



CIM Convective Interaction Media[®]
Liquid Chromatography Redesigned[™]



Rapid Chromatography
for Large Biomolecules

From Laboratory
to Industrial Scale!



The Company: BIA Separations

Experienced and Committed

What do major pharmaceutical companies and leading research institutions have in common? They have found that BIA Separations has reliable products and services that meet the research, production, and quality assurance needs of the biotechnology industry.

With more than 10 years of experience with our CIM Convective Interaction Media® platform technology and other chromatographic techniques, we have the experience to provide our customers the most reproducible and innovative solutions to meet their current and future separation needs.

Quality is Important

As an ISO 9001:2000 Certified Company, we are committed to pursuing the highest quality standards in our research, development, production, and customer service efforts. To facilitate the approval of our industrial partners purification processes, we have proactively obtained Drug Master File numbers for our strategic chemistries and will file them for our other cGMP CIM® monolithic columns in the future.

Solution Oriented

It is our goal to find the best solutions for our customers purification challenges. To facilitate this, we apply our experience with CIM® columns and other chromatographic techniques to design the ideal industrial purification process. Additionally, we use our expertise in liquid chromatography to develop and validate analytical methods. Our combined experience and expertise allows us to provide customized and comprehensive technical support.

Your Industrial Partner

BIA Separations provides products, technology, and experience to enable pharmaceutical and biotech companies to develop proprietary products and production methods. Our partnership leads to increased productivity and efficiency in drug discovery and the production of viruses, plasmids, and proteins.

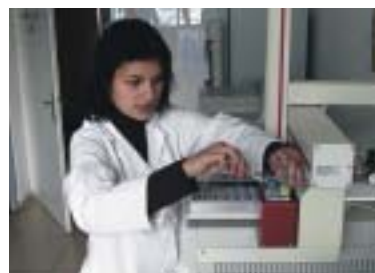
The Company is committed to chromatographic products of consistent and uniform quality in compliance with current Good Manufacturing Practice (cGMP) standards, the latest requirements of the regulatory authorities, and ISO standards.

State of the art solution

Because of their collaboration with BIA Separations, Boehringer Ingelheim Austria GmbH received the *2004 Frost & Sullivan Technology Leadership Award* for their innovative pDNA manufacturing process. The award committee stated: *"The company is leading technology providers in the race to capitalise on the huge demand for new therapeutics by investing in an area which has received scant attention – technology innovation in downstream processing."* The key to the pDNA downstream processing is our proprietary CIM® monolithic column platform technology.

Find out more!

Visit us at www.monoliths.com or contact us at sales@biaseparations.com to learn how BIA Separations can help you improve your efficiency in downstream processing and identify solutions for your purification challenges.



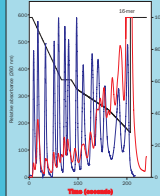
The Technology: CIM Convective Interaction Media®

Innovation

CIM® monolithic columns are an innovative, but well proven chromatographic media that will redefine your biomolecule purification at any scale; laboratory or industrial ... Operating at flow rates up to 10 times higher than comparable particle based supports, CIM® monolithic supports will ensure that your purification times and costs are significantly decreased. The fact that resolution is not compromised offers a significant advantage over other high-throughput media like membrane adsorbers.

Application

With the pore size adjusted to accommodate even the largest molecules and optimized for very high binding capacities at the highest flow rates, CIM® monolithic columns are an ideal chromatographic support for purifying your large biomolecules and nanoparticles like viruses and plasmid DNA.

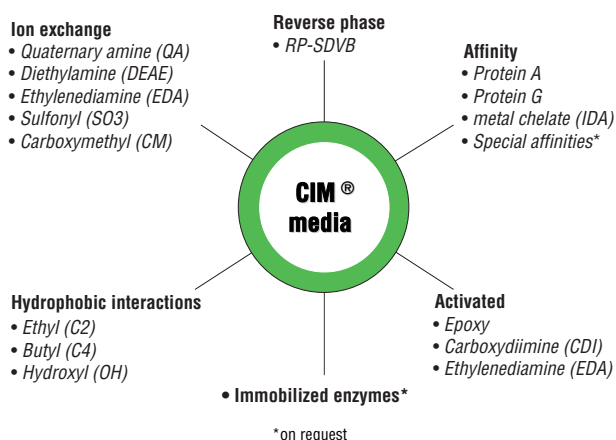
Speed	Capacity	Resolution	Simplicity
<p>Flow rates up to 10 times higher than particle based supports ...</p> <p>... acceptable pressure drops ...</p> <p>... resolution and binding capacity are not compromised!</p>	<p>High dynamic binding even at the highest flow rates!</p> <p>Up to 10¹⁴ virus particles/ml</p> <p>10 mg of pDNA/ml</p> <p>>10 mg of IgM/ml</p>	<p>Can your preparative column perform high resolution separations in 4 minutes?</p> 	<p>Assemble and connect the column in less than a minute!</p> <p>Air bubbles are no longer a worry!</p> <p>Simple linear scale up</p> <p>Conditioning within a minute</p> <p>Ability to combine different chemistries in a single column</p>

Unifying technology

Are you searching for a unifying technology for your vaccine and gene therapy targets? One that can be applied across all departments, saves time and money, while increasing productivity by an order of magnitude? CIM® monolithic chromatographic supports are the platform technology that achieves these goals by speeding up your process development, production, and quality assurance analysis.

All of the popular chemistries

CIM® monolithic columns are supplied in a number of different chemistries ... ion exchangers (strong and weak); weak hydrophobic interaction media; the most popular immunoaffinity ligands; metal affinity (IMAC) applications; to top things off, monoliths with functionalized groups for further ligand immobilization.

