Formulation Development of Antibodies Using Robotic System and High-Throughput Laboratory (HTL)

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ABSTRACT: Since each antibody has its unique physical chemical properties, optimal formulation for one antibody is likely not applicable for the others. To rapidly screen multiple antibody formulations, an automated system was constructed to perform sample preparation, testing, and data management. Using the automatic system, up to 500 liquid formulations can be prepared in deep well microplates and further distributed into standard microplates that can be stored under different stress conditions for degradation studies. In addition, the system can also be used to prepare samples in microplates for different analytical measurements such as UV spectroscopy, turbidity, dynamic light scattering (DLS), SEC-HPLC, RP-HPLC and CEX-HPLC, and automated lab-on-a-chip platform (ALP). The data generated using different techniques in the automatic system were comparable to those of the classical approaches. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2279–2294, 2010

Keywords: robotic system; high-throughput laboratory; liquid formulation development; analytical characterization; analytical techniques; antibody; microplates; stability study; data management

INTRODUCTION

Application of antibodies in therapeutics and diagnostics has increased significantly in the past 30 years. Administration of antibodies at high concentrations is often desirable and necessary. Development of stable formulations for antibody drugs, especially at high concentrations, is often challenging and time-consuming using classical approaches. Therefore, it was desirable to develop a high-throughput automated system to test multiple formulations and choose the best formulation for each antibody.

Different proteins drugs are degraded differently under normal or stressed storage conditions depending on their primary, secondary and tertiary structures, pI, hydrophobicity, and carbohydrate composition of the molecules.[1] Understanding degradation pathways is essential for formulation development of new antibody drugs. In classical approaches, analytical techniques such as turbidity, pH, size exclusion chromatography (SEC-HPLC), cation exchange chromatography (CEX-HPLC), reversed-phase chromatography (RP-HPLC), light scattering techniques, capillary electrophoresis, and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) are often used to detect degradation products of antibody drugs.[2]

Protein self-association or aggregation is one of the common decomposition pathways of antibody drugs, especially in high concentrations. Aggregation can lead to immunogenicity and/or loss of bioactivities of the antibodies.[3–6] Many factors, such as elevated temperatures, shaking, freezing/thawing, liquid/air, or solid/liquid interfaces can induce formation of aggregates. Addition of some excipients such as surfactants can prevent formation of aggregates.[3,7,8] Turbidity, SEC-HPLC and static or dynamic light scattering (DLS), and other emerging techniques are normally used to detect aggregates. Another common degradation pathway for antibodies is fragmentation, which may result in different biodistribution of the molecules, reduction in potencies of antibodies and faster clearance.[9] SDS–PAGE, SEC-HPLC, or RP-HPLC are often used for monitoring and quantification of the fragments.[10]

Oxidation occurs often in antibody drugs during storage or exposure to oxidants or light.[11,12] Methionine and cysteine are the amino acid residues most

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prone to oxidation.[11] Besides methionine and cysteine, other amino acids such as tryptophan, histidine, and tyrosine can be oxidized.[13] RP-HPLC and peptide mapping can be used to monitor oxidation.[14] Deamidation is also a common degradation pathway in antibodies.[15,16] Glutamine and asparagine are prone to deamidation.[17,18] Deamidation of these amino acids causes changes in charge heterogeneity of monoclonal antibodies.[19,20] Commonly seen deamidation degradation products include aspartic acid, glutamate, iso-aspartic acid, and cyclic imides.[19–21] Deamidation can be detected by isoelectric focusing (IEF) or CEX.[22]

Equipments and assays in high-throughput modes have been popularly used in different areas in the last 15 years. Automated sample preparation and analysis of a large number of samples in microplates have been used for DNA sequencing in the Human Genome Project.[23] Individual analytical technique such as UV, fluorescence measurements, light scattering, calorimetry, X-ray, FTIR, and capillary electrophoresis in high-throughput modes have already been developed and applied in many different fields.[24–34] Some of these techniques such as UV spectroscopy, iCE280 analyzer, FTIR, turbidity, intrinsic tyrosine fluorescence, ANS fluorescence, and nile red fluorescence measurements have already been used for monitoring degradation of proteins for formulation development.[35–38]

So far in the literature, only individual high-throughput techniques have been described. In this article, an automatic system that contains a complete sample preparation module using a robotic system, a wide range of analytical techniques in high-throughput laboratory (HTL) modes and a comprehensive data evaluation system was introduced. The automatic system has several advantages such as minimum requirements for sample amounts, high flexibility in preparing samples for degradation studies and formulation screening, characterizing antibodies in many formulations and selection of the best formulation candidates in a very short time. The data produced using the analytical techniques in the automatic system were compared to those of the classical approaches.

MATERIALS AND METHODS

Materials

All chemicals and excipients were USP, EP, FCC, or ACS grade. Dithiothreitol (DTT), trifluroacetic acid (TFA), and polyethyleneglycol 300 (PEG300) were from Fluka (Buchs, Switzerland). Acetonitrile, dipotassium hydrogen phosphate, potassium di-hydrogen phosphate, and potassium chloride were from Merck (Darmstadt, Germany).

Microplates used were from different vendors: 96-well standard plates from Eppendorf (Schönenbuch, Switzerland), Agilent (Waldbronn, Germany), and Nunc (Reinach, Switzerland), 96-well standard plates with transparent bottoms from Greiner (Reinach, Switzerland), 96-well standard plates with round bottoms from Nunc, 96-well plates (half area usable) with transparent bottom from Corning (distributed by Vitaris, Baar, Switzerland), 96 deep well plates from Whatman (Bottmingen, Switzerland). Peel-it-lite foils for covering the microplates were from Eppendorf and Agilent. Microplate sealers (easy sealer 133) were from Tecan (Männedorf, Switzerland). A thermocycler (master cycler) was from Eppendorf. The protein 200 HT-2 chip and the protein 200 HT-2 reagent kit were from Agilent. The MixMate shaker was from Eppendorf.

Methods

Set Up of the Automatic System

Task 1 (Preparation of Formulations). A robotic system (model: Star, Hamilton, Switzerland) was custom-designed to prepare different formulations in up to five 96 deep well microplates (Whatman) using different buffers, diluents, excipients (e.g., stabilizers, additives), and antibody stock solutions (see Fig. 1). The final volume of samples in each well was set to be 1.3 mL. Up to 21 sterile glass bottles (100 mL), and six sterile polypropylene boxes (130 mL) can be used to carry different buffers, diluents, excipients, water and antibody stock solutions for preparation of samples in different formulations. A Hepa filter and four UV lamps were installed to ensure sterility of samples (see Fig. 2A).

Two separate pipetting heads were used for sample preparation (Fig. 2B). One of them has eight individual channels which can aspirate and dispense solutions randomly into each well up to 1.3 mL and mix them multiple times. For highly viscous solutions, an extra shaking step using a MixMate shaker was added to ensure homogeneity of the samples. The final concentration of an antibody solution prepared may range from 10 to 150 mg/mL. The other pipetting head has 96 channels and transfers solutions from the deep well microplates to 96-well microplates (150 μL) for further studies (e.g., pH, freezing/thawing, shaking, oxidation, deamidation, aggregation) (Fig. 2C–E).

The robot can prepare simple serial dilutions (e.g., 1:2, 1:4, 1:8, 1:16, etc.) in 96-well plates very quickly and precisely. It takes <1 min for the robot to perform such dilutions. Preparation of 500 (5×96) different formulations takes a person roughly 1 week, but the robot only 5 h.