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Applications of Laser Fluorimetry to Microcolumn Liquid Chromatography

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Laser-induced fluorescence (LIF) is examined as a sensitive and selective detector for microcolumn liquid chromatography (LC). This detector employs a cw helium-cadmium laser (325 nm, 5-10 mW) as the excitation source together with a simple filter/photomultiplier optical system. Several flowcells are evaluated: (1) a flowing droplet; (2) an ensheathed effluent stream; and (3) a fused-silica capillary. The latter is judged to be the most promising for general use. The LIF detector is capable of sensing femtogram amounts of model solutes (coumarin dyes), and the response is linear over more than eight orders of magnitude in concentration. The potential of combining the high separation power of microcolumn LC with the high sensitivity of LIF detection is demonstrated through the analysis of multicomponent mixtures of polynuclear aromatic hydrocarbons and derivatized amino acids.

The identification and quantitation of individual components in complex samples presents a challenging analytical problem that demands continual improvement of existing methodology and instrumentation. This demand has been a primary motivation for the development of high-efficiency separation methods, such as gas (GC), supercritical fluid (SFC), and liquid (LC) chromatographic techniques. In particular, microcolumn liquid chromatography is rapidly gaining popularity for the separation of multicomponent mixtures of nonvolatile compounds. The microcolumns currently under development are of three general types: narrow-bore or microbore packed columns (1-4), semipermeable packed capillaries (5-7), and open tubular capillaries (8-11). Although the theoretical potential and the current state of the art are

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different for each column type, these microcolumns have demonstrated several distinct advantages over conventional LC columns. First, microcolumns are capable of achieving much higher chromatographic efficiency than their conventional counterparts, exceeding one million theoretical plates (4,11). Consequently, microcolumns will improve the separation of very complex samples or hard-to-resolve solutes. Alternatively, a given separation efficiency can be attained in a shorter analysis time using semipermeable packed capillary or open tubular capillary columns because of their high permeability (12). Another desirable attribute of microcolumns is their low volumetric flowrates (1-50 μL/min), which result in significantly reduced consumption of both sample and solvent. Although the economic and environmental advantages of reduced solvent consumption are immediately obvious, other benefits have become apparent with the continued development and use of microcolumn LC. For example, separations may be performed using novel mobile and stationary phases that are too expensive, rare, or toxic to be used with conventional columns (13). Furthermore, novel detection techniques can be implemented that are largely incompatible with conventional columns, among which flame- or plasma-based detectors (14-18) and mass spectrometry (19,20) are representative examples.

Despite the many advantages of microcolumn LC, there are several limitations that may ultimately restrict the practical application of this technique. Long analysis times, ranging from a few to many hours, are frequently necessary to provide resolutions of very complex samples. This problem is not characteristic of microcolumns themselves, but is inherent in all high-efficiency LC separations because of slow diffusional processes in the condensed phase. Another limitation of microcolumn LC is the stringent technological requirements imposed on ancillary chromatographic equipment such as pumps, sample injectors, connecting hardware, and detectors. Although significant improvements have been achieved for many components of the chromatographic system, there has been a conspicuous lack of sensitive, low-volume detectors. In most current applications of microcolumn LC, the detectors are merely miniaturized versions of those employed with conventional columns, such as UV-absorbance, fluorescence, and electrochemical detectors. Whereas such devices are adequate at the present stage of column development, the technological limitations and the consequences of further miniaturization become immediately apparent.

In order to achieve the full potential of microcolumn LC, it is necessary to develop new detection systems that are in compliance with the rigorous requirements of this analytical technique. Among the many possibilities, laser-based spectroscopic detectors appear to be particularly well suited for this application. The intensity of the laser radiation permits very high sensitivity to be achieved using those spectroscopic techniques in which the signal is proportional to source intensity; for example, fluorescence (21-23), phosphorescence (24-26), light scattering (27,28), and thermooptic or thermoacoustic measurements (29-31). Moreover, the highly collimated laser radiation can be readily focused into flowcells of nanoliter volume, as required for

microcolumn LC, without concomitant loss of radiant power. Other properties of laser sources, such as narrow spectral bandwidth, polarization, and temporal characteristics (pulsed or cw), have been favorably exploited for liquid chromatographic detection as well (32).

