INTRODUCTION

The technique of Gel Permeation Chromatography (GPC) was first described by J.C. Moore for the fractionation of synthetic polymers by molecular weight using crosslinked polystyrene gels in non-aqueous mobile phases. The separation mechanism for GPC is based on the size of the polymer in solution relative to the pore size distribution of the column’s packing material. This separation mode relies on the absence of all other separation mechanisms, such as hydrophobic interaction or ion-exchange.

Historically, packing materials for GPC have been largely organic polymers, such as hydrophobic styrene-divinyl benzene gels or hydrophilic hydrocarbyl gels. However, these organic polymer particles do not provide the highest efficiency due to their lack of rigidity and susceptibility to compressing under pressure.

The goal of this investigation was to evaluate the use of low dispersion chromatographic systems in combination with high efficiency essentially non-compressible rigid organic/inorganic packing materials. These hybrid materials have been synthesized to have pore sizes ranging from 45Å to 450Å, and were evaluated with regard to their ability to perform size-based separations. Initial evaluations were performed to determine the usable molecular weight range for each of the pore size materials and the results are compared to those of commercially available GPC materials of similar pore size.

METHODS

A. Materials Evaluated

The hybrid packing materials had pore sizes ranging from 45Å to 450Å. The 125Å, 200Å, and 450Å materials were 2.5 µm, and the 45Å material was 1.7 µm. These materials were evaluated in 4.6 x 150 mm stainless steel hardware.

Table 1. ACQUITY APC™ XT Materials

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Molecular Weight Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC XT 45 1.7µm</td>
<td>200-5,000</td>
</tr>
<tr>
<td>APC XT 125 2.5µm</td>
<td>1,000-30,000</td>
</tr>
<tr>
<td>APC XT 200 2.5µm</td>
<td>3,000-70,000</td>
</tr>
<tr>
<td>APC XT 450 2.5µm</td>
<td>20,000-400,000</td>
</tr>
</tbody>
</table>

HSPgel™ HR columns (6.0 x 150 mm) were chosen as the commercially available product that would possess a similar pore size. HSPgel™ HR columns are available with pore sizes of 45Å, 125Å, 200Å, and 450Å. These columns were evaluated in 4.6 x 150 mm stainless steel hardware.

Table 2. HSPgel HR Materials

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Molecular Weight Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSPgel HR 1.0</td>
<td>100-1,000</td>
</tr>
<tr>
<td>HSPgel HR 2.0</td>
<td>500-10,000</td>
</tr>
<tr>
<td>HSPgel HR 3.0</td>
<td>1,000-20,000</td>
</tr>
<tr>
<td>HSPgel HR 4.0</td>
<td>2,000-60,000</td>
</tr>
</tbody>
</table>

| Material Type | 10,000-400,000 |

B. Chromatographic Conditions

System: Prototype ACQUITY APC System using an ACQUITY TUV™ with a 5 mm stainless steel flow cell (1500 µL).

System variance: 2.70 µL

Detection wavelength: 254 nm

Column temperature: 30 °C

Mobile phase: 100% tetrahydrofuran (THF)

Injection volume: 3.4 µL (6.0 x 150 mm)

Table 3. Linear Polystyrene Standards Prepared in 100 % THF

<table>
<thead>
<tr>
<th>Material</th>
<th>Molecular Weight (Da)</th>
<th>Conc. (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>6,000 - 250,000 Da</td>
<td>0.020</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>2,520,000 - 474 Da</td>
<td>0.000</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>2,520,000 - 474 Da</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DISCUSSION

Traditional styrene-divinyl benzene gels come in a variety of pore sizes which gives them the ability to analyze samples having a wide range of molecular weights. Rigid hybrid materials with pore sizes of 45Å, 125Å, 200Å, and 450Å were evaluated in 4.6 x 150 mm stainless steel hardware. These materials were evaluated at 1.7 µm and 2.5 µm. The 45Å material was 1.7 µm. These materials were evaluated in 4.6 x 150 mm stainless steel hardware.

The choice of flow rate is at times a compromise between resolution, analysis time, and column operating limits. The recommended maximum flow rate for the HSPgel HR 3µm columns was 0.6 mL/min. The scaled flow rate of 0.3 mL/min was easily achieved on the APC XT materials and could be exceeded. From the flow rate study using the APC XT 4.5 µm column, the highest column efficiencies were achieved at flow rates greater than 0.5 mL/min (Figure 4). This can be observed by the increased resolution of those molecular weights in the polystyrene oligomers with the peak molecular weight of 474Å (Figure 5). Polystyrene with a peak molecular weight of 2.52 kDa was analyzed at 0.2 mL/min and 0.8 mL/min to determine the Mn, Mw, and Mw/Mn values. They were comparable to the values given for the standard and not significantly different at the two flow rates (Table 5).

CONCLUSION

- ACQUITY APC column materials with 45Å, 125Å, 200Å, and 450Å pores, demonstrated a separation range of 200 Da to 400 kDa for linear polystyrenes; comparable to the commercially available styrene- DVB gel family.

- The benefits of using a low dispersion ACQUITY APC system include: 6 mm ID columns packed with rigid hybrid particles for sized-based separations: a) Reduction in analysis times. b) Lower consumption of solvents. c) Maintenance of resolution for oligomers. d) Accurate determination of Mw values.

- Size-based separations using aqueous mobile phases have been achieved using ACQUITY APC AQ materials based on the same rigid, hybrid particles (Figure 6).

ACKNOWLEDGMENTS

The authors would like to thank Waters Corporation for their financial support. The authors also gratefully acknowledge the contributions of REACH Consulting, Inc. for their assistance in the development of the hybrid materials and the National Science Foundation for funding this research. We also acknowledge the helpful advice of Dr. John Gibson and Dr. John D. Wagoner.

REFERENCES

1. J. C. Moore; Gel Permeation Chromatography. I. A New Method for Practice of Gel Permeation and Gel Filtration Chromatography Hoboken, NJ: John Wiley and Sons, Inc. 2009


3. J. S. M. van den Anker, M. S. J. M. Jansen, G. M. J. van Eijk; Selection of SEC Columns for the Analysis of Synthetic Polymers J. Chromatogr. 1987; 432: 1-10

Figure 1. 50% Images Highlighting the Morphology Changes for the Different Pore Sizes.

Figure 2. HPSEC HR Columns: Calibration Curves Generated Using Linear Polystyrene Standards.

Figure 3. ACQUITY APC XT Columns: Calibration Curves Generated Using Linear Polystyrene Standards.

Figure 4. Flow Rate Study Using ACQUITY APC XT 45 1.7 µm.

Figure 5. Effect of Flow Rate on Polystyrene Mp 474 Da and Polystyrene Mw 2.92 kDa Using an ACQUITY AC 45 XT 1.7 µm Column.

Figure 6. PublUnstand Standards on ACQUITY APC AQ 200 2.5µm 100 mM NaNO3 in 80:20 HCl:acetonitrile (v/v).

Figure 7. Detection at 254 nm, Column Temperature: 40 °C.