

Silica Gel and Chemical Bonding for Peptide and Protein Purification

Reversed phase HPLC is very effective in peptide and protein separation and purification. AGC offers a wide range of spherical silica gels bonded with various chemistries to meet the separation and purification needs of the peptide and protein industries.

Chromatographic fine silica M.S.GEL™ is used in pharmaceutical and biotechnology industries for identification, separation, and purification of components.

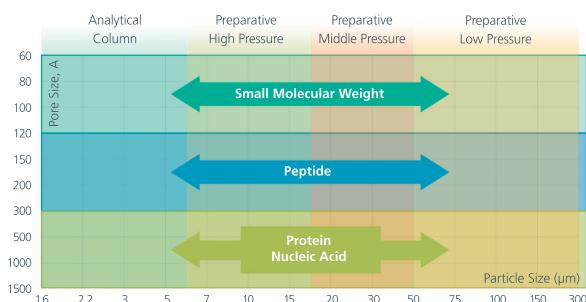
- Purification of peptides, oligonucleotides, and antibiotics (coloradolcin, benzanthrins, rosarimicin)
- Purification and analysis of synthetic biological components
- Separation and purification of medicines such as sedatives, analgesics, anesthetics, and steroids
- Separation of vitamins; identifying/quantifying amino acids, triglycerides, and sugar content
- Identification of molecular components such as nucleic acids, fats, carbohydrates, proteins, and vitamins

M.S.GEL™ Silica GEL

Without a good silica foundation, accurate, consistent results cannot be obtained.

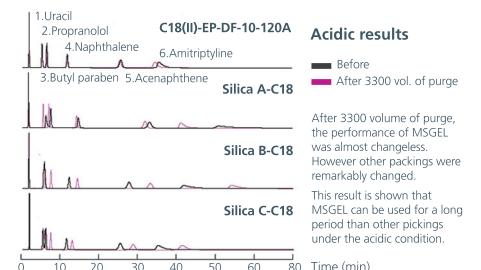
AGC's M.S.GEL™ range is found below.

Property	Benefit
Perfect spherical shape	Leads to better performance, low back pressure
Narrow particle size distribution	Better resolution and consistency
Free from balloon particle	Consistency, accuracy
High mechanical strength and chemically stable (pH range 2~11)	Long lasting, durable
Purity range of SiO ₂ is 99.9 – 99.99%	No impurity interference

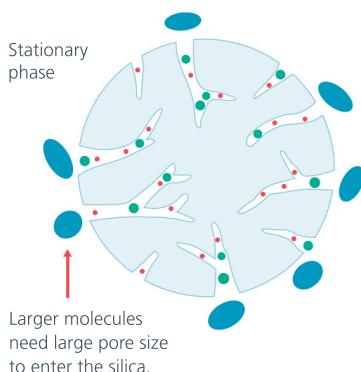


Chemical Stability

Chemical stability is very important for consistent and accurate separation/purification results. M.S.GEL™ fine silicas have very good stability over a pH range of 2-11.



Key Components: Chemical Bonding, Silica Pore Size and Particle Size



Principles of the Separation Process

It is important to understand the properties of each component:

- Size
- Hydrophobic/hydrophilic
- Polarity (+/-)
- Other (affinity/chirality)

Selecting the chemical bonding, particle size, and pore size

The selection process is dependent on the properties of the components:

- The chemical bonding/surface treatment and physical properties of the silica are optimized separately.
- Chemical bonding type is typically selected first
- After the chemical bonding is selected, the particle size and pore size of the silica is then considered.



Chemical Bonding

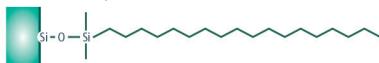
AGC offers various chemistries that can be bonded to our silica. For the best separation efficiencies of peptides and proteins, it is important to select not only the particle/pore size silica but also the right chemical bonding/ligand. In selecting the best ligand, there are many factors to consider:

- Molecular weight
- Hydrophobicity
- Isoelectric point*
- Combination of mobile phase

* Isoelectric point is the pH where a molecule has no electric charge.

