Chromalite[®] M Resins





Since 1981, Purolite® has grown into the world's premier resin-based separation, purification and extraction technology manufacturer and innovation leader, with manufacturing facilities, advanced research laboratories and over 1400 people employed world-wide.

Your solutions company

Purolite[®] Life Sciences focuses on any applications involving interactions with people, bringing innovative thinking and a distinguished history of resin technology expertise to the global Life Sciences marketplace. We supply premium quality APIs, enzyme carriers, immobilized enzymes, and agarose or synthetic chromatography resins for purification and separation, to support research and development and production-scale applications in pharmaceuticals, protein purification, food processing, bioprocessing and fine chemical markets.

"We provide solutions for our customers' most critical questions."

Quality

Purolite® maintains a global Quality Management System (QMS) which supports BSI requirements of ISO 9001. Compliance is monitored and maintained through a quality assurance and regulatory team, who conduct internal audits to ensure operations meet the guidelines and protocols for equipment and procedures. Our teams are given continuous training on quality processes to ensure batch-to-batch consistency, and the highest product quality.

Secure Supply through Manufacturing Excellence

Ensuring reliable availability of our resins is vital to customers, and of paramount importance to Purolite® Life Sciences. As a leading supplier of resin to the world's most regulated industries, we recognize that our resins are critical purification products. As such, a real-world security-of-supply system is in place to support your process requirements for business continuity. Supply risk is managed end-to-end, with a global network of qualified suppliers. Long-term supply agreements with periodic audits ensure consistency and 'fit for purpose' performance.

Purolite[®] has manufacturing facilities at 4 strategic locations in the USA, Asia and Europe, including the recent addition of one of the world's largest agarose resin manufacturing facilities located in Llantrisant, South Wales, UK.

Our agarose manufacturing facility includes stateof-the-art Siemens® automation systems and can securely supply 30% of the current annual global demand for agarose chromatography resins to the biopharmaceutical sector.

Our Romanian facility is FDA-inspected, with a total of 4 state-of-the-art clean rooms. These offer separate facilities for ligand/enzyme immobilization, removal of fines, solvent or purified water washing, screening, vacuum drying and packaging. Lifetech[™] and Chromalite[®] products are manufactured in a clean room dedicated solely to Life Sciences products.



100% focused on resin technology.



Global manufacturing at facilities in the UK, Romania, China and USA.



De-risked long-term supply through dual-sourcing.



25+ years of regulatory experience from FDA inspected cGMP facility.



Over 35+ years of experience in solving advanced R&D and purification challenges.

Purolite® technologies

Purolite® Life Sciences manufactures resins using three different technologies:

Batch Suspension is a robust, proven technology with broad particle distribution.

Typical particle size range - 100 - 1200 μm

UC <1.6

Seeding creates uniform particle size beads with a monodispersed particle size distribution. Small 'seeds' are subsequently grown (seeded) through repeated additional polymerization.

Typical particle size range - 3 - 50 μm

UC <1.1

Jetting produces resins with a very narrow particle size distribution, providing enhanced performance and column packing consistency.

Typical particle size range - 50 - 250 μm

UC <1.3





The Jetting Process

Chromalite[®] M products for high-resolution/ polishing applications are manufactured using a patented technology called 'Jetting'.

This process involves a monomer phase pumped through a specially engineered steel membrane or can into aqueous phase, forming a suspension with a narrow particle size distribution (UC <1.3). A high yield, economical process, it provides Chromalite[®] products with unique performance characteristics.



Patented, Innovative Manufacturing



PUROLITE® PATENT

S. R. Kosvintsev, Pat. 20140264984, 2013, Method of producing uniform polymer beads of various sizes. US Pat. 9028730 B2, 2015

Jetting - Advantages in Chromatographic processes



Customization is our strength

Our team of world-class researchers, polymer chemists and application scientists are experts at developing novel, customized products that meet the diverse requirements and expectations of our customers.

Whether you need a customized particle size, functional group, porosity or ligand density, Purolite[®] Life Sciences can help you.

"Customization is our strength."

Complete regulatory support

For over 35 years, Purolite® has supplied specialty resin technology to industries within complex regulatory environments including biotechnology, pharmaceutical, food, fine chemical and electric power generation.

The regulatory environment is ever changing, driven by increasing regulatory requirements, increasing development costs and times, and market pressures impacting pharma and food industries.

