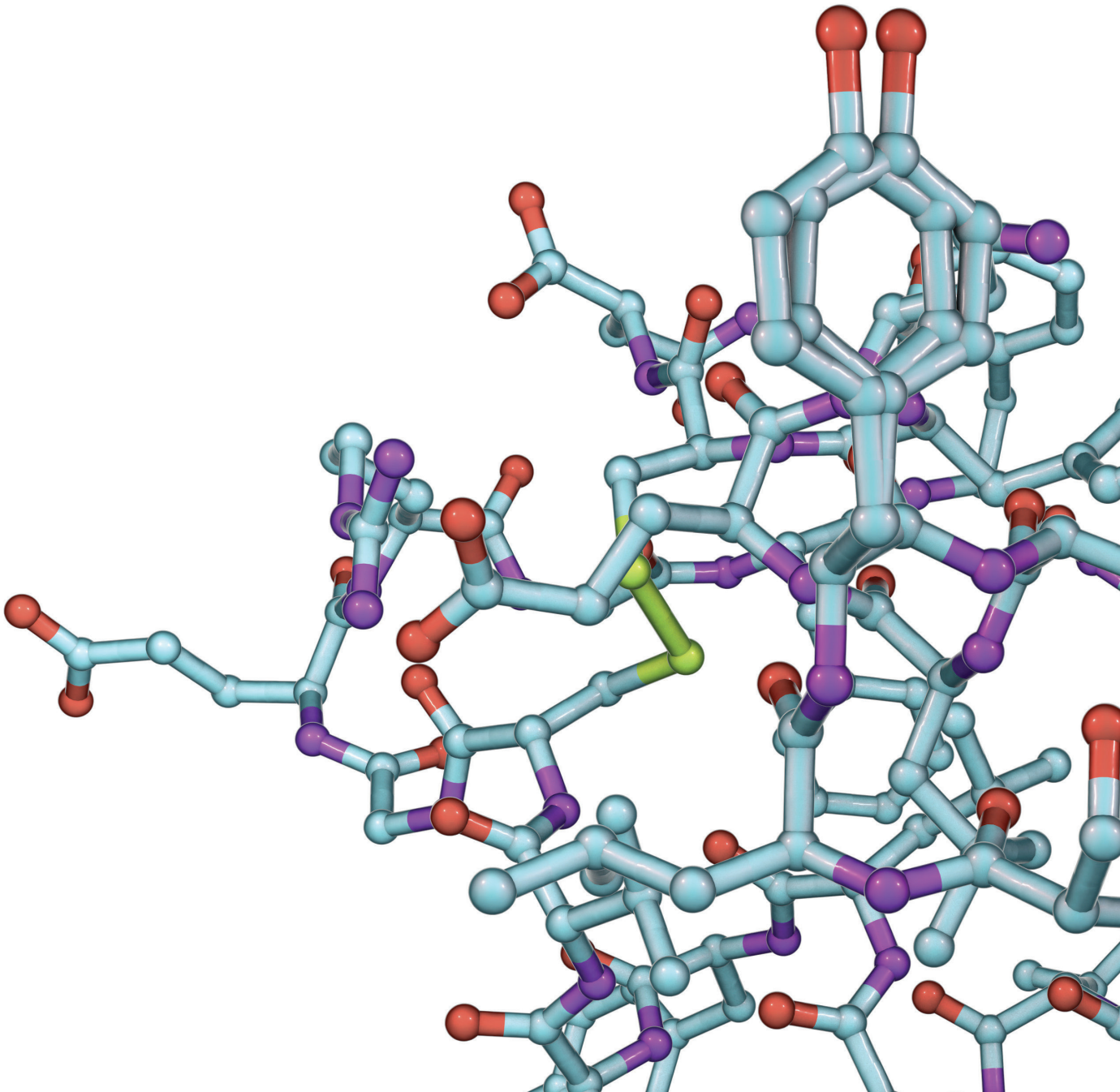
DAISOGEL
Purity. Sustained.

