ELSEVIER

Journal of Chromatography A, 828 (1998) 421–430

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatography of aromatic amines with photochemical derivatization and tris(bipyridine)ruthenium(III) chemiluminescence detection

Michael E. Bolden, Neil D. Danielson*

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Abstract

We have shown by flow injection that tris(bipyridyl)ruthenium(III) [$Ru(bpy)_3^{3+}$] chemiluminescence (CL) detection of some aromatic amines can be enhanced by on-line photochemical derivatization. Two of the aromatic amino acids, tryptophan, and tyrosine as well as the peptide phenylalanine-alanine and other primary aromatic amines such as L-dopa, phentermine, and tryptamine upon irradiation with UV light are found to give an increased CL signal on the order of 4–9 times that for nonirradiated compounds. For benzylamine, phenethylamine, and phenylalanine, the improved CL detectability upon photolysis is about 15–16 times better. Chemiluminescence detection limits of the photolyzed compounds are generally 2–20 pmol, significantly better than those by UV–Vis detection at 254 nm. GC–MS work has been done to identify the products of some of the photolysis reactions and explain the enhanced CL detectability. The fact that other aromatic amines without a one or two carbon spacer from the aromatic ring to the amine group such as aniline, *m*- and *p*-phenylenediamine, and *N,N′*-dimethylaniline did not show any CL signal improvement upon irradiation with UV light suggests that there is some selectivity in the reaction. CL detection of aromatic amino acids after on-line photochemical derivatization and HPLC has been shown.

Keywords: Derivatization, LC; Chemiluminescence detection; Detection, LC; Amines, aromatic; Tris(bipyridine)ruthenium(III); Amino acids

1. Introduction

The use of UV photolysis to modify organic compounds for enhanced detection by either electrochemistry, spectrophotometry, or luminescence often in conjunction with liquid chromatography has been previously demonstrated. Representative examples using these three detection modes are provided. Spirolactone, hydrochiorothiazide, and a degradation product can be detected in the low ng range after separation by reversed-phase HPLC with UV photolysis electrochemical detection (ED) [1]. Krull et al. have shown that biological pharmaceuticals can be converted to photo-derivatives prior to ED [2]. Aromatic amino acids have also been detected at lower levels after HPLC separation using ED [3]. A review of photoelectrochemical detection in conjunction with HPLC has been published [4]. Facile HPLC detection of barbitals at 270 nm is possible after on-line photochemical reaction [5]. Photodiode array detection of aromatic amines such as phenylenediamine and aminophenol was facilitated in terms of wavelength shifts and enhanced absorbance by on-line post column photochemical detection [6]. Methotrexate in the presence of H_2O_2 could be photochemically cleaved to the fluorescent 2,4-

*Corresponding author.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00833-4

diaminopteridine derivatives [7]. Several nitrogenous pharmaceuticals such as sulfonamides, lidocaine, and diazepam upon photochemical reaction showed enhanced fluorescence after reaction with *o*-phthalaldehyde and mercaptoethanol [8]. Metal ions such as iron, nickel, mercury, and cobalt after complexation with *n*-butyl-2-naphthylmethyldithiocarbamate can be separated by HPLC and then photochemically reacted on-line to a fluorescent product [9]. Fluorescence detection of certain aflatoxins can be enhanced after photochemical reaction at 50°C [10]. Fluorescence of two pyrethroid insecticides can be induced in a micellar media using photochemistry [11]. UV irradiated imipramine, a tricyclic antidepressant, generated a radical that could be directly detected by chemiluminescence (CL) and electron spin resonance (ESR) spectroscopy in a flow injection mode [12]. The production of H_2O_2 from aliphatic oxygenated organic analytes using on-line photochemistry was detected by luminol CL [13]. Photochemistry of various substituted anthraquinone and naphthaquinone derivatives also resulted in the production of H_2O_2 that could be determined by peroxylate CL [14]. The reagent H_2O_2 required for peroxylate CL could be photochemically produced and the flow injection analysis of substituted polyaromatic hydrocarbons was made [15]. Solid-state peroxylate CL has been adapted for the determination of various analytes that can produce H_2O_2 upon photochemical reaction. Quinones [16,17], aliphatic compounds such as glucose and ascorbic acid [18], and aromatic compounds such as caffeine, aniline, phenol, and L-dopa [18] could be detected in the 5–50 pmol range.

Renewed interest in the analytical possibilities of the tris(bipyridine)ruthenium(III) [$Ru(bpy)_3^{3+}$] CL reaction has been evident since the first report that aliphatic amines can react with $Ru(bpy)_3^{3+}$ to generate light [19]. Upon electrooxidation, an electron is removed from the nitrogen atom in the structure of the analyte resulting in a short lived radical intermediate. Through some reaction scheme, this radical will cause the formation of $Ru(bpy)_3^{2+}$* which will drop back to the ground state releasing light with a wavelength maximum of about 610 nm [20]. The CL response of aliphatic amines is inversely proportional to the oxidation potential with detection limits ordered tertiary<secondary<primary. Amino acids [21–24], amino alcohols [25], and pharmaceuticals often with tertiary amine functional groups such as antihistamines [26], diuretics [27], and alkaloids [28,29] have all been determined by either flow injection or HPLC with $Ru(bpy)_3^{3+}$ CL detection. Several of the previously cited papers described the CL detection as electrogenerated chemiluminescence (ECL) pioneered by Bord et al. [30]. ECL involving the oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ at a glassy carbon electrode in the flow cell has the advantages of good long term reproducibility and convenience as compared to external generation of $Ru(bpy)_3^{3+}$ which gives lower detection limits [31]. Using $Ru(bpy)_3^{2+}$ dissolved in the mobile phase, dansyl amino acid standards and oxalate in biological fluids have been determined by HPLC with ECL [32]. In a batch mode, Ce^{4+} has been used to oxidize $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ and a reactant such as pyruvic, malonic, or lactic acid to generate a CL response [33].

