Waters
Division of MILLIPORE

WATERS PREPPAK CARTRIDGES AND PREP GUARD-PAK INSERTS

CARE AND USE MANUAL

Address comments
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Waters Publications

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1 INTRODUCTION

This manual describes the Waters PrepPak® Cartridges and the Prep Guard-Pak™ Inserts.

Refer to the *Waters RCM 25 x 10 Operator's Manual* or the *PrepPak Holder Assembly Operator's Manual* for information and identification of module parts.

Description

Use the PrepPak Cartridges and Prep Guard-Pak Inserts in the RCM 25 x 10 cartridge module or the PrepPak Holder Assembly. Up to three PrepPak Cartridges with a Prep Guard-Pak Insert can be connected in series to provide columns of overall lengths of 100, 200 or 300 mm (Figure 1).

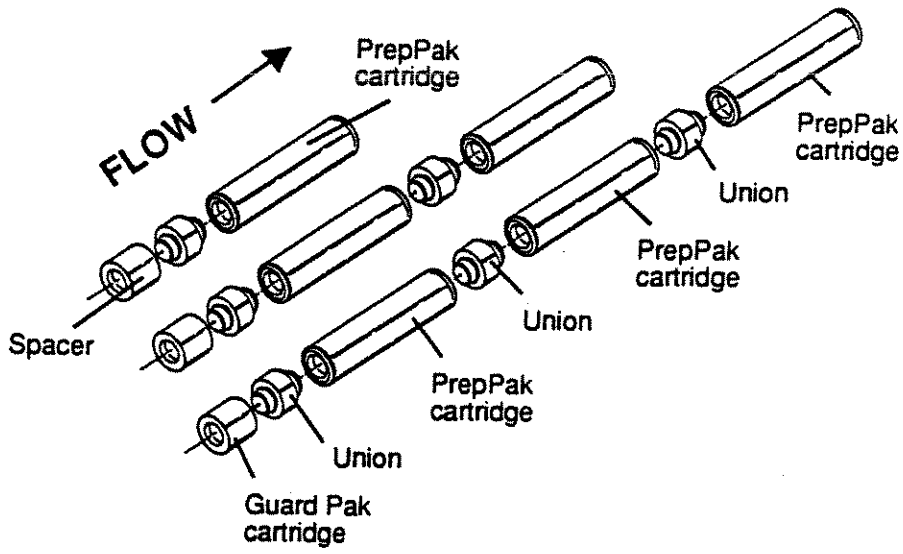


Figure 1 Waters PrepPak Cartridges and Prep Guard-Pak Inserts

You can use the PrepPak Cartridges in the holders with or without a Prep Guard-Pak Insert. The Prep Guard-Pak Inserts are used in front of PrepPak Cartridges, compressed in the holder. The inserts protect PrepPak Cartridges by absorbing sample impurities and filtering particulate matter that can foul PrepPak Cartridges.

The Prep Guard-Pak Inserts are packed with the same chemistries and particles sizes as the PrepPak Cartridges.

Segmented Column Technology

Build the column capacity required for your purification using Waters Segmented Column Technology. You can increase column capacity by:

- Adding extension tubes and 25 mm PrepPak Cartridges to the RCM 25 x 10 or the PrepPak Holder Assembly
- Changing to the 40 mm diameter cartridges for the PrepPak Holder Assembly

2 INSTALLING THE CARTRIDGE

Installation consists of cartridge insertion, compression, and conditioning. Follow the instructions in the *RCM 25 x 10 Operator's Manual* or the *PrepPak Holder Assembly Operator's Manual* to insert and compress the cartridges.

Conditioning

1. Condition the cartridge with 5 to 10 column volumes of the stronger-eluting component of the mobile phase. The cartridge volumes are:

Cartridge	Volume (mL)
PrepPak 25 x 100 mm	50
PrepPak 40 x 100 mm	125
Prep Guard-Pak 25 x 10 mm	5
Prep Guard-Pak 40 x 10 mm	12.5

2. Purge the cartridge with the starting or final mobile phase.

During conditioning, air is forced from the packed bed. A completely wetted bed contains no air.