FOR THE PURIFICATION OF RECOMBINANT PEPTIDES

PK SERIES SILICA GEL



ABOUT DAISOGEL

DAISOGEL is a leading bulk silica gel used in the chromatographic analysis and purification of active pharmaceutical ingredients and other high-value substances. It falls within the Pharmaceutical Related business of our parent company OSAKA SODA Co., Ltd.

DAISOGEL is proudly made in Japan under the direction of our dedicated manufacturing team at our Amagaski factory outside of Osaka.

Our products are manufactured under ISO 9001 controlled conditions using our leading-edge sol-gel method with GMP compliance based on ICH-Q7A.

Our superior manufacturing standards guarantee DAISOGEL is produced with the highest quality and purity to meet and exceed expectations of any major pharmaceutical company audit worldwide.

We have FDA Drug Master File registration for the following top-selling DAISOGEL phases:

- **File# 23227 for DAISOGEL ODS series**
- **File# 22317 for DAISOGEL C8 series**
- **File# 29201 for DAISOGEL C4 series**

Regulatory Support Files are also available upon request.

OUR COMMITMENT

Our quality is paramount in providing a consistent product to you. With locations in Japan, the US, and Europe, we also pride ourselves in our deep technical support built from over 40 years of chromatography experience.

We look forward to work alongside you to optimize your purification process with reliable products and support that will solve problems today and endure into the future, as embodied in our three core principles:

CONSISTENCY

QUALITY

SUSTAINABILITY

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MOLECULES WE SERVICE

PK SERIES ●

SMALL MOLECULES

CANNABINOIDS

FISH OIL / EPA

SYNTHETIC PEPTIDES

RECOMBINANT PEPTIDES / INSULIN

RECOMBINANT PROTEINS / EPO

PURITY. SUSTAINED.

Purification of synthetic and recombinant peptides has always been challenging. Recent advances in peptide synthesis and fermentation processes have resulted in even more complex impurity mixtures, as modern peptide therapeutics are prone to dimerization, self-aggregation, and fibrillation.

At DAISOGEL, we recognized the need for a silica gel that resolves these impurities in a sustainable, cost-effective manner. A silica gel robust enough to withstand the rigors of alkaline Cleaning-In-Place. A silica gel whose mechanical strength would preclude the generation of fines and hence sustain consistent operating pressures during large-scale purification in dynamic axial compression (DAC) columns. And a silica gel whose loading capability simply outperforms other similar medias on the market, providing the best economics for large scale peptide purification.

The PK Series has been developed based on our holistic design concept of chemical stability, mechanical strength, and high performance to provide you with the ultimate in sustainable purity.

ALKALINE DURABILITY
OF THE PK SERIES BONDED PHASES

Cleaning-In-Place (CIP) is a widely used procedure to remove self-aggregated, fibrillated peptides from the inlet side of the silica bed in the column. This procedure must be repeated periodically to maintain resolution, separation of peaks, and low column backpressure.

For cleaning severely blocked columns, the dual action of NaOH to cleave the fibrils and the desorption by the organic solvent (such as acetonitrile) is ideal. While the high pH 13 of NaOH cleans the column well, it historically damages the silica gel particles as well. Our PK Series solves this issue though our improved alkaline durability of the bonded silica.

The PK Series alkaline durability was tested by washing the columns with a 0.1 M NaOH CIP solution and monitoring the subsequent change in column backpressure.

DAISOGEL SP-100-10-C8-PK demonstrated the lowest increase in column backpressure compared to other silica gels, as seen in **Figure 1.0**.

Figure 2.0 depicts a test in which the actual column backpressure of various silica gels was monitored over the course of hundreds of column volumes of 0.1 M NaOH washes. DAISOGEL SP-100-10-C8-PK demonstrated the lowest column backpressure of the silica gel cohort.