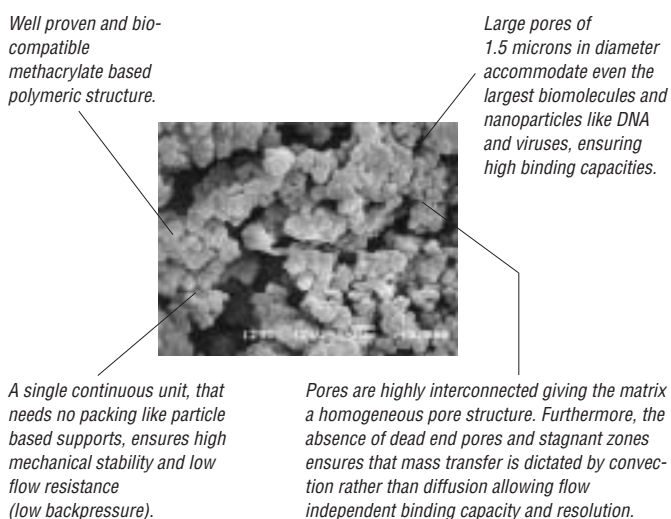


Structural characteristics

CIM® monoliths are continuous stationary phases that are cast as a single homogeneous piece that can be prepared in various dimensions. The matrix is composed of standard methacrylate polymers with a well proven track record in biochromatography.

With highly interconnected flow-through pores, the binding sites are easily accessible to the target molecules in the mobile phase so your binding (or resolution) does not depend upon the speed that the molecules travel through the media.

Furthermore, the pore size of CIM® monolithic supports has been adjusted to accommodate even the largest molecules (like viruses and DNA) without compromising the mechanical stability of the support and are optimized for very high binding capacities at the highest flow rates.



Rapid Method Development & Analytics

with Convective Interaction Media®



CIM® disk monolithic columns

CIM® disk monolithic columns are designed to increase your productivity in the laboratory by speeding up method development, analytics, in-process control, or simple laboratory scale purification.

Column characteristics:

- Disk diameter – 16 mm
- Disk thickness – 3 mm
- Disk volume – 0.34 ml
- Ring color – unique for each chemistry

Operating conditions:

- Flow rates – up to 10 ml/min
- Pressure – up to 50 bar
- Temperature – up to 50°C
- pH – 1 to 14

Fitted with standard 1/16" OD UNF 10–32 VALCO-type connectors, the column can be fitted to any LC, HPLC, or FIA system.

The housing can be supplied in two different plastic versions: POM (blue & white) and PEEK (brown).

Disks are easily placed into the housing allowing simple column handling; fast, efficient, and inexpensive screening of different chemistries.

The housing can accommodate up to 4 disks. By packing the same chemistry, the volume (capacity) and length can be increased; by packing different chemistries, one can perform Conjoint Liquid Chromatography (CLC).

Fast method development

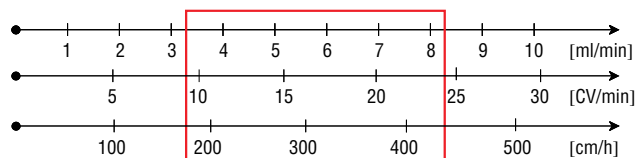
CIM® disk monolithic columns allow the fast development of the most robust method and avoids pitfalls when scaling up at a later stage ... Do more in a single day than you can do in a whole week!

This is all possible due to the high throughputs that CIM® disks allow. Work at flow rates as high as 30 column volumes (CV)/min (10 ml/min). Imagine how much time you save during each run and especially during column re-equilibration!

The table to the right demonstrates the time saved while developing a robust purification method. The sample is loaded in 5 CV, eluted using a linear gradient of 10 CV, and then the column is equilibrated with 5 CV.

Bottom line: You can do as much with CIM® in a single day than you could achieve with a particle based column in a whole week!

Start by increasing your productivity on the lab scale and carry it through to the industrial scale!



	CIM® monoliths	Porous particles
Column volume	0.34 ml	1 ml
Flow rate applied	4 ml/min (11.8 CV/min)	1 ml/min (1 CV/min)
Time – loading (5 CV)	0.4 min	5 min
Time – elution (10 CV)	0.9 min	10 min
Time – equilibration (5 CV)	0.4 min	5 min
Time – total per run	1.7 min	20 min
Time for 20 runs	0.6 h	6.7 h
Time for 100 runs	2.8 h	33.3 h

Rapid in-process control and analytics

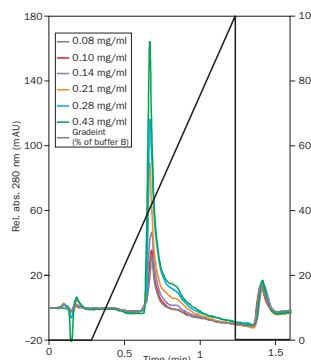
There are situations where speed is more important than resolution. For instance, consider your tricky fermentation process where the yield can vary by an order of magnitude. How can you determine the composition of your sample in a fast reliable manner to avoid over or under loading of your chromatography column?

CIM® disk monolithic columns allow you to do just that! Operating at high flow rates, you have a reliable result in minutes. The disks are a proven robust tool that are simple to use – all this at a very low cost!

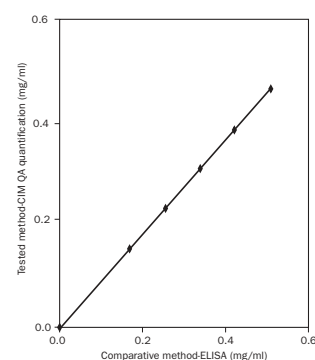
Replace your traditional time-consuming quantification methods like ELISA, real-time PCR, and in some cases plaque assays with CIM® chromatographic columns.

The first thing that you should think about when it comes to in-process control and analytics ...

Fast / Inexpensive / Robust – as CIMple™ as that!



Chromatography of purified ToMV on a CIM® QA monolithic disk at 6 ml/min.



