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IMPROVED MINIATURE FLOW FLUOROMETER FOR LIQUID CHROMATOGRAPHY

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SUMMARY

A simple, reliable, and inexpensive miniature flow fluorometer has been developed for use as a detector in liquid chromatography. The new instrument has been designed after approximately 15 years cumulative experience with a previously reported model. All of the desirable features of the earlier instrument are retained, and a number of simplifications and improvements have been added. These include three light-source alternatives for meeting a wide range of excitation requirements. For many applications a 1–10 ng limit of detection has been attained[2].

INTRODUCTION

The improved miniature flow fluorometer has evolved from an earlier version[1] after extensive use as a detector for liquid chromatographs in various programs at the Oak Ridge National Laboratory[3–9]. In many of those applications, the instrument was used to detect fluorescent Ce(III) generated by eluted components which reduce reagent Ce(IV) added to the column eluate. In other applications, the natural fluorescence of the eluted components was detected directly[8].

EQUIPMENT

The fluorometer (Fig. 1) consists of the fluorometer body and a box for electronic components and circuits; the electronic system includes an internal high-voltage supply which replaces the separate supply previously required. A schematic diagram of the instrument system is shown in Fig 2.

The fluorometer body (Figs. 1 and 3) is a machined aluminum block of simple design. The earlier design was assembled from two halves to permit milling rectangular slit optical apertures within the body; the improved version is a monolithic block with circular drill holes for all apertures. The use of a 1/2-in.-diameter side-viewing photomultiplier that fits inside the fluorometer body also yields additional savings

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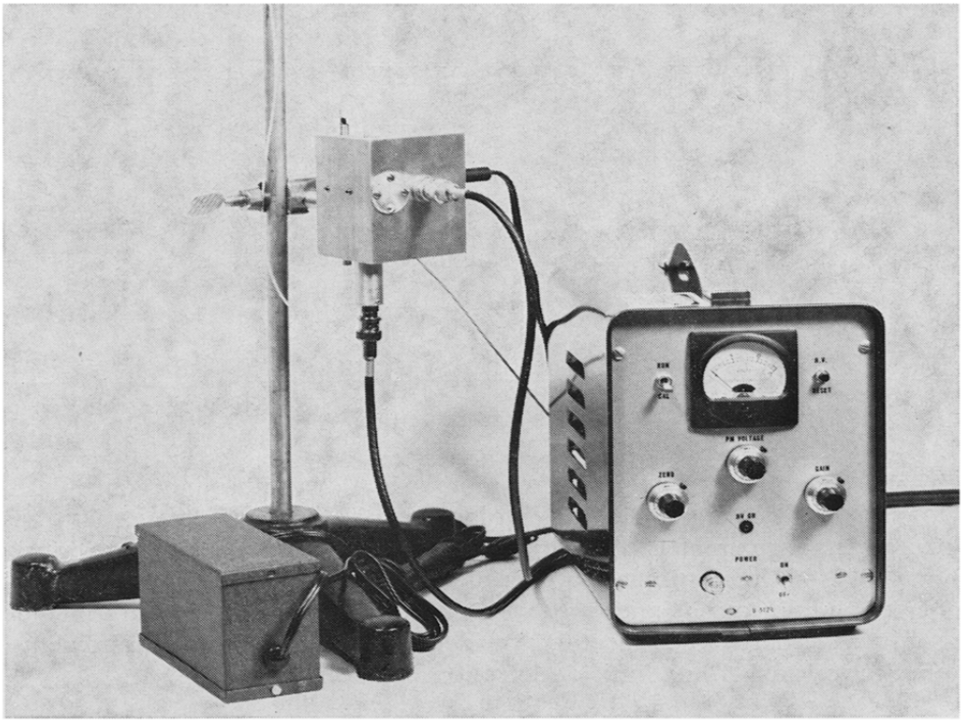


Fig. 1. The improved miniature flow fluorometer.

in fabrication costs. The $3 \times 2\frac{5}{8} \times 2$-in. block contains the desired light-source or adapter, an appropriate excitation filter, a quartz-tube flow cell, an appropriate blocking filter, the photomultiplier, and a photoconductor which compensates for changes in lamp intensity. The mounting accommodations for all of these components are machined into the aluminum block along with the required optical apertures; therefore, the problem of establishing or maintaining proper alignment of components does not exist. The fluorescence from the sample is observed at a right angle to the excitation radiation.

