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Gel Permeation Chromatography Column Packings—Types and Uses*

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Summary

Proper selection of the column packing material is an essential part of a successful gel permeation analysis. Generally the desirable properties of a packing are good chemical, thermal, and mechanical stabilities combined with good resolution and low resistance to liquid flow. Pore size distribution, particle size distribution, polar characteristics, as well as other physical parameters play a role in how well a packing will perform. This paper describes the different types of column packings which are commercially available, as well as briefly mentioning several packings used on an experimental scale. Physical characteristics of the various packings are compared. Suggested uses along with some limitations are given.

A variety of porous packing materials are available to the chromatographer, and it is the task of the individual to choose the one best suited to his needs. Generally, the desirable properties of a packing are good chemical, thermal, and mechanical stability combined with good resolution and low resistance to liquid flow. A combination of the above properties would allow one to use a column in different solvents, at different temperatures, and at various flow rates with little or no loss of resolution.

Most commonly used for synthetic polymer analysis are the crosslinked polystyrenes. These came about through the work of Moore

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(*1*) who demonstrated that by controlling the nature and amount of diluent present, cross-linked polystyrene beads of controlled pore sizes could be obtained. Table 1 illustrates the different recipes for obtaining certain pore sizes. These gels are sold under the name Styragel and are available only from Waters Associates, Inc., Framingham, Massachusetts. The gels can be used with most organic solvents; however, they cannot be used without specific precautions with acetone,

TABLE 1

Permeabilities of Gels with Various Diluents, all Made from 30% Styrene, 10% Divinylbenzene, 60% Diluent,[a] According to Moore (*1*)

Diluents, parts/100 parts of gel	Excluded molecular weight (M_e)
60 Toluene	7×10^3
30 Toluene, 30 diethylbenzene	1.5×10^4
60 Diethylbenzene	1.2×10^4
45 Toluene, 15 *n*-dodecane	1×10^5
30 Toluene, 30 *n*-dodecane	3×10^5
15 Toluene, 45 *n*-dodecane	2×10^6
10 Toluene, 50 *n*-dodecane	2×10^3
40 Diethylbenzene, 20 isoamyl alcohol	3.6×10^3
20 Diethylbenzene, 40 isoamyl alcohol	8×10^6
13.3 Diethylbenzene, 46.7 isoamyl alcohol	10^{10}
60 Isoamyl alcohol	Extremely high

[a] "Styrene" is a mixture of styrene and ethylvinylbenzene. "Divinylbenzene" is a mixture of about 54% divinylbenzene, 42% vinyl ethylbenzene, and 4% diethylbenzene.

alcohols, most acids, and water. The gels as sold have a particle range usually of 20–60 μ and are available in exclusion ranges of from 40–10^7 Å expanded chain length, i.e., from about 200 to 50,000,000 molecular weight. The upper exclusion limit is determined by the largest pores present. Molecules larger than this limit will be eluted at the interstitial volume, V_0, of the column. Styragel is a rigid gel as compared to soft gels such as reported by Heitz (*2*). The difference is in the degree of cross-linking. The soft gels will swell appreciably in many solvents and are limited to lower operating pressures than the rigid gels. Figure 1 shows the separation of a polystyrene made on soft gel. Rather remarkable separations can be achieved at low flow rates. The upper temperature limit of the Styragel packing is about 140°F.

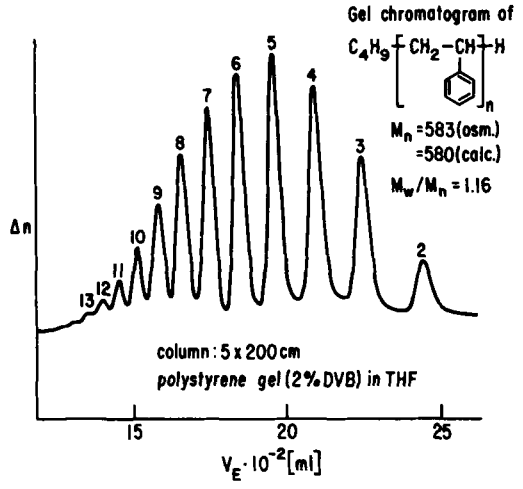

FIG. 1. Separation of polystyrene oligomers on soft polystyrene gel.

A series of soft cross-linked polystyrene gels is marketed by Bio-Rad Laboratories under the trade name Bio-Beads S. They have an operating range of 100 to 14,000 in molecular weight based on polystyrene dissolved in tetrahydrofuran. Their physical characteristics are given in Table 2.