A recent review [34] of the relationship of structural attributes of tertiary amines to the observed $Ru(bpy)_3^{3+}$ Cl signal has shown that the molecular geometry of the amine functional groups as well as the type of substituents are important. Aliphatic alkyl groups tend to enhance CL. Electron withdrawing substituents such as C=O attached to the nitrogen or α-carbon atom destabilize the radical intermediate reducing CL response. Nitrogen atoms incorporated in hetereocyclic rings such as piperidine are not free to adjust to a trigonal planar structure of the positively charged radical ion. Nicotinamide adenine dinucleotide (NAD^+) having a pyridine ring does not generate CL upon reaction with $Ru(bpy)_3^{3+}$ but the reduced form NADH does since the pyridine ring has been oxidized to a tertiary amine group [35]. Aromatic amines such as *N,N*-diethylaniline and aromatic substituted amines such as di- or triphenylamine tend to give a low $Ru(bpy)_3^{3+}$ CL response because the radical or positive charge on the nitrogen atom is effectively delocalized by the carbon–carbon double bonds. In fact, aromatic amines such as *N,N′*-dimethylaniline, *p*-anisidine, and α-naphthylamine as well as aromatic amines with nitro substituents have been reported to quench the emission of light by the excited $Ru(bpy)_3^{2+}$* complex [36,37].

The present study examines the effect of on-line photochemical derivatization of primary aromatic

amines prior to detection by flow injection analysis with $Ru(bpy)_3^{3+}$ CL reaction. Phenylalanine, tryptophan, tyrosine, and the peptide phenylalanine-alanine as well as other compounds such as benzylamine, L-dopa, phenethylamine, phentermine, and tryptamine upon irradiation with UV light were found to give an increased CL signal on the order of 4–16 times with detection limits in the 2–20 pmol range. Schiff base products identified by GC–MS that could be responsible for the enhanced $Ru(bpy)_3^{3+}$ CL are proposed. CL detection of aromatic amino acids after on-line derivatization and HPLC has been shown.

2. Experimental

2.1. Equipment

The following instrument diagrammed in Fig. 1 was used for the flow injection work with $Ru(bpy)_3^{3+}$ CL detection. An ISCO model μLC-500 HPLC syringe pump was used to transport the mobile phase and sample injection was made with a Rheodyne model 7125 injector with a 20-μl injection loop. The knitted open tubular (KOT) reactor with a volume of 0.89 ml was produced by weaving 2.0 m of 0.75-mm I.D. PTFE tubing into a metal wire grid (12×6 cm) similar to that reported previously [38]. The wire grid, with the PTFE tubing, was then placed before the mercury (254 nm) lamp source in an IBM model LC/9522 UV detector. One hundred ml of a 1 m*M* $Ru(bpy)_3^{2+}$ solution was oxidized to $Ru(bpy)_3^{3+}$ with a Princeton Applied Research Model 174A polarographic analyzer using a platinum gauze working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode. The $Ru(bpy)_3^{2+}$ reagent solution was bubbled with helium gas prior to and after application for 30 min of +950 V across the electrodes. Too high a voltage did not provide effective oxidation of the reagent and protection of the $Ru(bpy)_3^{3+}$ solution from the light was important for maximum signal generation. The resultant $Ru(bpy)_3^{3+}$ oxidizing reagent was transported via a Gilson Minpuls 3 peristaltic pump that utilized 1.0 mm I.D. Tygon pump tubing at a flow-rate of 0.3 ml/min. The connection between the Tygon pump tubing and the fused-silica capillary (322 I.D.×434 μm O.D.) was made with a male/male union in which a graphite ferrule was used to seal the Tygon tubing and a graphite ferrule used to seal the capillary tubing.

A modified Waters 420-AC fluorometer, with the excitation source removed, was used to detect the CL reaction signal produced inside the 8-μl flowcell. The fused-silica capillary, described above, was fitted through a stainless steel tee and into a stainless steel tube (1.0 I.D.×1.6 mm O.D.) that was attached to the 8-μl flowcell [39]. The $Ru(bpy)_3^{3+}$ oxidizing reagent would pass through this capillary and into the top of the flowcell while the mobile phase and analyte would come in through a stainless steel tubing (0.2 mm I.D.×1.6 mm O.D.) connected at the bottom of the flowcell (see Fig. 1). Enough excess