The low-pressure mercury lamp option (Ultra-Violet Products; Model 11SC-2) dissipates approximately 4.6 W, with an approximate 4% conversion efficiency for emission of the mercury spectral lines at 253.7, 312.5, 365, 404.7, 435.8, 546.1, and 577 nm. Of the total irradiance, approximately 90% is in the 253.7-nm line, with the remainder divided approximately equally among the next five longer wavelengths. An Ultra-Violet Products SCT-1 power supply drives the mercury lamp.

The halogen cycle incandescent lamp option uses the new miniature General Electric type 3027 bulb which dissipates 12 W and is mounted in an adapter with external cooling fins. Although the bulb is not made of quartz, it transmits 13% at 2800 Å, 40% at 3000 Å, 36% at 3200 Å, and 71% at 3400 Å[10]; thus, it is very useful with "cut-off" and "cut-on" interference filters for many types of fluorescence studies.

The quartz fiber optic option (Fig. 3) makes possible the use of almost any choice of external light source or monochromator. This option is used only when the

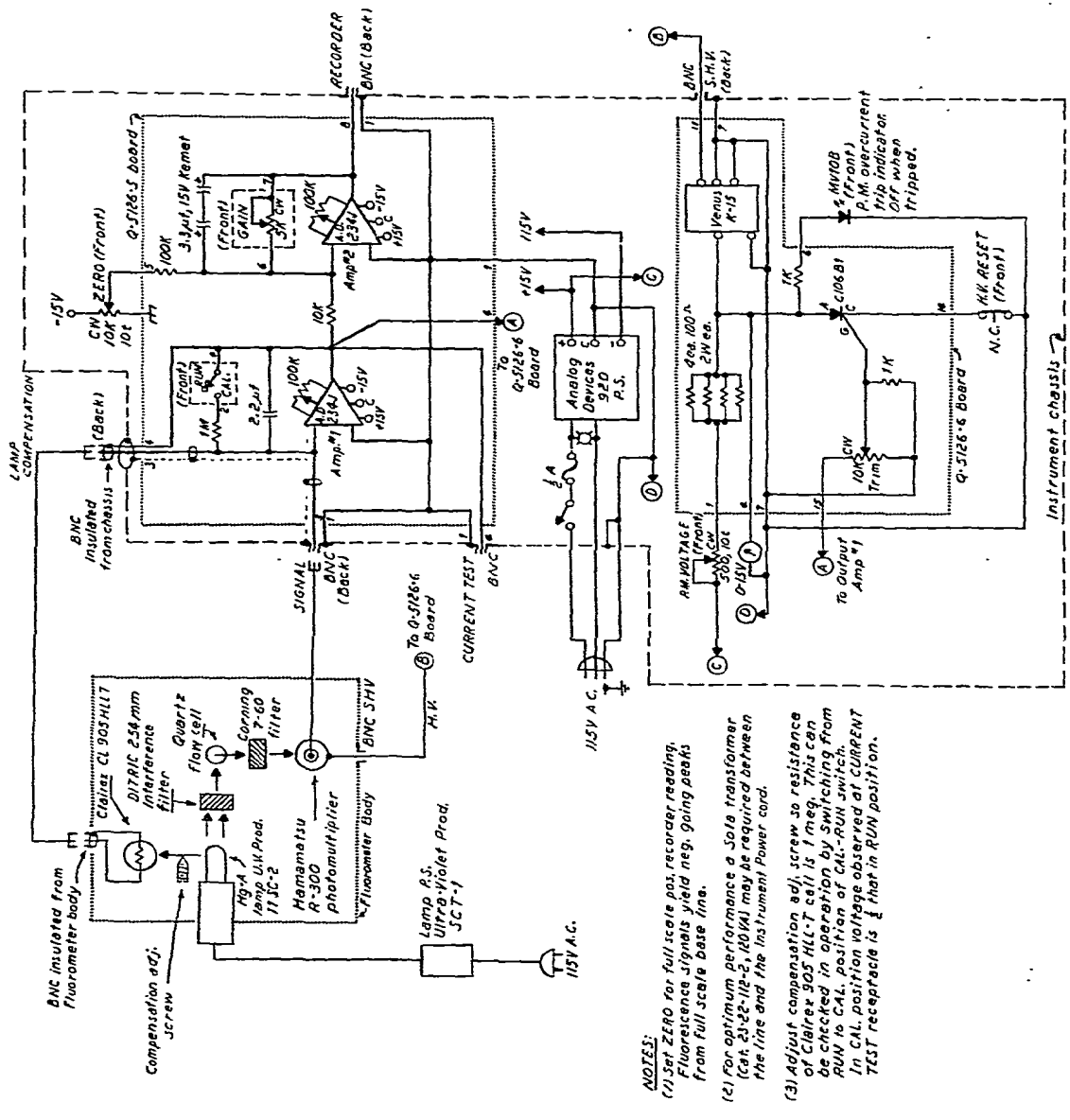


Fig. 2. Fluorometer schematic diagram.

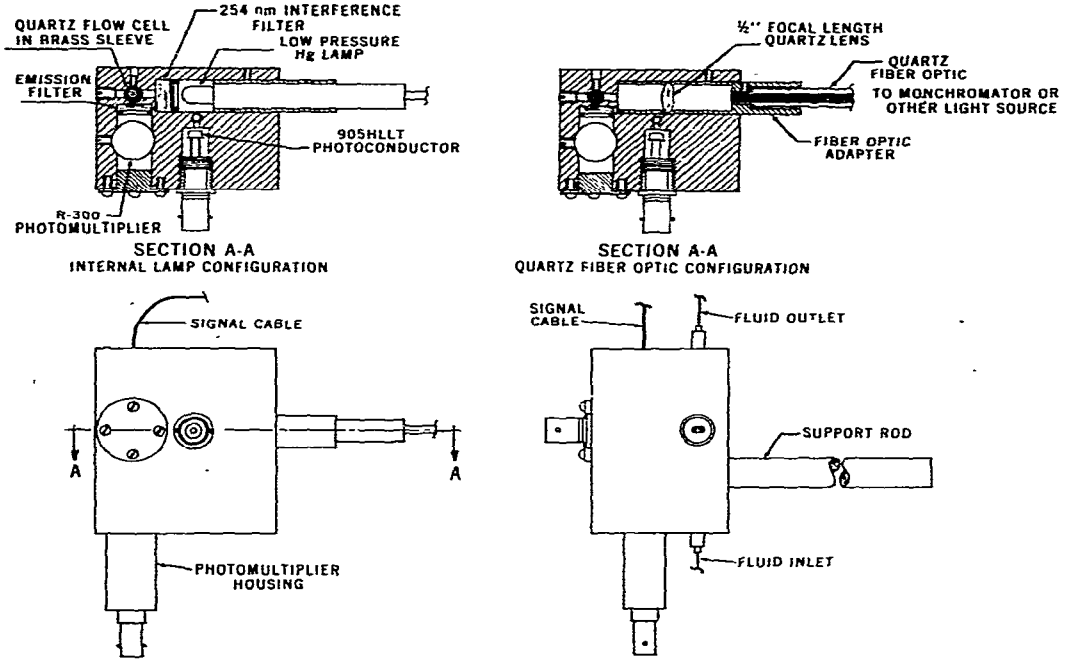


Fig. 3. Fluorometer body.

much more convenient internal lamps cannot supply the required excitation power at the desired wavelength.

The excitation filter is chosen to select the desired spectral band for exciting the sample. Because the excitation maximum for Ce(III) ions is at 260 nm, the instrument is operated with a 254-nm interference filter and the low-pressure mercury lamp for that application. The choice of a particular interference filter should be based on high transmittance in the pass band and high blocking (10^{-4}–10^{-6}) at longer wavelengths, rather than on a narrow pass band. Appropriate filters are available from Ditric Optics.

