

# TheReporter

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# A New, High Efficiency Resin for Reversed Phase HPLC

G. Lai

*The SUPELCOGEL TPR-100 column is the newest addition to Supelco's line of reversed phase columns. Based on a patented templating process, this packing overcomes the limitations of silica-based supports. The TPR-100 column exhibits the pH stability and inertness of a resin, as well as higher efficiency, better stability, and different selectivity than other resins.*

Silica is the most commonly used support for HPLC phases. However, this material has several drawbacks, including a narrow working pH range (pH 2-7), and the existence of free silanols and metal ion impurities. At readings below pH 2, hydrolysis of the bonded phase can occur, and above pH 8 the silica itself can dissolve. The presence of residual silanols can cause unpredictable retention, irreversible adsorption, poor peak shape and efficiency, and unsatisfactory reproducibility for silanophilic compounds. And much discussion recently has focused on the role of metal ion impurities in the silica as sites for chelation, another retention mechanism that leads to poor chromatography for susceptible compounds.

Because of these limitations, alternate supports are needed for some separations. The second choice is typically a resin- or polymer-based support. Most resins chosen for HPLC are not susceptible to chemical degradation by acids or bases, and have no limits in mobile phase pH. This is especially important when analyzing some compounds (e.g. amines and basic drugs), when a higher pH (pH minus  $pK_a \geq 2$ ) enables the separation of basic analytes in a non-ionized form. This pure reversed phase mechanism results in increased column performance and improved peak shape for most of the basic compounds. Additionally, HPLC resins do not have silanols or metal ions to act as adsorptive sites.

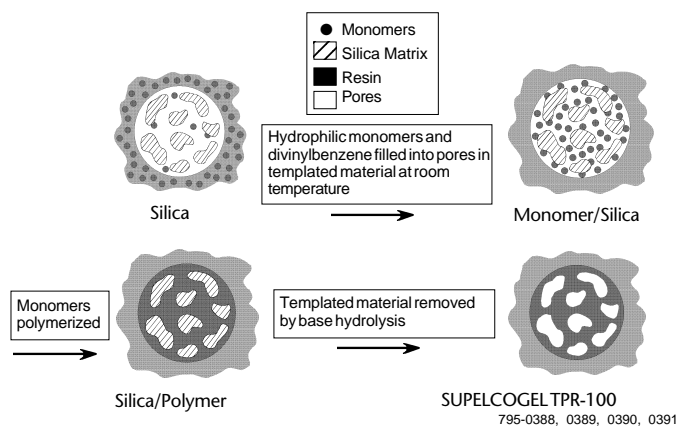
HPLC resins, however, often are characterized by low efficiency and short lifetime. These problems usually are attributed to the suspension polymerization technique by which most HPLC resins are made. In suspension polymerization, the particle grows out from widely distributed monomer droplets. Porogens are added to make the particle porous, but ultimately cause contamination. The grow-out rate is not uniform and a wide distribution of particle sizes results, creating the need for extensive sizing to obtain the desired particle size. Too narrow a distribution results in yield losses. Thus a wide distribution often is accepted, causing low efficiency of conventional resins. Other sources of low column efficiency are the presence of micropores ( $<20\text{\AA}$ ); "dead-end" channels; and inhibitors of mass transfer.

The SUPELCOGEL™ TPR-100 column overcomes these limitations. This new column is prepared by a patented templated polymerization<sup>▲</sup> (Figure A), rather than by the conventional

suspension polymerization process. First, a high efficiency silica particle (the same used to make HPLC phases) is imbibed with a monomer solution. Then the monomer solution is polymerized and crosslinked for stability. Finally, the starting silica bead is dissolved by a caustic solution. The result is an exact template of the silica particle, in resin. This template has the physical attributes of silica (narrow particle and pore size distributions), and the chemical attributes of a polymer (inertness and pH stability). Table 1 summarizes the differences in physical characteristics of the two polymerization techniques.

The SUPELCOGEL TPR-100 resin has advantages over other HPLC resins (Table 2). Because a mixture of hydrophobic and hydrophilic monomers is used, the TPR-100 particle is intermediate in polarity between the hydrophobic styrene-divinylbenzene polymers and the hydrophilic methacrylate polymers. The TPR-100 resin yields a different elution pattern, demonstrating its unique selectivity as well as its improved efficiency and symmetry compared with other

**Figure A. Templated Polymerization**



**Table 1. Templated vs. Suspension Polymerization**

Characteristic	Suspension Polymerization	Templated Polymerization
Particle size	1-1000µm	5µm
Contamination from porogen	possible	not possible
Pore size distribution (single)	wide (bimodal)	narrow
Pore size control	difficult	simple
Micropores ( $<20\text{\AA}$ )	present	absent
Sizing needed	yes	no
Monomer compatibility	some problems	no problems