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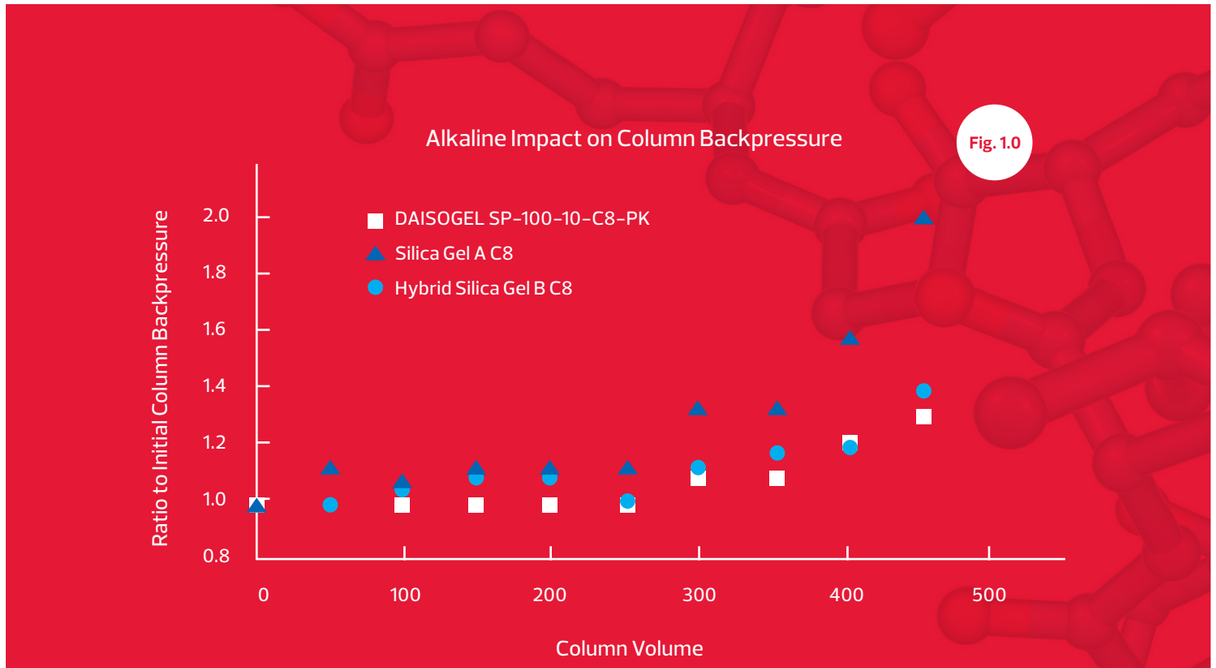
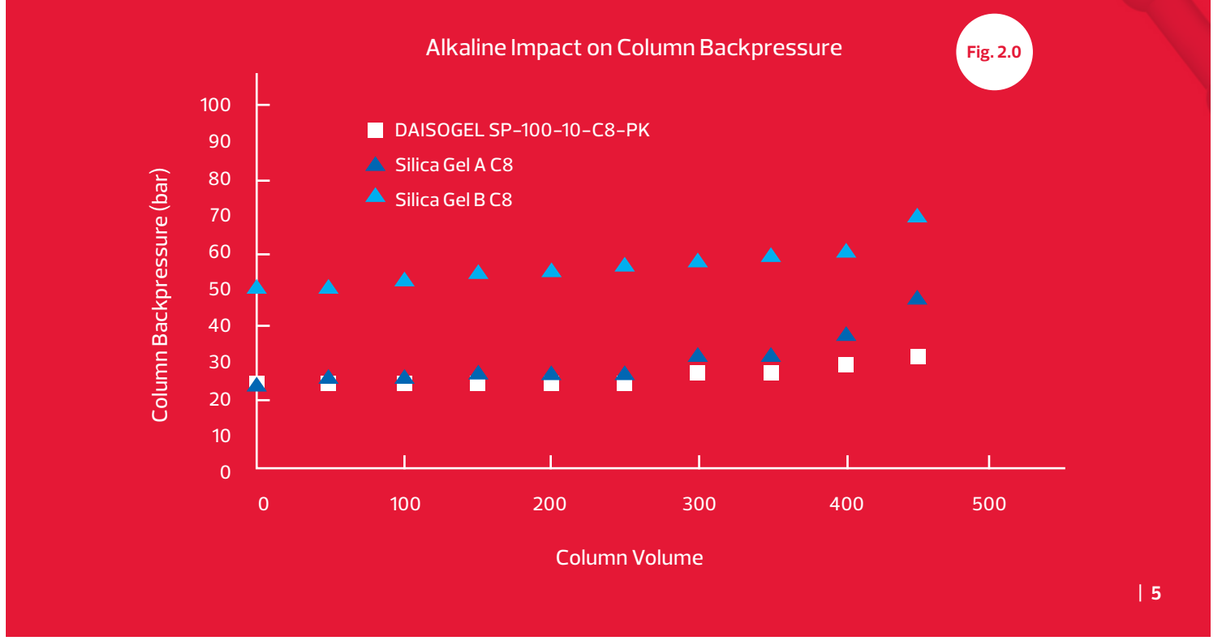


