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USE OF PTFE COILS IN POST-COLUMN PHOTOCHEMICAL REACTORS FOR LIQUID CHROMATOGRAPHY — APPLICATION TO PHARMACEUTICALS

A. H. M. T. SCHOLTEN, P. L. M. WELLING, U. A. TH. BRINKMAN and R. W. FREI*

Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)

SUMMARY

The advantages and the performance of PTFE reaction coils in photochemical reaction detectors for high-performance liquid chromatography (HPLC) are discussed. The excellent performance of these materials at irradiation wavelengths below 300 nm is based on a diffuse radiation transfer and an internal reflectance (light-tube) effect. Optimal coil designs can be obtained from signal vs. flow-rate curves for a particular application. Lamp performance has to be tested periodically and the reactor should be equipped with an efficient cooling system.

Application of the principle to the fluorimetric detection of clobazam and phenothiazines after HPLC separation is discussed. The sensitive detection of these pharmaceuticals and their metabolites in serum with a minimum of sample handling demonstrates the potential of photochemical reaction detectors.

INTRODUCTION

The merits of photochemical reactors as a means to improve the UV-visible or fluorescence detection properties of selected groups of compounds has been discussed by several groups of workers¹⁻⁴ in the recent past. Various designs have been proposed, such as a mercury lamp source with water cooling², and xenon and xenon-mercury sources with different types of air cooling^{1,3,4}. In all instances quartz capillaries were used as reaction coils to assure good transparency to the incident UV light. This appeared to be necessary as most of the compounds investigated so far had absorption maxima well below 300 nm, *viz.*, clobazam at 230 nm with a small shoulder at 280 nm, and thioridazine and its oxidation products at around 270 nm.

Quartz capillaries, unfortunately, are not readily available in different geometries (coil and helix diameter; length). Also, they are expensive and fragile, and tight connections are not easily made. It was therefore decided to investigate other reaction materials such as Pyrex glass and PTFE coils, because of their better accessibility and greater ease of handling, although possibly at a considerable sacrifice in sensitivity. In addition, attention was devoted to the optimization of the reactor design and to the application of this principle.

EXPERIMENTAL

Two photochemical reactors were used. The design of one of these has been described earlier⁴. With this one, a 200-W xenon-mercury lamp is used as a light source, and air cooling is effected by means of a vacuum cleaner sucking air through the reactor housing at a controlled rate; the reactor is surrounded by a reflecting aluminium shield. As an alternative, a reactor (Fig. 1) was constructed that differed from the earlier one in two respects: a glass mirror serves as reflection medium and a controllable-speed fan blows air through the reaction chamber. Unless otherwise indicated, the latter reactor was used in this study.

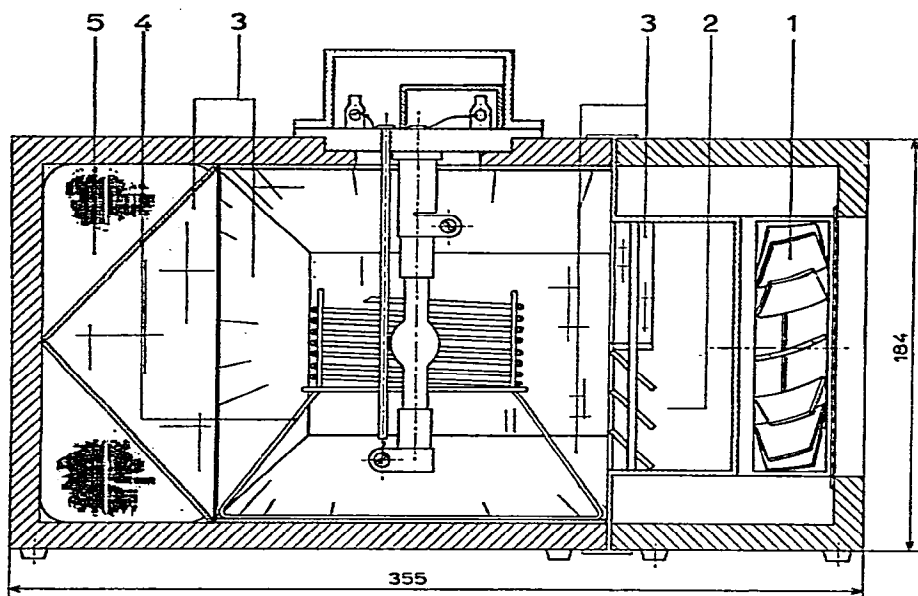


Fig. 1. Design of new photochemical reactor. 1 = Fan; 2 = streamliner; 3 = glass mirrors; 4 = standard for capillary; 5 = air outlet.

