High-efficiency, silica-based packings have been used in HPLC since the introduction of µBondapak® C18 (Waters Corp., Milford, MA) in 1973. Over the course of the last 26 years, many improvements have been made to the technology of these packings. Notable developments in recent years include the use of high-purity silicas such as Symmetry® packings (Waters)1 and the incorporation of a polar functional group into the ligand as applied in SymmetryShield™ packings (Waters).2 Both approaches have improved the peak shapes obtained by the user. In addition, packings with a polar functional group give different selectivities than classical packings3 and can be used in mobile phases with 100% water without difficulty. Furthermore, much progress has been made in the reproducibility of the synthesis of packings, as has been demonstrated in an independent investigation of the batch-to-batch reproducibility of Symmetry C18.4

A general problem with silica-based packings remains, however: Silica dissolves at alkaline pH values. Most manufacturers of silica-based bonded phases recommend that they should not be used above pH 8. While some progress has been made in understanding the dissolution of these phases at alkaline pH,5–7 a solution to this difficulty has not been found until recently.8

On the other hand, packings made from organic polymers, such as poly(divinylbenzene), are stable over a wide pH range, but suffer from low plate counts and often from distorted peaks. In addition, polymer-based packings are plagued by poor mechanical stability due to the fact that the packings swell and shrink in the presence of different solvents.

Over the last few years, solutions to this dilemma have been investigated. Hybrid organic-inorganic technology provides a novel solution. This technology and the performance of the resulting packings (XTerra™, Waters) are the subject of this article.

Technology

To design hybrid organic-inorganic particles, the developers followed a path that was first explored by Unger and co-workers.9 A tetraalkoxysilane and an alkyltrialkoxysilane are reacted with each other to form a precursor, and this precursor is then used to create the particles:

\[
(RO) \text{Si} + n (RO) \text{Si} \text{R}^* + (1.5 n + 2) \text{H}_2\text{O} \rightarrow \text{SiO}_2(\text{R}^* \text{SiO}_{1.5})_n + (3 n + 4) \text{ROH}
\]

As one can see, the final particle contains an alkyl group (R*) that is incorporated into the matrix of the packing. This approach yields a material that contains both inorganic units (SiO₂) and organic units (R′SiO₁₅), combined at the molecular level. The use of different alkyl groups as well as different ratios of the two starting materials was investigated. The best overall properties were achieved for a packing with the composition SiO₂(CH₃SiO₁₅)₀.₅. With this composition, every third silicon atom in the matrix is substituted with a methyl group. The carbon content of such a packing was determined to be about 7% carbon before any surface modifications. The packing is as hard as silica and, just like silica, does not swell or shrink in the presence of organic solvents. The pH stability of bonded phases based on these hybrid particles (see below) exceeds that of silica-based bonded phases.

A consideration in the design of the XTerra particle was to leave silanols on the surface of the packing that allow for further derivatization of the packing. This makes the design of specific packings possible, for example, C₁₈ and C₈ packings for reversed-phase chromatography. As expected, the surface concentrations achievable on the XTerra particle are limited to lower values than for classical silica due to the presence of the methyl groups on the surface of the packing. For the XTerra particles, the C₈ and C₁₈ surface concentrations are limited to about 2.5 µmol/m², while for classical silica packings, the surface concentration may reach 3.6 µmol/m². This is not a detriment, however, since the concentration of residual silanols is significantly reduced compared to classical packings, and a large portion of the surface is occupied by methyl groups instead of silanols.

The structures of two XTerra bonded phases are shown in Figure 1.

Much progress has been made in the reproducibility of the synthesis of packings.
The first structure shows the XTerra RP packing. A version with an extended chain length, the XTerra RP18 packing, is available as well. For these two packings, a carbamate group has been inserted into the chain. This surface function was selected based on the authors’ experience with SymmetryShield packings. The best peak shapes and wettability in 100% water are key properties of XTerra RP packings.

The second structure shows the XTerra MS C8 packing. It is made using a trifunctional silane, as is the XTerra MS C18 packing. These packings are designed for LC-MS applications, where very low bleed is a must. Both the XTerra RP packings and XTerra MS packings are fully endcapped.

As shown in the structures, the common side group is not an unbonded silanol group, but rather a methylsiloxane group. As a consequence of this, the residual activity of silanols is substantially lower on XTerra packings than on classical silica-based packings. This reduced activity results in improved peak shapes for basic analytes.

pH stability

An important feature of the packings is their significantly improved pH stability compared to classical silica-based packings. While the recommended range for silica-based packings is normally pH 2–8, XTerra packings can be used from pH 1 to 12. This expands the range of mobile phase conditions available to the chromatographer. It is possible to run basic analytes on a high-performance packing under nonionizing conditions. This provides new opportunities to manipulate selectivity, as well as the reproducibility of an assay. If one uses a packing at intermediate pH values, the retention of ionic, especially basic, analytes is highly susceptible to small changes in mobile phase pH. This is not so when the analytes are either fully ionized or not ionized at all, i.e., at low and high pH values. Consequently, the reproducibility of an assay improves at high and low pH, and XTerra packings provide this option.