Equilibrium between the mobile phase and the stationary phase is established when a stable baseline is produced.

Eliminating air

Spikes in the baseline after conditioning indicate that air remains trapped in the cartridge. Remove air from the system by disconnecting the holder from the detector and pumping the mobile phase at a higher flow rate.

If the air is trapped in the detector cell, add some restriction at the detector outlet to remove air. Refer to the detector manual for pressure limits before adding restriction.

3 OPERATIONAL GUIDELINES

This chapter presents:

- Solvent compatibility
- Chromatography guidelines
- Operating pressure
- Flow rate ranges
- Sample load guidelines

3.1 Solvent Compatibility

The holder end connector, spacer, and Prep Guard-Pak union are shipped with Viton® O-rings suitable for use with reverse-phase solvents. For use with normal-phase solvents, change the O-rings on the end connectors, spacer, and union to EPM O-rings. Replacement module O-rings for normal and reverse-phase solvents are in the Startup Kit. Refer to the *RCM 25 x 10 Operator's Manual* or the *PrepPak Holder Assembly Operator's Manual* for replacement instructions.

3.2 Chromatography Guidelines

When using PrepPak Cartridges and Prep Guard-Pak Inserts, observe the following guidelines:

- Do not exceed an operating pressures of 1500 psi (10 MPa, 100 atm).
- When using silica-based packings, stay within pH 2 to 8. Avoid concentrated acids and bases.
- Dedicate cartridges to specific applications to prevent sample cross-contamination.
- Filter samples and solvents before use.

3.3 Operating Pressure

The maximum mobile phase pressure limit is 1500 psi (10 MPa, 100 atm).

When flow begins, the pressure on the module rises due to the increases in the system pressure. This pressure is generated by flow through the cartridge, tubing, and connections.

When flow stops, the holder pressure may drop to a level lower than the initial setting because the O-rings and cartridge are compressed. Do not increase the compression if the cartridge registers greater than 200 psi (1.3 MPa, 13 atm) with no system flow.

Refer to *Appendix A, Pressure and Viscosity Charts*, for flow rate, pressure, and viscosity information.

3.4 Flow Rate Ranges

The following table lists typical flow rate ranges for PrepPak Cartridges on a chromatographic system with:

- Tubing with inside diameter at least 0.030 inches (0.76 mm)
- Semiprep detector cells
- High flow rate system pumps (45 to 300 mL/min)

Cartridge	Flow Range (mL/min)
PrepPak 25 mm	14 to 60
PrepPak 40 mm	35 to 150

A 25 x 100 mm cartridge may be used on an analytical system plumbed with 0.009-inch (0.23 mm) ID tubing at flow rates of 5 to 10 mL/min. Most of the observed backpressure of this system is due to the hydraulic resistance created by the 0.009-inch ID tubing, not the column.

In all cases, the operating pressure limit is 1500 psi (10 MPa, 100 atm).

3.5 Sample Load Guidelines

The following table presents sample-load guidelines for the PrepPak Cartridges:

PrepPak Cartridge	Loads
25 x 100 mm	25 mg to 1 g
40 x 100 mm	70 mg to gram quantities

For stacked cartridges, multiply the sample range by the number of PrepPak Cartridges used. The amount of sample that can be purified depends upon:

- Complexity of the sample
- Solubility of sample components
- Purification requirements

4 SEPARATION STRATEGY

This section describes how to scale up from an analytical separation to preparative separation.