Other organic polymers have been used. One which is commercially available is EM-Gel-OR, a cross-linked polyvinyl acetate. These gels, developed by Heitz (*3*) and manufactured by E. Merck, are produced by copolymerization of vinyl acetate and butanediol-1, 4-divinyl ether or divinyl esters of dicarboxylic acids. Up to an exclusion limit

TABLE 2

Physical Characteristics of Bio-Beads S

Type	Mesh size dry	Exclusion limit	Separation range, mol wt	Swollen bed volume (ml/g)[a]
S-X1	28–74 μ	14,000	600–14,000	9.8
S-X2	28–79 μ	2,700	100–2,700	6.2
S-X3	28–74 μ	2,000	Up to 2,000	5.1
S-X9	28–74 μ	1,400	Up to 1,400	4.2
S-X8	28–74 μ	1,000	Up to 1,000	3.9
SM-1	20–50 mesh	14,000	600–14,000	3.1
SM-2	20–50 mesh	14,000	600–14,000	2.9

[a] In benzene.

TABLE 3

Physical Characteristics of EM-Gel-OR

Gel type	Exclusion limit	Swelling factor
OR 750	ca. 750	ca. 2
OR 1,000	ca. 1,500	ca. 3
OR 5,000	ca. 5×10^3	ca. 4.5
OR 20,000	ca. 2×10^4	ca. 4.0
OR 100,000	ca. 1×10^5	ca. 4.3
OR 1,000,000	ca. 1×10^6	ca. 4.1

of 5000 molecular weight, the gels are homogeneously cross-linked. Higher limits are produced by polymerization in the presence of an inert diluent. The gels are lipophilic and could be used for adsorption chromatography. Table 3 shows the gels which have been prepared. The exclusion limits and swelling factors are for polystyrene in tetrahydrofuran. Only the lower four exclusion ranges are commercially available at the present. These gels are primary for use in polar organic solvents such as acetone, methanol, ethanol, and tetrahydrofuran. The upper limit of thermal stability is 100°C. The swelling porosity enables a separation of low molecular weight compounds even with gels of high exclusion limits. Figure 2 shows the separation of polystyrenes and oligophenylenes covering the molecular weight range of 92 to 8.3×10^5. The separation was done using EM-Gel-OR 1,000,000, an open glass column of 1 m $\times$ 1.5 cm, and THF as the solvent. Even though the gels swell, they are rigid, and columns can be operated with or without pressure.

Of increasing interest and popularity are the various glass or silica packings which are available on the market. Best known of these are the spherical porous silica beads of de Vries (4) manufactured by

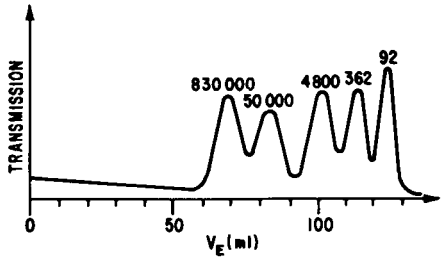

FIG. 2. Separations on Merckogel OR 1,000,000 0.020–0.055 mm, open glass column, 1 m length, 1.5 cm diameter. Solvent: THF.

TABLE 4

Physical Characteristics of Porasil[a]

Type	Surface area (m^2/g)	Average pore diameter (Å)	Excluded mol wt
A	480	100	6×10^4
B	200	100–200	2.5×10^5
C	50	200–400	4×10^5
D	25	400–800	1×10^6
E	4	800–1500	1.5×10^6
F	1.5	>1500	$>2 \times 10^6$

[a] Particle size ranges available: 36–75 μ, 75–125 μ.

Pechiney-Saint-Govain under the name Spherosil and sold in this country by Waters Associates under the name Porasil. Table 4 shows the types available and the upper pore size for each type. Two particle sizes are available, and the size of 75–125 μ is recommended for per-

