Journal of Chromatography, 360 (1986) 371–383
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 556

SOLID-STATE PEROXYOXALATE CHEMILUMINESCENCE DETECTION OF HYDROGEN PEROXIDE GENERATED IN A POST-COLUMN REACTION

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(Received February 10th, 1986)

SUMMARY

Solid-state chemiluminescence detection was used for the first time in conjunction with high-performance liquid chromatography (HPLC) and compared with liquid-phase chemiluminescence detection. The separation and detection of quinones commonly used in the wood pulping industry served as a model system. Hydrogen peroxide was produced by the eluting quinones in a post-column photochemical reaction. The reaction is photocatalytic in that up to 100 H_2O_2 molecules are produced for each analyte molecule. The H_2O_2 was subsequently detected using peroxyoxalate energy transfer chemiluminescence.

The two additional reagents, a suitable fluorophor and an oxalate ester, may be introduced by means of post-column reagent pumps. It is shown that one pump may be eliminated by chemically bonding the fluorophor, 3-aminofluoranthene in these experiments, to glass beads or silica gel at a small cost in sensitivity and peak width. Use of an oxalate ester (bis-2,4,6-trichlorophenyl oxalate) in the solid state allowed the elimination of the remaining post-column pump at an additional cost in sensitivity and resolution. In this ultra-simple solid-state system using no post-column reagent pumps, detection limits were typically less than 5 pmol on-column, or about five times poorer than the best results obtained with a three-pump liquid phase system.

The role of base catalysis of the chemiluminescence was also explored. As shown earlier for flow injection analysis, although addition of a catalyst enhances the detection sensitivity, the signal-to-noise ratio is only slightly improved; however, the catalyst does improve the precision and, as a buffer, reduces the susceptibility of the technique to interference from pH changes.

0021-9673/86/$03.50

INTRODUCTION

Bis-2,4,6-trichlorophenyl oxalate (TCPO) and other oxalate esters have been useful as reagents for exciting chemiluminescence since the original reports of Rauhut[1]. Excitation is thought to occur by an electron transfer mechanism in which annihilation of a radical ion pair leaves a fluorescent molecule in an excited state[2]. Detection schemes based on peroxyoxalate chemiluminescence have been designed for static systems[3], flow injection analysis[4], thin-layer chromatography[5] and more recently high-performance liquid chromatography (HPLC)[6]. When TCPO, H_2O_2 and a fluorophor are mixed together, chemiluminescence is observed. In most instances, a buffer, formed from triethylamine, imidazole or tris(hydroxymethyl)aminomethane (Tris) and a strong acid, is added to enhance the rate of light emission. Chemiluminescence decay rates also vary with the solvent composition and reagent concentrations[7].

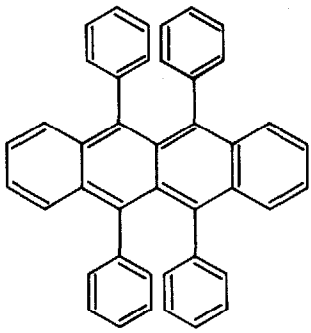
Peroxyoxalate chemiluminescence is useful in detecting either H_2O_2 or certain fluorophors. To detect fluorophors, H_2O_2 and TCPO are mixed with the sample in the presence of a catalyst. Chemiluminescence indicates that a fluorophor capable of accepting the chemi-excitation energy is present. Peroxyoxalate fluorophor detection in HPLC yields extremely low detection limits for dansyl amino acids[6] and amino polycyclic aromatic hydrocarbons (amino-PAHs)[8]. When H_2O_2 is the analyte, TCPO, a suitable fluorophor and the catalyst are added to the sample. Linearity over several orders of magnitude between the H_2O_2 concentration and the chemiluminescence intensity is observed in most instances[3–6,9,10].

Solid-state detection systems for peroxyoxalate H_2O_2 determinations offer several advantages over liquid-phase reagent addition. Typically, the cost is decreased by reducing the number of pulse free pumping systems necessary for reagent addition and by the use inexpensive light detection devices. As stability is better in the solid state than in solution, drift caused by reagent decomposition is reduced. Additionally, the less complex system is easier to operate and more reliable.

The first solid-state peroxyoxalate cells for flow injection analysis of H_2O_2[9] consisted of powdered TCPO through which a solution containing perylene and a catalyst flowed. As the samples and carrier solvent were spiked with these reagents, only one pump was necessary. Expanding on this idea, methods of immobilizing fluorophors capable of accepting peroxyoxalate excitation were developed[10]. When a detector cell containing an immobilized fluorophor is used, the carrier phase and analytical samples no longer need to be spiked with perylene.