For Life Sciences products, Regulatory Support Files (RSF) are available. Regulatory Support Files provide direct and detailed information on performance, stability, extractable compounds, and analytical methods for each resin.

"Regulatory expertise throughout the product life-cycle is essential to identify options for product development, optimize 'speed to market' and produce a product that meets customer needs."

Purolite[®] implements control documentation and processes at every level to ensure regulatory support to customers using our products.

Purolite[®] complies with required national and international regulations, as well as many voluntary specialty certifications. These include:

- GMO/TSE/BSE free
- ResAP(2004)3
- REACH regulations
- ISO 9001:2015 quality system specifications
- ISO 14001:2015 Environmental Management
 System requirements
- Halal and Kosher requirements

We also hold Drug Master Files with the US FDA, Japan, Canada and EU



Safety data

Purolite[®] Life Sciences maintains Material Safety Data Sheets (MSDS for the U.S. and ERSDS for Europe) on all of its bulk resins. These data sheets contain relevant information that you may need to protect your customers and employees against any known health or safety hazards associated with our products. Purolite[®] Life Sciences supplies copies of our Material Safety Data Sheets with all bulk resins. These describe precautions to be taken in the storage and handling of our products and in the maintenance of the health and safety of persons exposed to our products, the public and the environment with respect to our products.







Your trusted partners for resin technology solutions.

Purolite[®] continues to lead the future of chromatography with the expertly designed Chromalite[®] M range of methacrylic resins.



"Hydrophilic, macroporous methacrylic resins for reverse phase, ion exchange or affinity chromatography applications."

Introduction

The Chromalite[®] M range of chromatographic resins are hydrophilic, macroporous, methacrylic resins for large-scale applications. Based on a rigid polymeric backboned that ensures excellent pressure-flow properties, they have been designed for high performance, stability and reliability under the conditions of modern industrial bioprocesses.

Chromalite[®] M resins for ion exchange chromatography include both strong and weak functional groups in both anion and cation exchange processes. This document provides information on resin characteristics, advantages over competitor resins, column packing guidance and methodology, as well as maintenance and scale-up instructions.

These instructions guarantee the best possible performance of Chromalite® M resins.



Chromalite [®] M resins for ion			
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weak functional groups in			
both anion and cation			
exchange processes.			
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Chromalite® M Resins

Chromalite® M are designed to have intermediate hydrophilicity among the highly hydrophobic Chromalite® resins, based on styrene/divinylbenzene DVB and the highly hydrophilic Praesto® range based on agarose suitable for very large biomolecules.

Chromalite® M resins feature:

- A protein friendly matrix
- High chemical stability
- Suitability for high resolution, intermediate and capture purification
- Optimal porosity for small, medium or large molecules
- IEX and Epoxy functional groups for ligand coupling



Chromatography Resin General Features

Product range	Chromalite [®] Styrene / DVB	Chromalite® Methacrylate	Praesto [®] Agarose
	Highly Hydrophobic	Medium Hydrophilic	Highly Hydrophilic
Particle size (micron)	5 - 300	40 - 300	35 - 90
Matrix	Styrene / DVB	Methacrylate	Agarose
Method of separation	RP / IEX	IEX / Affinity	IEX / Affinity
Method of elution	Organic solvents / salt	Organic solvents / buffers / salt	Buffers / salt
Max. pressure	50 bar	10 bar	4 bar
Typical target molecule	100 Da - 5 kDa	1 - 250 kDa	20 - 500 kDa

Figure 1. Characteristics of Chromalite[®] M resins compared to other existing product ranges from Purolite[®] Life Sciences.

Chromalite® M product	Particle size (micron) ¹	Functional groups	Functional groups (meq/ml)	Dynamic capacity ²
MEP/F	40-90		>0.4	n.a.
MEP/M	75-125	Ероху	>0.5	
MEP/C	100-300		>0.6	
MS/F	40-90		>0.1	>40 mg/mL
MS/M	75-125	Sulfopropyl/Na+	>0.1	
MS/C	100-300		>0.1	
MCM/F	40-90		>0.4	>40 mg/mL
MCM/M	75-125	Carboxymethyl/Na+	>0.5	
MCM/C	100-300		>0.6	
MQ/F	40-90		>0.2	>20 mg/mL
MQ/M	75-125	Quaternary ammonium/Cl-	>0.3	
MQ/C	100-300	-	>0.4	
MDEA/F	40-90		>0.4	>30 mg/mL
MDEA/M	75-125	Diethylamine	>0.5	
MDEA/C	100-300		>0.6	

Table 1. Characteristics of functionalized Chromalite® M resins.

