



## Review article

## A review of post-column photochemical reaction systems coupled to electrochemical detection in HPLC

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## ABSTRACT

Post-column photochemical reaction systems have developed into a common approach for enhancing conventional methods of detection in HPLC. Photochemical reactions as a means of 'derivatization' have a significant number of advantages over chemical reaction-based methods, and a significant effort has been demonstrated to develop an efficient photochemical reactor. When coupled to electrochemical (EC) detection, the technique allows for the sensitive and selective determination of a variety of compounds (e.g., organic nitro explosives, beta-lactam antibiotics, sulfur-containing antibiotics, pesticides and insecticides). This review will focus on developments and methods using post-column photochemical reaction systems followed by EC detection in liquid chromatography. Papers are presented in chronological order to emphasize the evolution of the approach and continued importance of the application.

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## Contents

|   |   |
|---|---|
| 1. Introduction.....  | 1 |
| 2. Photochemical reaction systems.....                                      | 2 |
| 3. Photochemical reaction systems coupled to electrochemical detection..... | 3 |
| 4. Conclusion.....  | 7 |
| References .....  | 7 |

## 1. Introduction

Over the past three decades, post-column photochemical reaction systems in HPLC have progressed to become a common approach in executing analyses that otherwise may have been recognized as impracticable or difficult to accomplish. Altering the detection attributes of analytes, which exhibit poor optical and electrochemical detection properties, as a result of photolysis has significantly expanded the range of applications ordinarily achieved by conventional detection techniques.

Post-column chemistries which rely on the irradiation of matter have evolved into a highly applicable area of post-column reaction systems. Whether the photochemical reaction results in the degradation of an analyte into smaller molecules (photolysis) or the further reaction of smaller molecules with water (photohy-

drolisis), the improvements often sought after are in terms of sensitivity and/or selectivity of detection [1]. From the analytical chemist's perspective, the involvement of post-column photochemical reactions in quantitative analyses may be categorized as a form of derivatization (i.e., altering the chemical structure of the analyte). In comparison to chemical derivatization, post-column photochemical reactions offer several advantages, which also function to simplify the adaptation of photochemical reactors into the chromatographic system. These advantages are as follows:

- Since the primary reagent, photons, is generated via controllable electronic equipment at the time of use, the preparation and storage of chemicals is unnecessary. Also, the issues of reagent degradation, shelf-life, and disposal do not exist.
- Photons can be delivered at various intensities and wavelengths to enhance rates and specificity, respectively.
- The primary reagent, photons, can be delivered without the use of an additional pump or mixing tee [2–4]. This serves to significantly reduce any incompatibility observed with the mixing of

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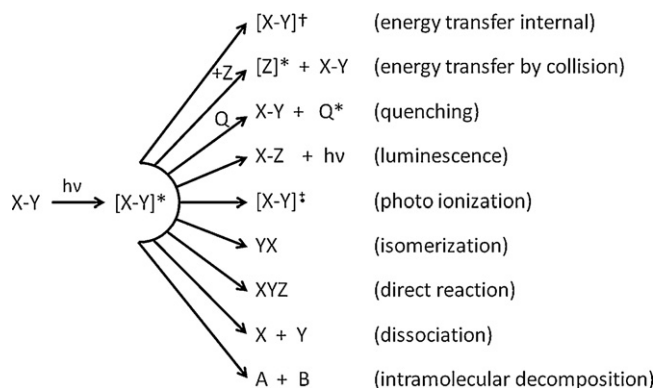


Fig. 1. A general classification of photochemical reactions.

solvents as well as to minimize the formation of artifacts that may potentially interfere with detection.

- Post-column photochemical reaction systems allow for the separation of analytes based on their original structure, which may simplify method development or conversion of existing methods.
- Post-column photochemical reaction systems eliminate the need for reaction completion, as long as the reaction is deemed reproducible.

Furthermore, the development of sophisticated post-column reactors has facilitated the incorporation of photochemical reaction systems into high performance liquid chromatography (HPLC), which allows for its use in routine analysis.

This paper will focus on reviewing post-column photochemical reaction systems with an emphasis on photolysis coupled to EC detection. Special attention will be given to the development of photochemical reactors used in combination with conventional detection techniques of HPLC. In addition, applications of significant advances of photochemical reaction systems with EC detection will be reviewed.

## 2. Photochemical reaction systems

The fundamental purpose of incorporating post-column photochemical reactors into a method of detection is to convert the starting analyte to a product or collection of products, which have significantly improved detection properties by fluorescence (FL), ultraviolet (UV), EC detection, etc. The unique aspects of photochemistry provide the foundation for a series of reactions (e.g., photolysis, photohydrolysis, intramolecular rearrangements, photodimerization, photoionization and/or electron transfer reactions), some of which are depicted in Fig. 1 [5]. Most commonly, post-column photolysis reactions are exploited in EC; where the analyte undergoes dissociation to form electroactive entities. For example, organic nitro compounds produce the nitrite anion, which is then oxidized at a glassy carbon electrode to form nitrate. Modification of the analyte's chemical structure is specific to the type of reaction the analyte is able to undergo under the conditions of the mobile phase. As a consequence when in comparison to the constituents of the sample matrix, this effect typically results in enhanced specificity and selectivity for the analyte and, frequently, increased sensitivity.

