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Application Note

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Comparison of Cation-Exchange Monoliths: CIM® SO3 and Multimodal CIM® SO4 Monoliths using a Standard Protein Mixture

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Abstract

This application note compares CIM® SO3 and CIM® SO4 cation-exchange monoliths. Both are strong cation exchangers, but SO4 ligand provides additional hydrophobic and hydrogen-bonding interactions. Under identical chromatographic conditions, SO4 showed increased retention of cytochrome c and lysozyme, consistent with multimodal behavior.

While with CIM SO3 higher lysozyme capacity was achieved at low salt, CIM SO4 maintained higher lysozyme capacity at > 200 mM NaCl concentration, allowing efficient operation under elevated salt conditions. Due to different ligands characteristics, it is recommended to refine CIM SO3 methods for CIM SO4 to exploit multimodal interactions and achieve optimal chromatographic performance.

Introduction

CIM SO3 and CIM SO4 are both polymethacrylate monoliths bearing sulfur containing anionic ligands: sulfonate ($-SO_3-$) for SO3 and sulfate ($-OSO_3-$) for SO4. The presence of anionic groups on the surface of the monoliths results in strong cation-exchange properties for both chemistries. A strong cation exchanger maintains a negative charge across the entire pH range attracting positively charged molecules at any operating pH value.

However, due to the differences in ligand properties and manufacturing procedures, CIM SO4 enables at least two additional interaction mechanisms: hydrophobic and hydrogen-bonding.

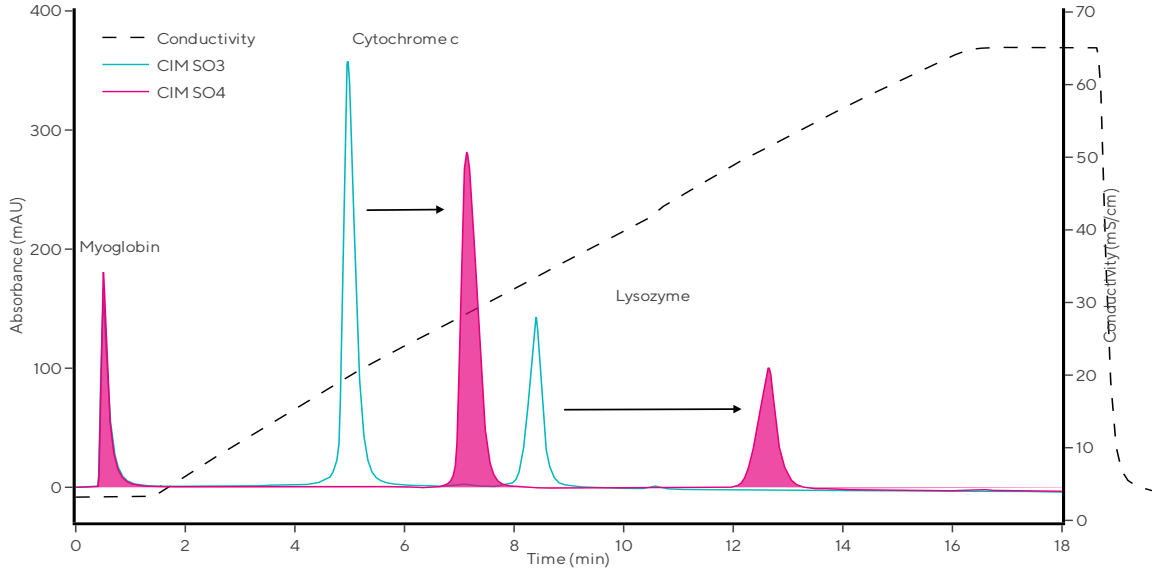
This application note presents the differences in chromatographic performance between these two CIM monoliths under the same chromatographic conditions. Experiments were performed using CIMmultus® 1 mL columns, but similar characteristics has been confirmed across other CIM monolith types and scales (CIMac, CIM® Monolithic Well Plates, CIM® Octa etc).

CIM SO4 monolith is suitable for purifying a broad range of biomolecules (proteins, viruses, VLPs, EVs etc.). In this study, we compared performance of CIM SO3 and CIM SO4 using three model proteins.

Separation of Cytochrome c and Lysozyme

Three standard proteins: equine skeletal muscle myoglobin (Myo), equine cytochrome c (Cyt) and chicken egg-white lysozyme (Lys) were separated in ascending NaCl gradient using CIMmultus SO3 1 mL column (2 µm channels) and CIMmultus SO4 1 mL column (2 µm channels). Chromatogram overlay is shown in Figure 1.

Figure 1: *Separation of Myo, Cyt and Lys on CIM SO3 and on CIM SO4 columns in linear ascending NaCl gradient.*



Both products bind Cyt and Lys, while Myo doesn't bind under these conditions. However, with CIM SO4, increased retention of Cyt and Lys compared to CIM SO3 was proven, leading to improved resolution between their elution peaks.

Chromatographic parameters are detailed in Table 1.