In both Fig. 1.0 and 2.0, DAISOGEL SP-100-10-C8-PK showed the lowest column backpressure.

Testing Conditions

Column Dimension: 4.6mm ID X 250mm L
Alkaline Condition: 100 mM NaOHaq/EtOH = 30/70, pH 13
Flow Rate: 2.0 mL/min
Temperature: 25°C

Note: All silica brands tested in this experiment were 10 µm in average particle size as measured by Osaka Soda.



ALKALINE DURABILITY
OF THE PK SERIES BARE SILICA

While silica gel has a naturally narrow pH tolerance range, not all bare silicas are created equal. During the development of the PK Series, we noticed significant variation in alkaline durability among different bare silica gel products and took steps to ensure that the PK bare silica gel was as alkaline-tolerant as possible.

To evaluate alkaline durability, we tested various bare silica gels by passing a weak NaOH solution across pre-packed columns until the column backpressure ratio spiked – an indicator of particle breakage and fine migration within the column.

Figure 3.0 illustrates the alkaline durability results of various bare silica gels compared to our SP-100-10-PK unbonded silica. SP-100-10-PK demonstrated the highest alkaline durability, enduring more than 170 column volumes of weak NaOH washing prior to showing any meaningful backpressure increase.

MECHANICAL STRENGTH
OF THE PK SERIES BARE SILICA

It is well known that packing, unpacking and re-packing bulk silica gel in DAC columns can mechanically stress the silica particles and reduce the media lifetime. Hence, it is critically important to employ a bare silica gel that is mechanically strong.

To that end, we have optimized one important step in the manufacturing process of our PK Series silica gel: calcination. This controlled thermal treatment results in a much stronger bead; in fact, the mechanical strength of the PK Series is simply superior to all other spherical silica gel-based products. This enhances its ability to withstand repeated DAC column packings and unpackings, resulting in longer media lifetime and reduced costs for you.

A common stress test to measure mechanical strength of a silica gel is to compress the dry silica for a given time and pressure and then determine the percentage of broken particles by visual inspection with a microscope. We compared a leading competitor spherical silica gel to our new SP-100-10-PK unbonded silica using this method. The competitor's silica gel suffered 13% broken particles, but our new SP-100-10-PK unbonded silica experienced only 11% broken particles, or 15% fewer broken particles than the competitor silica gel.