Laser-induced fluorescence (LIF) is one of the simplest and most promising applications of laser-based detection in liquid chromatography. Previous investigations with conventional LC columns have clearly indicated the high sensitivity and selectivity that can be attained (33-40). Based on these preliminary and highly promising results, several laboratories have undertaken concurrently the combination of microcolumn liquid chromatography and laser fluorimetric detection (41-44). In the present study, we have developed a sensitive low-volume LIF detector that is compatible with all of the microcolumns described previously, yet is simple and reliable to operate. Several flowcells were constructed and evaluated, including a fused-silica capillary (45), a miniaturized flowing droplet (33,46) and an ensheathed effluent stream (21,22,35). The LIF detector was optimized and characterized with respect to sensitivity, linear dynamic range, and dead volume. Finally, the outstanding performance of this detection system was demonstrated through the analysis of polynuclear aromatic hydrocarbon and derivatized amino acid samples.

Experimental Section

A schematic diagram of the liquid chromatography system and laser fluorescence detector is shown in Figure 1. The key components of this analytical system are described sequentially in what follows:

Liquid Chromatography System. The solvent delivery system was constructed of two 10-mL stainless-steel syringe pumps (MPLC Micropump, Brownlee Labs, Santa Clara, CA). By splitting the pump effluent between the microcolumn and a restricting capillary (1:20-1:2000), isocratic separations were achieved reproducibly at column flowrates as low as 0.005 μL/min, and gradient separations as low as 0.1 μL/min. Samples of 0.5 to 50 nL volume were introduced by the split injection technique with a 1- L valve injector (Model ECI4W1., Valco Instruments Co., Inc., Houston, TX). The injection valve, splitting tee, and microcolumn were maintained at constant temperature (± 0.3°C) in a thermostatted water bath.

Three different types of microcolumns were prepared. Packed microcolumns were fabricated from fused-silica tubing (Hewlett-Packard, Avondale, PA) of 0.20 to 0.32 mm inner diameter and 1 to 2 m length. This tubing was packed under moderate pressure (400 atm) with a slurry of the chromatographic material [Aquapore RP-300 (10 μm), Brownlee Labs; Micro-Pak SP-18 (3 μm), Varian Instrument Group, Walnut Creek, CA] in an appropriate solvent (3). In this manner, narrow-bore packed microcolumns reproducibly yielded 150,000 or more theoretical plates. Semipermeable packed capillary columns were prepared by loosely packing a Pyrex glass tube with an irregular silica adsorbent [LiChrosorb Si-60 (30 μm),

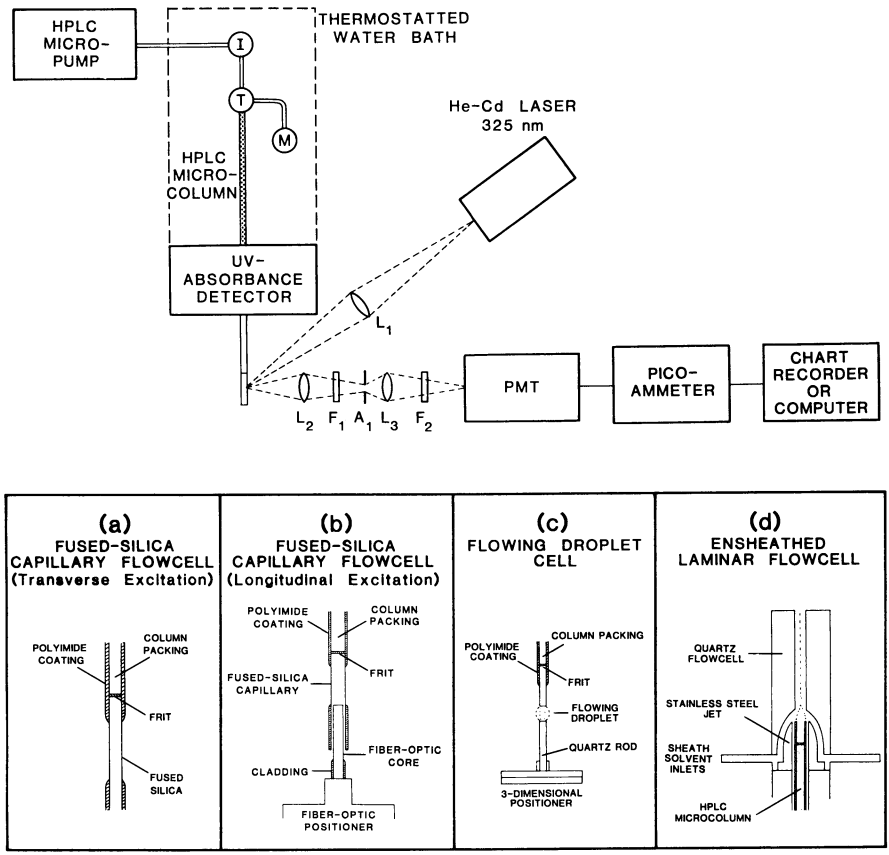

Figure 1. Schematic diagram of the liquid chromatography system and laser fluorescence detector with different flowcells: (a)-(d). I = injection valve, T = splitting tee, M = metering valve or restricting capillary, L = lens, F = filter, A = aperture, PMT = photomultiplier tube.