4.1 Scaling a Separation

When scaling an analytical separation to preparative separation, use the following isolation approach:

1. Define the objective by determining the:
   - Complexity of the sample mixture
   - Component to be isolated
   - Required quantity
   - Degree of purity
   - Properties of the components in the mixture
2. Perform crude separations. Simplify a complex sample through coarse separation techniques, such as liquid-liquid or solid-liquid extraction, crystallization, or precipitation. This step removes large quantities of extraneous material and/or isolates groups or classes of compounds. Centrifuge or filter the sample to remove particulates.
3. Develop the separation method. To optimize the separation for compounds of interest, make small scale injections of the sample mixture, varying the solvent composition.
4. Perform a loading study. Make progressively larger injections of the sample mixture on a small scale column, such as the 8 x 100 mm Radial-Pak™ Cartridge, to determine the effect of overload on resolution. This study defines the amount of sample that can be separated in a column of a given volume.
5. Scale up the separation. Translate the small scale parameters to the large scale system values. Adjust the:
   - Sample load
   - Flow rate
   - Gradient volume

4.2 Adjusting Sample Load and Flow Rate

When scaling the separation, use analytical and preparative columns:

- Of the same length
- Packed with the same particle size material

This section describes three equations to adjust the sample load and flow rate. Use Equation 1 to calculate the sample load and Equation 2 to calculate the flow rate. Alternately, use Equation 3 to calculate a scale factor, which you may use to obtain the preparative value. In all three equations, if you use preparative columns and analytical columns of the same length, both sample mass and flow rate are proportional to the square of the column cross section.

1. Use the following equation to increase sample load in proportion to the volume of the packed bed.

$$\frac{m_p}{m_a} = \frac{L_p}{L_a} \cdot \frac{r_p^2}{r_a^2}$$

L = column length
m = mass injected
r = column radius
p = preparative
a = analytical

2. Use the following equation to increase flow rate in proportion to the volume of the packed bed.

$$\frac{F_p}{F_a} = \frac{V_p}{V_a} = \frac{L_p}{L_a} \cdot \frac{r_p^2}{r_a^2}$$

V = column volume
F = flow rate
L = column length
r = column radius
p = preparative
a = analytical

3. Use the following equation to calculate the Scale Factor. Multiply the analytical sample mass and/or flow rate by the Scale Factor to obtain the preparative value.

$$SF = \frac{(r_p)^2 \cdot L_p}{(r_a)^2 \cdot L_a}$$

SF = Scale Factor
r = column radius
L = column length
p = preparative column
a = analytical column

You should obtain similar retention times and peak widths for both the analytical and the preparative column and thus, similar resolution.

4.3 Adjusting Gradient Parameters

When scaling the flow rate, the gradient time profile should be kept constant. However, due to pressure limitations on the preparative instrument or column, you may have to reduce the flow and adjust the gradient time table. Use the following formula to:

- Keep a constant ratio between the gradient volume and column volume
- Adjust the gradient table

$$\frac{t_p}{t_a} = \frac{V_p}{V_a} \cdot \frac{F_p}{F_a}$$

V = column volume
F = flow rate
t = times in gradient table
p = preparative
a = analytical

4.4 Adjusting Gradient Delay Volume

Before the gradient reaches the inlet of the column, there is an isocratic period caused by the delay volume between the point of mixing of the gradient and the column inlet. The isocratic period exists until the delay volume is purged.

In small columns, the delay volume results in a longer isocratic period than in larger preparative columns. To maintain the same separation for analytical and preparative columns, you must delay the gradient for the preparative column by the same number of column volumes. Use the following equation to calculate the additional gradient delay time for the preparative column:

$$t_d = \frac{V_d}{F_p} \cdot \left(\frac{V_p}{V_a} - 1\right)$$

t_d = additional isocratic delay time for the preparative gradient
V_d = delay volume of the instrument
V_p = volume of the preparative column
V_a = volume of the analytical column
F_p = preparative flow rate

5 CARTRIDGE STORAGE

PrepPak Cartridges and Prep Guard-Pak Inserts may be left compressed in the holder.

Reverse-phase

For extended storage of reverse-phase cartridges, flush buffers from the cartridge with an aqueous mobile phase, followed by a 100 percent organic solvent flush. Cartridges can be stored with the plastic end caps on or off.

Normal-phase

For storage of silica cartridges, purge the cartridges with 100 percent isopropanol or ethyl acetate.

