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# Evaluation of the potential usefulness of in situ fiber optic sensors for column monitoring in chromatographic separations and purifications

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**Abstract**

Fiber optic sensors show promise for real-time monitoring of local conditions inside chromatography columns. Experiments on a Sephadex G-50 column, with blue dextran as the test analyte, showed that insertion of a single fiber or a lattice of fibers did not interfere with column fluidics, provided the fiber diameter was within the same order of magnitude as the diameters of the particles composing the column packing. Experiments on a Sephadex G-50 column, with fluorescein as the test analyte, showed that column packing particles do not interfere with the sensor's ability to accurately reflect differences in fluorescence produced by fluorescein under different conditions (e.g., concentration, pH). Experiments with fluorescein on a G-50 column also showed that fiber optic sensors can be used to monitor local analyte concentrations within a column and to diagnose column problems which lead to reduced column efficiency.

*Keywords:* Fiber optic sensors; Detection, LC; Optical fibers; Fluorescence detection; Column monitoring; Fluorescein

## 1. Introduction

Column chromatography is one of the main tools used in the separation and purification of potentially therapeutic biological materials made in bioreactors [1]. The current technology for process monitoring consists of, at most, measurement of pH, pressure, conductivity and UV-absorbing species at inlet and outlet positions of the column. Although inlet and outlet monitoring provides information for corrective action downstream or in subsequent batches, the purification system as a whole remains passive. Furthermore, inlet and outlet monitoring fails to capture many of the important effects that occur within the column itself. These effects include boundary distortions or band broadening caused by nonuniformities in the packing medium [2] or non-equilibrium phenomena at the column inlet and outlet [3–7], development of reaction zones among interacting components in the column [8], and development of gradients and inhomogeneities during the chromatographic process [3–6,9–12]. All of these effects reduce the efficiency of a column.

Real-time monitoring of analytes and conditions inside a chromatography column should prove useful in column performance studies and in process or

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method development. It can also be useful for troubleshooting and identifying the precise location of column problems. Furthermore, a detailed knowledge of conditions inside the column and of the flow of the species of interest, as well as key impurities is the first step toward constructing intelligent large scale preparative separation systems. It is hoped that, ultimately, control of the process can be maintained at all times by means of sensing, feedback, and actuation operations acting on all important variables.

The work reported here is a preliminary evaluation of the suitability of fiber optic sensors for in situ, real time monitoring in column chromatography. For these studies, we constructed a chromatography column with a jointed wall through which an optical fiber could be inserted and we conducted several simple experiments that addressed fundamental concerns about the operation of a fiber optic sensor in situ. These concerns included disruption of column fluidics by the optical fiber, interference of packing medium with operation of the sensor, and discrepancy between fiber optic detection and conventional detection methods.

An ultimate goal of our work is to outfit single chromatography columns with many fiber optic sensors, each having the ability to monitor one or more different column conditions or analytes. We are taking a stepwise approach to achieve this goal, starting with a simple fiber optic sensor that excites fluorescence in a passing analyte and simultaneously collects the fluorescence for evaluation. Subsequent sensors will be based on optical fibers whose surfaces are derivatized with reagents that interact specifically with analytes of interest, as described by the examples in Refs. [13–16].

The sensor used for the preliminary evaluation reported here was kept as simple as possible and did not involve the complicating effects of chemical reactions or diffusion zones associated with more sophisticated sensors [17,18]. By using the same optical fiber to carry and distribute exciting light to the sampling region and to collect and carry the resulting fluorescence back to the detector system, we maximized the overlap between the excited volume and the collection volume, and thereby obtained the largest possible signal from the sample.

## 2. Experimental

A single chromatography column was fabricated from two lengths of clear polyvinyl chloride pipe, butted together lengthwise one on top of the other. The column arrangement and dimensions are shown in Fig. 1. The butt joint between the two pipe lengths served as a slot in the wall through which one or more optical fibers could be placed. A pair of soft elastomeric ring gaskets (DAP silicone sealant, Dow, Midland, MI, USA), with inside diameters matched to that of the column, prevented leakage at the joint without crushing the optical fiber(s). Through this joint, optical fibers could be passed either partially or completely across the column diameter. An external clamp, not shown in Fig. 1, held the two portions of the column firmly together.

