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바이오분야 분리용 막크로마토그래피 설계 방안

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How to Design Membrane Chromatography for Bioseparations: A Short Review

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요 약: 현재 바이오 분야에서 분리에 대한 수요가 급증함에 따라, 투과율 및 결합능 측면에서 높은 성능을 띠는 막크로마토그래피가 수지 크로마토그래피의 대체 분리 공정으로 부상하고 있다. 실증을 기반으로 하여 막 소재가 결정되는 기존 분리막 공정과 달리, 막크로마토그래피의 경우 분리하고자 하는 목표 물질에 적합한 분리 메커니즘 이해 그리고 이를 기반한 공정 설계가 필요하다. 본 논문에서는 생특이성을 활용하여 선택적으로 거대 분자를 포집하는 친화성 작용, 전하를 활용하여 생분자와 결합하는 이온 교환 작용 그리고 소수성을 활용하여 생분자와 결합하는 소수성 작용과 같은 막크로마토그래피 주요 분리 메커니즘들에 대해 다루고자 한다. 또한 본 논문에서는 단계적 측면에서 또는 소재 측면에 막크로마토그래피 기술 설계 시 고려해야할 변인들에 대해서 다루고자 한다.

Abstract: While there are increasing demands on biomolecules separation, resin chromatography lacks in terms of throughput and membrane chromatography is an alternative with high binding capacity and enhanced mass transfer properties. Unlike typical membrane processing, where the performance can only be empirically assessed, understanding how mechanisms work in membrane chromatography is decisive to design biospecific processing. This short review covers three separation mechanisms, including affinity interaction modes for selectively capturing bulk molecules using biospecific sites, ion exchange modes for binding biomolecules using net charges and hydrophobic interaction modes for binding targeted, hydrophobic species. The parameters in designing membrane chromatography that should be considered operation-wise or material-wise, are also further detailed in this paper.

Keywords: *membrane chromatography, affinity interaction, ion exchange interaction, hydrophobic interaction*

1. Introduction

The importance of the biomolecule separation has arisen due to the increasing demand for biopharmaceuticals such as recombinant proteins and therapeutic antibodies and separation/recovery processes in downstream line are subdivided into four steps, which are as follows: 1) First step, where solid-liquid phase separation takes place using centrifugation or filtration, 2) Second step, where impurities are removed utilizing different physicochemical properties based on affinity interaction 3) Third step, where unwanted components, sharing similar physicochemical properties with the target, are removed through the chromatographic process, and 4) Final step, a polishing step where gel filtration or crystallization of final product may be used[1].

In downstream processing, where high-resolution biomolecule separation is required, chromatography is widely used for purification. The chromatographic columns may include interaction modes like affinity inter-

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Table 1. Characteristics according to the Purification Mode of Chromatography

Chromatography mode	Molecular characteristics	Purification steps	Main features
Size exclusion	Size	Intermediate, polishing	Limited resolution, low capacity, low speed
Ion exchange	Charge	Capture, intermediate, polishing	High resolution, high capacity, high speed
Affinity	Biospecific sites	Capture, intermediate, polishing	High resolution, medium capacity, high speed
Hydrophobic interaction	Hydrophobicity	Capture, intermediate, polishing	Good resolution, good capacity, good speed
Reversed-phase	Lipophilicity	Intermediate, polishing	High resolution, low capacity, low speed

action, ion exchange, hydrophobic interaction, and reversed-phase chromatography as summarized in Table 1[2]. Conventional chromatographic technique, resin chromatography, generally suffer from a high-pressure drop because of colloidal substances being accumulated - fouling - during the biomolecule diffusion. The performance of resin chromatography is highly reliant on intraparticle diffusion since the binding happens inside the bead pores. With solute molecules being piled up, it slows the diffusion, hence the processing time increases. Furthermore, the solvent volume required for the elution step increases.

