

## BIOshell Fused-Core Columns for Ultra Efficient RPLC of Proteins and Peptides: Analysis of IgG fragments using a middle-down approach

Roy Eksteen and Hillel Brandes

### Abstract

Columns packed with fully porous particles have long been considered 'normal science' for HPLC practitioners. Since 2006, Fused-Core® or core-shell columns have caused a paradigm shift in the minds of liquid chromatographers who analyze small molecules (1). The benefits of porous-shell columns are now also available to scientists who are tasked to advance our knowledge about peptides, proteins and other biopolymers.

### Introduction

Fused-Core HPLC columns are based on the pellicular silica technology developed by J. J. Kirkland at E. I. du Pont de Nemours and Company in the late 1960s during the formative years of HPLC, and applied in this millennium to create ultra-efficient particles in the range of 2.7 to 5 micron (2). The latest innovations derived from this technology are now available as BIOshell™ Fused-Core Reversed Phase columns for biopolymer separations, featuring 2.7 and 5 micron packed C18 and CN columns for peptides and 3.4 micron packed C4 columns for high efficiency protein separations.

### Discussion

In 2007 Supelco introduced the groundbreaking Ascentis® Express Fused-Core columns. Like the particles in Ascentis Express columns, the particles in BIOshell columns are composed of a spherical solid glass core surrounded by a thin layer of nano-sized silica particles. Table 1 summarizes the characteristics and maximum operating conditions of the new BIOshell porous shell columns.

As shown in Table 1, to accommodate the larger size of biopolymers, the particles in BIOshell columns either have a 160 Å pore size suitable for peptides up to mass of about 20 kDa or a 400 Å pore size to provide unrestricted access to proteins with a molecular mass up to about 500 kDa. Practical operating guidelines, such as the ability to reliably operate BIOshell columns at very high temperature are also listed in Table 1. Like Ascentis Express columns for small molecular weight analysis, BIOshell and other columns packed with Fused-Core particles show significantly higher efficiency than columns packed with fully porous particles (3).

The data in Figure 1 (next page) demonstrates how BIOshell columns support life science researchers using a so-called middle-down, as opposed to a top-down or bottom-up approach to analyze the structure of a monoclonal antibody. In a middle-down approach IgG is fragmented into a few large fragments prior to further characterization. The novel proteolytic enzyme IdeS, commercially available as FabRICATOR®, cleaves an antibody into F(ab')<sub>2</sub> and scFc fragments, which have approximate molecular masses of 100 kDa and 25 kDa respectively. Using disulfide reduction the F(ab')<sub>2</sub> fragment can be further resolved into its light-chain (LC) and F(ab) components. Each of the components in the resulting mixture has a molecular mass of about 25 kDa.

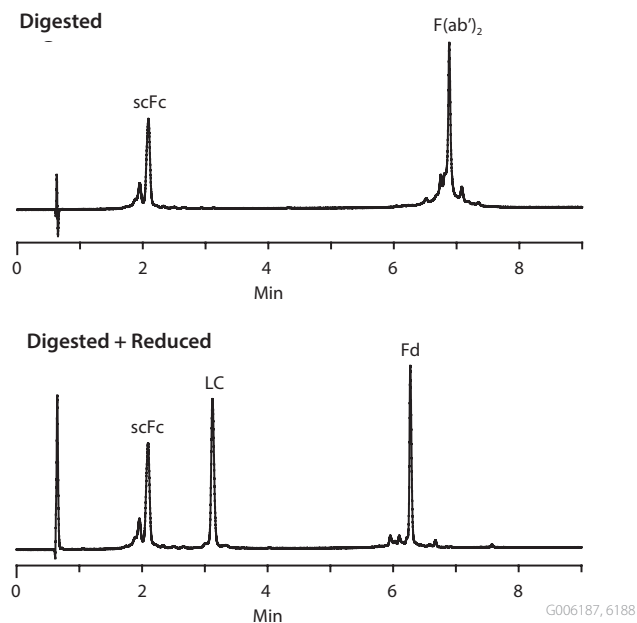
The top chromatogram in Figure 1 shows the simple reversed phase separation of the F(ab')<sub>2</sub> and scFc fragments following the digestion of IgG by IdeS, while the bottom trace shows the results following the reduction of F(ab')<sub>2</sub> into its LC and F(ab) fragments.

