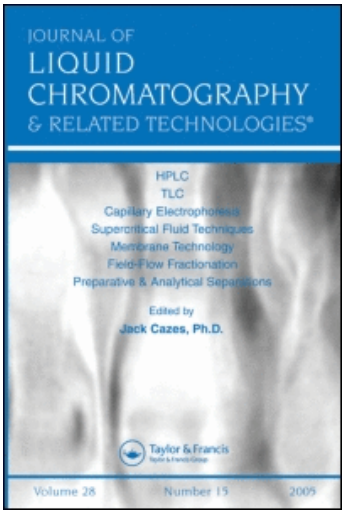

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INEXPENSIVE, LOW-DEAD VOLUME FLOW CELLS FOR MICROCOLUMN LC

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ABSTRACT

The dead volume of the detector flow cell is very important in packed microcolumn liquid chromatography. Most commercially available detectors are inadequate because of excessive extra column band broadening. In this work, the conversion of conventional liquid chromatographic UV detectors to low dead volume capillary detectors is described. Inexpensive flow cell conversion has been completed successfully on four different LC detectors.

INTRODUCTION

Since the advent of fused silica capillaries (1), packed microcolumn liquid chromatography (LC) has become increasingly popular (2-7). Indeed, it seems to be one of the most practical ways of obtaining very high numbers of theoretical plates ($>10^5$) in LC (8,9). Efficiency in packed microcolumn LC is very

dependent on extra-column band broadening effects. Consequently, very low volume injectors, detectors and fittings are absolutely essential. Currently, there is but one commercially available UV-visible detector of appropriate flow cell volume (~0.03 µl). Unfortunately, this detector is very expensive (10). The alternative is to convert the flow cell in conventional LC detectors to low-dead-volume analogues that maintain reasonable sensitivity. Some researchers have briefly reported flow cells for microcolumn LC (11-13). In this work, we describe a facile, inexpensive flow-cell conversion that has been completed successfully on four different LC detectors (i.e., Shimadzu SPD-2AM and SPD-6A, Waters model 440 and Gow-Mac model 080-20).

EXPERIMENTAL

The construction of the modified flow cell consists of three main steps; cell block construction, razor blade (slit) alignment and capillary placement. Minimal amounts of skill and equipment are necessary to construct a working "micro-volume" flow cell, but the most difficult step is acquiring and reproducing a duplicate cell.

The cell block must be precisely machined only so far as it must fit into the detector housing as well as the factory provided cell. The example we will describe will be for a Shimadzu SPD-2AM variable wavelength detector. Figure 1 shows the original factory microbore cell to the right and the machined cell to the left. The cell was cut out of a block of grey

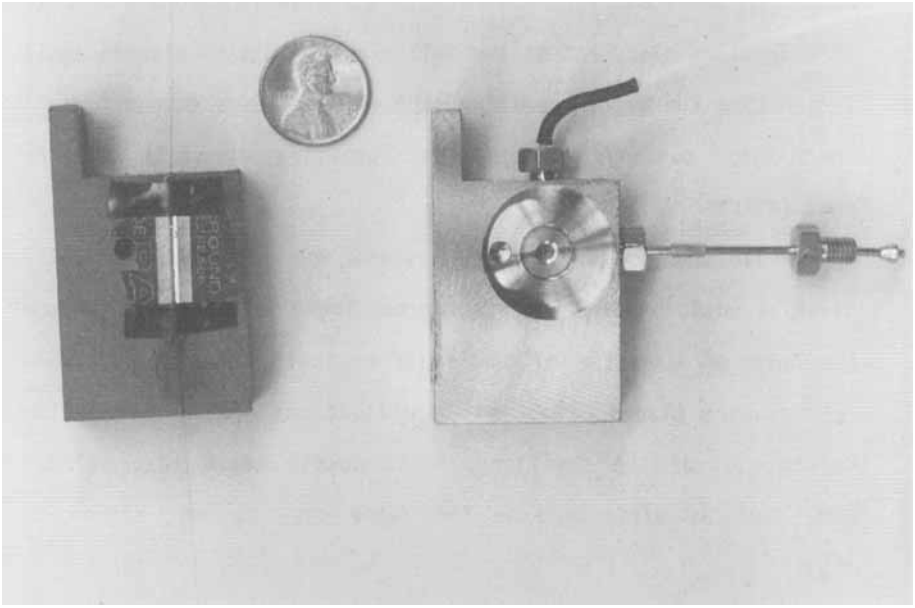

Figure 1. Photograph of factory supplied microbore flow cell with 0.5 µl volume capacity on the right and on the left, the machined cell completed with razor blades and capillary in place.

polyvinyl chloride (PVC). PVC was used because it was inexpensive and easy to machine.

