A New Post-Column Photochemical Reactor for Liquid Chromatography

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Photochemical reactions are sometimes added to the liquid chromatography repertoire as a means of improving overall assay selectivity and detection limits. Such reactions can augment optical as well as electrochemical detectors. This article briefly reviews the concept as it is applied to a new photocatalytic post-column reactor system based on a low-temperature UV lamp and titanium dioxide catalyst.

Although the USP/NF does not have a monograph on LC post column derivatization as yet, this technique is generally accepted and marketers of specialized reagents and equipment for post column derivatization have prospered. Of all the liquid chromatographic online post column derivatization methods, UV photolysis is the most appealing because of its simplicity, yet is used least often and is generally unfamiliar to analytical chemists. In spite of nearly 100 publications on this subject during the last 20 years(1,2) covering a wide range of pharmaceutical, clinical, food, environmental, forensic, and biotechnology applications, the few commercial UV photoreactors for LC are not widely used in spite of their potential advantages. ***F1*** schematically illustrates the normal instrumentation.

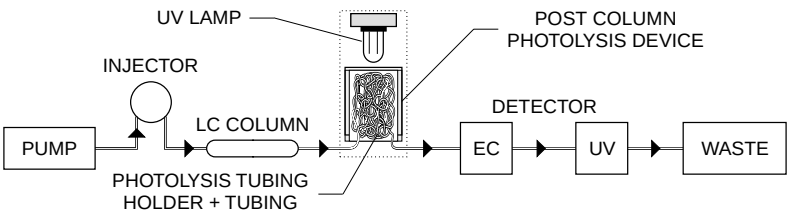
Operating problems which limited the routine use of these instruments in the early years included reactor coil heating and leaking and the need for long (10 to 30 meters) reactor coils with large (0.7 to 1.2 mL) dead volumes to provide adequate UV exposure time. Instrumentation has been relatively expensive and bulky, making it more difficult to fit with a variety of commercial instruments. Early experiments even required cooling the reactor coil with an icebath. The new compact BAS/Agrenetics PhotoBlaster® System 1 and LuxTube® Assembly eliminate these problems in a convenient, low-cost format. The goal was to make the instrumentation convenient for every laboratory using liquid chromatography.

An inorganic photocatalyst is immobilized on the inside wall of a precision bore fluoropolymer reactor coil. The reactor coil never contacts the hot UV lamp, and constant air cooling to below 30°C permits the use of acetonitrile and other organic modifiers and facilitates photocatalytic reactions instead of photothermolysis. Solutes transparent to UV light are not readily photooxidized but may be photocatalytically oxidized because the photocatalyst is activated although the solute may not be. Rapid photocatalytic reactions can be performed in short knitted coils (1.5 m) at room temperature with a dramatic decrease in dead volume; the potential is good for selective irradiation of individual analytes, and extended reactor coil life. The potential for using such coils with 1-2 mm diameter LC columns is quite good.

In 1976, Iwaoka et al.(3) were the first to use photohydrolytic action in an LC system employing a post-column photochemical reactor, and fused silica tubing. In 1980, Scholten et al.(4) published research on using PTFE coils to substitute for quartz capillaries in a photochemical reactor. Quartz capillaries have several disadvantages. They are not readily available in different geometries (coil, helix, 3-dimensional knit, diameter, length). They are expensive and fragile and leak-tight connections are difficult

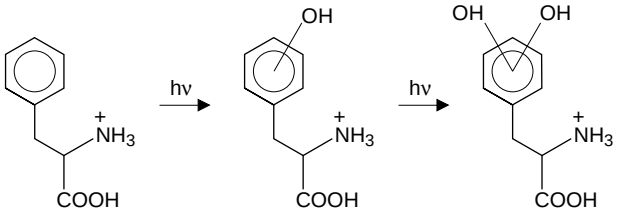
F1

Normal instrumentation of a UV photoreactor in an LC instrument.



F2

Photooxidation of phenylalanine.



to make. For a review of fluoropolymer tubing in photochemical reactors, see reference 5.

