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# Directing membrane chromatography to manufacture $\alpha_1$-antitrypsin from human plasma fraction IV

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ABSTRACT

The surging demand for plasma proteins, mainly driven by the growing market and the development of new therapeutic indications, is promoting manufacturers to improve the throughput of plasma proteins. Due to the inherent convective mass transfer, membrane chromatography has been proved to be an efficient approach for extracting a small amount of target proteins from large-volume feed. In this study, $\alpha_1$-antitrypsin (AAT) was extracted from human plasma fraction IV by a two-step membrane chromatography. An anion-exchange membrane chromatography (AEMC) was used to capture the plasma proteins in bind/elute mode, and the obtained effluent was further polished by a hydrophobic interaction membrane chromatography (HIMC) in flow-through mode. Under optimal conditions, the recovery and purity of AAT achieved 87.0% and 0.58 AAT/protein (g/g) by AEMC, respectively. After the precise polishing by HIMC, the purity of AAT was 1.22 AAT/protein (g/g). The comparison results showed that membrane chromatography outperformed column chromatography in both steps because of its high throughput. This two-step membrane chromatography could obtain an AAT recovery of 83.3% and an activity recovery of 91.4%. The outcome of this work not only offers an alternative process for protein purification from plasma, but also provides guidelines for manufacturing product from a large-volume feed with multi-components by membrane chromatography.

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## 1. Introduction

Plasma proteins, as life-saving medicine, are niche products in therapeutic protein market. Their surging demand mainly driven by the growing market and the development of new therapeutic indications, is promoting manufacturers to improve the throughput of plasma proteins [1]. Human $\alpha_1$-antitrypsin (AAT) is the main protease inhibitor in the plasma preventing connective tissues from degrading by elastase. It is usually used to cure lung emphysema induced by AAT deficiency. Manufacturers normally extract AAT from human plasma fraction IV precipitate obtained by ethanol fractionation process [2,3]. In order to adequately dissolve AAT and alleviate the negative impact of ethanol on AAT stability [4], fraction IV paste needs to be mixed with large-volume buffer before purification [5,6]. Similar with the protein extraction from fermentation broth with a low concentration of target product [7–9], the AAT purification has to deal with a large-volume dilute solution. Nowadays, column chromatography is a prevalent technique for AAT purification. Various resins based on ion-exchange, immunoaffinity as well as metal chelating have been introduced into AAT production [4,10]. However, due to the limitation of diffusive mass transfer in the column, the throughput is relatively low and thus it takes a long time to accomplish one purification cycle, where more AAT aggregates would form with increasing processing time [1]. Therefore, it is necessary to find a high-throughput method with high resolution to efficiently recover low dose AAT from the large-volume feed.

Membrane chromatography was introduced as an integrative technology for protein purification, where the transport of solutes to their binding sites takes place mainly by convection, thus diminishing both process time and buffer usage [11,12]. It is well known that a high flow rate may cause media deformation and high pressure drop in the diffusion-based column chromatography, while convective-based membrane adsorbers could maintain high binding capacity and high resolution at a high flow rate. Moreover, for labile biomolecules such as plasma proteins, therapeutic recombinant proteins and plasmid DNA, membrane chromatography is advantageous to keep their native conformation by reducing contact time with adsorbents [13,14]. Due to the easy scale-up, low chemical consumption and small space requirement of membrane

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chromatography, its flexibility for manufacturing facility was high [15,16]. Last but most significantly, membrane adsorbers could be integrated conveniently with the present equipment utilized for column chromatography, thus reducing the investment cost.

As an effective method to fast remove host cell proteins, DNA, virus during antibody purification in flow-through mode (i.e. the impurities are retained while the target product passes through) [17–19], membrane chromatography has been successfully applied in commercial-scale biopharmaceutical production for impurities removal. However, due to the low specific surface area and the resulting few binding sites on membrane adsorber, membrane chromatography operated in bind/elute mode (i.e. the target product is captured while the impurities flow through) for commercial-scale implementation did not gain wide industrial acceptance until 2012 [7]. Actually, for capturing a small amount of target products [8], the relative low binding capacity of membrane adsorbers would be sufficient. Therefore, thanks to its hydrodynamic advantage, membrane chromatography is especially suitable for dealing with large-volume feed containing low-concentration target biomolecules in bind/elute mode, which has attracted much attention in recent years. Vogel et al. described a robust anion-exchange membrane chromatography in bind/elute mode for directly capturing target proteins from cell culture broth (as a preliminary purification step), showing that membrane chromatography could greatly reduce operating time and achieve an excellent efficiency [7]. Mönster et al. reported a feasible way to purify target proteins from dilute cell lysate in one-step by ion-exchange membrane chromatography [8]. Ghosh and his colleagues proved the potential of purifying low-concentration immunoglobulin G or monoclonal antibody from diverse resources by membrane adsorbers [20–23]. It was also reported that membrane chromatography could be applied to rapidly extract whey proteins from dairy feed stream [24,25]. Therefore, it can be reasonable to hypothesize that membrane chromatography is potentially suitable to purify low dose AAT from its dilute feed, and to our best knowledge, there has been no previous study regarding purification of AAT by membrane chromatography.