The assembled column was fitted at top and bottom with medium pressure, 2.5 cm diameter flow adaptors (Fisher, Pittsburgh, PA, USA). These flow adaptors defined the column length, the outlet (bot-

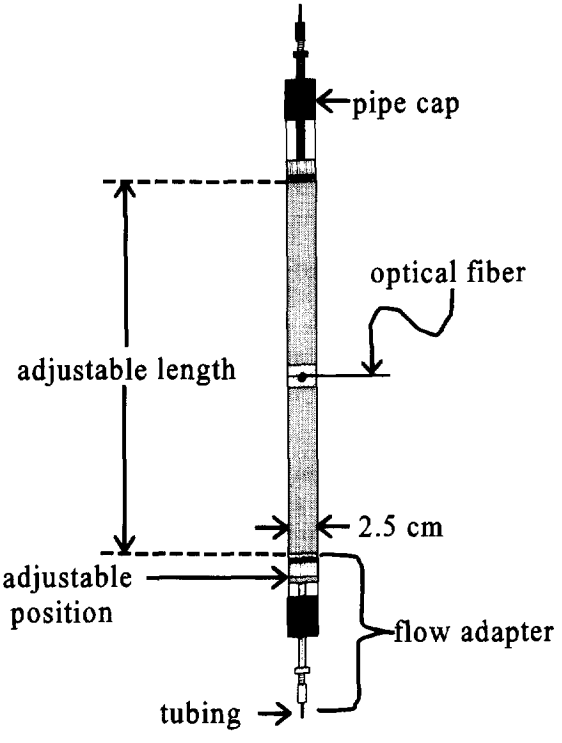


Fig. 1. Schematic diagram of column.

tom) adaptor serving as the support for the column packing and the inlet (top) adaptor directly contacting the top surface of the packing.

With this type of column, bed volumes within the range 90–160 ml were possible. Also, the optical fiber could be positioned at any location along the length of the column by the appropriate selection of column packing height and relative position of the bottom of the column with respect to the joint. After these selections were made for a given column, it was packed by gravity flow.

A Rheodyne Model 5020 low pressure injector (Rainin Instrument, Woburn, MA, USA) with 1-ml sample loop was used to inject analyte solution onto the top of the column. This loop was used for sample injection unless otherwise stated. Mobile phase (buffer) was pumped through the column at 1.5 ml/min by an Isco Wiz peristaltic pump (Lincoln, NE, USA).

An Isco Model UA-5 absorbance detector, with Type 6 optical unit operated at 280 nm, was connected to the column outlet to obtain elution profiles of emerging species. The detector signal was converted from analog to digital by a CIO-DAS08-PGH A/D board (Computer Boards, Mansfield, MA, USA). Control CB software from the same company was used for data acquisition (one datum every 6 s), display, and storage.

The fiber optic sensor was composed of three basic components: an argon ion laser light source (Omnichrome, Chino, CA, USA) emitting 514 nm light at 13.5 mW, a single fused-silica optical fiber (Quartz Products, Tuckerton, DE, USA) of 200/240 $\mu$m core/cladding diameters, and a PIN photodiode detector (EG&G Judson, Montgomeryville, PA, USA). Fig. 2 shows the system. The exciting light passed through a small hole in the parabolic mirror and entered the optical fiber's proximal end. The light travelled down the fiber and into the packed column, where, emerging from the fiber's distal end, it excited analyte in the sampling region. A portion of the analyte fluorescence entered the fiber's distal end and travelled back to the proximal end where it diverged, cone-like, onto the reflecting surface of the parabolic mirror. The mirror directed the fluorescence through a 550 nm cut-off filter through a focusing lens, and onto the photodiode detector. A Model 4000 chopper (Photon Technology International, Princeton, NJ, USA) and Model SR510 lock-in amplifier (Stanford Research Systems, Sunnyvale, CA, USA) were used to minimize noise and reduce background [19]. SR565 software from Stanford Research Systems was used for data acquisition (maximum rate of one datum every 0.05 s), display, and storage.