Amino-PAHs were chosen for immobilization because of their efficiency in accepting chemi-excitation and the presence of a readily derivitizable functionality. Use of amino-PAHs in liquid-phase systems is unacceptably dangerous because of their carcinogenic and mutagenic activity. Gübitz *et al.*[10] developed two procedures for bonding 3-aminofluoranthene (3AF) to glass beads and silica particles. The fluorescence spectrum of 3AF immobilized by the 3-glycidoxypropyltrimethoxysilane route (Fig. 1) is qualitatively the same as the solid-state spectrum of unbound 3AF. However, when the cyanuric chloride method is used, the product shows a substantial blue shift in the excitation and emission spectra and a lower chemiluminescence yield[10].

As H_2O_2 is selectively detected by peroxyoxalate chemiluminescence[1,3,4,9], it



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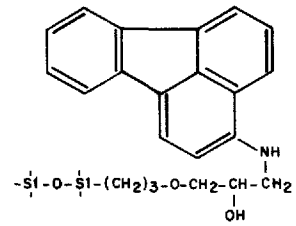

3-AMINO FLUORANTHENE
IMMOBILIZED ON 40μ GLASS BEADS

Fig. 1. Structures of fluorophors used in liquid-phase (rubrene) and solid-state peroxyoxalate H_2O_2 detection.

can be determined without separation methods. Also, the sensitivity and selectivity make H_2O_2 a suitable surrogate for HPLC detection of analytes from which it can be efficiently generated in a post-column reaction. As many enzymatic and photochemical reactions produce H_2O_2 and are, in principle, adaptable to dynamic flow systems, we are developing solid-state peroxyoxalate cells suitable for use in HPLC. HPLC detectors are subject to much more rigorous limitations with respect to dead volume than those used for flow injection analysis. Hence, much of our concern in adapting solid-state cells to HPLC is focused on minimizing the band broadening caused by the various components of the system. Band broadening in open-tubular and packed bed reactors has been discussed previously[11,12].

We have developed a detection scheme, the photocatalytic chemiluminescence (PCCL) detector, in which H_2O_2 is produced post-column by "type 1 photooxygenation" sensitizers[13]. Sensitizing analytes (typically quinones) catalytically generate H_2O_2 in a photochemical reaction, which is subsequently detected by peroxyoxalate chemiluminescence. While the H_2O_2 yield varies with reaction time, solvent composition and sensitizer, yields in excess of 100:1 peroxide molecules per sensitizer molecule have been observed under normal chromatographic conditions. As sensitization of type 1 photooxygenation is exhibited by a very limited class of compounds, the detection scheme is extremely selective. Most anthraquinone derivatives used in modern wood pulping processes are sensitively detected by PCCL.

In this paper, PCCL serves as a model system for the comparison of solid-state and liquid-phase peroxyoxalate H_2O_2 detection systems for HPLC applications. Band broadening, detection limits and operating characteristics are the basis of this comparison. In addition, the effects of using a base catalyst are explored. UV detection limits for some of the analytes that respond to PCCL detection also are presented.

EXPERIMENTAL

Chemicals

Quinone sensitizers were used as received from Aldrich, Sigma, Fisher and Pfaltz and Bauer. HPLC-grade solvents were purchased from Baker or Fisher. Baker HPLC-grade water was further purified by pumping it through a clean C-18 column before use. TCPO was synthesized as in the literature[14], with additional recrystallization from Uvasol benzene (Merck, Darmstadt, F.R.G.). TCPO and rubrene (Aldrich) solutions were prepared fresh daily in ACS reagent-grade acetone which had been purified on an alumina column (Merck aluminium oxide 90, 0.063–0.200 mm particles, 65 g per 500 ml in a column of 2.0 cm I.D.). This procedure enhanced the stability of the TCPO solutions and slightly reduced the chemiluminescence background. 3-Aminofluoranthene (Janssen, Beuse, Belgium) was immobilized on 40–80 μm controlled-pore glass beads (350 Å pore size; Serva Feinbiochem) via the 3-glycidoxypropyltrimethoxysilane route described by Gübitz *et al.*[10].

HPLC apparatus

Two HPLC systems were employed. System A consisted of a 250-ml capacity high-pressure syringe pump, designed and built in the Free University of Amsterdam machine shop, which delivered the eluent [methanol–water (88:12 to 95:5, v/v; Baker], at either 0.76 or 0.95 ml/min. A Rheodyne Model 7125 six-port injection valve (10-μl loop), modified so that the loop was filled from one of the ports, was used for sample introduction. Separations were performed on a 20 cm × 4.6 mm I.D. Zorbax (DuPont) C-18 column packed in the Free University of Amsterdam laboratory. A Waters Assoc. Model 440 254-nm single-wavelength detector was used for UV detection. Signals were recorded on a Kipp & Zonen Model BD-40 strip-chart recorder.