The development of photochemical reaction systems has resulted in three distinctive reactor designs, which differ primarily based on reaction kinetics [6]. Open tubular reactors (OTR) were considered to be the simplest type of photochemical reactor available consisting of a coiled or loosely knitted piece of tubing surrounding a light source. Initially, the use of photochemical reaction systems with loosely coiled tubing had contributed to a signifi-

cant decrease in resolution as a result of an additional reactor in an on-line system. It was determined that the band broadening of analytes as a result of the additional length of tubing inherent of the photochemical reactor is significantly minimized with crocheted or knitted tubing in comparison to loosely coiled tubing in the photochemical reactor as reported by Birks et al. (Fig. 2) [3]. A knitted open tubular (KOT) reactor coil in the photolysis chamber introduces secondary flow of the fluid passing through the coiled tubing serving to minimize dispersion of the analyte, thus decreasing the dispersion of analyte bands. Reactors which consist of knitted or crocheted open tubing have also been referred to as deformed open tubular reactors (DOTR) in the literature. Such reactors have been determined to be best suitable for reactions taking place in 30 s or less. Packed-bed reactors (PBR) were considered to be those reactors which contained a column packed with inert, non-retenting beads intended to maximize mixing and minimize peak broadening for reactions with residence times of 0.5–4 min. PBRs were recognized to be problematic as photochemical reactors due to the difficulty in irradiating packed-bed columns. Segmented stream reactors (SSR) were developed as an alternative type of photochemical reactor used for incorporating slower reactions (up to 20 min). SSRs were based on segmenting eluent with either air or other gas bubbles serving to strongly diminish the dispersion of analyte while also enabling extended residence times for slow reactions to occur. Published reports indicate that the majority of photochemical reactors currently in use are considered to be OTR or DOTR.

The majority of reactors used for post-column photolysis consist of a medium to high-powered mercury or xenon–mercury light source, polytetrafluoroethylene (PTFE) reaction coil and housing for temperature control. The housing also serves to protect users from UV radiation. In general, photochemical reactions used for the 'derivatization' of organic compounds require light in the 200–400 nm range. The intensity of the light source used governs the reaction time necessary for optimum sensitivity to be achieved for the desired analysis. This fact accounts for the frequent use of high or medium pressure mercury or mercury xenon arc lamps, which result in shorter reaction times for the analytes due to their

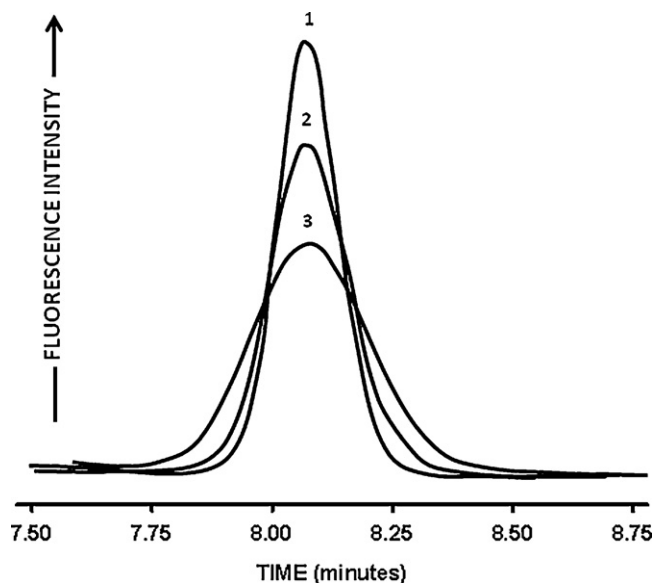


Fig. 2. Reduced band broadening as a result of crocheted tubing used in post-column reactors. Phenanthrene was eluted from a Zorbax (Dupont) C18 column with methanol:water (95:5) at a flow rate of  $0.76 \text{ mL min}^{-1}$ . Fluorescence of phenanthrene was monitored with a Kratos FS-970 Fluorometer ( $\lambda_{\text{ex}} = 280$ ,  $\lambda_{\text{em}} > 389 \text{ nm}$ ). (1) Direct coupling to the fluorometer. (2) Crocheted reactor with a volume of 2.36 mL. (3) Coiled reactor with a volume of 2.17 mL. Adapted from reference [5].

high intensities. Other light sources (e.g., deuterium and hydrogen lamps, incandescent lamps, low-pressure metal vapor lamps) may be applied for analyses which require longer irradiation wavelengths or longer reaction times in the photochemical reactor. Over the years, PTFE tubing has been the primary material used for photochemical reactor instrumentation. The inherent durability of PTFE tubing has made it applicable for use with a variety of reagents at a relatively inexpensive price. In addition, the flexibility of PTFE enables the tubing to be efficiently coiled, which has been demonstrated to be effective in minimizing additional band broadening [7,8]. Furthermore, a light-tube effect based on the diffusion of radiation transfer and internal reflectance serves to increase effective photon flux within the photochemical reactor [9]. This has led PTFE tubing to be an excellent means of introducing irradiation to the analyte despite the fact that the direct transmission of ultraviolet light by PTFE is considered to be inefficient.

