European Journal of Pharmaceutics and Biopharmaceutics 127 (2018) 37–43

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Protection of therapeutic antibodies from visible light induced degradation: Use safe light in manufacturing and storage

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ABSTRACT

As macromolecules, biologics are susceptible to light exposure, which induces oxidation of multiple amino acid residues including tryptophan, tyrosine, phenylalanine, cysteine and methionine. Pertaining to safety, efficacy and potency, light-induced oxidation of biologics has been widely studied and necessary precautions need to be taken during biologics manufacturing process, drug substance and products handling and storage. Proteins will degrade to varying extents depending on the protein properties, degradation pathways, formulation compositions and type of light source. In addition to UV light, which has been widely known to degrade proteins, visible light from indoor fluorescent lighting also can mediate protein degradation. In this report, we examine and identify wavelengths in the visual spectrum (400–700 nm) that can cause monoclonal antibody and histidine buffer degradation. Installation of safe lights which exclude the identified damaging wavelengths from visible spectra in manufacturing and storage areas can provide a balance between lighting requirement for human operators and their safety and conservation of product quality.

1. Introduction

During and after manufacturing of biologics, it is unavoidable that the drug substance and drug product are exposed to certain light sources, namely, ultraviolet (UV), sunlight or artificial fluorescent light. The light exposure can happen during manufacturing operations, including upstream cell culture, downstream purification, formulating, filling, visual inspection, packing, storage and transportation. It is well known that proteins are sensitive to light exposure which results in oxidation of proteins [1]. UV light induced-degradation pathways are well documented (for review, see Reference. [1]). The aromatic amino acids, tryptophan, phenylalanine, tyrosine and sulfur-containing cystine are able to absorb UV light at 250-–300 nm range and are excited to a the electronic excited state either to generate amino acid radicals, or to undergo non-radiative relaxation to triplet state and the released energy can be absorbed by oxygen to generate reactive oxygen species (ROS) which, in turn, can oxidize amino acids. In addition, tryptophan oxidation products, such as kynurenine (Kyn) and N-formylkynurenine (NFK) can generate secondary photooxidation effects. The NFK (absorption max at 261 and 325 nm and emission max at 434 nm [2]) and Kyn (absorption max at 258 and 360 nm and emission max at 480 nm [2]) can absorb light at longer wavelengths than Trp, not only generating color in drug substance but also thereby acting as photosensitizers to visible light causing additional damage to the protein [1,3]. Indeed, light-mediated protein degradation can involve multiple amino acid residues and occur from a combination of multiple degradation pathways.

Fluorescent lights are the most widely used indoor light sources in offices, laboratories and manufacturing areas. Commercial fluorescent lamps emit visible light and a small amount of UV light [4]. Multiple reports have suggested that biologics are sensitive to fluorescent light exposure with increasing oxidation and aggregation, loss of biological activity and changes in charge variant species [5–10]. During biologics manufacturing, storage and transportation, majority of light exposure occurs indoor. The guidelines from the International Conference on Harmonization (ICH Q1B) specifies that photostability testing includes both visible light (400-–700 nm) and UV-A light (320-–400 nm). For the visible light test, the ICH Q1B requires that the photostability studies consist of two parts, accelerated studies using high intensity lamps to determine the degradation pathway and confirmatory studies using room temperature room light (RTRL) to provide information for appropriate processing and handling of drug substance. Since therapeutic mAbs themselves do not directly absorb visible light, various photosensitizers have been proposed as the primary absorbers of visible light

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https://doi.org/10.1016/j.ejpb.2018.02.007
Received 14 November 2017; Received in revised form 2 February 2018; Accepted 6 February 2018
Available online 07 February 2018
0939-6411/

that mediate photooxidation of protein products. These photosensitizers can be impurities from polysorbate 80 [5] or histidine [6], or media components, such as riboflavin (vitamin B2) and pyroxidine (vitamin B6) [7]. Nevertheless, many marketed therapeutic antibodies have a "protect from light" warning label. On the other hand, there is not much discussion about the mitigation and in-process control strategies to achieve the goal of "protect from light". In this report, we investigate light sensitivity of proteins to examine and identify wavelengths in the visual spectrum (400–700 nm) that can cause monoclonal antibody degradation. New lighting technology such as LED lights and their impact on drug substance stability were also evaluated. Installation of safe light which excludes the identified damaging wavelengths from the visible spectrum in manufacturing and storage areas can provide a balance between the light required for operation and human safety and the conservation of product quality.

2. Materials and methods

2.1. Protein stocks

Three different therapeutic IgG4 molecules (mAbs 1 to 3) were used in this study. These mAbs were produced in CHO cells and affinity purified using protein A chromatography, additional polishing chromatography and final UF/DF to a formulation buffer containing histidine. Protein concentration in drug substance (DS) was determined by absorption at 280 nm.