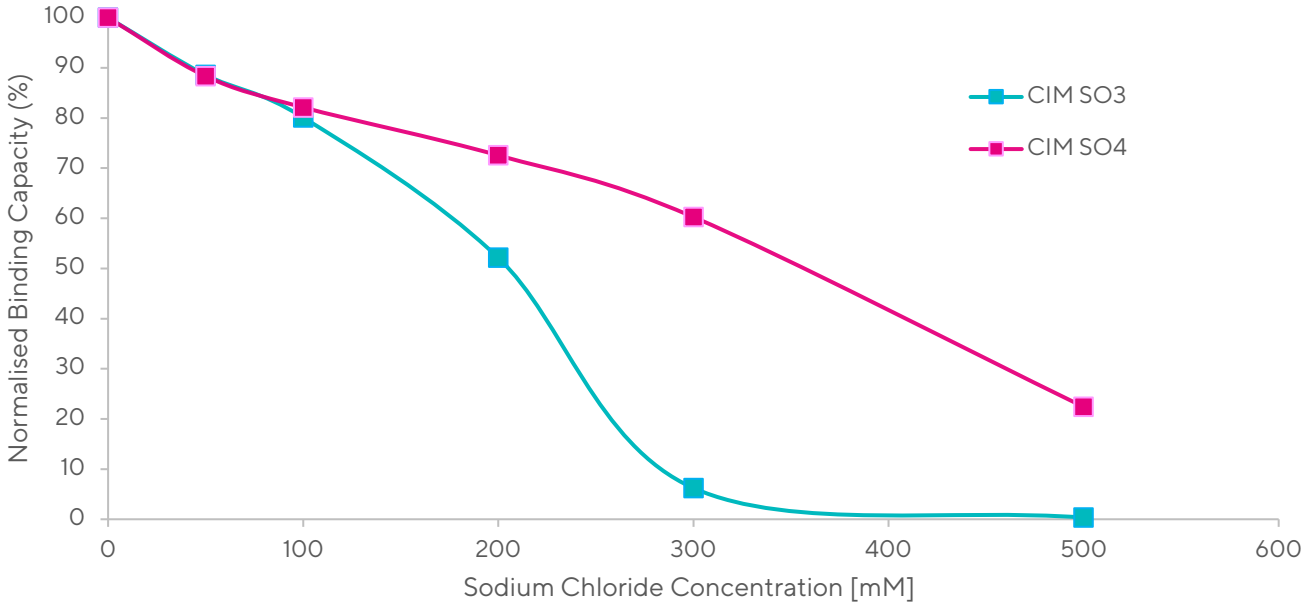
Table 1: *Chromatographic parameters for CIMmultus SO3 and CIMmultus SO4 1 mL column. tR = retention time; σ = conductivity; R = resolution between Cyt and Lys elution peaks.*

Column type	t_R Cyt [min]	t_R Lys [min]	σ Cyt (mS/cm)	σ Lys (mS/cm)	R
SO3	4.96	8.40	19.6	33.4	8.3
SO4	7.14	12.65	28.4	51.4	9.5

Lysozyme Binding Capacity

With increasing NaCl concentration in the loading sample, lysozyme binding capacity decreased for both products (Figure 2).

Figure 2: *Effect of sodium chloride concentrations in the loading sample on the normalized lysozyme binding capacity. Binding capacities were normalized to the maximum values observed in the absence of salt (100 %).*



Within 200-500 mM NaCl, CIM SO4 exhibited higher lysozyme binding capacity than CIM SO3. Electrostatic interactions are expected to contribute most of the binding energy to both columns, thus contributing most to the retention of lysozyme. Addition of salt leads to a decrease in electrostatic interactions, while hydrogen-bonding contribution is expected to be only weakly affected and hydrophobic interactions are expected to be even enhanced with increasing salt concentration.

In the absence of NaCl in the loading buffer, CIM SO3 obtained an approximately one-third higher lysozyme binding capacity than CIM SO4 and maintained this advantage under low-salt conditions. Capacity crossover, at which CIM SO4 surpassed CIM SO3 in lysozyme binding capacity occurred near 200 mM NaCl (data not shown).

Isothermal Titration Calorimetry

A study utilizing isothermal titration calorimetry to evaluate binding of proteins to CIM SO3 and CIM SO4 is thoroughly described in the poster Modulating and Understanding Retention of Proteins on Chromatographic Support by Changing Cation-Exchanging Ligand. Based on thermodynamic analyses of Lys adsorption under different binding conditions, study indicated additional stabilizing interactions provided by multimodal CIM SO4 compared to CIM SO3.

Conclusion

Although CIM SO3 and CIM SO4 are both strong cation exchangers, their chromatographic behavior differs under identical chromatographic conditions. Therefore, when using CIM SO4, it is not advisable to simply replicate conditions optimized for CIM SO3. To achieve optimal performance with CIM SO4, chromatographic methods should be developed independently to exploit not only electrostatic interactions but also hydrophobic and hydrogen-bonding interactions. As a unique multimodal strong cation-exchange monolith, CIM SO4 can facilitate binding and separation of biomolecules under chromatographic conditions that might better preserve biomolecule stability or better align with subsequent downstream steps, thereby improving overall purification performance.

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