APPLICATIONS

Utilizing Biphasic Salt and pH Gradients for Charge Variant Analysis

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Introduction

Cation exchange chromatography (CEX) is a favorable analytical method for characterization of proteins as it is non-denaturing and can be used to qualitatively and quantitively identify charge heterogeneity of a protein samples. CEX has particular use for monoclonal antibodies (mAbs) and is commonly used as a reference technique orthogonal to other analytical methods such as intact mass and peptide mapping. Along with capillary electrophoresis, CEX is the preferred method for QC lot release assays as it is transferable and robust.

Two mobile phase approaches are commonly used in CEX. The first is a simple salt gradient- the mobile phase composition consists of a buffer such as sodium phosphate and sodium chloride. The pH of the mobile phase is below the isoelectric point of the protein, such that the protein will have an overall net positive charge. Increasing the ionic strength of the mobile phase will displace the protein from the stationary phase. The more surface charge a protein has, the higher concentration of salt will be required to displace the protein by the increasing of competitive binding of the buffer counterions (e.g. sodium ions) and the stationary phase. This standard salt gradient approach is used in both preparative and analytical chromatography. Because CEX using salt gradients has a non-linear response to solvent strength, extensive method development can be required to optimize a salt gradient method appropriately.[1]

Alternatively, another mobile phase approach which has gained popularity in recent years is the utilization of a pH gradient, or isoelectric chromatofocusing.[2] Ionic strength of the elution buffer remains constant, and changes of pH modulate elution and proteins behave similarly to the way their isoelectric point or pI would predict. This method can more readily be implemented for so-called “platform methods,” as method parameters such as ionic strength and pH do not have to be optimized.[3]

Commonly, a preformulated buffer such as the CX-1 gradient buffers from Thermo Fisher® is used for pH gradient mobile phase. Although this is a viable approach for development, there may be some concern in implementing a preformulated buffer for applications used further downstream in development. Another approach that has been utilized in the literature in the combination of a biphasic salt and pH gradient.[4] Here, we use two Good’s buffers, MES and HEPES, along with a moderate amount of NaCl, to effectively separate charge variants of monoclonal antibodies with varying isoelectric points. Results were then compared to a pH gradient method using the CX-1 buffers.

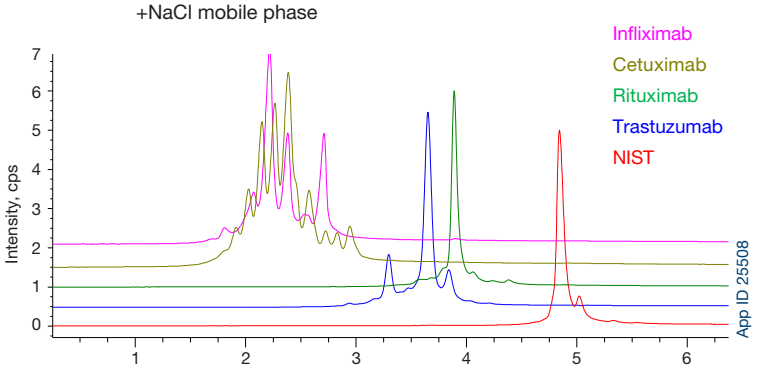
Materials and Methods:

Trastuzumab, cetuximab, rituximab and infliximab were purchased from Myoderm® (Norristown, PA, USA). NIST® mAb was purchased from National Institute of Standards and Technology (Gaithersburg, MD, USA). Thermo CX-1 gradient buffers were purchased from Thermo Fisher Scientific® (Waltham, MA, USA). All other chemicals were purchased from Sigma-Aldrich® (St Louis, MO).

Chromatographic Conditions:

Column: bioZen 6µm WCX
Dimensions: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: Method 1:
20 mM MES, pH 5.6
20 mM HEPES + 150 mM NaCl, pH 8.0
Method 2:
A: CX-1 Gradient Buffer A, pH 5.6
B: CX-1 Gradient Buffer B, pH 10.2
Gradient: 0-100**% B** in 30 minutes
Flow Rate: 30 °C
Detection: UV @ 280 nm

Figure 1. Various mAbs run using a linear gradient and MES/HEPES +NaCl mobile phase



Using a simple linear gradient from 0-100% B, the biphasic method provides good separation of various antibodies. Acidic and basic variants are clearly separated on infliximab (pI=7.6) as well as separation of basic variants on NIST mAb (pI= 9.2). Although not linear in response, elution order of the mAbs is similar to what isoelectric points would predict.

