Tutorial

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Chemiluminescence detectors for liquid chromatography

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In this tutorial we describe the construction of chemiluminescence detectors for high performance liquid chromatography (HPLC), comprising the components required to deliver the chemiluminescence reagent, a coiled-tubing flow cell, photomultiplier tube and detector housing, and various options for data acquisition. We also discuss two state-of-the-art commercially available chemiluminescence detectors for HPLC and other flow analysis methodology.

Keywords: chemiluminescence detection; chemiluminescence flow cell; high performance liquid chromatography; flow injection analysis; luminol; potassium permanganate

Design Considerations

In general, liquid-phase chemiluminescence detection involves combining analyte and reagent solutions and measuring the subsequent emission of light with a suitable photodetector.[1–9] In flow analysis (including liquid chromatography), this normally involves merging the sample carrier (or post-column eluate) stream with a chemiluminescence reagent solution at a T- or Y-connector; the reacting mixture then flows through a detection cell, often consisting of a transparent coil of tubing that has been mounted against a photomultiplier tube within a light-tight housing.

The emission process is identical to other modes of luminescence (such as fluorescence) – radiative relaxation of an excited molecule to its ground electronic state – but in the case of chemiluminescence, the excited species is an intermediate or product of a chemical reaction, and the time required to reach maximum intensity is therefore dependent on the physical processes of solution mixing and the kinetics of the chemical reaction.[10,11] Wavelength selection, essential for most fluorescence and phosphorescence procedures, is normally not required for chemiluminescence detection, due to the absence of scattered incident light, and because the reaction leads to only one luminophore (with some notable exceptions[12–14]). The selectivity of chemiluminescence is derived from the limited number of analytes that produce an intense emission with the reagent.[10] A conventional fluorescence detector (with the excitation source turned off) can be used to measure the light produced by a chemical reaction, but it is not normally the best option.

The flow rate and the length of tubing (and therefore the time required for the solution to travel) from the confluence point to the flow-cell should be optimized so that the most intense portion of the transient emission occurs within the flow-cell. For relatively long-lived chemiluminescence reactions, a mixing tube (0.5–0.8 mm internal diameter) of at least 10 cm (and possibly much longer) is required to mix the solutions prior to entering the flow cell and allow sufficient time to attain an intense emission. The luminescence may occur throughout the entire detection zone – and continue as the reacting mixture travels down the waste line – in which case the optimum volume of the flow cell exposed to the photodetector may be a compromise between sensitivity and post-column band broadening. For fast chemiluminescence reactions, the solutions should be merged very close to or even within the detection zone.[15] Under these circumstances, efficient and reproducible mixing of reactant solutions in this zone is critical and has a greater influence on luminescence intensity than the overall flow cell volume. Irrespective of the reaction, the light is emitted in all directions. Sensitivity can therefore be enhanced by directing stray light from the flow cell towards the photodetector surface.[16] Flow cells in which the reacting mixture is propelled into an enclosed shallow space, before exiting *via* tubing to waste, have been used with some success[17,18] because they allow a greater volume of solution to be in contact with a transparent flat surface facing the photomultiplier window. Unlike coiled-tubing flow cells, however, this configuration does not provide a well-defined path for solution flow, and therefore the discrepancy between the rates of solution flow through different areas of the cell may contribute to band broadening, particularly with longer-lived chemiluminescence reactions.[15]

In the following sections of this tutorial, we discuss the manifold used to deliver the chemiluminescence reagent(s), construction of a simple coiled-tubing flow cell and detector housing, selection of the photomultiplier tube, and some options for data acquisition, drawing from our own experiences for illustrative examples.