System B consisted of a Kratos Spectroflow 400 HPLC pump with a Rheodyne 7125 injector (20-μl loop) and a Zorbax ODS column (25 cm × 4.6 mm I.D.), packed by DuPont. For plug injection work, the analytical column was removed and a 25 cm × 4.6 mm I.D. column packed with 40-μm glass beads was placed between the pump and the injector. The glass bead column acted as a pulse damper. HPLC mobile phases were methanol and water mixtures, 80–100% methanol (Fisher). Plug injections were in methanol–water (87.5:12.5, v/v) (uncatalysed) or methanol–Tris buffer (pH 7.9; 0.021 *M*) (87.5:12.5, v/v). Flow-rates were 1.0 ml/min for this system. Peak areas were recorded on a Shimadzu CR-3A integrating recorder.

Mobile phases for both systems were shaken with air before use.

Photochemical reactors

All reactors were prepared from PTFE tubing obtained from Small Parts (U.S.A.). The reactors were crocheted into cylinders[15] that fit over a fluorescent

poster ("black") lamp. Lamps of this type are available for approximately $25 at hardware stores. While in use the reactors were wrapped in foil to increase the photon flux and cooled with a muffin fan. Two of the reactors were prepared from 30-gauge tubing (Cat. No. STT-30, 0.006-in. wall, 0.012-in. I.D.). The first of these reactors is 9.8-m long and has a residence time of 69 s at 0.76 ml/min. Connections were made by the method previously described by Huber *et al.*[16]; Swagelok was used on the inlet side and Valco on the exitside. A second STT-30 reactor, 29-m long, with a 187 s residence time at 0.76 ml/min, had Swagelok fittings at both ends. The third reactor, 10.4 m of 24 gauge tubing (Cat. No. STT-24), had a residence time of 213 s at 0.76 ml/min. It was connected with Swagelok fittings. A "bypass", consisting of 20 cm of STT-30 tubing and Swagelok fittings, was used to compare band broadening caused by the fittings to that due to the crocheted PTFE tubing. To demonstrate the reduction in band broadening achieved by crocheting the reactors, 29-m of STT-30 tubing (8 cm coil) connected with Swagelok fitting was compared with the 29-m reactor.

Post-column reagent addition

System A post-column reagents were added via two 50-ml capacity high-pressure syringe pumps. TCPO solutions (0.85–1.0 g/l) were added at 0.28 ml/min. The other pump, when used, delivered rubrene (40–45 mg/l), Tris (pH 7.4–9.6; 0.25–1.5 m*M*) or a mixture of these two reagents at 0.15 ml/min. When rubrene alone was used, the solvent was 100% acetone. If Tris was present, the solvent was acetone–water (99:1, v/v). Reagents were pre-mixed in a Valco three-way LDV mixing tee before joining the reactor effluent via a Valco three-way ZDV mixing tee. In systems with one post-column pump, only the ZDV tee piece was necessary.

System B utilized an Isco Model 314 syringe pump to add mixed solutions of TCPO (0.85–0.95 g/l) and rubrene (40–45 mg/l) at 0.33 ml/min. Catalyst (Tris, pH 7.9, 0.5 m*M*, buffered with nitric acid) for HPLC experiments was delivered by a Sage Model 341 low-pressure syringe pump. The Tris solutions were prepared in acetone–methanol–water (6:3:1, v/v) to facilitate mixing and prevent jamming of the plastic syringes. Mixing was effected by a ZDV three-way tee piece for one post-column pump system and by a four-way ZDV mixing tee when two pump systems were in use.

In both systems, the concentrations of TCPO and rubrene were lowered as the water content in the mobile phase increased to prevent precipitation in the mixing tees and flow cells.

Chemiluminescence detection

For all solid-state chemiluminescence detection, the packed quartz cells were mounted in an Oriel 3075 sample compartment (Oriel, Stanford, CT, U.S.A.). An RCA IP-28 photomultiplier tube inside an Oriel 77781 PMT housing, detected the emitted light. PMT voltages ranged from 800 to 850 V. An Oriel 7070 PMT detection system provided the high-voltage source and signal output to the chart recorder.

Chemiluminescence detection utilizing the following system configurations were compared. Further references in the text and tables will carry the letter of the HPLC system and the Roman numeral corresponding to the chemiluminescence apparatus used; see Fig. 2 for schematic diagrams of these systems.