Research in the development of photochemical reactors in the early 1980s initiated several attempts to improve sensitivity and efficiency of reaction detectors executing photolysis [8,9]. The optimization of irradiation times, knitted geometries and lamp intensities had been investigated by several research groups [9–11]. Chemometric techniques such as multivariate optimization using factorial experimental design to assess the effects of mobile phase pH and UV irradiation time have been used to optimize response in photochemical reaction systems coupled to EC [12]. The use of titanium dioxide ( $\text{TiO}_2$ ) as a photo-catalyst with a low temperature UV lamp in order to achieve improvement in product yield and rate of product formation has been demonstrated more recently [13–15]. Several helical tubing geometries with various coil diameters have also been investigated for the purpose of further minimizing band broadening in order to subsequently increase sensitivity [11]. As a consequence of both public demand and advances in reactor development, several photochemical reactors have become commercially available for purchase. Published reports have indicated the PHRED<sup>TM</sup> (Aura Industries Inc., New York, NY), Beam-Boost<sup>TM</sup> (ICT, Frankfurt, Germany), and Photoblaster<sup>TM</sup> (Agrenetics, Wilmington, MA) reactors as those most frequently incorporated in on-line automated systems [16]. In general, these photochemical reactors are equipped with low-pressure mercury lamps and knitted PTFE tubing coils of 0.33 mm internal diameter.

The first reported use of a photochemical reaction system was in 1976 by Iwaoka and Tannenbaum [17], who applied post-column photolysis following liquid chromatography to the analysis of N-nitrosamines. They utilized a photochemical reactor consisting of Pyrex glass capillary tubing and residence times which spanned over several minutes resulting in significant band broadening. Analytes were subjected to photolysis to produce the end product nitrite for subsequent colorimetric analysis by the Griess reaction. Hansen et al. [18] also applied this method for the determination of N-nitrosoproline in bacon. Following in 1983, Shuker and Tannenbaum [19] developed a modified photohydrolysis detector for use with colorimetric detection using the Griess reagent following reversed phase chromatography. Light from a high-intensity discharge metal halide lamp irradiated N-nitroso analytes in aqueous solution to produce nitrite. The photohydrolysis detector consisted of tubing wound around a water jacket which was cooled with tap water designed to prevent the tubing and light source from overheating. They also applied this system to the determination of nonvolatile N-nitroso compounds in biological fluids, but they reported a notable loss of resolution with the photohydrolysis detector. More recently, Bellec et al. [20] applied photohydrolysis using a KOT reactor with colorimetric detection by the Griess reagent for the determination of N-nitrosamines in gastric juice and alcoholic beverages.

The number of applications of photochemical reaction systems for quantitative analysis has significantly grown over the past three

decades. The development of efficient and sensitive photochemical reactors has enabled analysts to incorporate the reaction scheme into routine and automated analysis systems. As a result, conventional detection methods for HPLC such as FL, UV, and EC techniques have experienced an increase in the range of analytes for which may now be routinely determined. A selected number of recent applications incorporating photolysis coupled with FL, UV and chemiluminescence (CL) detection following HPLC are reviewed in Table 1 [21–33]. The remaining portion of this review paper will focus on the development of photochemical reaction systems coupled to EC detection and a thorough review of relevant applications.

### 3. Photochemical reaction systems coupled to electrochemical detection

Research into photochemical reaction systems quickly resulted in photolytic derivatization techniques being coupled to EC detection in liquid chromatography. A variety of techniques including both pre-column and post-column derivatizations, as well on-line and off-line methods, have been utilized over the years. Post-column photochemical reaction systems coupled to EC detection have been reported for use in the areas of environmental, toxicological and pharmaceutical applications.

It was the work of Snider and Johnson [34] which first revealed the application of photolysis to N-nitrosamines in order to generate nitrite for EC detection at a platinum working electrode. Referred to as a photo-electroanalyzer, the photochemical apparatus consisted of a photolysis cell constructed of quartz tubing coiled at 1.5 in diameter surrounding a 500-W, high pressure xenon arc lamp in combination with two chromatographic columns and an amperometric detector. They investigated the possibility of organic nitro compounds as electroactive photoproducts and suggested that the compounds were potential interferents in the analysis.

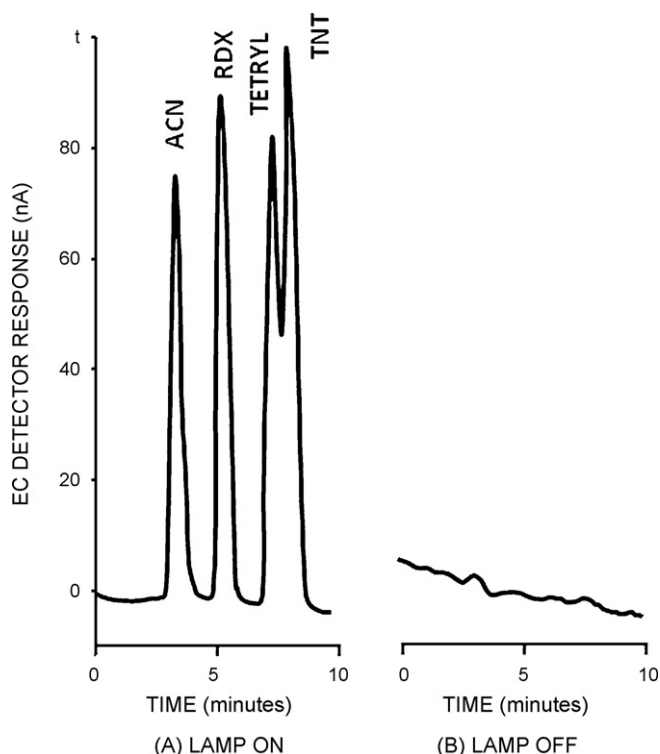
Krull et al. [35] applied photolysis coupled to oxidative EC detection following HPLC, or HPLC-hv-EC, to the determination of organic nitro compounds. This work included a variety of organic nitro compounds used as explosives, including alkyl nitrate esters ( $\text{R-ONO}_2$ ), aromatic nitro ( $\text{C-NO}_2$ ), aliphatic nitro ( $\text{C-NO}_2$ ), cycloaliphatic N-nitro (N-nitramines), and related nitro derivatives. Nitrite, determined to be released from parent nitro compounds by photolysis, was recognized to be readily detected by oxidative EC detection on-line. The use of a post-column photochemical reactor in this manner offered additional selectivity by means of comparing 'lamp on' vs. 'lamp off' chromatograms. Fig. 3 shows HPLC-hv-EC single-electrode chromatograms of three organic nitro explosives standards (i.e., royal demolition explosive, RDX; 2,4,6-trinitrotoluene, TNT; methyl-2,4,6-trinitrophenylnitramine, TETRYL) with the photolysis lamp on/lamp off approach. The photolysis apparatus applied by Krull and co-workers consisted of a mercury discharge lamp with Teflon<sup>TM</sup> tubing in a basic wrap around configuration held in an ice-water bath. A schematic diagram of the on-line photochemical reactor in a LC-EC system setup used by Krull and co-workers is shown in Fig. 4. Prior to this work, organic nitro compounds had only been studied by EC detection in the reductive mode due to their inability to be oxidized directly. Interference of dissolved oxygen when using EC in the reductive mode made this approach difficult to apply in a routine fashion. Hence, HPLC-hv-EC was considered to be a major advancement for the analysis of organic nitro compounds.

