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Research Paper

Effect of ambient light on IgG1 monoclonal antibodies during drug product processing and development

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ABSTRACT

Photostability studies are standard stress testing conducted during drug product development of various pharmaceutical compounds, including small molecules and proteins. These studies as recommended by ICH Q1B are carried out using no less than 1.2×10^6 lux-hours in the visible region and no less than 200 W h/m^2 in UV light. However, normal drug product processing is carried out under fluorescent lamps that emit white light almost exclusively in the >400 nm region with a small UV quotient. We term these as ambient or mild light conditions. We tested several IgG1 monoclonal antibodies (mAbs 1–5) under these ambient light conditions and compared them to the ICH light conditions. All the mAbs were significantly degraded under the ICH light but several mAbs (mAbs 3–5) were processed without impacting any product quality attributes under ambient or mild light conditions. Interestingly we observed site-specific Trp oxidation in mAb1, while higher aggregation and color change were observed for mAb2 under mild light conditions. The recommended ICH light conditions have a high UV component and hence may not help to rank order photosensitivity under normal protein DP processing conditions.

1. Introduction

Protein molecules are highly complex active pharmaceutical ingredients that require special care during drug substance (DS) and drug product (DP) processing. After producing the protein in different cell culture systems (*Escherichia coli* or CHO cells) and further purification, typical DP processing for monoclonal antibodies includes freeze–thaw, dilution and filling steps [1]. Proteins are known to be photosensitive molecules, especially to UV light in the 200–400 nm range [2,3]. This photosensitivity comes from various side chains and amino acid residues in the protein, including Trp, Tyr, His, Phe and disulfide bonds. At 280–300 nm, the absorption coefficient of Trp is roughly 28 times that of the disulfide bond and about 4 times that of Tyr [4]. The protein molecule is normally processed in the GMP setting under ambient light conditions that includes fluorescent, metal halide and/or LED lamps. In addition to the above DP processing steps, the protein in the final DP configuration has to be inspected prior to label/pack activities under ambient laboratory light conditions. A robust protein formulation should not only stabilize the protein for long-term storage (shelf life) but also prevent additional damage to the protein during DP processing. For proteins, especially monoclonal antibodies (mAbs), some of the product quality attributes that potentially change during processing and over time (and thus limit shelf life) include high molecular weight species (HMWS), charge variants, oxidation, and color. Hence there is an onus on the pharmaceutical scientist to understand the impact of various processing conditions, including effect of light in the GMP processing areas, on the protein in addition to other physico-chemical challenges that the protein endures over the shelf life.

Photostability testing is an integral part of the stress test for new DS and DP as indicated in ICH Q1B [5]. Photostability under these ICH conditions is usually carried out using no less than 1.2×10^6 lux-hours of visible light and no less than 200 W h/m^2 of UV light. Relatively little data is available regarding the

Abbreviations: ACN, acetonitrile; DTT, dithiothreitol; FS, fluorescence; His, L-Histidine; ICH, International Conference on Harmonization; mAb, monoclonal antibody; Met, L-Methionine; Phe, Phenylalanine; TFA, trifluoroacetic acid; Trp, L-Tryptophan; Tyr, L-Tyrosine.

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photosensitivity of proteins exposed directly to visible light with limited or no UV exposure (ambient or mild light conditions) or in the absence of vitamins [6–9]. Various groups have recently reported UV light mediated degradation of proteins, especially those UV conditions seen under the ICH recommendations [10–12]. We have recently reported that mAb1, which is a humanized IgG1 monoclonal antibody, is sensitive to ICH light conditions [13] and can initiate antibody-catalyzed water oxidation pathway (ACWOP) [14]. Surface exposed Trp in mAb1 efficiently converts light energy into various reactive oxygen species (ROS) such as singlet oxygen, superoxide, hydroxyl radicals and hydrogen peroxide. We also recently reported that an IgG1 mAb increased in acidic variants and Fc Met oxidation when exposed to ambient light during small-scale cell culture processes, especially when incubated in media and/or harvested cell culture fluid (HCCF) [15]. Interestingly, after the mAb is purified from media or HCCF and processed as drug substance and drug product, the photosensitivity may still be retained due to the intrinsic nature of the protein and/or its formulation components [3,15,16].