I. A Kratos FS-970 fluorimeter with the emission filter removed and the ex-

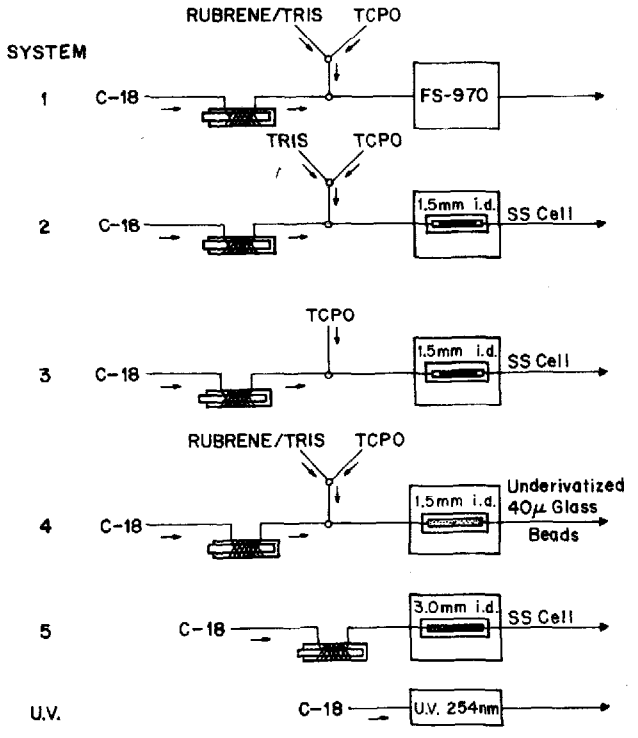

Fig. 2. Schematic diagrams of post-column apparatus for the various post-column detection systems.

citation source turned off. PMT voltages were in the range 1000–1360 V with a time constant in the range 1–5 s. All reagents were delivered in the liquid phase via two post-column pumps.

II. 3-Aminofluoranthene immobilized on 40–80 μm CPG glass and dry packed in a 3 cm × 1.5 mm I.D. (5 mm O.D.) quartz tube is placed in the Oriel cell holder[17]. This cell has no inlet frit, and the first and last 4 mm of the cell are packed with underivatized glass beads. The underivatized glass prevents chemiluminescence in parts of the cell that are not directly exposed to the PMT. One post-column pump delivers TCPO at 0.28 ml/min. No catalyst is used.

III. This system is identical with system II except that a second post-column pump and mixing tee are added to the system for delivering Tris buffer (pH 8.0; 0.5 m*M*) at 0.15 ml/min.

IV. The 1.5 mm I.D. cell is packed with underivatized 40–80 μm CPG glass. TCPO and rubrene/Tris are added in the liquid phase by two post-column pumps. The flow-rates are 0.28 and 0.15 ml/min, respectively.

V. The 3.0 cm × 3.0 mm I.D. cell previously described by Van Zoonen *et al.*[17] for flow injection analysis is used. Finely ground TCPO (80%) is mixed with glass beads (20%) and packed in the first 1 cm of the cell. 3-Aminofluoranthene bound to glass beads fills the remaining 2 cm.

HPLC system B uses the Kratos FS-970 exclusively.

RESULTS AND DISCUSSION

A discussion of the photooxygenation chemistry involved in peroxide production will be given in a forthcoming paper[13].

Solid state vs. liquid phase fluorophor

Sigvardson *et al.*[8] reported a correlation between the oxidation potential of the fluorophor and its chemiluminescence efficiency. In addition to providing indirect support to the proposed chemiluminescence mechanism, this also helped explain the high sensitivity of peroxyoxalate chemiluminescence toward amino-PAHs. Unfortunately, when the liquid phase fluorophor rubrene (Fig. 1) was replaced with immobilized 3-aminofluoranthene, the low detection limits for amino-PAH detection did not translate into sensitivity enhancements of a similar magnitude for peroxide detection. Table I shows a comparison of a 1.5 mm I.D. cell packed with immobilized 3-aminofluoranthene (system A-III) and the same cell packed with underivatized glass beads (A.IV) but with rubrene added to the Tris buffer. The TCPO and Tris concentrations and solvent composition are identical in this comparison. While the use of the immobilized fluorophor resulted in a 20% reduction in detection limits, this is far less than would be expected based on the difference in chemi-excitation efficiency between rubrene and 3-aminofluoranthene.

A possible explanation of this behavior is the presence of a large excess of fluorophor relative to the amount of intermediate available to excite it. When 3AF is immobilized on silica, higher loading densities are achieved[10]; however, no increase in sensitivity is observed when this material is used in place of glass beads. This lack of gain in sensitivity on increasing the fluorophor loading also has been reported for flow injection analysis[10].

Effect of catalyst

Another source of ambiguity in the peroxyoxalate literature is the role of the catalyst. Van Zoonen *et al.*[9] reported that the catalyst buffer afforded only small

TABLE I

COMPARISON OF DETECTION LIMITS IN HPLC FOR LIQUID-PHASE AND SOLID-STATE FLUOROPHORS

HPLC conditions: 95% methanol at 0.76 ml/min. Reaction time, 213 s. TCPO (0.90 g/l) in acetone at 0.22 ml/min. A-III: Tris buffer (pH 7.4)–acetone (1:99) at 0.12 ml/min. A-IV: rubrene (45 mg/l) is added to the Tris buffer–acetone solution and delivered at 0.12 ml/min. Final Tris concentration is 0.5 m*M* in both instances.