The photolytic apparatus used by the Krull co-workers was further applied to the determination of organothiophosphate agricultural chemicals [36], as well as pharmaceuticals such as phenobarbital, cocaine, and methylphenidate [37,38]. Initial work performed by Krull utilized a simple wraparound configuration of Teflon<sup>TM</sup> tubing surrounding the light source, resulting in severe

**Table 1**

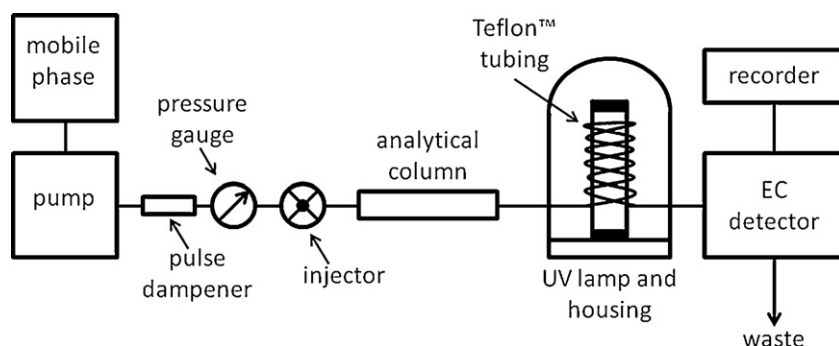
A selected number of recent applications of photolysis coupled with fluorescence (FL), ultraviolet (UV) and chemiluminescence (CL) detection following HPLC.

| Detection | Compounds                            | Application                             | Year | Reference |
|-----------|--------------------------------------|---|------|-----------|
| CL        | Aromatic amines                      | Amino acids                             | 1998 | [21]      |
|           | Chloramphenicol, antibiotic          | Pharmaceutical formulations             | 2000 | [22]      |
|           | Citric, oxalic, malic tartaric acids | Beverages                               | 2004 | [23]      |
|           | N-nitrosamines                       | Groundwater                             | 2005 | [24]      |
|           | N-methylcarbamate pesticides         | Water, fruits                           | 2007 | [25]      |
| UV        | Naphthodianthrones                   | Phytopharmaceutical extracts            | 2003 | [26]      |
|           | Busulfan                             | Plasma, water                           | 2004 | [27]      |
| FL        | Sulindac, metabolites                | Serum                                   | 1995 | [28]      |
|           | Rofecoxib                            | Plasma                                  | 1999 | [29]      |
|           | Alfatoxins                           | Pistachios, peanuts, fig                | 2002 | [30]      |
|           | Alfatoxins                           | Agricultural commodities                | 2002 | [31]      |
|           | Organophosphorus pesticides          | Environmental water, vegetables, grains | 2005 | [32]      |
|           | Alfatoxins                           | Pistachios                              | 2009 | [33]      |

**Fig. 3.** Photolysis coupled to electrochemical detection following HPLC (single-electrode chromatograms) of three explosives: RDX, TNT and TETRYL standards using a RP-C8 (10  $\mu$ m, 25 cm  $\times$  4.1 mm ID) column with 50:50 (methanol:0.1 M NaCl) mobile phase at 1.4 mL min<sup>-1</sup>, glassy carbon electrode at +1.0 V. (A) Photolysis lamp on (B) photolysis lamp off. Adapted from reference [35].

band broadening and peak distortion. Through the use of a KOT reactor coil in the photolysis chamber, Selavka and Krull [39] were able to significantly improve sensitivity and selectivity resulting in a substantial increase in resolution for the work in determining nitro-based high explosives and water gel formulation sensitizers in 1986. As a result of these developments, Righezza et al. [40] applied the improved post-column photolysis technology with EC detection following HPLC for the determination of N-nitrosamines.