Comparison of semi-quantitative ELISA method with that developed on a CIM® disk

Preparative and Industrial Applications


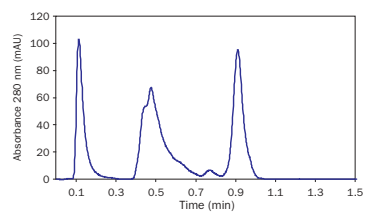

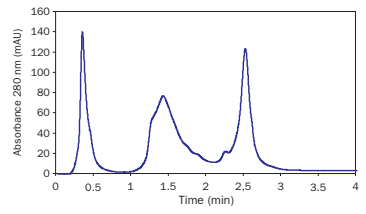

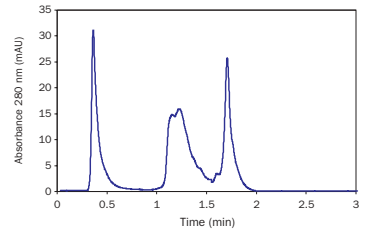

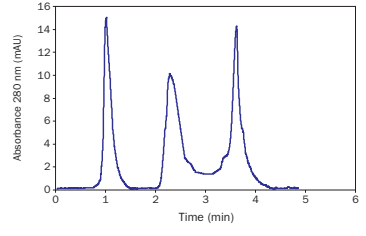

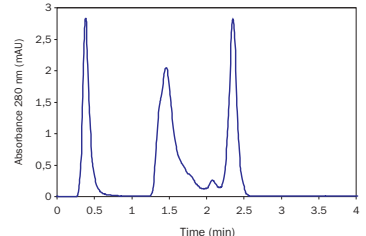
with Convective Interaction Media®



Simple Scale-up

Once you have developed your method on a CIM® disk monolithic column, you can easily scale it up to the preparative or industrial scale. With a range of available volumes up to 8 liters, you are sure to find the right column for your process. Furthermore, scale-up is straightforward since all columns perform like the CIM® disk monolithic columns. As shown below, the resolution is pre-

served regardless of the column used. The columns are designed to meet the most stringent demands of biochemists and process engineers; operating pressures from 10 to 50 bar, temperatures up to 50°C, pH 1–14 and they can be used with all common polar solvents. All preparative and industrial scale CIM® columns are supplied in ready to use housings (cGMP compliant if requested).

	Column	Characteristics	Performance
0.34 ml disk		<p><i>Use:</i></p> <ul style="list-style-type: none"> • Method development • Small lab-scale preparative work <p><i>Approximate housing dimensions:</i></p> <ul style="list-style-type: none"> • 7 cm (length) × 3 cm (diameter) <p><i>Recommended flow rates:</i></p> <ul style="list-style-type: none"> • 3–8 ml/min 	
8 ml column		<p><i>Use:</i></p> <ul style="list-style-type: none"> • Small scale preparative work • First step of scale-up <p><i>Approximate housing dimensions:</i></p> <ul style="list-style-type: none"> • 10 cm (length) × 3.5 cm (diameter) <p><i>Recommended flow rates:</i></p> <ul style="list-style-type: none"> • 10–40 ml/min 	
80 ml column		<p><i>Use:</i></p> <ul style="list-style-type: none"> • Medium scale preparative work • Optional step for scale-up <p><i>Approximate housing dimensions:</i></p> <ul style="list-style-type: none"> • 17 cm (length) × 5 cm (diameter) <p><i>Recommended flow rates:</i></p> <ul style="list-style-type: none"> • 40–250 ml/min 	
800 ml column		<p><i>Use:</i></p> <ul style="list-style-type: none"> • Large pilot scale • Small industrial scale units <p><i>Approximate housing dimensions:</i></p> <ul style="list-style-type: none"> • 25 cm (length) × 15 cm (diameter) <p><i>Recommended flow rates:</i></p> <ul style="list-style-type: none"> • 400–2000 ml/min 	
8000 ml column		<p><i>Use:</i></p> <ul style="list-style-type: none"> • Large industrial scale units <p><i>Approximate housing dimensions:</i></p> <ul style="list-style-type: none"> • 54 cm (length) × 37 cm (diameter) <p><i>Recommended flow rates:</i></p> <ul style="list-style-type: none"> • 2000–10000 ml/min 	

Column figures are not to scale!

Note: The Chromatogram for the 8000 ml column is an extrapolation

From: Milavec Žmak et al., J. Chrom. A, 2003, 1006, 195

Preparative and Industrial Applications

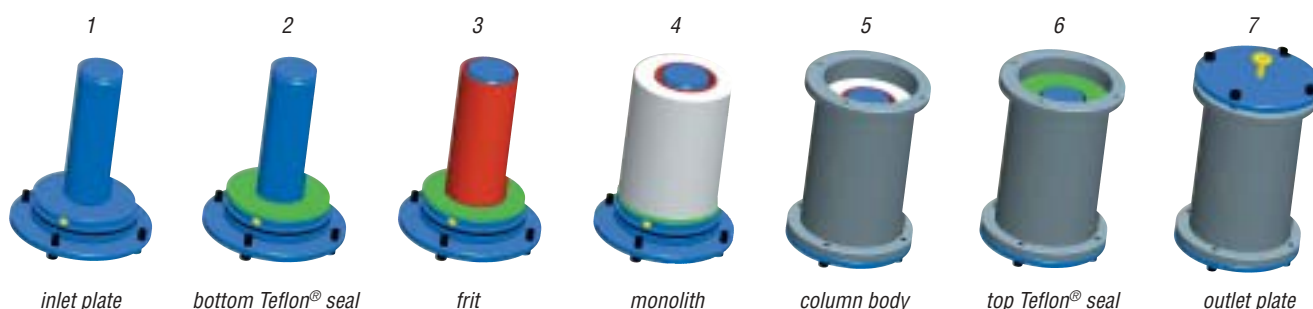
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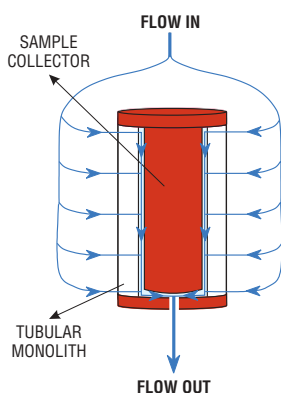
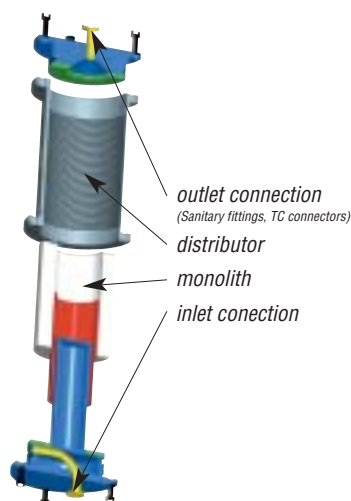
CIM® tube monolithic column structure

The preparative and industrial scale CIM® tube monolithic columns are typically placed on an inlet plate and have a frit on the inner side. Two Teflon seals placed on the top and bottom of the monolith ensure

sealing, while the whole monolith is covered by the column body and outlet plate. CIM® tube monolithic columns are designed for simple handling and robust processing and guarantee customer satisfaction.



CIM® tube monolithic column flow distribution & collection



CIM® tube monolithic columns operate in radial flow mode (see figure on the left) to ensure that the chromatographic layer remains short for any volume.

