Journal of Membrane Science 451 (2014) 312–318

Contents lists available at ScienceDirect

Journal of Membrane Science

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

Qualitative and quantitative examination of non-specific protein adsorption on filter membrane disks of a commercially available high throughput chromatography device

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ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form
23 September 2013
Accepted 24 September 2013
Available online 15 October 2013

Keywords:
High throughput process development (HTPD)
Granulocyte colony stimulating factor (GCSF)
Transtuzumab
Non-specific protein adsorption
Contact angle measurement

ABSTRACT

High throughput process development (HTPD) platforms provide time and resource effective solutions for optimization of resin as well as membrane based chromatography processes. They enable successful implementation of Quality by Design by allowing us to investigate more variables and wider ranges than would be possible using traditional lab-scale based experiments. This paper presents an investigation about non-specific protein adsorption that was found to occur on the membrane disks used in a commercially available high throughput device. Both qualitative as well as quantitative analytical tools were used in this investigation. Two biotherapeutic protein molecules, granulocyte colony stimulating factor (GCSF) and Transtuzumab were selected as model proteins in this study. Qualitative assessment was performed using Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). An empirical model based on contact angle measurement was developed for quantitative assessment of the amount of adsorbed protein. The results indicated that while significant non-specific adsorption was observed in case of highly hydrophobic GCSF molecule, adsorption was minimal in the case of Transtuzumab. The paper proposes a novel use of contact angle measurement for quantitative estimation of adsorbed protein. The study also highlights the importance of accounting for non-specific adsorption when using these HTPD devices.

1. Introduction

Miniaturization of chromatography platforms has several merits including reduction of solvent consumption, higher productivity and significant savings in space and resource requirements [1–5]. Accurate prediction of operating conditions for the laboratory and production scale chromatography processes is a crucial target for high throughput process development (HTPD) based chromatography platforms [6–12]. In our earlier investigations, we report on creation of a resin as well as membrane based microplate HTPD platform for defining characterization space for chromatography processes [7–11]. We demonstrated that while the proposed HTPD platform was capable of accurately predicting the operating conditions for optimal step recovery, significant differences were observed between the step recoveries obtained with granulocyte colony stimulation factor (GCSF) when using the HTPD platform vs. the traditional lab scale. These differences were the motivation for this investigation about non-specific protein adsorption on the filter disks of resin and membrane based HTPD devices.

Non-specific protein adsorption is known to be a key factor that contributes to membrane fouling and is caused by irreversible or reversible protein adsorption onto the membrane surface [13–17]. Physicochemical properties of the protein and membrane as well as hydrodynamics during the operation significantly affect the amount of protein that gets adsorbed on the membrane surface. Surface energy, hydrophobicity, and ionic or electrostatic interactions could be the driving forces that result in protein adsorption [18–20]. Simultaneous action of these occurs in real applications, thus making mechanistic modeling of non-specific protein adsorption a complex task.

Many researchers have investigated protein adsorption on membrane surfaces using various microscopy tools such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) [21–24]. These tools are based on visual observation and thus offer qualitative assessment of protein adsorption. If used for quantitative measurements of porosity and surface topography, they require additional confirmation using a tool such as Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). The

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http://dx.doi.org/10.1016/j.memsci.2013.09.052

Table 1
Process steps for performing an ion exchange chromatography using HTPD platform.

Sr. no.	Step	Duration (min)	Number of cycles	Incubation at revolutions per minute (RPM)
1	Equilibration of the resin	5	3	1100
2	Protein loading	30	2	1100
3	Removal of unbound protein using equilibration buffer wash	5	3	1100
4	Elution	5	3	1100

latter has been used widely for physicochemical characterization of membrane surface during fouling. These tools offer reliable estimation of surface characteristics such as pore distribution and surface roughness. Belfer et al. have studied fouling of PES membrane using Bovine Serum Albumin (BSA) as a model protein and reported that BSA undergoes structural modification during adsorption [25]. Quantification of the adsorbed protein was done by removing the protein with the aid of surfactant followed by quantification using SDS-PAGE and other spectroscopic methods. However, loss of protein during resolublization is a critical bottleneck in this approach and likely to result in inaccurate quantification of the adsorbed protein.