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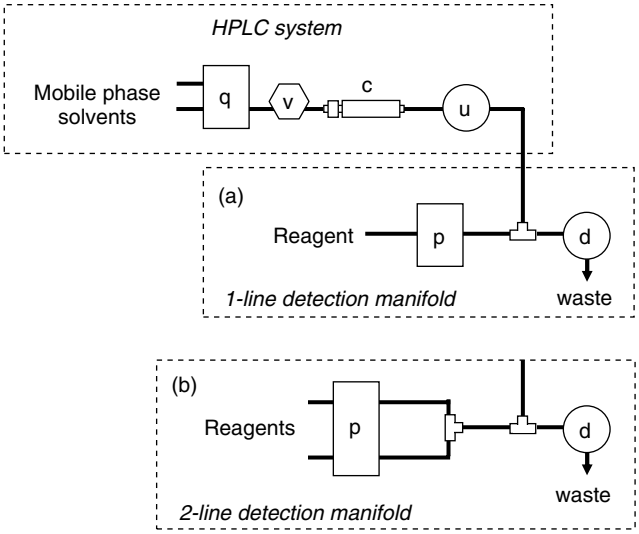

Figure 1. (a) Example manifold for HPLC with chemiluminescence detection. (b) An alternative detection manifold in which two reagent solutions are merged prior to mixing with the column eluate (this particular manifold is useful for on-line preparation of an unstable reagent). Key: q = quaternary pump; v = injection valve; c = column; u = UV-visible absorbance detector or other non-destructive detector (optional); p = peristaltic pump; d = flow-through chemiluminescence detector.

Reagent Pump and Manifold Components

The application of chemiluminescence detection in liquid chromatography requires that one or more reagents are merged with the column eluate stream to initiate the light-producing reaction.[1,8] Chemiluminescence detectors constructed in the manner described below do not generate significant backpressure and therefore the flow manifold to deliver the reagent(s) can be constructed from reasonably inexpensive low-pressure components, such as those commonly used in flow injection analysis (e.g. peristaltic pump, PTFE tubing and polymer T- or Y-fittings), as shown in Figure 1.

Peristaltic pumps are a convenient and commonly used option for the propulsion of reagents, suitable for most applications, but it should be noted that the pulsation caused by their mechanical action can, to some extent, reduce precision and contribute to baseline noise.[19] Chemiluminescence reagents are commonly prepared in aqueous solution, and therefore PVC peristaltic pump tubing is adequate, but some are prepared in other solvents, such as acetonitrile, for which silicon or other specialized pump tubing is required.

A range of other pumps have been used to deliver chemiluminescence reagents in flow analysis systems.[19–22] Syringe pumps can provide a smoother flow than peristaltic pumps, but require periodic refilling. This can be automated to coincide with column re-equilibration or to alternate with a second syringe, resulting in a near-continuous flow of solution. A smooth, continuous flow of solution can be generated with the reciprocating piston pumps developed for commercial high performance liquid chromatography (HPLC) systems,[22–27] but the compatibility of the pump materials with the chemiluminescence reagent (which often contains a strong oxidant in acidic or alkaline solution) must be considered.

Flow Cell

Coiled-tubing flow cells were first introduced for chemiluminescence detection in flow injection analysis by Rule and Seitz in 1979.[28] The cells were simple to construct, compatible with photodetector geometry, and when the analyte and reagent are mixed just prior to entry into the flow cell, enabled measurement of the light emitted during the first few seconds of the reaction.[28–30] This approach to chemiluminescence detection was adopted for HPLC in the 1980s, for the determination of analytes such as morphine,[31] acetylcholine[32] and corticosteroids.[33] Other designs have been explored,[34–40] but the coiled-tubing flow cell remains the most prevalent reactor employed for chemiluminescence detection in HPLC.