Changing Prep Guard-Pak Inserts

When cartridge stacking is used, the first PrepPak Cartridge in series may be used as a guard column, with or without a Prep Guard-Pak Insert. The frequency with which you replace the Prep Guard-Pak Insert or lead PrepPak Cartridge depends upon the sample. Monitor the pressure build-up to determine when the insert or cartridge should be changed. If components are colored, visually inspect the cartridges by holding them up to the light to see how far the absorbed, discolored material penetrates the cartridge.

APPENDIX A PRESSURE AND VISCOSITY CHARTS

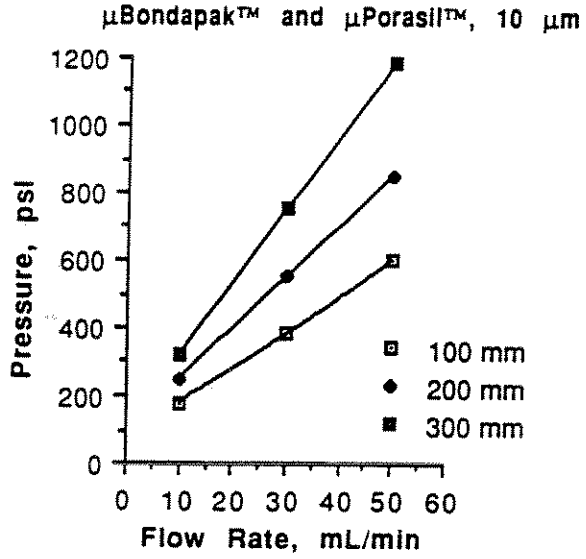
Use these charts for reference only. The pressure you observe is a function of tubing length and diameter, and can vary from system to system.

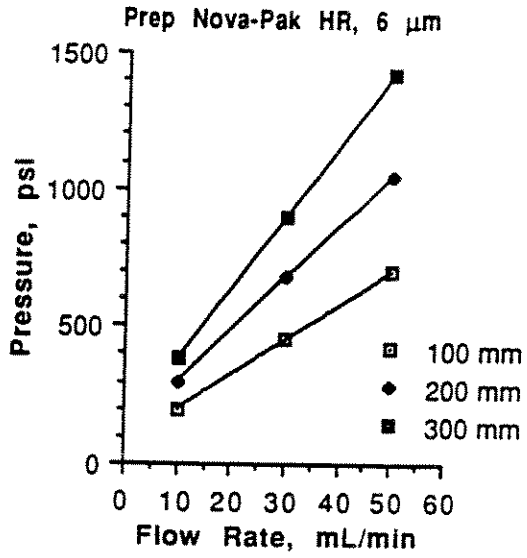
The data for the pressure graphs in this appendix were generated using a Delta Prep 3000 with 0.040-inch (1.02 mm) ID tubing. The solvent used has a viscosity of 1 centipoise.

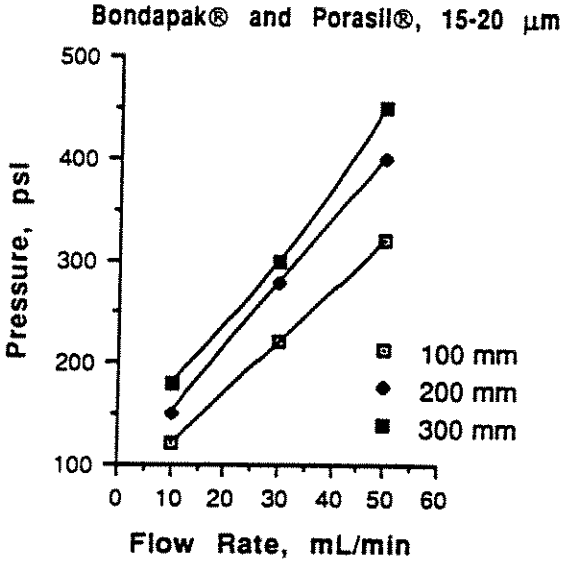
Use the graphs and Table 1 as guides for estimating system pressure. For example,

- If the mobile phase has a viscosity of 2 centipoise, expect twice the pressure.
- If the mobile phase has 0.5 centipoise viscosity, expect one-half the pressure.

