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# A simple and efficient purification platform for monoclonal antibody production based on chromatin-directed cell culture clarification integrated with precipitation and void-exclusion anion exchange chromatography

Quan Chen[a], Sarah Maria Abdul Latiff[b], Phyllicia Toh[b], Xinying Peng[a], Aina Hoi[b], Mo Xian[a], Haibo Zhang[a], Rui Nian[a,*], Wei Zhang[b,*], Pete Gagnon[b]

[a] CAS Key Laboratory of Biobased Materials, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, 266101 Qingdao, China
[b] Bioprocessing Technology Institute, Agency for Science, Technology and Research, 20 Biopolis Way, 138668, Singapore

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ABSTRACT

Protein A affinity chromatography, featured by its robustness and high-specificity, is still dominant as a first capture step for the purification of immunoglobulin G monoclonal antibodies (IgG mAbs). However, the material and operational costs of protein A are universally recognized as high, and its productivity is also limited as column mode. In order to overcome these limitations, industry is increasingly considering the use of non-protein A-based processes for IgG purification. In this study, sodium citrate precipitation (SCP) was developed as the primary purification step, and chromatin-directed cell culture clarification was demonstrated to significantly elevate the purification capability. Additional 0.05% (w/v) of Tween 20 was shown to effectively reduce the residual free antibody light chain (LC) during precipitation. The resuspended IgG was further polished by void-exclusion anion exchange chromatography (VEAX), which supported protein loading without buffer adjustment. The non-histone host cell protein (nh-HCP) content in the final product was about 5 ppm and histone HCP below limit of detection (LOD). DNA was reduced to less than 1 ppb, and aggregates/free LC less than 0.1%. The overall IgG recovery was 87.2%. A simple and efficient purification platform with only one-column step was therefore established, providing a more promising alternative to the current prevailing protein A-based purification platforms.



## 1. Introduction

Over the last three decades, therapeutic immunoglobulin G monoclonal antibodies (IgG mAbs) have grown to become the dominant product class within the biopharmaceutical market (Buss et al., 2012; Ecker et al., 2015; Gagnon, 2012). IgGs are large heterotetrameric molecules, with molecular weights of approximately 150 kDa, and composed of two kinds of polypeptide chain, namely the heavy chain (HC, ~50 kDa) and the light chain (LC, ~25 kDa). When an IgG-producing cell line is generated, DNA fragments encoding LC and HC are usually either placed on two separate vectors and co-transfected to host cells or on a single vector with multiple promoters, where the expression of LC and HC is under the control of their own promoters (Birch and Racher, 2006). It is reported that LCs have helped in a significant role for correct antibody folding and assembly. LC overexpression was found to be associated with higher cell viability, higher IgG productivity, and lower aggregation (Bhoskar et al., 2013; Chusainow et al., 2009; Ho et al., 2012; Schlatter et al., 2005). Therefore, most IgG-producing cell lines are designed to manufacture excess amount of free LCs, which are then released to culture media together with full-length

*Abbreviations:* IgG, immunoglobulin G; mAb, monoclonal antibody; CHO, Chinese hamster ovary; HCP, host cell proteins; nh-HCP, non-histone host cell proteins; HC, heavy chain; LC, light chain; CCS, cell culture supernatant; c-d CCS, chromatin-directed clarified CCS; CV, column volume; VEAX, void-exclusion anion exchange chromatography; SEC, size exclusion chromatography; DBC, dynamic binding capacity; NTU, nephelometric turbidity units; SC, sodium citrate; SCP, sodium citrate precipitation; CMC, critical micelle concentration; MuLV, murine leukemia virus; MVM, minute virus of mice; LOD, limit of detection; RP-HPLC, reversed phase-HPLC; HBS, HEPES buffered saline; bp, base pairs; DAD, diode array detector; CAD, charged aerosol detector; DI, de-ionized.

* Corresponding authors.
*E-mail addresses:* nianrui@qibebt.ac.cn (R. Nian), zhang_wei@bti.a-star.edu.sg (W. Zhang).

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IgGs and required to be removed in the following purification processes.

In recent years, significant improvements in cell culture productivity have greatly challenged purification technology to keep pace in the meantime (Gagnon, 2012). Affinity chromatography using *Staphylococcal* protein A is extensively used as the initial antibody capture step from the cell culture supernatant (CCS), and has become the industrial standard (Ghose et al., 2005; Tarrant et al., 2012). Nevertheless, protein A chromatography has long been reported with its inherent disadvantages including high cost (over $20,000 per liter), relatively low binding capacity, and protein A ligand leachability concerns (Tao et al., 2014). Precipitation is a promising alternative technique of interest because precipitating protein in solid format supports the utmost degree reduction of process volume, thus benefiting the subsequent purification steps (Gagnon, 2012; Low et al., 2007). Centrifugation is traditionally used for the recovery of protein precipitates, which relies on bulky expensive hardware and is difficult to implement in current industrial processes. Recently, membrane filtration has been developed and applied to both ammonium sulfate (Ghosh, 2004; Wang et al., 2009; Venkiteshwaran et al., 2008) and polyethylene glycol (PEG) precipitation (Kuczewski et al., 2010, 2011) for effectively removing the supernatant. Disposable dead-end filtration units were demonstrated in lab-scale method development with many successes. Precipitation with tangential flow filtration system can be used for scale up and industrial manipulations (Venkiteshwaran et al., 2008). So far, the purification of IgGs from bovine serum (Venkiteshwaran et al., 2008), human serum (Wang et al., 2009), and mammalian CCS has been achieved (Venkiteshwaran et al., 2008; Wang et al., 2009). However, non-specific precipitation and relatively low protein recovery are also commonly observed and hinder its further application. IgG recoveries range from 85% to 93%, while purity averaging about 85% (Gagnon, 2012).