Compound	*Detection limit (pmol)*	
	Liquid phase (A-IV)	*Solid state (A-III)*
Anthraquinone-2,6-disulfonate (AQDS)	1.4	1.1
Menadione (vitamin K-3)	3.5	3.0
Anthraquinone (AQ)	2.6	2.0
2-*tert.*-Butylanthraquinone (tBAQ)	1.9	1.5

TABLE II

COMPARISON OF DETECTION LIMITS

Signal-to-noise ratio = 3. On-column detection limits under the following conditions: mobile phase, 95% methanol at 0.76 ml/min; reaction time, 69 or 187 s; see Fig. 2 for solvent and flow conditions of post-column reagents; TCPO, 0.92 g/l (all systems); rubrene, 45 mg/l, Tris buffer (pH 8.0) 0.5 m*M* when used.

Compound	t_R *(min s)*	*Detection limit (pmol)*					
		Short reactor (9.8 m)		*Long reactor (29 m)*			*UV (254 nm)*
		A-I	*A-II*	*A-I*	*A-II*	*A-III*	
Anthraquinone-2,6-disulfonate (AQDS)	1 44	0.39	1.6	0.14	0.76	0.28	0.17
Menadione (vitamin K-3)	4 14	0.84	2.0	0.38	1.1	0.58	0.36
9,10-Anthraquinone (AQ)	5 27	0.68	1.8	0.28	0.85	0.43	0.19
2-*tert.*-Butylanthraquinone (tBAQ)	7 24	0.53	1.5	0.26	0.73	0.33	0.23
Sodium anthraquinone-β-sulfonate (NaAQBS)	1 47	0.41	1.2	0.20	0.53	0.28	0.09
9,10-Phenanthrenequinone (PQ)	4 02	2.7	10	1.3	3.7	1.6	0.32
2-Methyl-1-nitroanthraquinone (MNAQ)	4 24	1.5	4.3	0.78	2.1	1.1	0.20
2-Methylanthraquinone (MAQ)	6 19	0.56	1.6	0.24	0.79	0.39	0.22

improvements in detection limits for solid-state H_2O_2 detection in flow injection analysis. On the other hand, Weinberger[7] found strong catalyst effects and sharp pH optima in HPLC fluorophor detection. These apparently conflicting observations may be due to the difference in cell sizes and resulting residence times, and/or the large difference in the relative concentrations of the chemiluminescence reagents used in the two systems. In the light of these reported observations, we explored the effect of catalyst on H_2O_2 detection in HPLC flow cells.

Table II shows that the detection limits improve by no more than a factor of two to three between systems A-II and A.III, where catalyst addition is the only change. Fig. 3a demonstrates the effect of catalyst addition in the liquid-phase system B, operated in a plug injection mode. Once again, the detection limits are improved only slightly by the buffer. Least-squares linear regression slopes of 18.3 and 6.4 area units per picomole injected were calculated for the catalysed and uncatalysed curves, respectively. Log–log plots (Fig. 3b) show the same data over a wider concentration range. Both the catalysed and uncatalysed systems are seen to have linear dynamic ranges greater than three orders of magnitude.

Other experiments showed that increasing the concentration of the catalyst afforded no improvement in detection limits. The signals increase substantially with increasing pH; however, the noise from the chemiluminescence background also increases. The net result is a relatively small improvement in detection limit. Enhanced detection limits are only observed when the pH of the buffer is greater than 7.4. This

