Journal of Chromatography A, 1459 (2016) 78–88

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Mechanistic understanding of fouling of protein A chromatography resin

Mili Pathak, Anurag S. Rathore *, [1]

Department of Chemical Engineering, Indian Institute of Technology, New Delhi, India

ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form 28 June 2016
Accepted 30 June 2016
Available online 1 July 2016

Keywords:
Protein A chromatography
Resin lifetime
Column reuse
Monoclonal antibodies

ABSTRACT

This paper aims to provide a thorough understanding of how fouling of Protein A resin takes place. Binding and mass transport properties of widely used agarose-based Protein A resin, MabSelect SuRe™, have been examined to understand the mechanism of resin fouling. There could be various factors that impact resin fouling. These include product/impurity build-up due to components in the feed material and ligand degradation due to the use of harsh buffers. To unravel their contributions, cycling studies were performed with and without product loading. The results presented in this paper provide a lucid understanding of the causative factors that limit Protein A chromatographic resin lifetime. The capacity fall for protein A resin at the end of 100th cycle due to use of feed material was found to be five times greater than that without using feed material. Compared to the fresh resin, the cycled resin samples shows 24% reduction in particle porosity and 51% reduction in pore mass transfer coefficient. Transmission electron microscopy (TEM) was used to qualitatively monitor accumulation of foulants on the cycled resin. Fouled resin sample contained a dense residue in the interior and exterior of resin particle both as a film at the bead surface and as granules. The surface activation energy increased five times in the case of fouled resin sample. The major event in fouling was identified as the non-specific adsorption of the feed material components on resin, signaling that pore diffusion is the rate limiting step. It is anticipated that these findings will assist in development of a more robust and economical downstream manufacturing process for monoclonal antibody purification.

1. Introduction

Chromatographic separations continue to be the backbone for purification of biotherapeutic products. Their popularity and ubiquity are a result of the unparalleled selectivity, resolution, scalability, and ruggedness that they offer when compared to other process options. However, process chromatography steps are known to account for a significant portion of the processing cost, often the majority of that of downstream processing [1]. In order to keep the process economically viable, it is customary to reuse chromatography media with the number of cycles anywhere from 50 cycles to 200 cycles [2]. As per regulatory guidance, it is critical that performance of the chromatographic resin remains at par so that predetermined quality and safety attributes can be achieved throughout the resin lifecycle [3,4]. However, the capacity of the resin to provide clearance to the host cell impurities, primarily host cell proteins (HCP) and host cell DNA (HCD), reduces with cycling and this has been known to impact both product recovery and product quality.

With the growth in the number of monoclonal antibodies and Fc fusion proteins gaining approval, Protein A chromatography has long established its prominent place in purification processes of these products [5–7]. This mode of chromatography has been successfully employed over the decades to purify antibodies from a variety of sources including mammalian cell culture and transgenic plants [8]. One of the long recognized challenges with the Protein A ligand has been its limited stability under the strongly alkaline conditions that are routinely used in chromatography column clean-in-place (CIP) procedures [9–11]. As a result, old generations of Protein A chromatography resin was typically carried out with high concentrations of chaotropic agents such as urea or guanidine hydrochloride, sometimes at acidic pH [9,12]. Over time, researchers observed that limited exposure to mild alkaline conditions can be successfully employed for regeneration of

* Corresponding author at: Department of Chemical Engineering, Indian Institute of Technology, Hauz Khas, New Delhi, 110016, India.
E-mail address: asrathore@biotechcmz.com (A.S. Rathore).
[1] Web: www.biotechcmz.com.

http://dx.doi.org/10.1016/j.chroma.2016.06.084
0021-9673/

Protein A resins [7,13–15]. More recently, resin manufacturers have attempted to engineer the Protein A ligand such that it becomes more alkaline tolerant and this has resulted in the launch of products such as MabSelect SuRe™ [16–19]. Despite the significant advancements that the industry has made in design of Protein A resins, what has remained the same is that these resins are expensive and thus require to be cycled and as a result the need for understanding how the resin gets fouled and what can be done to accomplish its longevity remains. As per a recent survey of users of Protein A chromatography, it has been reported that this resin is used anywhere from 50 to 200 cycles in the industry [2]. After product elution, the resin is typically subjected to a strip condition to remove any residual product from the column prior to its regeneration (CIP) [20]. There are numerous sources which can result in variability of performance of a Protein A chromatography step, including variation in the elution, strip, and regeneration procedures and in the composition of the feed material (concentration of product, lipids, host cell proteins, nucleic acids, and cell culture media constituents) [20]. The latter is particularly significant as the Protein A column is used as a capture step and as a result faces a feed that contains a myriad of impurities. In view of the complexity of this step, an improved understanding of the fouling of Protein A resin would be critical for improving resin lifetime.