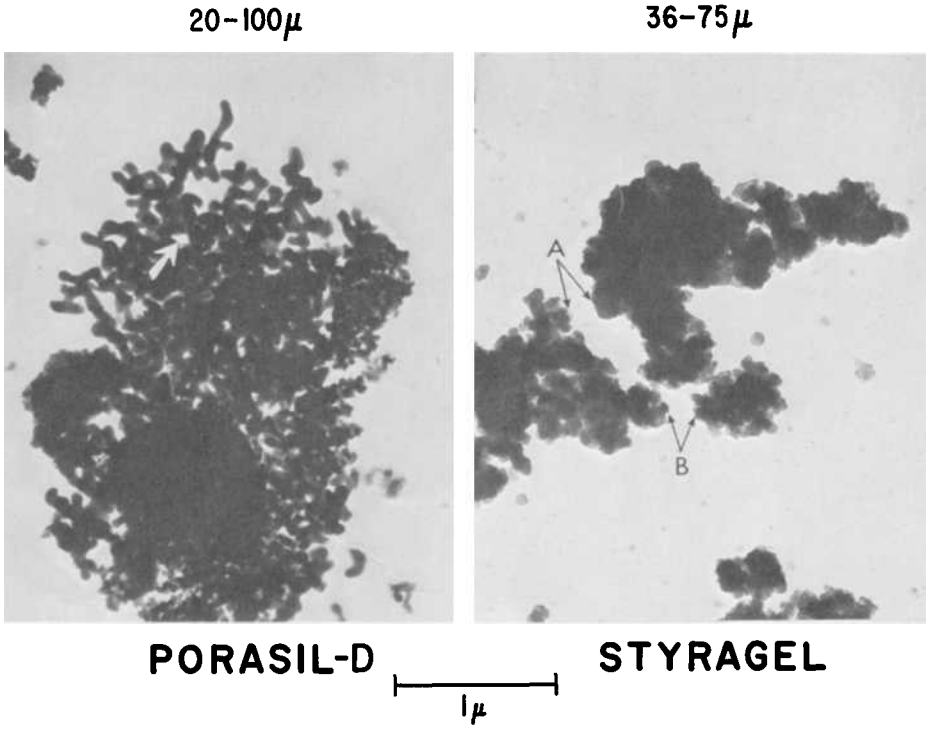

FIG. 3. Electron photomicrographs of Porasil-D and Styragel.

meation chromatography. Figure 3 is an electron photomicrograph of Porasil and Styragel. The Porasil appears to be made of overlapping saddlelike structures which have a smooth surface. These structures fused together form a porous particle. The size of the pore marked by the arrow is about 550 Å, which agrees with the designated pore size. The Styragel appears to be made up of very fine, almost round particles. These particles are fused together to form agglomerates with rough textured surfaces. The agglomerates, in turn, are joined to form the macro particles which appear to the eye as very small spheres. The manner in which the agglomerates go together leads to the formation of voids. The entrances to the two voids pointed out by the arrows are about 1500 Å. Since the Porasil-D has an upper exclusion limit of about 1×10^6 in molecular weight and the Styragel of about 3×10^6, this void size is reasonable. This structural difference may explain why Yau (*5*) observed that separation on porous glass appears to be controlled by steric exclusion while separation on Styragel appears to be influenced by lateral diffusion as well as steric exclusion.

Other glass packings are the Corning Porous Glass packings reported in the literature by Haller (*6*). The pores are all within ±15% of the reported value. These are porous glass granules with a packing density of 0.3 to 0.7 g/cc. Table 5 gives the characteristics of this material. Due to the small range of pore size, good resolution is obtained over a relatively narrow range as shown in Fig. 4. Best results are obtained using more than one column in series. Another commercially available porous glass with a wider pore size distribution than the glasses made

TABLE 5

Physical Characteristics of Corning Glass Packings[a]

Pore diameter (Å)	Exclusion limits,[b] mol wt	Operating range[b]
75	28,000	300–28,000
125	48,000	650–48,000
175	68,000	1,050–68,000
240	95,000	1,150–95,000
370	150,000	5,000–150,000
700	300,000	15,000–300,000
1,250	550,000	40,000–550,000
2,000	1,200,000	120,000–1,200,000

[a] Particle sizes available: 36–75 μ, 75–125 μ, 125–175 μ.

[b] Based on dextrans in distilled water.