The stability of the packings has been investigated at both acidic and basic pH. At acidic pH, the stability of XTerra MS C18 packing has been compared to some of the best silica-based packings on the market. The same protocol was used for all types of packings. The columns were exposed to 1% trifluoroacetic acid (TFA) at pH 1.2 at 50 °C for a given period of time, then washed with water and methanol for 20 min each. The columns were then tested using a standardized test condition with a mobile phase of methanol/pH 7.0 potassium phosphate buffer 65/35 vol/vol. The results are shown in Figure 2. The retention time of acenaphthene is plotted as a percentage of the initial value versus the time the columns were exposed to the TFA solution. As one can see, the MS C18 packing works as well or better than classical silica-based packings under acidic conditions.

More important is the stability of the packings at alkaline pH. Most silica-based packings begin to fail rapidly around pH 8. However, XTerra packings can be used to pH 12.

Silica-based packings fail rapidly at very high pH values. The compatibility of XTerra packings with high pH can best be demonstrated with column durability tests under realistic use conditions. Column lifetime at pH 11.5 was tested using a pyrrolidine buffer. The column tested was a 4.6 × 150 mm, 5 µm XTerra RP18 column protected with a guard column. The mobile phase was acetonitrile/50 mM pyrrolidine HCl buffer at pH 11.5 50/50 vol/vol. The column was run continuously at 30 °C at 1 mL/min, and a mixture of basic analytes—methamphetamine (pK 9.5), nor- triptyline (pK 9.7), and propranolol (pK 9.5)—was injected continuously. The results are shown in Figures 3 and 4. The column lasted for more than 45 days at pH 11.5 without deterioration of performance as measured by plate count and peak asymmetry.

![Figure 1: Structures of XTerra bonded phases.](image1)

![Figure 2: Exposure of packings to acidic hydrolysis. The MS C18 packing compares favorably to some of the better silica-based packings that are commercially available.](image2)
or shifts in retention (other than those due to changes in mobile phase composition).

Application of extended pH range

The packings’ extended pH stability allows compromise-free chromatography of basic analytes under nonionizing conditions. This is a powerful tool for the manipulation of the selectivity of a separation, which is not possible with classical silica-based packings. This can best be demonstrated by looking at the shifts in retention found for acidic and basic analytes as a function of pH. This was studied using XTerra RP<sub>18</sub> packing with a mobile phase of acetonitrile/20 mM buffer 35/65 vol/vol. The results are shown in Figure 5. Various buffers were used to cover the entire pH range from pH 1 to 12 (shown on the x-axis). On the y-axis, the logarithm of the retention factor is shown. The retention factor varied from as little as 0.2 to a high of around 50, spanning over two orders of magnitude.

The analytes used were a mixture of basic, acidic, and neutral compounds. The retention of the neutral analyte, toluamide, is not affected by the pH changes. The acidic analyte, ibuprofen, changes from high retention below pH 4 to a retention factor of less than 1 at pH 9. Conversely, the basic analytes nortriptyline, doxepin, lidocaine, and imipramine are weakly retained at acidic pH and reach high retention at pH 9–10. Acetaminophen contains a phenol group, which is a very weak acid. It is ionized above pH 8, reducing the retention of the analyte. For every analyte, the pH range over which retention changes spans over 4 pH units. Outside this range, the retention is not affected by pH shifts. Obviously, a pH shift is a very powerful tool if analytes of different charge need to be separated. For example, ibuprofen and toluamide overlap at alkaline pH, but a shift to even a weakly acidic pH results in a clean separation. Even for compounds of the same charge, pH shifts lead to shifts in elution order. At acidic pH, doxepin elutes before imipramine and nortriptyline. Conversely, at alkaline pH nortriptyline and doxepin elute very close to each other, and imipramine elutes last. At intermediate pH, around pH 7 and 8, the best separation between the three tricyclic antidepressants occurs. Stable, i.e., pH-independent retention is observed at both ends of the pH scale. At pH values above 9, basic analytes have lost the positive charge and their retention factor stabilizes. Under these circumstances, the control of the mobile phase pH does not need to be as stringent as at intermediate pH values. This has advantages for the ruggedness of a separation.

Conclusion

XTerra packings are based on a hybrid technology that combines the advantages of silica-based packings with the extended pH range of polymeric packings. The surface chemistry of the packings is identical to the surface chemistry of classical silicas. This makes their use as simple as that of any other reversed-phase packing. On the other hand, the pH stability of the packings extends the pH range accessible for reversed-phase packings. This opens up new options...
for the manipulation of the selectivity of a separation. In addition, the increased pH stability also results in improved ruggedness of the packing under normal operating conditions. The columns are offered in four different surface chemistries (RP8, MS C8, RP18, and MS C18), four particle sizes (2.5, 3.5, 5, and 7 µm), and 270 columns.

References

The authors are with Waters Corp., 34 Maple St., Milford, MA 01757-3696, U.S.A.; tel.: 508-478-2000; fax: 508-872-1990.

For more information: www.waters.com