FLOW INJECTION ANALYSIS WITH FRONT-SURFACE ILLUMINATION LASER INDUCED FLUORESCENCE SPECTROMETRY FOR ANALYTICAL APPLICATIONS

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ABSTRACT

In this contribution we describe for the first time a flow injection analysis manifold with a front-surface illumination laser induced fluorescence system. An argon-ion laser was used for excitation. This system employs the principle of zone merging and a laser colinear arrangement for the determination of 0.01-1 pg of sodium fluorescein by injecting 10 μl sample volumes. After optimization of the flow injection conditions, the detection limit of 3 fg of sodium fluorescein was four orders of magnitude better than that obtained by a well established conventional fluorescence method. 100 measurements per hour were possible with good precision.

INTRODUCTION

An area of current interest is the use of laser induced fluorescence detection (LIFD) in capillary electrophoresis[1-4]. The detection of very small amounts of fluorescent compounds is of great interest in chemical, biological and biomedical sciences. As well, for all practical purposes there have been indeed many and their number is steadily increasing of detectors applicable to flow injection analysis (FIA)[5-7] as its is possible to couple it with almost any commercially available instrumentation[8].

Applicattion of lasers to the detection of rhodamine[9] and porphyrins[10] using different FIA manifolds have been previously reported. However, in this work a more modern approach to collect fluorescence in a colinear arrangement at 180° to the direction of the laser beam is described. This system offered, among other advantages, high sensitivity, low sample consumption, high frequency of sample analysis, larger probe volume and it was very easy to assembly. Studies reported below employ sodium fluorescein in aqueous medium as a model analyte to demonstrate the succesful application of front-surface illimination (FSI) - LIFD as a detector for FIA, characterizing sampling rate, analytical response and limit of detection (LOD).

EXPERIMENTAL SECTION

Apparatus

The experimental arrangement for the FIA-fluorescence detection system is shown in Fig. 1. The apparatus is equipped with an air-cooled argon-ion laser (Spectraphysics, Toulouse, France; Model 161-C). This laser is well adapted to the excitation at 460 nm of the sodium fluorescein assay procedure[11]. In the present work, the colinear arrangement was found to ensure optimum collection of the emitted photons and it was very easy to assembly. The beam of coherent light was filtered with a band pass 450 nm to 490 nm filter (Carl Zeiss, Oberkochen, Germany; Model BP 450-490) reflected by a 460 chromatic beam splitter (Carl Zeiss, Oberkochen, Germany; Model FT 460) and condensed on the glass tubing by means of a fluorite objective (Carl Zeiss, Oberkochen, Germany; Model Neofuar). The emitted light was collected by the same objective. A 0.2 cm diameter plane mirror was placed on the opposite side of a 2-cm length of 1.0 mm i.d. glass tubing in order to improve the collection efficiency. After crossing the chromatic beam splitter, the emission was filtered through a 514.5 nm long wave pass filter (Carl Zeiss, Oberkochen, Germany; Model LP-514), a circular variable filter wheel which provides complete

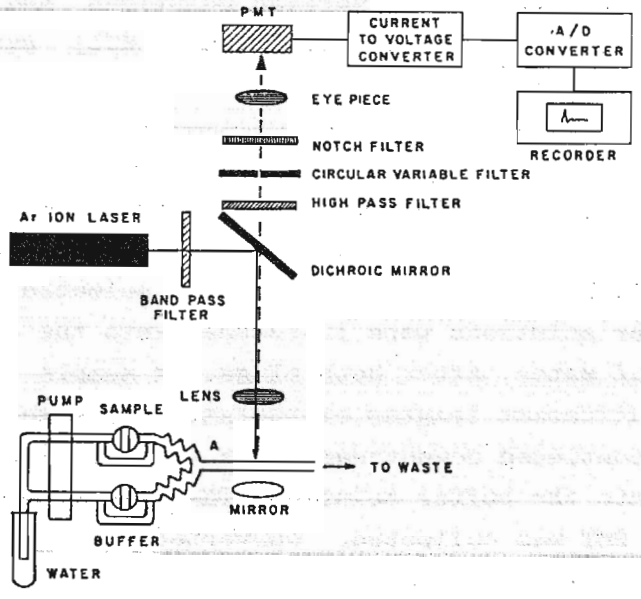

Fig. 1 Experimental arrangement for FSI LIFD: PMT, photomultiplier tube.

coverage of the 400 to 700 nm spectral region (Carl Zeiss, Oberkochen, Germany), and a costum made notch filter (Andover Co., Mass., USA). This last one suppresed the 488 nm line that the glass tube reflected toward the detector. A 1 cm focal length lens (Melles Griot) was placed after the notch filter to collect the signal on the quartz window of a Hamamatsu photomultiplier (PMT) (Spex, Edison, NJ, USA; Model R9285) operated at 750 V with a Bertan high voltage power supply (Bertan, Hicksville, NY, USA). The output of the PMT was connected to a 485 Keithley picoammeter (Keithley, Cleveland, OH, USA) operated at 200 nA for weak signals. The fluoresecence signals were monitored on a strip chart recorder (Varian-Techtron, Springvale, Australia; Model; 9176) which was connected to the output of the picoammeter.

Reagents

All chemicals were of certified grade unless specified differently. Water was deionized (Millipore, Bedford, MA, USA).

Sodium fluorescein standard and working solutions and the boric acid buffer (0.05M in boric acid and 0.05M in KCl) were prepared as described previously[11].