We recently reported a chromatin-directed cell culture clarification method, and such advance removal of chromatin heteroaggregates allowed protein A chromatography to achieve comparable dynamic binding capacity (DBC) with loading of purified IgG (Gagnon et al., 2014, 2015). It also enabled the reduction of non-histone host cell proteins (nh-HCP) in the eluted IgG pool from several hundred ppm to less than 10 ppm. This new technology is based on a synergistic effect mediated by allantoin and fatty acid. Fatty acid associates with allantoin by hydrogen bonding while conserving its charge and hydrophobicity. The allantoin-fatty acid complexes will then associate with nucleosomes mainly through their histone component. The follow-on addition of a multimodal solid-phase adsorbent eliminates most contaminants and residual additives.

Void-exclusion anion exchange chromatography (VEAX) represents a new chromatographic mode, distinct from so-called bind-elute and flow-through modes, of conducting chromatography on electrostatically charged porous particle media packed in columns (Nian et al., 2013). The IgG, positively charged, is forcibly passed through the void volume while contaminants either bind or diffuse through the pore volume. Fractionation performance is independent of load composition, so there is no need to equilibrate the load. Even 4 M guanidine was tolerated as shown in our previous study (Nian et al., 2013). It supported 99% removal of various HCP from CCS or protein A purified IgG, 99.5% removal of DNA and 99.7% removal of endotoxin. Meanwhile, it also reduced 3.5 log of minute virus of mice (MVM) and 3.0 log of murine leukemia virus (MuLV) (Nian et al., 2013). Practical limitation is the low load volume capacity, and the load volume is limited to the interparticle (void) volume of a packed column, which is about 35–40% of gravity-settled bed volume.

In this study, a novel seamless non-protein A chromatography-based IgG purification platform was developed. Chromatin-directed cell culture clarification was first integrated with sodium citrate precipitation (SCP), and then the resuspended antibody was directly applied at high concentration to VEAX to produce therapeutic pure end-product. The benefit of surfactant for better LC removal during SCP was also demonstrated.

## 2. Materials and methods

### 2.1. Reagents and equipment

All chemicals were purchased from Sigma-Aldrich. Chromatography resins were packed in XK series columns (GE Healthcare). UNOsphere™ Q was obtained from Bio-Rad Laboratories (cat.156-0105). WorkBeads™ 40 TREN$^{high}$ was obtained from BioWorks (cat. 40-603-010). MabSelect SuRe™ (cat. 17-5438-02) and Capto™ adhere (cat. 17-5444-01) were purchased from GE Healthcare. Chromatography experiments were carried out on ÄKTA™ Avant 25 (GE Healthcare).

### 2.2. Cell culture production

An IgG1 mAb (biosimilar Trastuzumab, Herceptin®) was expressed in CHO (DG44, Life Technologies, A11000-01) cells using a tricistronic vector developed by Ho et al. (2012). Recombinant antibody was produced according to a procedure described by Nian et al. (2016). Culture was harvested at 14–17 day with viability around 50%.

### 2.3. Cell culture clarification

Harvested cell culture broth was centrifuged at 4,000 × *g* for 20 min at room temperature, and the supernatant was passed through 0.22 μm dead end filter membrane (Nalgene® Rapid-Flow Filters, Thermo Scientific, cat. 295-3320). The CCS was then stored in 4 °C fridge for short-term usage or in −20 °C freezer for long-term storage.

Chromatin-directed cell culture clarification was performed following the protocol reported by Gagnon et al. (2014, 2015). Generally, 1.0% of allantoin (w/v) and 0.4% of caprylic acid (v/v) were added to CCS for chromatin extraction. pH was adjusted to 5.3 with 1.0 M acetic acid, and the mixture was mixed for 2 h. WorkBeads TREN pre-equilibrated with 50 mM MES, 150 mM NaCl, pH 5.3 was added at 5% (v/v) and continuously mixed for 4 h or longer if necessary. Solids were removed by centrifugation at 4,000 × *g* for 20 min at room temperature, and the supernatant was followed by filtration through a Sartoclear® PC1 Cap depth filter capsule (multilayer 25 cm$^2$ with nominal retention of 1 μm and 0.3 μm) (Sartorius-Stedim, Göttingen, Germany).

### 2.4. IgG purification by protein A and Capto adhere chromatography

Purified IgG employed in this study was obtained from protein A and Capto adhere chromatography. Protein A affinity chromatography was conducted with 53 mL of protein A media packed in a XK 26/20 column (10 cm bed), run at linear flow rate of 280 cm/h (volumetric flow rate 25 mL/min). The column was equilibrated with 5 column volumes (CV) of 50 mM HEPES, 120 mM NaCl, pH 7.0 (HBS). 1500 mL of CCS was loaded and washed with 10 CV of HBS. Antibody was eluted with 5 CV of 100 mM acetic acid, pH 3.5. Protein collection started when UV absorbance at 280 nm reached 50 mAU and collection stopped when UV absorbance descended below 50 mAU. The pH of the eluted IgG was neutralized by addition of 1.0 M Tris (pH unadjusted). The column was cleaned with 20 CV of 0.1 M NaOH, equilibrated and stored in 20% ethanol. Host cell contaminants were further removed by titrating the protein A eluate to pH