Krull et al. (6-13) have demonstrated the ability to use a post-column, on-line, photolytic or photochemical reaction-detection approach for a wide variety of organic and inorganic compounds. In this approach, analytes eluting from the LC column are photolyzed in a knitted fluoropolymer reaction coil as a function of the wavelength of the light and residence time within the reactor. Newly generated photoproducts are then eluted from the reactor and detected downstream by UV, PDA, FL, chemiluminescence, MS, and EC detectors. In the ideal case, a nonfluorescent substance becomes highly fluorescent in the reactor, leading to excellent signal-to-noise ratios. In many instances, electrochemically inactive analytes are converted into one or more photoproducts having favorable oxidative and/or reductive properties. At other times, analytes already possessing some EC activity can be converted to new photoproduct(s) with improved EC detection. The mechanisms of these photoconversions have been discussed for specific analyte classes, mainly organic (12,14). In summary, molecules may fragment or rearrange to improve detectability. There also are indirect photochemical detection schemes in which a reagent is added. The reagent can couple to the photoexcited analyte or the reagent can absorb the photon and then react with the analyte. These indirect schemes are generally less attractive. Adding a reagent is something to be avoided. ***T1*** is a tabulation (with references) for representative published applications of photochemical reactors coupled to LC. One should be cautious in noting that the actual performance achieved is quite dependent on reactor coil length, lamp intensity, mobile phase conditions, column diameter, temperature, and flow rate. These parameters are not independently adjustable, but are related to finding an adequate set of conditions.

The use of a photocatalyst, TiO_2, to improve the selectivity and sensitivity of fluorescence detection of various nitrogenous pesticides with on-line LC post column UV photolysis was attempted by Patel and Moye (28). A constantly stirred, aqueous suspension of TiO_2 was infused post-column into a water:methanol solvent system, but prior to the photochemical reactor. Only five pesticides were tested in this manner, and the observed increase in response was relatively small. The TiO_2 suspension plugged up the system, stopping further work (personal communication).

Because of the usually poor response of most amino acids to EC detection, chemical, photochemical, and even radiochemical derivatization methods have been applied, with varying degrees of success (29-31). Usually only two aromatic amino acids, tyrosine (Tyr) and tryptophan (Trp), as well as those containing sulfur, such as cysteine (Cys), show any significant EC activity underivatized, except under extreme conditions (0.1 M NaOH) where all amino acids can be anodically oxidized, albeit with relatively poor detection limits. Relatively little has been reported about photochemical derivatizations of amino acids, though some recent success has been reported (8,9, 32). Phenylalanine (Phe) appears to be the best candidate for photochemical derivatization, resulting in a mixture of known, hydrolytic products, the Tyr isomers (o-, m-, and p-) and L-dopa. All four of these have improved EC properties compared with the Phe itself (7,8,32), but the catechol L-dopa is by far the most desirable product since it can be detected at the lowest potential (***F2***).

Recently, Kaufman (33,34) studied the use of TiO_2 in various forms in an on-line LC post column photochemical reactor for the EC determination of Tyr and Phe. In the case of Phe, detection limits were two orders of magnitude lower with the TiO2 electron transfer photocatalyst compared to a system without TiO2. It is expected that many aromatic substances (especially phenols) would behave similarly.

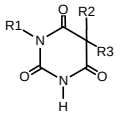
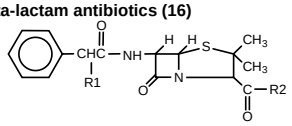
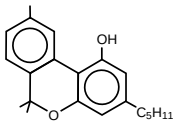
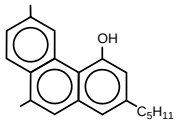
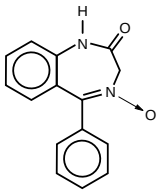
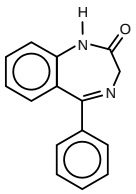
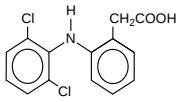
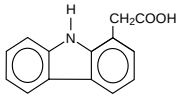
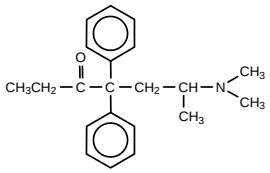
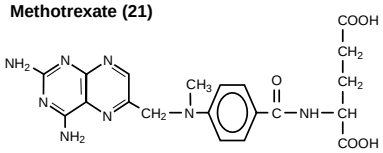
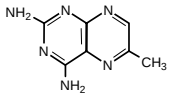
Recently Kissinger and coworkers explored the use of photochemical reaction detection to the determination of 3-nitrotyrosine, by converting it to the easily oxidized L-dopa (35,36). The purpose of these experiments was to optimize determination of nitrotyrosine in biological materials as a potential marker for peroxynitrite (-OONO), a putative candidate for cytotoxicity related to nitric oxide (NO).

