Column Packings for High Performance
Liquid Chromatography
TSKgel LLC/LSC/IEC Type

TSKgel
PACKING MANUAL

TOSOH CORPORATION

Safety Precautions

To help protect your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product.

[Notational Conventions]

Notation	Explanation
⚠WARNING	Alerts the user to the potential for serious injury or death.
⚠CAUTION	Alerts the user to the potential for damage to hardware or bodily harm.

⚠ WARNING

■ **Keep away from fire.**

Take proper precautions when using flammable solvents. There is the potential for fire, explosion, or poisoning.

⚠ CAUTION

■ **Use only in well ventilated areas.**

In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

■ **Do not spill solvents.**

Spillage and leakage can cause fire, electric shock, poisoning, injury, and corrosion. When cleaning up a spill, wear appropriate protective gear.

■ **Wear eye protection and protective gloves.**

Organic solvents and acids should not come in direct contact with the skin.

■ **Handle package with care.**

Inappropriate handling may cause rupturing and splattering.

■ **Only use this product as intended.**

This product is for separation and purification, do not use for any other purpose.

■ **Confirm compounds are safe.**

Check that obtained compounds and solutions after separation and purification are safe.

■ **Proper disposal.**

Dispose of in accordance with local laws and regulations.

NOTE

Keep this manual for future reference.

CONTENTS

1. Introduction

The TSKgel bulk materials have been optimized for HPLC. These materials were designed for analytical separation of various chemicals. They comprise 30 grades covering LLC, LSC and IEC. Their specifiifications are given in Table 1. Please read this packing manual carefully and pack the column correctly in order to make effective use of its high performance.

2. General information on packing

In the high-performance liquid chromatography, it is necessary to pay a good deal of attention to the column size and the packing method in order to obtain the maximum performance of the packing material employed. To obtain high resolution in the liquid chromatography, its is necessary to pack the gel in the column as uniformly and compactly as possible. Further, it is necessary that the packing is carried out under optimum conditions selected in accordance with various physical properties of the gel such as the particle diameter, particle shape, density, rigidity, etc.

3. Column size and packing temperature

3.1 Column diameter

In the column chromatography,the column diameter has much to do with the resolution and,if the column diameter is too small,separation behaviors will be disturbed by the wall effect of the inner wall of the column.Generally speaking, it is proper to use columns which inner diameter is at least 4mm.

3.2 Packing temperature

In the liquid chromatography,packing the column at the same temperature as the operating temperature of the column will generally produce better results because the gel volume and the density of packing solvent are subject to change with the temperature. As the particles of TSKgel are uniformed in size for ease of packing,you can carry out the packing at the room temperature (25℃ or higher). However,if the operating temperature of the column is substantially higher or lower than the packing temperture,it could degrade the column the performance ; so, it is necessary to pack the column at temperature less different from such operating temperature.

4. Preparation of gel slurry

The packed condition of gel has much to do with the separation. How to prepare the slurry of TSKgel in order to obtain the maximum separation performance is described as below.

4-1 The porous polymer types of TSKgel as shipped are in the swelled condition through imbibition of distilled water of a mixed solution of water and methanol, and silica types of TSKgel as shipped are in the dry condision.

4-2 In order to produce a uniformly fluidal slurry,add the slurrying solvent as listed in Table 2 and, after stirring well with a medicine spoon, transfer the slurry into a beaker, and settle down the gel.If fine particles float in the solvent,remove them by decantation.Make it sure to completely remove floating fine particles by repeating decantation.

4-3 Again add the slurrying solvent, stirr well,and pour the slurry into a measuring cylinder. Then,after allowing the gel to sufficiently precipitate,take a necessary volume which shall be approximately 1.2 times the volume of the column to be packed with the gel.

4-4 Prior to pouring the gel into the column,wash and displace the gel with the slurrying solvent. To wash the gel,set a glass filter (spec. G-4) on a suction bottle, as illustrated in Fig.1, and after sufficiently dispersing the gel in the packing solvent on the glass filter, filter out the solvent by sucking. You can wash the gel quickly and satisfactorily by repeating this operation 3 to 5 times.