PTFE capillaries of 0.35, 0.5 and 0.8 mm I.D. and 1/16 in. O.D. were purchased from Omnifit (Biolab, Cambridge, Great Britain); a capillary of 0.3 mm I.D. and 0.76 mm O.D. was obtained from Eriks (Alkmaar, The Netherlands). Quartz capillaries of 0.7, 0.8 and 1.1 mm I.D. were used. The helix diameter of all capillaries was 6 cm. An Altex-100 pump (Altex, Berkeley, CA, U.S.A.) was used and detection was effected with a Perkin-Elmer Model 204A fluorescence spectrophotometer. All experiments except for the applications were performed using plug injection, that is, without a column being installed. The reflectance-spectrometric studies were carried out with a Zeiss PMQII spectrophotometer (Zeiss, Oberkochen, G.F.R.) equipped with a densitometer attachment for fluorescence analysis.

Clobazam was obtained from Hoechst-Roussel Pharmaceuticals (Somerville, NJ, U.S.A.). Thioridazine, mesoridazine and sulphoridazine were gifts from Sandoz (Basle, Switzerland). The structure and main characteristics of these model com-

pounds, three of which (the phenothiazines) show native fluorescence, have been recorded previously⁴. Reagent-grade solvents (methanol and acetonitrile) were degassed in an ultrasonic bath before use.

RESULTS AND DISCUSSION

PTFE capillaries

Experiments were performed with quartz, Pyrex glass and PTFE capillaries of various internal diameter and length. All experiments carried out with Pyrex glass capillaries gave negative results, *i.e.*, no fluorescence (clobazam) or increase in fluorescence signal plus shift of emission maxima (phenothiazines) occurred upon irradiation. This is according to expectation as the transparency of Pyrex glass becomes negligible at wavelengths below about 300 nm.

In view of the above, the results of experiments with PTFE coils were all the more surprising, as the PTFE material does not permit direct transmission of light below about 300 nm either. Nevertheless, for short (see below) residence times, the intensity of fluorescence signals was comparable to or higher than those observed with quartz capillaries. Also, improved peaks with better symmetry and less tailing were obtained with PTFE than with quartz capillaries, as can be seen in Table I, which summarizes data on peak shape and asymmetry for various types of coils and various residence times, using clobazam as a model compound. The improved peak shapes, *i.e.*, flow characteristics, with PTFE coils, which were also observed with the phenothiazines, may be caused by the different wetting characteristics of the two types of coil material used.

Another interesting result appears when the signal intensity for clobazam is considered as a function of flow-rate (residence time) and mobile phase composition (Fig. 2). In an earlier study⁴ with this compound, and using quartz capillaries, it was observed that the fluorescence signal still increased rapidly after irradiation times of about 90 sec. For example, a 3-fold increase in signal resulted from irradiating for 400 instead of 50 sec. With PTFE coils, the intensity of the fluorescence signal increased with residence times up to about 20 sec. Subsequently, a sudden decrease in fluorescence intensity occurred, as can be seen in Fig. 2. This decrease was observed for all methanol-water mixtures tested after approximately the same irradiation time, and also with acetonitrile-water mixtures. [With the new reactor (Fig. 1) and a new lamp, *i.e.*, when using much more intense radiation, a breakdown also occurred in quartz capillaries, at reaction times of about 60 sec.]

The sudden decrease in signal, which was also observed with the three phenothiazines, reserpine and naphthalene, suggests a rapid breakdown of the molecular structure of the original product and/or the reaction products formed upon irradiation. From thin-layer chromatographic experiments with the phenothiazines, which are presently being carried out in our laboratory, we can conclude, however, that even after irradiation times of 20 min the original compound and the fluorescent reaction products are still present. Also, many polymeric and carbon-containing degradation products have been formed after such prolonged irradiation times. Probably this implies that the rapid decrease in fluorescence can be partly attributed to phenomena such as light absorption (filter effect), scattering due to particulate matter and possibly some quenching effects.

