Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 886–888

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Considerations on the quinine actinometry calibration method used in photostability testing of pharmaceuticals

Clayton Anderson de Azevedo Filho[a,*], Daniel de Filgueiras Gomes[b], João Paulo de Mélo Guedes[a], Rayana Muniz Falcão Batista[a], Beate Saegesser Santos[a]

[a] Núcleo de Pesquisas em Nutrição Parenteral, Centro de Ciências da Saúde, Departamento de Ciências Farmacêuticas, UFPE, Recife, Brazil
[b] Centro de Informática, Departamento de Sistemas da Computação, UFPE, Recife, Brazil

ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form 28 October 2010
Accepted 10 November 2010
Available online 18 November 2010

Keywords:
Photostability chamber
Photodegradation
Quinine actinometry
Exposure surface

ABSTRACT

This paper addresses two critical issues concerning the guidelines adopted by the ICH on the photostability testing: the quinine actinometry method and the light/radiation exposure map distribution of the photostability chamber. Using a qualified non-commercial photostability chamber tests were performed using quinine and physical actinometry and compared the results to those which are used as the basis of the ICH guidelines. The statistical analysis on the results showed that: (i) the calibration curve of the quinine solution depends on its concentration and on its location in the chamber; (ii) the quinine actinometry method currently recommended by the ICH guidelines should not be generalized to any photostability chamber.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Photostability testing is typically performed under controlled conditions, often in a chamber where exact exposure levels of the light spectrum are delivered for precise analysis of the effects. To perform photostability testing it is necessary to know the spectral and intensity distribution of the radiation source to ensure that a precise amount of radiation is homogeneously distributed on the surface area where the product will be exposed [1–5]. Thereby, it is necessary to guarantee a thorough and secure qualification of the photostability chamber in order to prove that this equipment is suitable to photostability testing [6]. There are two experimental ways to qualify the photostability chamber and to determine the correct applied radiation dose: (i) by using chemical actinometry and (ii) by applying a physical device (physical actinometry or radiometry) on which the (radiation) number of photons in a defined space can be fully determined [6–8].

Quinine actinometry is an adopted standard method for calibrating the intensity of UV radiation (300–400 nm) of the radiation light sources used in photostability testing. This methodology is reported in the U.S. Food and Drug Administration (FDA)/National Institute of Standard and Technology (NIST) studies and is the basis for different official parameters that guide the stability testing of pharmaceuticals [1–3,6]. The known paper of Yoshioka et al. [9] proposed quinine actinometry as a universal standardized method for calibrating UV intensity in light sources [9]. This paper presented a joint study of seven different laboratories and showed that there is a linear correlation between the quinine absorbance and the integrated UV radiation. The referred study was performed using a 5% quinine solution which was exposed to different UV sources and the authors suggested that being this solution too concentrated it would be more interesting to use a 2.5% quinine solution instead. The slope of both regression curves was compared and considered similar so that they could be used indistinctively, however these results were not compared statistically by the authors. These results were later used as the basis of the Q1B photostability testing guidelines described by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [1]. Although the main topics are addressed by these guidelines, there is still a great deal of fundamental questions concerning the photostability testing that remain unclear. Different authors have pointed out some of these questions in the literature since the publication of the ICH guidelines [10,11]. An example is the quinine chemical actinometry. Not only this method is poorly described in the guide but also it was shown by Baertschi et al. that its reproducibility depends on the experimental conditions (measurement time interval, pH, temperature, oxygenation, lamp emission spectrum) [10–12]. The main purpose of the present study is to show experimentally some divergences that arise when applying the quinine actinometry using different quinine solution concentrations.