For 25 x 100 mm PrepPak Cartridges on an analytical system with 0.009-inch (0.23 mm) ID tubing, expect 100 psi (0.67 MPa, 6.7 atm) per mL/min flow. This pressure is the result of the small ID tubing.







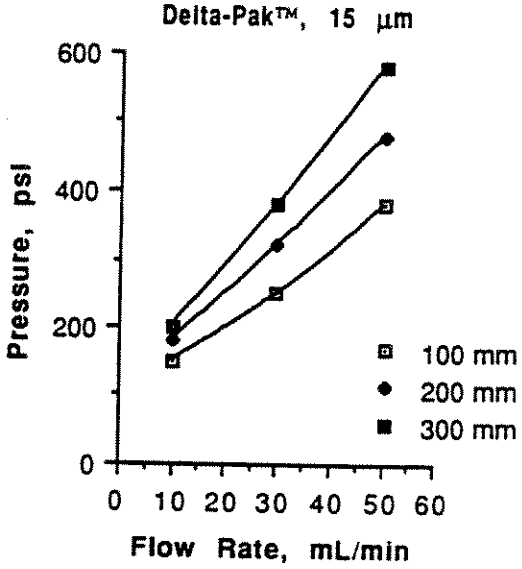


Figure 2 Pressure vs. Flow Rate Graphs for 25 mm Cartridges

Use these charts for reference only. The pressure you observe is a function of tubing length and diameter, and can vary from system to system.