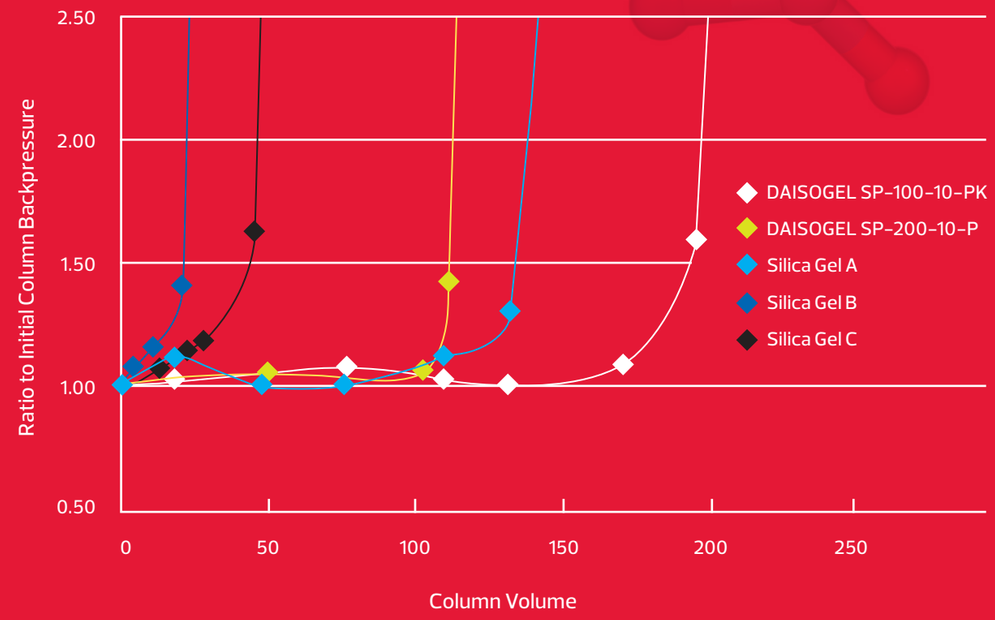
Dry silica compression stress test

15%

FEWER BROKEN PARTICLES
with PK Series silica gel

Alkaline Durability of Bare Silica Gel

Fig. 3.0



LOADABILITY
OF THE PK SERIES BONDED PHASES

Loadability is critically important when designing a scaled-up active pharmaceutical ingredient (API) purification process. Your process economics greatly depend on how much API you can load and purify in one run.

To demonstrate the superior loadability of our new PK Series silica gel, we performed a binding capacity and breakthrough study using insulin. We packed small columns with various silica gels typically used for insulin purification. We equilibrated each column with a mobile phase including an organic modifier. We then fed the columns with the same organically-modified mobile phase but now containing 10 mg/mL recombinant human insulin. The insulin is retained on the stationary phase until the column is saturated, at which point the insulin breaks through and is detected by UV monitoring. Longer breakthrough times equate to higher binding capacity for insulin and, hence, higher loadability.

As shown in **Figure 4.0**, the SP-100-10-C8-PK showed the highest binding capacity and loadability compared to three other common insulin purification silica gels.

PK Series provides the highest binding capacity and loadability for recombinant human insulin.

Testing Conditions

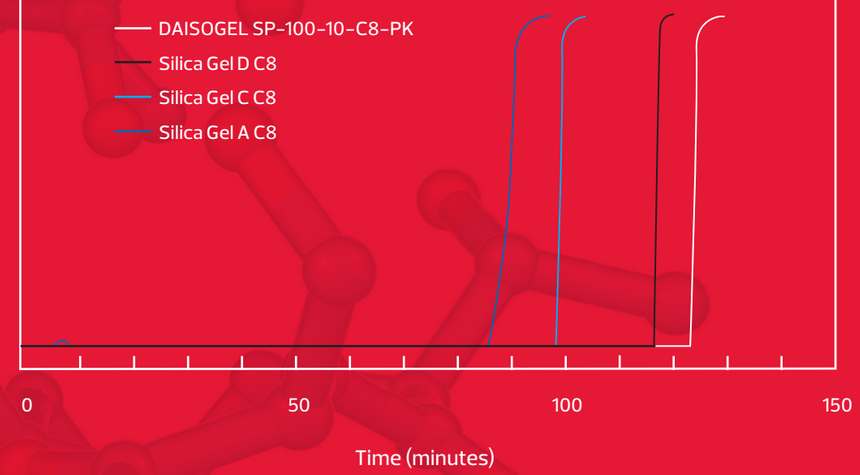
Column Dimension: 4.6 mm ID X 250 mm L
Adsorption Equilibration: ACN / 0.5% TFA aq.=10/90
Solute: Human Insulin (Recombinant, Wako, Japan)
Concentration: 10 mg/mL
Feed: Solute dissolved in ACN / 0.5% TFA aq.=10/90

Flow Rate: 0.5 mL/min
Temperature: 30°C
Detector: UV 290 nm
Dead Volume: 9 min

Note: All silica brands tested in this experiment were 10 µm in average particle size as measured by Osaka Soda.

Recombinant Human Insulin
Breakthrough on Various Silica Gels

Fig. 4.0



Product Name	Breakthrough Time (min)
SP-100-10-C8-PK	124
Silica Gel D C8	117
Silica Gel C C8	99
Silica Gel A C8	86

RESOLUTION
WITH THE PK SERIES BONDED PHASES

Resolution is undoubtedly of critical importance for your separation. The resolving power of your silica gel is one of the key elements to drive the highest recovery of your molecule.