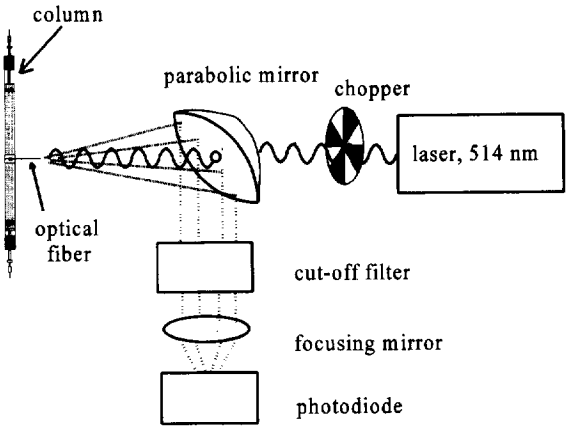


Fig. 2. Schematic diagram of fiber optic sensor.

The column packing medium was Sephadex G-50 gel filtration beads, 101–303 $\mu$m in diameter, from Sigma (St. Louis, MO, USA). Blue dextran, fluorescein and cytochrome *c* obtained from Sigma were used as test analytes. Polypropylene fiber screen and stainless steel wire screen were obtained from Small Parts (Miami Lakes, FL, USA). Buffers were freshly made with water purified by a Milli-Q filtering system (Millipore, Milford, MA, USA).

## 3. Results and discussion

### 3.1. Column reproducibility

Before evaluating the performance of in situ fiber optic sensors, we needed to establish the reproducibility of test analyte flow in control columns (no fiber) prepared in our laboratory. Run-to-run reproducibility was evaluated on a single column; replicate injections of blue dextran (4 mg/ml), an unretained compound, were made, and the elution profiles were obtained by conventional UV absorbance at the column outlet. Phosphate buffer (0.100

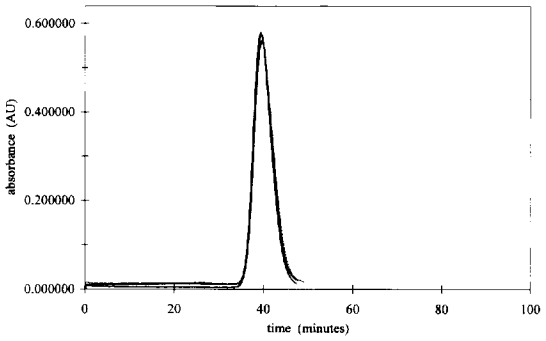


Fig. 3. Run-to-run reproducibility. Elution profiles from six replicate injections of blue dextran on a single 29-cm Sephadex G-50 gel filtration column are shown. Blue dextran concentration was 4 mg/ml and injection volume was 1 ml. Elution buffer was 0.100 *M* phosphate, pH 7.

*M*, pH 7) was used for elution. The resulting elution profiles are shown in Fig. 3, on the same axes. The superimposability of the elution profiles demonstrates the high level of run-to-run reproducibility.

Column-to-column reproducibility was evaluated on separate columns, packed using the identical conditions and procedures. In Fig. 4, elution profiles for blue dextran injection onto five columns are presented on the same axes. The column-to-column reproducibility was only slightly less than the run-to-run reproducibility. This excellent reproducibility provided an ideal background against which we could observe even the smallest perturbation caused by the presence of an optical fiber.

### 3.2. Effect of fiber on column fluidics

The effect of a single fiber on column fluidics was evaluated by comparison of elution profiles for columns with and without a fiber across the diameter. In Fig. 5, blue dextran elution profiles (obtained by UV absorbance at the column outlet) for columns with and without optical fiber are shown on the same axes. Clearly, fibers having diameters nearly three times the average Sephadex G-50 particle size did not alter column fluidics.