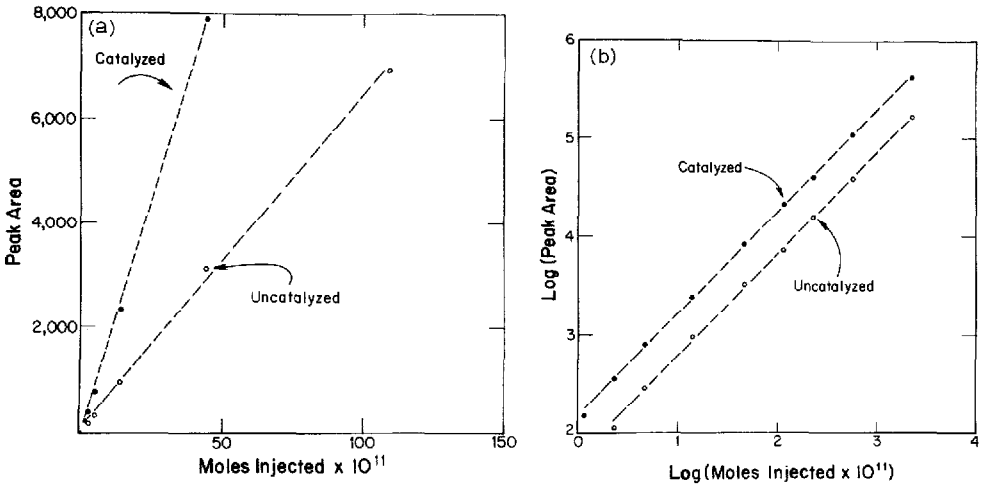

Fig. 3. (a) H_2O_2 calibration graphs in plug injection mode using system B-I. The solvent is 87.5% methanol with the remaining 12.5% water (uncatalysed) or 0.021 m*M* Tris buffer (pH 7.9). Flow-rate, 1.0 ml/min. Points are averages of 5–7 replicate injections. Slopes and standard deviations of the least-squares lines are 183 ± 1.4 and 64 ± 1.1 for the catalysed and uncatalysed system, respectively. (b) Data obtained under the same conditions as in (a) but plotted in a log–log format to illustrate the wide dynamic range of the chemiluminescence detection. Slopes and standard deviations of the least-squares lines are 1.038 ± 0.005 and 1.039 ± 0.010 for the catalysed and uncatalysed system, respectively.

pH behavior is similar to that reported in H_2O_2 detection by flow injection analysis[9].

The precision is, however, improved significantly by the use of a catalyst. For the calibration graphs in Fig. 3, the average of the relative standard deviation (R.S.D.) for the catalysed system is 4.4%. In comparison, the R.S.D. for the uncatalysed curve is 9.2%. At every concentration, the R.S.D. of the uncatalysed system exceeds that of the catalysed system.

As an alternative to the liquid-phase catalyst, various anion exchangers were investigated for potential as solid-state catalysts. Amberlite CG-400 (Rohm and Haas, U.S.A.) resin in the acetate and hydroxide forms in both layered and mixed beds with immobilized 3AF gave poorer detection limits than the uncatalysed system. We are unsure whether the decrease in sensitivity is caused by a chemical effect or by the opacity of these media. Aminex A-28 HPLC packing material (Bio-Rad Labs., U.S.A.) resulted in a high back-pressure, which caused the quartz cells to burst.

A conclusion that can be drawn from these experiments is that while the catalyst offers only small improvements in detection limits, it plays an important role in other aspects of the system. In addition to improvements in precision, the buffering capacity of the catalyst can be important in the application of flow injection analysis and HPLC H_2O_2 detection to samples of environmental interest. Examples of potential problems are acid rain samples in flow injection analysis and samples with complex matrices in HPLC. For the latter, it is possible for compounds with acidic or basic functional groups to co-elute with the analyte and cause pH-induced shifts in the sensitivity of the chemiluminescence response. Similar effects would be expected with precipitation samples of varying total acidity in an flow injection analysis mode. In either of these instances, elimination of the catalyst/buffer or insufficient buffering capacity will result in inaccuracy and irreproducibility.

TABLE III

BAND BROADENING CONTRIBUTIONS

Mobile phase: 95% methanol at 0.76 ml/min in all instances.

System component	*Contribution to variance (μl^2)*	*System*
Detector cells		
1.5 mm I.D. cell packed with derivitized or undervitized 40–80 μm glass beads	1200	A-IV, A-II
Solid-state flow injection analysis cell, 3.0 mm I.D.	4000	A-V
Reactors and connections		
9.8-m STT-30 reactor, Swagelok/Valco plumbed (normal union)	600	
29-m STT-30 reactor, Swagelok/Swagelok plumbed (normal unions)	1400	
29-m STT-30 tubing (8-cm coil), Swagelok/Swagelok plumbed (normal unions)	6500	
0.2-m bypass, Swagelok/Swagelok plumbed (normal unions)	570	
0.2-m bypass, Swagelok/Swagelok plumbed ("bored-out" Swagelok unions)	235	

Band broadening

One of the most important criteria in judging an HPLC reaction detector is its effect on chromatographic performance. Table III presents the contribution to peak variance, expressed in volume units (μl^2), by the components of the systems compared in this paper (refer to Fig. 2 for diagrams of the post-column apparatus). In the calculation of variance (σ^2), chromatographic peaks were assumed to be Gaussian, and the standard deviation of the peak was measured at half-height ($\sigma = w_{1/2}/2.354$). Because peak variances are additive, the variance of an individual component can be determined from the change caused by its removal from the system[12].