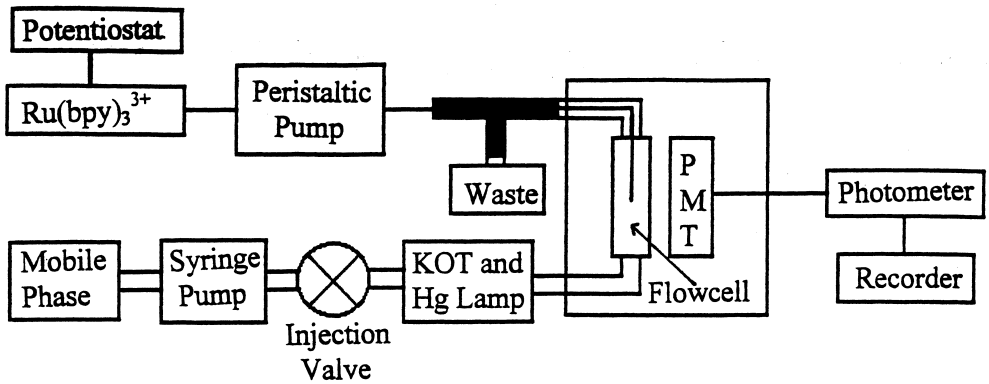

Fig. 1. Instrument diagram for flow injection analysis with on-line photochemical derivatization and $Ru(bpy)_3^{3+}$ CL detection.

space was left within the stainless steel tube to allow the waste solution to pass between the capillary and stainless steel walls and exit at the stainless steel tee. A Hamamatsu PMT model R928YP2547 with maximum sensitivity in the visible light region was used throughout the work. Readout of all data was made on a Linear Instruments recorder.

For the HPLC work, the same instrument as diagrammed in Fig. 1 was used except a Bischoff Model 2200 pump was substituted and the HPLC column was placed between the injector and the KOT photolysis reactor. The HPLC column 15 cm× 4.6 mm I.D. in dimensions was packed with 3-μm C_{18} modified silica microspheres and sold as the Platinum EPS model by Alltech Associates.

The gas chromatograph–mass spectrometer used to identify the photochemical reaction products produced off-line was the Hewlett-Packard G1800A GCD system, with an electron ionization detector. It utilized a HP-5 (crosslinked 5% phenyl/methylsilicone) capillary column, 30 m×0.25 mm, with a 0.25-μm film thickness. The carrier gas was 99.999% helium gas, from AGA (Maumee, OH, USA), at a flow-rate of 1.0 ml/min. The temperature program started at 40°C with an isothermal time of 2.0 min, then a ramp rate of 20°C/min until a temperature of 250°C was reached. A solvent delay of 3.0 min was also utilized. Typical injection volumes were on the order of 0.2 μl.

A Perkin-Elmer (Norwalk, CT, USA) Model LS 50B spectrofluorometer with 1-cm pathlength cuvettes was also used to characterized the off-line photolysis products.

2.2. Reagents

Tris(2,2′-bipyridyl)ruthenium(II) hexachloride (Aldrich, Milwaukee, WI, USA) prepared at 1 m*M* in a 0.05 *M* sodium sulfate supporting electrolyte solution was used in all studies. Buffered solutions were prepared as mobile phases for the samples. The pH 6.5, 7.0, and 8.0 solutions were prepared by dissolving 3.0 g each of sodium phosphate, mono- and dibasic, in 1.0 l of distilled, deionized water, and adjusting the pH with 1 *M* hydrochloric acid or 1 *M* sodium hydroxide as necessary. The pH 9.0 buffer used was a 0.1 *M* carbonate buffer adjusted with the HCl or NaOH solutions as necessary. Water was purified in our laboratories with a Barnstead E-Pure system (Muskegon, MI USA). The phenylalanine-alanine, phentermine, phenethylamine, phenylalanine, tryptophan, tryptamine, and tyrosine were purchased through Sigma (St. Louis, MO, USA). The L-dopa was obtained from Aldrich).

3. Results and discussion

The primary amines studied all had either a one or two carbon spacer separating the amine group from the aromatic ring. Phenylalanine, tyrosine, and phetermine can be considered related to phenethylamine in structure. One dipeptide phenylalanine-alanine was also studied. Benzylamine and L-dopa are analogous in structure. Finally, tryptamine and tryptophan based on the amine group attached through a two carbon chain to an indole ring were studied. Other aromatic amines examined such as aniline, *N*-methylaniline, *N,N′*-dimethylaniline, *m*-phenylenediamine, *p*-phenylenediamine, and atropine which did not contain this functionality of a primary amine separated by one or two aliphatic carbons from the aromatic ring were found to exhibit no CL signal intensity improvement upon irradiation.

The CL signal intensity profiles in the off-line and on-line modes as a function of photolysis time were characterized. For the off-line photolysis experiments, samples in acetonitrile having a volume of 6.3 ml were added to quartz cuvettes (2-cm pathlength) that could be stoppered and placed in front of the same mercury lamp used for the on-line flow injection work. For the on-line work, flow injection analysis was used and the photochemical reaction time was controlled by the flow-rate. A comparison of the CL signal intensities for benzylamine and phenethylamine in the off-line and on-line modes as a function of photolysis time is shown in Fig. 2. Both curves show a similar slope profile up to about 5 min but the off-line data shows a decrease in slope about 5 min before the on-line signal levels off at 12 min. In the off-line mode, the CL signal will continue to increase steadily over at least a 3-h time period. The CL signal for the off-line quartz cell mode is actually about 1.5 times higher at 15 min than that for the

