Liquid Chromatography Problem Solving and Troubleshooting

Question

I have noticed over the last few years articles where polymer reversed-phase materials have been used. For the most part, I don't see any advantages in using these types of columns in the reported applications. Clearly, there must be cases where the columns offer advantages over conventional reversed-phase materials or the manufacturers would not continue to sell this type of product. Could you provide a little background about reversed-phase polymer columns and some hints where the columns would be most useful?

Answer

Historically, as separation materials, poly(styrene-divinylbenzene) polymeric packings have been in use for many years. In the earlier accounts, they were introduced as ion-exchange resins following conversion to their cation and anion forms and later as non-modified size exclusion packings for carrying out molecular weight determinations (1). In addition to these more classical applications, a few manufacturers have produced materials that are highly cross-linked with controlled pore sizes in the range of 100 to 300 Å as well as solid materials. For the most part, these types of columns have not been used to the extent that columns packed with chemically modified silica have because of efficiency limitations caused by poor mass transfer kinetics. Greater details about these effects can be found elsewhere (2) and in other articles from these authors.

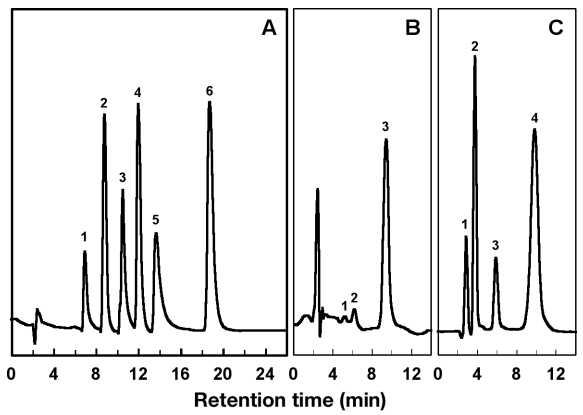

Figure 1. Application of highly cross-linked poly(styrene-divinylbenzene) reversed-phase columns. Chromatograms showing the separation of: (A) dihydroxy (peaks 2, 4, and 6) and hydroxyamino (peaks 1, 3, and 5) benzenes at an eluent pH of 5.2; (B) hydrochlorothiazide (peak 3) from impurities (1 and 2) at an eluent pH of 3.0 (B); and (C) cytosine nucleosides (peaks 1, 3, and 4) from a degradation product (peak 2) at an eluent pH of 2.5.

In more recent literature, a number of investigators have developed a variety of specialty polymer-based packings that are targeted for performing highly selective separations. In this instance, the polymerization process is carried out in the presence of a template molecule (i.e., the target analyte) to produce a shape selective cavity in the polymer matrix once it is removed by washing. A common application of these types of packings is the resolution of optical isomers, especially those of biomedical and pharmaceutical interest. Additional applications can be found in a recent review of the pharmaceutical literature (3) and other similar past reviews in this series by the same authors.

The most important advantage of highly cross-linked poly(styrene-divinylbenzene) reversed-phase packings is their ruggedness when using extremely harsh eluent conditions. Most conventional silica-based reversed-phase materials, such as the commonly used octyl and octadecyl phases, have useful pH ranges from approximately 3.5 to 8.0. Although separations can be carried out beyond this range, when they are, most materials degrade rapidly because of a combination of phase loss and dissolution of the silica matrix. Poly(styrene-divinylbenzene) can be used over a much broader pH range (i.e., ~ 0–13). Likewise, high concentrations of secondary eluent additives also do not cause column breakdown as they sometimes can with silica-based packings. However, the trade-off for the polymer columns is much lower plate counts for equivalent dimensions and particle size.

Figure 1 shows three examples of separations carried out using highly cross-linked poly(styrene-divinylbenzene) columns. The first chromatogram (Figure 1A) is for a series of highly polar, simple dihydroxy and aminohydroxy

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Roger Gilpin
Associate Editor

benzenes. Although the analytes can be separated on the polymer column, this application represents a case where there is no advantage in using this type of column because (*i*) the eluent conditions are relatively mild (i.e., pH 5.2) and (*ii*) the polar analytes are not compatible with the highly hydrophobic nature of the packing, which results in considerable peak tailing. In the second chromatogram (Figure 1B), the polymer column probably offers a small advantage in terms of stability because the separation is carried out at pH 3.0, but clearly the efficiency of the column is not as good as that obtainable on conventional silica-based reversed-phase materials. In the third chromatogram (Figure 1C), a separation of cytosine nucleosides is carried out using a pH 2.5 eluent. In this latter example, the poly(styrene-divinylbenzene) column offers measurable stability advantages over conventional silica-based materials, and this is a good example of the type of assay that will benefit most from the use a polymer column.

References

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