This means that backpressure remains low and the residence (contact) time is short in order to reduce the amount of undesired transformation of biomolecules due to possible oxidative degradation or enzymatic attack.

Since monoliths exhibit flow unaffected characteristics, this does not compromise the end result when scaling up from disks to tubes (Podgornik et al., *J. Biochem. Biophys. Methods*, 2004, 60, 179).

From the end user's point of view, there is no difference in the performance between disk (axial flow) and tube (radial flow) monolithic columns.

Choose a format to your liking

Although you may not find the exact column volume for your process, this is not a problem as you can prepare columns of intermediate volumes by connecting several CIM® tube columns in parallel.

This principle has proven very effective during the pre-clinical production of a viral vaccine.



New generation CIM® high throughput columns



Characteristics:

- Volume: 8 ml
- Flow rate: up to 400 ml/min (50 CV/min!)
- cGMP compliant

The next generation of CIM® QA high throughput monolithic columns are ideal for applications like virus and DNA removal during the production of monoclonal antibodies and recombinant proteins. The columns are well characterized, cGMP compliant, and supported by a DMF. The columns are supplied in stainless steel housings and disposable housings will be available in the future.

Purifying Viral Vectors & Vaccines

with Convective Interaction Media®



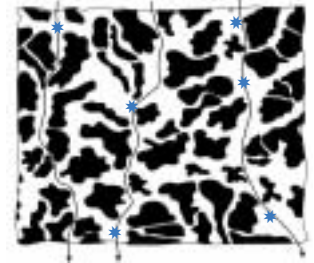
High binding capacity at high speed



Particle support

CIM® QA supports have high binding capacities on the order of 10^{14} virus particles per ml of support even at high throughputs, due to their unique structure. CIM® supports have a flow-through pore structure that provides high surface accessibility (1.5 μm diameter channels), resulting in high binding capacities, and ensures that mass transfer is not limited by diffusion.

The result is that CIM® media outperforms conventional particle based supports that only adsorb viruses on the beads' outer surface (due to their small pores) – meaning their binding capacities are 1–2 orders of magnitude lower. Even modifications to particle supports (like perfusion particles or those with tentacles) are unable to compete with CIM® media since mass transfer is still limited by diffusion resulting in decreased binding capacity at higher flow rates.



CIM® monolith

Truly friendly to virus particles

The pores of CIM® media are large enough (1500 nm) to handle even the largest virus particles.

Furthermore, viruses experience low shear forces while traveling through CIM® monoliths meaning that high recoveries of even the most labile products can be obtained. This is especially important for virus purification due to the vulnerability of the proteins and carbohydrates extending from the capsid.

CIM® monolithic columns have already been employed for the purification of therapeutically relevant viruses.

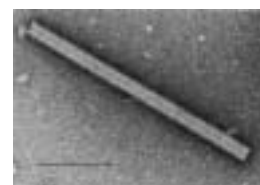
“Tom Ouellette of the Biopharmaceutical Development Program, SAIC/NCI-Frederick ... went on to present data on the purification of Herpes virus on CIM DEAE, achieving excellent fractionation and 100% activity recovery of this extremely labile product. He emphasized that the apparent low shear characteristics of monoliths are especially important for virus purification due to the vulnerability of the proteins and carbohydrates extending from the capsid.”

From: Gagnon, Genet. Eng. News, 2006, 26(17), Oct. 1, 44–46.

Tomato Mosaic Virus (model system)

Tomato Mosaic Virus (ToMV) is a useful model that demonstrates the effectiveness of CIM® supports for the purification of viruses used for human therapeutics.

ToMV has been used to compare different methods of virus purification like conventional gradient centrifugation as well as other chromatographic techniques (particle-based columns or membrane absorbers).



Size 300 × 18 nm
Shape Rod-like
Surface Non-enveloped
Genome ss RNA, linear
pI 4.6

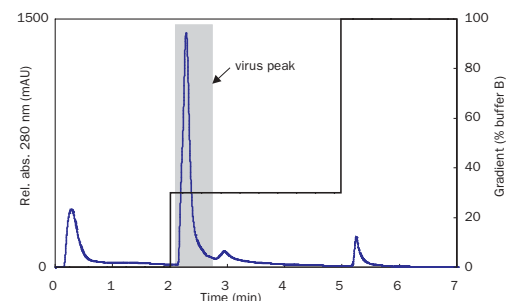
ToMV purification with CIM® supports

Before loading the sample onto a CIM® QA disk monolithic column, the plant material was homogenized and clarified by centrifugation. The sample was then loaded and eluted with 0.3–0.45 M NaCl at high flow rates (6 ml/min or 17.5 CV/min!).

Results:

- VPs infective after purification
- Dynamic binding capacity: 12.6 mg or $\sim 2 \times 10^{14}$ ToMV particles/ml
- 75% virus recovery
- 99% of host DNA removed
- Host cell proteins removed

Sample 1 ml clarified plant homogenate in buffer A
Buffer A 20 mM NaAc
Buffer B 20 mM NaAc, 1.5 M NaCl
pH 5.5
Flow rate 6.0 ml/min
Column CIM® QA disk



Purification of ToMV from 1 ml of homogenized and clarified plant material

Purifying Viral Vectors & Vaccines

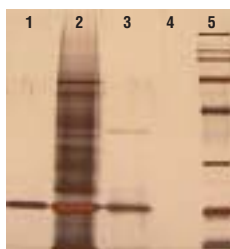
with Convective Interaction Media®



ToMV purification: CIM® vs. gradient centrifugation

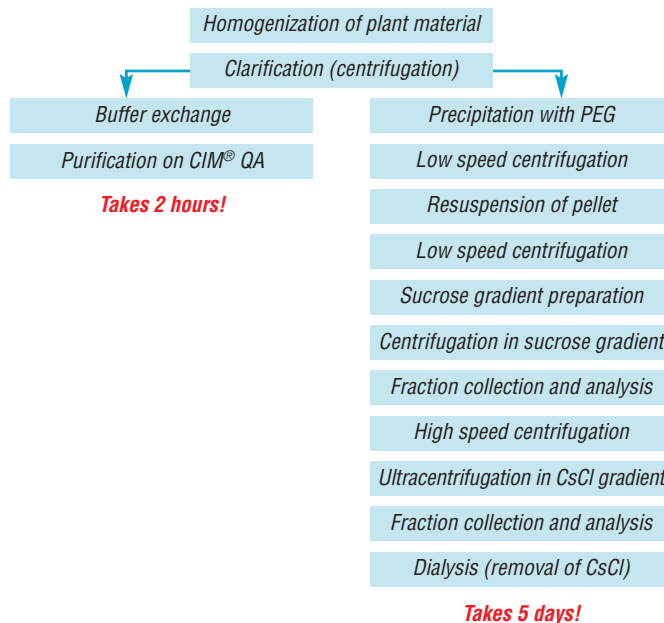
By using CIM® monolithic columns, the entire ToMV purification procedure took **2 hours** while the conventional purification protocol based on gradient **centrifugation** requires approximately **5 days**.