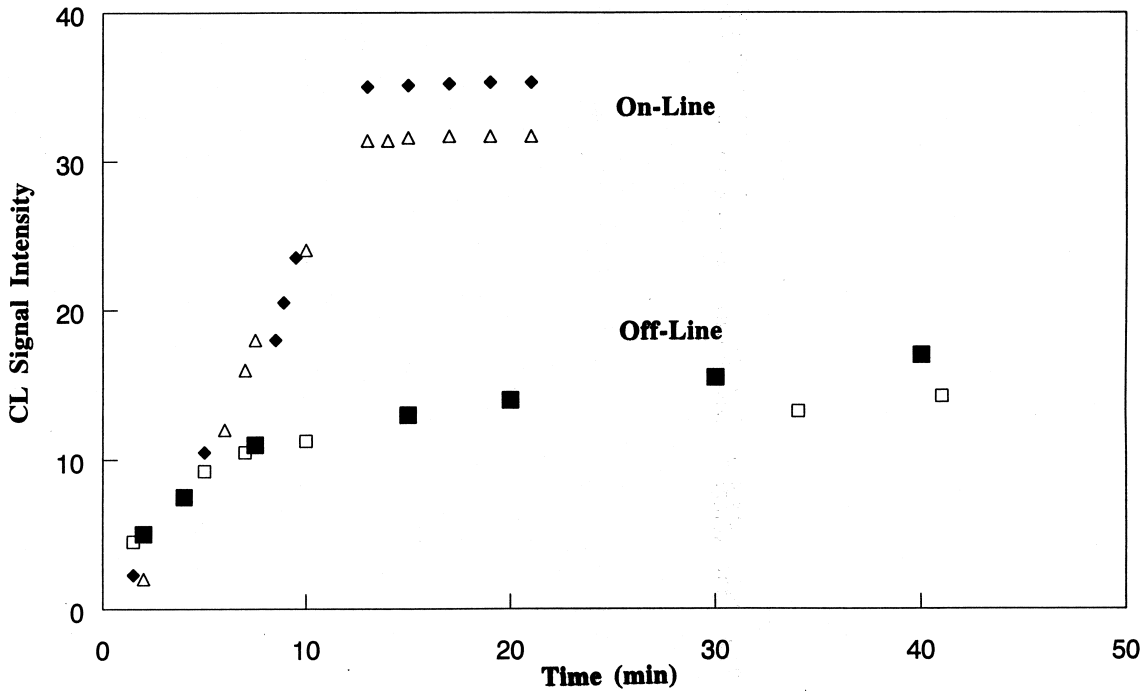

Fig. 2. CL signal intensity (mm where 1 mm=0.46 CL detector units) versus time for benzylamine and phenethylamine irradiated in the off-line batch and on-line flow modes at pH=7.0. Open symbols: benzylamine; dark symbols: phenethylamine. See text for explanation.

on-line PTFE reactor mode due to the greater efficiency of light transmission. In Fig. 2, the off-line data have been normalized to the on-line data by dividing the former by a factor of four to show the comparable rate of CL increase near the beginning of the photolysis reaction. Certainly off-line photolysis of the aromatic amines in a quartz cell for long photolysis times could provide lower detection limits than those possible with the on-line PTFE coil reactor. For example, the off-line photolysis CL signal at 2 h for phenylalanine is about 3 times higher than that at 10 min. However the convenience, reproducibility, and smaller sample size inherent in the flow injection method was deemed more important and future work was done in this mode.

A plot of CL signal intensity versus photolysis time in the on-line mode for structurally similar compounds of other aromatic amines is shown in Fig. 3. Phenylalanine structurally similar to phenethylamine gave a similar CL enhancement in terms of slope (5.3 for the former compound compared to 5.8 for the latter). Tyrosine and L-dopa which differ from phenylalanine only by the addition of hydroxyl groups, formed a second group of compounds with similar irradiation results. These two compounds gave a CL signal intensity-time slope of about 3.2. Compounds with the indole functionality such as tryptophan showed the lowest CL signal-time slope of 1.5.

Using an irradiation time of 5 min at pH=7, a time that produced a good signal increase without significant band broadening, a comparison of detectability for irradiated and nonirradiated samples at the μM level was made (Table 1). It was found that benzylamine, phenethylamine, and phenylalanine exhibited the best detectability improvement of 15–16 times upon irradiation. Phenylalanine–alanine and tryptophan showed the least detectability improvement of 4–6 times upon irradiation. The other