To check the effect of a lattice of fibers (pertinent to future work with multiple sensors) on column fluidics, we performed some tests using polypropylene and stainless steel screens. The screens, cut into discs and placed in the column at the joint, caused little to no perturbation of the column fluidics. Fig. 6 shows the elution profiles for blue dextran injected onto these columns. Only the finest screen (with the smallest open area), showed a noticeable effect on the elution profile. Similar experiments with cytochrome *c*, a compound that is retained by Sephadex, showed that the retention characteristics of the column were not disrupted by the presence of screens.

The above results are very reassuring for the field of in situ column monitoring. Apparently, not only individual optical fibers, but multiple fibers in the

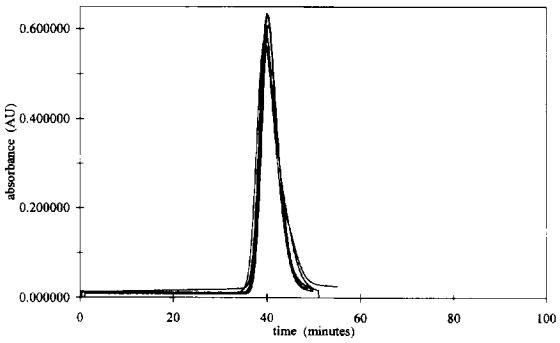


Fig. 4. Column-to-column reproducibility. Elution profiles from single injections of blue dextran on five different but identically-packed gel filtration columns are shown. Conditions were as in Fig. 3.

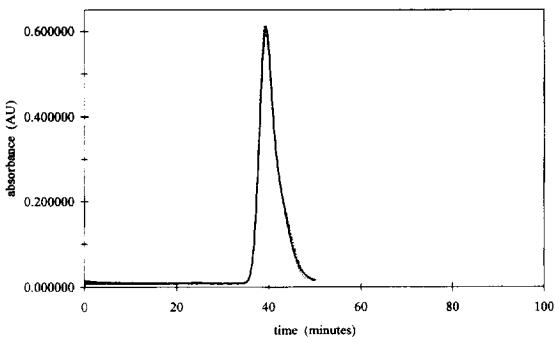


Fig. 5. Effect of single fiber on column fluidics. Elution profiles of blue dextran without fiber (———), with 200-$\mu$m diameter fiber (- - -), and with 600-$\mu$m diameter fiber (– · · –) in column. Conditions were as in Fig. 3.

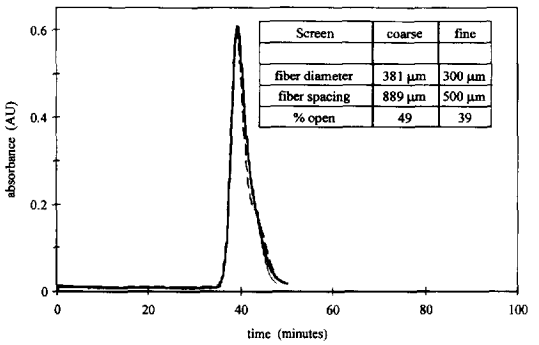


Fig. 6. Effect of screen on column fluidics. Elution profiles of blue dextran without screen (———), with coarse screen (———) and with fine screen (– – –) in column. Conditions were as in Fig. 3.

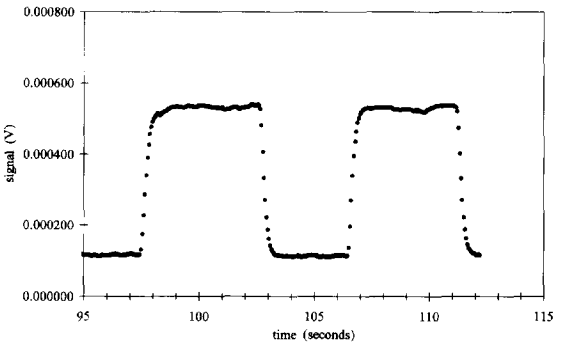


Fig. 7. Response time and reversibility of sensor in tee cell. Data were collected as flow was switched between buffer and fluorescein solution.

form of a lattice can be inserted without a negative effect on column performance. This means that fiber optic sensors can be used in situ without need for either compensatory action or correction factors.