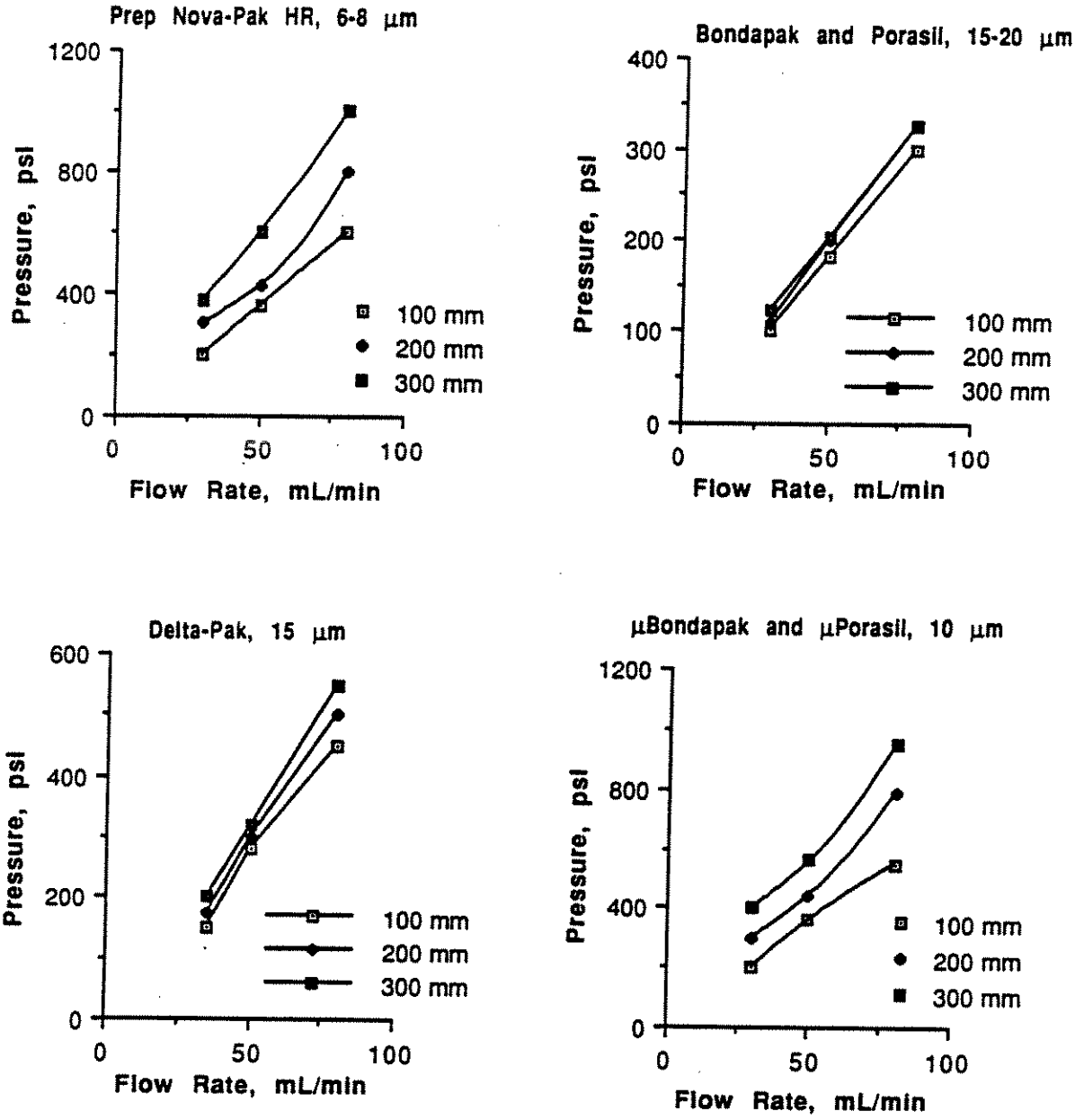


Figure 3 Pressure vs. Flow Rate Graphs for 40 mm Cartridges

Use these charts for reference only. The pressure you observe is a function of tubing length and diameter, and can vary from system to system.

Table 1 Viscosity Table

Mobile Phase	Viscosity centipoise, 20°C	Mobile Phase	Viscosity centipoise, 20°C
n-Pentane	0.235	Acetone	0.32
n-Hexane	0.33	Dioxane	1.54
n-Heptane	0.42	Nitromethane	0.65
Isooctane	0.50	Acetonitrile	0.37
n-Propylchloride	0.35	*n*-Propanol	2.3
Benzene	0.65	Ethanol	1.2
Methylene chloride	0.44	Methanol	0.6
Tetrahydrofuran	0.46	Water	1.0
Methyl ethyl ketone	0.4		