The Shimadzu SPD-2AM has two light paths to the photodetectors; one for the reference and one for the flow cell. For the reference cavity of the cell, 1/8 inch holes were countersunk to a depth of 6mm from the front and back. The reference hole was completed by connecting the two countersunk holes with a #79 (0.37mm) drill bit (Krivo Industrial Supply, Chicago, IL). The capillary flow cell hole was drilled with a

7/16 inch drill bit (see Figure 2). The size of the hole drilled for capillary placement on the cell block should be significantly larger than the actual capillary and also be large enough for all the light passing through the capillary to fall on the photodetector.

Razor blades were used as slits for the machined cell. Since it would be advantageous for the light to pass only through the internal diameter of the capillary cell, the razor blades (slits) were placed so as to block light not coming through the hollow i.d. of the capillary. Thin double edged razor blades were used as slits because they were easy to cut, shape and align.

Figure 3 shows the sequence of A) fixing the first razor blade (slit) to the cell with silicone glue; B) placing the capillary next to the first slit with cyanoacrylate adhesive (Mil-A-46050C, Type II Cl.2, Threebond of America, Inc., Torrence, CA); and C) placing the second blade (slit) up against the capillary and adhering to the cell block with silicone glue.

Polymide coated, fused silica capillary tubing (50 µm i.d. x 150 µm o.d. and 250 µm i.d. x 350 µm o.d., PolyMicro Technologies, Inc., Phoenix, AZ) was used to fabricate micro flow cells. 10 mm of the polyimide coating in the middle of a 20cm length of capillary was removed with concentrated sulfuric acid. This cleared portion of the capillary was used as the actual flow cell and appropriately placed over the cell block hole as shown in Figure 3B and 3C.

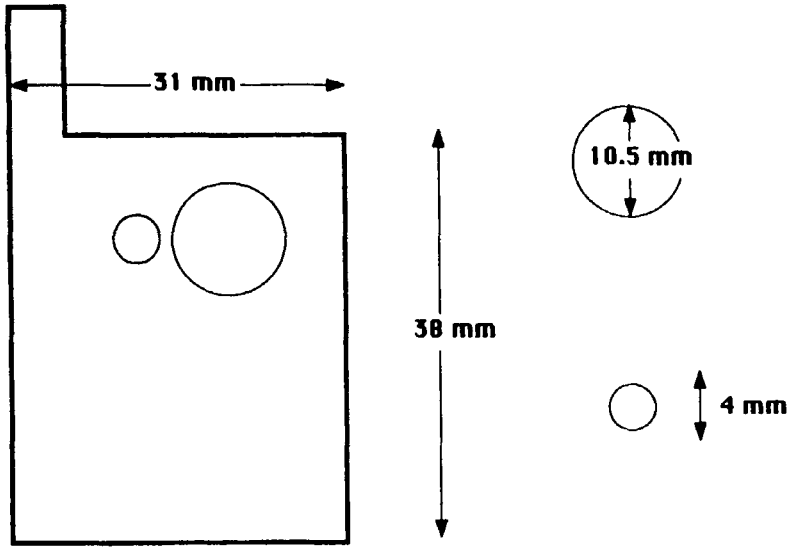

Figure 2. Shape and dimensions of the machine PVC cell block for the Shimadzu SPD-2AM.