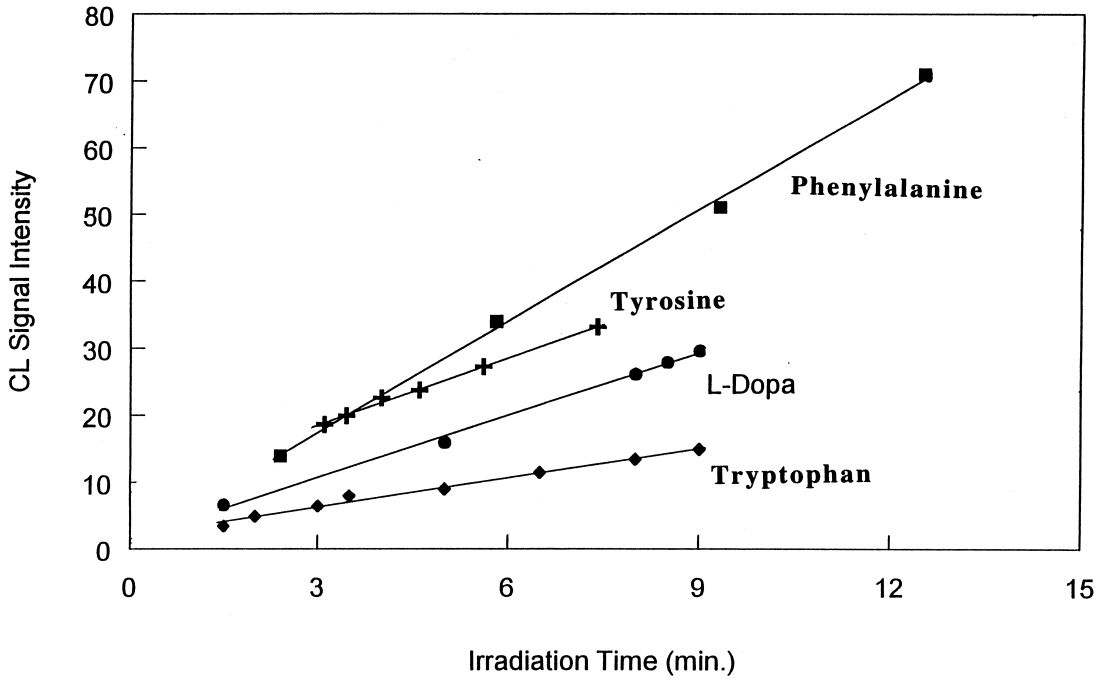

Fig. 3. CL signal intensity (mm) versus on-line irradiation time (mm) at pH=7.0 for phenylalanine, tyrosine, L-dopa, and tryptophan.

compounds exhibited detectability improvements from 7–9 times upon irradiation. Tertiary amines such as atropine and *N,N′*-dimethylbenzylamine were found to exhibit no CL signal intensity improvement upon irradiation.

GC–MS work of some of the off-line photolysis reaction mixtures has permitted identification of the likely CL products. We have shown that benzylamine upon irradiation with UV light will convert to *N*-(phenylmethyl)benzenemethanamine (PMBMA) as shown by the GC–MS trace in Fig. 4A. No PMBMA was found by GC–MS in a nonirradiated sample of benzylamine. Increasing the irradiation time to 4 h did not significantly increase the yield of the product however evidence of the production of benzaldehyde was noted. It is likely that benzaldehyde is reacting with benzylamine to form PMBMA which, not being a primary amine, would be expected to produce a much greater CL signal than the original analyte. A GC–MS study of phenethylamine upon photolysis in acetonitrile showed a somewhat different product profile (Fig. 4B). Benzaldehyde is formed again which can react with phenethylamine to generate *N*(phenylmethyl)benzeneethanamine (PMBEA). A nonirradiated sample of phenethylamine showed by GC–MS the expected major peak at 8.55 min and one unidentified tiny peak at 17.1 min. Increasing the reaction time to 4 h did show a decrease in the

Table 1
Ratio of CL detectability (no photolysis)/CL detectability (on-line photolysis) at the micromolar level at pH=7.0

Compound	CL Detectability improvement ratio due to photolysis
Benzylamine	16.3
Phenethylamine	15.4
Phenylalanine	14.9
Tyrosine	9.2
L-Dopa	9.0
Tryptamine	8.0
Phentermine	7.4
Tryptophan	5.7
Phenylalanine–alanine	4.3

