The Effect of Buffer Type and pH on the Capacity of ProTain™ as an In-Line Protein Removal System

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The type of buffer, specifically its strength as a Lewis base, and the pH of the mobile phase play a significant role in determining the actual protein binding capacity of the ProTain™ system inserts. In this work, a range of pH of 2 - 9, was examined with the following buffers: trifluroracetate, acetate, phosphate, and carbonate.

Introduction

The unique surface chemistry of porous zirconia allows for a strong, three faceted interaction with serum proteins[1]. Electrostatic, ligand exchange, and hydrophilic/hydrophobic interactions all play a role in the material's capacity to absorb proteins. These mole cular interactions are governed both by buffer type and the pH of the mobile phase. Control of both the mobile phase type and pH is imperative in the process of maximizing the capacity of the material. To further investigate these effects, a range of pH, from 2 to 9, was examined with the following buffers: trifluroracetate, acetate, phosphate, and carbonate. Please note that buffers were tested only at pH conditions where there was sufficient buffer capacity. Want more details about buffering capacity? Visit www.zirchrom.com to use our free buffer wizard.

Experimental

A ProTain™ 20 mm x 4.6 mm i.d. cartridge and holder was tested without an analytical column on an Agilent 1100 HPLC. After conditioning the cartridge with the desired mobile phase for 15 minutes, repeated 5 µl injections of bovine serum (protein load = 3.5 mg/ml) were made until a protein peak was observed eluting from the cartridge using UV detection. The injections were continued until the protein peak area became constant. The peak area of all of the injections was then used to back calculate how much protein had become adsorbed onto the ProTain™ Cartridge. The experimental conditions were as follows:

Column:	ProTain Cartridge/Holder, 20 mm x 4.6 mm i.d. (Part Number: PT01-0246/850-00-2)
Mobile Phase:	50/50 ACN / 20 mM Indicated buffer and pH
Temperature:	30 ºC with Metalox™ 200-C column heater
Flow Rate:	1 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 280 nm

Figure 1 shows that the combination of acetate or phosphate and pH conditions between 5 and 7 result in the highest protein capacity. The maximum protein capacity for a 20 mm x 4.6 mm i.d. ProTain™ cartridge, under the conditions tested, was 5 mg.

		Buffer Type			
		TFA	*Acetate*	*Phosphate*	*Carbonate*
Mobile Phase pH	*2*	+		++	
	3		+	++	
	5		+++	+++	
	7		+++	+++	+
	9		+	+	+

Figure 1: Loadability Matrix for the ProTain Media Inserts

Capacity: + = 0 – 0.2 mg
++ = 0.2 – 1.0 mg
+++ = 1.0 – 5.0 mg
Black Areas: Not tested due to lack of buffer capacity at pH condition.

ZirChrom's ProTain™ system can be incorporated in front of any type of analytical column to offer a selective, cost effective, and simple method of reducing matrix interferences for the HPLC analysis of small molecules in bio-samples. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for further details.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

References

1) Sun, L.; Carr, P. W. *Analytical Chemistry* **1995**, *67*, 2517-2523

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