Membrane chromatography (MC), also termed as membrane adsorber, has been investigated and developed over the past two decades to overcome the problems conventional chromatography face, particularly for large volume processing, antibody polishing, and purifying viruses and plasmid DNA. Comparatively faster binding behavior allows the mobile phase to process at high speeds, and this allows to diminish the operation cost. Also, such a high flow rate in MC prevents products from being biologically decayed because of proteolysis or denaturation. Other advantages include reduced buffer usage due to low effective volume, the reduced pressure drop with simple operating facilities, and high scalability. Moreover, membrane chromatography is recommended for single-use, so cleaning or regeneration is no longer required, lowering the risk of cross-contamination. Comparison between the conventional chromatography and MC are made in Table 2[3].

Table 2. Comparison of Chromatography Stationary Phases

	Resin	Membrane
Flow rates	Low	High
Pressure drop	High	Low
Dominant transport	Diffusion	Convection
Binding capacities	High	Low
Resolution	High	Moderate

Much research on MC has been reported, using interaction modes such as affinity or ion exchange for applications like protein purification, mAb purification, and plasmid purification[1]. Nevertheless, further research and development are needed due to a lack of understanding in binding capacity, configurational design, and physical properties of the membrane as membrane thickness and ligand density. Following article will briefly cover the overall interaction modes in MC, how separation works in this platform, and the variables that need to be taken care of when designing MC.

2. Separation Mechanisms in MC

2.1. Basic principles

In MC, the basic concept of a membrane being a permselective medium between two phases[4], still stands. Yet, tuning solubility- or diffusivity-selectivity to separate the desired component from the mixture, is not applied in MC. In the principal membrane process, the nominal pore size, inferred from the permeant size, is graded based on what kind of application the mem-

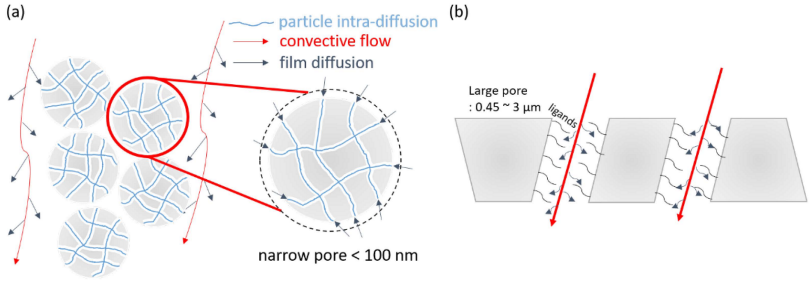

Fig. 1. Transport Mechanisms in (a) resin chromatography and (b) membrane chromatography[6].

brane goes through and follows the theoretical model - either the solution-diffusion model or the pore-flow model[5]. As for MC, the pore size should comparatively be bigger than the permeant size to make the convective flow possible, thus accelerating the solutes' mass transfer. It is the binding kinetics that sorts out the targeted species from the mixture. Due to the adsorptive interaction between the brush-like ligands - a stationary phase - and permeants - a mobile phase, desired components are immobilized; the pores in the matrix are chromatographic columns and the rest is the scaffold to withstand the high flow rate.

MC has been garnering attention as an alternative platform to conventional resin chromatography to refine biological products. As demonstrated in Fig. 1 (a) - resin chromatography - the feed should seep into pores to interact with the effective binding site, forming intricate fluidic streamline; it slows the process. To mitigate such geometric restriction, MC, shown in Fig. 1 (b), feed-in convective flow is solely aligned to the direction of applied pressure, making the mass transfer readily faster form and avoid any backpressure, and this convection predominantly allows the target molecules to be bound to specific sites in MC. For MC to fully be formulated as a separation process, knowing the physicochemical properties of desired species and utilizing them to tune the right ligands, is imperative.

2.2. Mechanism

However, biological products dealt in MC, vary in forms and come from many sources and to design the process, one should be aware of the parameters like 1) the chemical nature of the target materials in feed, 2) the optimal operational environment (i.e. pH, concentration, temperature, etc.) and 3) the desired quality of the product (i.e. the maximum acceptable level or the chemical nature of the final product)[7]. With the above parameters in mind, the separation mechanisms (or operations modes) of MC are decided.