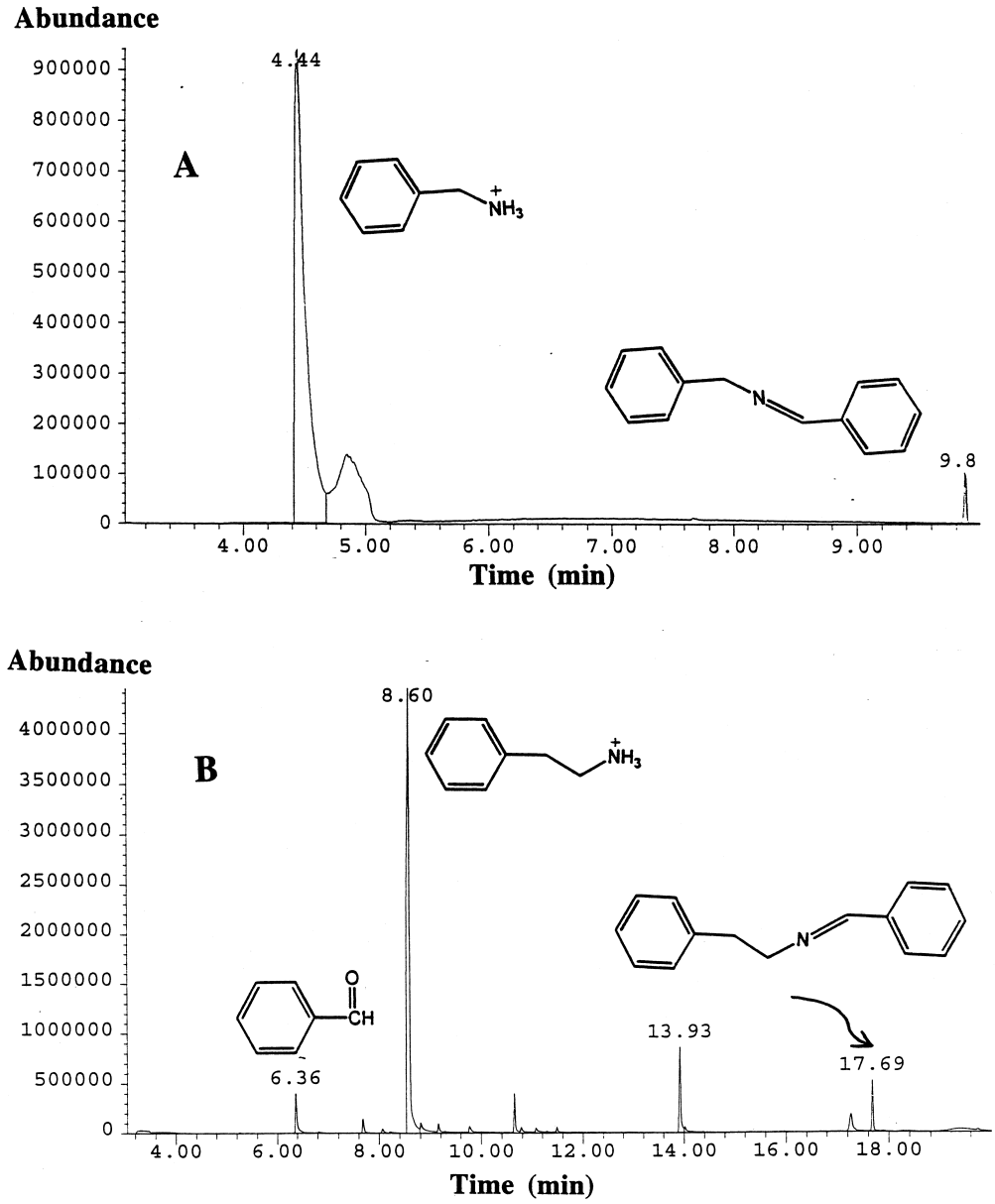

Fig. 4. (A) GC–MS chromatogram for irradiated (3.5 h) benzylamine; (B) GC–MS chromatogram for irradiated (2 h) phenethylamine. Identification of the peaks was at the 90% quality level or higher.

benzaldehyde peak and an increase in the PMBEA peak. This photolysis product again having a tertiary amine substituent should have a greater CL reactivity than the original primary aromatic amine. We have shown that mixing phenylalanine with benzaldehyde before injecting it into the flow injection instrument does give a markedly enhanced CL signal.

It seems possible that the other investigated analytes such as phenylalanine could undergo a similar reaction although GC–MS studies of even esterified

aromatic amino acids was inconclusive. We did show that fluorescence spectra (both signal intensity and wavelength) of phenethylamine and phenylalanine solutions after UV photolysis for 4 h were similar. For phenylalanine, excitation at 389 nm and emission at 456 nm were observed as the wavelength optima while comparable data for phenethylamine were at 363 nm and 422 nm. For 100 mg/l solutions, substantial fluorescence emission signals of about 300–400 units on a 1000 unit scale were found. An analogous study with benzylamine as expected showed a similar fluorescence profile with excitation and emission wavelength optima of 384 nm and 475 nm. No fluorescence at these visible wavelengths was observed for any of the unphotolyzed phenethylamine, phenylalanine, and benzylamine solutions. Previously it has been established that phenylalanine upon photochemical reaction can form hydroxylated products such as tyrosine and L-dopa [40,41]. We cannot rule out the formation of these products however they would not be expected to react with $Ru(bpy)_3^{3+}$ to give an enhanced signal.

It has been stated by Kagen that a bond can be produced between an aromatic carbon and a nitrogen in the photochemical synthesis of indolines [42]. In this manner a primary aromatic amine, that is only marginally CL reactive, could possibly undergo a photochemical cyclization process, forming a more CL reactive secondary amine. We have shown by GC–MS that an aromatic amine such as tyrosine may undergo this cyclization process although the yield was low. For tyrosine, a molecular mass of 193 u was found for the photochemical product. This corresponds to a loss of two hydrogen atoms which would be expected for the cyclization reaction. A mass of 134 u was also found that would correspond to the loss of the carboxylic group and a hydrogen. The few remaining fragments were consistent with the expected molecule. The fluorescence spectra of an UV photolyzed solution of tyrosine showed excitation and emission wavelength maxima at 278 nm and 313 nm, respectively. Since fluorescence in this wavelength region is observed for unphotolyzed tyrosine the evidence is still not strong for the formation of some different fused ring product for photolyzed tyrosine. In the case of tryptamine and tryptophan, evidence of a cyclization or a Schiff base type reaction could not be established by GC–MS after off-line irradiation.

It has been shown by Brune and Bobbitt that the signal produced in the $Ru(bpy)_3^{3+}$ CL reaction with primary aliphatic amines such as amino acids will increase with pH up to about 10 [22]. An on-line photolysis study of CL signal as a function of pH for three aromatic amines is shown in Table 2. For all three compounds, a consistent increase in CL was noted as the pH increased but at pH 9.5, the baseline was very noisy and unstable. The ratio of photolyzed CL signal to unphotolyzed signal shown in parentheses was independent of pH for all three compounds. No significant difference in CL signal trend for unphotolyzed and photolyzed aromatic amines with pH can be concluded.