Photochemical reaction systems coupled to EC detection following HPLC have further been applied to several pharmaceutical drugs, food additives and for the forensic determination of abusive drugs [41]. Selavka et al. [42] applied this continuous photolytic derivatization technique to several penicillin derivatives and cephalosporin prior to EC detection in the oxidative mode. They separated the beta-lactam derivatives by conventional reversed phase chromatography and then photolytically degraded the antibiotics to a stable anionic species, presumed at the time. Signal response ratios determined for the beta-lactam derivatives and cefoperazone under both lamp on and lamp off conditions revealed that penicillin derivatives experienced no electroactivity at oxidative potentials without exposure to UV irradiation. A pre-column chemical derivatization method was also investigated as a means to improve chromatographic and detection properties for amino alcohols and amino acids by photolysis coupled EC detection [43]. Over the next several years, Krull and co-workers continued to expand the number of applications for photolysis coupled with EC detection by exploring degradation patterns of several analytes of interest [44–47]. Specifically, organoiodide, organobromide, and organochloride compounds were investigated. Cyclic voltammetry coupled to a photolysis arrangement as a mechanistic tool suggested that the EC response was a result of the fragmented halogen counterpart [48]. In the early 1990s, Dou and Krull [49,50] described the use of photolysis with EC detection for the determination of

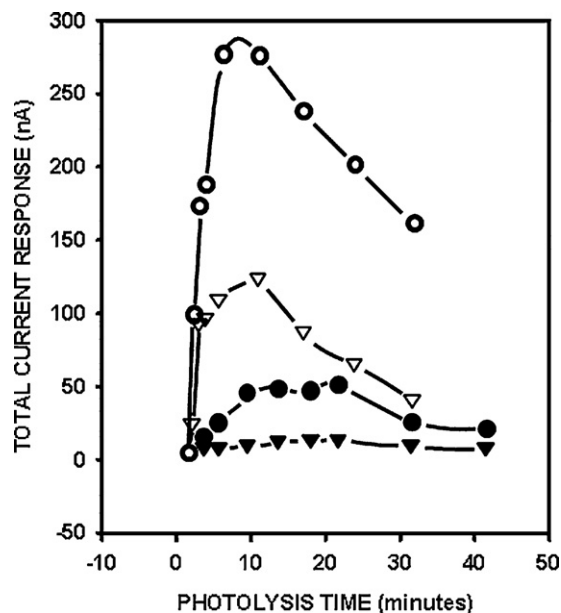
**Fig. 4.** Schematic diagram of a HPLC-photolysis-electrochemical detection system. Adapted from reference [36].



aromatic and sulfur-containing amino acids, peptides and proteins following HPLC. The Krull co-workers [51,52] also described the possibility of peptide mapping for peptides containing nonelectroactive amino acids by the use of UV irradiation to generate electroactive species. Dou and Krull [53] also applied the technique for phenylalanine determination in human urine as a method of monitoring the amino acid in phenylketonuria (PKU) patients and for the determination of inorganic anions such as dichromate and chromate [54].

As the availability of commercial photochemical reactors increased, the number of publications began to dramatically increase as well. Bachman and Stewart [55] applied photolysis coupled to oxidative electrochemical detection for a large assortment of cardiovascular drugs, following the method originally proposed by Krull and co-workers in the mid 1980s. Childress et al. [56] successfully applied photolysis coupled to electrochemical detection for the determination of deoxynivalenol, a naturally occurring toxic fungal metabolite found in grains such as wheat, corn, rye and barley. The determination of aspicillin by Yamazaki et al. [57] was applied for a variety of body fluids yet to be fully investigated by Krull in recent reports at the time. Ofner and Wintersteiger [58] attempted to minimize possible plasma interferences by photolysis with EC detection using chlordiazepoxide as a model substance. This resulted in the minimization of interferences and allowed for the determination of chlordiazepoxide at the nanogram level. Subsequently in 1995, Macher and Wintersteiger [59] applied photolysis following EC detection for the determination of diuretics, specifically, hydrochlorothiazide, butizide, bendroflumethiazide, chlortalidone, furosemide, and etacrynic acid. Wintersteiger et al. [60] also investigated the influence of photolysis coupled to EC detection for chlorophenoxy acid herbicides in comparison to their detection directly at oxidative potentials. Studies revealed that lower background currents and a more stable baseline ensued due to the use of photolysis coupled with EC detection resulting in superior detection limits. Lihl et al. [61] incorporated an on-line automated pre-column exchange system coupled to a photolytic EC detection system for a range of penicillin derivatives in bovine muscle tissue. This approach served to increase ease in sample handling as well as selectivity and sensitivity for the analysis. Within the same year, Galletti and Bocchini [62] applied photolysis coupled to oxidative EC detection for the determination of aspartame, an artificial sweetener commonly used in diet colas and pharmaceutical products.

After more than a decade of a lack of significant changes to the photolysis apparatus initially used by Krull and co-workers, the optimization of post-column photolysis coupled to EC detection was explored by Kissinger and co-workers. The use of  $\text{TiO}_2$  as a photo-catalyst, which coats the inside of the KOT reaction coil of the photochemical reactor, was suggested to improve the yield and rate of product formation [14]. In addition, different photolysis lamps and reactor tubing lengths were evaluated in terms of photolysis efficiency [13]. The comparison of the  $\text{TiO}_2$  coated photoreactor and unmodified photoreactor was evaluated by monitoring the sum of electroactive photolysis products of phenylalanine in water as a function of photolysis time in both reactors. Fig. 5 presents the results of current response at two separate applied potentials for both reactors. The rate of which products were formed, as well as current response, were observed to be highest for the  $\text{TiO}_2$  coated photoreactor. Subsequently, Kissinger and co-workers [15] compared UV, oxidative EC and post-column photolysis with EC detection for the determination of 3-nitro-L-tyrosine in biological matrices. Results from the comparison studies revealed lower limits of detection in addition to a more selective analysis of 3-nitro-L-tyrosine using post-column photolysis with  $\text{TiO}_2$  coated knitted reaction coil coupled to EC detection following HPLC. Improvements with regards to the photolysis system were imple-



