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Optimization of Protein Purification Conditions on Sartobind® Lab Membrane Adsorbers

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Abstract

In this Application Note we show the optimization of conditions for binding and elution of proteins on Sartobind® Lab Ion Exchange membrane adsorbers. We demonstrate that dynamic binding capacity and recovery are affected by salt concentration and pH of the binding buffer but are not affected by flow rate. Further, we show binding capacities for various proteins after buffer optimization.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

Introduction

The following experiments with Sartobind® Lab membrane adsorbers show how optimization of conditions for binding and elution can lead to very high recovery of target proteins in a matter of minutes. Dynamic binding capacity and recovery are affected by salt concentration and pH of binding buffer but are not affected by flow rate. Diffusion has been nearly eliminated by use of a macroporous membrane as the chromatographic matrix. Each Sartobind® Lab unit is supplied ready-for-use in a convenient pressure filter format for purification by syringe or pump. With the included adapters, these units can also be used on liquid chromatography systems. They are intended for research and discovery applications, with larger Sartobind® capsules and cassettes that use the same matrix available for convenient scale up to clinical and commercial production.

Results

Cytochrome P-450 Purification

Purification of cytochrome P-450 from *E. coli* was performed under different conditions. First the solution was directly applied to a Sartobind® S Lab unit (strong acidic cation exchanger, 0.41 mL MV) at a concentration of 12.3 µg/mL using 25 mM phosphate buffer plus 50 mM NaCl, pH 7.1, for equilibration. Due to the elevated salt concentration only low binding of the target protein could be achieved (data not shown). Diluting the protein feed 1:2 with 25 mM phosphate buffer containing 10 mM NaCl resulted in an increased binding and 80% recovery in the eluate fractions. Dilution of the protein 1:5 with 25 mM phosphate buffer containing 10 mM NaCl further increased binding to achieve an optimal recovery of 95%. In gerneral Sartobind® ion exchange membranes behave exactly as conventional resins in terms of binding at different pH and elution. However, slightly lower ionic strengths should be chosen for loading, when compared with those used for resins. If possible, as illustrated in our results, binding should be performed at 10 mM rather than 50 mM salt.

Conditions

Protein	Cytochrome P-450 from recombinant *E. coli,* 49 kDa MW, pI 9.4		
	undiluted	1:2 dilution	1:5 dilution
Concentration	12.3 µg/mL	6.15 µg/mL	2.46 µg/mL
Volume	1,500 mL	3,000 mL	7,500 mL
Buffer	A1 = 25 mM phosphate + 50 mM NaCl pH 7.1	A2 = 25 mM phosphate + 25 mM NaCl pH 7.1	A3 = 25 mM phosphate + 10 mM NaCl pH 7.1
Washing	50 mL of A1	500 mL of A2	500 mL of A3
Elution	A1 + 500 mM NaCl	A1 + 500 mM NaCl	A1 + 500 mM NaCl
Elution volume	5 mL	20 mL	20 mL
Membrane volume	0.41 mL	2.8 mL	2.8 mL

Results

	1:2 dilution		1:5 dilution	
	Feed	**Eluate**	**Feed**	**Eluate**
Volume	3,000 mL	20 mL	7,500 mL	20 mL
Volume reduction	–	99.3%	–	97.3%
Concentration	6.15 µg/mL	740 µg/mL	2.46 µg/mL	856 µg/mL
Concentration factor	–	120x	–	350x
Target protein	18.45 mg	14.75 mg	18.45 mg	17.53 mg
Total protein	1,600 mg	18.4 mg	1,600 mg	19.5 mg
Target recovery	–	80%	–	95%
Purity determined by SDS-PAGE	–	>80%	–	>90%
Total time needed	35 – 37 minutes		80 – 85 minutes	

Green Fluorescent Protein Purification

Green fluorescent protein was simultaneously purified and concentrated 138-fold on a Sartobind® Q Lab unit (strong anion exchanger, 0.41 mL MV).

Conditions

Protein	Green Fluorescent Protein form jellyfish *Aequorea victoria*, 26 kDa MW, pI 5.0
Initial concentration	0.0038 mg/mL
Volume	500 mL
Flow rate	18 mL/min (connected to a peristaltic pump)
Loading	10 mL of buffer A (10 mM Tris-HCl, 1 mM EDTA, pH 7.5)
Washing	10 mL of buffer A
Elution	3.6 mL of 500 mM ammonium sulfate in buffer A

Results

	Feed	Eluate	Results
Volume	500 mL	3.36 mL	99.3% volume reduction
Concentration	0.0038 µg/mL	0.527 µg/mL	138.7-fold concentration
Protein	1.9 mg	1.9 mg	100% recovery
Total process time	30 minutes		

Protein Binding Capacities on Sartobind® S | Q Lab

Various standard proteins have been applied to the Sartobind® IEX membranes to determine binding capacities and recoveries. Capacities for each protein on S or Q adsorbers are listed below in mg/mL. The average recovery found here was about 95%.

Protein	pI	Sartobind® S, mg/mL	Sartobind® Q, mg/mL	Optimum pH (load)
Saccharase	4.0	–	73	4-9
BSA	4.8	–	47	5-6
Ovalbumin	5.9	–	69	6-8
Globulin	7-8	–	66	6-7
Peroxidase	7.2	86	–	4
LDH muscle	8.5	29	–	6-7
Papain	8.8	91	–	5-9
Lysozyme	10.5	73	–	7

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