* Corresponding author at: Av. Prof. Arthur de Sá, s/n, Cidade Universitária, 50740-521 Recife, PE, Brazil. Tel.: +55 81 2126 8511; fax: +55 81 2126 8511.
E-mail address: claytonazevedo@hotmail.com (C.A. de Azevedo Filho).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.11.012

2. Experimental

The present study was performed in a photostability chamber equipped with one ultraviolet lamp with 22 W UV power in accordance to the Guidance for Industry Q1B Photostability Testing of New Drug Substances and Products by ICH [1]. The temperature in the chamber was kept at 28 ± 1 °C. A radiometer model MRU-201 (Instrutherm™ – Brazil) maximum range 200 W h/m^2 was used to determine the ultraviolet irradiance and the average value was estimated with at least 10 measurements. Lamp emission spectra were collected on a spectrophotometer model HR4000 (Ocean Optics™ – Germany) and the absorption spectra were registered in a 10 mm quartz cuvette on a Cary 50 Bio spectrophotometer (Varian Inc. – USA) and. Quinine monohydrochloride anhydrate with a 90% purity grade was purchased from Sigma–Aldrich (Germany) and was used to prepare. Solvents and other reagents were of the highest purity commercially available. Chemical calibration was performed using quinine monohydrochloride in 2% and 5% (w/v) aqueous solution sealed in glass vials.

A confidence interval of 95%, with alpha = 5% and $n - 2$ degrees of freedom, has been constructed for the absorption curves of the 2% and 5% (m/v) quinine solutions in order to compare with the results obtained by Yoshiokaĭs group [9]. Two different situations were evaluated: (1) the agreement of the confidence interval of the 2% and 5% (m/v) curves obtained by us with the 5% (m/v) concentration curve obtained by Yoshioka and (2) the agreement confidence interval of the 2% and 5% (m/v) curves in relationship to each other measured in the same photostability chamber. In the statistical analysis we assume that there is an agreement between the results of both curves only when there is a confidence interval overlap for the slopes and for the linear coefficient of the curves.

3. Results and discussion

3.1. UV irradiance intensity and surface exposure mapping

The photostability chamber used in the present study is in accordance to the ICH (option 2) guideline [1]. The emission spectrum and the band profile of the UV lamp agree with the manufacturerĭs description and also to the ICH recommendations, having a spectral distribution from 320 to 400 nm and maximum at 362 nm. Theoretical estimative of the direct incidence of UV radiation (based on data of the UV power emission supplied by the manufacturer) was calculated by dividing the UV power output by the total sample exposure area. The estimated irradiance is I = 12.3 W/m^2.

Although there is no official recommendation on the location of the sample in the chamber some authors have reported a high variability of radiation and light dose depending on the type of the photostability chamber used and on the positioning of the sample inside the chamber [2,3,6]. Taking this information into account we performed the surface exposure mapping in order to characterize this parameter. This procedure is necessary to ensure a homogeneous irradiance distribution to the analyzed sample, and in the present study, to ensure the repeatability of the quinine actinometry system. The chamber irradiation intensity mapping in the UV region (measured in W/m^2) is shown in Fig. 1. We observe a non-uniform distribution in the intensity of UV radiation on the sample exposure area and this is probably related to the location of the lamp in the chamber. This central region presents an irradiation intensity which corresponds to an irradiance of I = 14.45 W/m^2, showing a good agreement with the theoretical estimative.

Observing the UV irradiation mapping we observe that, as expected, the further away from the center of the radiation source the lower the irradiation intensity. It is interesting to observe the difference in uniformity of the distribution of UV radiation, even with the chamber walls covered with aluminum sheets to provide better light distribution. This distribution profile shows that only a restricted area may be used to induce a uniform photodegradation kinetics of the sample. As each photostability chamber has its own design one has to estimate the working area which will guarantee a homogeneous radiation output to the drug, thus, providing reproducible degradation kinetics. In Fig. 1, we observe an ellipsoidal gradient graph shape for the lamp used and the difference in intensity average between the central region (I = 14.45 W/m^2) and peripheral region (I = 6.2 W/m^2) is approximately 55%. Nevertheless, the central value of the gradient shape graph is close to the theoretical value calculated considering only the direct incidence of light. This suggests that the UV radiation is practically not reflected by the surface inside the chamber, i.e., the degradation due to UV radiation is resulting by direct incidence. This inhomogeneous surface mapping, also observed by other authors, corroborates that to ensure the reproducibility of the photostability testing and to guarantee a reliable comparison among different chambers, a surface exposure mapping should always be performed. We strongly suggest that the ICH review the Guideline Q1B to include this recommendation.