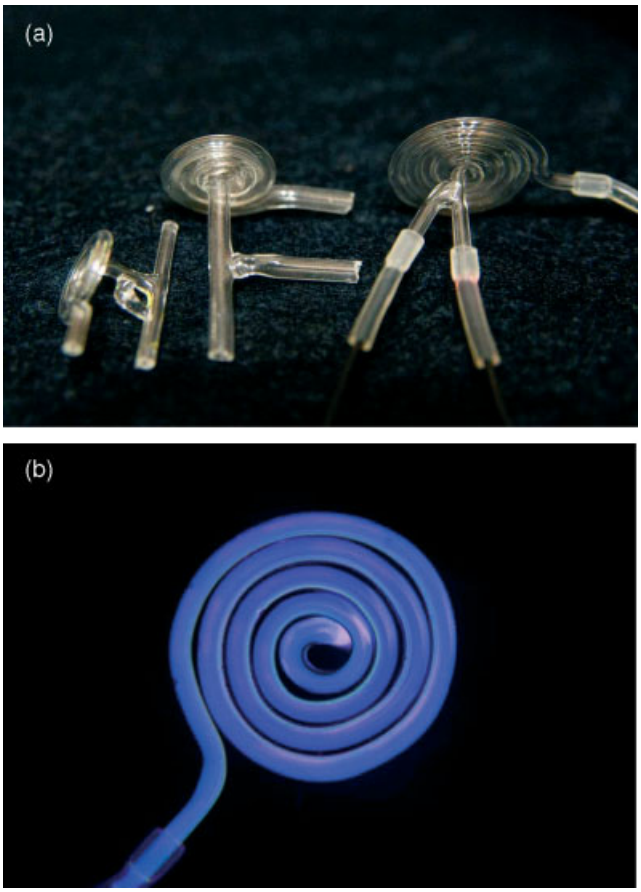

Figure 2. (a) Glass flow cells with integrated T- or Y-piece. (b) A peroxyoxalate chemiluminescence reaction in a glass flow cell.

The coil must be inert to mobile phase solvents and chemiluminescence reagents, and transparent to the wavelengths of emission. Fluoropolymer tubing such as polytetrafluoroethylene (PTFE) is commonly used. Glass flow cells have also been utilized,[31,41–45] and although they are more difficult to construct, the confluence point can be integrated into any part of the cell (Figure 2). Very few analytically useful chemiluminescence reactions that emit light at wavelengths beyond the UV cut-off of glass are known, but fused silica flow cells have been used to examine chemiluminescence in that region.[46]

In our laboratory, we have constructed flow cells for relatively fast chemiluminescence reactions (with reagents such as acidic potassium permanganate and tris(2,2′-bipyridine)ruthenium(II)) by mounting a coil of transparent PTFE-PFA polymer tubing (0.8 mm i.d.; 50928, Lachat) on a thin metal backing plate. Prior to creating the coil, one end of the tubing is pushed through a slit in the backing plate and attached to a polypropylene

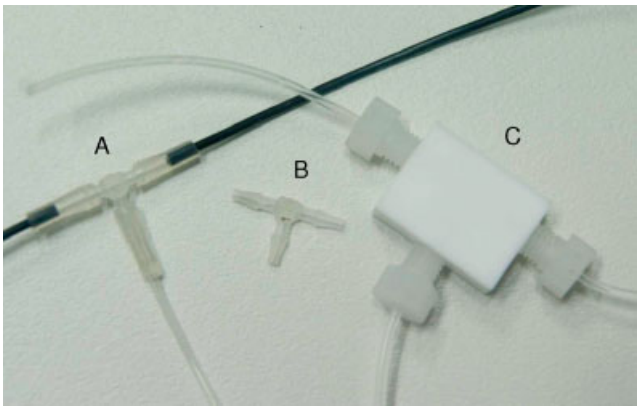

Figure 3. (A) T-piece connected to manifold tubing with silicone pump tubing, (B) a T-piece, and (C) a T-piece with screw-in fittings.

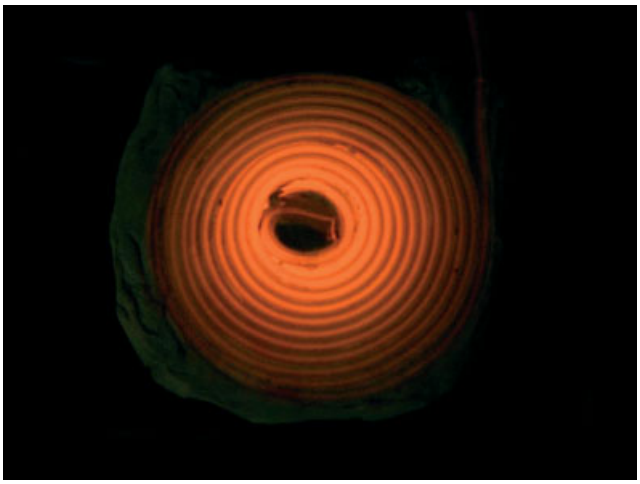

Figure 4. Chemiluminescence from the reaction between tris(2,2′-bipyridine)ruthenium(III) and codeine in a flow cell constructed from a coil of PTFE tubing mounted on a backing plate with Blu-Tack®.

