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## Note

# Liquid chromatography of aliphatic trialkylamines with post-column chemiluminescent detection using tris(2,2'-bipyridine)ruthenium(III)

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Liquid chromatography (LC) has had difficulty with the direct determination of aliphatic amines due to their low molar absorptivity in the ultraviolet spectrum. Derivatization has been employed for their detection, and a number of different reagents have been examined for primary and secondary amines. Among these are ninhydrin[1], *o*-phthalaldehyde[1–3], 5-dimethylaminonaphthalene-1-sulphonyl chloride[3], and 4-chloro-7-nitrobenzo-1,2,5-oxadiazole[3]. However, very few derivatization reactions are known for tertiary amines. Addition of a 2% citric or malonic acid solution prepared in acetic anhydride to a tertiary amine will produce a colored solution upon heating[4]. An analogous reaction with aconitic acid will produce a fluorescent derivative[5]. However, this reaction must be carried our for 20 min in a non-aqueous solvent and would be unsuitable for use in conjunction with reversed-phase high-performance liquid chromatography (HPLC) having an organic–water mobile phase. Tertiary amines possessing an ethyl group have been detected using chloranil after thin-layer chromatography[6].

We have recently characterized the chemiluminescent reaction of trialkylamines with tris(2,2'-bipyridine)ruthenium(III) [$Ru(bpy)_3^{3+}$] by flow injection analysis[7]. A yellow-orange light was generated upon mixing the amine and $Ru(bpy)_3^{3+}$ in an aqueous solution buffered between pH 4 and 7. The signal response of trialkylamines was a factor of 100 and 1000 greater than that for secondary and primary amines, respectively. No chemiluminescent reaction with aromatic amines such as aniline, diphenylamine, or triphenylamine was observed. Therefore, the $Ru(bpy)_3^{3+}$ chemiluminescent method is complimentary to existing reaction schemes for the detection of amines.

The feasibility of $Ru(bpy)_3^{3+}$ as a reagent for the post-column reaction of aliphatic trialkylamines after LC separation is demonstrated in this communication. Separations of trimethylamine through tri-*n*-hexylamine will be presented with approximate detection limits and linear range data.

## EXPERIMENTAL

### *Apparatus*

Because of the short lifetime (< 1 s) of the chemiluminescent reaction, mixing of the column eluent and $Ru(bpy)_3^{3+}$ at the detector was required. Fig. 1 shows a

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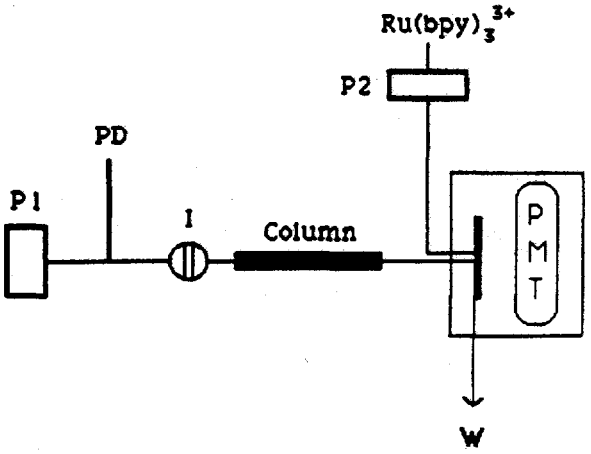


Fig. 1. Schematic diagram of the HPLC system with the post-column chemiluminescent reactor. P1 = HPLC pump; PD = pulse dampener; I = injector; P2 = peristaltic pump; PMT = photomultiplier tube; W = waste.

schematic of the HPLC post-column reactor system. The HPLC system consisted of the following components. A LDC Milton Roy minipump Model 396 (Laboratory Data Control, Riviera Beach, FL, U.S.A.) was equipped wityh a pulse dampener consisting of a 52 cm × 4.6 mm I.D. stainless-steel tube. A Rheodyne Model 7010 injector (Rheodyne, Berkeley, CA, U.S.A.) with a 20-$\mu$l sample loop was used. The column was an IBM 25 cm × 4.5 mm I.D. cyano silica column (IBM Instruments, Danbury, CT, U.S.A.). The oxidized $Ru(bpy)_3^{3+}$ was pumped by a Gilson Minipulse 2 peristaltic pump (Gilson, Worthington, OH, U.S.A.) at 1.0 ml/min to the detector. The two streams were mixed in a dual inlet Pyrex spiral T-mixing cell positioned in front of the photomultiplier tube (PMT) window[7]. The chemiluminescence generated upon mixing in the cell was detected by a Hamamatsu Model R 372 PMT (Hamamatsu, Middlesex, NJ, U.S.A.) powered at 800 V with a Model EU-701-30 GCA/McPherson Instrument photomultiplier module (GCA/McPherson Instruments, Acton, MA, U.S.A.). The entire photomultiplier unit was enclosed in a blackened wooden box to minimize stray light. The PMT current was measured with a Keithley 617 programmable electrometer (Keithley Instruments, Cleveland, OH, U.S.A.). A Fisher Recordall Series 5000 (Houston Instruments, Austin, TX, U.S.A.) Model D5117-5AQ strip chart recorder was used to record the electrometer signal.

