Journal of Chromatography B, 1032 (2016) 61–78

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Cutting-edge capillary electrophoresis characterization of monoclonal antibodies and related products

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ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 12 May 2016
Accepted 17 May 2016
Available online 18 May 2016

Keywords:
Monoclonal antibodies
Biosimilar
Fc-fusion protein
Antibody
Drug conjugate
Capillary gel electrophoresis
Capillary zone
Electrophoresis
Capillary isoelectric focusing
Mass spectrometry

ABSTRACT

Out of all categories, monoclonal antibodies (mAbs), biosimilar, antibody-drug conjugates (ADCs) and Fc-fusion proteins attract the most interest due to their strong therapeutic potency and specificity. Because of their intrinsic complexity due to a large number of micro-heterogeneities, there is a crucial need of analytical methods to provide comprehensive in-depth characterization of these molecules. CE presents some obvious benefits as high resolution separation and miniaturized format to be widely applied to the analysis of biopharmaceuticals. CE is an effective method for the separation of proteins at different levels. capillary gel electrophoresis (CGE), capillary isoelectric focusing (cIEF) and capillary zone electrophoresis (CZE) have been particularly relevant for the characterization of size and charge variants of intact and reduced mAbs, while CE–MS appears to be a promising analytical tool to assess the primary structure of mAbs and related products. This review will be dedicated to detail the current and state-of-the-art CE-based methods for the characterization of mAbs and related products.

1. Introduction

Monoclonal antibodies (mAbs) are highly complex tetrameric glycoproteins that must be extensively analytically and structurally characterized to become drug candidates [1,2]. This is also true for biosimilar [3,4] and biobetter antibodies [5], for glyco-engineered antibodies [6] and for IgG-related products such as Fc-fusion proteins and peptides [7,8], antibody-drug conjugates [9], bi- and multi-specific antibodies [10] and antibody mixtures [11,12].

A plethora of separation techniques based both on liquid chromatography and electrophoresis are used for antibody characterization and homogeneity assessment [13]. These orthogonal analytical methods aim to separate the antibody main isoform from microvariants and process related impurities such as host cell proteins (HCPs) [14]. A correct primary structure assessment and extensive glyco-profiling are the cornerstone of antibody characterization. A combination of intact, middle-down, middle-up and bottom-up mass spectrometry (MS) techniques is the workflow of choice to characterize the amino acid sequence and the major post-translational modifications (PTMs) of antibodies [15]. IgG glycans represent an average of only 2–3% of the total antibody mass, but they are one of the critical quality attributes for therapeutic candidates [16]. Micro-variants are commonly observed when mAbs are analyzed by charge-based separation techniques such as isoelectric focusing gel electrophoresis (IEF), capillary IEF (cIEF), imaged cIEF (icIEF), and ion exchange chromatography (CEX/AEX). Many of the modifications leading to the formation of acidic and basic species have been identified by analyzing fractions collected from chromatography-based methods, with the aim of further analyzing their impact on safety, pharmacokinetics and pharmacodynamics (PK/PD). Those located in antibody complementarity-determining regions (CDRs) are often considered to be the most critical because they are surface exposed and often involved in the antigen binding [17].

In the past decade, several hundreds of papers have been published on mAbs analytical and structural characterization, and the trend will certainly continue to expend in the future. Multiple and complementary liquid chromatography, electrophoresis, spectroscopic methods and MS methods are used at all stage of mAbs discovery, preclinical and clinical development. Because of its high

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http://dx.doi.org/10.1016/j.jchromb.2016.05.028
1570-0232/

resolving power, simple instrumentation, miniaturized format and superior separation efficiency, capillary electrophoresis (CE) represents a powerful techniques to characterized biomolecules [18]. The different electrophoretic modes that can be employed such as capillary gel electrophoresis (CGE) [19], cIEF, icIEF [20] and capillary zone electrophoresis (CZE) [18], exhibits attractive opportunities for the characterization of mAbs and related products at different levels such as intact or reduced charge- or size-variants, isoforms, glyco-profiling and PTMs and also impurity studies. As the main handicap of CE is known to be the poor UV-detection limit, CE is fully suitable for coupling to different very sensitive detection methods such as laser induced by fluorescence (LIF) [19] and MS [21,22]. Some reviews assessed that CE-based methods as efficient techniques for the characterization and quality control of biomolecules are fully implemented in biopharmaceutical industry [13,23–29]. Here we review the current and state-of-the art electrophoretic methods relevant in biopharmaceutical field of applications from the year 2010–2015. We focused on the characterization of mAbs and the related product as biosimilars, ADCs and Fc-fusion proteins which represents today the fast growing class of biotherapeutic products.