The recovery of ToMV purified on a CIM® monolithic support is higher, while the purity of ToMV (in terms of host proteins and DNA) is comparable to the conventional method. During both processes, ToMV preserves its infectivity.



Silver stained SDS-PAGE gel:

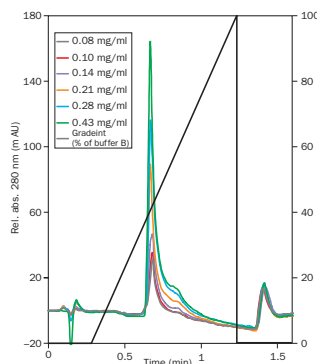
- 1: ToMV purified by the conventional method
- 2: Starting material
- 3: First elution fraction from the CIM® disk
- 4: Negative control
- 5: Marker proteins (Biorad, Broad-range)



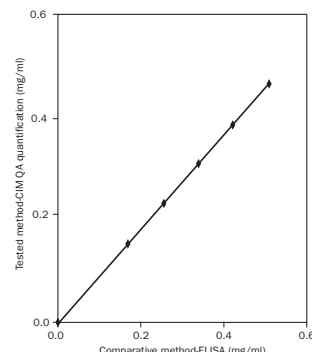
Virus quantification for in-process control

CIM® disk monolithic columns are an excellent tool for virus quantification and in-process control. The calibration curve can be prepared in a matter of minutes, while the concentration values closely match those determined by ELISA.

The method enables quantification of purified ToMV in only a few minutes and the results are comparable with the semi-quantitative ELISA measurement which takes 2 days to obtain. A CIM® based quantification method could be used as a preliminary method to quickly estimate the virus concentration and decide what further actions to take. After preliminary screening, a more accurate quantification method like real-time PCR can be used to determine the exact virus concentration.



Chromatography of purified ToMV on a CIM® QA monolithic disk at 6 ml/min.



Comparison of semi-quantitative ELISA method with that developed on a CIM® disk

CIM® is a unifying technology for your vaccine and gene therapy targets that speeds up your process development and production; enabling quantification for in-process control and quality assurance analysis.

Productive Purification of Plasmids

with Convective Interaction Media®



Plasmid DNA – characteristics and challenges

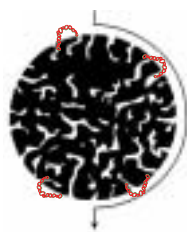
Plasmid DNA is a closed-loop double stranded DNA that occurs naturally in bacteria. They are very suitable vectors (delivery vehicles) for gene therapy applications where highly pure pDNA is needed. It must be virtually free from impurities like genomic DNA, RNA, proteins and endotoxins and >90% must be in the supercoiled (sc) pDNA form. (sc) pDNA is unstable and can quickly undergo an irreversible transformation to the open circular (oc) form. This means that the culture supernatant needs to be processed quickly.

A purification process that uses high flow rates, has high binding capacities, is scalable and cGMP compliant must be employed to process the large amounts of plasmids needed. But, does such a process exist?



Plasmid DNA: supercoiled (left drawing), different types (right photo)

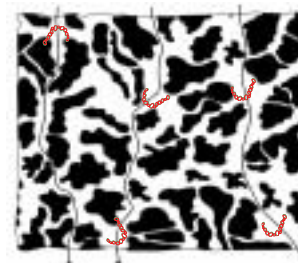
Outperforming particle based supports



Particle support

CIM® DEAE supports provide high binding capacities up to 10 mg of plasmids/ml even at high throughputs. Their flow-through pore structure not only provides high surface accessibility (1.5 µm diameter channels) and high binding capacities, but ensures that mass transfer is not limited by diffusion. CIM® monolithic supports are an ideal media for purifying your plasmids of any size.

Conventional particle based supports are only able to bind plasmids on their outer surface – meaning their binding capacities are several orders of magnitude lower than CIM®. Even improved particle supports (like perfusion particles or those with tentacles) are unable to compete as their mass transfer is limited by diffusion resulting in decreased binding capacity at higher flow rates.



CIM® monolith

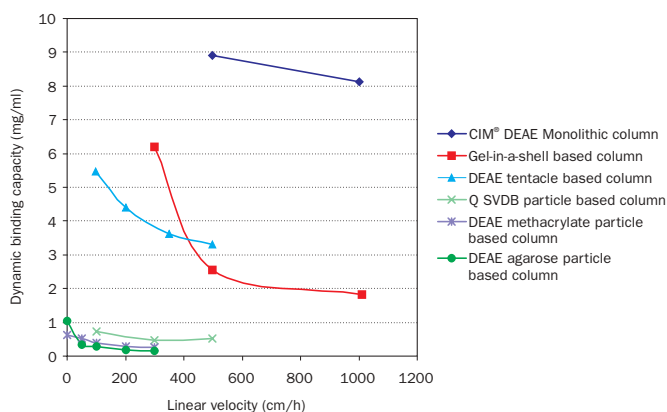
High binding capacity at high speed

When the pDNA binding capacity of CIM® DEAE supports are compared to particle based supports, no media could compete with the overall performance of CIM® DEAE monolithic supports.

The capacity of particle based media, optimized to handle (much smaller) protein molecules, was 10–20 times lower than CIM® DEAE supports.

Modified particle supports (perfusion type or those with tentacles) are also no match for CIM®; diffusion limitations cause a severe drop in binding capacity at higher flow rates although the number of binding sites is higher.

CIM® media maintain a high capacity even at industrially relevant mobile phase velocities! The result is up to a 15-fold increase in productivity.