 1 F = 40-90 is manufactured by jetting to obtain narrow particle size distribution (UC < 1.3), whereas M = 75-125 and C = 100-300 are manufactured by suspension (UC < 1.6)

² Determined by frontal analysis at a flow rate of 300 cm/h, using an 8.0 mg/ml solution of lysozyme in 20 mM sodium citrate buffer, pH 5.0 – Chromalite[®] MCM and MS, or a 5.0 mg/ml solution of BSA in 20 mM Tris buffer, pH 8.5 - Chromalite[®] MQ and MDEA. The actual loading capacity in a real working situation will depend on the nature and concentration in the sample, and the degree of resolution required.

Chemistry for Chromalite® M

Chromalite[®] M resins are initially manufactured from an epoxy-functionalized resin (Chromalite[®] MEP) and from that resin different IEX resins are obtained (Chromalite[®] MS, Chromalite[®] MCM, Chromalite[®] MQ and Chromalite[®] MDEA).

Chromalite[®] MEP is an excellent resin for ligand coupling, since it reacts well in mild conditions (usually water or buffers) with hydroxy, thiol and amino groups, so customers can bind target molecules with ease. The technology applied by Purolite® to couple ligands is simple and avoids the use of expensive and rare spacers. This strategy offers different benefits compared to similar products on the market:



The backbone of the Chromalite[®] M resins is stable and can be used in a temperature range of 2-60°C. Higher temperatures as 80°C can be used but for very short periods.



Figure 2. Product range for Chromalite® M resins.

pK_a values for ion exchange groups



Figure 3. pK values for Chromalite $^{\rm \$}$ M IEX resins, and their operational pH range

Key Benefits of Chromalite® M Ion Exchange Resins

- Large porosity (typical 1000Å) suitable for biomolecules
- Hydrophilic, methacrylic matrix for minimal non-specific binding
- Robust matrix suitable for industrial chromatography and large columns configurations
- Minimal swelling
- Chemical stability (pH 1-14)
- Wide range of particle sizes available (customized particle sizes available upon request)
- Patented 'Jetting' manufacturing process for high resolution, intermediate purification and polishing applications



Porosity

The matrix of Chromalite[®] M is designed with a macroporosity of about 1000Å (Figure 4), which is suitable for large molecules up to 250kDa. Our polymerization technology allows us to achieve a narrow pore distribution within the matrix, thus reducing tailing and improving separation or proteins.



Pore size distribution

Figure 4. Porosity profile of Chromalite® M.

Blue: Relative number of pores of the size e.g. Pores of size 1000 Å have a relative abundancy of 38, compared to pores of size 100 Å, which have a relative abundancy of 4; this means that there are nearly 10 times as many pores of 1000 Å as pores of 100 Å.

Grey: (Read right to left) Cumulative volume of all pores e.g. all pores of 1000 Å and above have a total volume of approximately 0.225 cc/g resin and all pores of 100 Å and above have a total volume of approximately 0.425 cc/g resin. The highly porous structure of Chromalite® M allows for diffusion of large biomolecules up to 250kDa. It offers the ideal balance between optimal porosity and high mechanical stability.

Electron microscopy of Chromalite[®] MDEA/C.

Particle size

Resolution in a chromatographic process depends on particle size. Purolite[®] Life Sciences offers Chromalite[®] M resins in three different particle size.

Grade	Particle size (micron)	Manufacture Process	Typical UC	Application
F	40-90	Jetting	< 1.3	 High resolution Intermediate Purification Polishing
М	75-125	Suspension or Jetting	< 1.6	 Intermediate Purification Capture
С	100-300	Suspension	< 1.6	Capture

Table 2. Chromalite $^{\ensuremath{\mathbb S}}$ M resins are available in three different particles sizes.

Figure 5. Effect of particle size distribution of Chromalite® M resins on resolution





UC definition: Uniformity coefficient (UC) is where monodispersity is defined as:

 $UC = \frac{D_{60}}{D_{10}}$

A uniformity coefficient of 1 represents
 monodispersity. D₁₀ and D₆₀ are diameters that
 corresponds to the 10% and 60% the particle size
 distribution (PSD) respectively and the Uniformity
 Coefficient (UC) is calculated as D₆₀/D₁₀.