E. Merck Reagents, Darmstadt, F.R.G.], and subsequently extruding the packed capillary with a glass-drawing apparatus to 70 μm i.d. and 25 m length (7). Open tubular capillary columns were fabricated from fused-silica tubing of 10 to 100 μm i.d. and 5 m length (Scientific Glass Engineering, Inc., Austin, TX), and were used without further surface modification. The latter microcolumns were employed solely to ascertain compatibility with the laser fluorescence detector and to optimize flowcell design.

A variable-wavelength UV-absorbance detector (Model Uvidec 100-V, Jasco Inc., Tokyo, Japan) was modified to permit "on-column" detection with packed and open tubular fused-silica microcolumns, as described previously (3,45,47). The UV-absorbance detector was placed in series before the laser fluorescence detector (see Figure 1), and was used for comparisons of sensitivity, selectivity, and dead volume (44).

Laser Fluorescence Detector. A helium-cadmium laser (Model 4240B, Liconix, Sunnyvale, CA) was chosen as the excitation source because of its stability and convenient wavelengths (325 and 442 nm). The UV laser radiation (325 nm, 5-10 mW cw) was isolated with a dielectric mirror and was focused on the miniaturized flowcell with a quartz lens. Sample fluorescence, collected perpendicular to and coplanar with the excitation beam, was spectrally isolated by appropriate interference filters and then focused on a photomultiplier tube (Centronic Model Q 4249 B, Bailey Instruments Co., Inc., Saddle Brook, NJ). The resulting photocurrent was amplified with a picoammeter (Model 480, Keithley Instruments, Inc., Cleveland, OH), and finally was displayed on a stripchart recorder (Model 585, Linear Instruments Corp., Reno, NV).

Several miniaturized flowcells were evaluated, including a fused-silica capillary, a suspended flowing droplet, and an ensheathed effluent stream (Figures 1a-d). The fused-silica capillary flowcell was formed by removing the protective polyimide layer from a short section of fused-silica tubing. When the LIF detector was used alone, the flowcell was simply an extension of the column itself (0.20 to 0.33 mm i.d.), thereby eliminating dead volume from connecting tubes and unions. However, when the UV-absorbance and LIF detectors were employed in series, it was necessary to use a capillary of smaller diameter in order to reduce laminar dispersion between the detectors. In such cases, a fused-silica capillary of 0.035-0.100 mm i.d. and 1 m length was attached to the microcolumn outlet with PTFE (Teflon) tubing, and formed the flowcells for both UV-absorbance and LIF detectors. By varying the inner diameter, flowcells were constructed with illuminated volumes from 1 to 100 nL, and corresponding optical pathlengths of 0.035 to 0.33 mm. Illumination was achieved in either the transverse (Figure 1a) or longitudinal (Figure 1b) direction with respect to the flowcell axis. The latter method, which employed a UV-transmitting optical waveguide inserted directly into the capillary flowcell, was more easily aligned and permitted control of the illuminated volume without requiring a change in capillary diameter. The flowing droplet cell, illustrated in Figure 1c, was a straightforward miniaturization of the windowless fluorescence flowcell described for conventional

liquid chromatography by Diebold and Zare (33). This cell was formed by suspending a flowing droplet of the microcolumn effluent between a fused-silica capillary and a quartz rod of similar outer diameter. By varying the diameter and spacing of the capillary and rod, the droplet volume could be adjusted from approximately 50 to 200 nL, with corresponding optical pathlengths of 0.15 to 0.5 mm. The ensheathed effluent flowcell, shown schematically in Figure 1d, was both structurally and functionally similar to those described by Hershberger et al. (35) and Dovichi et al. (21,22). This flowcell was obtained from a commercial flow cytometer (System 50, Ortho Diagnostic Systems, Inc., Westwood, MA), and consisted of a quartz tube of square cross section having a 0.25 mm square central bore. The effluent was introduced directly from the fused-silica microcolumn, which extended just beyond a conical stainless-steel jet at the bottom of the quartz tube. The sheath solvent, which was of similar composition to the column effluent, was supplied by a gas-pressurized liquid reservoir through two stainless-steel tubes perpendicular to and slightly below the column inlet. Turbulence and mixing of the effluent and sheath streams were not observed when pulseless, laminar flow conditions were maintained. The optical pathlength and effective volume of the effluent stream were controlled by hydrodynamic focusing, by varying the relative flowrates of the column effluent and the sheath solvent. In this manner, the pathlength was readily varied between 5 and 20 μm, with corresponding illuminated volumes of 20 to 300 pL (calculated values). The large volume of sheath solvent employed, typically 0.5 to 5.0 mL/min, eliminated the advantage gained by microcolumns in reducing solvent consumption.