**Fig. 5.** Comparison of the photo-catalytic activity of a p25  $\text{TiO}_2$  coated Teflon reactor and an open-tubular Teflon reactor: (○) p25  $\text{TiO}_2$  coated Teflon reactor at an applied potential of +1000 mV vs. Ag/AgCl; (●) open-tubular Teflon reactor at an applied potential of +1000 mV vs. Ag/AgCl; (▽) p25  $\text{TiO}_2$  coated Teflon reactor at an applied potential of +600 mV vs. Ag/AgCl; (▼) open-tubular Teflon reactor at an applied potential of +600 mV vs. Ag/AgCl. Each data point represents the sum of the peak currents of the detectable products at the photolysis time. Adapted from reference [14].

mented specifically for the analysis of 3-nitro-L-tyrosine and have not been optimized for other applications.

Within the past several years, the range of compounds applicable with regards to photochemical reaction systems coupled to EC detection has progressively increased. Bocchini et al. [63] compared photolysis following EC detection with regards to UV detection following HPLC for the determination of diallyl thiosulfinate (allicin) in garlic. Detection limits were reported to be an order of magnitude lower for EC detection following photolysis in comparison to UV detection. Wu et al. [64] utilized post-column photolysis coupled with EC detection for the analysis of alkyl organoiodide mixtures for the purpose of evaluating the purity of acetic acid products. In 2003, Schulte-Ladbeck and Karst [65] developed a post-column UV irradiation EC detection method for the quantitative trace analysis of several peroxide-based explosives. Rancan et al. [66] described a procedure for the determination of imidacloprid, a frequently used insecticide, and its metabolites using photolysis with EC detection following HPLC. Following this, they applied photolysis with EC detection for the determination of thiamethoxam residues in honeybees [67]. Goger et al. [68] compared photolysis coupled with EC detection with EC detection alone to the determination of phenylurea pesticides. It was reported that the electroactivity of several pesticides was significantly influenced by irradiation with UV light, resulting in a decrease in signal intensity.

Recently, Marple and LaCourse [69,70] introduced an automated on-line, solid phase extraction (SPE) unit coupled to HPLC-UV followed by photolysis with EC detection, which they called photo-assisted electrochemical detection (PAED), for the analysis of 14 nitro explosives. Marple and LaCourse's method was implemented as an enhanced version of Method 8330 of the U.S. Environmental Protection Agency (EPA). A comparison of limits of detection (LOD) determined for all 14 nitro explosives by PAED and UV detection to risk-based target levels is presented in Table 2 [69]. In addition to confirmation of the analyte's identity using EC/UV response ratios, the PAED approach offered higher sensitivity, especially for

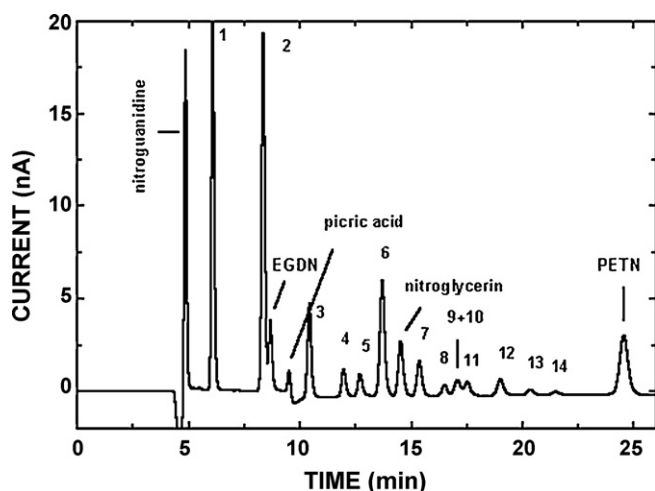
**Table 2**

A comparison of limits of detection of 14 nitro-based explosives determined by PAED and UV detection following HPLC to risk-based target levels. High melting explosive (HMX), royal demolition explosive (RDX), 1,3,5-trinitrobenzene (1,3,5-TNB), 1,3-dinitrobenzene (1,3-DNB), nitrobenzene (NB), methyl-2,4,6-trinitrophenylnitramine (TETRYL), 2,4,6-trinitrotoluene (TNT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 2,6-dinitrotoluene (2,6-DNT), 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 2,4-dinitrotoluene (2,4-DNT), 2-nitrotoluene (2-NT), 4-nitrotoluene (4-NT), 3-nitrotoluene (3-NT). Adapted from reference [69].