Resolution increases when particle size decreases as shown in Figure 5.

Resolution can be expressed as dependent on three primary factors – the number of theoretical plates, the retention time and the separation factor:

$$R = \frac{\sqrt{N}}{4} \times \frac{k'}{k'+1} \times \frac{\alpha - 1}{\alpha}$$

Where:

- R = Resolution
- N = number of theoretical chromatographic plates
- k' = retention time
- α = separation factor

An increase in the number of theoretical chromatographic plates is therefore correlated with an increase in resolution and, since N is inversely proportional to particle size, a decrease in particle size results in an increase in resolution. This decrease in particle size is also correlated with an increase in pressure, according to the equation:

 $\Delta P=150 \times L \times \left(\frac{\eta \times u}{d_p^2}\right) \times \left(\frac{(1-\varepsilon)^2}{\varepsilon^3}\right)$

Combining the constants into one term, k, we can express the equation as:

 $\Delta P = \left(\frac{k}{d_p^2}\right)$

Demonstrating that the pressure is inversely proportional to particle size.





Figure 6. Mechanical stability of Chromalite® MDEA/C (100-300 micron) compared to Relisorb[™] DA400/SS (50-150 micron) and Sepabeads® FPDA13 (90-250 micron) and Chromalite® MEP/F (40-90 micron) compared to Toyopearl® HW-65C (50-100 micron).

Mechanical stability is determined by suspending the resins in water in a bead beater, mixing at 15800 rpm, and measuring the absorbance at 600 nm of the supernatant after 5 minutes. A lower mechanical stability will result in more fragmentation of the beads and increased absorbance due to small bead fragments in suspension.

Chromalite[®] resins are manufactured using either jetting technology (F grade, 40-90 micron) or by classical suspension polymerization (M grade, 75-125 micron and C grade, 100-300 micron). Figure 6 shows that both technologies ensure strong and robust matrices are obtained, and show high mechanical stability compared to other commercial products.



Swelling properties in water and organic solvents

High rigidity and mechanical stability of Chromalite[®] M resins allow them to operate at pressures up to 20 bar without deformation. In fact, as shown in Figure 7, the change in bed volume between various water, salts, water miscible and non-miscible solvents is negligible.

Chromalite[®] M resins can be used in organic solvents or mixtures of organic solvents.





Figure 7. Relative swelling of Chromalite® MEP/M in different aqueous solutions (light blue), in water miscible organic solvents (blue) and in water immiscible organic solvents (dark blue) with swelling in water set as a reference at 100.

Chromalite[®] M resins are robust and highly cross-linked, thus minimizing the swelling in different media as shown in Figure 7. This minimal swelling is a benefit during chromatographic operations that require changes in elution phase. The maximum variation of swelling is 30 %, with a minimum observed in 0.02 M Kpi pH 8.0 or 0.02 M citrate pH 5.0, and a maximum observed in organic solvent as toluene.

Stability to pressure

Chromalite[®] M resins are designed to withstand high pressures, as shown in Figure 8. Testing with Chromalite[®] M resins has been conducted up to pressures of 9 MPa without loss of performance (equivalent to 90 bar or approx. 1300 psi).

In operative conditions, Chromalite® M can be used at flow rates up to 1400 cm/hr with a linear increase of pressure as shown in Figure 8, Therefore, indicating excellent stability of resin to compression without any deformation.



The graph shows that all Chromalite[®] resins have excellent pressure stability over flow and show a linear behaviour.



Figure 8. Pressure-flow comparison. Column size: 10 mm ID x 60 cm L; Mobile phase: distilled water Temperature: 25 °C.



Figure 9. Pressure-flow comparison. Chromalite® */C resins: 100-300 µm, Chromalite® */M resin: 75-125 µm, Toyopearl® HW-65C: 50-100 µm, Toyopearl® SP650M: 65 µm, Bio-Rad Nuvia™ S: 85 µm.

Nuvia resin from Bio-Rad is a gel bed made of acrylamide and vinylic polymers with a compression ratio of 1.15-1.18.

