Optimization on Pore Size of C8-Bonded Silica Reversed-Phase for Insulin Purification
DAISOGEL C8-Modified Phases
Designed for Insulin Purification

- 200 Å Enlarged Pore Diameter
  Suitable for Medium-Weight Molecules, e.g. Insulin

- Available in 10-20 μm Spherical Particles
  having Ultra-High Purity
  High Resolution and Excellent Peak Shape
  Applicable for Preparative Purification

- High Surface Bonding Density (C8 > 3.5 μmol/m²)
  Combined with New Endcapping Technology (Patented)
  Improvement in Phase Stability against
  Extreme Acidic and Alkaline Environments
Bare Silica Gel Matrixes
Pore Distribution Region Comparison

Differential Pore Volume

Cumulative Surface Area

Incremental pore volume was estimated according to B.J.H. method from desorption branch of N₂-physisorption isotherm at 77K.

Cumulative surface area was estimated according to B.J.H. method from adsorption branch of N₂-physisorption isotherm at 77K.
DAISOGEL SP-200-10-C8-BIO
Improvement in Phase Stability

Column Dimension: 6 mm I.D. x 250 mm Length;
Mobile Phase: CH₃CN/1% TFA (pH=1) = 10/90;
Temperature: 70°C; Flow: 0.9 ml/min; Time for Purge: 20 h.

Column Dimension: 6 mm I.D. x 250 mm Length;
Mobile Phase: CH₃CN/20mM Na₃PO₄-NaOH (pH=12) = 10/90;
Temperature: 40°C; Flow: 1.7 ml/min; Time for Purge: 5 h.
Loading Capacity Evaluation

Column Dimension: 4.6 mm I.D. x 250 mm Length

- **Adsorption**
  - Equilibration: 0.5% TFA aq.;
  - Solute: Human Insulin (Recombinant, Wako, Japan);
  - Concentration: 10 mg/ml;
  - Feed: Solute Dissolved in 0.5% TFA aq.;
  - Flow Rate: 0.5 ml/min; Temperature: 30°C; Detector: UV 290 nm.

- **Desorption/Elution**
  - Equilibration: 0.5% TFA aq.;
  - Elution: CH$_3$CN/H$_2$O/TFA = 30/70/0.1;
  - Flow Rate: 0.5 ml/min; Temperature: 30°C; Detector: UV 300 nm.
Loading Capacity Evaluation
Conceptional Illustration of Theoretical Adsorption-Elution Isotherm

Feed (Insulin 10 mg/ml in TFA aq.)

→ Adsorption

Equilibration

→ Elution

30% CH₃CN Added

Insulin Adsorbed

Breakthrough

Insulin Eluted

UV Absorbance

Time on Stream
Loading Capacity of Insulin Breakthrough Curve Comparison

SP-120-10-C8-BIO
SP-200-10-C8-BIO
K-Sil-100-10-C8
Loading Capacity of Insulin
Expanded Pore Diameter Correlation

Optimum Pore Range for Insulin Molecule
19 - 25 nm

Loading Capacity
= \( F \times C_0 \times \frac{T_b}{CV} \)

F: Flow Rate;
C_0: Concentration;
T_b: Breakthrough Time;
CV: Column Volume.
Cumulative surface area was estimated according to B.J.H. method from adsorption branch of N$_2$-physisorption isotherm at 77K.
Alkaline Durability Evaluation

Column Dimension: 4.6 mm I.D. x 250 mm Length

- **Alkalic Durability Evaluation**
  Mobile Phase: Ethanol/0.1 N NaOH \(aq\). \((pH=13)\) = 70/30;
  Flow Rate: 2.0 ml/min; Temperature: ambient.
  Elution was collected every 50 CV and Si dissolved was analyzed by ICP.

- **Chromatographic Evaluation after Alkali Purged**
  **Aromatic Standards**
  Mobile Phase: \(CH_3OH/H_2O = 60/40\) (100 Å) or 55/45 (200 Å)
  Flow Rate: 1.0 ml/min; Temperature: 40ºC; Detector: UV 254 nm;
Alkaline Durability (pH=13)  
SP-200-10-C8-BIO for Aromatic Standards
Alkaline Durability (pH=13)
K-Sil-100-10-C8 for Aromatic Standards
Alkaline Durability (pH=13)
Comparison for Aromatic Standards

Theoretical Plates

Peak Asymmetry

SP-200-10-C8-BIO
K-Sil-100-10-C8
Alkaline Durability (pH=13)
Comparison of Si Dissolved in Mobile Phase

![Graph showing comparison of Si dissolved in mobile phase for two different materials: SP-200-10-C8-BIO (red bars) and K-Sil-100-10-C8 (black bars). The x-axis represents purged column volume in ml, ranging from 50 to 250, and the y-axis represents Si dissolved in mobile phase (mg), ranging from 0 to 250. The graph illustrates the Dissolution of Si in the mobile phase as a function of the purged column volume.]
Summary

- **SP-200-C8-BIO** exhibited the highest loading capacity for insulin estimated adsorption breakthrough point.

- The optimum pore size for insulin molecular diffusion seems to be 19 - 25 nm.

- **SP-200-C8-BIO** also exhibited better durability in alkaline (pH=13) environment even compared with K-Sil-100-10-C8.

- **SP-200-C8-BIO** seems to be the best packing material for insulin purification.