

# Separation techniques (Proteins)

## Monolithic support Chromatography

### Monolithic support

Biochromatography on Convective Interaction Media

Convective Interaction Media (CIM®) are evolutionary chromatographic and bioconversion supports based on a highly cross-linked porous monolithic polymer, offering exceptional chemical stability and flow characteristics. They are available in different forms and chemistries, making them suitable for ion-exchange, hydrophobic interaction, reversed phase, affinity chromatography and bioconversions on an analytical and preparative scale.

- ◆ Faster separations
- ◆ High volumetric throughput
- ◆ High capacity for very large biomolecules
- ◆ Low back pressure

Characteristics :

CIM® Matrix : poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) or poly(styrene-co-divinyl benzene)

Number of Disks per housing : up to 4

Operating Pressure : up to 50 bar (5Mpa)

Operating temperature : up to 50°C (122°F)

pH : 1-14

Disk dimensions : diameter 16 mm ; thickness 3 mm ; active bed volume 0.34 ml

Solvents : 0-100% of common polar solvents

Ionic strength : 0-8 M, all common buffers and salt

### CIM® Disk monolithic column

The CIM® Disk Monolithic Column consists of a CIM® Disk in a specially designed housing. The CIM® housing provides low dead volume, excellent sample distribution, simple handling, and can be easily connected to any LC/HPLC or FIA system. The CIM® Disk consist of a CIM® matrix and a non-porous, self-sealing fitting ring which ensures only axial flow through the disk and prevents any sample and mobile phase leakage or by-pass. The CIM® matrix has a well defined bimodal pore size distribution providing excellent separations at low back pressures.

- ◆ Very fast analyzes
- ◆ Flow-unaffected high dynamic binding capacity
- ◆ Chemically and mechanically stable
- ◆ Up to 1000 analyzes

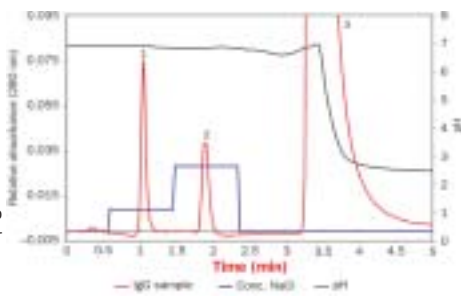
Operating flow rates : up to 10 mL/min (Recommended flow rate : 5 mL/min)



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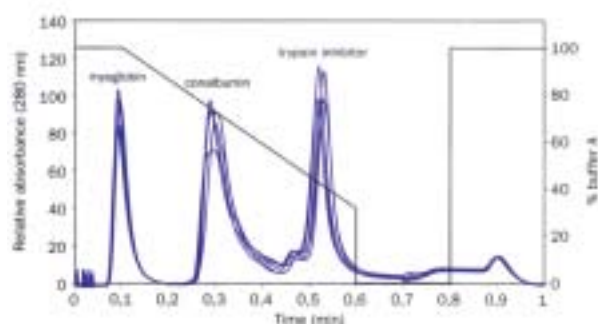
### Separation of immunoglobulin (IgG) sample on CIM® QA disks

Binding buffer : 20 mM Tris-HCl pH 7.0  
Elution buffer 1 : 20 mM Tris-HCl, 1M NaCl, pH 7.0  
Elution buffer 2 : 0.1 M Glycine-HCl pH 2.0  
Flow rate : 4 ml/min  
Sample concentration : 16 mg IgG/ml of binding buffer  
Injection volume : 250 µl  
Peak 1 : Transferrin, peak 2 : Albumin, Peak 3 : IgGs



Courtesy of Dr. Andrea Buchacher and Dr Karmen Branovic, Octapharma, Austria

### Separation of a test protein mixture (180 to more than a 1000 injections)



Key features :

- ◆ Easily exchangeable : a disk is easily replaced with an other one in a minute
- ◆ Adjustable column length : working on a laboratory scale has never been so easy ! simply place several disks in a single housing and the capacity will increase accordingly
- ◆ Conjoint Liquid Chromatography (CLC) : the more important advantage : you can combine several kind of separation in one single column (Affinity with ion exchange for instance).