Analytical Methodology. An N.B.S. Standard Reference Material (SRM 1647) containing sixteen polynuclear aromatic hydrocarbons was diluted twenty-fold with methanol/methylene chloride (1:1) prior to analysis.

Amino acids were derivatized with 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride) according to the procedure of Tapuhi and coworkers (48-50). A 10^{-3} M stock solution of twenty common L-amino acids (Sigma Chemical Co., St. Louis, MO) was prepared by dissolving the carefully weighed standards in 0.1 M aqueous hydrochloric acid. Aliquots of this solution were transferred to conical vials, evaporated to dryness, and redissolved in 500 μL aqueous buffer (0.04 M lithium carbonate, pH 9.5). A 500 μL volume of dansyl chloride solution (5×10^{-3} M in acetonitrile) was added, and the derivatization was allowed to proceed in the dark at 35°C for one hour. The reaction was terminated by the addition of 2% methylamine hydrochloride, and the derivatized sample was analyzed immediately by microcolumn liquid chromatography with UV-absorbance and LIF detection.

Organic solvents employed in this investigation were high-purity, distilled-in-glass grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI); water was deionized and doubly distilled in glass (Mega-Pure System, Corning Glass Works, Corning, NY).

Results and Discussion

Evaluation of Flowcell Performance. There are many important factors to be considered in the evaluation of flowcell performance for microcolumn liquid chromatography. Foremost among such criteria is sensitivity; i.e., the signal-to-noise ratio that can be obtained for a standard sample injection. If we assume that the flowcells are uniformly illuminated in the laser beam and that the fluorescent emission is collected from each cell with the same efficiency, then the signal intensity should be primarily a function of the optical pathlength of each flowcell. Accordingly, the flowing droplet cell, which had the longest pathlength (0.15-0.5 mm), provided the largest photocurrent, while the fused-silica capillary (0.05-0.32 mm) and the ensheathed effluent (0.005-0.02 mm) flowcells yielded proportionately smaller responses. However, the background noise level also appeared to be nearly proportional to pathlength, indicating that the predominant source of noise was fluorescent or scattered (Rayleigh and Raman) light from the effluent itself, rather than from cell walls. Specifically, Raman scattering from the solvent seemed to be the major component of background interference, particularly when the detected emission wavelength was near the excitation wavelength. Thus, while the flowing droplet and ensheathed effluent flowcells were able to eliminate or discriminate against light originating from cell walls through spatial filtering, this did not translate directly to an improvement in signal-to-noise ratio. Indeed, this figure of merit was approximately the same for each of the flowcells investigated when appropriate spectral filtering was employed. Consequently, other performance criteria, such as dead volume, simplicity of operation, and other factors dictated the preference in flowcell design.

It is imperative that the volume of the flowcell and associated connections does not contribute excessively to the dispersion of the chromatographic peaks. Moreover, it is desirable to have a single flowcell design that will accommodate microcolumns of different types and sizes, and will adapt to their different volumetric requirements. The flowing droplet cell, which could be varied from 50 to 200 nL, was found to be suitable for packed microcolumns of 0.5 mm or greater inner diameter, but to suffer from reduced sensitivity and stability for columns of smaller bore. On the other hand, the very small volume of the ensheathed effluent flowcell (20-300 pL) made it suitable for open tubular and semipermeable packed capillary columns, but somewhat impractical for columns of larger bore because of reduced sensitivity and increased consumption of ensheathing solvent. In contrast, fused-silica capillary cells of varying dimensions could be readily interchanged to provide a suitable compromise between sensitivity and dead volume for all of the microcolumns under study.

Another important consideration is the compatibility of the flowcell with the normal range of operating conditions in liquid chromatography. First, the flowcell should be compatible with a wide variety of solvents for both normal- and reversed-phase separations. Second, it should readily accommodate variations in solvent flowrate to permit both high-efficiency and high-speed

applications. Furthermore, for optimal analysis of complex samples, the flowcell should allow gradients in either solvent composition or flowrate to be employed. Finally, it is desirable for the detector cell to be insensitive to temperature and pressure fluctuations in order to minimize background noise. Although our investigations encompassed a wide range of operating conditions, the results can be briefly summarized in the following manner: For a fixed set of operating conditions (solvent composition, flowrate, temperature, pressure), the flowing droplet and ensheathed effluent flowcells could be readily optimized. However, because these cells have flexible boundaries in the optical region, they were strongly dependent upon the physical properties of the effluent and could not readily tolerate changes in operating conditions without reoptimization. Hence, these cells were not compatible with gradients in either solvent composition or flowrate. Moreover, both the flowing droplet and ensheathed effluent flowcells were sensitive to pressure and flow fluctuations from the pump, mechanical vibration, and bubble formation. Furthermore, these cells were essentially destructive; i.e., they caused dilution or mixing of the column effluent such that another detector could not readily be employed in series. In contrast, the fused-silica capillary cell exhibited only nominal dependence upon the chromatographic conditions.