APPLICATIONS

In comparison to the CX-1 gradient buffers, the biphasic mobile phases produce similar profiles for trastuzumab. **Figure 2** shows a slight improvement in resolution between basic variants with the biphasic buffer. Additionally, overall peak heights are slightly improved. **Table 1** shows the relative peak areas of acidic and basic variants as comparable.

Figure 2. Comparison of Trastuzumab with CX-1 buffers (red) and MES/HEPES + NaCl (blue)

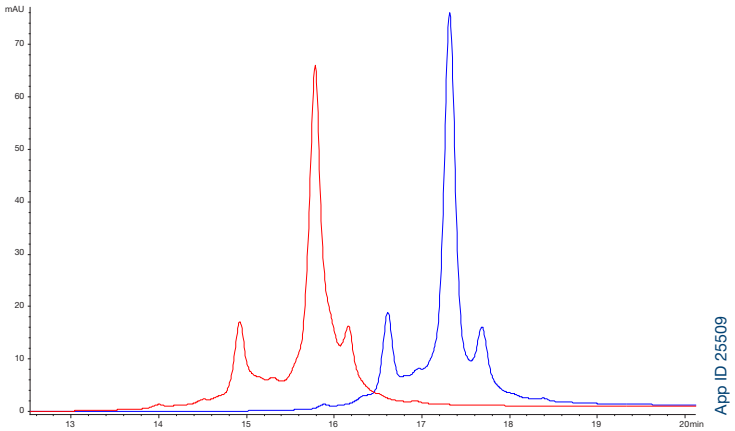


Table 1.
Comparison of Acidic and Basic Variants, Trastuzumab

Sample	Method	Acidic Variants (%Area)	Main Peak (%Area)	Acidic Variants (%Area)
Trastuzumab	CX-1 Method	23.6	62.5	13.9
	MES/HEPES + NaCl	24.5	59.0	15.9

Conclusion:

Although mobile phase for pH gradient can be made with preformulated buffer concentrates, an alternative approach is a simple biphasic salt and pH gradient. Utilizing two Good's buffer components and a moderate concentration of sodium chloride, excellent separation can be obtained for various mAbs. This biphasic approach does not require the extensive method development that a traditional salt gradient might require, while still providing a separation similar to preformulated pH gradient buffer concentrates.

References

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3. Farnan, D.; Moreno, G. T. Multiproduct High-Resolution Monoclonal Antibody Charge Variant Separations by pH Gradient Ion-Exchange Chromatography Anal. Chem. 2009, 81(21), 8846–8857. doi: 10.1021/ac901408j.
4. Patel, B. A., et al. On-Line Ion Exchange Liquid Chromatography as a Process Analytical Technology for Monoclonal Antibody Characterization in Continuous Bioprocessing Anal. Chem., 2017, 89(21), 11357–11365. doi: 10.1021/acs.analchem.7b02228.

bioZen™ Ordering Information

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
				—	—	/3pk	—	ea
bioZen 2.6 µm Glycan	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	AJ0-9800	—	AJ0-9000
				—	—	/3pk	—	ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJ0-9803	—	AJ0-9000
		—				/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJ0-7605	AJ0-7606	KJ0-4282
				—	—	/3pk	—	ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJ0-9806	—	AJ0-9000
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJ0-9806	AJ0-9808	AJ0-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJ0-9812	AJ0-9814	AJ0-9000
	50 x 4.6	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**300 x 4.6**		**for 4.6 mm**	**Holder**
	—			—		—	/3pk	ea
bioZen 1.8 µm SEC-2	—	—	00F-4769-E0	—	00H-4769-E0	—	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
					—	—	/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJ0-9400	KJ0-4282

Sample Preparation Ordering Information

Format	bioZen Solid Phase Extraction	Sorbent Mass	Part Number	Unit
Microelution 96-Well Plate				
	bioZen N-Glycan Clean-Up	5 mg/well	8M-S009-NGA	1/box

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