### *3.3. Inherent response time and reversibility of sensor*

Prior to conducting experiments with the fiber optic sensor inside the chromatography column, we needed to establish that the sensor responded rapidly and reversibly to a test analyte outside, and independent of, the column. For this, a small flow cell was constructed from a clear plastic tee and the distal end of the optical fiber was placed in this cell for measurement. The flow in the cell could be switched by stopcock from carbonate buffer to 1.4 $\mu M$ fluorescein, and vice versa, in less than half a second.

In the flow cell, the fiber optic sensor responded rapidly to the changes from buffer to fluorescein and back. This is shown in Fig. 7 where the fluorescence intensity, measured by the fiber optic sensor and expressed as voltage, is plotted against time. The vertical edges of the boxcar-shaped profiles prove that the sensor exhibited a fast and reversible response to stream switching in the small cell. The change from minimum to maximum signal level and vice versa was achieved within 1.0 s, with most of the change requiring only 0.5 s. The signal reached the asymptotic value in the remaining 0.5 s, an interval no doubt arising from diffusional mixing at the boundary between the two streams. Thus, we can conclude that the response time of the sensor itself is less than 1.0 s and probably closer to 0.5 s.

### *3.4. In situ operation of sensor*

The ability of a fiber optic sensor to operate reliably in situ, surrounded by the column packing, required demonstration. One question was whether sensors of this type would be able to make an accurate measure of analyte concentration in the column, without being impaired by excessive scattering and reflection of light in the sampling region or at the fiber surface. Another question was whether sensors of this type could accurately monitor known differences in fluorescence intensity of an analyte passing through the column. A final question was whether sensors of this type could be used to track movement and dispersion of the analyte, as well as inhomogeneities, in the column. These questions were addressed by several different experiments, whose results are reported below.

#### *3.4.1. In situ reproducibility*

We evaluated the reproducibility of the fiber optic sensor's response in a 17-cm-long column by making replicate injections of a test analyte and monitoring elution profiles with an in situ fiber optic sensor. Fluorescein, injected as a 1.00 m$M$ buffered solution, was used as the test analyte, and 0.100 $M$ carbonate

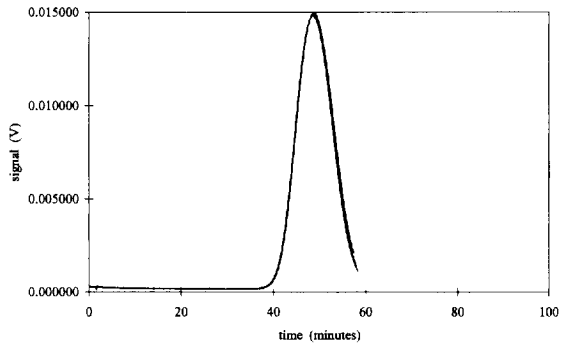


Fig. 8. Reproducibility of in situ fiber optic sensor response. Elution profiles from three replicate injections of 1 m*M* fluorescein on a single column are shown.

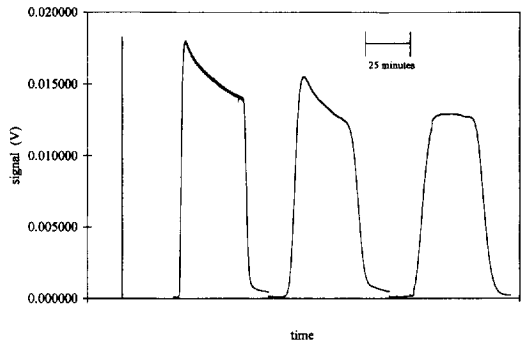


Fig. 9. Response of sensor to alternating streams of 100 $\mu M$ fluorescein and buffer. The sharp spike at left shows the signal amplitude of the solution prior to its introduction to the column. The boxcar profiles that follow were obtained with optical fibers positioned, respectively, at the top of the column (just downstream from the inlet flow adaptor), at the bottom of the column (just upstream from the outlet flow adapter) and at the column outlet (just downstream from the outlet flow adaptor).

buffer (pH 10.0) was used for elution. Elution profiles obtained by an optical fiber positioned 2/3 of the way down the column are shown in Fig. 8. The superimposability of the elution profiles from these replicate injections of analyte demonstrates the reproducibility of the sensor response.