To demonstrate the resolving power of our PK Series silica gel, we compared the ability of SP-100-10-C8-PK to separate a prominent API from an impurity against a competitor's top C8 silica gel.

To generate the sample, a pure insulin solution was left overnight on the benchtop, thereby enriching the solution with a ubiquitous impurity, des-amido insulin. We then injected the impurity-enriched insulin sample onto the two columns and measured the R_s factors of each. The R_s factor is a quantitative indicator of resolution, or how far apart the insulin and des-amido peaks are pulled apart. Results are shown in **Figure 5.0** and **Figure 6.0**. The SP-100-10-C8-PK showed better separation between the two peaks and higher R_s, thereby confirming its better resolution.

For an insulin separation

22.5%

HIGHER R_S than the competitor

Testing Conditions

Column Dimension: 4.6 mm ID X 250 mm L
Mobile Phase: ACN/$H_2$0/TFA = 30/70/0.1
Flow Rate: 1.7 mL/min
Temperature: 30°C
Detector: UV 220 nm

Solute: Hydrolyzed Human Insulin (Recombinant, Wako, JP)
Source: Human Insulin cDNA Expressed in Yeast
Concentration: 5 mg/mL
Injection: 5 mL

Note: All silica brands tested in this experiment were 10 µm in average particle size as measured by Osaka Soda.

Fig. 5.0 DAISOGEL SP-100-10-C8-PK
R_s = 1.85

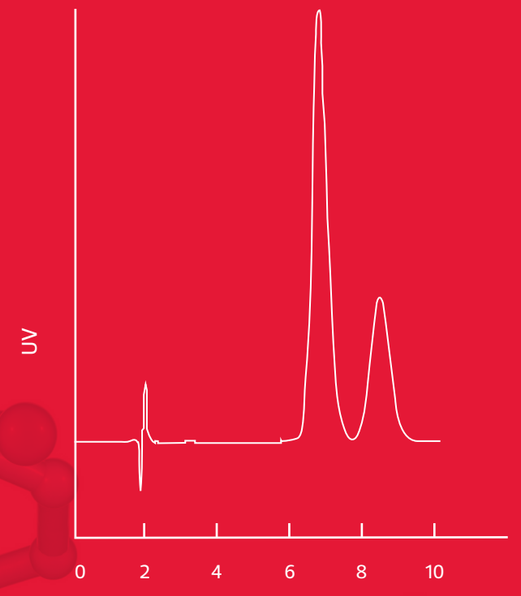
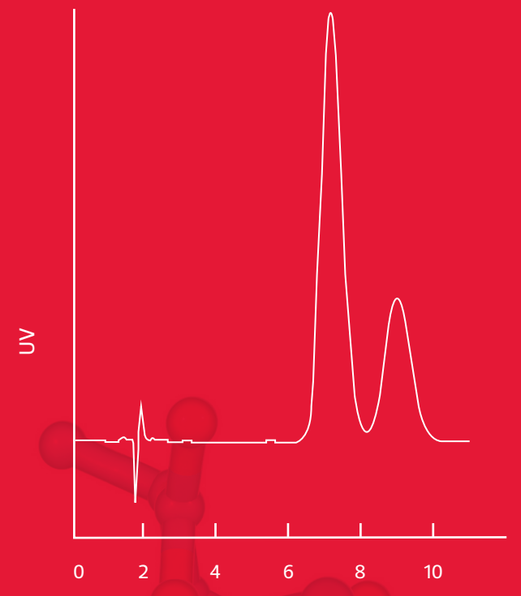


Fig. 6.0 Silica Gel A C8
R_s = 1.51



$$R_s = 2(t_2 - t_1)/(w_1 + w_2)$$

t_1: Retention Time of Former Peak
t_2: Retention Time of Latter Peak
w_1: Width of Former Peak
w_2: Width of Latter Peak

OVERLOAD
OF THE PK SERIES BONDED PHASES

For real-world API separations at preparative or large-scale, OSAKA SODA understands that you will employ "overload" conditions in order to maximize your batch recovery and yield. This is also why overloading is our primary parameter for testing the performance of PK Series bulk silica gel.