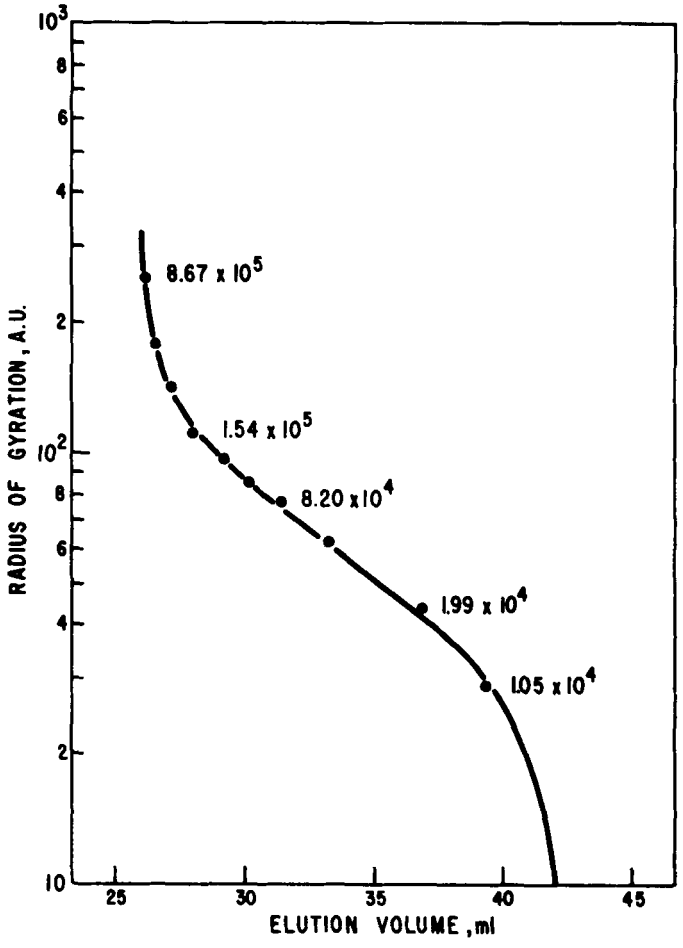

FIG. 4. Radius of gyration vs elution volume of polystyrene in butanone-isopropanol. Small size porous glass.

by Haller's process is Bio Glass sold by Bio-Rad Laboratories, Richmond, California. The physical characteristics of the Bio Glass packings are given in Table 6.

Another glass-type packing which only recently became available is EM-Gel-Si. These packings are irregularly shaped, crushed silica particles. They are manufactured by E. Merck and like the EM-Gel-OR are marketed in the United States by Waters Associates Inc. These

TABLE 6

Physical Characteristics of Bio Glass Packings[a]

Type	Exclusion limit	Separation range, mol wt	Average pore diameter (Å)
200	30,000	3,000–30,000	200
500	100,000	10,000–100,000	500
1,000	500,000	50,000-500,000	1000
1,500	2,000,000	400,000-2,000,000	1500
2,500	9,000,000	800,000-9,000,000	2500

[a] Particle sizes available: 147–297 μ, 147–187 μ, 125–147 μ, 74–147 μ, 44–74 μ, <44 μ.

materials exhibit high-surface area and have active sites typical of silicalike materials. Table 7 lists the physical characteristics of the materials which are available.

All of the glass packings mentioned have some polar sites present and care must be used to prevent tailing. In some cases a liquid modifier such as a silylating agent is required. Cooper and Johnson (*7*) have described the effect of treatment with hexamethyldisilazane on porous glass packings. Recently Bombaugh et al. (*8*) described the GPC analysis of polyvinyl alcohol using Porasil columns with water as the carrier solvent. A temperature of 65°C and a flow rate of 1 ml/min was employed. Figure 5 illustrates the chromatograms obtained for three different polymerization systems. Polyvinyl alcohol is normally permanently retained on Porasil. The separation was made possible by development of a deactivated Porasil in which the polar sites are blocked. Columns in use up to 6 months have shown no increase in adsorption.

For molecules which are soluble in water or electrolyte solutions, one can use Sephadex packings. Sephadex is dextran crosslinked to give a three-dimensional network of polysaccharide chains. Because of Sephadex's high content of hydroxyl groups, the beads swell consider-

TABLE 7

Physical Characteristics of EM-Gel-Si

Gel type	Exclusion limit	Surface (m^2/g)	Pore size (Å)	Pore volume (ml/g)
150	ca. 50,000	120–170	120-220	0.81
500	ca. 400,000	35–65	300-700	0.78
1,000	ca. 1×10^6	10–20	700-1300	0.70

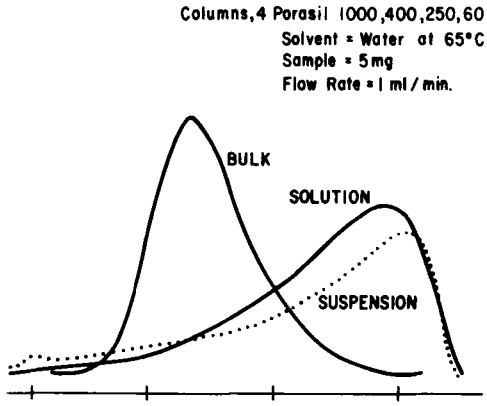

FIG. 5. GPC chromatograms of polyvinyl alcohol from various polymerization methods.

ably in water and electrolyte solutions. Table 8 lists the grades available and some of their characteristics. Sepharose is an agarose gel which complements Sephadex by extending the separation range to include viruses and high molecular weight proteins, polysaccharides, and nucleic acids.