Several flow-cell designs have been tested. The highest sensitivity and signal-to-noise ratio have been obtained with a cell consisting of a 2.5 cm × 4 mm O.D. by 0.5 mm wall commercial quartz tubing with ends smoothly pulled down to 1.6 mm O.D. Each end of the flow cell is connected to 1.6 mm O.D. PTFE tubing. The quartz-to-PTFE connection is made by etching the end of the PTFE tube with commercial etchant, abutting the PTFE tube to the quartz tube, covering the joint w th a short length of No. 16 PTFE, and sealing it by shrinking a sleeve of heat-shrinkable plastic over it. The tubulated flow cell is mounted in a brass tube with appropriately cross drilled excitation and emission apertures. These flow-cell assembles are tested for leaks at 30 p.s.i.g. before they are installed in the instrument. At a small sacrifice in sensitivity and signal-to-noise ratio, a flow cell having a volume of only 15.7 μl (less than one-tenth the volume of the optimum cell) has been used. It is fabricated by slightly expanding the ends of 1.6 mm O.D. PTFE tubes; these ends were then pushed inside a length of 3 mm O.D. by 0.5 mm wall quartz tube to leave a clear gap of

about 5 mm. The quartz-to-PTFE seal is again made with heat-shrinkable plastic tube.

The emission filter that defines the band of wavelengths passed to the photomultiplier is selected to block the excitation wavelengths while passing as much of the emitted light as possible. Since the fluorescence emission spectrum of Ce(III) corresponds almost precisely with the pass band of the Corning 7-60 filter, that filter material is used in many applications.

The photomultiplier is a Hamamatsu R-300 1/2-in.-diameter side-viewing tube with an S-5 photoresponse characteristic. This response spectrum imposes an ultimate 185–650-nm limit on the detectable fluorescence emission spectrum. The voltage divider is contained in the 5/8 in. O.D. photomultiplier housing tube; a coaxial high-voltage cable is connected at one end of the tube, and a miniature coaxially shielded signal cable emerges from the opposite end.

A Clairex CL 905HLL-T photoconductor senses the emission of the light source and adjusts the gain of the electronic circuit to compensate for variations in lamp intensity. An aperture-adjusting screw determines the amount of light falling on the photoconductor; the aperture screw is set initially so that the photoconductor resistance is 1 MΩ. Subsequent checks and adjustments can be made during normal operation through use of the Run-Calibrate switch on the electronic chassis, as described below.

The principal components in the electronic chassis are a ±15-V d.c. power supply (Analog Devices 920), two high-quality chopper-stabilized operational amplifiers (Analog Devices 234-J), and a miniature d.c.-to-d.c. converter (Venus Scientific K-15) which generates the high voltage for the photomultiplier from the 15-V power supply. The amplifier electronics and a high-voltage power supply that has an over-current safety system are implemented on individual printed circuit boards (Fig. 2) which plug into sockets on the chassis.

Amplifier 1 is operated as a current-to-voltage converter; the signal current from the photomultiplier is taken directly to the summing junction of the amplifier, and the photoconductor in the fluorometer head is connected as the feedback resistor. Thus, when the intensity of the mercury lamp decreases, the signal current also decreases proportionately; since the resistance of the photoconductor increases proportionately, the output of amplifier 1 remains essentially unchanged. The resistance of the compensating photoconductor can be checked while the system is operating by switching the toggle switch on the front panel from the Run to the Calibrate position. In the Calibrate position, the photoconductor is paralleled by a 1-MΩ resistor; since the desired resistance of the photoconductor is 1 MΩ, the voltage observed at the Current Test receptacle with the switch in the Calibrate position should be exactly one-half the value observed when the switch is in the Run position If this is not the case, the photoconductor resistance can be adjusted by the aperture screw at the top of the fluorometer body.