TABLE I

PEAK SHAPES FOR CLOBAZAM IN PTFE AND QUARTZ CAPILLARIES

Conditions: flow-rate, 0.9 ml/min; eluent, methanol-0.01 M sodium acetate (1:1); clobazam, 290 ng/ml; plug injection, 20 μ l; λ_{ex} = 356 nm, λ_{em} = 395 nm; recorder, 2 mV; sensitivity range, 1.0; gain 3. Measurements performed with "old" reactor and "old" lamp.

Capillary material	Dimensions	Peak height (mm)	Peak width (sec) at		Asymmetry factor at		Residence time (sec)
			0.6 h	0.1 h	0.6 h	0.1 h	
PTFE	1 m, 0.5 mm I.D., 1/16 in. O.D.	137	10.3	25.5	1.3	2.0	13
	2 m, 0.5 mm I.D., 1/16 in. O.D.	183	12.4	28.4	1.1	1.5	29
Quartz	1.5 m, 0.8 mm I.D., 1/16 in. O.D.	102	24.0	48.6	1.0	1.4	51
	1 m, 0.7 mm I.D., 3.8 mm O.D.	90	17.2	35.7	1.5	1.9	26
	1 m, 0.8 mm I.D., 3.0 mm O.D.	85	21.3	46.8	1.9	2.6	36
	1 m, 1.1 mm I.D., 3.0 mm O.D.	81	37.2	81.6	2.0	2.5	64

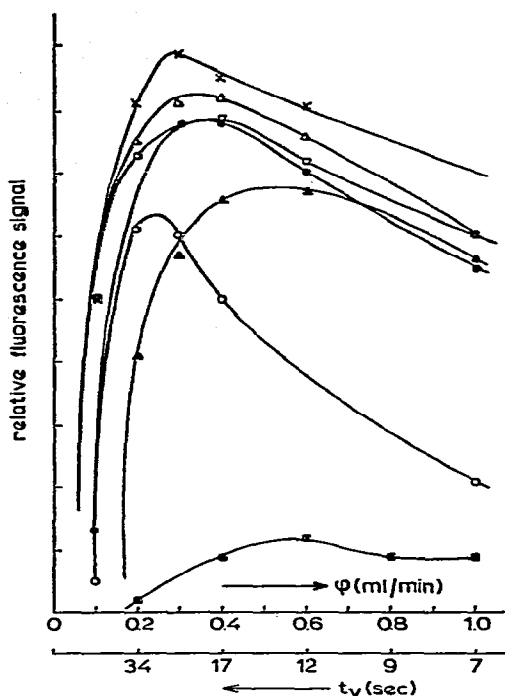


Fig. 2. Influence of carrier stream composition and time of reaction, t_r , on the intensity of the fluorescence signal for clobazam. Carrier stream: methanol-0.01 *M* sodium acetate solution. (▲, 2:8, ●, 4:6, ×, 1:1, △, 6:4, □, 8:2); ■, 0.01 *M* sodium acetate; ○, methanol. Conditions; 6-m PTFE capillary of O.D. 0.76 mm and I.D. 0.3 mm.

The apparently excellent transparency of PTFE towards UV light, probably based on a diffuse radiation transfer, was further investigated by irradiating clobazam spots applied to silica gel thin-layer plates with a medium-pressure mercury lamp for 60 min, the spots being either non-covered, or covered with PTFE or quartz. Very similar fluorescence yields were obtained in all three instances (Fig. 3), with only a minor decrease for the PTFE-covered sample. As the experiments with the Pyrex glass coils demonstrate that irradiation with light having $\lambda > 300$ nm does not contribute much to the photochemical process, one may conclude that, even for the PTFE-covered sample, reaction proceeds by means of irradiation with the 254, 265, 280 and/or 290 nm mercury lines. As additional proof of the transmittance of UV light by PTFE, diffuse reflectance measurements of clobazam spots on silica gel covered with a piece of PTFE showed that the spots display absorption at around 230 nm, the short-wavelength maximum of clobazam. As regards the high efficiency (high signals, rapid decomposition) of PTFE coils, this can be explained by the "light-tube" effect of this material. This results in an internal multiple reflectance of the radiation and hence in an amplification of the photochemical effect. The light-conducting properties of PTFE tubing are well known as an undesirable phenomenon in fluorescence detection.