From: Urthaler et al. (Boehringer Ingelheim), J. Chrom. A, 2005,1065, 93–106

Productive Purification of Plasmids

with Convective Interaction Media®

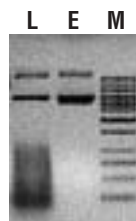


RNase free process for purifying transfection grade pDNA

Using CIM® media, the isolation of plasmid DNA no longer requires the addition of ribonuclease A (RNase). A 2-step purification solution is all you need to purify your transfection grade pDNA on any scale.

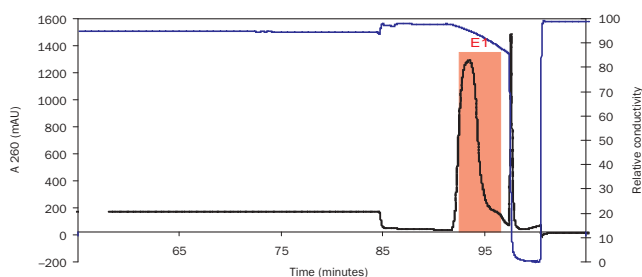
The process employs a CIM® OH column which captures the pDNA from the fermentation pool and separates it from the majority of RNA. This is followed by the polishing step which uses a CIM® DEAE column (weak anion exchanger). Employing the 8 ml columns of these chemistries, up to 13 mg of highly pure pDNA can be isolated.

The purified plasmid DNA solution retains its biological activity (confirmed by bacterial transformation) and complies to the highest standards of product purity with RNA, proteins, genomic DNA, and endotoxins being under the detection limit.

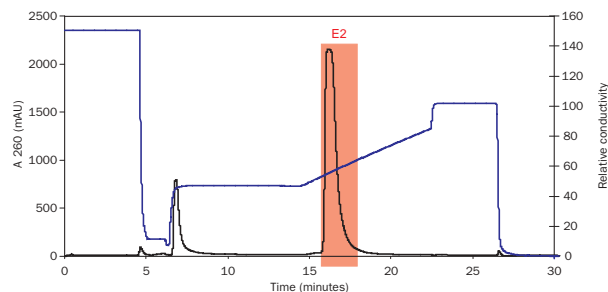


RNA	Not detected
Genomic DNA	Not detected
Proteins	Not detected by BCA
Transformation	Successful
A260/A280	1.85
Endotoxins	< 0.1 EU/mg pDNA

Quality of purified plasmid DNA – Biologically Active with impurities under the detection limit



Sample Cleared bacterial lysate (*E. Coli*)
Buffer A 50 mM Tris-HCl, 10 mM EDTA, 3.0 M (NH₄)₂SO₄ (pH 7.2)
Buffer B 50 mM Tris-HCl, 10 mM EDTA (pH 7.2)
Flow rate 20 ml/min
Column CIM® OH 8 ml tube



Sample DNA fraction eluted from step 1
Buffer A 50 mM Tris-HCl, 10 mM EDTA (pH 7.2)
Buffer B 50 mM Tris-HCl, 10 mM EDTA, 1.5 M NaCl (pH 7.2)
Flow rate 20 ml/min
Column CIM® DEAE 8 ml tube

References & State of the art solution

Boehringer Ingelheim Austria GmbH developed a pDNA purification process that was quickly moved to pilot scale using an 80 ml CIM® monolithic column and then transferred to production using a cGMP 800 ml CIM® monolithic column—clearly demonstrating that CIM® makes this an economical and scaleable cGMP process which results in high quality plasmid DNA suitable for therapeutic use. By using CIM® instead of particle-based supports, there was a 15-fold increase in productivity.

Because of their collaboration with BIA Separations, Boehringer Ingelheim received the *2004 Frost & Sullivan Technology Leadership Award* for their innovative pDNA manufacturing process. The award committee stated: “*The company is leading technology providers in the race to capitalise on the huge demand for new therapeutics by investing in an area which has received scant attention – technology innovation in downstream processing.*” The key to the pDNA downstream processing is CIM® monolithic column platform technology.

Industrial relevant media

CIM® supports come in volumes up to 8000 ml and are cGMP compliant – a Drug Master File for the DEAE chemistry is already in place.



Purifying Proteins the Simple Way

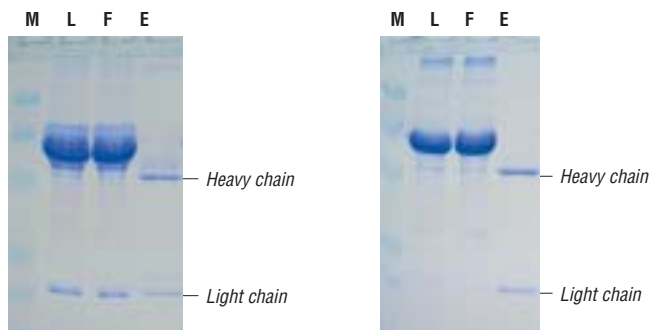
with Convective Interaction Media®



Immunoglobulin G – IgG

CIM® Protein A and Protein G media are ideal for preparative chromatography and high throughput screening of samples due to the high flow rates they allow. While Protein G binds most IgGs strongly at neutral pH, mouse IgG1, human IgG3, and rat IgGs bind only weakly to Protein A under the same conditions. Mouse IgG1 can bind to Protein A under alkaline, high salt conditions.

Results on the right performed with mouse mAbs anti-chicken IgY produced by hybridoma cells (Serum Free Media) demonstrate that a high purity is obtained with CIM® Protein A and Protein G columns. Dots Immunobinding Assay (DIBA) indicated that purified mAbs retain biological activity!