4-5 Using the same type solvent,prepare the washed gel into a slurry having a right concentration of gel and, at this time, deaerate the gel slurry and the solvent thoroughly in order to drive out air from pores of the gel. This deaeration and the dispersion of gel in the solvent will be carried out simultaneously in about 5 minutes through subjection to ultrasonic waves (generally, 22 to 28 kHz). For porous polymer type gels, select a solvent whose characteristic to swell the gel is slightly less than that of the solvent to be used in the measurement (separation), in order to utilize the characteristic of these gels to swell and shrink in the solvent.It is important that the solvent used as above shall have a good affinity with the gel and also that the gel shall be thoroughly dispersed in the solvent.

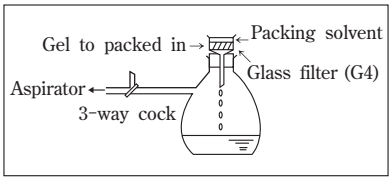

Fig. 1 Gel washing apparatus

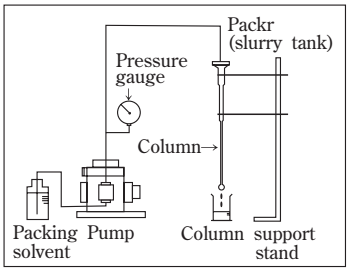

Fig. 2 Setup of packing apparatus

5. Packing method

5-1 As illustrated in Fig.2,set up Solvent Reservoir, Pump, Slurry Reservoir, Column, etc.

5-2 Prior to starting the packing operation,set the pump's flow rate with the eluent(packing solvent). The optimum flow rate for packing varies depending on the grade and particle size of gel, the column size,the type of slurrying solvent, and the gel concentration in the slurry. It is necessary that the lineal velocity of the solvent for packing is greater than the flow velocity of solvent at which the packed column is used for actual measurement. (Flow the packing solvent at a velocity at least two times the velocity of solvent you will flow for measurement.)

5-3 After giving a good stirring to the gel slurry for uniform gel concentration, carefully transfer the entire volume of the slurry into Gel Packer in one uninterrupted pouring operation, paying attention not to let air bubbles enter the slurry. (If the introduction is made in two or more pours, there will be two or more gel layers one over another in the introduced slurry causing the gel concentration in the slurry to become less uniform.) Also,make it sure to completely remove air bubbles if any from the introduced slurry by lightly tapping the packer and the column several times because presence of even a few small bubbles will cause degradation of the packed column.

5-4 Connect the lead pipe from the pump to the packer and flow the packing solvent at the optimum flow rate to the column via the packer. Because unevenness in the flow rate of the solvent will cause the column efficiency to degrade, do not change the velocity of flow during the packing. So, the pump shall be a constant-flow type.

5-5 In order to equilibrate the gel bed in the column, flow the packing solvent until the volume of effluent from the column exceeds the sum of the internal volume of the column and the internal volume of the packer.

5-6 Then,stop the pump and, after ascertaining that the pressure gauge reads 0MPa, detach the column from the packer.Then, introduce small volume of gel remaining in the packer into a separate end fitting up to the level same as the column top and quickly attach the end fitting to the column top.

5-7 Take out the last residue of gel from the packer into a beaker and keep it in the slurried condition in the beaker for future use.

5-8 Mount the packed column on a chromatographic measuring apparatus (so that the measuring solvent will flow in the same direction as the solvent flow when the column was being packed) and conduct a column efficiency test by an ordinary method,by referring to Table 3. To exchange the packing solvent with the measuring solvent for this test, flow the measuring solvent of a volume of at least 3 times the column volume at a flow rate not exceeding the flow rate for measuring.

5-9 Should the packed column fail to satisfy the efficiency test,detach the outlet end fitting of the column and pump out the gel from the column.Either repack this pumped out gel after washing,or keep it in the slurried condition for future use.In the case of porous polymer type gel, remember that the pore size will be possible to change if dried.To repack the column with the pumped out gel,make it sure to completely remove fine gel particles floating in the solvent by repeating decantation.