Procedure

Working and buffer solutions were transferred to the corresponding loops of the injector resting in the sampling position. On switching the injector, the selected volumes of sample and buffer solutions were introduced into the corresponding carrier stream of water. After both plugs, of sample and buffer, passed through different lengths of tubing, they mixed at point A in Fig. 1 and continued downstream while the sample plug is mixed and dispersed into the buffer solution. The resulting fluorescence signal from the PMT was collected, converted to a voltage and displayed on a recorder.

RESULTS AND DISCUSSION

The introduction of samples and buffer solutions as previously described in the procedure gives rise to a peak response as illustrated in Fig. 2. The peak height and shape greatly varied according to the parameters governing FIA (sample and buffer volumes, carrier stream flow rates and length and diameter of tubing). The highest peaks with least tailing were obtained under the conditions listed in Table I.

The volumes of sample and buffer solutions influenced both the peak height and peak shape for a constant flow-rate of carrier solution. But variations on the sample volumes changed more dramatically the analytical signal. Since the buffer is nonfluorescing, the chemical inhomogeneity of larger sample plugs within the buffer solution contributed significantly to broadening the peak signals. Thus, by using a relatively reduced sample volume an appropriate mixing of the sample and buffer plugs was obtained and good sensitivity and reproducibility were achieved.

Tube diameters of 0.5, 1.0 and 1.5 mm were examined. Increasing

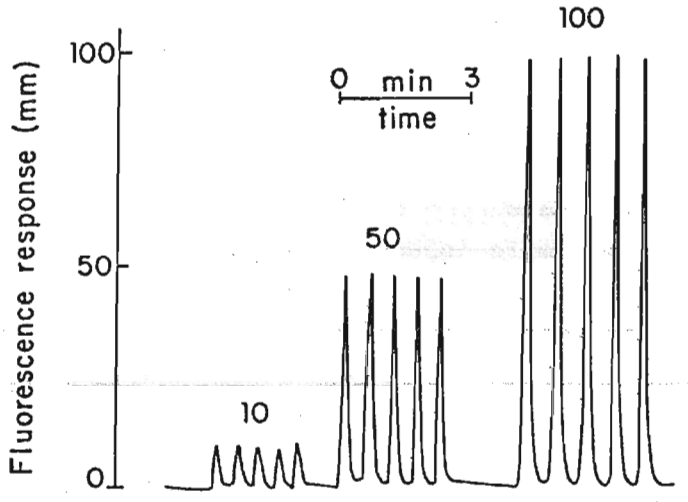

Fig. 2 Quintuplicate recorded signals obtained by injecting 10-μl of sodium fluorescein samples in the FIA-FSI LIFD system shown in Fig. 1. 10, 50 and 100 indicate ng of sodium fluorescein/l. Conditions as specified in Table I and throghout the text.

the i.d. diameter from 1.0 to 1.5 decreased the analytical signal but only to small extent, because of increased dispersion of the sample zone. Whereas, smaller and thiner peaks were obtained with a tube diameter of 0.5 mm i.d., probably due to an innapropriate dilution of the sample plug within the buffer solution.

Changes in each carrier solution flow-rate within the interval 0.5 to 2.5 ml/min caused small variations in the measured signals. However, the relation-ship between both carrier flow-rates must allow an appropriate dilution of the sample plug within the buffer solution. Therefore, the flowing-rates of 1.2 and 1.4 ml/min for the sample and buffer solutions were found to be in a good compromise in order to obtain a good sensitivity.

TABLE I Experimental FIA parameters for the determination of sodium fluorescein by FSI LIFD.

Parameter	Value
Sample volume	10 μl
Buffer volume	100 μl
Carrier of sample flow rate	1.2 ml/min
Carrier of buffer flow rate	1.4 ml/min
Length of tubing from sample injection to point A	20 cm
Length of tubing from buffer injection to point A	20 cm
Length of tubing from point A to detector	200 cm
Internal diameter	1 mm

Due to the manifold characteristics, the distances of tubing from the injector to point A (Fig. 1) were different. Therefore, the length of tubing were optimized to ensure an appropriate mutual overlaping of both, sample and buffer, plugs. The distance of tubing from point A to the detection point did not significantly changed the analytical signal, and therefore it was kept as short as the instrumental allowed.

The linearity of the fluorescence signal was evaluated from a plot of peak height (in mm) as a function of sample concentration. The signal was linear over the 1.0 to 100 ng/l range (0.01-1 pg) (slope = 0.98, correlation coefficient = 0.9995). The LOD based on S/N = 2 was found to be 0.3 ng/l (3 fg) of sodium fluorescein, which is nearly 4 orders of magnitude lower than that using a well established conventionalfluorescence detection method[11]. The relative standard deviations for the determinations of 10 and 50 ng/l of sodium fluorescein, obtained from ten replicate analysis were 2.8 and 2.4 %, respectively. The sample measurements throughput readily achieved was about 100 per hour.

Conclusions and Future Directions

The FIA- FSI LIFD system here described can be applied to a variety of analytes. Even though the separation that could be achieved by FIA could not mactch to that of capillary zone electrophoresis, the increased speed on analysis would be of a great advantages for the evaluation of single or limited number of components in biological and biomedical samples. In the future, the application of other data treatment procedure should allow even lower detection limits to be achieved, as well the use of a wide range of other data treatment procedure should allow even lower detection limits to be achieved. The use of various kind of lasers (specially in colinear arrangements) and other FIA manifolds will surely make possible the determination of many chemical species at sub pg levels. In either case, the FSI LIFD would allow further flexibility in the way of collecting the fluorescence emission and of removing interferences.

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