When the length of the PTFE capillaries was changed from coils of length 5.6 m (30 windings) to 1.9 m (10 windings), the maxima of the fluorescence intensity

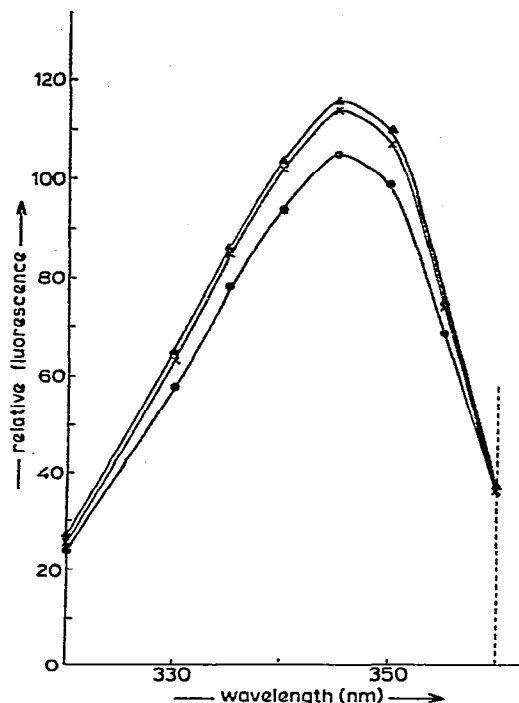


Fig. 3. Measurement of relative fluorescence of a sample of clobazam spotted on a silica gel thin-layer plate after 1-h irradiation with a mercury lamp, using a non-covered spot (×) or a spot covered with quartz (▲) or PTFE (●).

curves shifted to lower flow-rates while the general shape of the curves remained essentially the same (Fig. 4). However, one should realise that the sudden decrease in signal intensity occurs after practically the same (residence) time in all instances, as can be seen in Fig. 4. It is also interesting that variations of the flow-rate have a much larger effect on the residence time with coils of 10 than 30 windings. This is of importance when selecting conditions for analytical separations. It will then be necessary to operate under optimal conditions at a given flow-rate. Therefore, for each application a set of fluorescence intensity *vs.* flow-rate curves will have to be constructed, from which optimal conditions can be read.

Reactor parameters

In the photochemical reactor, two different designs were used to effect efficient cooling. With the older system⁴, temperatures measured just outside the capillary coils were 6–8°C lower than the temperature of the carrier stream inside the capillaries. In the new design, the temperature differences were distinctly smaller (2–4°C), which demonstrates the superiority of the new cooling system. We have observed that temperature changes slightly influence the peak height and shape of the fluorescence intensity *vs.* flow-rate curves such as are shown in Figs. 2 and 4. Using conditions in which peak heights increase with decreasing flow-rate (right-hand branch of the curves), higher fluorescence signals are obtained at higher temperatures; however,

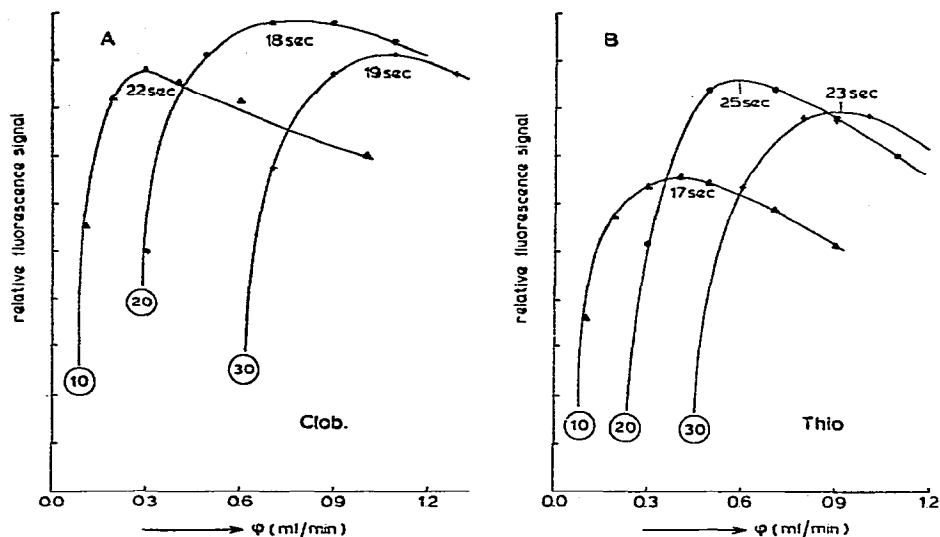


Fig. 4. Fluorescence intensity vs. flow-rate curves for (A) clobazam and (B) thioridazine constructed with 10-, 20- and 30-winding capillaries. Carrier stream, methanol-0.01 M sodium acetate solution (1:1). Ten windings corresponds to a length of capillary of 1.9 m.

under breakdown conditions (left-hand branch) a higher temperature effects a more rapid signal decrease. Consequently, curves constructed at different temperatures intersect each other in the neighbourhood of their maxima.