We evaluated the overload capability by measuring the R_s factor when separating an insulin peak from the des-amido insulin impurity peak under increasingly overloaded conditions, comparing SP-100-10-C8-PK to a competitor's top C8 media.

Figure 7.0 shows the results on SP-100-10-C8-PK, still providing R_s of 1.82 at a 50 µL overload injection.

Figure 8.0 shows Silica Gel A C8 with lower R_s of 1.68 at the same injection volume.

The higher R_s translates to a larger amount of API that can be purified in one run. This is a very important consideration for process economics and determining the productivity of your purification process.

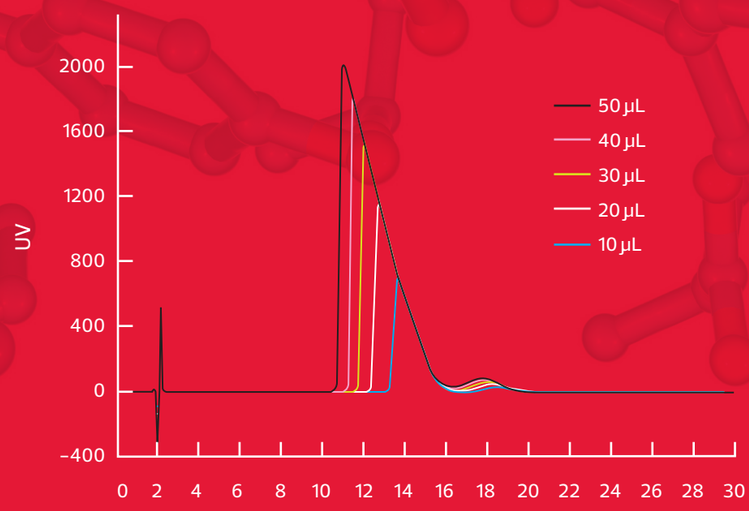
For an insulin overload separation

8.3%

HIGHER R_S than the competitor

Fig. 7.0

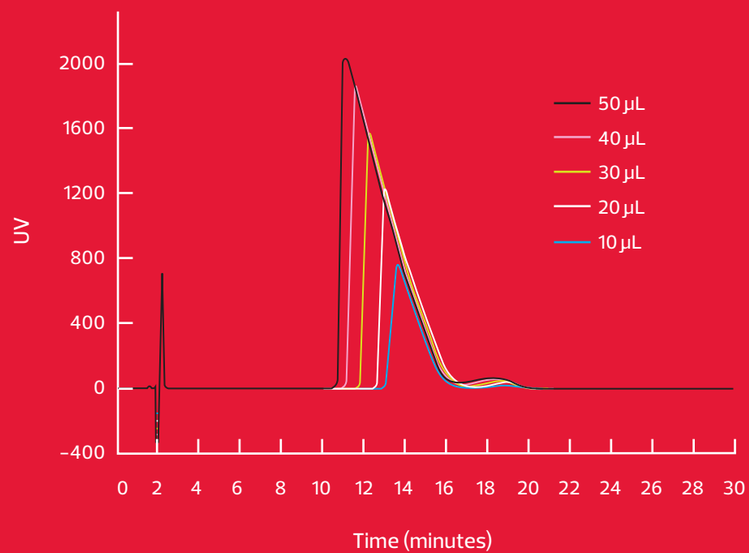
DAISOGEL SP-100-10-C8-PK
Feed concentration was 10 mg/mL insulin



Injection (µL)	R_s
10	2.21
20	2.11
30	2.01
40	1.94
50	1.82

Fig. 8.0

Silica Gel A C8
Feed concentration was 10 mg/mL insulin



Injection (µL)	R_s
10	2.04
20	1.98
30	1.89
40	1.77
50	1.68

EXTRACTABLES + LEACHABLES

OF THE PK SERIES BONDED PHASES

Since reversed-phase stationary phases are typically used as the final polishing step in the downstream purification of a peptide-based API, it is of utmost importance that the API does not become contaminated by the gel during the step. It is incumbent on the silica gel manufacturer to ensure that the extractables and leachables profile of their bonded phase is well characterized under different extraction conditions.