2.2. Light exposure

Formulated drug substance (30 mL) was filled into a 30-mL disposable bioprocess bag with the same contact film as the commercial manufacturing containers. In order to keep results consistent, the air in the headspace of the bag was removed after filling. Two parallel light exposure tests were conducted, one with room temperature (16° to 24 °C) and room light (RTRL, about 500 to 1,000 Lux) and the other with room temperature and high intensity light. The RTRL was carried out in a photostability chamber (Caron, Model 6545–2) with the temperature set at 24 °C and light intensity set at 1 kLux. The chamber is equipped with daylight (white) lamps whose output was similar to the D65/ID65 emission standard referenced in ISO 10977 (light spectrum is in Supplementary Fig. 1A, supplied by manufacturer). The high intensity light experiment was carried out in a fume hood with an air flow rate of 100 cubic feet per minute to prevent material heating due to light. The light source was either an LED white lamp (15 W) or compact white fluorescent lamp (13 W). To generate colored light, either colored filters (Arbor Scientific, Kit 33-0190), or a LED blue or red lamp (15 W, PowerPAR™ LED lamps) were used. The transmittance spectra of the color filters and output spectra of LED lamps are included in the Supplementary Figs. 1 and 2 (supplied by manufacturer). A polycarbonate glass sheet (2.4 mm in thickness) was purchased from a hardware store and used to filter out UVA light. Light intensities were measured by light meters (Sper Scientific UVA/B light meter Model 850009 and Extech HD400 for UVA and visible light, respectively).

2.3. Analytical methods

Size-Exclusion ultra performance liquid chromatography (SE-UPLC) was used to measure mAb monomer, high molecular weight aggregate (HMW) and low molecular weight species (LMW). Imaged Capillary Isoelectric Focusing (iCIEF) was used for measurement of charge variant species. Reducing and non-reducing Caliper was used to determine antibody purity. UV–visible spectral scanning (300–700 nm) was done with a BioTek Epoch 2 model with 5 nm intervals.

2.4. Reactive oxygen species (ROS) level detected by dihydroethidine (DE)

DE (Thermo-Fisher, Cat.# D11347) was dissolved in DMSO as 1 mg/ml. The stock solution was added to the test solution in a 1:200 volumetric ratio and 100 μL sample was added in a 96-well plate, incubated at room temperature in the dark for two hours before reading fluorescence intensity on a ChemiDoc MP Imaging system (Bio-Rad) with 488/605 nm for excitation and emission and the mean fluorescence intensity was analyzed with Image Lab software.

2.5. Tryptic peptide mapping and LC–MS/MS

Tryptic peptide mapping with LC–MS/MS was performed to measure the relative abundance of oxidized methionine (Met) and tryptophan (Trp) residues in mAbs. Approximately 0.6 mg of protein was evaporated to dryness under vacuum using a Savant SpeedVac centrifugal-evaporator and subsequently dissolved in 80 μL of 8.0 M guanidine-HCl (50 mM Tris, pH 8.0) followed by reduction using 200 mM dithiothreitol (DTT) at 37 °C for 20 min and then S-alkylated by adding 400 mM iodoacetamide (IAM) at room temperature in the dark for 15 min. Samples were desalted and buffer exchanged (into 50 mM Tris/HCl, 1.0 M urea, 1.0 mM $CaCl_2$, pH 7.6) using Zeba gel spin-columns (ThermoFisher, Pierce catalog # 0089883: 0.5 mL, 7 kDa MWCO) and then were diluted 2.5-fold (in 50 mM Tris/HCl, 1.0 mM $CaCl_2$, pH 7.6) to give protein concentration of ~2 mg/mL (50 mM Tris/HCl, 0.4 M urea, 1.0 mM $CaCl_2$, pH 7.6) and digested using trypsin with a protein:trypsin ratio of 25:1 (w/w) at 37 °C for 150 min. Digestion reaction was quenched by lowering the pH to 2–3 with 1.0 M HCl.

Digested samples were analyzed by LC-MS tryptic mapping using a mass spectrometer (ThermoScientific Orbitrap Velos, San Jose, CA) coupled with an UPLC instrument (Waters Acquity, Milford, MA) equipped with a UV photodiode array and a fluorescence detectors connected in series. About 40 μL protein digest was loaded onto an UPLC system with a BEH300 (C18, 2.1 × 100 mm, Catalog No. 186003686) reversed-phase column (Waters, Milford, MA) and separated at the flow rate of 0.2 mL/min. The eluents were 0.02% trifluoroacetic acid in water (mobile phase A) and 0.02% TFA in 80% (v/v) acetonitrile (mobile phase B). The column temperature was set to 45 °C. The percentage of mobile phase B increased from 1% to 45% over 100 min.

LC-MS was run using electrospray ionization acquiring sequential MS full-scans with data-dependent acquisition of tandem mass spectra (MSMS) for the ten most abundant parent ions. LC-MS data were acquired in positive ion mode in a mass to charge ratio (m/z) range of 200–2000, capillary temperature of 275 °C, and a source voltage of 5000 V. MSMS data were acquired using 15–35% normalized collision energy, 0.25 activation Q and 100 ms activation time. The peptides containing oxidized methionine were identified by mass-to-charge values at 10 ppm error and confirmed by MS/MS. The relative quantities of methionine oxidation products were calculated from the peak areas in the extracted ion chromatograms of the corresponding peptides.

3. Results

3.1. Multiple mAbs are sensitive to visible light

In our stability studies, drug substance stability was tested by holding at room temperature protected from light and room temperature exposed to room light conditions (RTRL) to distinguish temperature and light effects. For example, mAb 1 DS was highly sensitive to room light exposure with an increase in high molecular weight aggregate (HMW) but relatively stable when exposed only to room temperature (Fig. 1A and B). Extended light exposure generated brown color in the material (Fig. 1C). Further analysis by mass spectrometry showed that certain Try and Met residues were oxidized (Supplementary Table 1). Similar results were obtained for high intensity light