### *3.4.2. In situ response time and reversibility*

We evaluated response time and reversibility of the sensor in the column by alternately pumping 100 $\mu M$ fluorescein and 0.100 *M* carbonate buffer through the column. The sample injection loop was not used for these experiments. Although the scale of these experiments was much greater than for the small flow cell experiments described earlier, the results were expected to be qualitatively the same.

Fig. 9 shows typical results. At the left, as a reference, is a sensor reading of the fluorescein solution in the reservoir, prior to being pumped onto the column. To the right of this are the boxcar profiles obtained by optical fibers positioned at the top of the column (within the packing medium), at the bottom of the column (within the packing medium), and at the column outlet (external to the packing medium), respectively. The salient differences between these results and those obtained in the flow cell are discussed below.

First, the change from minimum to maximum signal took $<1$ s in the flow cell but took 3 min in the column. The longer change time exhibited in the column was expected as a natural consequence of chromatographic band broadening, and was not taken to indicate an inherent change in the sensor response.

Second, the boxcar profiles from the flow cell were level, but the boxcar profiles from inside the column were sloped. Examination of the column revealed that some of the fluorescein had precipitated, and was present as a distinct red coloration in the column that could be removed by extensive washing with buffer. We suggest that this precipitate scattered both the exciting light and the sample fluorescence, leading to progressive signal reduction and, hence, the observed slopes in the profiles. On the other hand, the boxcar profile obtained by the optical fiber positioned at the column outlet was level, which would be consistent with absence of precipitation (and no light scattering) outside the column packing.

It should be emphasized that the precipitation described above was observed only when large volumes of fluorescein were applied to the column. No precipitation was observed when the injector was used to introduce small volumes of sample that were then considerably diluted during elution.

### *3.4.3. In situ concentration experiments*

For the concentration experiments, a series of buffered fluorescein solutions was prepared. The concentrations used spanned five orders of mag-

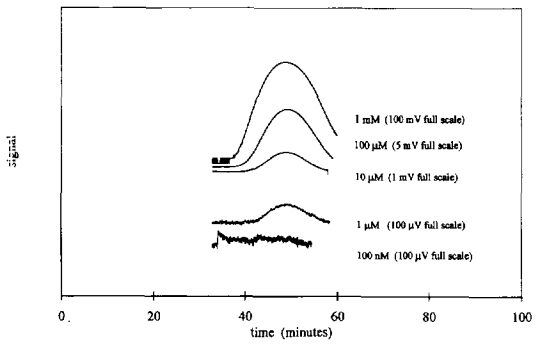


Fig. 10. Elution profiles for five different concentrations of fluorescein. Profiles were obtained with an optical fiber positioned 2/3 of the way down the column.

nitude. Each member of the series was injected separately and eluted with 0.100 *M* carbonate buffer. The elution profile for each concentration was obtained by an optical fiber positioned 2/3 of the way down the length of the column.

Fig. 10 shows the peaks obtained with the fiber optic sensor; peak heights increased with analyte concentration. Signal amplitude vs. analyte concentration is plotted in log–log form in Fig. 11. Were the slope of this plot equal to one, a linear relation between signal and concentration over the whole range would be indicated. However, the slope is slightly less than one, indicating a lag in signal increase per concentration increase at the higher concentrations. This lag is not surprising at the higher concentrations, and is probably due to fluorescence quenching.

### 3.4.4. *In situ pH experiments*

For the pH experiments, a series of buffered, 100 $\mu M$ fluorescein solutions spanning the pH range 3–10 was prepared along with elution buffers of matching pH. Sodium chloride was added to maintain constant ionic strength. Each member of the series was chromatographed separately. Two different buffers were used to achieve the buffer capacity needed over the wide pH range; carbonate buffer was used for pH 10 and 9, while phosphate buffer was used for pH 9 and lower. Runs on both buffers at pH 9 confirmed that the change of buffer did not influence the observed fluorescence. The optical fiber was positioned 2/3 of the way down the length of the column for each run in the series.