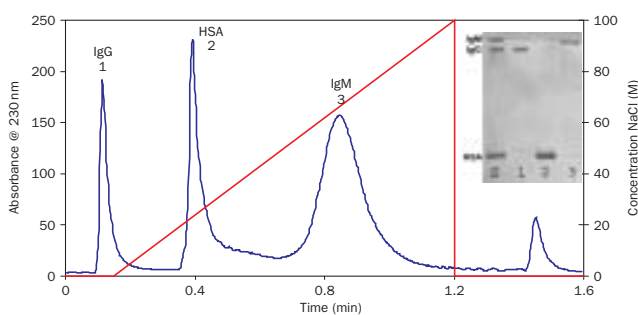
Sample	Mouse mAbs anti-chicken IgY	Sample	Mouse mAbs anti-chicken IgY
Binding buffer	20 mM Tris HCl (pH 7.4)	Binding buffer	20 mM Tris HCl (pH 7.4)
Elution buffer	0.5 M acetic acid (pH 2.5)	Elution buffer	0.1 M Glycine-HCl (pH 2.0)
Flow rate	3.0 ml/min	Flow rate	3.0 ml/min
Column	CIM® Protein A disk	Column	CIM® Protein G disk

Immunoglobulin M – IgM

IgM is a high molecular weight protein (950 kDa), which usually consists of five subunits and is by far the largest antibody. IgM antibodies appear early in the course of an infection, usually do not reappear after further exposure and do not cross the human placenta. These two biological properties make IgM useful for the diagnosis of infectious diseases.

Due to its size, the capacity of particle based media is low. Furthermore, affinity chromatography is not suitable as the conditions (long exposure to low pH) required significantly decrease IgM activity. CIM® EDA weak anion exchange media provides excellent results in a very short time!

Sample	0.3/1/1 mg IgG/IgM/HSA/ml	pH	7.0
Buffer A	20 mM Phosphate	Flow rate	4.0 ml/min
Buffer B	20 mM Phosphate + 1 M NaCl	Column	CIM® EDA disk

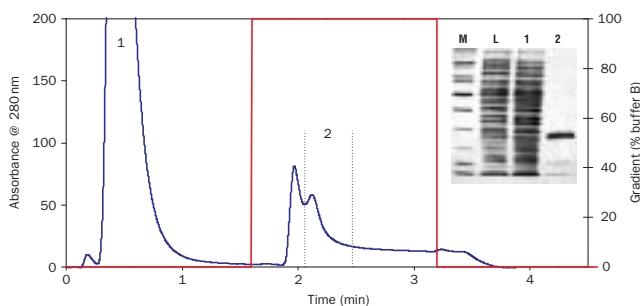


Separation in a single minute!

His-tagged proteins

GFP-His6 is a recombinant green fluorescent protein with 6 histidine tags on the N-terminus and a molecular weight of 29 kDa. CIM® IMAC media are an efficient tool for the isolation and purification of His-tagged proteins. Purities of ~90% and a dynamic binding capacity of ~30 mg/ml have been achieved.

Sample	0.2 ml GFP-His6 cleared cell lysate
Buffer A	20 mM phosphate + 0.5 M NaCl + 20 mM imidazole
Buffer B	20 mM Phosphate + 0.5 M NaCl + 250 mM imidazole
pH	7.4
Flow rate	3 ml/min
Column	CIM® IDA-Cu disk

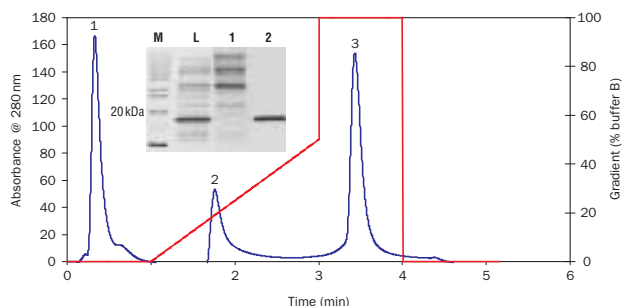


From: Peterka et al., J. Chromatogr. A, 2006, 1109, 80–85

His-containing proteins

LK-801 is a tumor necrosis factor- α (TNF- α) analog with surface-exposed histidine residues and a molecular weight of 17 kDa. CIM® IMAC media are a powerful tool for the isolation and purification of His-containing proteins. Purities of ~90% and dynamic binding capacities of ~20 mg/ml and recoveries of ~80% have been achieved.

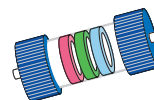
Sample	200 μ l of TNF LK801 cleared cell lysate
Buffer A	20 mM Phosphate + 0.2 M NaCl
Buffer B	20 mM Phosphate + 0.2 M NaCl + 100 mM imidazole
pH	7.1
Flow rate	3.0 ml/min
Column	CIM® IDA-Cu disk



Lane 1: load. Lane 2: flowthrough. Lane 3: elution (LK801)
From: Peterka et al., J. Chromatogr. A, 2006, 1109, 80–85

Conjoint Liquid Chromatography

with Convective Interaction Media®



What is this?

Conjoint Liquid Chromatography (CLC) is one of the most innovative and advantageous features of CIM® monolithic columns. CLC is the possibility of placing supports with different functional groups into one housing—preparing a CLC Monolithic Column. This enables extremely fast multidimensional chromatography. Now! It is no longer

necessary to purchase a large variety of chromatographic columns. Furthermore, there are no extra column effects, such as peak broadening, giving much sharper resolution. The idea is even applicable on an industrial scale.

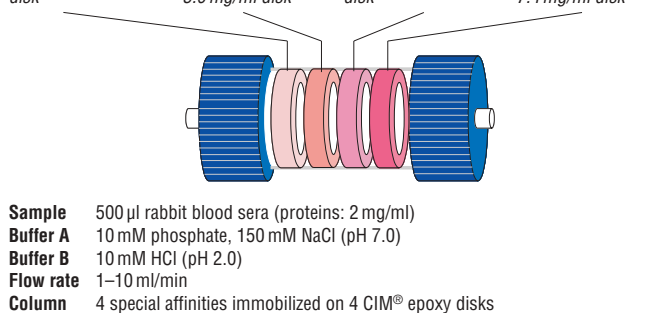
CIM® CLC: Superior performance – Cost saving!

Selective fractionation of polyclonal antibodies

The CLC method can be successfully used for the fast and quantitative fractionation of complex biological mixtures such as pools of polyclonal antibodies from blood sera as well as effective for semi-preparative separations. For example, rabbits immunized with complex protein-peptide conjugates. Several disks with different affinity functionalities, prepared by immobilizing protein and peptide ligands onto CIM® epoxy disks, were placed in the same housing and the different antibodies were selectively captured within a few minutes. The captured antibodies were then eluted one at a time.