Because of its operational simplicity, compatibility with many solvents as well as gradient elution, compatibility with a wide range of microcolumn types and sizes, and nondestructive nature, the fused-silica capillary flowcell was judged to be the most versatile and useful among those investigated.

Characterization of LIF Detector. In a previous study (44), the LIF detector was characterized with the fused-silica flowcell using transverse excitation. The limit of detection, measured at a signal-to-noise ratio of seven (99.9% confidence level) for three replicate measurements, was determined to be 2.3×10^{-15} g of coumarin 440 (1.3×10^{-17} moles) injected in a 25 nL sample. Whereas detection limits as much as an order of magnitude lower have been reported for laser fluorimetry (22), such results were obtained under rather rigorous conditions. In contrast, the detection limit demonstrated in the previous study (44) was achieved with a relatively low-power laser and simple optical system using a representative solute analyzed under normal chromatographic conditions. Hence, this high sensitivity can be routinely achieved under practical operating conditions.

The fluorescence signal was found to be linearly related to solute concentration over at least eight orders of magnitude, extending from the detection limit (5×10^{-10} M) to the solubility limit (2×10^{-2} M) of coumarin 440 in methanol. It was suggested that the small diameter of the capillary flowcell effectively reduced nonlinear behavior in both the absorption and emission processes, thereby reducing both inner and outer filter effects and extending the linear response into very high concentrations (44). This extraordinary linear range facilitates the simultaneous quantitation of both major and minor components in complex sample matrices.

In Chromatography and Separation Chemistry; Ahuja, Satinder;
ACS Symposium Series; American Chemical Society: Washington, DC, 1986.

There are several important sources of band broadening in the LIF detector, including both volumetric and temporal contributions. These contributions were assessed independently in order to establish the major sources of dispersion and to minimize their effects (44). The volumetric variance, given by the second statistical moment of the chromatographic peak, was determined to be from 0.06 nL^2 to 0.06 μL^2 for capillary flowcells of 0.035 to 0.33 mm diameter, respectively. This variance was predominantly due to laminar dispersion occurring between the microcolumn frit and the illuminated region (see Figure 1a). The temporal dispersion, given by an exponential time constant of 0.7 ms, was equivalent to 0.0001 nL^2 in variance units. Thus, both volumetric and temporal variances were sufficiently small for almost any application in microcolumn liquid chromatography, including both high-speed and high-efficiency separations.

Applications. Many molecules of environmental and biochemical significance are inherently fluorescent, among which the polynuclear aromatic hydrocarbons (PAH) are a representative example. Although such compounds may occur in very complex sample matrices, they can be efficiently separated by microcolumn LC and sensitively detected by laser fluorimetry. An exemplary chromatogram is shown in Figure 2, wherein a Standard Reference Material (SRM 1647) containing sixteen polynuclear aromatic hydrocarbons has been analyzed. This separation was achieved on a fused-silica microcolumn (0.2 mm i.d., 1.3 m length) containing a 3-μm octadecylsilica packing material (N=150,000) using an isocratic mobile phase of 92.5% aqueous acetonitrile. For comparison of sensitivity and selectivity, the chromatograms obtained with UV-absorbance and laser-induced fluorescence detection are illustrated in Figure 2. Several important features of LIF detection become apparent: Whenever fluorescence detection is applicable, the sensitivity is generally far superior to UV-absorbance and other common detection methods, frequently by several orders of magnitude. However, not all solutes of interest are naturally fluorescent, and very similar molecules may have widely differing absorption coefficients and quantum yields. This becomes evident when comparing the relative response of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, two six-ring polynuclear aromatic compounds present in equimolar amounts in the Standard Reference Material. In this case, fluorescence of the alternate PAH benzoperylene is significantly lower than that of the non-alternate indenopyrene due to differences in the absorption coefficient (51) and to quenching by the solvent acetonitrile. This high degree of selectivity can be advantageous for the simplification of complex chromatograms and for the reduction of background interferences.