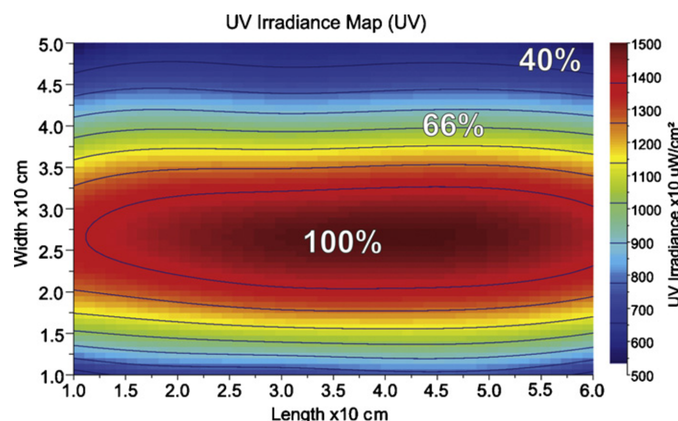

Fig. 1. UVA irradiation intensity mapping (W h/m^2) of the photostability chamber. The numbers indicate the irradiance percentages.

3.2. Quinine actinometry method

The absorbance *versus* irradiation plots of the 2% and 5% (w/v) UV exposed quinine solutions are depicted in Fig. 2 and the results of the angular and linear coefficients of both curves are described in Table 1, which also presents Yoshiokaĭs data [9]. Two main considerations may be drawn when applying the statistical analysis

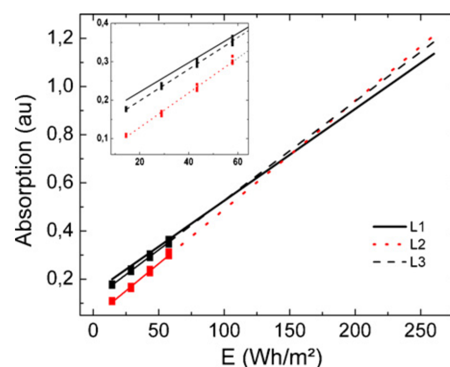

Fig. 2. Absorption at 400 nm *versus* energy density (E) calibration curve of UV degraded quinine solutions. (L_1) 5% quinine solution reported by Yoshioka et al. [9]; (L_2) 2% and (L_3) 5% quinine solutions. The insert shows the projection of the calibration curves of the UV degraded quinine solutions.

Table 1
Results of the calibrating curve coefficients obtained in this work and by Yoshioka et al. [9].

	Slope ($W h/m^2$)	Intercept	Adjusted R^2
Quinine solution 2% (w/v)	$0.00451 \pm 7.2 \times 10^{-5}$	0.03868 ± 0.00285	0.992
Quinine solution 5% (w/v)	$0.00411 \pm 6 \times 10^{-5}$	0.11633 ± 0.002368	0.993
Yoshioka et al.	$0.00381 \pm 47 \times 10^{-5}$	0.145 ± 0.009	

described in the present study. First, the agreement of the confidence interval of the 2% and 5% (m/v) curves obtained by us with the 5% (w/v) concentration curve obtained by Yoshioka et al. [9] is not statistically comparable with 95% of confidence. This implies that Yoshiokaїs results should not be generalized to any UV lamp. The curve shown in Yoshiokaїs work (and plotted in Fig. 2) corresponds to an average angular coefficient obtained from seven slopes estimated for seven different lamps and cannot be generalized without implying large errors. Second, the agreement of the confidence interval of the 2% and 5% (w/v) curves in relationship to each other and measured in the same photostability chamber indicates that the quinine actinometry is concentration dependent. As can be seen in Table 1, there is no overlap at 95% confidence intervals of the 2% and 5% (w/v) quinine curves, so, it is not possible to say that these curves are statistically comparable. Analyzing Fig. 2 we see that the curves meet at one single point (at $I = 194.13\ W h/m^2$) and differences between the curves are more evident under $150\ W h/m^2$ and above $200\ W h/m^2$. This dependence with the concentration has a direct impact on the ICH guideline. Yoshiokaїs work uses quinine solutions at 5% (w/v) while the ICH and FDA recommends the use of 2% (w/v) quinine solutions as a reference for conducting drug photostability studies. Although this value may be considered negligible for some substances it may also result in a great difference for highly photosensitive compounds. These will show great decomposition rates even at smaller radiation doses and may be completely degraded in very short time periods, requiring a reduced exposure time to fully monitor its photodegradation kinetics.