In the present investigation, non-specific adsorption of the protein molecule on the filter disks of a commercially available HTPD device was studied using various surface characterization tools. Two model proteins, GCSF and Transtuzumab, which significantly differ in their physicochemical properties were selected. GCSF is a single chain polypeptide containing 174 amino acid residues (molecular weight = 19.1 kDa) whereas Transtuzumab is a recombinant DNA derived monoclonal antibody with 1328 amino acids and molecular weight of 148 kDa. Modification of the physical properties of the membrane surface due to protein adsorption was characterized by using SEM, AFM, ATR-FTIR and contact angle measurement. Microscopic images of blank membranes vs. used membranes (exposed to protein solution) were used to study pore distribution and roughness, respectively. Our results clearly demonstrate negligible adsorption of Transtuzumab and significant adsorption with GCSF. Further, ATR-FTIR studies for the case of GCSF confirm the signature protein bands corresponding to the alpha helical structure of protein. Finally, quantitative measurement of the amount of protein adsorbed on the filter disks was performed using a semi empirical model based on contact angle measurements [26].

2. Experimental

2.1. Materials

High throughput resin based chromatography platform used in this study involved use of the PreDictor™ plates prefilled with 6 μl of CM Sepharose FF and 50 μl Capto Q resin matrices from GE Healthcare (Uppsala, Sweden). Glacial acetic acid, sodium acetate (anhydrous), sodium chloride, mono sodium phosphate and disodium phosphate, acetonitrile (HPLC grade), and trifluoroacetic acid (TFA) were purchased from Merck Chemicals India [7].

2.2. Methods

2.2.1. Preparation of the feed material for experimentation

Granulocyte colony stimulating factor (GCSF) and Transtuzumab monoclonal antibody were the two model proteins used in this investigation. Refolded GCSF protein was initially concentrated using ultra filtration. After adjusting pH to 4.00, concentrated protein solution was buffer exchanged into respective buffers. The buffer exchanged samples were used as an input for the experimentation. Transtuzumab was purified using protein A (MabSelect SuRe) and eluted in citrate buffer. After low pH treatment, the feed material was buffer exchanged into appropriate buffers as required by the experimental design.

2.2.2. HTPD experimentation

Automation in liquid dispensing was achieved using multichannel Eppendorf Research® pro (Eppendorf AG, Germany) electronic pipette. Ika MTS 2/4 digital shaker (IKA® India Private Limited, India) was used to fully suspend the sample/buffer in the medium during incubation at various stages of the experiment. Storage solution was removed from the plate using the vacuum. Ion exchange resins were equilibrated using 200 μl of respective equilibration buffer (3 cycles). After resin equilibration, pre-treated protein solution was added into the wells and incubated for 1 hour. Three cycles of the equilibration buffer wash were given to remove any unbound protein. Salt based elution was performed using the respective buffer containing 1 M NaCl. Three cycles, each of 200 μl elution buffer, were performed to completely elute the protein from the resin. Liquid fractions from PreDictor™ plate were collected by vacuum filtration at −300 mbar gauge for 10 s using Whatman® vacuum manifold (GE Healthcare Bio-Sciences). All filtrate fractions except the initial buffer washes were collected in Costar UV readable microtiter plates (Corning Incorporated, NY) and absorbance was read at 280 nm using Epoch microplate spectrophotometer (BioTek ® Instruments Inc. Winooski, VT, USA). Table 1 enlists the sequence of steps followed in the experimentation. Control experiment to check the non-specific adsorption of the protein on the filter membrane disk of the HTPD device was performed by removing the resin from the respective wells. Removal of the resin was followed by the gentle washing of the wells with Mili Q to achieve complete resin removal.

2.2.3. Column scale chromatography

Preparative scale ion exchange chromatography experiments were performed using an ÄKTA Purifier 10 chromatography system (GE Healthcare Bio-Sciences, Uppsala, Sweden). Omnifit chromatography column (6.6 × 150 mm^2) was used to pack the CM Sepharose FF and Capto Q resins for case studies I and II, respectively. Protein solution was injected into the chromatography column using a sample loop of varying capacities like 10–2000 μl (GE Healthcare Bio-Sciences). The output from the chromatography column was monitored using UV detection at 280, 260 and 215 nm and pH/conductivity online detectors. The pressure drop across the packed resin bed was monitored by pressure transducers installed in the ÄKTA Purifier 10 system. All data were recorded and viewed in Unicorn 5.2 software (GE Healthcare Bio-Sciences). The purified protein samples were collected in 15 ml polypropylene BD® centrifuge tubes using a Frac-920 fraction collector (GE Healthcare Bio-Sciences) connected to the ÄKTA Purifier 10 system. Measurement of absorbance at 280 nm (A280) for protein concentration determination was performed using an Epoch microplate spectrophotometer (BioTek® Instruments Inc. Winooski, VT USA).