To allow a simple comparison of pressure/flow relationships it is often easier to compare backpressure over a fixed column length, generally per metre. This can be calculated from any previously determined value using the following equation:

 $\Delta P=150 \times L \times \left(\frac{\eta \times u}{d_{2}^{2}}\right) \times \left(\frac{(1-\varepsilon)^{2}}{\varepsilon^{3}}\right)$

= Mobile phase viscosity (constant)

= Interstitial void volume intrinsic to

= Empty column linear velocity (constant)

= Change in pressure

= Mean particle size

= Column Length (constant)

ΔP

1

η

u

ε

dp2

Combining the constants into one term, k, we can express the equation as:

∆P=L×k

Hence a resin that generates a back pressure of 1 bar at a column length of 10 cm would generate a back pressure of 2 bar at 20 cm. Figure 9 shows the back pressures expressed per metre of column height, with selected competition resins as a comparison.

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Batch to batch repeatability

The production of Chromalite® M resins ensures the consistency of material between batches, securing the required performance from initial laboratory trials through pilot scale up to full production. A case study is shown in Figure 10. With a target capacity of 35 g/L protein and 99.2 % protein recovery, Chromalite® MEP functionalized with a proprietary ion exchange ligand was successfully produced at 3000 L per batch scale with results consistent with initial laboratory trials.



Figure 10. Batch to batch repeatability during development and scale up of Chromalite[®] M resins. The lines represent the protein capacity (%) for laboratory and production batches respectively. The protein recovery (%) is reported by the bars in the right axis for laboratory (green columns) and production batches (purple columns).

Stability to NaOH

Chromalite[®] M resins are characterized by high physical resistance, as shown by our mechanical stability data, Figure 6. Alkaline stability is also a pre-requisite for use of chromatographic resins in biomolecule purification, since Cleaning in Place (CIP) is a routine procedure applied during the separation process to avoid resin fouling, binding of impurities and microbiological contamination.

Industrial CIP utilizes rigorous cleaning agents like 0.5 M NaOH and even 1 M NaOH.

As shown in Figure 11, Chromalite[®] M resins are fully stable to 1M NaOH treatments at room temperature and can also tolerate short treatments up to 60°C at 0.5M NaOH, thus showing excellent alkaline stability.



Figure 11. Stability of Chromalite[®] M resins to NaOH treatments. Washes conducted with 1 M NaOH at a flow rate of 1 mL/min for 5 bed volumes. Chromalite[®] MQ/M: 100-300 µm, Relisorb[™] IDA400: 75-200 µm, Toyopearl[®] Q-600C: 50-150 µm (data from Tosoh).

The stability of ligand binding to Chromalite® MEP resins was tested over NaOH treatments. Figure 12 shows that the ligand is strongly bound and do not show any leaching.

This means that the binding to epoxy groups is highly stable and does not cause any leaching, so Chromalite[®] MEP is an optimal resin to make custom ligand coupling



Figure 12. Stability of Chromalite® MEP/C functionalized with specific ligand. Particle size: 100-300 $\mu m.$

Dynamic binding capacity (DBC)

The dynamic binding capacity (DBC) is an expression of the maximum protein loading of a resin without significant losses of protein passing through the resin unbound. A resin with a low DBC will bind a lower quantity of protein before major breakthrough occurs.

Purolite[®] DBC testing is carried out using standard proteins: lysozyme for cation exchange resins and BSA for anion exchange resins. These standard proteins act as excellent models for the behaviour of other proteins and provide essential information to aid in the selection of an ion exchange resin. The breakthrough curves shown on the next page represent the standard Purolite[®] testing and show the high dynamic binding capacities that can be achieved with the Chromalite[®] methacrylic chromatography range.





Figure 13. Breakthrough chromatograms for Chromalite® M resins.

Column size: 5 mm ID x 20 cm L; Sample concentration: 5 mg/mL BSA (66 kDa) or 2.5 mg/mL lysozyme (15 kDa); Loading buffers: BSA 0.02 mol/L Tris-HCl (pH = 8.5); Lysozyme 0.02 mol/L citrate (pH = 5.0) Flow rate: 50 cm/h (double check); Detection: UV @ 280 nm The breakthrough point is directly related to the dynamic binding capacity quoted for these resins, with a 10% breakthrough generally used as the signifier of a completely loaded resin. The gradient of the breakthrough curves relates to the pore size distribution and the size of the model protein.

