

# TheReporter

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# Improved Gel Filtration of Proteins: Biocompatible SigmaChrom™ GFC Columns

M. Hommer, N. Lai, K. Pardue

New high performance SigmaChrom GFC-100 and GFC-1300 gel filtration columns are well suited for separating and isolating peptides and proteins, in HPLC or FPLC systems. The two columns span an optimal separation range of 3000 to 600,000 dalton, with exclusion limits of ~100,000 dalton and ~1,300,000 dalton, respectively, for globular proteins. Constructed of inert polyetheretherketone polymer and filled with a polysaccharide-based packing, the biocompatible columns are excellent alternatives to silica-based stainless steel gel filtration columns. Typical applications and calibration curves for globular proteins are shown.

**Table 1. Characteristics of SigmaChrom GFC Columns**

Packing:	crosslinked polysaccharide, 12-15µm
Column:	polyetheretherketone
Column Dimensions:	30cm x 7.5mm
Plates/Meter:	>30,000
Peak Symmetry:	0.7-1.3
Empty Column Volume:	13.25mL
Flow Rate:	typical: 0.5mL/min maximum: 1.0mL/min
Maximum Back Pressure:	250psi (1.72MPa)
pH:	3-12
Maximum Protein Capacity:	5.5mg
Optimal Separation Range:	GFC-100: 3-70 x 10 <sup>3</sup> dalton GFC-1300: 10-600 x 10 <sup>3</sup> dalton
Protein Exclusion Limit:	GFC-100: 100 x 10 <sup>3</sup> dalton GFC-1300: 1300 x 10 <sup>3</sup> dalton
Typical Recovery:	Mass: >90% Enzyme Activity: >80%
Typical Separation Time:	15-30 min

In gel filtration (GFC) and gel permeation (GPC) chromatography (better known as size exclusion chromatography), the *fractionation range* – the molecular weight range of the largest molecule that is fully included by the pores in the packing particles to the smallest molecule that is fully excluded, for a particular analyte type and shape (e.g., globular proteins) – is a function of the pore size in the particles. Within the fractionation range, *peak resolution* is determined by the pore volume – the greater the pore volume, the more proteins of similar molecular weight that can be resolved. In new, high performance SigmaChrom GFC-100 and SigmaChrom GFC-1300 columns, the mobile phase occupies ~85% of the column volume, and interparticle porosity is ~35%. Thus, the pores occupy half the total column volume. Additional characteristics of these columns are summarized in Table 1.

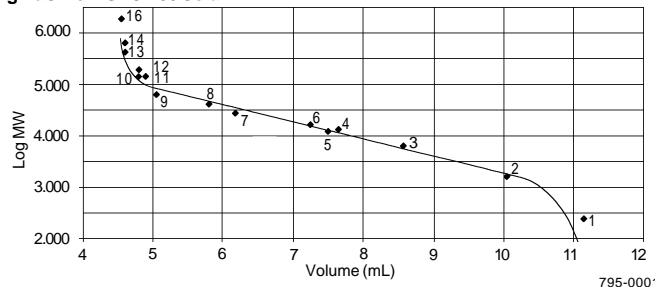
**Figure A. Molecular Weight Calibration Curves for SigmaChrom GFC Columns (Globular Proteins)**

Columns: 30cm x 7.5mm, 12-15µm particles  
 Cat. Nos.: 54750-U (GFC-100), 54751 (GFC-1300)  
 Mobile Phase: 50mM Tris-HCl/100mM KCl, pH 7.5  
 Flow Rate: 0.5mL/min  
 Det.: UV, 280nm  
 Inj.: 20µL, 1-10mg/mL each analyte

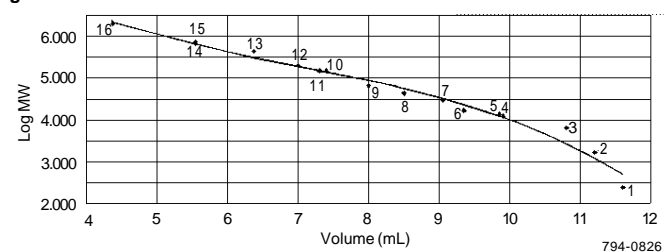
Molecule	MW	Molecule	MW
1. Cytidine	243	9. Albumin	66,000
2. Neurotensin	1673	10. Alcohol dehydrogenase	150,000
3. Aprotinin	6500	11. γ-Globulins	150,000
4. Cytochrome c	12,400	12. β-Amylase	200,000
5. Ribonuclease A	13,690	13. Apoferritin	443,000
6. Myoglobin	16,900	14. Thyroglobulin	669,000
7. Carbonic anhydrase	29,000	15. α <sub>2</sub> -Macroglobulin*	750,000
8. Ovalbumin	43,500	16. Blue dextran	2,000,000

\*GFC-1300 column only

**SigmaChrom GFC-100 Column**



**SigmaChrom GFC-1300 Column**



A sigmoidal calibration curve is obtained by plotting the logarithm of molecular weight (MW) versus the elution volume (Ve) for molecules of known weight (Figure A). The optimal separation range is defined by the linear portion of the curve. By using the curve, the elution volume for a protein of similar shape, but unknown weight, can be used to determine MW. The calibration curves in Figure A demonstrate the excellent separation characteristics of the SigmaChrom GFC columns – a function of their very large pore volumes. The large pores and large pore volume in the GFC-1300 column allow the separation of medium

to large proteins. The linear portion of the curve extends from cytochrome c to thyroglobulin. The capability for separating a very broad MW range of proteins makes the GFC-1300 column the preferred general purpose column, providing best results for large proteins. The smaller pores in the GFC-100 column provide optimum resolution for large peptides and small proteins, from about 3000 to 70,000 dalton. Note that despite similar weights, cytochrome c, ribonuclease A, and myoglobin elute in the expected order in the middle of the linear portion of the curve.