Fig. 12 shows peak height plotted as a function of pH. The inflection in the curve is centered around 6.4, the known p$K_a$ of fluorescein. Also shown on the axes are values reported in the literature for fluorescence intensity of fluorescein solution as a function of pH [20]. The good agreement between the two curves verifies that the in situ fiber optic sensor is able to accurately monitor changes in fluorescence intensity.

### 3.4.5. *Column profiling experiments*

The fiber optic sensor's ability to reveal column heterogeneity radially and along the length was evaluated by means of column profiling, i.e., collect-

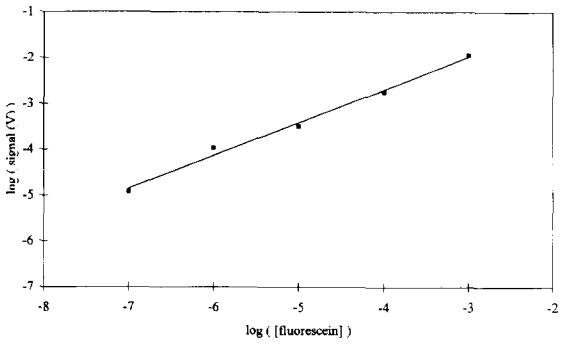


Fig. 11. Log–log plot of fluorescence maxima (in volts) from elution profiles in Fig. 10 versus fluorescein concentration (molar). Best fit line is shown through symbols representing experimental data.

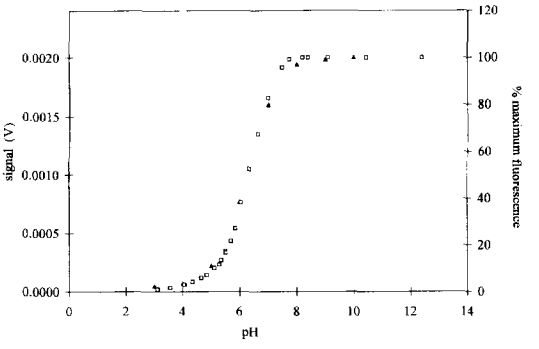


Fig. 12. Fluorescence maxima (in V) as a function of pH. Our data (▲), data of Diehl and Markuszewski (□) [20].

ing elution profiles at different positions in a column. With fluorescein as the test analyte, replicate runs were made with the optical fiber successively positioned as follows inside the column: top, 1/3 of the way down, 2/3 of the way down, and bottom. An additional optical fiber sensor was positioned at the column outlet (after the flow adaptor).

Elution profiles collected at the radial center of a column are shown together in Fig. 13. The peak obtained at the top of the column (left), before significant band broadening had occurred, was relatively sharp. The peak obtained 1/3 of the way down indicated the expected band broadening. The next band broadening of significance occurred as the band exited the column through the flow adaptor, and was detected at the column outlet. This is an expected effect of the band's exit through the flow adaptor [5] (all peaks in Fig. 13 were confirmed to be of equal area). Elution profiles obtained near the wall are presented in Fig. 14, and show the same trends as the profiles obtained at the radial center.

It is also interesting to see what can be learned by comparison of center and wall elution profiles at a given position down the length of the column. Were conditions inside the column uniform, elution profiles obtained at the center would be identical to those obtained near the wall, for any given position along the length. Real conditions, however, might include column packing nonuniformities [2] as well as flow perturbations from the inlet or outlet flow

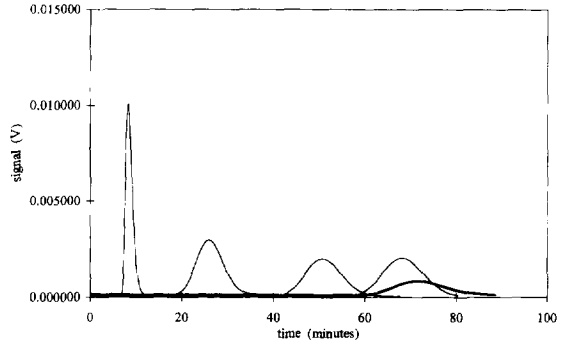


Fig. 13. Elution profiles obtained with optical fibers' distal ends positioned at the column's radial center. Profiles were obtained (from left to right) at the top, 1/3 of the way down, 2/3 of the way down and at the bottom of the column. Elution profile shown by bold line was obtained with optical fiber positioned at the column outlet.