2. Capillary gel electrophoresis (CGE)

2.1. Technical considerations

Since the 90s, CGE has known a significant concern for the characterization of proteins. Currently, this method is recognized as an essential analytical technique especially in the biopharmaceutical industry for the characterization of mAbs. CGE is a method based on the same mechanism as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After complete reaction between SDS and proteins, SDS-protein complexes with the same negative charge are formed allowing electrophoretic separation in a sieving medium only based on their hydrodynamic radius. SDS-PAGE has been used for size-based protein separation routinely. However, this methods is also known as labor-intensive, time consuming and lacking reproducibility. The adaptation of SDS-PAGE to a capillary format permits to reduce drawbacks and brings some advantages as ease of handling, automated procedure, short analysis time and improved resolution. The transfer of SDS-PAGE to CGE is based on the replacement of the traditional slab gel by soluble polymers used as replaceable molecular sieve. This allows the separation based on hydrodynamic radius differences directly into the capillary. Proof that this method is now routinely established in industries, several companies commercialized sieving kits to perform CGE. In this electrophoretic mode and to assure that the separation is strictly based on the size differences, electroosmotic flow (EOF) has to be suppressed. The vast majority of reported CGE analysis (Table 1) were performed using bare fused silica capillary (BFS). This can be explained by the use of commercial sieving kits which contain some additives to suppress EOF. For example, the Beckman Coulter SDS-Mw gel buffer includes a high concentration of Tris-borate to eliminate EOF. However, other strategies have been described to suppress EOF and to reduce possible adsorption of protein on the inner capillary wall. The article published by Szabo et al. reported the use of a linear polyacrylamide (LPA) neutral coating to enhance separation efficiency for rapid and high resolution glycan analysis of mAbs [30]. Other neutral capillary coated are described as hydroxypropyl cellulose (HPC) [31], Polyvinyl alcohol (PVA) [32,33] and some commercial solution [34–43].

CGE is classically coupled with UV or fluorescence detection. Using a UV detection, applied wavelengths are generally 220 nm and more rarely 200 nm, 214 nm and 280 nm. Concerning fluorescence detection, laser-induced fluorescence (LIF) has been employed to improved sensitivity. The most common condition for CGE-LIF analysis consist to the use of a laser operating at 488 nm for fluorescence excitation with the resulting emission signal monitored at 520 or 560 nm. CGE-LIF of 8-Aminopyrene-1,3,6-trisulfonic-acid (APTS) labeled N-glycan appears to be a methods of choice for the determination of mAbs N-glycan profiles [35,36]. Indeed, APTS reaction with proteins allows the derivation of a fluorophoric group and the addition of three negative charges in the glycan moety. With these modifications, electrophoretic separation can be obtained faster, with a greater efficiency and a higher sensitivity. Other strategies have been described to label N-glycan. Michels et al. developed a fluorescent derivatization method using 3-(2-furoyl)-quinoline-2-carboxaldehyde (FQ) as fluorogenic reagent for mAb analysis [44]. The same group described a quantitative impurity analysis of mAb size heterogeneity also using FQ labeling [45]. More recently, Reusch et al. performed a comparative study of APTS and 8-aminonaphthalene-1,3,6-trisulfonate (ANTS) labeled glycan. All of the methods showed excellent precision and accuracy. Some differences were observed, particularly with regard to the detection and quantitation of minor glycan species [35].

2.2. Applications

For the characterization of mAbs and their related products, CGE is commonly used for protein size heterogeneity, purity, assessment of product fragment impurities characterization and aggregation and N-glycan profiling. Table 1 summarized applications of CGE in mAbs and related products in 2010–2015. Size heterogeneity represents one of the most important application of CGE for mAbs characterization. In 2012, Shi et al. proposed a comparison between SDS-PAGE and CGE in mAb purity analysis. This work based on the size heterogeneity analysis reported several advantages of CGE especially in terms of accuracy and resolution of size determination as well quantitative performance [46]. Visser et al. performed a comparability study between innovator approved product and biosimilar candidate of rituximab [47]. Using different methods including the size heterogeneity of intact mAb with CGE, they concluded that biosimilar candidate and originator rituximab are pharmacologically comparable with regard to antitumor activity, pharmacokinetic exposure and B-cell depletion. The same year, Glover et al. described a size heterogeneity study of intact and reduced mAbs to assess the compatibility and the stability between pertuzumab and trastuzumab in intravenous infusion bags for coadministration [48]. Using different method as CGE-LIF, CZE and icIEF, they obtained results demonstrating no observable differences for the analysis of pertuzumab/trastuzumab mixtures store up to 24 h at either 5 °C of 30 °C (Fig. 1). As Glover et al., numerous of publications described size heterogeneity of reduced mAbs (Table 1). Indeed, changes in molecular composition result in alterations of functional performance, therefore quality control and validation of therapeutic or diagnostic protein is essential. Applying reduction step in the sample preparation using a reducing reagent like tris(2-carboxyethyl)phosphine (TCEP) or dithiothreitol (DTT) which allow to reduce disulfide bonds of mAbs; light chain, heavy chain and post-translational modification (PTM) can be determined. Cherkaoui et al. described a CGE method to evaluate the chemical integrity of mAbs during the coupling to microparticles surface by tracking reduction fragments [49]. They followed with a great accuracy the reduction of mAbs in all intermediate isoforms down to single heavy and light chains. In 2013, Yin et al. used CGE-LIF with reducing conditions for the characterization of mAbs between *in vitro* and *in vivo* time-course studies [50]. They demonstrated that *in vivo* mAbs modification cations are not fully represented by *in vitro* phosphate buffer or plasma incubation. To further improve the performance of CGE, Szekely et al. proposed a generally applicable multi-capillary SDS-gel electrophoresis pro-