By alkylation of most of the hydroxyl groups of Sephadex G-25, a material which swells in organic solvents is obtained. This is Sephadex

TABLE 8

Physical Characteristics of Sephadex

Type	Particle size (μ)	Excluded mol wt[a]	Bed volume (ml/g)
Sephadex G-10	40–120	7×10^2	2
G-15	40–120	1.5×10^3	3
G-25[b]	50–150	5×10^3	5
G-50[b]	50–150	1×10^4	10
G-75[c]	40–120	5×10^4	15
G-100[c]	40–120	1×10^5	20
G-150[c]	40–120	1.5×10^5	30
G-200[c]	40–120	2.0×10^5	40
Sepharose 6B	40–210	1×10^6	—
4B	40–190	5×10^6	—
2B	60–250	2×10^7	—
Sephadex LH-20	25–100	4×10^3	—

[a] Based on dextrans.
[b] Available in fine, 20–80 μ; coarse, 100–300 μ.
[b] Available in superfine, 10–40 μ.

LH-20 which can be used with polar organic solvents as well as water and has the same separation range as G-25.

Other column packings for aqueous systems are the Bio-Gel P and Bio-Gel A packings of Bio-Rad Laboratories. The Bio-Gel P is obtained by polymerizing varying concentrations of acrylamide and methylene bisacrylamide. Each is readily hydrated for use in a wide variety of aqueous media. Four particle size ranges are available: 50–100 mesh for large columns where speed is important, 100–200 mesh for general purpose work, 200–400 mesh for high resolution chromatography, and —400 mesh for thin layer chromatography. Table 9 lists the physical characteristics of Bio-Gel P.

TABLE 9

Physical Characteristics of Bio-Gel P[a]

Type	Exclusion limit	Separation range, mol wt	Hydrated bed volume (ml/g)	Water regain (g/g)
P2	1,800	200-1,800	3.8	1.5
P4	4,000	800–4,000	5.8	2.4
P6	6,000	1,000-6,000	8.8	3.7
P10	20,000	1,500-20,000	12.4	4.5
P30	40,000	2,500-40,000	14.8	5.7
P60	60,000	3,000-60,000	19.0	7.2
P100	100,000	5,000-100,000	19.0	7.5
P150	150,000	15,000–150,000	24.0	9.2
P200	200,000	30,000-200,000	34.0	14.7
P300	400,000	60,000-400,000	40.0	18.0

[a] Particle sizes available: 147–297 μ, 74–147 μ, 28–74 μ (P2 through P10 only), <28 μ.

Bio-Gel A complements Bio-Gel P in that it extends the upper operating range from 400,000 to 1.5×10^8 molecular weight. Bio-Gel A is agarose, the nonionic constituent of agar. The use of agarose eliminates the undesirable side effects associated with agar: ion exchange and adsorption, electroendosmosis, and specific chemical interactions. These agarose gels have a useful temperature range of 0–30°C. The physical characteristics are shown in Table 10.

To summarize, it would seem that acceptable packing materials are now available for almost any polymer solvent system one may desire to work with. While the glass packings offer greater stability and ease of packing, the cross-linked polymers give the least amount of peak spreading or the highest plate counts. Problems of polarity

TABLE 10
Physical Characteristics of Bio-Gel A[a]

Type	Exclusion limit	Separation range, mol wt	Approximate % agarose in gel
A-0.5 m	5×10^5	10^4 to 5×10^5	10
A-1.5 m	1.5×10^6	10^4 to 1.5×10^6	8
A-5 m	5×10^6	10^4 to 5×10^6	6
A-15 m	1.5×10^7	4×10^4 to 1.5×10^7	4
A-50 m	5×10^7	10^5 to 5×10^7	2
A-150 m	1.4×10^8	10^6 to 1.5×10^8	1

[a] Particle sizes available: 147–297 μ, 74–147 μ, 28–74 μ.

can be encountered with both glass and polymer packings, and one must be alert for signs of adsorption when working with unfamiliar systems. Lightly cross-linked gels may be of interest where low flow rates and low pressures can be used. Extraordinary separations have been achieved with such gels.

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