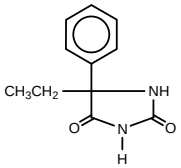
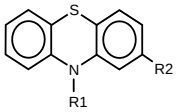
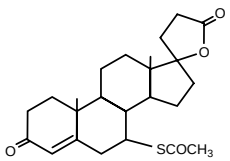
$$NO + O_2^- \longrightarrow {}^-OONO \longrightarrow NO_3^-$$

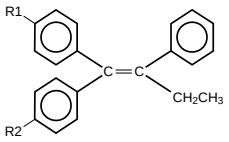
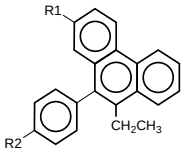
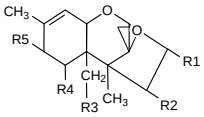
Peroxynitrite reportedly has a half-life in solution of about 1 s. Its role (if any) in biology is difficult to ascertain. An excellent short review by Fukuto and Ignarro summarizes this chemistry (37). Peroxynitrite

T1

Representative published applications of photochemical reactors coupled to LC.

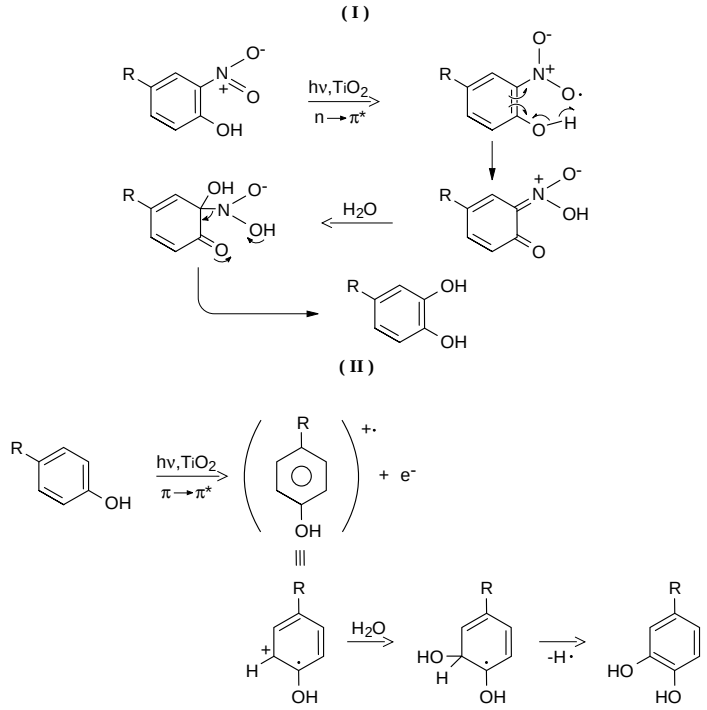
Analyte/Detectable Product(s)	Detection Mode	Conditions
Barbiturates (15)  No products determined	Electrochemistry Dual parallel glassy carbon working electrodes W1 = +1,100 mV W2 = +850 mV	40% MeOH 60% 0.2 M NaCl pH 7.0 Optimized Residence time: 2.4 min. Optimized
Beta-lactam antibiotics (16)  No products determined	Electrochemistry Glassy carbon working electrode +1.10 V vs. Ag/AgCl	Gradient: 35% to 65% MeOH: 0.2 M NaCl in H_20 Optimized for background noise. Residence time: not reported. Optimized
Cannabinol (17)  Suspected product 	Fluorescence Ex 258 nm Em 362 nm	60% isooctane 40% dioxane Not optimized Residence time: 1 min. Optimized
Demoxepam (18)  Suspected product 	Fluorescence Ex 380 nm Em 460 nm	50% MeOH: 50% 0.05 M sodium phosphate pH 8.0 Optimized for pH Residence time: 100 sec. Optimized
Diclofenac (19)  Suspected product 	Fluorescence Ex 288 nm Em 360 nm	0.06 M sodium phosphate pH 6.6 32% (v:v) ACN. Not Optimized Residence time: 2 min. Optimized
Methadone (20)  No product determined	Ultraviolet 254 nm	55% ACN 35% MeOH 10% ammonium nitrate pH 9.5 Not Optimized Residence time: 65 sec. Not Optimized
Methotrexate (21)  Suspected product 	Fluorescence Ex 370 nm Em 417 nm	500 mL 0.05 M sodium phosphate pH 6.2 35 mL ACN 28 mL DMF 0.75 mL 30% H_2O_2 Optimized for organic content, pH, ionic strength, and H_2O_2 concentration. Residence time: 3.2 sec. Optimized