Detector cell variances were compared with the Kratos FS-970 fluorimeter which was taken as the arbitrary zero, as peaks exhibited the lowest total variance in it for a given post-column configuration. Anthraquinone and menadione were chosen as model compounds for PCCL and UV detection because they respond to both and exhibit good peak shape. Reactor variances and the variance reductions due to crocheting the tubing and drilling out Swagelok unions were determined using both UV and fluorescence detection. Phenanthrene was the model fluorescent compound (λ_{ex} = 280 nm, λ_{em} > 389 nm).

In comparing detector cell performance, one must consider that the peaks are already broadened by the post-column reactors. Additional broadening in the detector cell often causes little additional loss in resolution. With PCCL detection, the 1.5 mm I.D. cells in systems A-II and A-III caused only slight losses in performance (Table III). Band broadening in the solid state cells is kept low by the use of uniform packing material.

In the solid-state cell for flow injection analysis, described by Van Zoonen *et*

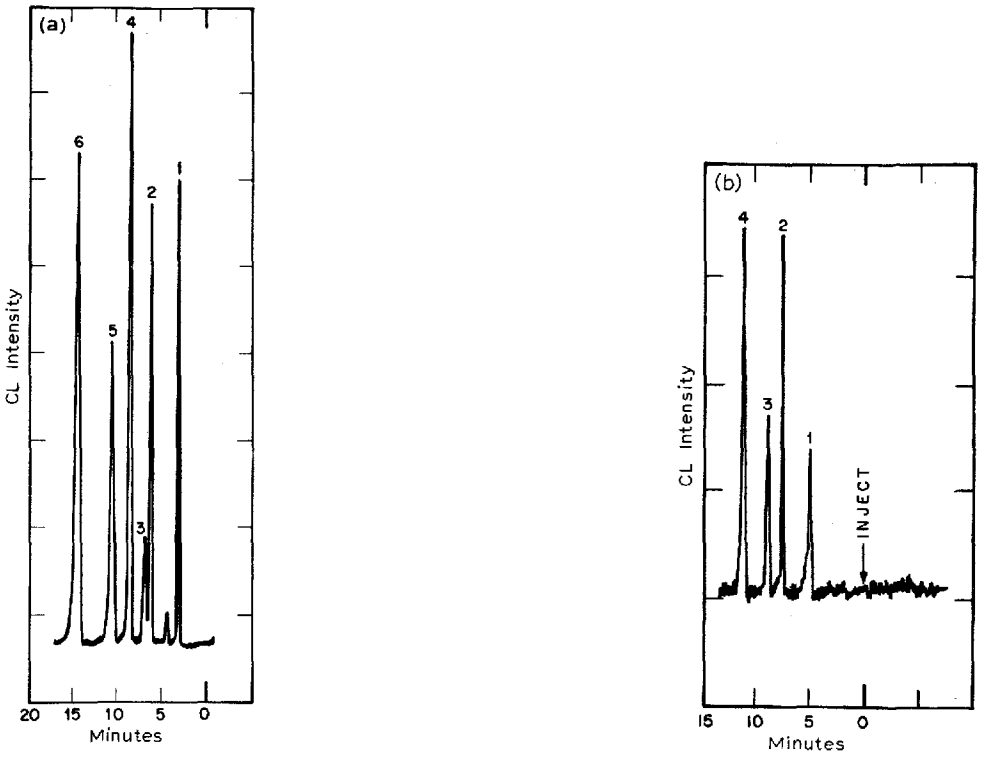

Fig. 4. (a) Chromatogram of a mixture of quinones using the one-pump, solid-state system (A-V). Conditions: 88% methanol at 0.76 ml/min, reaction time 69 s. Peaks: 1 = sodium anthraquinone-2-sulfonate (260 pmol); 2 = 2-methyl-1,4-naphthoquinone (vitamin K_3) (430 pmol); 3 = 2-methyl-1-nitroanthraquinone (230 pmol); 4 = anthraquinone (460 pmol); 5 = 2-methylanthraquinone (260 pmol); 6 = 2-*tert.*-butylanthraquinone (420 pmol). (b) Chromatogram of a mixture of quinones using the three-pump, liquid-phase system (A-I). Conditions: 95% methanol at 0.76 ml/min, reaction time 187 s, TCPO (1.0 g/l) in acetone at 0.24 ml/min, Tris (pH 7.4, 0.5 m*M*). Peaks: 1 = anthraquinone-2,6-disulfonate (2.1 pmol); 2 = 2-methyl-1,4-naphthoquinone (14 pmol); 3 = anthraquinone (5.3 pmol); 4 = 2-*tert.*-butylanthraquinone (8.4 pmol).

al.[17], the TCPO is consumed with use and generates an increasing dead volume. System A-V utilizes this cell, shortening of the PTFE capillaries (0.010 in. I.D.) being the only modification. Glass beads, approximately 20% by volume, are mixed with the ground TCPO to improve the packing and flow characteristics. The peak variance for this solid-state system was substantially larger than for the liquid-phase systems. Fig. 4a demonstrates, however, that despite its relatively large variance, it is possible to obtain good chromatograms with the solid-state cell. For this very simple, one-pump configuration, detection limits are degraded by approximately a factor of five as compared with the three-pump liquid-phase system. At the high polarity modifier concentrations necessary for the photochemistry and chromatography, TCPO consumption is rapid, and cell lifetimes of only about 2.5 h were obtained under these conditions. Peak variance increased significantly with use. In HPLC systems with lower modifier contents, cell lifetimes would be increased owing to the reduced solubility of TCPO. However, the sensitivity is also reduced in systems with higher water contents[7,9,17].