The silica backbone is naturally very inert, therefore nothing can be extracted or leached from it. The only potential concern arises from the chemical compounds that are bonded to the silica surface. Here we look at the C8 bonded phase: the octyl silane (C8 ligand) and its dimer.

The quality of the manufacturer's bonding chemistry, bonding process, and washing process can be established in an extraction test. A smaller amount of ligand extracted during the test corresponds to a higher quality bonding process.

In **Figure 9.0**, we show the results of a standard toluene extraction for SP-100-10-C8-PK and a competitor's top C8 phase. The PPM of methoxylated C8 monomer and dimer were measured. The PK Series product demonstrated a significantly lower PPM concentration of both the monomer and the dimer.

The amount of extractables and leachables also depends on the mobile phase composition used during the purification process. Therefore, we tested the extractables of SP-100-10-C8-PK under various extraction conditions that mimic typical mobile phase profiles.

As seen in **Figure 10.0**, SP-100-10-C8-PK had a lower extractables PPM of monomer and dimer than the competitor when extracted with n-Propanol under acidic conditions.

Fig. 9.0 Extractables in Standard Toluene Extraction

Product Name	Concentration (PPM) Methoxylated C8 Monomer	Concentration (PPM) Methoxylated C8 Dimer
SP-100-10-C8-PK (toluene 100%)	97.34	10.51
Silica Gel A C8 (toluene 100%)	249.04	49.29

Experimental Procedure: 1 gram of bonded silica from each product was extracted with 5 mL of toluene. The same detection method as described in Fig. 10.0 was used.

Fig. 10.0 Extractables Under Various Extraction Conditions

Product Name	Concentration (PPM) Methoxylated C8 Monomer	Concentration (PPM) Methoxylated C8 Dimer
SP-100-10-C8-PK		
1. 0.1% TFA (pH=2.0): n-propanol = 40:60	0	0
2. 0.1% TFA (pH=2.0): acetonitrile = 40:60	0	0
3. 100 mM $(NH_4)_2SO_4/H_2SO_4$ (pH=3.2): n-propanol = 40:60	94.98	5.79
4. 100 mM $(NH_4)_2SO_4/H_2SO_4$ (pH=3.2): acetonitrile = 40:60	0	0
5. 0.1 M NaOH (pH=13.0): n-propanol = 40:60	510.24	0
6. 0.1 M NaOH (pH=13.0): acetonitrile = 40:60	438.56	0
7. 20 mM NH_4OAc / CH_3COOH, 20 mM NH_4Cl: n-propanol = 40:60	91.62	5.77
8. 20 mM NH_4OAc / CH_3COOH, 20 mM NH_4Cl: acetonitrile = 40:60	66.38	0
Standard C8 Ligand Mixture (40 mg/L)	40.00	40.00
Silica Gel A C8		
1. 100 mM $(NH_4)_2SO_4/H_2SO_4$ (pH=3.2) + n-propanol	106.81	5.98

Experimental Procedure: 5 grams of bonded silica from each product was extracted using 50 mL of the above mobile phases.

1. Filtration (remove silica)
2. Evaporation of organic solvent
3. Add 50 mL of toluene
4. Wash by separation funnel (50 mL of H_2O x 3)

Detection Method

GC: GC-2010 (Shimadzu)
Column: CBP-1-M25-025 (Shimadzu)
Temp.: 150°C (1 min.)→10°C/min.→320°C (2 min.)
Carrier gas: N_2

DAISOGEL
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ORDERING INFORMATION

Product Name	Bonded Phase	Pore Size (Å)	Particle Size (µm)	Pore Volume (mL/g)	Surface Area (m^2/g)	% of Carbon
SP-100-8-ODS-PK	C18	100	8	0.9	320	18.0
SP-100-10-ODS-PK	C18	100	10	0.9	320	18.0
SP-100-8-C8-PK	C8	100	8	0.9	320	11.0
SP-100-10-C8-PK	C8	100	10	0.9	320	11.0
SP-100-8-C4-PK	C4	100	8	0.9	320	8.0
SP-100-10-C4-PK	C4	100	10	0.9	320	8.0

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