Analyte/Detectable Product(s)	Detection Mode	Conditions
Nirvanol (20)  No products determined	Ultraviolet 254 nm	30% ACN 70% 20 mM sodium phosphate pH 7 Not Optimized Resident time: 75 sec. Not Optimized
Nitrate (22) NO_3^- Suspected product NO_2^-	Electrochemistry Dual parallel glassy carbon working electrodes W1 = +1,100 mV W2 = +1,000 mV vs. Ag/AgCl	10% MeOH 90% 0.050 M sodium phosphate pH 6.8 0.005 M TBAHS Optimized for pH Residence time: 2.8 min. Optimized
Organobromides (23) R-C-Br Suspected Products $R\text{-}C^+ + Br^-$	Electrochemistry Dual parallel silver working electrodes W1 = +250 mV W2 = +200 mV vs. Ag/AgCl	55% MeOH 45% 0.1 M NaOAc pH 5 Optimized Residence time: 5 min. Not Optimized
Organochlorides (23) Ph-Cl Suspected Products $Ph+ + Cl^-$	Electrochemistry Dual parallel silver working electrodes W1 = +350 mV W2 = +300 mV vs. Ag/AgCl	55% MeOH 45% 0.2 M NaOAc pH 5 Optimized Residence time: 5 min. Not Optimized
Organoiodides (24) R-C-I Suspected product $R\text{-}C^+ + I^-$	Electrochemistry Dual parallel glassy carbon working electrodes W1 = +1,000 mV W2 = +850 mV vs. Ag/AgCl	40% MeOH 60% 0.2 M NaCl Not Optimized Residence time: 1.8 min. Optimized
Phenothiazines (18)  No products determined	Fluorescence Ex 380 nm Em 460 nm	50% MeOH 50% 0.05 M sodium phosphate pH 8.0, Not Optimized Residence time: 100 sec. Not Optimized
Spirolactone (25)  Suspected product - $HSCOCH_3$	Fluorescence Ex 288 nm Em 360 nm	0.06 M sodium phosphate pH 6.6 32% (v:v) ACN Not Optimized Residence time: 2 min. Optimized

Analyte/Detectable Product(s)	Detection Mode	Conditions
Tamoxifens (26)  Suspected product 	Fluorescence Ex 265 nm Em 340 nm	100 g H_2O 1,000 g MeOH 15 g propionic acid 20 g 1% ammonia Not Optimized Residence time: 1.89 sec. Not Optimized
Tricothecenes (27)  No products determined	Electrochemistry Dual parallel glassy carbon working electrodes W1 = +1,100 mV, W2 = +850 mV vs. Ag/AgCl	15% MeOH 85% 0.05 M NaCl Optimized Residence time: 2.4 min. Optimized

F3

Mechanism for production of a catechol from a nitrophenol (I) and from a phenol (II).



can nitrate tyrosine and perhaps other aromatics such as γ-tocopherol as reported by Christen et al. (38). Phenols and nitrophenols are interesting candidates for photochemical reactions of analytical value. Nitro compounds have long been studied in this manner and shown to produce nitrite which is electrochemically oxidizable (2). Phenols can generate catechols and p-hydroquinones and aromatic nitrophenols can do so with even greater facility. The classical photochemistry is depicted in ***F3*** where [I] depicts the generation of a catechol from a nitrophenol and [II] shows the less facile conversion of a phenol to a catechol.

While photochemical reactions are analytically advantageous in numerous unique situations, the most practical examples are those where fluorescence has been enhanced due to an extension of conjugation or electrochemical detection has been enhanced via hydroxylation of an aromatic ring. Post-column photolysis can also be useful in a qualitative sense. Comparison of "lamp on" and "lamp off" chromatography is often quite useful in characterizing a sample. While not the subject of this brief review, one can also explore photodegradation of compounds by placing the reactor coil in front of the LC injection valve and examining chromatograms as a function of illuminating the sample for various times. The photo products are themselves chromatographed and can potentially be identified by LC/MS, diode array absorbance detection, and/or voltammetry. Precolumn photolysis is also diagnostically useful in understanding the mechanism of a post-column detection scheme and in optimizing conditions as a preliminary method is validated.

Acknowledgments

Peter Kissinger is indebted to Prof. Harry Morrison of Purdue University for helpful photochemical advice over the years and to Prof. Adam Heller of the University of Texas for enlightening discussions of the form and function of TiO_2.

PhotoBlaster and LuxTube are trademarks of Agrenetics.

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