Because most common UV lasers operate at fixed wavelengths, the range of application of laser-induced fluorescence may be somewhat limited. The He-Cd laser provides additional versatility because two excitation wavelengths are available, 325 nm and 442 nm. This feature is illustrated in Figure 3, taken from the recent work of Guthrie, Jorgenson, and Dluzneski (41), where a solvent-refined coal fluid has been analyzed using an open tubular

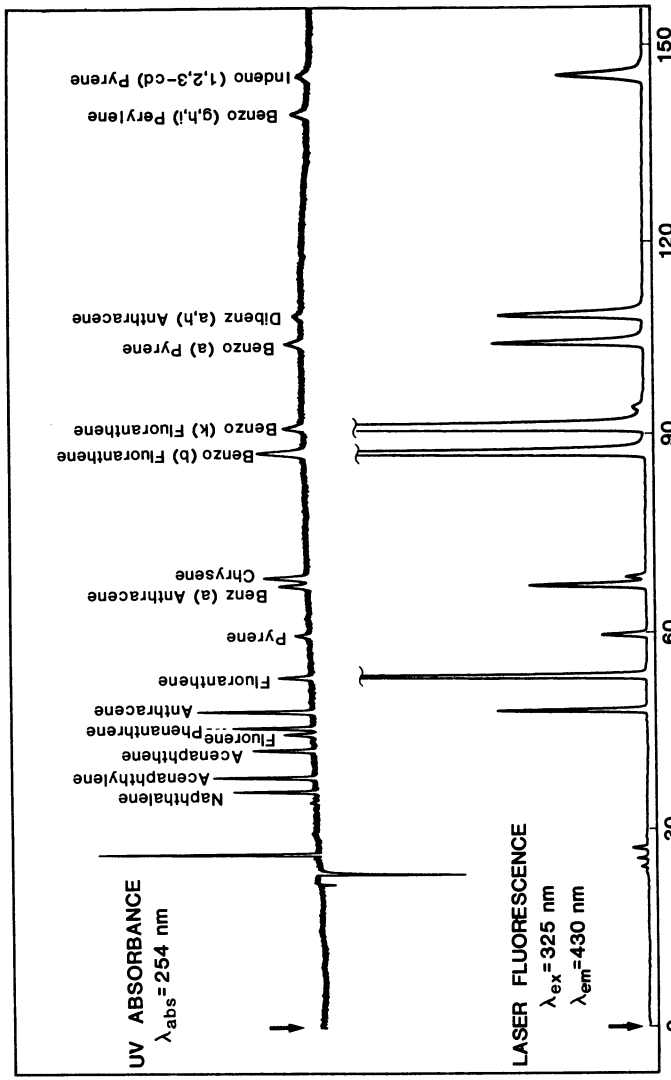

Figure 2. Chromatogram of polynuclear aromatic hydrocarbon standards. Column: fused-silica capillary (0.2 mm i.d., 1.3 m length) packed with Micro-Pak SP-18 (3 μm); Mobile phase: 92.5% aqueous acetonitrile at 1.2 μL/min; Sample injection: 50 nL, 0.2–2.5 ng per component. Reproduced with permission from Ref. 43. Copyright 1984, American Assoc. for the Advancement of Science.

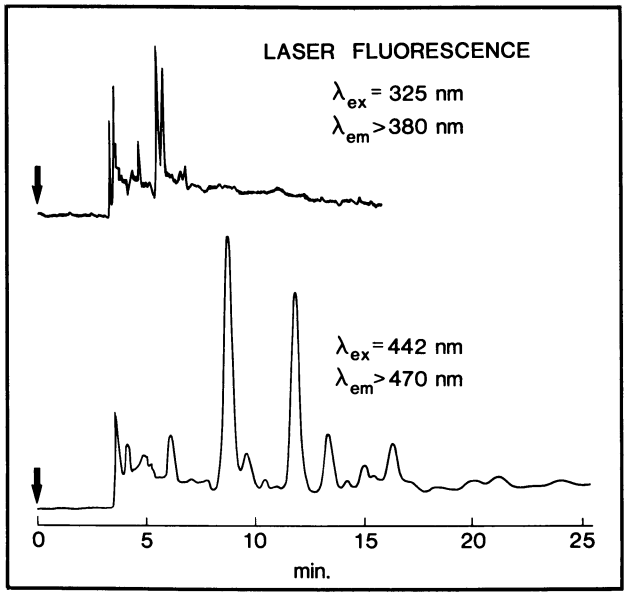

Figure 3. Characterization of a solvent-refined coal sample by laser fluorimetric detection with dual excitation wavelengths. Column: open tubular glass capillary (16 μm i.d., 2.3 m length) with octadecylsilane bonded phase; Mobile phase: 50% aqueous acetonitrile , 425 psi. Reproduced with permission from Ref. 41. Copyright 1984, Preston Publications.

capillary column with a chemically bonded octadecylsilane stationary phase. It is evident that UV excitation at 325 nm (0.30 mW) is appropriate for the detection of small polynuclear aromatic hydrocarbons, while visible excitation at 442 nm (4.1 mW) permits greater sensitivity for PAH compounds of higher molecular weight. The complementary information contained in these chromatograms provides a powerful analytical tool for the characterization of complex coal-derived samples.

