Why Uncoated Capillary Precolumns Enable Injection of Large Volumes, continued.

...Continued from page 11.

which is the main problem in using glass capillaries, did not damage the stationary phase because the inlet was uncoated. Indeed, when the sample liquid was spreading in the uncoated inlet only, peaks were sharp. The explanation was rapidly at hand. Solutes pass much more rapidly through the inlet if the latter is uncoated (low retention power) and are focused at the entrance of the coated section. Actually they pass through the inlet at low temperature, are stopped in the inlet of the separation column, and wait there until temperature has increased further to enable the separation process to start. It took some scratching of my beard to give this child a name, also because English is the third foreign language in my country. We finally called the uncoated inlet with negligible retention power a "retention gap."

The maximum injection volume

Having learned this, we wanted to explore the usefulness of the retention gap, i.e. how long an uncoated precolumn could be and what would be the limit to the injection volume then. It could be experimentally confirmed that the focusing effect, hence the shortening of the initial bands, was about equal to the ratio of the retention powers in the uncoated inlet and the coated column. Thus, the longer the retention gap or the thicker the coating in the separation column, the more efficient was reconcentration. The retention power of an uncoated inlet corresponded to that of a column coated with a film of around 1 nm thickness. Hence, combined with a separation column with a 1 µm film of stationary phase, the initial bands would be shortened by a factor of 1000. This was breathtaking: as some 20 cm of residual band length can be tolerated in the separation column, the initial band could be 200 m long — presupposing, of course, that the uncoated column inlet was that long. We were more modest and first used a 5 m uncoated inlet to inject twice the total volume of an ordinary 10 µl on-column syringe. As this was immediately successful, we had a 100 µl on-column syringe made, prepared a 50 m deactivated glass capillary and connected it to a 15 m separation column. Eagerly we injected 200 µl of a very dilute sample. The first observation was that the pen of the recorder did not want to return from the solvent peak. The minutes passed and the fear grew that we had flooded the whole gas chromatograph. But finally, after some 35 min, the pen came down very rapidly. Many extremely sharp peaks followed (mostly solvent impurities), showing that reconcentration of the initial bands had worked. With a column temperature closer to the solvent boiling point, the width of the solvent peak was reduced to hardly 10 min. This was a milestone we celebrated with a cake.

The next step (after having carried out the food analyses we are paid to do) was to determine the lengths of the flooded zones per injection volume or how much could be injected into an uncoated precolumn of given size. For example, a 10 m x 0.53 mm ID or a 15 m x 0.32 mm ID precolumn had a capacity to safely retain 50-100 yl of sample liquid. Using 60 m x 0.32 mm ID precolumns, we could, in fact, inject 400 pl.

Concurrent solvent evaporation

We immediately started using the technique for our work, e.g. for the analysis of surface and ground waters. The gain in sensitivity and the advantages for sample preparation were spectacular. Although, as expected for on-column techniques, the samples needed to be reasonably clean to avoid excessively rapid contamination of the precolumn. Some practical problems had to be solved, of course. First of all, a method was needed for joining the uncoated precolumn with the separation column. After having a hard time with butt connectors and fused joints, the press-fits were a great relief (1986). In 1984, we started transferring whole HPLC fractions on-line into GC, comprising 200-350 pl of (normal phase) eluent (HPLC served for sample preseparation or clean-up at high resolution). Since transfer of even larger volumes was desirable (some 400-800 yl), we returned to some basic development work. The sample liquid in the flooded precolumn provides solvent effects to focus the volatile sample components. However, not all of the solvent is needed for this. As the sample was introduced at conditions causing a large proportion of the solvent to evaporate simultaneously (partially concurrent evaporation), the first peaks were still sharp and perfect in size, but for a given precolumn the transfer volume could be increased several times or the precolumn could be shortened. When samples were introduced at a speed such that all solvent evaporated concurrently, an uncoated precolumn of merely 1-3 m in length could receive virtually unlimited volumes of sample-at the expense, of course, of the solvent effects: components eluted below about 150°C were lost. In 1985, we introduced a 10,000 ul volume-but it took 83 min. This was good enough for a record, but the solvent peak required nearly 1 m of chart paper! Furthermore, the FID soon became black like a chimney.

The early vapor exit

On the four automated LC-GC instruments, which perform more than half of our analyses today, partially and fully concurrent