All commercially available GFC supports show some deviation from ideal size exclusion behavior, but ionic and/or hydrophobic secondary interactions can be overcome with the proper mobile phase conditions. For the SigmaChrom GFC columns, plots of distribution coefficient versus mobile phase ionic strength indicate that a minimum ionic strength of 0.120 (e.g., 0.05M potassium phosphate) is required to prevent ionic interactions between analytes and packing particles. Hydrophobic interactions with small molecules can be overcome by adding alcohol to the mobile phase. Secondary interactions on gel filtration columns are discussed in Bulletin 891 (available on request).

Ideal size exclusion chromatography is critical for accurate determination of molecular weight, but secondary interactions can be helpful in isolating and/or purifying analytes (1). Figure B shows that, by using a mobile phase of low ionic strength, secondary interactions are used to separate two analytes of similar weight, NADP (MW 765) and NAD (MW 663). The negatively charged molecules are repelled from the matrix, and are separated by differences in their hydrophobicity. Under ideal SEC conditions, these analytes would have eluted from the column in the inclusion volume (~11.6mL) and would not have been separated. Note that under the conditions in Figure B other compounds are retained on the column past the inclusion volume, due to hydrophobic interactions with the support.

We also have used a SigmaChrom GFC-1300 column in a semi-preparative isolation of cytochrome c peroxidase, traditionally isolated from bakers' yeast by low pressure techniques. Relative to the low pressure protocol, the procedure shown in Figure C is faster and eliminates several steps. The procedure is described in detail in Bulletin 883 (available on request).

Biocompatible SigmaChrom GFC-100 and GFC-1300 columns are excellent alternatives to silica-based stainless steel gel filtration columns, for separating or isolating a wide range of biomolecules in HPLC and FPLC® systems. Each lot of packing is tested to ensure quality, and each column is individually tested for efficiency and peak symmetry.

#### Reference

- Mant, C.T. and R.S. Hodges (Eds.), *High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation* CRC Press, Boca Raton, FL (1991) p 125.

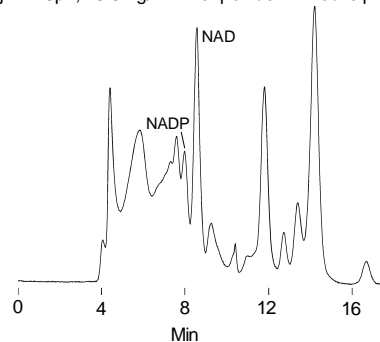
Reference not available from Supelco.

#### Trademarks

FPLC, Sepharose – Amersham Pharmacia Biotech  
SigmaChrom – Sigma-Aldrich Co.

### Figure B. GFC with Secondary Interactions Resolves NAD from NADP

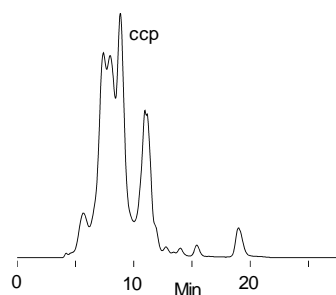
Column: **SigmaChrom GFC-1300, 30cm x 7.5mm, 12-15µm particles**  
Cat. No.: **54751**  
Mobile Phase: 0.001M potassium phosphate/  
0.002M sodium phosphate, pH 7.2  
Flow Rate: 1mL/min  
Det.: UV, 280nm  
Inj.: 10µL, 13.3mg/mL liver powder in mobile phase



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### Figure C. Semi-Preparative Isolation of Cytochrome c Peroxidase by GFC

Column: **SigmaChrom GFC-1300, 30cm x 7.5mm, 12-15µm particles**  
Cat. No.: **54751**  
Mobile Phase: 50mM Tris-HCl/100mM KCl, pH 7.2  
Flow Rate: 1mL/min  
Det.: UV, 280nm  
Inj.: 100µL concentrated crude ccp preparation from DEAE Sepharose® CL-6B



794-0503

### Ordering Information:

SigmaChrom GFC-100 Column	<b>54750-U</b>
SigmaChrom GFC-1300 Column	<b>54751</b>
Replacement Frits, pk. of 2	<b>54770</b>
GFC-100 Top-Off Gel, 1mL	<b>custom</b>
GFC-1300 Top-Off Gel, 1mL	<b>custom</b>
<b>Molecular Weight Standards Kits</b>	
1 vial each of 5-7 proteins in MW range indicated.	
MW 6500-66,000	<b>MWGF70-1KT</b>
MW 12,000-200,000	<b>MWGF200-1KT</b>
MW 29,000-700,000	<b>MWGF1000-1KT</b>

For descriptions of kits and individual molecular weight standards, refer to the Supelco catalog.