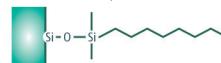
ODS (C18)

Separation/purification of pharmaceuticals, nutraceuticals, cannabis/CBD oil, acidic, neutral, and basic compounds



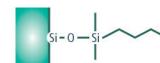
C8

Separation/purification of pharmaceuticals, acidic, neutral, and basic compounds



C4

Peptides and proteins



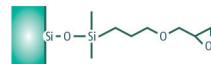
Diol

Separation / purification of pesticides, herbicides, pharmaceutical metabolites, natural products (polar), peptides, proteins, and other polar biomolecules.



Epoxy (Glycidoxypipropyl)

Separation/purification of insulin; chiral HPLC



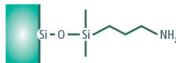
Phenyl

Aromatic compounds, antibiotics, lipids, ring-structured compounds



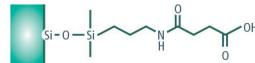
WCX CM (Carboxymethyl)

Separation/purification of proteins, protein aggregates, charge isomers of monoclonal antibodies, PEGylated proteins, and peptide digests.



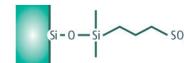
NH2 (Aminopropyl)

Sugars, alcohols, vitamins, nucleosides, oligonucleotides, and anionic compounds



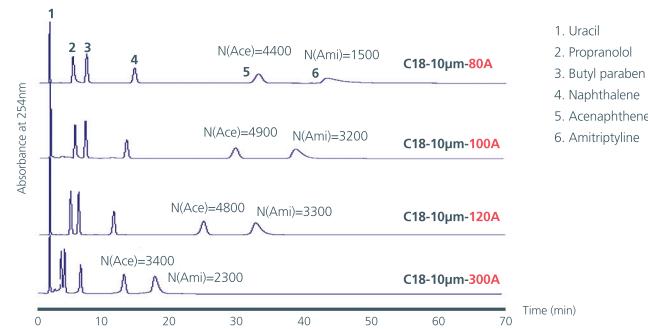
SCX SP (Sulfopropyl)

Amine and polyamine containing compounds, nucleotides, and peptides



Pore Size and Particle Size

Effect of pore size on separation



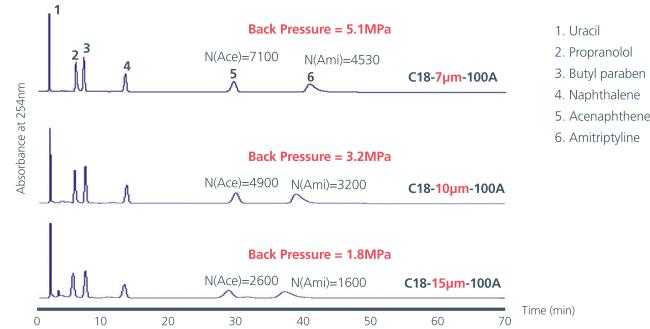
Conditions: Column:4.6mmx150mm, Eluent:65:35 methanol /20mM KH₂PO₄/K₂HPO₄ (pH7.0). Flow rate:1.0mL/min, Detection:UV254nm at 23°C.

- AGC SI offers pore size ranging from 60A to 1500A. For peptide and protein separation, common pore sizes range between 60A to 300A.
- Optimum pore sizes are dependent on the MW of the peptides and proteins:

MW range	100 - 5,000	200 - 10,000	5,000 - 40,000	>20,000
Optimum pore size	60A - 100A	120A	200A	300A

- Selecting the correct pore size is crucial for high resolution and high yields.

Effect of particle size on separation



Conditions: Column:4.6mmx150mm, Eluent:65:35 methanol /20mM KH₂PO₄/K₂HPO₄ (pH7.0). Flow rate:1.0mL/min, Detection:UV254nm at 23°C.

In general:

- AGC SI offers a wide range of silica 1.6 – 300μm particle size. For peptide and protein separation, common particles sizes range from 3 to 10μm.
- The smaller the particle size, the better the separation efficiency.
- This can be observed above, as pore size remains constant, the separation and peak performance is improved with smaller particle sizes.
- Back pressure is increased with smaller particle sizes.
- M.S.GEL™ grades have a narrow particle size distribution which prevents/reduces back pressure.

AGC

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