Easily surmised from the term "membrane chromatography", the separation mechanism of MC is based on exploiting the differences in interaction strength between the chromatographic medium and permeants in the mixture. Targeted species should alone favor the interaction with the medium so that removing the impurities and binding the target can both be achieved. For this reason, one of the features of ideal membrane support in MC is to be either neutral or hydrophilic for support to be chemically stable in aqueous surroundings and to stop molecules from non-specifically interacting with the support itself[8]. Specific interactions between the ligands and permeants between MC are as follows: 1) Affinity Interaction 2) Ion Exchange and 3) Hydrophobic Interaction.

2.2.1. Affinity interaction

In bioprocessing streamline, MC has been applied to protein capture or purification. As inferred from the word 'capture', the primary goal of protein capture is to selectively remove bulk impurities and to hook such impurities in diluted conditions, chromatographic media should possess high binding capacity (high selectivity), which is why affinity MC is used in this step[6].

Affinity MC separates components using the bio-

specific interaction, meaning that when designing the ligands, it is on point to the targeted molecules; the premise of this interaction is that affinity between the target and anchored ligands is perfectly steric fit[7]. This implies, if carefully calculated, even weak, non-covalent interaction can partake in retaining desired components and this lowers the risk of denaturing the product in elution.

Affinity ligands used in the following mode, include Protein A or G, antibodies, immunoaffinity, dyes, peptide, metal, etc., which may covalently be anchored to stationary phase through hydroxyl, amino or carboxylic functional group. It is noteworthy that if affinity MC shows a poor performance owing to its low ligand accessibility, a spacer arm can be introduced to extend the ligand length to enhance ligand surface area.

One of the representative ligands used in affinity MC is metal[8]. Immobilized metal ions, coupled with chelating agents, works as an electron acceptor (Lewis acid). These species are likely to immobilize biomolecules having an electron donor (Lewis base), amino acid residues like histidine or tryptophan. Metal ions affinities to retained biomolecules are in the following orders: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$[8,10-12]. These metal ions are apt for interacting not only with nitrogen in amino or imino groups but also with oxygen and sulfur[4]. Also, the use of the right chelating agents is crucial because the number of coordination sites in metal ions is limited, which serve as open sites to be bound onto chromatographic media as well as to be binding sites for biomolecules.

2.2.2. Ion exchange

Among mentioned interactions, ion exchange MC is perhaps a largely developed technique in both lab and production scale due to its exceptional throughput. Applied in the purification step with relatively high binding capacity, it allows the following mode to suffice in large volumes while keeping the biological activity of retained products intact. Retention is achieved through ionic bonding, meaning that the target should play its role as either anion or cation so that the ligands would do vice versa.

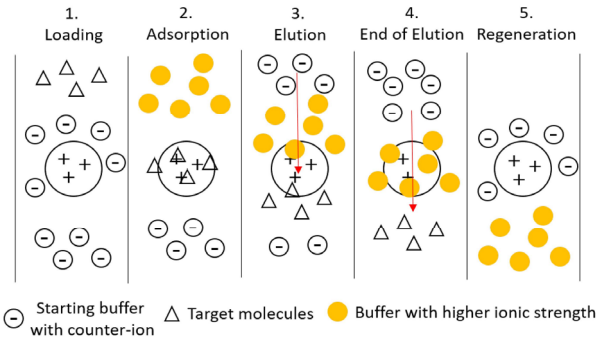

Fig. 2. Ion exchange chromatogrphy, in principle.

As to strong cation or anion ligands, like quaternary ammonium group or sulfonic acid group, they are independent of pH of the mobile phase, whereas weak cation or anion, such as carboxymethyl or diethylaminoethyl group, their binding capacity relies on pH. Thus, in the case of weak cation/anion ligands, controlling the pH environment of the mobile phase is important to augment the ionic binding strength; buffer engages in the electrostatic interaction between target and ligands, as exhibited in Fig. 2.

In principle, retention happens when ligand charge is equivalent to the net charge - relative to the difference between isoelectric point and pH - of molecules. Yet, because of the charges being not well-distributed in the molecule surface and with the steric hindrance being involved, there is no direct relationship between net charge and retention. To define the extent of retention, therefore, either stoichiometric displacement model or electrostatic interaction models can be used.