**Table 2. Chromatography of Polar Compounds on Resin-Based HPLC Columns**

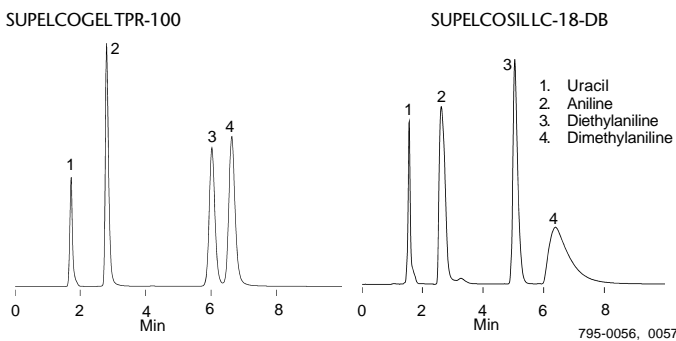
	SUPELCOGEL TPR-100	PLRP-S	TSK OD-2PW	PRP-1	ODP-50	DE-613
<b>Retention (k')</b>						
4-Hydroxybenzoic acid	0.59	0.71	1.38	0.84	1.31	3.26
Acetylsalicylic acid	1.47	2.20	1.55	2.64	1.81	4.73*
p-Acetophenetidine	1.85	3.06	1.69	3.61	2.11	4.73*
Salicylic acid	2.74	4.67	4.94	5.53	6.73	12.13
<b>Efficiency</b>						
4-Hydroxybenzoic acid	6126	1323	36781	401	1766	4229
Acetylsalicylic acid	5930	932	3860	826	3161	*
p-Acetophenetidine	5741	1115	2814	1470	4103	*
Salicylic acid	5335	749	3409	1584	2139	4432
<b>Symmetry</b>						
4-Hydroxybenzoic acid	1.03	1.74	1.07	1.19	1.92	1.26
Acetylsalicylic acid	0.98	1.43	1.17	0.64	1.39	*
p-Acetophenetidine	0.98	1.33	1.54	0.95	1.55	*
Salicylic acid	1.01	1.31	1.36	0.90	2.00	1.52

Columns: 15cm x 4.6mm ID  
(except PRP-1, 15cm x 4.1mm ID, and DE-613, 15cm x 6.0mm ID)  
Mobile Phase: acetonitrile:0.1% TFA (30:70)  
Flow Rate: 1mL/min

\*Coelution.

**Figure B. Anilines**

Columns: 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: acetonitrile:water (60:40)  
Flow Rate: 1mL/min  
Temp.: ambient  
Det.: UV, 254nm  
Inj.: 10µL



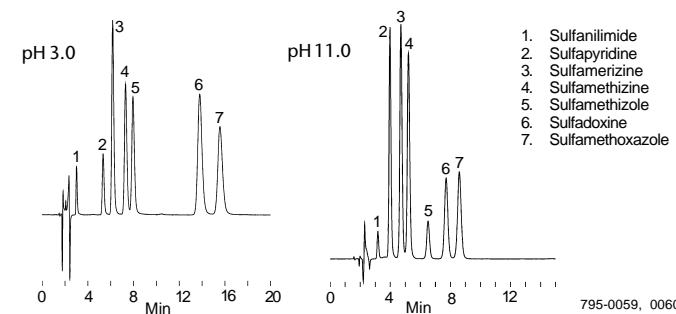
resin columns. Another benefit of the monomer chemistry of the TPR-100 resin is stability. Repeated gradients do not change the bed structure, allowing this resin to be used with 100% aqueous or 100% organic mobile phases without shrinking or swelling. Pressures up to 5000psi are accommodated.

The inertness of the resin is demonstrated in Figure B. Note the severe peak tailing of N,N-dimethylaniline and aniline on the silica-based SUPELCO SILLC™ LC-18-DB column. The SUPELCOGEL TPR-100 column yielded excellent peak shape for both compounds. The overall elution pattern is similar on both phases. The sulfa drug separation in Figure C illustrates the importance of using an unlimited mobile phase pH range to obtain selectivity and curtail the use of solvents. At pH 3, the drugs are more hydrophobic and require 20% acetonitrile for elution. However, by using a mobile phase of pH 11, less organic solvent is needed, and the selectivity is different.

Consider the stability and efficiency of the resin-based SUPELCOGEL TPR-100 column for your future HPLC analyses.

**Figure C. Sulfa Drugs**

Column: SUPELCOGEL TPR-100, 15cm x 4.6mm ID, 5µm particles  
Cat. No.: 59154  
Mobile Phase: acetonitrile:25mM KH<sub>2</sub>PO<sub>4</sub>, 20:80 (pH 3.0) or 9:91 (pH 11.0)  
Flow Rate: 1mL/min  
Temp.: ambient (pH 3.0) or 30°C (pH 11.0)  
Det.: UV, 254nm  
Inj.: 10µL

**Ordering Information:**

Description	Cat. No.
SUPELCOGEL TPR-100 Column 15cm x 4.6mm ID, 5µm particles	59154

▲US Patent No. 4,933,372, Sigma-Aldrich Co.

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