T-piece (RZ-06365-77, Cole-Parmer) with a small section of silicon tubing (Figure 3A). 'White-white' silicon pump tubing (116-0497-10, DKSH) has an ideal internal diameter (1.02 mm) to slip over the barbed connector and tubing and firmly hold them together. The other solution lines can be attached at this time. Screw-in T- or Y-pieces can also be used (Figure 3C). The attachment of fittings to the tubing increases the minimum length between confluence and detection points, but this is only an issue when trying to get the utmost sensitivity from particularly fast chemiluminescence reactions.

The tubing on the other side of the plate is then shaped into a coil using an adhesive material to hold it in place. Figure 4 shows the emission of light from a chemiluminescence reaction in a flow cell of this type. We normally prepare the coil with a diameter corresponding to that of the end-on window of our photomultiplier tubes (~29 mm), wound tightly to allow emission from as close as possible to the centre of the detection zone. The coil is then mounted against the photodetector window within a light-tight housing.

Chemiluminescence detectors containing coiled-tubing reactors are also commercially available.[47,48] An examination of papers describing HPLC with chemiluminescence published over the last decade revealed that over 60% of studies were conducted

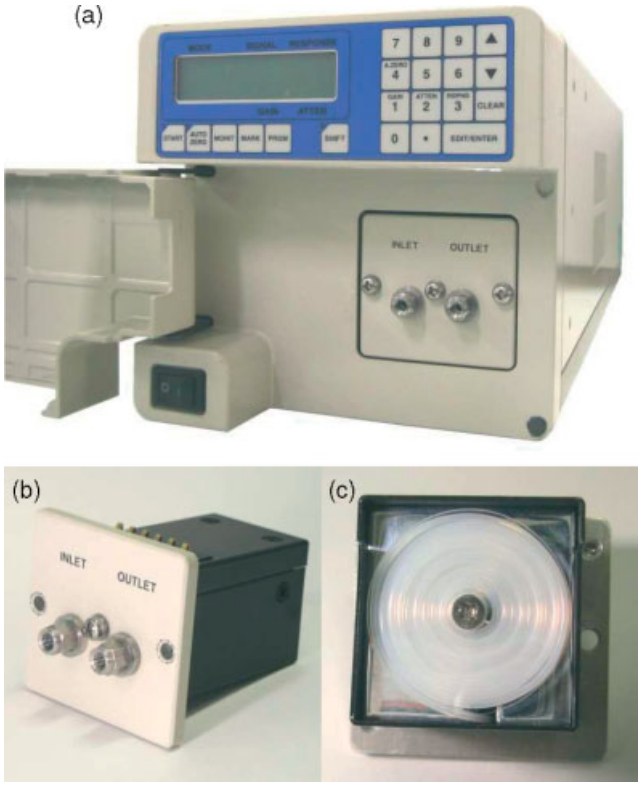

Figure 5. (a) The Jasco CL-2027 chemiluminescence detector with front door open. (b) Flow-cell module removed from detector. (c) Rear view of flow-cell module showing the coiled-tubing detection zone. Courtesy of JASCO Corporation.

using commercially available detectors; the most popular are those developed by Jasco Corporation.

The Jasco model CL-2027 detector is shown in Figure 5. The flow cell module is contained within a detector unit that provides programmable control of parameters such as photomultiplier tube voltage (gain), attenuation, response speed, and flow-cell temperature.[47] Digital filtering is available to improve signal-to-noise ratios, and bulkhead fittings and standard analogue outputs allow convenient connection to any liquid chromatography system.

In the standard configuration (Figure 6a), a single solution line runs from the inlet fitting through a hole in the rear of the flow cell module, where it is coiled to form the detection surface, and finally connected to the outlet fitting. This design, where the column eluate and chemiluminescence reagents are combined prior to the mixed solution entering the module, is appropriate for medium- to long-lived chemiluminescence reactions. In an alternative (custom) configuration designed for fast reactions (Figure 6b), the chemiluminescence reagent is pumped through an inlet into the module, where it is merged with the column eluate at a T-connector close to the entrance of the detection coil.[49] In either configuration, the tubing can be replaced to provide the optimum conditions for any particular procedure.

