Improved Adsorption and Purification of Active Pharmaceutical Compounds

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Purolite Life Sciences
Over three decades, Purolite has grown into the world’s premier resin technology manufacturer and innovation leader, with production plants and advanced research labs across the globe.

- 1,400 Employees
- 45+ Global Sales Offices
- 4 Production Plants
- 5 R&D Centers
Purolite Life Sciences offers the widest portfolio of high-quality chromatographic resins, enzyme carriers, ion exchange resins, APIs, adsorbents, and specialty resins.

We develop standard and customized solutions for our customer’s needs.
Resin Technology Experts

36 years experience & 100% focused on resin innovation.

Purolite Life Sciences offers you the highest level of resin technology expertise and process understanding available.

Access to our team of industry-leading experts, who are here to assist you with any challenge.

We believe in one-on-one customer support.
25 Years' Regulatory Expertise

Producing pharmaceutical APIs in our FDA-inspected cGMP production site.

Production is carefully controlled to ensure that our products meet the stringent criteria of highly regulated industries.
What are adsorbents

1. Spherical polymeric resins with polystyrenic or polymethacrylate based matrices
2. Stable in various solvents and wide pH ranges
3. Particle size from 3 microns (analytical reversed phase) to 1 mm (industrial size separators)
4. Highly porous structure with high surface area and selective size exclusion of large molecules
5. Highly hydrophobic — replaces solvent extraction applications with minimum waste
### Interaction factors between resin and molecule

<table>
<thead>
<tr>
<th>FORCES AVAILABLE</th>
<th>STRENGTH (KJ/MOL)</th>
<th>DISTANCE (NM) BETWEEN RESIN AND MOLECULE</th>
<th>POLYMERIC RESIN STRUCTURE NEEDED</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>van der Waals</td>
<td>0.4 – 4.0</td>
<td>0.3 – 0.6</td>
<td>Any interaction such as</td>
<td>Weakest interaction: controls molecule orientation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>electrostatic bonding</td>
<td></td>
</tr>
<tr>
<td>Hydrogen bonds</td>
<td>12 – 30</td>
<td>0.3</td>
<td>Hydroxyl or primary amine</td>
<td>Strong interaction with polar and protic molecules</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>groups</td>
<td></td>
</tr>
<tr>
<td>Ionic interactions</td>
<td>20</td>
<td>0.25</td>
<td>Sulfonate, carboxylate and</td>
<td>Interaction with ionizable groups of molecules; Interaction is driven by pH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>amine groups</td>
<td></td>
</tr>
<tr>
<td>Hydrophobic interactions</td>
<td>&lt; 40</td>
<td>Varies</td>
<td>Polystyrenic functionality</td>
<td>Strong interaction with molecules having aromatic groups or alkyl chains</td>
</tr>
</tbody>
</table>
# Advantages and disadvantages of Synthetic Adsorbent as a Purification Tool

## Mechanism

**SYNTHETIC ADSORBENT**
- Hydrophobic interaction; (Solid phase extraction)

**SOLVENT EXTRACTION**
- Difference of solubility toward two solvents

**CRYSTALLIZATION**
- Solubility change with temperature

## Advantage

**SYNTHETIC ADSORBENT**
- Easy to scale up
- Chromatographic separation enable to separate multicomponent
- High recovery
- Various system can be applied to give best efficiency
- Non-solvent system can be developed

**SOLVENT EXTRACTION**
- Availability of abundant basic data
- Low cost of equipment

**CRYSTALLIZATION**
- Availability of basic data
- Easy to give high purity

## Disadvantage

**SYNTHETIC ADSORBENT**
- High cost of equipment
- Large amount of eluent is sometimes required

**SOLVENT EXTRACTION**
- Heavy use of organic solvent
- Multi-step extraction is required for multi-component separation

**CRYSTALLIZATION**
- Molecule which does not crystallize can not purified.
- High cost of energy

## Application examples

**SYNTHETIC ADSORBENT**
- Fermentation broth
- Plant extracts

**SOLVENT EXTRACTION**
- Fermentation products
- Synthetic medicine

**CRYSTALLIZATION**
- Synthetic medicine
- Sugar

## Capital cost

**SYNTHETIC ADSORBENT**
- Sometimes high

**SOLVENT EXTRACTION**
- Medium

**CRYSTALLIZATION**
- Low
PORE EFFECT of an adsorbent

Larger molecules (purple figures) are excluded from entry into the resin due to pore size.