The photolysis CL detection limits by flow injection analysis at pH=8 of the irradiated amines were found to be at the 0.02–0.2 μg/ml or 2–20 pmol level (Table 3) with one exception (phenylalanine–alanine or Phe-Ala). From previous work [21], it has been shown that peptides do not give low $Ru(bpy)_3^{3+}$ CL detection limits (about 10–100 times poorer than amino acids). Because methacetamide gave almost no CL signal, this weaker CL response of peptides is likely due to the proximity of the carbonyl group to the secondary amine in the peptide

Table 2
Study of CL signal as a function of pH

	CL on-line photolyzed signal[a] (Ratio of photolysis/no photolysis CL signals)			
Compound	pH 6.0	pH 7.0	pH 8.0	pH 9.5
Tryptophan	44 (4.5)	208 (4.6)	395 (4.3)	658 (4.6)
Phenylalanine	158 (6.8)	624 (6.9)	958 (7.2)	1159 (7.0)
Benzylamine	184 (6.7)	473 (7.0)	932 (6.9)	1253 (7.2)

[a] CL signal measured as mm where 1 mm=0.46 detector units.

Table 3
Flow injection analysis detection limits of aromatic amines taken using CL (with photolysis) and UV detection (without photolysis) at pH 8.0

Compound	Detection limits as μg/ml (pmol)		
	CL	UV (214 nm)	UV (254)
Tryptophan	0.02 (2)	0.01 (1)	0.8 (80)
Phentermine	0.04 (5)	0.07 (6)	0.2 (17)
Tryptamine	0.07 (9)	0.02 (3)	0.4 (51)
Benzylamine	0.06 (11)	0.05 (13)	4.5 (825)
Phenylalanine	0.09 (11)	0.3 (38)	5.2 (360)
Phenethylamine	0.08 (13)	0.06 (10)	4.9 (796)
L-Dopa	0.20 (20)	0.07 (7)	4.7 (477)
Tyrosine	0.2 (21)	0.04 (4)	0.3 (32)
Phenylalanine–alanine	1.8 (150)	0.4 (33)	5.5 (458)

bond. Therefore any possible route such as UV photolysis to cause an improvement in the detection limit for peptides such as Phe-Ala is important. The CL detection limits are always significantly lower and often orders of magnitude better than those taken at 254 nm. In addition, the CL detection limits for benzylamine, phenethylamine, phenteramine, and tryptophan are comparable to those taken using UV–Vis at 214 nm without photolysis. UV detection after photolysis of the amino acids always resulted in similar or slightly reduced signals at both these wavelength optima. L-Dopa, Phe–Ala, tryptamine, and tyrosine are detected at levels about 3–5 times lower using UV at 214 nm than with CL but phenylalanine is detected better by CL than UV at 214 nm. Of course, selective detection using $Ru(bpy)_3^{3+}$ CL is a potential advantage over UV detection at 214 nm. The CL detection limits are comparable to those previously published for aromatic amines including L-dopa determined by solid state peroxylate CL [18] and those previously reported for aromatic amino acids determined by photolytic ED [3]. In comparison to derivatization of amines with an aryldialdehyde such as naphthalenedialdehyde [43] for fluorescent detection, the CL detection limits in Table 3 are comparable for tryptophan but about five and ten times higher for phenylalanine and tyrosine, respectively.

A chromatographic separation of the aromatic amino acids was compared with and without on-line photolysis with CL detection (Fig. 5). Without on-line photolysis, detection of the aromatic amino acids

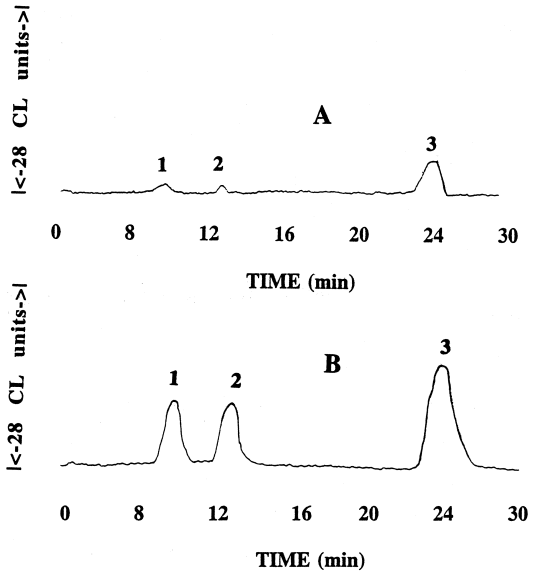

Fig. 5. HPLC chromatograms of aromatic amino acids with CL detection without (A) and with (B) on-line photolysis. Peak identification: 1=tyrosine, 2=phenylalanine, 3=tryptophan. Mobile phase: water–acetonitrile (83:17) at 0.25 ml/min.

tyrosine and phenylalanine is difficult. Detection of tryptophan with a secondary amine group is definitely enhanced using on-line photolysis before CL detection. Peaks are somewhat broad due to the low mobile phase flow-rate used to maximize the photolysis time. Future HPLC work is directed toward using narrower bore columns which have better compatibility for low mobile phase flow-rates.

Recently we have found that off-line UV photolysis of sulfa drugs such as sulfadiazine, sulfanilamide, sulfacetamide, and sulfamerazine which show no CL response through direct reaction will generate products that can react with $Ru(bpy)_3^{3+}$ to give a CL signal. Optimum photolysis times at pH= 7 are long (about 15 h) and detection limits high in the 50–100 μg/ml range. However the possibility of UV photolysis to broaden the scope of $Ru(bpy)_3^{3+}$ reactivity with other aromatic amines has been established.

Acknowledgements

Support by a NIH AREA grant is gratefully

appreciated. Funding of the GC–MS instrument was made possible by a Separation Science Consortium grant from the Ohio Board of Reagents.