*Chemicals*

All chemicals used were reagent grade or better. $Ru(bpy)_3Cl_2 \cdot 6H_2O$ was obtained from GFS Chemicals (Columbus, OH, U.S.A.) and was used without further purification. The water used was obtained from a Barnstead Nanopure distillation unit (Sybron/Barnstead, Boston, MA, U.S.A.). The acetonitrile used was HPLC grade (Mallinckrodt, St. Louis, MO, U.S.A.). The trialkylamines were obtained from commercial sources.

*Procedure*

All solutions were made fresh before use. A 1 m*M* $Ru(bpy)_3^{2+}$ solution was

allowed to oxidize for 30 min for each 100 ml needed at + 1.35 V with an IBM Model EC/225 voltammetric analyzer with a standard three-electrode arrangement (working: Pt gauze, auxiliary: Pt wire, reference: saturated calomel electrode). The $Ru(bpy)_3^{3+}$ solution was buffered with 10 m*M* sodium acetate at pH 5.8. Based upon previous work[7], most of the trialkylamines showed an optimum chemiluminescent response at pH 5.8.

The mobile phases used with the cyano column were 0.10 *M* sodium acetate (pH 5.8)–acetonitrile (45:55) (mobile phase A) and 0.10 *M* sodium acetate (pH 5.8)–acetonitrile (90:10) (mobile phase B). A pH of 5.8 was chosen since it would be compatible with the pH of the $Ru(bpy)_3^{3+}$ buffer solution. The mobile phases were degassed with helium prior to use.

## RESULTS AND DISCUSSION

The separation of amines such as phenylthiohydantoin amino acids[8] and drugs[9] have been previously carried out on a cyano silica column. Therefore, this type of column was chosen for the separation of trialkylamines. The retention of the trialkylamines was dependent upon the ionic strength of the mobile phase and was practically independent of the mobile phase pH. The retention times using unbuffered 0.10 *M* sodium nitrate, 0.10 *M* sodium acetate at pH 4.6, and 0.10 *M* sodium acetate at pH 5.8 did not change by more than 5% for the same percentage of organic modifier. As the ionic strength increased, the retention time decreased for the trialkylamines. It was also noted that mobile phases containing acetonitrile resolved the trialkylamines better than did the same percentage of methanol.

Fig. 2 shows the separation of a mixture of $C_2$ through $C_6$ trialkylamines. The five peaks are totally resolved from each other in just under 30 min. Fig. 3 shows the separation of trimethylamine, triethylamine, and tri-*n*-propylamine in about 10 min. Improved resolution between trimethylamine and triethylamine was not possible

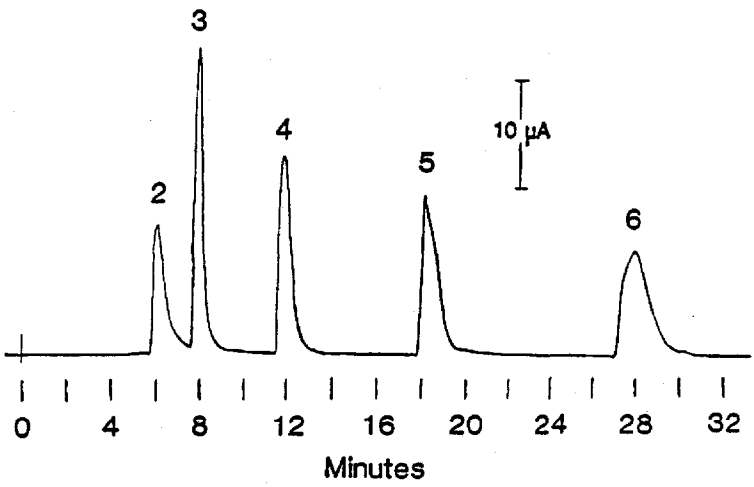


Fig. 2. Separation of 115 ppm triethylamine, 110 ppm tri-*n*-propylamine, 121 ppm tri-*n*-butylamine, 213 ppm tri-*n*-pentylamine, and 320 ppm tri-*n*-hexylamine. Peak numbers correspond to number of carbons in alkyl group. Mobile phase: 0.10 *M* sodium acetate (pH 5.8)–acetonitrile (45:55). Flow-rate: 1 ml/min.