As per the present approach, the biotherapeutic manufacturer first optimizes the chromatography process, including the cleaning and sanitization steps [21,22]. Next, cycling studies are performed at laboratory scale using scale-down models of the process and resin lifetime is established [23,24]. Finally, this target is verified at scale by collecting appropriate data during commercial manufacturing. As is evident, this is quite a resource and time intensive tedious approach. Moreover, a clear determination of fouling is lacking and multiple attributes including step yield, product quality, impurity levels, and binding capacity are typically monitored to assess deterioration in resin performance over cycling [25].

Several mechanisms have been reported in the literature that can contribute to decay in performance of a chromatography resin over repeated uses [26–28]. It is known that mAb aggregates and other biological impurities and/or residual product can bind irreversibly to the resin and either block pores and/or block access to surface ligands [25]. An additional consideration, in the case of Protein A resins, is that of leaching or degradation of the Protein A ligand over time, particularly as a result of harsh cleaning conditions [20]. In general, the maximum number of reuses for any given chromatographic stationary phase is product specific and depends on a variety of factors including the resin used, the placement in the process, the level and nature of impurities the resin comes in contact with, the product itself and the nature of the strip, cleaning, and column storage solutions used [20].

This paper aims to provide a thorough understanding of how fouling of Protein A resin takes place. It attempts to answer several critical questions including – How does the hydrodynamics of the chromatography column change with fouling? What is the contribution of the processing buffers vs. feed material? How is the intraparticle mass transfer impacted by fouling? We expect the results presented here to be of interest to those working on purification of monoclonal therapeutic products.

2. Theory

2.1. van Deemter equation: contribution of mass transfer

The contributions from film and pore mass transfer can be estimated under unretained conditions by using the methodology that has been described in the literature [29]. The Height Equivalent to Theoretical Plate (HETP) under unretained conditions can be expressed as:

$$H = 2Z + \frac{2(1-\varepsilon_i)\varepsilon_p \mu}{\left[\varepsilon_i + (1-\varepsilon_i)\varepsilon_p\right]^2} \left| \frac{R}{3K_f} + \frac{R^2}{15D_p} \right| \quad (1)$$

Where, H is the HETP, Z is the axial dispersion parameter, μ is the linear flow velocity (cm s^{-1}), R is the average particle radius (cm), K_f is the film mass transport coefficient, D_p is the pore mass transport coefficient, ε_i and ε_p are the intra and inter-particle porosities. The film mass transfer coefficient (K_f) can be calculated using the following correlation [30]:

$$K_f = \frac{ShD_m}{d_p} \quad (2)$$

$$Sh = 2 + 1.45Re^{1/2}Sc^{1/3} \quad (3)$$

Where, Sh is the Sherwood number, Re is the Reynolds number ($=\mu d_p/\eta$), Sc is Schmidt number ($=\eta/D_m$), D_m is molecular diffusivity ($cm^2\ s^{-1}$), d_p is particle diameter (cm), and η is kinematic viscosity ($cm^2\ s^{-1}$).

Molecular diffusivity can be calculated using the stop-flow method [31].

$$D_m = \left(\sigma_1{}^2 - \sigma_2{}^2\right)/2\tau \quad (4)$$

Where, σ_1 and σ_2 are the variance in solute (mAb) peak due to flow interruption, and τ is the time duration for which the flow was interrupted. Once K_f is known, the HETP vs. u plot can be used to determine the intercept and slope and thereby calculate Z and D_p [29].

2.2. Adsorption kinetics

The kinetics of mAb adsorption on Mabselect SuRe™ resin during resin cycling, were evaluated using pseudo first-order [32], pseudo-second-order [33], and Elovich [34,35] kinetic models.

A linear form of pseudo-first-order model as described by Lagergren is as follows [32]:

$$\ln(q_e - q) = lnq_e - k_1 t \quad (5)$$

Where, k_1 ($1\ min^{-1}$) is the pseudo-first order rate constant and q_e (mg) is equilibrium value of q. A linear plot of $ln(q_e - q)$ against time t can then be used to obtain k_1 and q_e from slope and intercept, respectively.

The linear form of pseudo-second-order kinetics can be expressed as [36]:

$$\frac{t}{q} = \frac{1}{k_2 q_e{}^2} + \frac{t}{q_e} \quad (6)$$

Where, q_e (mg) is the equilibrium adsorption capacity and k_2 ($mg^{-1}\ min^{-1}$) is the rate constant. The plot of t/q versus t can then be used to estimate q_e (mg) and k_2 ($mg^{-1}\ min^{-1}$) experimentally from the slope and intercept, respectively.

The Elovich equation has also been used successfully to describe second order kinetics and assumes that solid surfaces are energetically heterogeneous [35]. Though the equation is not supported by an underlying mechanistic model, it has been extensively used for describing a chemisorption process [37]. The linear form of this equation is given by [35]:

$$q_t = 1/\beta \ln\left(\alpha\beta\right) + 1/\beta \ln(t) \quad (7)$$

Where, α is initial adsorption rate ($mg\ g^{-1} min^{-1}$), β is related to the extent of surface coverage and the activation energy for chemisorption ($g\ mg^{-1}$) [38].