Smaller hydrophobic molecules are adsorbed onto the surface (orange figures).

Hydrophilic small molecules such as salts (blue figures) freely flow through the structure of the resin.
## Features of PuroSorb™ and Macronet® adsorbent based on chemical structure

<table>
<thead>
<tr>
<th>POLYMER MATRIX</th>
<th>FEATURES</th>
<th>PHYSICAL PROPERTIES</th>
<th>CORRESPONDING PAD AND MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic: Polystyrene crosslinked with divinylbenzene</td>
<td>Widely used in food and pharmaceutical applications. Hydrophobic strength and capacity increase with surface area.</td>
<td>Moderate hydrophobicity Specific gravity = 1.01 to 1.03</td>
<td>PuroSorb™ PAD350 PuroSorb™ PAD400 PuroSorb™PAD550 PuroSorb™PAD600 PuroSorb™ PAD900 PuroSorb™PAD1200 Macronet™ MN200 Macronet™ MN202 Macronet™ MN250 Macronet™ MN270</td>
</tr>
<tr>
<td>Aromatic: Modified brominated polystyrene</td>
<td>Polishing organic substances at low concentrations. Bromination gives higher specific gravity, enabling its use in up-flow operations.</td>
<td>High hydrophobicity Specific gravity = 1.18 to 1.20</td>
<td>PuroSorb™ PAD428</td>
</tr>
<tr>
<td>Aliphatic: Methacrylate</td>
<td>more hydrophilic than polystyrene; but hydrophobic for very hydrophobic compounds</td>
<td>Low hydrophobicity Specific gravity = 1.09 to 1.11</td>
<td>PuroSorb™ PAD610 PuroSorb™ PAD950</td>
</tr>
<tr>
<td>Functionalized Aromatic: Ion exchange</td>
<td>Low number of functional groups as sulfonic groups or dimethyl amine for easy regeneration with acids or bases.</td>
<td>Low hydrophobicity Specific gravity = 1.05 (weakly basic) to 1.20 (strongly acidic)</td>
<td>Macronet™ MN100 Macronet™ MN102 Macronet™ MN500 Macronet™MN502</td>
</tr>
</tbody>
</table>
## Typical characteristics of PuroSorb™ and Macronet® adsorbents