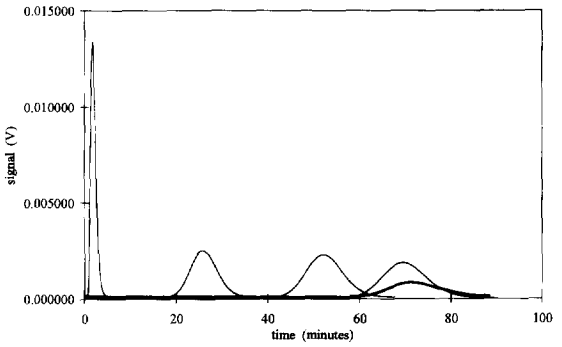


Fig. 14. Elution profiles obtained with optical fibers' distal ends positioned near column wall. Optical fibers were positioned along column length as in Fig. 13.

adaptors [3–7]. These factors could result in the development of flow differences between the center and wall regions of the column as the analyte moves down and exits the column.

Center and wall profiles for the position at the top of the column are shown in Fig. 15; the figure reveals that the analyte band was moving more slowly at the radial center than at the wall. Post mortem inspection of the flow adaptor at the top of the column showed that the replaceable porous disc had become slightly clogged in its central area. The resultant retardation of flow through the clogged portion of the disc would explain the observed difference between wall and center at the top of the column. Center and wall profiles for the bottom position are shown in Fig. 16; the figure reveals that the analyte band was moving faster at the radial

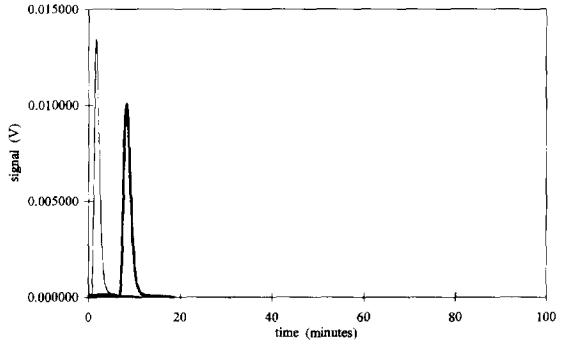


Fig. 15. Elution profiles obtained with optical fiber positioned at the top of the column distal end at radial center (———) and near the wall (———).

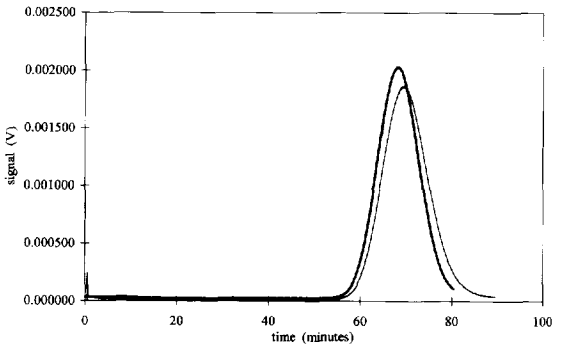


Fig. 16. Elution profiles obtained with optical fiber positioned at the bottom of the column distal end at radial center (———) and near the wall (———).

center than at the wall. This difference may have developed from nonuniform column packing.

Clearly, column profiling procedures described here demonstrate the usefulness of in situ sensors for tracking movement and dispersion of the analyte in the column and for revealing spatial inhomogeneities.

## 4. Conclusions

The work described here demonstrates that in situ fiber optic sensors are very promising for monitoring the chromatographic process. Our experiments showed that the column packing does not adversely affect the operation of the sensor. Both concentration changes and pH-induced fluorescence changes were able to be accurately measured. Furthermore, fiber optic sensors placed along the length of the column were able to track the progress of the fluorescent analyte through the column, showing the development of band broadening and radial heterogeneity, and also showing the perturbations caused by the flow adaptors.

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