High concentration protein formulations and bulk drug substance (BDS) sometimes have substantial color, which to some extent may be dependent on light exposure and can be a challenge during technical development. Vijayasankaran et al. have demonstrated that lowering the concentration of B-vitamins and iron in the cell culture media led to reduction of color in the final BDS [17]. Prentice et al. reported recently that hydroxocobalamin (OH-Cbl) is formed from cyanocobalamin (Vitamin B12) in the presence of light [18]. This OH-Cbl associates with the protein during cell culture process and results in a pink colored protein product (IgG1, IgG4 and Fc fusion proteins). Derfus et al. have also recently reported that a red colored IgG4 is caused by OH-Cbl in addition to disulfide reduction in the antibody [19]. This red color in the IgG4 was independent of light exposure and maybe due to a different mechanism. In addition to the above literature recently Butko et al. have reported that advanced glycation end products could also lead to color in monoclonal antibodies [20] while Li et al. used a combination of absorption spectroscopy and mass spectrometry to identify UV light induced tryptophan oxidation products as the root cause in color changes in a stressed mAb formulation [21].

Most photochemical studies reported in the literature are focused on light sources that are either a combination of excessive UV and visible light (200–600 nm) or exclusively UV light (<305 nm). Some studies such as that reported by Stroop et al. [8] and Qi et al. [9] use fluorescent lamps and photosensitive His-based buffers. In this report, we investigated different IgG1 mAbs in their respective formulation buffers under ambient or mild light that is typically encountered during drug product processing steps (e.g. fluorescence lamps). In addition, we compared the changes in product quality attributes between the ICH light and ambient light studies to elaborate on relevant stress conditions that may be more applicable under realistic DP processing conditions. While product quality for all mAbs was affected by ICH light conditions irrespective of their formulation components, several mAbs (mAbs 3–5) could be processed under white fluorescent lamps without significant changes to product quality. Interestingly, white fluorescent light did impact the product quality of mAb1 and mAb2. While mAb1 was oxidized in His based buffers, we report significant color changes in mAb2 in succinate and His based buffers. Results from our studies indicate that mAbs have different sensitivities under these processing conditions and that the light source (e.g. bulb type), illuminance (lux hours) and especially UV irradiance (W h/m^2) play a key role in the photosensitivity of mAbs. Additionally, our data clearly demonstrate that ICH light conditions are rather harsh for protein molecules and need to be reconsidered in order to be useful to rank order protein photosensitivity. Rank ordering photosensitivity under ambient or mild light conditions is potentially useful in determining appropriate exposure limits during DP production and determining relevant limits to critical quality attributes.

2. Materials and methods

2.1. Materials

mAbs 1–5 are IgG1 monoclonal antibodies that possess different complimentary determining regions (CDRs) and were expressed in Chinese Hamster Ovary (CHO) cell lines. All mAbs were purified by a series of chromatography methods including affinity purification by protein A chromatography and ion-exchange chromatography. mAb1 was formulated at 50 mg/mL in 20 mM Histidine HCl at pH 6.0, while mAb2 was formulated at 150 mg/mL in 200 mM arginine succinate or sodium succinate, pH 5.5 or in 20 mM Histidine acetate, pH 5.5 as liquid formulations. mAbs 3–5 were formulated at 10 mg/mL in sodium succinate and in histidine acetate at pH 5.5. All formulations contained 0.02% polysorbate 20 unless noted otherwise.

2.2. Spectroradiometer

An EPP 2000 spectroradiometer was purchased from StellarNet Inc. (Tampa, FL). It features a fiber optic cable, CR2 cosine receptor, USB2EPP interface, and SpectraWiz software. It was used to measure the irradiance (in $Wm^{-2}\,nm^{-1}$) in the rooms of interest. The spectroradiometer can measure light between the wavelengths of 190–850 nm.

2.3. Light exposure studies

Different light exposure studies were carried out as described below.

(1) **ICH conditions** – Samples in glass vials were exposed to light in an Atlas SunTest CPS + Xenon Test Instrument (Chicago, IL) with an irradiance level of 250 W/m^2, a total UV dose of 538 W h/m^2 and a total visible dose of 1.32×10^6 lux hours over a 24 h period. Control vials were wrapped with aluminum foil and treated similarly.

(2) **Ambient or mild light conditions** – mAb1 samples in glass vials were placed on laboratory benches with an illuminance of about 300–750 lux, and in a laminar flow hood with an illuminance of 3000 lux. To generate higher light intensities, mAbs 1–5 were placed in a custom ambient light box with a fluorescent (FS) light bulb (fitted with a canopy) that emits light in the 400–750 nm as reported earlier [15]. Samples were exposed to different intensities (1000–10,000 lux) for various periods of time (0–14 days). Control vials were wrapped in foil and treated similarly.

2.4. Quantification of hydrogen peroxide

The amount of hydrogen peroxide generated in mAb1 and mAb2 samples after exposure to light was measured using the Amplex® Ultra Red Assay (Invitrogen, Carlsbad, CA) following the manufacturer's recommended procedure as reported earlier [13].

2.5. Fenton reaction

Fenton reactions for mAb1 were carried out by adding H_2O_2 and either Fe^{2+} or Fe^{3+} in the absence and presence of L-Met as previously reported by Grewal et al. [16].