### CIM® Tube monolithic column

CIM® Tube Monolithic Columns are designed for laboratory and industrial scale purification and bioconversion. It consist of a CIM® Tube modified with the appropriate active group(s) seated in a specially constructed tubular housing. The housing is made of stainless steel and provides low dead volume, excellent sample distribution, simple handling and it can be easily connected to any LC/HPLC or FIA system. The design of the housing forces liquid to flow in a radial direction from the outer surface of the CIM® Tube, through the porous tube wall where it is then collected in the hollow center of the tube.

Operating flow rates : up to 40 mL/min for 8 mL tubes, up to 250 mL/min for 80 mL tubes, up to 2 L/min for 800 mL tubes

Key features :

- ◆ Fast large scale separations of biomolecules
- ◆ High flow unaffected dynamic binding capacity :
  - Up to 200 mg H SA on 8 ml CIM® Tubes
  - Up to 2 g on 80 ml CIM® Tubes
  - Up to 20 g on 800 ml CIM® Tubes
- ◆ Ideal for preparative purifications : tubes are made on the same material as the analytical CIM® Disks. Thus, the purification can be performed on the same type of support by applying similar separation conditions
- ◆ Conjoint Liquid Chromatography : due to its unique tube in a tube design, it is possible to construct CLC Tube Monolithic Columns for your large scale multidimensional chromatographic purifications

### Fast linear gradient separation of a mixture of three test proteins on an 80 ml CIM™ DEAE tube Monolithic column

Conditions

Buffer A : 20 mM Tris-HCl buffer, pH 7.4

Buffer B : 20 mM Tris-HCl buffer + 1 M NaCl, pH 7.4

Flow rate : 160 ml/min

Gradient : 0-70% buffer

B in 37 s

Detection :

UV at 280 nm

Sample

1. Myoglobin

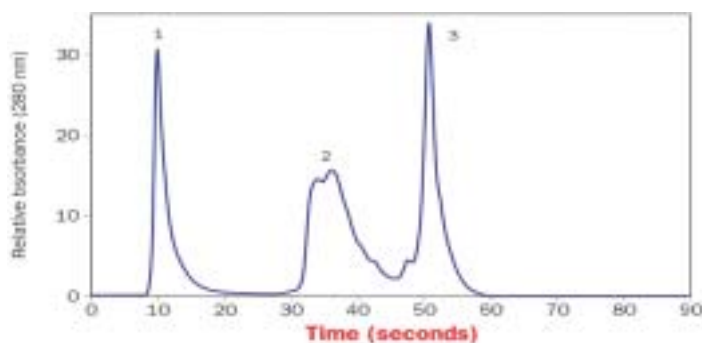
2. Conalbumin

3. Soybean Trypsin

Inhibitor

Injection volume : 1000

µl

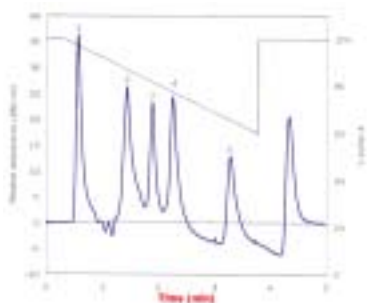


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### Conjoint liquid Chromatography

(combination of a weak anion and strong cation CIMGEL™ support in the same column) separation of a mixture of 5 proteins



Mobile Phase :

Buffer A : 20 mM Tris-HCl buffer, pH 8.0

Buffer B : 20 mM Tris -HCl buffer + 2 M NaCl

Column Amersham Pharmacia XK™ 16 column

Column packed with : 2 CIMGEL™ DEAE-16 and 2 CIMGEL™ SO3-16

Flow rate : 3 ml/min

Gradient : 0-40 % buffer B in 3.5 min

Detection : UV at 280 nm

Injection volume : 20 ml

Sample : 1. Myoglobin (0.6 mg/ml)