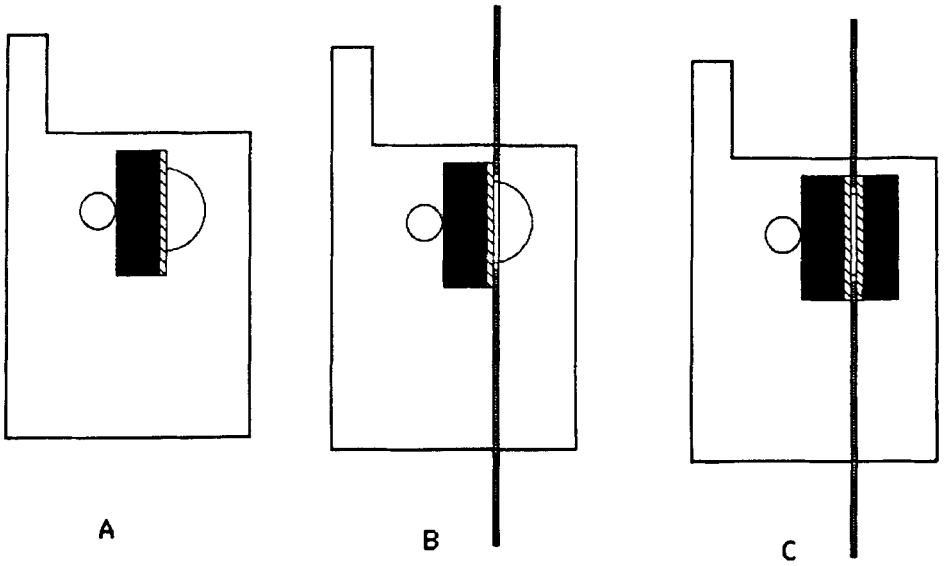

Figure 3. Steps used in making a working 0.013 µl capillary flow cell. Step A shows the first razor blade secured over half of the capillary hole. Step B shows the capillary glued next to the first razor blade. Note that the polyimide coating was retained except for the area that the light must pass. Step C shows the second razor blade secured against the capillary.

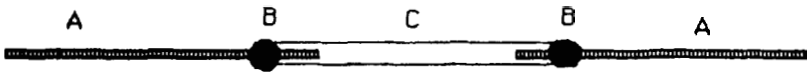

Figure 4. The connections involved in making the 0.24 µl (250 µm pathlength) flow cell. A is the 50 µm i.d., capillary with the polyimide coating. B is the epoxied joint described in the text. C is the 250 µm i.d. capillary with the polyimide coating removed.

For a 0.013 µl flow-cell, the 50 µm i.d. capillary was used. For a 0.24 µl flow-cell, the smaller 150 µm o.d. capillary was epoxied to the cleared segment of the 350 µm o.d. capillary with Epo-tek (Epoxy Technology, Inc., Bitlerica, MA). This is shown in Figure 4.

A Shimadzu model 5A Liquid Chromatograph was used in conjunction with a packed C_{18} (packing material from Advanced Separations Technologies, Inc., Whippany, NJ) microcolumn as previously described (14,15). A Valco (Houston, TX) 0.2 µl internal loop injector was used. Solutions of diphenylamine (Fisher Scientific, Plano, TX, thrice recrystallized), phenanthracene and pyrene (used as received, Supelco, Inc., Bellefonte, PA) were used to measure efficiency. Measurements were made at 254 nm.

RESULTS AND DISCUSSION

The efficiency of microcolumn LC is critically dependent on the dead-volume of the detector flow-cell. This is clearly illustrated by the data in Table 1. Using a commercially available microbore flow cell (0.5 µl volume) the microcolumn system appears to be quite inefficient (Table 1). Using a

Table I. Effect of Flow Cell Size on Chromatographic Efficiency in Microcolumn LC[a].

	Cell Volume		t_r[b] (min)	$W_{1/2}$ (cm)	N
A.	0.5 µl (commercial)	1)	33.18	0.14	14600
		2)	40.09	0.155	17400
		3)	45.44	0.18	16600
B.	0.24 µl (250 µm i.d. tubing)	1)	32.72	0.9	34500
		2)	39.72	1.0	41200
		3)	45.39	1.2	37300
C.	0.013 µl (50 µm i.d. tubing)	1)	33.60	0.08	46100
		2)	41.01	0.1	48900
		3)	47.14	0.11	47900

[a]Compounds used were (1) diphenylamine (0.525 mg/ml), (2) phenanthracene (0.15 mg/ml and (3) pyrene (0.25 mg/ml). 0.2 µl of each solution was injected into a C_{18} packed microcolumn (50 cm X 250 µm i.d.). The mobile phase was 85:15 V:V Acetonitrile:Water.

[b]Chart speed was 0.217 cm/min.

converted flow cell containing a 250 µm i.d. fused silica capillary, the plate count more than doubles (Table 1). By changing to a 50 µm i.d. fused silica capillary, the efficiency increases to a level obtained with a single commercial detector (Table 1).

The low-dead-volume flow cells (see Experimental Section) can be made in less than one day for under twenty dollars. The sensitivity with the 0.24 µl flow cell is better than that of the 0.013 µl capillary cell. This difference is due both to

pathlength differences and the lens effect of the cylinderical capillaries. The detection limit for phenanthracene (measured with the 0.24 µl flow cell) was found to be 1.33 µg/ml and 0.7 µg/ml for 0.013 µl flow all at 254 nm. No other alteration of the detector is necessary. Furthermore, the detector can be quickly and easily converted back to its original state.

ACKNOWLEDGMENTS

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