Table 1. Characteristics and Operating Conditions of BIOshell Core-Shell Columns

BIOshell Product Line	Nominal Values						Phase Characteristics and Operating Conditions					
Physical Parameter	dp (µm)	Core (µm)	Shell (µm)	Pore Size (Å)	SBET (m <sup>2</sup> /g)	Capacity vs. Porous	Bonded Phase Ligand	End Capped	Tmax °C	pH Range	Pmax (bar)	Frit (µm)
2.7 µm A160 Peptide C18	2.7	1.7	0.5	160	80	75%	di-isobutyl-octadecylsilane	No	100	1 - 8	600	2
2.7 µm A160 Peptide CN	2.7	1.7	0.5	160	80	75%	di-isopropyl-cyanopropylsilane	Yes	80	1 - 8	600	2
5 µm A160 Peptide C18	4.7	3.5	0.6	160	60	59%	di-isobutyl-octadecylsilane	No	100	1 - 8	600	2
5 µm A160 Peptide CN	4.7	3.5	0.6	160	60	59%	di-isopropyl-cyanopropylsilane	Yes	90	1 - 8	600	2
3.4 µm A400 Protein C4	3.4	3.0	0.2	400	15	31%	dimethylbutylsilane	Yes	90	2 - 9	600	2

**Figure 1. Analysis of antibody fragments after IdeS digestion followed by disulfide reduction**

column: BIOshell A400 Protein C4, 10 cm x 2.1 mm I.D., 3.4  $\mu$ m  
 mobile phase: (A) 80:20, (water, 0.1% TFA):(acetonitrile, 0.1% TFA);  
 (B) 50:50, (water, 0.1% TFA):(acetonitrile, 0.1% TFA)  
 flow rate: 0.3 mL/min  
 temp.: 90 °C  
 det.: UV at 215 nm  
 inj.: 1  $\mu$ L (sample dilution in mobile phase A)  
 gradient: 30 to 70% B in 12 min



#### References

1. T.S. Kuhn, The Structure of Scientific Revolutions, Univ. of Chicago Press, LCCN 62019621.
2. J.J. Kirkland, Superficially Porous Supports for Chromatography, US Patent 3505785.
3. S. Fekete et al., J. Chrom. A, 1236 (2012) 177.

#### Featured Products

Pore Size Å	Particle Size ( $\mu$ m)	I.D. (mm)	L (cm)	C4	C18	CN
<b>BIOshell Fused-Core HPLC Columns</b>						
400	3.4	2.1	5	66824-U	—	—
400	3.4	2.1	10	66825-U	—	—
400	3.4	2.1	15	66826-U	—	—
400	3.4	4.6	5	66827-U	—	—
400	3.4	4.6	10	66828-U	—	—
400	3.4	4.6	15	66829-U	—	—
160	2.7	2.1	3	—	66901-U	66965-U
160	2.7	2.1	5	—	66902-U	66966-U
160	2.7	2.1	7.5	—	66903-U	66967-U
160	2.7	2.1	10	—	66904-U	66968-U
160	2.7	2.1	15	—	66905-U	66969-U
160	2.7	3.0	3	—	66906-U	66970-U
160	2.7	3.0	5	—	66907-U	66971-U
160	2.7	3.0	10	—	66908-U	66972-U
160	2.7	3.0	15	—	66909-U	66973-U
160	2.7	4.6	5	—	66913-U	66974-U
160	2.7	4.6	10	—	66915-U	66975-U
160	2.7	4.6	15	—	66917-U	66976-U
160	5	2.1	3	—	67001-U	67061-U
160	5	2.1	5	—	67002-U	67062-U
160	5	2.1	7.5	—	67003-U	67063-U
160	5	2.1	10	—	67004-U	67064-U
160	5	2.1	15	—	67006-U	67065-U
160	5	3.0	3	—	67007-U	67066-U
160	5	3.0	5	—	67008-U	67067-U
160	5	3.0	10	—	67011-U	67068-U
160	5	3.0	15	—	67012-U	67069-U
160	5	4.6	5	—	67013-U	67071-U
160	5	4.6	10	—	67014-U	67080-U
160	5	4.6	15	—	67015-U	67081-U
<b>BIOshell Guard Cartridges</b>						
400	3.4	2.1	3	66830-U	—	—
400	3.4	4.6	3	66831-U	—	—
160	2.7	2.1	3	—	66918-U	66977-U
160	2.7	3.0	3	—	66919-U	66978-U
160	2.7	4.6	3	—	66921-U	66979-U
160	5	2.1	3	—	67016-U	67082-U
160	5	3.0	3	—	67017-U	67083-U
160	5	4.6	3	—	67018-U	67084-U

#### Universal Guard Holder

Description	Cat No.
BIOshell Guard Cartridge Holder (cartridge not included with holder)	66841-U

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