Molecules that are not amenable to LIF detection because of their low absorption cross sections or low fluorescence quantum yields may be analyzed through a variety of indirect fluorescence techniques. For example, non-fluorescent solutes may be detected by the extent to which they enhance (52) or quench (53) the emission of a fluorescent dye added to the mobile phase. Alternatively, simple displacement of the dye molecule in the column effluent by a non-fluorescent solute will result in a reduction of the fluorescence intensity, which can provide nearly universal detection capability with adequate sensitivity for many applications (54). Finally, non-fluorescent molecules may be rendered detectable through the incorporation of a fluorescent label that is optimized for the laser excitation wavelength. Derivatization methods are particularly attractive because they introduce an additional dimension of chemical and spectroscopic selectivity that can simplify analyses in a predictable and reproducible manner.

A wide variety of fluorescent molecular probes have been demonstrated to be suitable for excitation by the He-Cd laser: 4-bromomethyl-7-methoxycoumarin has been employed for the detection of carboxylic and phosphoric acids (44,55), 7-chlorocarbonyl-methoxy-4-methylcoumarin for hydroxyl compounds (42), 7-isothiocyanato-4-methylcoumarin for amines and amino acids (55), 7-diazo-4-methyl-coumarin for a variety of aromatic compounds (55), and terbium chelate molecules with long fluorescence lifetimes (~ 1 ms) for protein analysis (56). In this study, we examined the utility of 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride) as a sensitive and selective reagent for the determination of biogenic amines and amino acids.

The use of dansyl chloride as a derivatization reagent for amino acids and peptides is a well established and well accepted methodology. Nevertheless, improvements in the separation and detection of these derivatives may have a significant impact on clinical and biomedical research. The fluorescence excitation and emission spectra of the dansyl amino acids are shown in Figure 4, whereupon the laser excitation wavelength (325 nm) and filter emission wavelength (546 nm, 10 nm FWHM) have been indicated. It is evident that this fluorescent label is not excited optimally by the He-Cd laser, yet there appears to be sufficient overlap to permit sensitive detection. The analysis of twenty common amino acids derivatives (50-100 pmol each) is demonstrated in Figure 5. This separation was achieved using a fused-silica microcolumn containing Aquapore RP-300, a wide-pore octylsilica packing material of 10-μm nominal diameter. The mobile phase consisted of an optimized gradient from 5% to 32% 2-propanol in a formic and acetic acid buffer (pH 3.2). The chromatographic separation shown

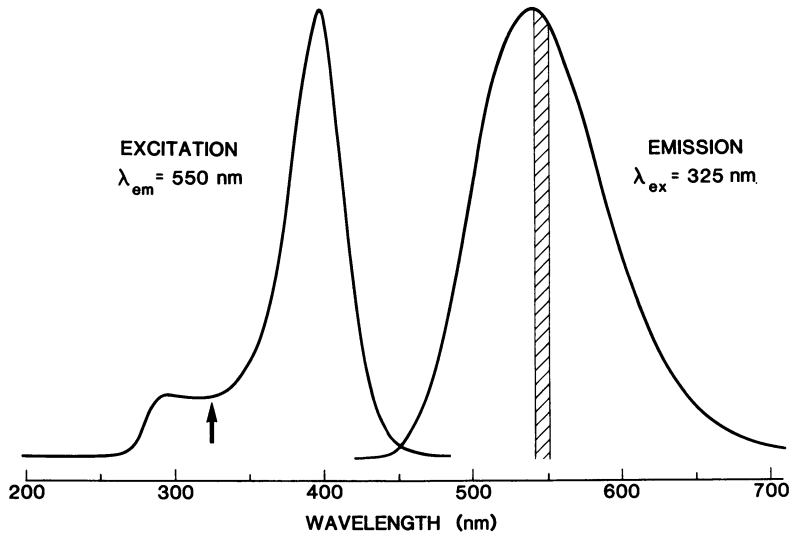

Figure 4. Fluorescence excitation and emission spectra of dansyl amino acids.

