator

Illumination time (min)	$[H^+]$ (M)	pH	Color
0	7.91×10^{-14}	13.1	Pink
5	9.56×10^{-14}	13.0	Pink
10	1.17×10^{-13}	12.9	Pink
15	1.58×10^{-13}	12.8	Pink
20	2.28×10^{-13}	12.6	Pink
25	4.32×10^{-13}	12.4	Pink
30	1.48×10^{-12}	11.8	Pink–orange
32	8.01×10^{-12}	11.1	Pink–orange –yellow
34	2.37×10^{-8}	7.6	Yellow
35	1.26×10^{-7}	6.9	Yellow
36	3.29×10^{-7}	6.5	Yellow
37	5.75×10^{-7}	6.2	Yellow
38	1.03×10^{-6}	6.0	Yellow
39	1.13×10^{-6}	6.0	Yellow

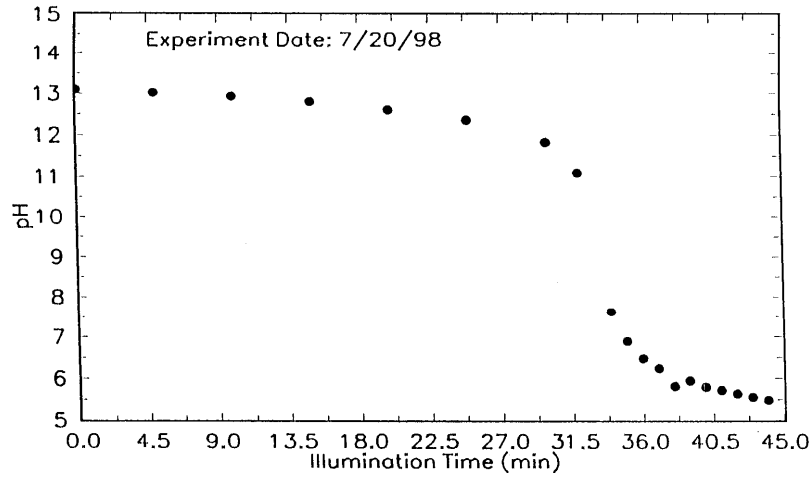

Fig. 4. Photochemical titration of a 0.1 M 2-NB and 0.03 M NaOH in 50% H_2O per 50% ethanol solution.

allow the end point to be determined by constructing a simple pH titration curve; a plot of pH versus illumination time. In order to more easily determine the end point, a first derivative plot of the data (dpH/dt) can be constructed as in Fig. 5. This makes determination of the end point very simple. The pH measurements were less tedious than with the 2-NB photolysis described earlier because readings were more stable since the pH is initially very basic and is unaffected by CO_2 dissolution from the air.

A total of three replicate measurements were made using the photochemical titration method as described herein. The endpoint of the titration was reached in our solar simulator under the conditions employed in 34 ± 2 min. Thus, the target light dose of 200 W-h^{-1} m^{-2} for UVB and UVA as specified in the ICH guidelines [2] is delivered to samples by our illumination system in 34 min. We must emphasize that the time to reach the end point for the 2-NB photochemical titration using our solar simulator system is likely to be significantly different than with most other illumination systems used for drug photostability testing. This is because our system focuses the output from the 1 kW Xe arc lamp down to a small area (ca. 5 cm^2). Most illumination systems are set up to illuminate a much larger area. Thus, measured irradiances for other illumination systems are likely to be much lower than those reported here and the time required to reach the endpoint will be accordingly longer.

The use of acid-base indicators to determine the end point for 2-NB photochemical titrations was somewhat problematic. Some of the indicators (e.g., phenolphthalein) absorbed photons in the same wavelength region as 2-NB and slowed the reaction. Other actinometers (e.g., bromcresol green) produced a colored product when the endpoint was reached which was masked by the yellow color of the 2-nitrosobenzoic acid product from 2-NB photolysis.

It was found that effective use of the phenolphthalein indicator is possible because the actinometer solution changed from pink to yellow at the endpoint of the titration as determined by pH measurements and the construction of a titration curve. Phenolphthalein is pink at $pH > 10$ and changes to colorless at $pH < 8$; the yellow color at the endpoint was due to the presence of 2-nirosobenzoic acid in the solution.

An estimated correction factor was determined in order to account for the screening effect of phenolphthalein upon the photolysis of 2-NB and a correspondingly reduced amount of NaOH was added to actinometer solutions. This was done because the absorption spectrum for the phenolphthalein indicator overlaps that of 2-NB at high pH (Fig. 6). Under the experimental conditions reported here, at 400 nm the absorbance due

to phenolphthalein is less than 10% of that for 2-NB and at 300 nm it is less than 1% of that for 2-NB. We estimate that the addition of phenolphthalein absorbs approximately 5% of the incident photons from 300 to 400 nm and thus slows the 2-NB actinometer photolysis by about 5%. As anticipated, by the determination of the end point from the titration curve, it was found to require an approximately 5% longer period of illumination to reach the end point when phenolphthalein was added than when phenolphthalein was omitted from the 2-NB actinometer solution. Phenolphthalein does not directly photolyze under the conditions employed in our experiments.