<table>
<thead>
<tr>
<th>RESIN</th>
<th>POLYMER MATRIX</th>
<th>PORE DIAMETER (Å)</th>
<th>PORE VOLUME (mL/g)</th>
<th>SURFACE AREA (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAD350</td>
<td></td>
<td>50</td>
<td>0.7</td>
<td>550</td>
</tr>
<tr>
<td>PAD550</td>
<td></td>
<td>130</td>
<td>1.7</td>
<td>950</td>
</tr>
<tr>
<td>PAD600</td>
<td>Polystyrene cross-linked with DVB</td>
<td>90</td>
<td>1.3</td>
<td>850</td>
</tr>
<tr>
<td>PAD400</td>
<td></td>
<td>360</td>
<td>1.0</td>
<td>700</td>
</tr>
<tr>
<td>PAD900</td>
<td></td>
<td>220</td>
<td>1.9</td>
<td>850</td>
</tr>
<tr>
<td>PAD1200</td>
<td></td>
<td>400</td>
<td>1.7</td>
<td>600</td>
</tr>
<tr>
<td>MN200</td>
<td></td>
<td>700</td>
<td>0.4</td>
<td>1,100</td>
</tr>
<tr>
<td>MN202</td>
<td></td>
<td>220</td>
<td>0.3</td>
<td>950</td>
</tr>
<tr>
<td>MN250</td>
<td></td>
<td>380</td>
<td>0.6</td>
<td>1,100</td>
</tr>
<tr>
<td>MN270</td>
<td></td>
<td>80</td>
<td>0.5</td>
<td>1,200</td>
</tr>
<tr>
<td>PAD610</td>
<td>Methacrylic polymer</td>
<td>300</td>
<td>1.2</td>
<td>490</td>
</tr>
<tr>
<td>PAD950</td>
<td></td>
<td>120</td>
<td>0.6</td>
<td>450</td>
</tr>
<tr>
<td>PAD428</td>
<td>Polystyrene cross-linked with DVB functionalized with bromine</td>
<td>100</td>
<td>1.0</td>
<td>600</td>
</tr>
<tr>
<td>MN100</td>
<td>Polystyrene cross-linked with DVB functionalized with strong acid cation exchanger</td>
<td>650</td>
<td>0.4</td>
<td>1200</td>
</tr>
<tr>
<td>MN102</td>
<td>Polystyrene cross-linked with DVB functionalized with weak base exchanger</td>
<td>350</td>
<td>0.4</td>
<td>800</td>
</tr>
<tr>
<td>MN500</td>
<td></td>
<td>750</td>
<td>0.2</td>
<td>500</td>
</tr>
<tr>
<td>MN502</td>
<td></td>
<td>650</td>
<td>0.3</td>
<td>660</td>
</tr>
</tbody>
</table>

PAD600, PAD400, PAD900, and PAD1200 are polydivinyl benzene with no polystyrene present.
Important aspect of the resin matrix

Solubility is a measure of hydrophobicity therefore:

- Brominated Styrenic > Styrenic > Methacrylic

Expected Capacity would also be as follow (best with PAD428):

- Brominated Styrenic > Styrenic > Methacrylic

Expected regeneration efficiency (best with PAD610 and PAD950):

- Brominated Styrenic < Styrenic < Methacrylic
Chromalite particle size selection

<table>
<thead>
<tr>
<th>Chromalite size range</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 micron</td>
<td>Analytical (HPLC)</td>
</tr>
<tr>
<td>10, 15 micron</td>
<td>Preparative (HPLC)</td>
</tr>
<tr>
<td>15, 35, 50, 75 micron</td>
<td>Process/polishing of proteins, small molecules and synthetic biomolecules, recombinant proteins, front-end capture, desalting, purification, SPE</td>
</tr>
<tr>
<td>75 and 150 micron</td>
<td>Large scale purification, desalting, polishing of proteins and synthetic biomolecules</td>
</tr>
</tbody>
</table>
Particle size effect on resolution

PCG1200F15
15 μm

PCG1200F
35 μm

PCG1200M
75 μm

PCG1200C
120 μm

Plates

>3000

>1800

>900
Control of porosity for efficient separations

Source 15 RPC
D50=14.7um, UC=1.00
SA: 423 m2/g
PV = 0.62 ml/g
Porosity: 200-1000 A

PCG1200F15
D50= 15.2 um, UC=1.04
SA: 730 m2/g
PV = 1.42 ml/g
Porosity: 1200 A
Relationship between pore size and molecule size

DBC at 50% represents 50% of the compound binding on the resin when using a 5/50mm column at 2.4 (flow 0.42mL/min) or 6 min (flow 0.17mL/min) compound residence time. The liquid phase is 0.1% trifluoroacetic acid in water.
The relationship between paracetamol recovery and pore diameter for the tested resins. Agilent Bond-Elut ENV result not shown for clarity; trendline based on Purolite Chromalite sorbents (excluding PCG1200MHEMA) only.