Amplifier 2 provides zero and gain adjustments for interfacing with a variety of standard recorders. Because workers in our programs want to record peaks in the same format (and often on the same chart) as transmittance and absorbance peaks from other instruments, we use zero adjustment to place the base line at positive full scale on the recorder; fluorescence peaks are recorded as down-scale (negative) deflections of the recorder pen. In situations not demanding ultimate sensitivity, amplifier 2 could be an inexpensive, integrated circuit amplifier.

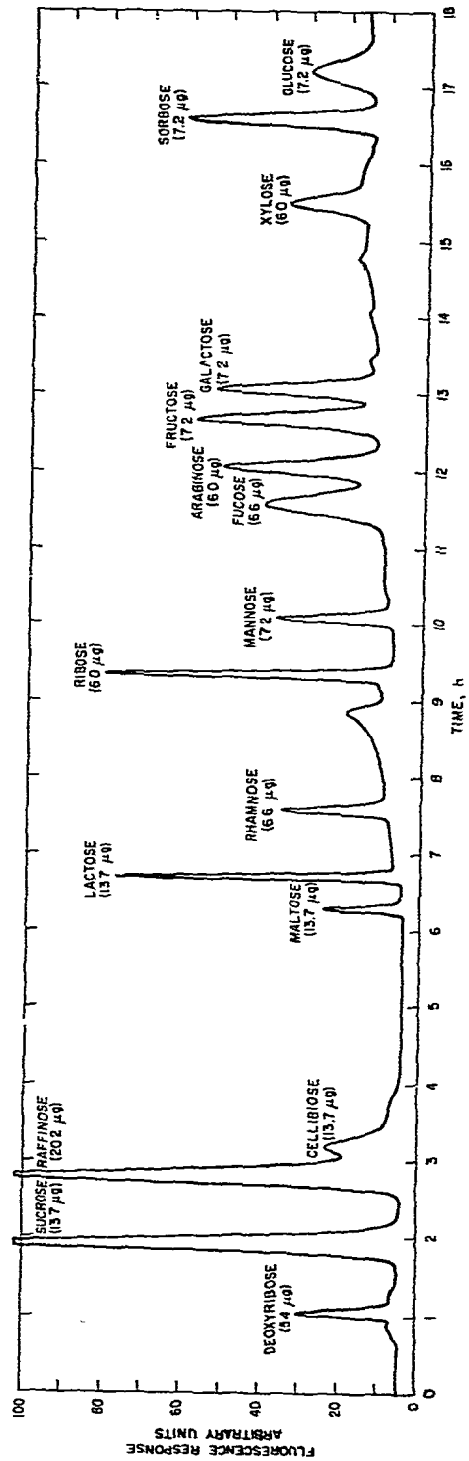


Fig. 4. Fluorescence response in anion-exchange separation of 16 carbohydrates.

The photomultiplier voltage is adjustable from about 300 V to 1500 V from a front-panel dial. A silicon controlled rectifier monitors the photomultiplier current and, if the current exceeds that selected by an adjustable trip point, shorts the d.c.-to-d.c. converter input to ground, thus reducing the output voltage and protecting the photomultiplier tube.