The objective of the present work is to establish an efficient separation method for obtaining pure AAT from human plasma fraction IV. The feed stream was obtained by dissolving human plasma fraction IV precipitate in an appropriate buffer and then removing lipoproteins (by PEG precipitation) [1]. First, preliminary purification of AAT was carried out by anion-exchange membrane chromatography (AEMC) in bind/elute mode. Second, the partially purified AAT was then polished by hydrophobic interaction membrane chromatography (HIMC) to further improve AAT purity. Sample loading conditions including pH, ionic strength, loading volume as well as flow rate were optimized systematically. Moreover, the elution gradients were adjusted to obtain the optimal separation resolution of AEMC and the corresponding results were compared with those of anion-exchange column chromatography (AECC). Then, ammonium sulfate (AS) concentration and flow rate were optimized to develop a precise polishing process by HIMC in flow-through mode, and the separation efficiency was also compared with that of hydrophobic interaction column chromatography (HICC).

## 2. Materials and methods

### 2.1. Materials

Human plasma fraction IV precipitate and mixed plasma were kindly provided by Shandong Taibang Biological Products Co. Ltd, China. A Q membrane coin (with quaternary ammonium) (MSTG18Q16, 0.35 mL), a housing (MSTG18H16)

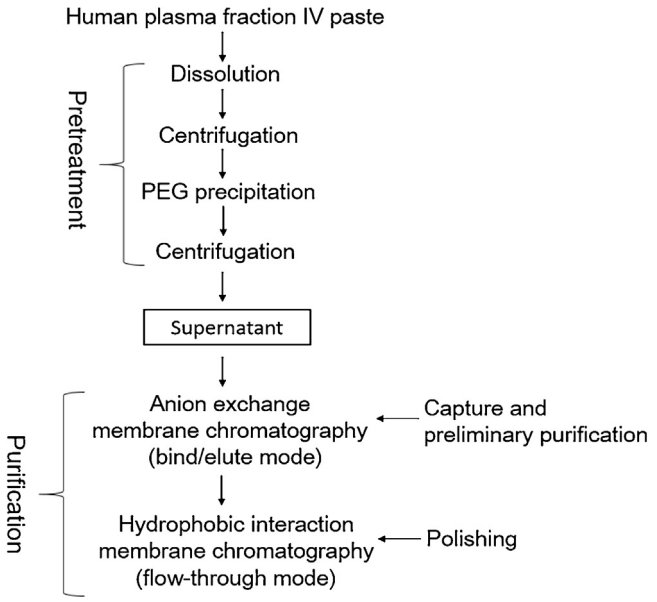


**Fig. 1.** Flowsheet diagram of AAT purification from human plasma fraction IV (bind/elute mode: the target product is captured while the impurities flow through; flow-through mode: the impurities are retained while the target product passes through).

and a Mustang Q XT Membrane (with quaternary ammonium) (XT5MSTGQPM6, 5 mL) were purchased from Pall Corporation, USA. A Sartobind® Phenyl hydrophobic interaction membrane adsorber (96HICP42EUC11-A, 3 mL) was bought from Sartorius Stedim Biotech, Germany. Standard AAT, Trypsin and Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA) were purchased from Sigma–Aldrich, USA. A HiPrep Q XL 16/10 column (with quaternary ammonium) (20 mL), a HiTrap Phenyl FF (high sub) column (1 mL) and a HiTrap Phenyl FF (low sub) column (1 mL) were purchased from GE, USA. Amicon Ultra-15 Centrifugal Filters with a molecular weight cut-off (MWCO) of 10,000 Da were bought from Millipore, USA. Chemicals used for buffer preparation, sample analysis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were of analytical grade.

### 2.2. Pretreatment of human plasma fraction IV precipitate

Fig. 1 shows the flowsheet diagram of AAT purification from human plasma fraction IV precipitate. Human plasma fraction IV precipitate was dissolved with sodium phosphate buffer (PB, 20 mM, pH 7.0) by stirring for six hours at 4 °C (1 kg paste in 12.5 L buffer). After the dissolution was completed, large particles were removed by centrifugation at 5000 rpm for 20 min. The 11% (w/v) PEG4000 was added to the supernatant with stirring for 1 hour and the pH was then adjusted to 5.2 with 1 M acetic acid. After stirring for 30 min at 4 °C, the PEG precipitate was removed by centrifugation at 9000 rpm for 30 min. Then, the pH of supernatant was adjusted to 6.5 by 1.0 M NaOH and the supernatant was finally micro-filtered through 0.22 μm membranes. The clarified solution was stored at −80 °C, and thawed at 4 °C before use. The concentration and purity of AAT in feed solution were approximately 0.1 mg/mL and 0.1 AAT/protein (g/g), respectively. The properties of main proteins in human plasma fraction IV are shown in Table S1 [1,26–29].

### 2.3. AEMC and AECC

A Q membrane coin containing 16 layers anion-exchange membranes was housed in the filter holder and then was integrated

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