*Paracetamol*: non steroid antiflammatory drug (NSAID)
Separation of small APIs & Peptides

**Conditions**
Column: 25x250mm  
Room temperature  
Detection: UV 1monitor, 220 nm  
Flow rate 7.5 ml/min  
Eluent «A»: 10mM citric acid, 10% ethanol, water for injections.  
Eluent «B»: 50% ethanol  
Sample: Mixture of bradykinin, bacitracin, gramicidin C

**Comparison of results**
peak 1 – Bradykinin (MW1059)  
peak 2 – Bacitracin (MW 1421)  
peak 3 – Gramicidin C (MW 1140)

PCG600 and PCG900 are optimal for small/medium molecules
Polymeric compared to silica - Peptides

Separation of hexa- (1st peak) and octapeptide (2nd)

Very strong 15 microns polymeric resin

Performances of 15 micron are same as 5 micron silica
Performance comparison between PCG1200F15 15 µm and C8 Silica (15 µm) – Insulin Purification

**Column:** 25x250 mm column

**Adsorbent:** PCG1200F15 (15 µm)

**Sample:** 10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin

**Buffer A:** 0.1 M acetic acid / 10% ethanol in water

**Buffer B:** 50% ethanol, water
Enzyme immobilization

Macroporous styrene

Hydrophobic interaction

Adsorbed enzyme
Process for cephalosporins manufacture

**Fermentation**
- Cephalosporin C

**Extraction**
- Cephalosporin C

**Purification 1**
- PAD700 (scavenger)
- PAD550 (adsorber)
- IEX (decolorization)

**Enzymatic hydrolysis 2 (min. 1000 cycles)**
- 7-ACA

**Enzymatic synthesis 3 (500 cycles)**
- beta-lactams derivatives of Cephalosporin C (i.e. Cephatriaxon, Cephazolin)

**Purification 4**
- beta-lactams derivatives of Cephalosporin C (i.e. Cephatriaxon, Cephazolin)

**Enzyme immobilization**
- Immobilized CCAC

**Isolation**
- Enzyme CCAC

- Fermentation

**Enzyme immobilization**
- Immobilized SynPGA

**Isolation**
- Enzyme SynPGA

- Fermentation

**Extraction**
- Cephalosporin C

**Purification 1**
- PAD428 (polisher) OR PAD550 (polisher) OR MN270 (polisher)

**Enzymatic hydrolysis 2 (min. 1000 cycles)**
- 7-ACA

**Enzymatic synthesis 3 (500 cycles)**
- beta-lactams derivatives of Cephalosporin C (i.e. Cephatriaxon, Cephazolin)

**Purification 4**
- beta-lactams derivatives of Cephalosporin C (i.e. Cephatriaxon, Cephazolin)

**Cefalexin**
Process for penicillins manufacture

Fermentation

Penicillin G

Enzymatic hydrolysis 5 (min. 1000 cycles)

Immobilelized EcPGA ECR8205 (small beads)

Enzyme immobilization

6-APA

Enzymatic synthesis 6 (500 cycles)

Immobilized SynPGA ECR8205 (big beads ECR8405 (big beads)

Enzyme immobilization

beta-lactams derivatives of Penicillin G (i.e. amoxicillin or ampicillin)

Re-crystallization

beta-lactams derivatives of Penicillin G (i.e. amoxicillin or ampicillin)

Isolation

Fermentation

Ampicillin

6-APA

Amoxicillin
Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure

Selective removal of toxins with adsorbent
Elution improvement with solvation parameters

![Graph showing the effect of solvents on resin swelling.](image)

Resin Swelling (% of its maximum swelling)

Solvent concentration (% in water)

- Methanol
- Ethanol
- 2-Propanol
- Acetone

Steam < caustic wash < methanol < ethanol < Isopropanol or n-propanol

Purolite®
Jetting Technology
Two methods of producing uniform particle size beads

Classical Jetting
• Bottom vibrating plate
• Large volume production
• ~180 – 600 µm direct
• < 1.1 UC (uniformity coefficient)
• Simple operation

Cross Flow Membrane (CFM) / Can Jetting
• Oscillating (can) membrane
• Small volume capacity
• ~15 - 250 µm direct
• < 1.2 UC (uniformity coefficient)
• Patent pending
• Simple operation
Thank you!
Purification Challenges — Think Purolite First!

Questions?

Purolite®
Booth 80K10