From: Ostryanina et al., *J. Chrom. A*, 2002, 949, 163–171

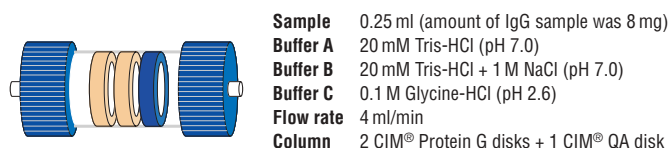
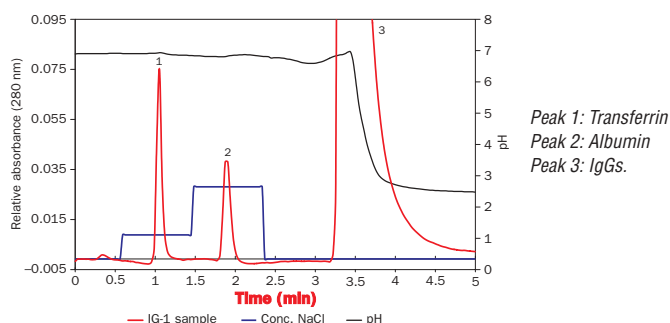
Bradykinin (BK) 1.5 mg/ml disk
Bovine serum albumin (BSA) 3.0 mg/ml disk
Succinylated BSA (BSA-S) 3.0 mg/ml disk
Conjugate of BK with BSA (BSA-S-BK) 7.4 mg/ml disk



Quantification of impurities in IgGs

Transferrin and albumin are often present in IgG concentrates and are considered impurities. It is important to determine their concentration in order to obtain a well-characterized biological product. Two CIM® Protein G disks and one CIM® quaternary amine (QA) monolithic disk were placed consecutively into one housing forming a CLC monolithic column allowing a complete separation of all three proteins in less than 5 minutes. Under the applied binding conditions, the IgGs were captured on the Protein G disks while transferrin and albumin were bound to the QA disk. Subsequently, transferrin and albumin were eluted separately by a stepwise gradient using sodium chloride, whereas the IgGs were released from the Protein G ligands by applying a low pH. This validated method permits the quantification of albumin and transferrin in IgG concentrates.

From: Branović et al. (*Octapharma*), *J. Immunol. Meth*, 2002, 271, 47–58

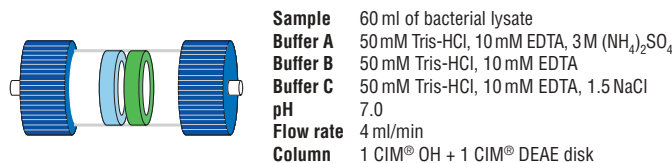
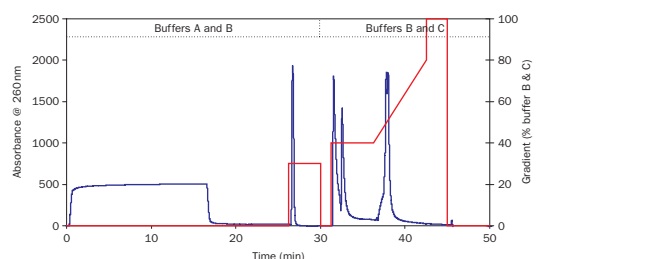


Transfection grade plasmid DNA

To achieve the required purity for pDNA, several chromatographic steps are commonly needed with each one contributing to dispersion and decreasing resolution. This problem can be overcome by combining the capture and purification steps into a single CIM® CLC column.

Specific advantages of the CIM® CLC approach:

- **Same product purity**
- **Higher yield**
- Considerable **reduction in buffer consumption**
- Considerable **reduction in time and effort!**



Services & Other Formats Upon Request

with Convective Interaction Media®



Major services

Joint process development: If you are looking for a partner to help develop a purification process for your pDNA, virus, or protein, BIA Separations is the right choice. BIA Separations and Boehringer Ingelheim co-developed a process for the contract manufacturing of plasmid DNA (pDNA). The manufacturing process produces pDNA for clinical trials and market supply. In addition, BIA Separations is developing small and medium scale kits for the isolation of pDNA on a laboratory scale.

Contract Research: BIA Separations contract research laboratory develops and validates analytical methods, performs analysis for pharmacokinetic studies, and isolates and identifies drug impurities using HPLC and GC.

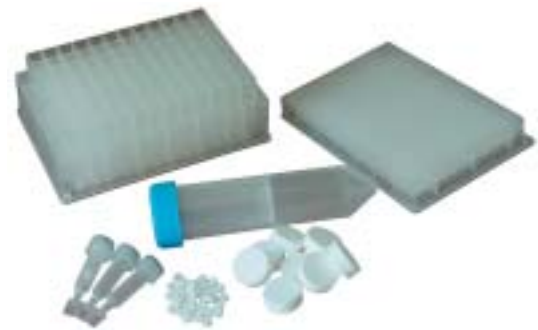
Designing Industrial Purification Processes: BIA Separations application laboratory utilizes its expertise in liquid chromatography, monolith and particle based, to design the most efficient, cost effective, and optimized process for biomolecule and traditional small molecule purification.

Are you missing a format from our product list?

The range of products in the market devoted to screening and other applications is almost endless – and so are the possible formats that BIA Separations' monolithic supports can be made into:

- 96-well plates
- 384-well plates
- Large centrifuge spin columns
- Small centrifuge spin columns
- Mini disks (34 µl)
- Micro disks (3.4 µl)

... and many more ... If you are interested in a specific format, do not hesitate to contact us. We are prepared to listen and discuss possible technical solutions as well as OEM business solutions.



Markets served

BIA Separations is focused on serving corporate and academic research and production scientists working in the biotechnology sector. In order to meet their needs, BIA Separations has estab-

lished a worldwide network of partner distributors serving North America, Europe, Australia, and Asia. BIA Separations directly serves regions where we do not have a partner distributor.

Information and specifications contained herein are, to the best of our knowledge, accurate and represented in good faith. They are intended to help you start working with the new separation technology and are subject to change without notice.

BIA Separations shall not be liable for errors contained herein or for incidental or consequential damages in connection with the performance or use of CIM®.

For more information on our products, visit our home page at:

<http://www.biaseparations.com> and <http://www.monoliths.com> or contact your local distributor.

CIM® technology is covered by US patents 4889632, 4923610, 4952349, 5972218, 6319401, 6736973B, 6664305 and foreign equivalents. Other patents pending.

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We reserve the right to alter specification details etc. without prior notice or liability.



CIM Convective Interaction Media[®]

Liquid Chromatography Redesigned[™]



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