References

[1] W.J. Bachman, J.T. Stewart, J. Chromatogr. Sci. 28 (1990) 123.
[2] C.M. Selavka, I.S. Krull, I.S. Lurie, J. Chromatogr. 23 (1985) 499.
[3] L. Dou, I.S. Krull, Anal. Chem. 62 (1990) 2599.
[4] W.R. LaCourse, I.S. Krull, Trends Anal. Chem. 4 (1985) 118.
[5] C. Wolf, R.W. Schmid, J. Liq. Chromatogr. 13 (1990) 2207.
[6] V. Andrisano, R. Gotti, A.M. DiPietra, V. Cavrini, Chromatographia 39 (1994) 138.
[7] J. Salamoun, M. Smrz, F. Kiss, A. Salamounova, J. Chromatogr. 419 (1987) 213.
[8] B.M. Patel, H.A. Moye, R. Weinberger, Anal. Lett. 22 (1989) 3057.
[9] Y.T. Shih, P.W. Carr, Anal. Chim. Acta 159 (1984) 211–228.
[10] H. Joshua, Application Note, Aura Industries, Staten Island, NY, 1997.
[11] J.-J. Adam, A. Coly, Analyst 121 (1986) 1545–1549.
[12] S. Suzuki, H. Nakazawa, M. Fujita, S. Ono, M. Susuki, S. Takitani, M. Sonoda, Y. Sakagishi, Anal. Chim. Acta 261 (1992) 39.
[13] M.S. Gandelman, J.W. Birks, J. Chromatogr. 242 (1982) 21.
[14] J.R. Poulsen, J.W. Birks, Anal. Chem. 62 (1990) 1242.
[15] R.E. Milofsky, J.W. Birks, Anal. Chem. 62 (1990) 1050.
[16] J.R. Poulsen, J.W. Birks, G. Gubitz, C. Gooijer, N.H. Velthorst, R.W. Frei, J. Chromatogr. 360 (1986) 371.
[17] J.R. Poulsen, J.W. Birks, P. van Zoonen, C. Gooijer, N.H. Velthorst, R.W. Frei, Chromatographia 21 (1986) 371.
[18] I. Aichinger, G. Gubitz, J.W. Birks, J. Chromatogr. 523 (1990) 163.
[19] J.B. Noffsinger, N.D. Danielson, Anal. Chem. 59 (1987) 865.
[20] F.E. Lytle, D.M. Hercules, J. Am. Chem. Soc. 91 (1969) 2253.
[21] L. He, K.A. Cox, N.D. Danielson, Anal. Lett. 23 (1990) 195.
[22] S.N. Brune, D.R. Bobbitt, Anal. Chem. 64 (1992) 166.
[23] W.A. Jackson, D.R. Bobbitt, Anal. Chim. Acta 285 (1994) 309.
[24] K. Uchikura, M. Kirisawa, Anal. Sci. 7 (1991) 971.
[25] S. Yamazaki, K. Ozaki, K. Saito, T. Tamimura, J. High Resolut. Chrom. 18 (1995) 68.
[26] J.A. Holeman, N.D. Danielson, Anal. Chim. Acta 277 (1993) 55.
[27] J.A. Holeman, N.D. Danielson, J. Chromatogr. A 679 (1994) 277.
[28] G.M. Greenway, A.W. Knight, P.J. Knight, Analyst 120 (1995) 2549.
[29] N.W. Barrett, R.D. Gerachi, D.L. Hampson, R.A. Russell, Analyst 121 (1996) 827.
[30] I. Rubenstein, C.R. Martin, A.J. Bard, Anal. Chem. 55 (1983) 1580.
[31] W.-Y. Lee, T.A. Nieman, Anal. Chem. 67 (1995) 1789.
[32] D.R. Skotty, W.-Y. Lee, T.A. Nieman, Anal. Chem. 67 (1995) 1789.
[33] Z.K. He, H. Guo, L.J. Yum, Q.Y. Luo, Y.E. Zeng, Analyst 122 (1997) 1343.
[34] A.W. Knight, G.M. Greenway, Analyst 121 (1996) 101R–106R.
[35] T.M. Downey, T.A. Nieman, Anal. Chem. 64 (1992) 261.
[36] C.P. Anderson, D.J. Salmon, T.J. Meyer, R.C. Young, J. Am. Chem. Soc. 99 (1977) 1980.
[37] C.R. Bock, J.A. Connor, A.R. Gutiemez, R.J. Meyer, D.G. Whitten, B.P. Sullivan, J.K. Nagle, J. Am. Chem. Soc. 101 (1979) 44815.
[38] J.R. Marsh, N.D. Danielson, Analyst 120 (1995) 1091.
[39] J.A. Holeman, N.D. Danielson, J. Chromatogr. Sci. 33 (1995) 297.
[40] L. Dou, I.S. Krull, J. Pharm. Biomed Appl. 8 (1990) 493.
[41] A.D. Kaufman, P.T. Kissinger, J.E. Jones, Anal. Chim. Acta 356 (1997) 177.
[42] J. Kagen, Organic Photochemistry, Academic Press, London, 1993.
[43] P. de Montigny, J.F. Stobaugh, R.S. Givens, R.G. Carlson, K. Srinivasachar, L.A. Sternson, T. Higuchi, Anal. Chem. 69 (1987) 1096.