The present considerations add to the results and comments reported by Baertschi et al. which demonstrated strong evidences that a more thorough study should be performed on the quinine system [10,11]. We also agree that the quinine actinometry system is not yet completely validated to be applied as the ideal method for photostability testing and the ICH guidelines should be reviewed to include more detailed and reliable experimental conditions to ensure the reproducibility of this actinometry system.

4. Conclusions

We performed tests using quinine and physical actinometry and compared the results to those which are used as the basis of the ICH guidelines. The results point out that the calibration curve of the quinine solution depends on its concentration and on its location in the chamber and we suggest that the quinine actinometry method currently recommended by the ICH guidelines should not be generalized to any photostability chamber. Moreover we suggest that to ensure the reproducibility of the photostability testing and to guarantee a reliable comparison between different chambers, a surface exposure mapping should always be performed and this measurement should be recommended by the ICH guidelines.

Acknowledgements

The authors are grateful to Prof. Waldeciro Colaço, Prof. Adriana Fontes and Prof. Patrícia Farias for helpful discussions; to Prof. Cid B. de Araújo and Prof. José Almiro da Paixão for the use of the spectrophotometer and the financial support of the following agencies: FACEPE, CAPES, PROPESQ/UFPE and CNPQ.

References

[1] ICH, Guidance for industry Q1B photostability testing of new drug substances and products, Fed. Regist. 62 (1996) 27115–27122.
[2] H.H. Tønnesen, Photostability of Drugs and Drug Formulations, second ed., CRC Press LLC, New York, 2004.
[3] J.T. Piechock, K. Thoma, Pharmaceutical Photostability and Stabilization Technology, Informa Healthcare, New York, 2007.
[4] K.C. Waterman, R.C. Adami, Accelerated aging: prediction of chemical stability of pharmaceuticals, Int. J. Pharm. 293 (2005) 101–125.
[5] H.H. Tønnesen, Formulation and stability testing of photolabile drugs, Int. J. Pharm. 225 (2001) 1–14.
[6] S.R. Thatcher, R.K. Mansfield, R.B. Miller, C.W. Davis, S.W. Baertschi, Pharmaceutical photostability: a technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability – Part 2, Pharm. Technol. 25 (2001) 58–60.
[7] J.M. Allen, S.K. Allen, S.W. Baertschi, 2-Nitrobenzaldehyde: a convenient UV-A and UV-B chemical actinometer for drug photostability testing, J. Pharm. Biomed. Anal. 24 (2000) 167–178.
[8] H.J. Kuhn, S.E. Braslavsky, R. Schmidt, Chemical actinometry, Pure Appl. Chem. 76 (2004) 2105–2146.
[9] S. Yoshioka, Y. Ishihara, T. Terazono, N. Tsunakawa, M. Murai, T. Yasuda, Kitamura, Y. Kunihero, K. Sakai, Y. Hirose, K. Tonooka, K. Takayama, F. Imai, M. Godo, M. Matsuo, K. Nakamura, Y. Aso, S. Kojima, Y. Takeda, T. Terao, Quinine actinometry as a method for calibrating ultraviolet radiation intensity in light-stability testing of pharmaceuticals, Drug Dev. Ind. Pharm. 20 (1994) 2049–2062.
[10] S.W. Baertschi, Commentary on the quinine actinometry system described in the ICH draft guideline on photostability testing of new drug substances and products, Drug Stab. 1 (1997) 193–195.
[11] S.W. Baertschi, K.M. Alsante, H.H. Tønnesen, A critical assessment of the ICH guideline on photostability testing of new drug substances and products (Q1 B): recommendation for revision, J. Pharm. Sci. 99 (2010) 2934–2940.
[12] S.R. Thatcher, R.K. Mansfield, R.B. Miller, C.W. Davis, S.W. Baertschi, Pharmaceutical photostability: a technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability – Part 1, Pharm. Technol. 25 (2001) 98–110.