5-10 If the column packed as above has failed to satisfy the efficiency test, repack the column by changing the flow rate of the packing solvent. Whether the packing has been carried out satisfactorily or poorly can be judged by the shape of the elution curve peak. Generally,where the peak is leading,slow down the flow rate. Where the peak is tailing, either speed up the flow velocity or decrease the slurry concentration.

5-11 In the case of ion exchange gels,increasing the salt concentration is effective in remedying the trailing peak.

6. Caution for packing

6-1 As the back pressure of the pump will build up during the packing operation, pay good attention to tightening of all connections on the packing apparatus.

6-2 As the packing solvents are mostly harmful to the human body, conduct the packing at a well ventilated location.

7. Remarks

For any questions regarding the contents of this manual or any additional information,kindly contact your local representative of TOSOH.General packing conditions and column efficiency test conditions of TSKgel are attached hereto as reference data.For examples of analysis using TSKgel packed columns, see our catalogs and data books which will be sent to you upon request.

References

Table 1 TSKgel (Bulk materials)

(1) Polymer gels for partition adsorption

Type name	Type description	Application
TSKgel Styrene-250	Styrene gel	Reversed-phase chromatography : pharmaceuticals, surface active agents, etc.
TSKgel Styrene-60	Styrene gel	
TSKgel Acetate-60	Vinyl acetate gel	normal phase chromatography : vitamins, etc.
TSKgel Ether-250	Polyethylene glycol gel	

(2) Polymer gels for ion exchange

TSKgel SCX	Strong cation exchange gel	Amion acids, polyamines, nucleosides
TSKgel SAX	Strong anion exchange gel	Carbonic acids
TSKgel Suger AX	Strong anion exchange gel	Monosaccharides, Disaccharides, Suger-alcohol

(3) Silica gels for adsorption

TSKgel Silica-150	Spherical silica gel	Steroids, aromatic fatsoluble vitamins
TSKgel Silica-60	Spherical silica gel	

(4) Chemically bonded type silica gels for partition adsorption

TSKgel ODS-120A	ODS bonded Silicagel (C_{18})	Medium and low molecular weight chemicals, Drugs, Peptides, and Proteins
TSKgel ODS-120T	Fully end capped, ODS bonded silicagel (C_{18})	Peptides, Low molecular weight proteins
TSKgel ODS-80TM	Fully end capped, ODS bonded silicagel (C_{18})	Medium and low molecular weight chemicals, Drugs, Peptides, and Low molecular weight proteins
TSKgel TMS-250	Fully end capped, ODS bonded silicagel	High molecular weight proteins
TSKgel NH_2-60	NH_2 bonded silicagel	Saccharides
TSKgel OH-120	OH bonded silicagel	Steroids
TSKgel Amide-80	Amide bonded silicagel	Saccharides, sugar-alcohol

Packed units of above gels are :

a) TMS-250 ····· 10 μm (5g)
b) Amide-80 ····· 5 μm (5g, 10g)
c) Sugar AXI ····· 8 μm (5g)
d) Sugar AXG ····· 10 μm (5g)
e) Others ····· 5 μm (5g, 10g), 10 μm (10g)