Changes in the 'spectral power distribution' of polychromatic illumination sources such as Xe arc lamps over time [15] will result in some error when any chemical actinometric method is used unless these changes are quantified and corrections made to the actinometry data. This is also the case when radiometric measurements are employed. Only spectroradiometric methods allow changes in spectral power distribution to be detected. However, reliable spectroradiometric measurements require very expensive instrumentation and sophisticated labor-intensive calibration techniques.

Drug photostability testing as currently practiced is subject to several significant uncertainties. These include, but are not limited to, differences in the spectral distributions of illumination sources, the effect of ageing upon the spectral distributions of illumination sources (discussed above), variations in irradiance as a function of sample position in light cabinets, poor temperature control in many illumination systems, humidity effects, choice of construction materials for sample containers, and reflective effects on surfaces (e.g., tablets). The significance of any experimental uncertainty associated with chemical actinometry is likely to be quite small in comparison to the other sources of uncertainty listed above.

4. Conclusions

The 2-NB photochemical titration actinometry method presented here is readily applicable to drug photostability testing. Like other chemical actinometry methods, it has the significant advantage when compared with instrumental methods, of allowing determination of absolute photon flux incident upon drug samples. It is simple to use, the reagents are inexpensive and readily available from several sources, and it requires no sophisticated instrumentation. It shares the disadvantages of other chemical actinometry methods when polychromatic illumination sources are used in that an estimate of the weighted-average photon

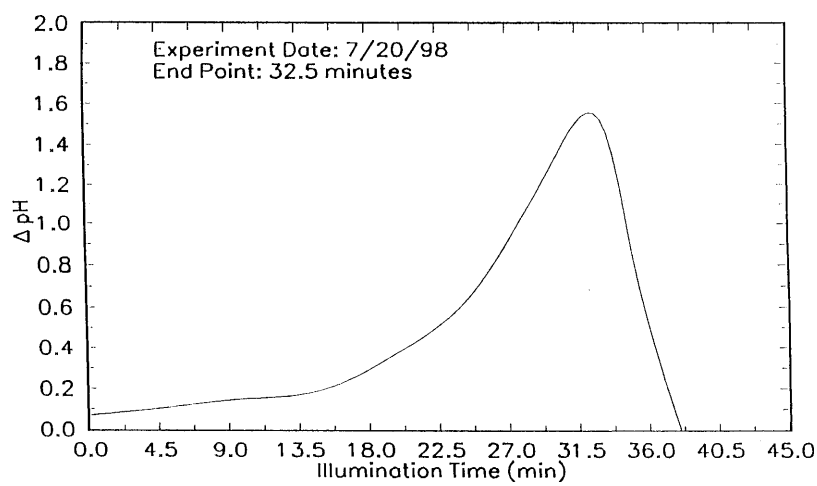

Fig. 5. First derivative plot {dpH/dt vs. illumination time) of the photochemical titration data shown in Fig. 4.

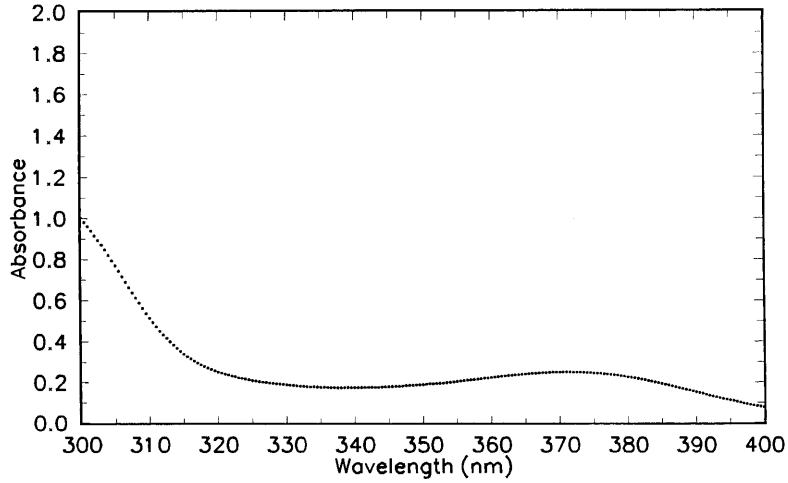

Fig. 6. Absorption spectrum for 20 μl of 0.1% phenolphthalein indicator solution adjusted to pH 12 with NaOH in a 1-cm cuvette diluted with CH_3CH_2OH/H_2O to a total volume of 4.0 ml.

energy must be made for each set of illumination conditions (i.e., type of lamp, filters, etc.).

Several important points should be reiterated: A calculation of the weighted-average photon energy must be carried out for each illumination system to be employed and the proper amount of NaOH must be added to the actinometer solution based upon the illumination source used and the desired sample irradiance. This will require the exclusive use of illumination systems for which spectroradiometric data are available; either from the manufacturer or by measurement in-house. If the phenolphthalein indicator is used in order to visually determine the end point, the amount of added base should be reduced by approximately 5% in order to account for screening of the 2-NB actinometer by phenolphthalein. It is also essential that the vessels used for the actinometer solution and drug samples be constructed of UV quartz. The vessel containing the actinometer solution must also be properly masked in order to reach the specified irradiance at the end point. If these precautions are followed, the photochemical titration method using 2-NB developed herein should provide an excellent method for the measurement of irradiance during drug photostability testing.

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