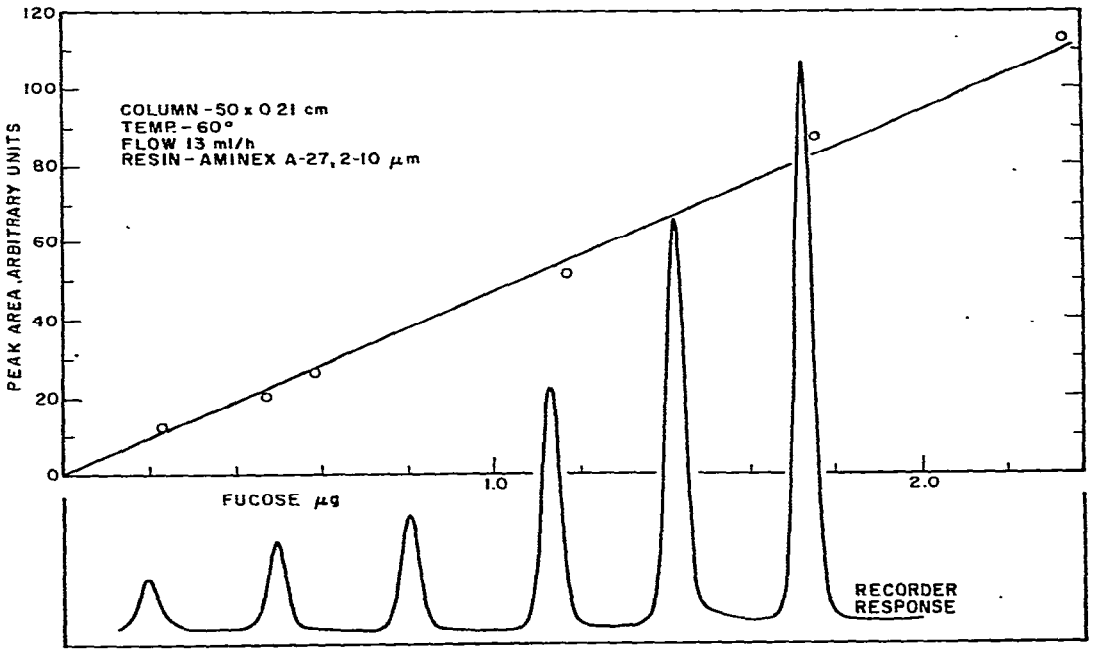
Unless line voltages are clean and stable, it has been necessary to provide power through a Sola transformer (Catalog No. 23-22-112-2; 120 V-A) for operation at high sensitivities. Since the capacity of the Sola transformer is adequate, power for the mercury-lamp supply is also taken from that source.

INSTRUMENT APPLICATIONS

The miniature flow fluorometer has been used in a large number and variety of applications. A cross section of these applications is summarized and referenced below.

In a liquid-chromatographic analysis of neutral carbohydrates in serum glycoproteins, Mrochek *et al.*[3] used the instrument in a cerate oxidimetric system[4]. Fig. 4 shows results they obtained in separating and detecting 16 carbohydrates. A highly linear response to eluted amounts of fucose, ranging from 0.23 to 2.3 μg (Fig. 5), is shown, and a sensitivity to 1 nmole of fucose is demonstrated.

Katz *et al.*[5] compared the sensitivity of fluorescence detection with UV photometry for 12 substituted aromatic acids using the fluorometer in a cerate oxidimetric



LINEAR RESPONSE OF CERATE FLUORESCENCE DETECTOR FOR FUCOSE ELUTED WITH 0.5 M BORIC ACID

Fig. 5. Linear response of cerate fluorescence detector for fucose eluted with 0.5 *M* boric acid.

system. For 11 of the compounds, they found the ratio of fluorescence response to UV response to range from 2 to greater than 50; for one aromatic acid (2-hydroxybenzoic), the ratio was only 0.8.

Katz *et al.*[6] used dual monitoring by UV absorption and fluorescence produced by cerate oxidation to obtain sensitive and wide-ranging detection capabilities in a comparative serum and urine analysis by anion-exchange chromatography.

In another report, Katz *et al.*[7] found that the cerate oxidative detector system, using the reported fluorometer, provides better sensitivity and more effective peak resolution than other monitors for eluted carbohydrates, but the system is less specific than most of the earlier detectors.

Mrochek *et al.*[8] used the detector in a study of acetaminophen metabolism in man. Fluorescence and UV absorbance chromatograms as a function of time after ingestion are presented in their report.

In a personal communication[9], Katz reported that the fluorometer was used extensively for about a year in studies of coal-derived liquids on both a preparative and an analytical scale in series with a UV detector. The solvents used included heptane, hexane, isooctane, and various alcohols. In this application, a wide-band emission filter was installed, and the instrument was used to monitor the natural fluorescence of polycyclic aromatic hydrocarbons. Although the fluorometer was operated at reduced sensitivity, the response was generally as good or better than the UV detector. One particular advantage reported was that the fluorometer was less sensitive to flow disturbances.

ACKNOWLEDGEMENT

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