2. Conalbumin (1.5 mg/ml)

3. Cytochrome C (1.0 mg/ml)

4. Soybean Trypsin inhibitor (2.0 mg/ml)

5. Lysozyme (1.0 mg/ml)

### CIMGEL® monolithic column

CIMGEL® Monolithic support were developed to improve the performance and simplify the packing and repacking of standard liquid chromatography columns. You no longer need to be concerned with air bubbles during column and sample preparation. It consists of a CIM® matrix and a non-porous, self-sealing fitting ring which ensures only axial flow through the disk and prevents any sample and mobile phase leakage or by-pass. The CIMGEL® matrix has a well defined bimodal pore size distribution providing excellent separations and low back pressure. It comes in a 16 mm and a 26 mm size.

Characteristics :

CIM® Matrix : poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate)

Maximum number of supports : 5 per column

pH : 1-14

Dimensions : 16 mm : diameter 16 mm ; thickness 3.1 mm ; active bed volume 0.34 ml

26 mm : diameter 26 mm ; thickness 6.2 mm ; active bed volume 2.0 ml

Solvents : 0-100% of common polar solvents

Ionic strength : 0-8 M, all common buffers and salt

Key features :

- ◆ Designed for 16 mm and 26 mm ID columns
- ◆ Extremely easy column packing and repacking
- ◆ No more air bubbles
- ◆ Conjoint Liquid Chromatography
- ◆ No Shrinking/swelling while working with different mobile phases

### CIM® Disk monolithic Column

Description	Cat.#
Quaternary amine (CIM® QA)	210.5113
Diethylamine (CIM® DEAE)	210.5114
Ethylenediamine (CIM® EDA)	210.5116
Ethyl (CIM® C2)	210.8130
Butyl (CIM® C4)	210.8135
Sulfonyl (CIM® SO3)	211.6157
Carboxymethyl (CIM® CM)	211.6170
Reversed Phase (CIM® RP-SDVB)	220.2000
Epoxy (CIM® Epoxy)	213.7175
Protein A, low ligand density (CIM® Protein A LLD)	217.1001
Protein A, high ligand density (CIM® Protein A HLD)	217.1002
Protein G (CIM® Protein G)	217.1011
Special affinities	217.1201
Immobilized enzymes	217.1256

### CIM® Disk "Scale Up"

4 CIM® QA Disks (210.5113)	245.5113
4 CIM® DEAE Disks (210.5114)	245.5114
4 CIM® EDA Disks (210.5116)	245.5116
4 CIM® C2 Disks (210.8130)	245.8130
4 CIM® C4 Disks (210.8135)	245.8135
4 CIM® SO3 Disks (211.6157)	245.6157
4 CIM® CM Disks (211.6170)	245.6170
4 CIM® RP-SDVB Disks (220.2000)	245.2000
4 CIM® Epoxy Disks (213.7175)	245.7175
4 CIM® Protein A LLD Disks (210.1001)	245.1001
4 CIM® Protein A HLD Disks (210.1002)	245.1002

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### Housing and Accessories for CIM® Disks

Complete housing for CIM® disks*	222.0830
Retaining fitting with distributor	222.0831
Housing cylinder	222.0832
Screw cap	222.0834

\*To be used at moderate temperatures up to 50°C and with aqueous mobile phases (buffers).

Complete housing for CIM® disks, **PEEK Type	222.0850
Retaining fitting with distributor, **PEEK Type	222.0851
Housing cylinder, **PEEK Type	222.0852
Screw cap, **PEEK Type	222.0854

\*\*PEEK type, is autoclavable and compatible with most organic solvents!