Retained species are obtained through elution by simply increasing the salt concentration of eluents, which leads to salts intervening the attraction between the target and ligands and subsequently the target falls off from the stationary phase. The salt-tolerant ligands like primary amine, on the other hand, allow both adsorption and elution to happen at high salt concentrations. This is due to the following ligands having both electrostatic interaction and hydrogen bonding, which increases the total free energy interaction between ligands and molecules, thus improving the binding affinit

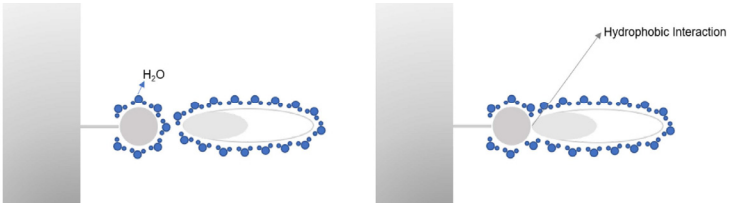

Fig. 3. Scheme representation of hydrophobic interaction chromatography[15].

under relatively high conductivity[13,14].

2.2.3. Hydrophobic interaction

Along with ionic exchange MC, another interaction used in the purification step is hydrophobic interaction, where more precise clearance of remaining impurities in the dilute condition is required. By grafting hydrophobic ligands, such as aromatic rings, short alkyl, or polyester chains, on the hydrophilic surface, interaction happens between the hydrophobic ligands on the stationary phase and hydrophobic molecules in the mobile phase. The driving force of the following interaction is not adsorption, but for both ligands and molecules to lessen the contact with the surrounding water molecules (or hydration number), the direct contact to one another is promoted as portrayed in Fig. 3.

Since water displacement is why retention works in hydrophobic interaction, multiple parameters like the salt type/concentration, pH, temperature of mobile phase, and the nature of stationary phase should be controlled to enhance the performance; the retention is derived from the solvent effect[15]. As for the salt type, it has been shown that the retention merely follows the Hofmeister series, shown in Fig. 4, and "salting in" has a positive effect on precipitating the molecules as well as increasing the molar surface tension of water, thus leading to better retention[17]. It is fair to point out although the retention factor is somewhat related to the surface tension of the mobile phase, surface tension increment does not necessarily lead to enhancing the performance, meaning that other factors, aside from surface tension of the mobile phase, are involved.

Salt concentration and pH of the mobile phase also play a role in the retention factor, as it is directly related to the water molecule binding to permeants. Unlike ionic exchange MC, by increasing the salt concentration, molar surface tension of water increases and electrostatic interactions likely reduce, hence improves the performance. As to pH, it was found that equating pH to isoelectric point triggered more release of surrounding water molecules in permeants, making hydrophobic interaction more pronounced[18].

Because of hydrophobic interaction being an enthalpy-driven process, temperature substantially affects the retention; the higher temperature, the better the performance[19,20]. Yet the denaturation can easily occur in biomolecules when changing the temperature, so tuning the optimal temperature range is advised.

3. How to Design MC

3.1. Operational steps in MC

MC process consists of three steps: 1) loading, 2) washing, and 3) elution. Initially, the feed is injected into the platform. After the targets are occupied to stationary phase, unfettered mobile species should be washed through a loading buffer, then followed by elution (desorption), which releases the bound species through elution buffer, thus recovering the desired components with high yield. It is worth keeping in mind that when designing MC, both steps, loading, and elution, should be considered to gauge the possibilities of products being denatured or to ascertain if ligands are compatible throughout the process.

The breakthrough curve, shown in Fig. 5, is a concentration profile throughout the operation. Because of its nonidealities in actual flow, the curve is broadened in the loading step due to the flow maldistribution or

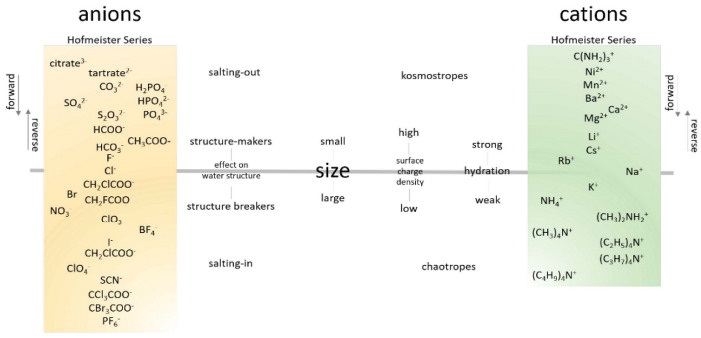

Fig. 4. Hofmeister series of ions in water[16].