As regards reflector material, in the early design an aluminium casing was used, which served as both a heat- and a UV-radiation reflector. In the new reactor, glass mirrors were installed instead of an aluminium shield. Comparison of the two designs under equal temperature conditions (51–52°C in the carrier stream) resulted in about the same signal intensities for thioridazine (Fig. 5) and for clobazam. Fig. 5 also depicts the results of a series of experiments in which the glass mirror was covered with either black cardboard or aluminium foil. The signal intensities for the glass mirror are lower, which is probably due to the UV absorption by the glass. The operating temperatures were found to be essentially the same in all three instances (glass, cardboard and aluminium).

Ageing of the light source and, therefore, the temperature and UV characteristics of the light source can be expected to exert a considerable influence on the reactor performance and should therefore be tested at regular intervals. The efficiency of an old lamp (several hundred working hours) compared with a new lamp for thioridazine can be seen in Fig. 5. Ageing influences the signal intensity and even the position of the peak maximum of the curves. Not surprisingly, a higher UV intensity (new lamp) results in a higher maximal signal and a more rapid decrease, which starts at shorter reaction times.

Application

The photochemical reactor was coupled with a reversed-phase HPLC system and tested with clobazam and desmethylclobazam, and with three phenothiazines as model compounds.

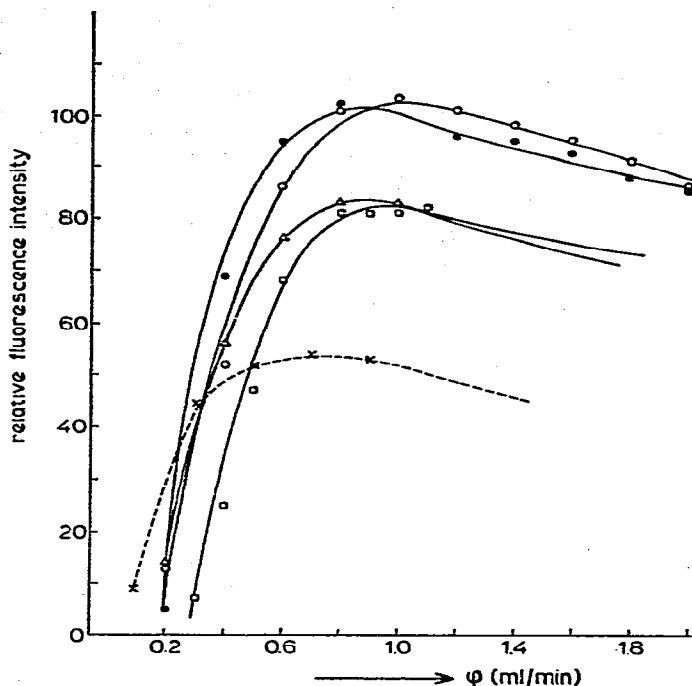


Fig. 5. Comparison between the new photochemical reactor equipped with glass mirrors (Δ) or mirrors covered with aluminium foil (\circ) or cardboard (\bullet), and the older design* (\square). The broken curve indicates the performance of a much used lamp installed in the new reactor using glass mirrors. Model system, methanol-0.01 *M* sodium acetate solution (1:1) with thioridazine as test compound.

For clobazam and its major metabolite, methanol-0.01 *M* sodium acetate (6:4) was used as the mobile phase at a flow-rate of 1.0 ml/min on a 15-cm 5- μ m LC-18 reversed-phase column (Supelco, Bellefonte, PA, U.S.A.) with a residence time of 15 sec (20 windings). The detection limits, calculated for a 2:1 signal/peak-to-peak noise ratio, were 20 pg for clobazam and 50 pg for desmethyloclobazam. With both compounds, linear calibration graphs were obtained (r , 0.999) in the investigated range of 2.5-90 ng/ml. An application of this principle to clobazam and its metabolite in serum has been shown earlier⁴.

