Why Uncoated Capillary Precolumns Enable Injection of Large Volumes

by Dr. Konrad Grob

This issue of Koni's Korner deals with uncoated capillary precolumns or desolvation precolumns. The development of the retention gap technique for introduction of large volumes of sample was an exciting experience of which I would like to give a summary here. Uncoated precolumns are used for two totally different purposes: for on-column injection of large volumes and as a garbage bin (guard column, disposable inlet) for the analysis of samples with non-evaporating by-products ("dirty" samples).

The "Retention Gap"

During the first two years of using on-column injection, we were puzzled by occasional splitting of peaks that eluted at several ten degrees above the oven temperature during injection. In 1981, using glass capillaries, we saw how the injected sample liquid moved rapidly along the capillary wall and deeper into the column. In a 0.32 mm ID column, 2ul easily "flooded" 50 cm of the column inlet. Even worse, sample liquids not wetting the stationary phase (e.g. solutions in methanol on apolar silicones) just left a droplet here and there (as water on a window pane) and entered the column up to several meters for every microliter injected. It was obvious this would not produce the sharp initial bands required. Flooded zones of 20-40 cm seemed to be the maximum for avoiding noticeable peak broadening. A paper by W.L. Saxton (HRC 1984) confirmed this conclusion. This enables injections up to 1-2ul of a wetting sample.

During these experiments, we were puzzled by certain columns that did not produce broad or split peaks even when we injected 5ul It took some time and several cups of coffee to discover these were the columns which we had prepared with 0.5-lm of uncoated inlet. We realized that straightening the end sections of the columns,

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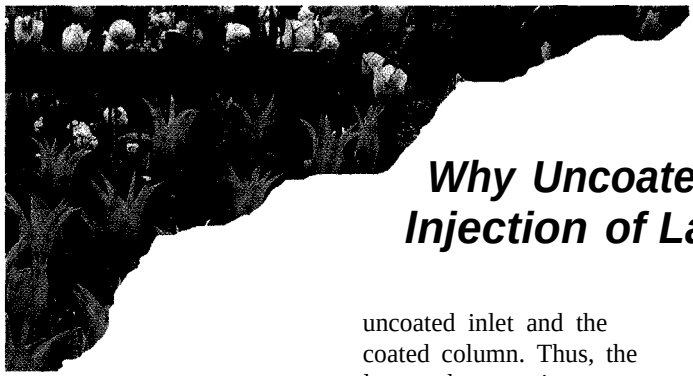

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which is the main problem in using glass capillaries, did not damage the stationary phase because the inlet was uncoated. Indeed, when the sample liquid was spreading in the uncoated inlet only, peaks were sharp. The explanation was rapidly at hand. 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

evaporation are still the key techniques. However, a further improvement was important: the early vapor exit. When more than 50-100ul of solvent are introduced, discharge of the vapors through the whole column becomes slow and the flame detector turns into waste incinerator. A separate outlet is needed for the solvent vapors. The earlier this exit is positioned, the shorter the path is for the vapors and the faster is their release. On the other hand, the inlet must be long enough to retain the solutes, i.e. to achieve solvent/solute separation. The latter is achieved either by solvent trapping in the flooded zone (uncoated precolumn) or by the (less efficient) stationary phase trapping in the coated ("retaining") precolumn (see Figure1). Partially concurrent evaporation provides solvent trapping and usually produces perfect peaks even for components eluted immediately after the solvent. For fully concurrent evaporation, however, just stationary phase trapping is available (often reinforced by phase soaking), which restricts the analysis to solutes eluted several tens of degrees at least above the column temperature during solvent evaporation. With the early vapor exit, evaporation rates went up to typically 100-400 @/min. The new record for concurrent evaporation (from 1989) stands at 20,000 ul of a hexane solution introduced in 20 min.

The future

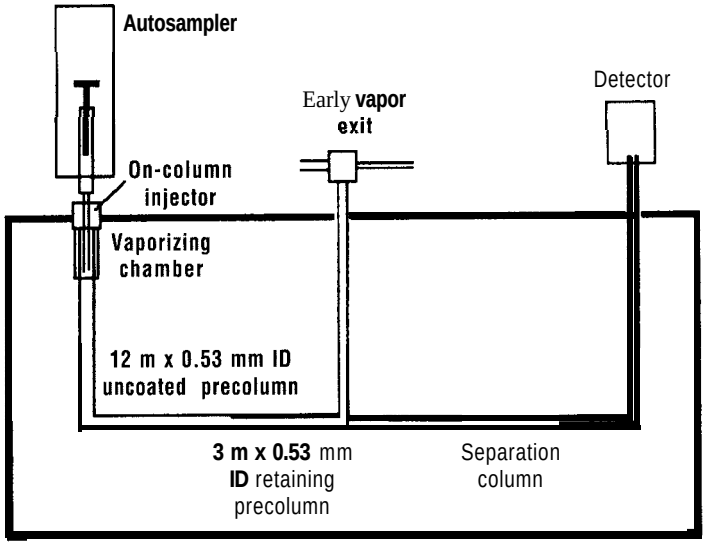
Presently the injection of volumes larger than 10u1 is a subject at most meetings dealing with capillary GC. Two approaches are in the focus of the interest: Programmed Temperature Vaporizing (PTV) injection by the solvent split technique and large volume on-column injection. The PTV technique is relatively robust regarding the injection of "dirty" samples", but the most volatile as well as the high boiling and labile components tend to be lost. The on-column technique avoids such losses and the results are highly quantitative, but the uncoated precolumn is sensitive to contamination by non-evaporating sample byproducts and to attack by aggressive components like water (humidity).

The future will show which technique wins, but the end of the development has not been reached yet. I believe that the on-column/retention gap technique provides the better basis and can still be improved. The first step has been made by the European leader in GC instrumentation, CE Instruments. Because the adjustment of appropriate conditions requires some understanding of the background, a computer-guided instrument for sample volumes up to 250ul was designed. A standardized precolumn system ("Uncoret" - composed of an uncoated and a retaining precolumn in one piece, 15m x 0.53mm ID) is used and the software has evaporation rates for the most commonly solvents in its memory. It automatically adjusts the autosampler injection speed and closes the vapor exit at the appropriate moment for the analysis. In Europe, a good number of instruments are in use for trace analysis of fairly clean samples, and probably about half of them are in commercial laboratories doing water analysis.

The next step is the addition of a small bore, probably permanent, hot vaporizing chamber above the precolumn system (Figure 1) that serves as a filter for retaining "dirt" and for vaporizing non-wetting samples. This adds to the on-column system the robustness against "dirt" of the PTV but maintains the better and more reliable means for solvent/solute separation. In summary, GC is an excellent technique for trace analysis, but the small injection amounts (typically l-3ul) are as appropriate as wooden wheels on a sports car.

Figure 1:

System for Large Volume Injection.



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