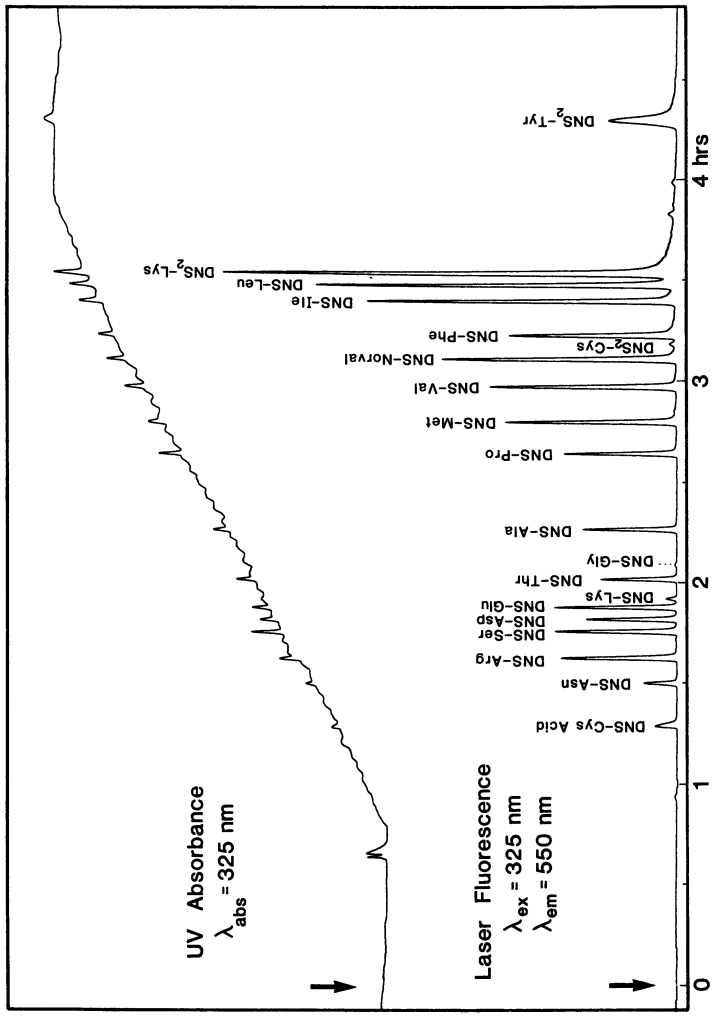

Figure 5. Chromatogram of dansyl amino acid standards. Column: fused-silica capillary (0.32 mm i.d., 1.3 m length) packed with Aquapore RP-300 (10 μm); Mobile phase: linear gradient from 5% to 32% 2-propanol in 0.05 M formic acid/0.06 M acetic acid buffer (pH 3.2) in 200 min, 2.0 μL/min, 55°C; Sample injection: 130 nL, 50-100 pmol per component.

here required four hours for completion when run at optimum flowrate (2.0 μL/min), yet it clearly reveals the high resolving power of microcolumn LC. It is possible and frequently desirable to compromise separation efficiency to achieve faster analysis time; a slightly inferior chromatogram, still with baseline resolution, can be obtained in less than two hours. For purposes of comparison, the separation was monitored using both UV-absorbance and laser fluorescence detection. The UV-absorbance chromatogram (Figure 5, upper trace) shows relatively poor sensitivity for the dansyl amino acids, even at the optimum absorbance wavelength. Because this detector is highly sensitive to changes in absorbance and refractive index of the mobile phase during gradient elution, the small peaks of interest are superimposed upon a steeply sloping baseline. In contrast, the fluorescence chromatogram (lower trace) demonstrates a superior signal-to-noise ratio with the peaks of interest superimposed upon an absolutely flat baseline. The high sensitivity of the LIF detector permits the determination of all dansyl amino acids, as well as some impurities therein, at the subpicomole level.

Summary

The development of suitable detectors is currently one of the limiting factors in the routine practical application of microcolumn liquid chromatography. The laser-induced fluorescence detector described herein appears to fulfill the rigorous requirements because of its high sensitivity (a few femtograms of analyte detected), remarkable linearity (greater than 10^8 dynamic range), and small detection volume (0.06 nL^2 to 0.06 μL^2 for flowcells of 35 to 330 μm i.d., respectively). Although many improvements are possible, such refinements would involve a sacrifice in the inherent simplicity, reliability, and affordability of the present detection system. This system can already be applied to a wide variety of interesting analytical problems, of which the separations of polynuclear aromatic hydrocarbons and dansyl amino acids are only representative examples. It would appear that the combination of laser-induced fluorescence detection and microcolumn liquid chromatography shows exceptional promise for the analysis of complex samples of biochemical and environmental origin.

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