| Explosive   | Risk-based target level ( $\mu\text{g mL}^{-1}$ ) <sup>a</sup> | Limit of detection ( $\mu\text{g mL}^{-1}$ ) |     |       |                     |        |                            |       |
|-------------|--|--|-----|-------|---------------------|--------|----------------------------|-------|
|             |  | No precon                                    |     |       | Precon <sup>b</sup> |        | Precon <sup>b</sup> matrix |       |
|             |  | 8330 <sup>a</sup>                            | UV  | PAED  | UV                  | PAED   | UV                         | PAED  |
| HMX         | 1800   | 2  | 2   | 0.007 | 0.04                | 0.0007 | 0.2                        | 0.004 |
| RDX         | 0.6  | 0.6  | 2   | 0.02  | 0.1                 | 0.002  | 0.6                        | 0.006 |
| 1,3,5-TNB   | 1.8  | 1  | 1   | 0.06  | 0.07                | 0.008  | 0.3                        | 0.05  |
| 1,3-DNB     | 2.36   | 0.9  | 1   | 0.5   | 0.06                | 0.03   | 0.1                        | 0.07  |
| NB          | 15.2   | 1.5  | 0.9 | 0.3   | 0.05                | 0.04   | 0.2                        | 0.02  |
| Tetryl      | 370  | 1.2  | 2   | 0.03  | 0.2                 | 0.007  | 0.1                        | 0.02  |
| 2,4,6-TNT   | 2.2  | 1.6  | 2   | 0.1   | 0.09                | 0.02   | 0.2                        | 0.06  |
| 2-A-4,6-DNT | –  | 1.6  | 2   | 1     | 0.3                 | 0.3    | 0.5                        | 0.3   |
| 4-A-2,6-DNT | –  | 0.9  | 2   | 0.8   | 0.3                 | 0.2    | 0.5                        | 0.2   |
| 2,4-DNT     | 37   | 2  | 0.8 | 0.8   | 0.2                 | 0.1    | 0.2                        | 0.1   |
| 2,6-DNT     | 60.8   | 1.2  | 0.8 | 0.8   | 0.2                 | 0.1    | 0.2                        | 0.2   |
| 2-NT        | 2.2  | 2  | 3   | 0.3   | 0.3                 | 0.1    | 1                          | 0.08  |
| 3-NT        | 370  | 2  | 4   | 1     | 0.4                 | 0.2    | 2                          | 0.2   |
| 4-NT        | 370  | 2  | 5   | 3     | 0.4                 | 0.4    | 2                          | 0.2   |

<sup>a</sup> From Method 8330.

<sup>b</sup> 2 mL sample injection.



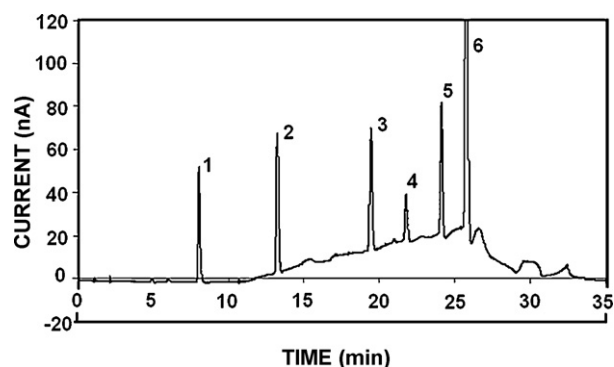
**Fig. 6.** Optimized separation of explosives using PAED following HPLC-UV. Conditions: mobile phase: 50% methanol in 20 mM acetate buffer, pH 4.5; flow rate: 1.0 mL min<sup>-1</sup>; guard column: Phenomenex SecurityGuard with 4 mm × 3.0 mm C8 cartridge; column: C18, 5  $\mu\text{m}$ , 4.6 mm × 250 mm; column oven temperature: 30 °C; electrode: 1.0 mm glassy carbon; reference electrode: Ag/AgCl; applied potential: 1.0 V vs. Ag/AgCl. (1) High melting explosive (HMX), (2) royal demolition explosive (RDX), (3) 1,3,5-trinitrobenzene (1,3,5-TNB), (4) 1,3-dinitrobenzene (1,3-DNB), (5) nitrobenzene (NB), (6) methyl-2,4,6-trinitrophenylnitramine (TETRYL), (7) 2,4,6-trinitrotoluene (TNT), (8) 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), (9) 2,6-dinitrotoluene (2,6-DNT), (10) 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), (11) 2,4-dinitrotoluene (2,4-DNT), (12) 2-nitrotoluene (2-NT), (13) 4-nitrotoluene (4-NT), (14) 3-nitrotoluene (3-NT) [71].

**Table 3**

Limits of detection of additional organo nitro explosives by PAED and UV detection. Pentaerythritol (PETN), nitroglycerin, ethylene glycol dinitramine (EGDN), nitroguanidine, picric acid. Adapted from reference [71].

| Explosive      | Limit of detection ( $\mu\text{g L}^{-1}$ ) |           |      |
|----------------|---|-----------|------|
|                | UV-254 nm                                   | UV-220 nm | PAED |
| Nitroguanidine | 0.2   | –         | 0.02 |
| EGDN           | –   | 40        | 0.2  |
| Picric acid    | 2   | 3         | 0.4  |
| Nitroglycerin  | –   | 50        | 0.3  |
| PETN           | –   | 60        | 0.3  |

nitramines, and enhanced selectivity. Earlier research in the 1980s was deterred by the loss of signal when assaying 'real-world' environmental samples. The use of automated on-line SPE overcomes this problem with the additional benefits of preconcentrating the analytes of interest and eliminating manual sample preparation procedures. Detection limits for HMX, RDX, and tetryl using a 2 mL injection with on-line SPE and PAED were 4, 6, and 20 ng L<sup>-1</sup> in sample matrix, see Table 2. Marple and LaCourse [71] applied PAED to the determination of nitro-containing explosives in groundwater and soil samples. Additional nitro explosives such as pentaerythritol (PETN), nitroglycerin, ethylene glycol dinitramine (EGDN), nitroguanidine and picric acid were incorporated into the analysis by PAED following HPLC-UV without any modification of their originally proposed method. Fig. 6 shows a chromatogram of a mixture of 19 explosives, which highlights the increased sensitivity achieved by PAED for early eluting nitramines high melting explosive (HMX) and RDX, as well as other nitro explosives such as TETRYL, TNT, and nitroglycerin. A summary of detection limits for additional nitro explosives investigated by PAED and UV following HPLC is listed in Table 3 [71]. New column technologies introduced