For example, BSA (bovine serum albumin) is a larger protein (66 kDa) than lysozyme (14 kDa), hence it diffuses into small pores more slowly than lysozyme. This means that the breakthrough increases more gradually, as the diffusion gradient is shallower than for lysozyme and small pores will fill with BSA more slowly, meaning that some BSA will pass through the column despite available pores for binding. By comparison, lysozyme is much smaller and therefore has an increased diffusion gradient, meaning that it is less likely to pass through the column in the presence of available binding sites.

This results in a breakthrough curve where no lysozyme passes through until all binding sites are full, at which point all lysozyme passes through, hence the very steep breakthrough curves.

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In antibiotic purification, chromatographic separation occurs sequentially using anion exchange chromatography (Chromalite® MDEA), hydrophobic interaction chromatography (e.g. Chromalite® PCG1200M) and anion exchange chromatography to purify a preparation containing lipopeptides. Figure 14 shows the process.

Figure 14. – Flow process showing industrial separation of antibiotics



Chart: International Labour Organization

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		-15.X E		
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		15.x c		• •
		15.X C		• •
		15.x c		

Preparation for Packing

We recommend to pack Chromalite® M resins at a pressure from 0.5 to 3 bar (7 to 45 psi) across the bed length. Recommended columns for packing are glass, acrylic or stainless-steel columns. Chromalite® M resins can be packed by simple gravitational settling, although it is preferable to use pressure for bed consistency. For optimal performance, pack the resins at a high flow rate and pressure.

Chromalite® M resins are provided with strict control of fines, especially for materials manufactured using Jetting technology. Removal of fines is not required, and resins are ready-to-use as provided. Magnetic or manual stirring of settled resin or a packed bed may damage the resin and generate fines. Re-suspension of Chromalite[®] M resin material before use can be achieved by gently shaking the container before opening. Packed column beds can be removed by gently pumping liquid into the column with the opposite end open.

Chromalite® M resins are supplied in 20 % EtOH and should be packed in 20 % EtOH. Chromalite® M resins are supplied ready-to-use and no pre-treatment is necessary besides suspension in 20 % EtOH.

Equilibration of resins in the buffers

The optimal buffer for packing depends on the application. We recommend to equilibrate and pack in the buffer with the highest ionic strength that will be used for the separation.

Table 3 shows a list of suggested typical buffers for packing.

Chromalite [®] MEP	Chromalite [®] MS	Chromalite [®] MCM	Chromalite [®] MDEA	Chromalite [®] MQ
1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM
phosphate, pH 7	citrate, pH 5	citrate, pH 5	Tris, pH 8	Tris, pH 8
1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM	1 M NaCl in 50 mM
Tris, pH 7	acetate, pH 5	acetate, pH 5	phosphate, pH 8	phosphate, pH 8

Table 3. Typical buffers recommended for packing Chromalite® M resins

Preparation of resin slurry

The slurry concentration is calculated as the volume of settled resin divided by the total volume of the slurry, and the slurry concentration is adjusted as follows:

- a) Resuspend the resin slurry and transfer the homogeneous slurry to a graduated cylinder
- b) Allow the slurry to settle (approx. 1 hour) for best results.
- c) Determine the settled resin volume and adjust the slurry concentration to 30 - 50 % by adding or removing packing buffer.
- d) For packing a column of a given volume, use approximately 1.2 x the column volume of resin.

Ion exchange purification of biological molecules like proteins and peptides is generally highly selective, due to minor differences in molecule structure resulting in significant differences in retention. This facilitates the use of relatively short columns, should the technique be used to its fullest extent. We recommend the use of bed heights between 5 and 15 cm for the lowest backpressure and highest productivity.

Best practice will utilize columns with good distribution of flow at the inlet and outlet of the column, as well as a pressure stability in excess of 0.3 mPa (3 bar, 43 psi). Filters to prevent the flow of particles should also be in place to prevent increases in backpressure. Additional considerations for column selection regarding microbial control may also be required if strict controls on sterility are necessitated by the process.

Elution

The elution step involves an increase in the ionic strength of the elution buffer, either continuous or stepwise. This increase in ionic strength weakens the interaction between adsorbent and bound molecule, resulting in elution from the column. pH gradient-based elution is usually unsuccessful due to the buffering effects of both adsorbed molecules, and for weak ion exchangers, the functional groups on the resin. Stepwise pH-based elution can be more successful, even as the change in pH is delayed slightly from the buffer front – caused by these buffering effects.

