Journal of Chromatography B 1073 (2018) 27–33

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Rapid preparative separation of monoclonal antibody charge variants using laterally-fed membrane chromatography

Rahul Sadavarte, Pedram Madadkar, Carlos DM Filipe, Raja Ghosh*

Department of Chemical Engineering, McMaster University, Hamilton, Ontario, L8S 4L7, Canada

ARTICLE INFO

Keywords:
Monoclonal antibody
Charge variants
Oxidation variants
Separation
Analysis
Membrane chromatography

ABSTRACT

Monoclonal antibodies undergo various forms of chemical transformation which have been shown to cause loss in efficacy and alteration in pharmacokinetic properties of these molecules. Such modified antibody molecules are known as variants. They also display physical properties such as charge that are different from intact antibody molecules. However, the difference in charge is very subtle and separation based on it is quite challenging. Charge variants are usually separated using ion-exchange column chromatography or isoelectric focusing. In this paper, we report a rapid and scalable method for fractionating monoclonal antibody charge variants, based on the use of cation exchange laterally-fed membrane chromatography (LFMC). Starting with a sample of monoclonal antibody hIgG1-CD4, three well-resolved fractions were obtained using either pH or salt gradient. These fractions were identified as acidic, neutral and basic variants. Each of these fractions contained intact heavy and light chains and so antibody fragmentation had no role in variant generation. The separation was comparable to that using column chromatography but was an order of magnitude faster.

1. Introduction

Monoclonal antibodies (mAbs) belonging to the IgG1 subclass occupy a major portion of the therapeutic antibody market [1,2]. Most of the currently used IgG1 mAbs have almost identical Fc regions allowing their use for diverse applications by modulation of the variable Fab region [2]. Purification and polishing steps employed during mAb processing often destabilize the protein and in extreme situations leads to protein aggregation [3–5], or chemical modifications like oxidation [6,7], deamidation [8], racemization [9], and hydrolysis [9]. The final mAb formulation for therapeutic use is required to be substantially free of such degradation products as they tend to have lower biological activity, altered pharmacokinetic profiles, and in some case, these may induce severe immunogenic responses [10]. Therefore, mAb formulations often contain sugars and polyols [11,12], polymers such as PEG [13], surfactants [14,15], amino acids [16], and other additives to stabilize the complex structure of the protein.

According to International Conference on Harmonization (ICH) Q6 B specifications, undesired physical and chemical changes to a monoclonal antibody throughout its lifetime are categorized as either process-related or product-related [17]. These guidelines recommend thorough characterization of mAb products to test for such changes. Impurities resulting due to physical degradation (aggregation, fragmentation, etc) are easier to detect. The more challenging task is to identify chemical modification to mAb molecules. Chemical modification usually does not affect the antibody backbone structure but alters properties such as hydrophobicity [18,19], surface charge [18], isoelectric point (pI) [20–26] and local conformation [27,28]. Since chemical modifications frequently produce changes in charge, these modified molecules are often called charge variants. Various pathways leading to formation of monoclonal antibody charge variants have been comprehensively described [19,29].

Stracke et al. [30] have reported significant differences in binding of oxidized and non-oxidized IgG1 mAb to FcRn receptors, i.e., the oxidized mAb bound weakly and was cleared faster, resulting in shorter half-life in mouse models. Boswell et al. [31] in their study reported differences in tissue distribution and consequently pharmacokinetic properties of mAb variants injected in rat model. The effect of antibody variants on serum half-life and clearance rate in mouse models has also been reported by Igawa et al. [32]. It is now widely acknowledged that antibody variants affect the pharmacokinetic properties of a mAb drug and therefore any efficient method for separation and analysis of such variants would be an extremely valuable tool for manufacturing these molecules [30–32].

Presently, mAb variants are separated and analyzed by column chromatography or electrophoresis. Column-based methods typically include ion exchange chromatography (IEX) [33,34], hydrophobic interaction chromatography (HIC) [35] and reverse phase-high

* Corresponding author.
E-mail address: rghosh@mcmaster.ca (R. Ghosh).

https://doi.org/10.1016/j.jchromb.2017.12.003
Received 12 October 2017; Received in revised form 25 November 2017; Accepted 2 December 2017
Available online 05 December 2017
1570-0232/ © 2017 Elsevier B.V. All rights reserved.

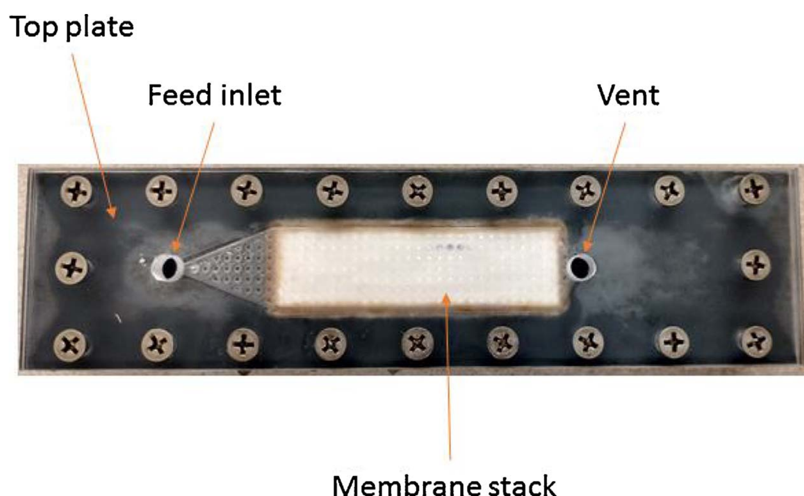

Fig. 1. Top view of the 1 mL bed volume laterally-fed membrane chromatography (LFMC) device.