Blind fitting	222.0837
CIM® disk extractor	222.0901
Biocompatible, extra-low dead volume mixing tee (strongly recommended for fast gradient mixing)	231.0850
PEEK In-line high-pressure filter	231.0851
Replacement frits for In-line filter	231.0852
Replacement O-rings for retaining fitting	231.0853

### CIM® 8 ml Tube Monolithic Columns

Quaternary amine 8 ml tube monolithic column (CIM® QA-8)	410.5113
Diethylamine 8 ml tube monolithic column (CIM® DEAE-8)	410.5114
Ethylenediamine 8 ml tube monolithic column (CIM® EDA-8)	410.5116
Ethyl 8 ml tube monolithic column (CIM® C2-8)	410.8130
Butyl 8 ml tube monolithic column (CIM® C4-8)	410.8135
Sulfonyl 8 ml tube monolithic column (CIM® SO3-8)	411.6157
Carboxymethyl 8 ml tube monolithic column (CIM® CM-8)	411.6170
Reversed Phase 8 ml tube monolithic column (CIM® RP-SDVB-8)-PEEK Housing	420.2000
Epoxy 8 ml tube monolithic column (CIM® Epoxy-8)	413.7175
Protein A, low ligand density 8 ml tube monolithic column (CIM® Protein A-8 LLD)	417.1001
Protein A, high ligand density 8 ml tube monolithic column (CIM® Protein A-8 HLD)	417.1002
Protein G, 8 ml tube monolithic column (CIM® Protein G-8)	417.1011
Special affinities 8 ml tube monolithic column	417.1201
Immobilized enzymes 8 ml tube monolithic column	417.1256

### CIM® 80 ml Tube Monolithic Columns

Quaternary amine 80 ml tube monolithic column (CIM® QA-80)	610.5113
Diethylamine 80 ml tube monolithic column (CIM® DEAE-80)	610.5114
Sulfonyl 80 ml tube monolithic column (CIM® SO3-80)	611.6157
Epoxy 80 ml tube monolithic column (CIM® Epoxy-80)	613.7175

\*80 ml tube are packed in a stainless steel housing of the medical grade quality and specially designed for good sample distribution

### CIM® 800 ml Tube Monolithic Columns

Diethylamine 800 ml tube monolithic column (CIM® DEAE-800)	810.5114
Epoxy 800 ml tube monolithic column (CIM® Epoxy-800)	813.7175

\*800 ml tube are packed in a stainless steel housing of the medical grade quality and specially designed for good sample distribution

### CIMGEL™ Monolithic Units ("bulk monoliths")

Diethylamine CIMGEL™ (CIMGEL™ DEAE-16) ; 0.34 ml	210.5114-16
Diethylamine CIMGEL™ (CIMGEL™ DEAE-26) ; 2 ml	210.5114-26
Sulfonyl CIMGEL™ (CIMGEL™ SO3-16) ; 0,34 ml	211.6157-16
Sulfonyl CIMGEL™ (CIMGEL™ SO3-26) ; 2 ml	211.6157-26
Epoxy CIMGEL™ (CIMGEL™ Epoxy -16) ; 0.34 ml	213.7175-16
Epoxy CIMGEL™ (CIMGEL™ Epoxy -26) ; 2 ml	213.7175-26

### CIMGEL™ "Scale-Up"

Diethylamine CIMGEL™ (CIMGEL™ DEAE-16) ; 1 ml	210.5114-16.3
Diethylamine CIMGEL™ (CIMGEL™ DEAE-26) ; 10 ml	210.5114-26.5
Sulfonyl CIMGEL™ (CIMGEL™ SO3-16) ; 1 ml	211.6157-16.3
Sulfonyl CIMGEL™ (CIMGEL™ SO3-26) ; 10 ml	211.6157-26.5
Epoxy CIMGEL™ (CIMGEL™ Epoxy -16) ; 1 ml	213.7175-16.3
Epoxy CIMGEL™ (CIMGEL™ Epoxy -26) ; 10 ml	213.7175-26.5

Column packing kit for 16 mm ID columns (consisting of two 13x2.00 O-rings and one disk extractor tool)	200.1001
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Column packing kit for 26 mm ID columns (consisting of two 24x2.00 O-rings and one disk extractor tool)	200.1010
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