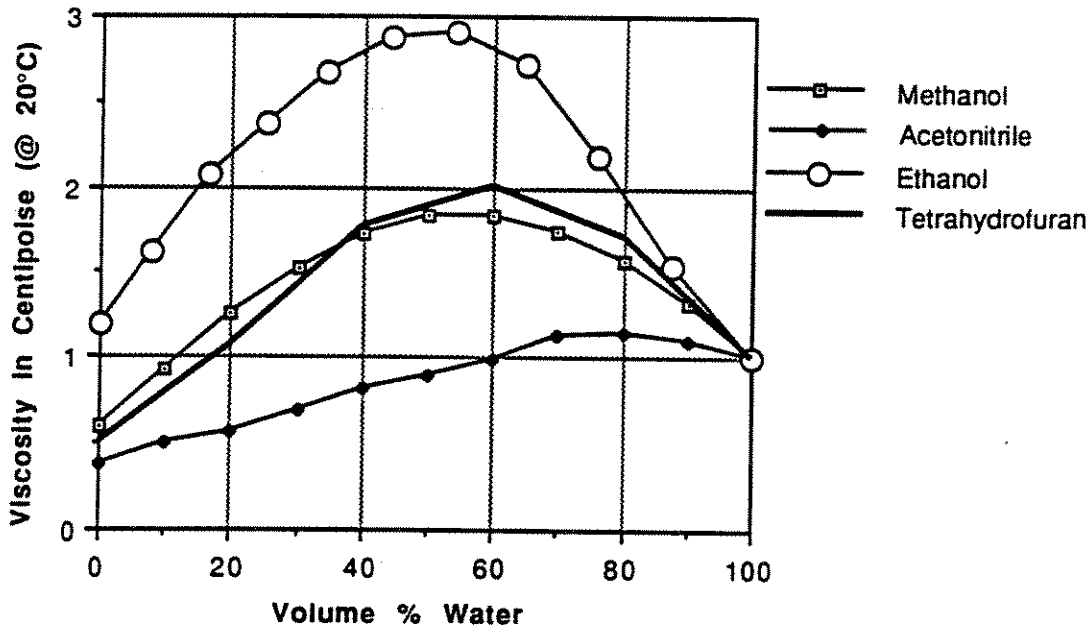


Figure 4 Viscosities of Aqueous Mixtures

APPENDIX B ORDERING INFORMATION

Contact Waters to order these parts. Include part number, description, and quantity of each item.

Call: 1-800-252-4752

Write: Millipore Corporation
Waters Chromatography Division
34 Maple Street
Milford, MA 01757
Attn: Customer Sales

To avoid duplication, clearly mark confirming orders placed after a telephone order with the word CONFIRMING.

TELEX: 174166 or 174128

FAX: 1-508-872-1990 Attn: Customer Sales
International: Contact the nearest subsidiary.

Table B-1 Part Numbers

Cartridge Packings	Radial-Pak Cartridges 8 x 100 mm	PrepPak Cartridges 25 x 100 mm	PrepPak Cartridges 40 x 100 mm	Prep Guard-Pak Inserts 25 x 100 mm
Porasil® 125 Å, 15-20 µm	25842	38502	37672	38512
Bondapak® C_{18} 125 Å,15-20 µm	25841	38503	37676	38514
µPorasil™ 125 Å, 10 µm	85720	38504	37680	38516
µBondapak™ C_{18}, 125 Å, 10 µm	85721	38505	37684	38518
Delta-Pak™ C_{18} 100 Å, 15 µm	25846	38506	37688	38520
Delta-Pak C_{18} 300 Å, 15 µm	25845	38507	37692	38522
Delta-Pak C_4 100 Å, 15 µm	25848	38508	37696	38524
Delta-Pak C_4 300 Å, 15 µm	25847	38509	37700	38526
Prep Nova C_{18} HR 60 Å, 6 µm	25843	38510	37704	38528
Prep Nova Silica HR 60 Å, 6 µm	25844	38511	37708	38530
Bondapak C_{18} 300 Å, 15-20 µm	38581	38575	37712	38580
Bondapak C_8 300 Å, 15-20 µm	38582	38576	37720	38578

Base Assemblies	8 x 100 mm	25 x 100 mm	40 x 100 mm
RCM base	82887	27577	27577
Chamber	–	33994	27578
Extension tubes, 100 mm	38846	22180	22365

APPENDIX C WARRANTY/SERVICE INFORMATION

Warranty

Millipore Corporation, including its Waters Chromatography division (Waters), warrants its high performance liquid chromatography cartridges in accordance with the following terms and conditions:

Waters will replace without cost any cartridge that fails to perform satisfactorily if notified within 90 days form your receipt. Any cartridge returned must have a Return Authorization Number granted by the Waters Customer Service Department. Approval is subject to the following exclusions:

- Physical damage to the cartridge because of misuse or abuse.
- Chemical damage to the packing material because of use with incompatible solvents or buffers, or an incorrect pH.
- Physical damage to the packing material because of operation at incorrect temperatures or pressures.
- Particulate build-up or precipitation in the cartridge causing high internal pressure which has occurred because of improper solvent or sample filtration practices.

Service and applications assistance

Waters Chromatography Division's staff of experienced service specialists provide maintenance assistance on both preventative and/or corrective levels. For complete information and assistance, please call Waters Service Department at 1-800-252-4752. For solutions to particular applications questions, Waters team of technical support personnel are available to help you with specialized support. They may be contacted at 1-800-252-4752 in Milford, MA.

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