Table 2 TSKgel packing conditions

Particle dia. of gel : 5 μm
Column : 4.6 mm ID 25cm

Type	Slurry concentration (%)	Slurry solvent	Packing solvent	Packing flow rate (mL/min)	Packing pressure (kg/cm^2)	Remark
TSKgel Styrene-250	35～45	aq*1 : MeOH 6 : 4	aq*1 : MeOH 6 : 4	1.2～1.8	13～20	Reversed phase
TSKgel Styrene-60	35～45	aq*1 : MeOH 2 : 8	aq*1 : MeOH 1 : 9	2.0～4.0	8～20	Reversed phase
TSKgel Acetate-60	35～45	MeOH	aq*1 : MeOH 8 : 2	1.2～1.8	10～20	
TSKgel Acetate-60	20～40	n-Hexane : EtOH*3 5 : 5		5.0～10.0	20～40	Normal phase
TSKgel Ether-250	35～45	0.5% sodium acetate : MeOH 9 : 1		1.2～1.8	10～20	
TSKgel Ether-250	30～40	n-Hexane : EtOH*3 7 : 3		1.5～2.0	4～10	Normal phase
TSKgel SCX	35～45	0.5% sodium acetate : MeOH 9 : 1		1.5～2.0	15～30	Na^+type
TSKgel SAX	35～45	aq*1	0.5% sodium acetate	2.0～4.0	15～30	
TSKgel Sugae AXI	30～45	0.2mol/L boric acid buffer pH8.5		0.4～0.6	3～5	
TSKgel Augar AXG	30～45	0.2mol/L boric acid buffer pH8.5		0.6～1.0	3～5	
TSKgel Silica-150	15～25	Acetone	n－Hexane	5.0～10.0	15～35	
TSKgel Silica-60						
TSKgel ODS-120A	15～25	Chloroform	MeOH ↓ MeOH : aq*1 5 : 5	2.5～3.5 ↓ 1.2～2.0	15～35	
TSKgel ODS-120T					25～35	
TSKgel ODS-80TM				3.5～4.5 ↓ 1.2～1.8	35～40	
TSKgel TMS-250	10	MeOH	MeOH : aq*1 5 : 5	2.0～3.0	4～5	10 μm(7.5cm column)
TSKgel NH_2-60	15	MeOH	MeOH ↓ MeOH : aq*1 5 : 5	—	35～40	Constant pressure packing
TSKgel OH-120	15～25	THF : Acetone 5 : 5		2.5～5.0	25～45	
TSKgel Amide-80	30～35	aq*1 : Acetonitrile 65 : 35		2.0～3.0	40	

aq*1=water, MeOH*2=Methanol, EtOH*3=Ethanol

Noto : Remember that, where you use a different pwrticle-diameter gel and/or a different size column than the above, you have to change the packing flow rate and the packing pressure.

Table 3 Test conditions of TSKgel Column Efficiency

Type	Eluent	Sample	Theoretical plate number (TP/column)	Remark
TSKgel Styrene-250	Water : Methanol 5 : 5	Phenol	1500	Reversed phase
TSKgel Styrene-60	Water : Methanol 1 : 9	Phenol	1500	Reversed phase
TSKgel Acetate-60	Water : Methanol 7 : 3	Nicotinic acid amid	1500	—
TSKgel Acetate-60	Hexane : Ethanol 8 : 2	Benzene	2000	Normal phase
TSKgel Ether-250	1/15mol/L phosphoric acid butfer	Nicotinic acid amid	1500	—
TSKgel Ether-250	n-Hexane : Ethanol 8 : 2	Benzene	4000	Normal phase
TSKgel SCX	Distilled water	Ethylene glycol	2000	Cation exchange type
TSKgel SAX	Disilled water	Ethylene glycol	2000	Anion exchange type
TSKgel Sugar AXI	0.2mol/L boric acid buffer pH8.5	Benzyl alcohol	4500	
TSKgel Sugar AXG	0.2mol/L boric acid buffer pH8.5	Benzyl alcohol	3300	
TSKgel Silica-150	n-Hexane : Ethanol 8 : 2	Benzene	5000	—
TSKgel Silica-60	n-Hexane : Ethanol 8 : 2	Benzene	4000	—
TSKgel ODS-120A	Methanol : Water 7 : 3	Naphthalene	8000	—
TSKgel ODS-120T	Methanol : Water 7 : 3	Naphthalene	8000	—
TSKgel ODS-80T_M	Methanol : Water 7 : 3	Naphthalene	15000	
TSKgel TMS-250	Methanol : Water 5 : 5	Phenol	1000	Column size 4.6mm×7.5cm
TSKgel NH_2-60	Methanol	Benzene	5000	—
TSKgel OH-120	n-Hexane : Ethanol 8 : 2	Benzene	5000	—
TSKgel Amide-80	Acetonitrile : Water 7 : 3	0.2%Manitol	8000	Sample Vol.20 μL RI Detector

Colum size : 4.6mmID×25cm　　Sample loading : 10 μL
Flow rate : 1.0mL/min　　Detection : UV254nm

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