performance liquid chromatography (RP-HPLC) [36]. While these techniques give good resolution, separation time tends to be long. Moreover, the resin could be easily fouled and the need for frequent column cleaning increases the effective separation time even further. Electrophoretic methods include isoelectric focusing [20,21], capillary electrophoresis [22–24] and 2D-gel electrophoresis [25,26]. These methods rely on the use of specific buffers with carefully chosen ionic strength and pH that allow subtle changes on a protein's surface to be detected. Even though these methods provide good resolution, they are cumbersome and time consuming. Moreover, an electrophoretic method is tailored specifically for a particular protein and the operational parameters and strategies are not globally applicable.

Column chromatography is widely used for both analytical and preparative bioprocessing because of its directness and versatility. The main drawback of resin-based columns is that mass transfer is limited by diffusion which slows down the separation. By contrast, the mass transport in membrane chromatography [37], which has been proposed as alternative to packed-bed chromatography, takes place predominantly by convection, which allows faster separation. Thus, separation time with membrane chromatography could be an order of magnitude lower than with equivalent column chromatography. However, up until recently, membrane chromatography was not considered suitable for high-resolution separations. Using a new technique called laterally-fed membrane chromatography (LFMC) it is now possible to combine high-speed with high-resolution in membrane chromatography [38–42]. For instance, using this technique, it has been possible to perform fast analytical [41] and preparative [42] separation of monoclonal antibody aggregates. Our study on preparative separation of monoclonal antibody aggregates [42] demonstrated that sharp, well-resolved mAb monomer/aggregate peaks could be obtained at very high flow rates using a stack of cation exchange membranes as chromatographic media. In the present study, we use of an LFMC device based on the same membrane and a similar design to fractionate variants present in old samples of hIgG1-CD4, which is an IgG1 mAb. As mentioned earlier, monoclonal antibodies of the IgG1 subclass undergo degradation through various pathways and which alters their properties. This converts a homogenous mAb sample into a non-homogenous variant-containing mixture. The separation strategy used in the current study was to bind non-homogenous mixture of hIgG1-CD4 molecules, irrespective of the extent of chemical modification, on a stack of cation exchange membrane in the presence of slightly acidic buffer and sequentially elute these out in order of increasing pI using either salt or pH gradient. Our results showed that fast, high-resolution separation of the different variants could be achieved using our LFMC devices. The results obtained are discussed.

2. Materials and methods

2.1. Materials

Purified mAb hIgG1-CD4 monomer (batch 12, 23rd March 1999) was kindly donated by the Therapeutic Antibody Centre (Oxford, United Kingdom). The monomer mAb as received was found to be free from variants based on orthogonal chromatographic and electrophoretic testing [43] but prolonged storage and handling (e.g. freeze-thaw) of the material resulted in the formation of variants and some aggregates. Based on storage and handling logs, the number of freeze-thaw cycles was 15. Sodium phosphate monobasic (S0751) and sodium phosphate dibasic (S0876) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride (SOD 002.205) was purchased from Bioshop (Burlington, ON, Canada). Buffers were prepared using purified water (18.2 MΩ cm) obtained from a Diamond™ NANOpure (Barnstead, Dubuque, IA) water purification unit. Amicon® Ultra-4 Centrifugal Filters (fitted with Ultracel-50 membrane) purchased from Millipore (Billerica, MA) were used for buffer exchanging and concentrating mAb samples. For comparison of the results obtained using cation exchange membrane chromatography, control experiments were carried out using 1 mL analytical scale HiTrap™ SP FF and Hi Trap™ SP HP columns (GE, Mississauga, ON, Canada) which were used as per the manufacturer's recommendation.

2.2. LFMC device

The LMFC devices used in this study were designed according to the details provided elsewhere [42]. A 1 mL bed volume device (see Fig. 1) was used for analytical separations while a 4.69 mL bed volume device was used for preparative separations. Briefly, these devices consisted of a top plate with an inlet port leading to the top channel of the device, and a bottom plate with an outlet leading out from the bottom channel, the inlet and outlet being located at opposite sides of the device. A frame which housed a rectangular stack of Sartobind® S membrane was sandwiched between the top and the bottom plates. The membrane bed height of the 1 mL LFMC device was 2.75 mm while the length and width of the membrane in the stack were 38 mm and 10 mm respectively. The corresponding dimensions of membrane stack in the 4.96 mL LFMC device were 3.3 mm, 70 mm and 20 mm respectively. The channels in the top and bottom plates were provided with pillars for supporting the membrane stack as well as for ensuring uniform liquid distribution in these channels.

Download English Version:

https://daneshyari.com/en/article/7615482

Download Persian Version:

https://daneshyari.com/article/7615482

Daneshyari.com