For the three phenothiazines a separation was achieved with methanol-7.6 *M* sodium acetate (8:2) as the mobile phase and using a 10-cm 5- μ m LiChrosorb RP-8 column (Merck, Darmstadt, G.F.R.). The mobile phase was made 0.01% in ammonium peroxodisulphate in order to obtain a well defined system for the photochemical reaction (see also ref. 4). With this mobile phase a fluorescence intensity vs. flow-rate curve was measured, from which it was concluded that a reaction coil of 40 windings had to be used at a flow-rate of 1.0 ml/min; this corresponds to a reaction time of about 25 sec. Under these conditions the detection limits of the phenothiazines were about 0.5 ng. Linear calibration graphs (r , 0.999) were obtained in the 0.15-11.5 μ g/ml range. The gain factors after irradiation were 8-10 for thioridazine and 2-3 for mesoridazine and sulphoridazine.

In an application with serum samples, 1 ml of human serum was spiked with

mesoridazine (2.3 ppm) and thioridazine (6 ppm). After addition of 1 ml of methanol and shaking, the sample was centrifuged about 7 min at 7 g, and 20- μ l aliquots were injected on to the analytical column. The chromatogram obtained is shown in Fig. 6. For both compounds the recoveries were of the order of 90%. Sulphoridazine can not be detected in such an experiment, since its peak is hidden under the serum peak.

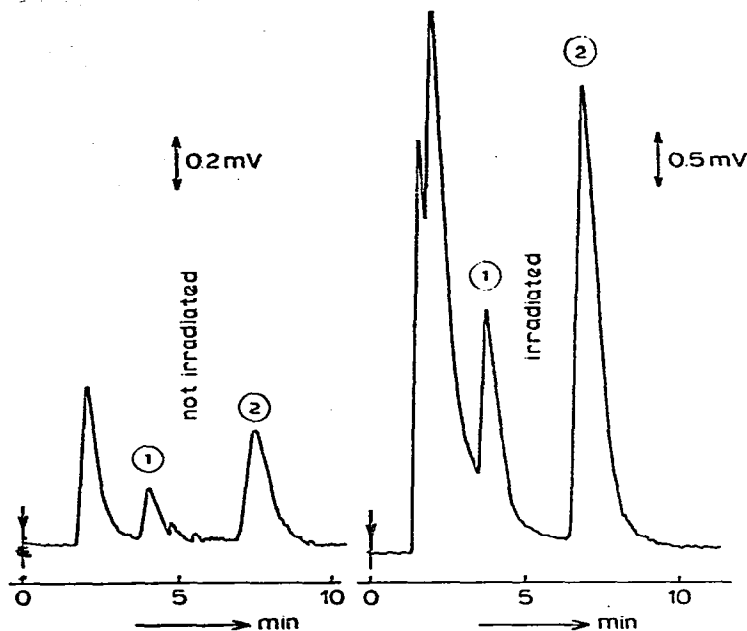


Fig. 6. High-performance liquid chromatogram of human serum spiked with 2.3 ppm of mesoridazine (1) and 6 ppm of thioridazine (2). The wavelengths selected were optimal for thioridazine, *viz.*, $\lambda_{ex} = 325$ nm and $\lambda_{em} = 435$ nm, and $\lambda_{ex} = 340$ nm and $\lambda_{em} = 370$ nm for the non-irradiated and irradiated experiment, respectively. Detection conditions: gain, 3; sensitivity range, 10. For further details, see text.

A separation of all three phenothiazines in serum can be achieved with methanol-7.6 M sodium acetate (3:1) as the mobile phase. At a flow-rate of 1 ml/min sulphoridazine, mesoridazine and thioridazine elute after 4.5, 5.5 and 14 min, respectively.

CONCLUSION

PTFE coils are highly suitable for use in photochemical reactors. An effective energy transfer occurs even in the 200-300-nm region and ensures rapid reactions and, therefore, intense signals after relatively short residence times. The low cost and ready availability of PTFE capillaries with a variety of tube diameters and their ease of installation add to the advantages of PTFE over quartz reaction coils. For a particular application it is strongly recommended to construct a series of fluorescence intensity *vs.* flow-rate curves and to check these at regular intervals in order to select and maintain optimal working conditions. As for the design of the photochemical

reactor, this does not appear to be very critical provided that the reactor is equipped with a reproducible and efficient cooling system.

Current research has demonstrated that PTFE capillaries can be recommended for the non-segmented flow systems discussed in this paper and for segmented carrier streams to ensure symmetrical band shapes and relatively little band broadening. In the future, attention will be mainly directed at the dependence of band broadening on (air or solvent) segmentation.

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