Waters
Lab Highlights
An Internal Communication of Applications and Techniques
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Post-Column Reaction Detection
I. Use a KOT* to Improve System Performance

To meet the ever-growing concerns about environmental air and water quality and contamination of our food supply, on-line, post-column chemical conversion or derivatization of LC analytes may be used to enhance detection sensitivity and/or specificity well below the ppm level, down to ppb or even ppt [1].

Post-column reaction systems using chemistries which require more than a second or two of reaction time, however, present a particular design problem. Usually a reagent solution is added to the column effluent through a low-volume mixing tee. Then, the combined stream is passed through a length of small-diameter tubing with a volume calculated to allow sufficient time for the analyte and reagent to react reproducibly before the desired derivative enters the detector cell. For example, if the mobile phase flow rate is 1 mL/min and reagent is added at 0.5 mL/min, then a tube 20' long with an I.D. of 0.018" and a volume of one mL permits a 40 second reaction time.

To reduce band spreading to an acceptable level, one might favor using 0.009" or even smaller I.D. tubing, but this would require 80' of tubing in the example just cited. The back pressure in and space taken up by this much 0.009" tubing would border on the impractical. And, there would still be a sizeable contribution to extra-column band broadening under normal laminar flow conditions.

An elegant solution to this problem uses a knitted open tube [KOT] of TFE capillary tubing in place of a straight length or coil of metal tubing [2,3]. The effect of the geometry of this KOT on band-broadening is dramatic, when compared to the performance of straight or helically coiled tubing, as shown in Figures 1 and 2 [4].

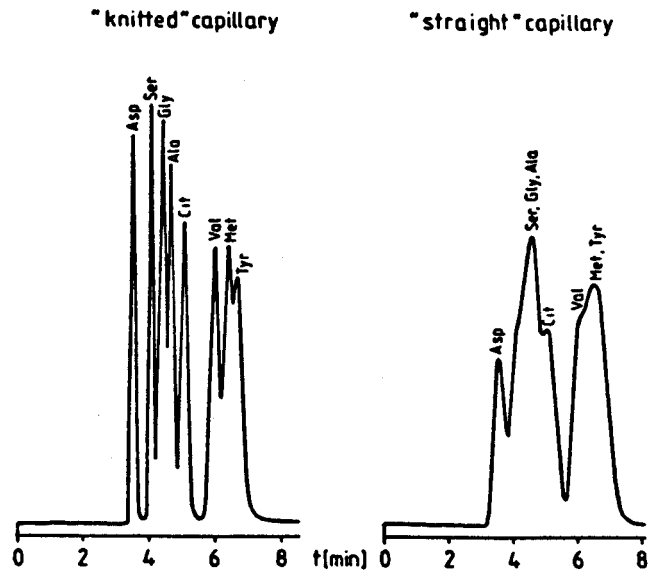

Figure 1. Same separation run using a KOT *vs.* using a straight capillary of equivalent dimensions demonstrates dramatic benefit of KOT technology. [adapted from Ref. 4]

* KOT = Knitted Open Tube

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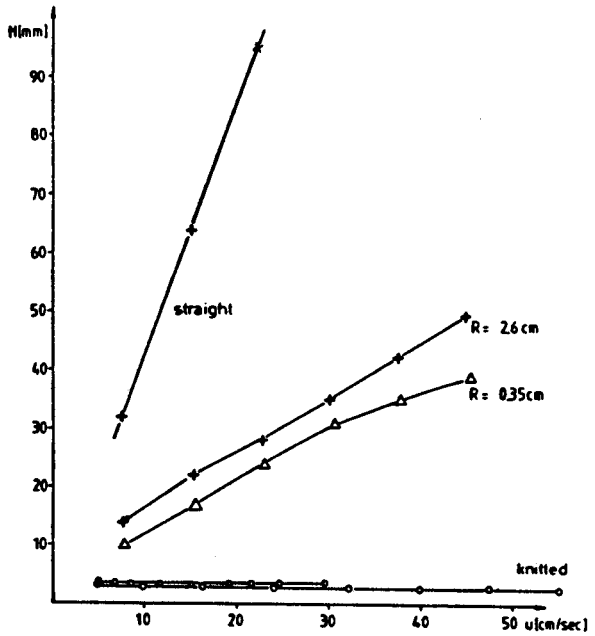

Figure 2. Plot of plate height (H) *vs.* linear velocity (μ) showing peak dispersion in straight (ideal), coiled (radius, R, of 2.6 and 0.35 cm, respectively), and knit open tubes. For reference, one mL/min = 15 cm/sec. [adapted from Ref. 4]

In a KOT, each successive small-diameter loop is bent in a plane nearly perpendicular to that of the previous loop. This alternate right- and left-hand twisting induces secondary flow via centrifugal force inside the tube which, in turn, promotes more radial mixing and less axial mixing, thereby keeping the sample band in a narrow linear zone.

If you are presently doing any post-column reaction methods, you should consider using, in place of homemade spirals of steel or plastic tubing, the most recent refinement of KOT technology (one mL "ninhydrin" coil, Waters P/N 07291), which embodies proprietary design and fabrication concepts [5]. This coil was first developed for the post-column reaction of amino acids with ninhydrin but now is used for several other post-column reaction schemes. Its application to carbamate pesticide analysis will be illustrated in subsequent Lab Highlights in this series.

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References:

1. U.A. Th. Brinkman, *Chromatographia*, **24**, 190-200 (1987).
2. U.D. Neue, Ph.D. Dissertation, U. Saarlandes, Saarbrücken, FRG (1976).
3. H. Engelhardt and U.D. Neue, *Chromatographia*, **15**, 403 (1982).
4. B. Lillig and H. Engelhardt, Chapter 1 in *Reaction Detection in Liquid Chromatography*, I.S. Krull, Ed., Marcel Dekker, New York (1986), 23-24.
5. U.D. Neue and T. Dourdeville, personal communication.