Table III also indicates the reduction in peak spreading achieved by crocheting the PTFE reactors relative to coiled tubing. Subsequent to this collaboration, we developed a method for reducing the dead volume in Swagelok unions[15]. Variances for the bypass connected with "normal" and "bored-out" Swagelok unions are given in Table III. In these modified unions, the ends of the 1/16-in. O.D. stainless-steel capillaries nearly touch each other. Huber *et al.* have reported a similar modification of Swagelok mixing tees[16]. ZDV Valco mixing tees, with a make-up flow, do not contribute to broadening when compared with a ZDV union.

Detection limits

As with band broadening, there are often competing goals in designing a reaction detector that affect its performance in terms of detection limits. In this instance, simplicity of operation, cost and control of hazardous chemicals were improved with a slight decrease in performance. Table II shows a comparison of the post-column systems previously described. Detection limits are given as the analyte concentration resulting in a signal-to-noise ratio of three.

Elimination of one post-column pump by using the solid-state fluorophor was accomplished at the cost of a reduction in detection limits by about a factor of three. With the catalysed, immobilized fluorophor system (A-III), the detection limits are less than a factor of two poorer than those in the liquid-phase system (A-I). As the design of the solid-state HPLC cell has not been investigated in depth, it is likely that solid-state detections limits could be further reduced by improving its design.

Table II also lists the UV (254 nm) detection limits obtained under the same chromatographic conditions. The UV detector was operated in the normal way (*i.e.*, no post-column reactor or reagents added). In terms of sensitivity, the PCCL detector offers no advantage over conventional UV detection. The detection limits given by both methods are fairly low, as quinones typically are strong absorbers in this region of the spectrum. For example, 2-*tert.*-butylanthraquinone absorbs with a log ε of 4.73 at its λ_{max} of 255.5 nm[18]. The advantage of PCCL detection of these compounds over UV detection is the large increase in selectivity[15].

CONCLUSIONS AND FUTURE DIRECTIONS

Use of a catalyst improves precision and can add an important buffering capacity to both solid-state and liquid-phase systems. Band broadening (in σ_v^2) is significantly larger (by a factor of *ca.* 2–3) in a solid-state reactor, although corresponding losses in detection limits were found to be minor. The major advantage of a solid-state reactor is its handling simplicity and economy, as additional pumps and mixing devices can be eliminated. Further improvements in cell design are possible to reduce σ^2 values but, in general, the feasibility of this concept for HPLC detection has been demonstrated. The solid-state TCPO addition has the additional advantage of higher stability compared with TCPO solutions, which are sensitive to solvent impurities[19] and decompose in the presence of peroxides, trichlorophenol, methanol and water. This aspect is currently being explored by pumping TCPO solutions to a PCCL system from a large solid-state TCPO bed mounted off-line from the eluent stream[20].

In conclusion, solid-state peroxyoxalate cells have potential for expanded use

in HPLC and flow injection analysis detection. Our miniaturized solid-state fluorophor cell can be coupled to HPLC with only a smal loss in resolving power. As the solid-state cell applies to H_2O_2 detection in general, the results presented here should be transferable to other HPLC applications. Solid-state systems are currently being applied to glucose determinations via enzymatic oxidation[21] in flow injection analysis and as an HPLC detector for anilines in a quenching mode[22]. Immobilized alcohol oxidase, in a post-column bed reactor, coupled with solid-state peroxyoxalate chemiluminescence is a potentially sensitive and class-selective detection scheme for alcohols. As more peroxide-producing, post-column reactions are exploited, the limitations of these cells with respect to chemical interferences such as quenching and pH-induced sensitivity changes will be better understood. At this time they appear to be a simple, cost-effective and selective alternative to electrochemical and liquid-phase chemiluminescence detection systems for hydrogen peroxide.

ACKNOWLEDGEMENTS

The authors thank NATO for a collaborative research grant (No. 0259/83) and Z.W.O. in Den Haag, The Netherlands, for a visitors grant to J.W.B. This work was partially supported by a grant from the U.S. Environmental Protection Agency (No. R-810717-01-0).

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