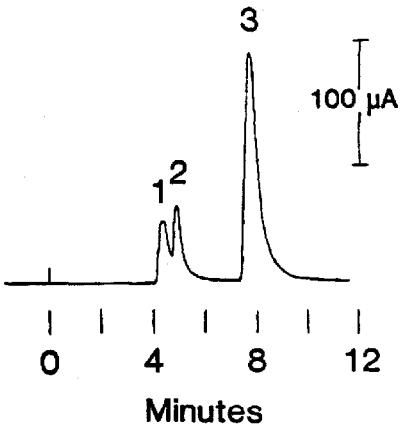


Fig. 3. Separation of 961 ppm trimethylamine, 1010 ppm triethylamine, and 1604 ppm tri-*n*-propylamine. Peak numbers correspond to number of carbons in alkyl group. Mobile phase: 0.10 *M* sodium acetate (pH 5.8)–acetonitrile (90:10). Flow-rate: 1 ml/min.

since mobile phases with less than 6% acetonitrile were not reproducible for lower concentrations of samples. The peak tailing is likely due to the 2 mm I.D. of the tubing used to construct the flowcell. Efforts are underway to design a flowcell to eliminate the extra column band broadening.

Table I shows the calculated detection limits and linear ranges for each of the compounds in the two mixtures studied. The detection limit calculation was based upon the average of three trials. The relative standard deviation (R.S.D.) values for these data ranged from 10–20%. The detection limits for this post-column method are good, with all of the compounds examined having a detection limit around 10–100 pmoles. The detection limits of triethylamine and tripropylamine are about 10–15 times higher using mobile phase A compared to mobile phase B. This is probably

TABLE I

DETECTION LIMITS AND UPPER LINEAR RANGE DATA FOR THE TRIALKYLAMINES

| | *Detection limits** | | | *Upper linear range*** (*pmoles* × $10^{-3}$) |
|---|---|---|---|---|
| | *ppb* | *ng* | *pmoles* | |
| *Mobile phase A: 0.10 M sodium acetate (pH 5.8)–acetonitrile (45:55)* | | | | |
| Triethylamine | 210 | 4.2 | 41.6 | 228 |
| Tri-*n*-propylamine | 300 | 6.1 | 42.6 | 145 |
| Tri-*n*-butylamine | 600 | 12.2 | 65.8 | 130 |
| Tri-*n*-pentylamine | 1180 | 23.6 | 104 | 187 |
| Tri-*n*-hexylamine | 2360 | 47.1 | 175 | 238 |
| *Mobile phase B: 0.10 M sodium acetate (pH 5.8)–acetonitrile (90:10)* | | | | |
| Trimethylamine | 330 | 6.7 | 11.3 | 202 |
| Triethylamine | 20 | 0.4 | 4.0 | 200 |
| Tri-*n*-propylamine | 21 | 0.4 | 2.8 | 224 |

* Detection limit has been calculated as two times the noise of the background signal.

** Upper linear range limit is the point at which the deviation from the line was 10%.

due to the higher organic content of mobile phase A which reduces the chemiluminescent signal. The detection limit for tri-*n*-propylamine of 2.8 pmoles for mobile phase B is only a factor of 10 greater than that determined previously by flow injection analysis[7]. Linear range plots consisted of three or more points for the two mixtures of trialkylamines. Calculating from the detection limit data, the linear range values varied from approximately three orders of magnitude for both tri-*n*-pentylamine and tri-*n*-hexylamine in the mobile phase A to nearly five orders of magnitude for tri-*n*-propylamine in mobile phase B. The R.S.D. values at the upper linear range were about 5%.

In conclusion, the application of $Ru(bpy)_3^{3+}$ as a post-column chemiluminescent reactor for aliphatic trialkylamines has been shown. HPLC separation and $Ru(bpy)_3^{3+}$ chemiluminescent detection of other tertiary amines, such as triethanolamine and aminocarboxylates, should also be feasible.

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