**Fig. 7.** PAED following HPLC-UV for the determination of RDX and RDX degradation products. Conditions: mobile phase: gradient 2–50% methanol in 10 mM acetate buffer, pH 4.5; flow rate: 1.0 mL min<sup>-1</sup>; guard column: 7.5 mm × 4.6 mm C18 cartridge; column: C18, 5  $\mu\text{m}$ , 4.6 mm × 250 mm; electrode: 1.0 mm glassy carbon; reference electrode: Ag/AgCl; applied potential: 1.0 V vs. Ag/AgCl. (1) Methylene dinitramine (MEDINA), (2) ethylene dinitramine (EDINA), (3) trinitroso-explosive (TNX), (4) dinitroso-explosive (DNX), (5) mononitroso-explosive (MNX), (6) royal demolition explosive (RDX) [72].

**Table 4**

Applications of photochemical reaction systems coupled to electrochemical detection [34–72].

| Applications                  | Limit of detection (LOD) |                            | Reference |
|-------------------------------|--------------------------|----------------------------|-----------|
|                               | ng mL <sup>-1</sup>      | pg injection <sup>-1</sup> |           |
| Aliphatic-nitro explosives    | 0.02                     | 2                          | [71]      |
| Allicin                       | 10                       | –                          | [63]      |
| Aromatic amino acids          | 50                       | 1000                       | [49]      |
| Aspartame                     | 500                      | –                          | [62]      |
| Aspoxicillin                  | 1                        | 100                        | [57]      |
| Barbituates                   | 10                       | 500                        | [37]      |
| Benzodiazepines               | –                        | 100                        | [41]      |
| Chlorodiazepoxide             | 3                        | 60                         | [58]      |
| Chlorophenoxy acid            | 0.002                    | 0.04                       | [60]      |
| Deoxynivalenol                | 10                       | 1,000                      | [56]      |
| Diuretics                     | 100                      | 600                        | [59]      |
| Hydrochlorothiazide           | 2                        | 300                        | [55]      |
| Imidacloprid                  | 0.6                      | –                          | [66]      |
| Inorganic anions [dichromate] | 300                      | 15,000                     | [54]      |
| Nitramines                    | 0.6                      | 130                        | [39]      |
| N-nitrosoamines               | 0.8                      | 1,000                      | [34]      |
| N-nitrosoamines               | –                        | 20                         | [40]      |
| Nitro-esters                  | 1                        | 100                        | [10]      |
| Nitro explosives              | 0.007                    | 0.7                        | [69]      |
| 3-Nitro-tyrosine              | 0.1                      | 2                          | [15]      |
| Organobromides                | 0.4                      | 80                         | [47]      |
| Organochlorides               | 80                       | 16,000                     | [47]      |
| Organoiodides                 | 0.1                      | 20                         | [45]      |
| Organoiodides                 | 0.6                      | 30                         | [64]      |
| Organothiophosphates          | 0.2                      | 4                          | [36]      |
| Organic nitro compounds       | 50                       | 1,000                      | [35]      |
| Penicillins                   | 6                        | 1,200                      | [61]      |
| Penicillins                   | 30                       | 6,000                      | [42]      |
| Peptide [Peptide 520]         | 500                      | 10,000                     | [51]      |
| Peroxide-based explosives     | 700                      | 7,000                      | [65]      |
| Phenylalanine                 | 90                       | 1,800                      | [53]      |
| Protein [RNase A]             | 700                      | 14,000                     | [50]      |

by commercial manufacturers have produced columns that offer baseline resolution of the 14 explosives tested for in Method 8330.

Most recently, Fedorowski and LaCourse [72] have utilized PAED following HPLC-UV for the determination of RDX and RDX degradation products. Specifically, both N-nitroso and ring cleavage degradation products of RDX were analyzed. Fig. 7 presents the separation of RDX and respective N-nitroso degradation products trinitroso-explosive (TNX), dinitroso-explosive (DNX), mononitroso-explosive (MNX) and ring cleavage product methylene dinitramine (MEDINA) using an on-line SPE unit coupled to PAED following HPLC-UV. Furthermore, the determination of RDX and respective degradation products has been applied for analysis in environmental (microcosm and groundwater) as well as biological (urine) matrices.

#### 4. Conclusion

Over a period lasting three decades, photochemical reaction systems coupled to EC detection in HPLC have progressed into an analytical technique which has proven to be adaptable for routine analysis, including use in automated systems. Over the years, the photochemical reactor has been optimized for liquid chromatography with advances in coil design to reduce band broadening effects, and as a result, several photochemical reactors have now been commercialized. Although photochemical reactors offer the ability to be coupled with numerous modes of detections (e.g., UV, FL, and CL) in HPLC, EC detection has benefited the most by the addition of a post-column photochemical reactor on-line, which further expands the range of analytes able to take advantage of EC detection's inherent sensitivity and selectivity. A comprehensive list of applications and respective detection limits of photochemical reaction systems coupled to EC detection is provided in Table 4. The

result has served to facilitate research in pharmaceutical, toxicological and environmental applications that may have otherwise been considered impracticable by conventional methods.

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