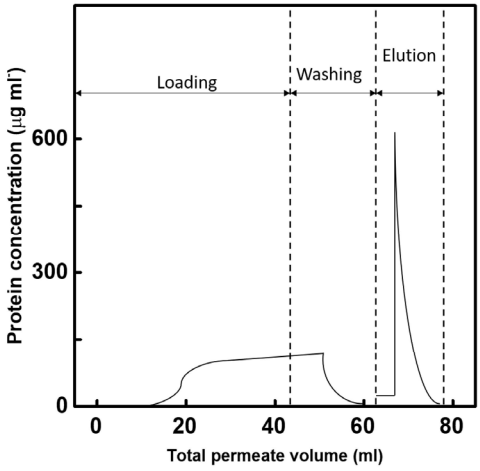

Fig. 5. Breakthrough curve, a concentration profile throughout the MC process.

dead volume mixing[8]. It is advised that before reaching its equilibrium, when the binding sites are occupied and no further sorption occurs, the product loss to permeate side should be as minimized as possible in the loading step.

3.2. Required traits in MC materials

As for membrane support, the required characteristics in MC are straightforward: 1) It should be microporous with a high flow rate, letting large biomolecules readily interact with ligands, 2) It should be hydrophilic/neutral to restrict interactions to happen between ligands and the permeants only, 3) It should be chemically/physically stable to endure harsh conditions and 4) It should have functional groups to easily anchor or activate the desired ligands. Widely used materials that meet the above requirements are regenerated cellulose, polysulfone, and polyamide[21-23].

To control the favorable interaction modes in MC, various strategies could be introduced with regards to functionalization, and the polymer-grafting method is by far a common technique to tailor the surface properties of the stationary phase. The following method can be classified into two: 1) “grafting to” method, where it simply functionalizes the reactive group of pre-synthesized material, or 2) “grafting from” method, where it initiates the polymerization from the surface of the material using UV irradiation, plasma treatment, photo-initiator, etc[24-28]. It is important to point out that while “grafting to” is a relatively facile method with better knowledge of molecular weight or polydispersity, its grafting density is lower than the “grafting from” method, which is why the “grafting from” method is preferable in MC[29].

4. Summary

For better throughput and yields, MC has become an alternative to resin chromatography for biomolecules separation. Unlike conventional membrane processing, where - due to the dynamic nature of mass transfer - mechanisms behind separating permeants *via* membrane matrices are empirically understood, MC is a “custom-built” separation process; it is crucial to design from the very start, knowing the physicochemical properties of the target/impurities and opting out which interaction modes (or ligands) suit for the process. In this

article, three separation mechanisms/operation modes of MCs were reviewed: 1) Affinity Interaction, where bulk impurities like protein were selectively captured, utilizing biospecific sites, 2) Ion Exchange, where molecular charges of mobile phase were employed to be bound to stationary phase and 3) Hydrophobic Interaction, where hydrophobic ligands are grafted onto membrane matrices to hook hydrophobic molecules, so as a compound, it can lessen its surface area in the aqueous phase. Operational steps are taken in MC and some of the required traits as MC materials (or membrane supports) were also dealt with in this article. Operational steps are 1) loading, 2) washing, and 3) elution and it is important to stress that preserving the chemical properties of target molecules must be considered in the steps of loading and elution, where products are prone to be denatured. Furthermore, when designing MC, materials should suffice as follows: they should be 1) microporous, 2) hydrophobic/neutral, 3) chemically stable in harsh conditions and 4) capable of anchoring functional groups to bind specific biomolecules, which can be achieved through functionalization or polymer-grafting methods.

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