A linear gradient is the most common tool for initial screening. This is followed by a stepwise -gradient-stepwise method once the approximate concentration of eluent that desorbs the molecule(s) of interest is identified, and can be selected for the shallow gradient. This approach results in the highest resolution of the molecule(s) of interest and the highest speed and productivity of the overall process.

Rather than using pH-based elution, the standard method exploits the high ionic strength of a sodium chloride solution. Typically, a gradient will be used up to a maximum of 0.5 - 1.0 M over roughly 10 column volumes. For scale-up processes, a stepwise approach is usually preferred, owing to the reduced complexity and higher reproducibility. It also reduces buffer consumption and increases productivity, as well as raising the concentration of the final product.

Recommended buffers can be found in Table 4. and Table 5.

Table 4. Suggested buffers for use with Chromalite® MDEA and MQ

Buffer Concentration	Concentration	рКа (25 °С)	Buffering Range
L-Histidine	20 mM	6.0	5.5-6.0
Imidazole	20 mM	6.95	6.2-7.8
Piperazine	20 mM	5.3,9.7	5.0-6.0, 9.5-9.8
bis-Tris	20 mM	6.5	5.8-7.2
Triethanolamine	20 mM	7.8	7.0-8.3
Diethanolamine	20 mM	8.9	8.4-8.8
Tris	20 mM	9.5	7.0-9.0
Glycine	20 mM	9.9	7.5-8.9
Piperidine	20 mM	11.1	10.5-12.0
Ethanolamine	20 mM	9.5	6.0-12.0

Table 5. Suggested buffers for use with Chromalite® MCM and MS

Buffer Concentration	Concentration	рКа (25 °С)	Buffering Range
Citrate 50 mM	20 mM	3.1	4.2-5.2
Formate 50 mM	20 mM	3.8	3.0-4.5
Acetate 50 mM	20 mM	4.8	4.8-5.2
MES 50 mM	20 mM	6.2	5.5-6.7
Phosphate 50 mM	20 mM	7.2	6.7-7.6
HEPES 50 mM	20 mM	7.6	7.6-8.2
BICINE 50 mM	20 mM	8.4	7.6-9.0

Chromalite[®] M resins are suitable for use in laboratory columns at flow rates up to 2000 cm/h and are compatible with all major HPLC and FPLC systems. This equipment is suitable for both small-scale preparatory applications and for initial screening experiments (Table 6). Table 6. Packing and operating conditions for use of Chromalite[®] M resins in laboratory scale using a column 1.0 cm (ID) x 20.0 cm (L).

Resin	Grade	Particle size (micron)	Packing velocity flow rate (cm/h)	Operating velocit (cm/h)	y ml/min
	F	40 - 90	450-600	100-300	1.3 - 3.9
Chromalite [®] MEP, MQ, MDEA, MS and MCM	Μ	75 - 125	900-1200	100-600	1.3 - 7.8
MS and MCM	С	100 - 300	900-1200	100-600	1.3 - 7.8

On scale-up, the increased restrictions on pressure of the larger equipment and challenges of process control mandate the use of reduced flow rates, generally in the range of 300-1000 cm/h linear velocity. For packing of process columns, we recommend packing at a linear velocity of 1.5 x the intended operating velocity. For example, a column of 1 m ID and 2 m length, intended to run at 300 cm/hr would be packed at minimum 450 cm/hr or 3.9 mL/min.

Placing your order

How to order

Resin Volume	Container	
25ml resin	75ml bottle	
100ml resin	250ml bottle	
500ml resin	1 litre bottle	
1 litre resin	1 litre bottle	
5 litres resin	10L Jerrycan	
10 litres resin	20L Jerrycan	

Chromalite® M Ordering Information

Chromalite ®	Product
Chromalite® MS/F	LS04412
Chromalite [®] MS/M	LS04422
Chromalite® MS/C	LS04432
Chromalite® MQ/F	LS04111
Chromalite [®] MQ/M	LS04121
Chromalite® MQ/C	LS04131
Chromalite® MCM/F	LS04512
Chromalite® MCM/M	LS04522
Chromalite® MCM/C	LS04532
Chromalite® MDEA/F	LS04311
Chromalite® MDEA/M	LS04321
Chromalite® MDEA/C	LS04331
Chromalite [®] MEP/F	LS04010
Chromalite [®] MEP/M	LS04020
Chromalite [®] MEP/C	LS04030

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