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 (coloradocin, benzanthrin, rosamycin)
- 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.

<table>
<thead>
<tr>
<th>Property</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfect spherical shape</td>
<td>Leads to better performance, low back pressure</td>
</tr>
<tr>
<td>Narrow particle size distribution</td>
<td>Better resolution and consistency</td>
</tr>
<tr>
<td>Free from balloon particle</td>
<td>Consistency, accuracy</td>
</tr>
<tr>
<td>High mechanical strength and chemically stable (pH range 2-11)</td>
<td>Long lasting, durable</td>
</tr>
<tr>
<td>Purity range of SiO&lt;sub&gt;2&lt;/sub&gt; is 99.9 – 99.99%</td>
<td>No impurity interference</td>
</tr>
</tbody>
</table>

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 (Glycidoxypropyl)
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, F(ab)2ylated proteins, and peptide digests.

NH2 (Aminopropyl)
Sugar, alcohol, vitamin, nucleosides, oligonucleotides, and anionic compounds

SCX SP (Sulfopropyl)
Amine and polypeptide containing compounds, nucleotides, and peptides

Pore Size and Particle Size

Effect of pore size on separation

<table>
<thead>
<tr>
<th>MW range</th>
<th>Optimum pore size</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 - 5,000</td>
<td>60A - 100A</td>
</tr>
<tr>
<td>200 - 10,000</td>
<td>120A</td>
</tr>
<tr>
<td>5,000 - 40,000</td>
<td>200A</td>
</tr>
<tr>
<td>&gt;20,000</td>
<td>300A</td>
</tr>
</tbody>
</table>

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:

Effect of particle size on separation

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.

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