Global FIA has recently developed a detector that contains a white Teflon disk with an engraved spiral channel that is sealed with a sapphire plate and mounted against the window of a photomultiplier tube (Figure 7).[15,50] The use of precisely machined channels enables flow-cell configurations that could not be achieved with tubing. For example, the introduction

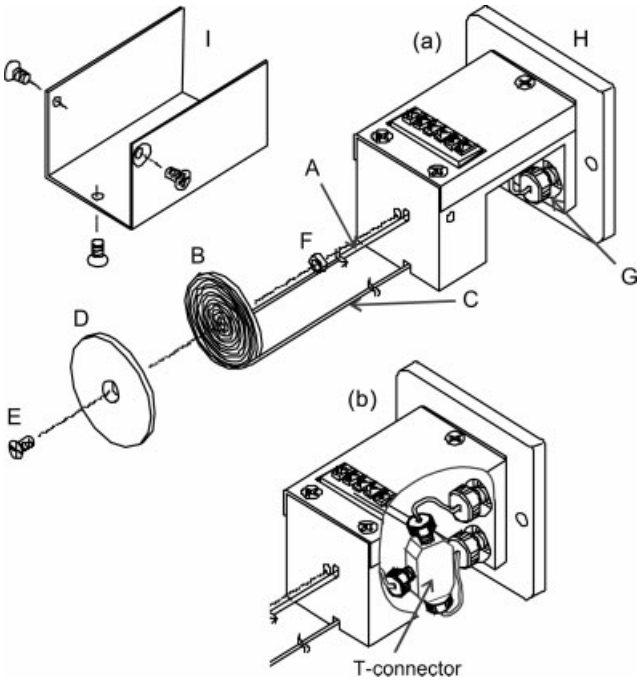

Figure 6. Exploded view of the flow-cell for the Jasco CL-2027 chemiluminescence detector. (a) Standard flow cell configuration (A: inlet tubing, B: coiled PTFE tubing flow cell, C: outlet tubing, D: window plate, E: screw, F: spacer, G: inlet fitting (outlet fitting not shown), H: external panel), I: cover. (b) Optional configuration with T-connector to mix sample and reagent just before the coil. Courtesy of JASCO Corporation.

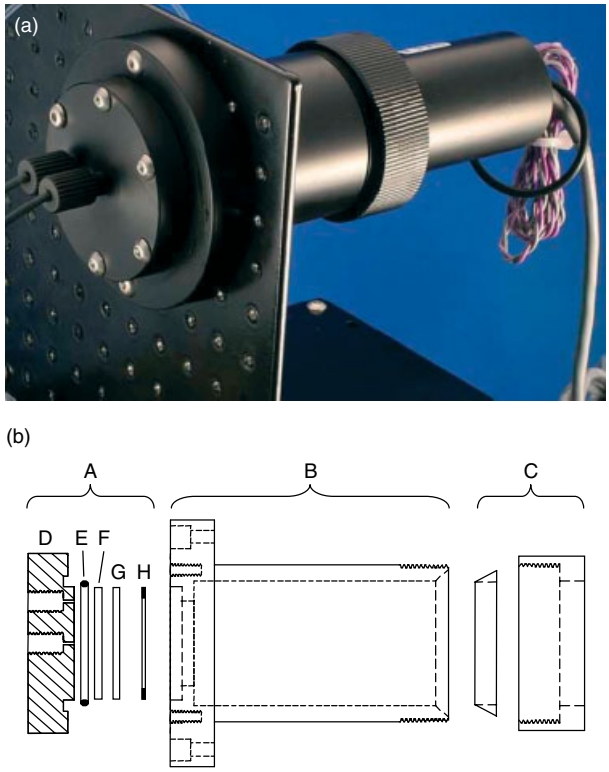

Figure 7. (a) GloCel chemiluminescence detector (shown with single-inlet configuration and with photon-counting module). Courtesy of Global FIA (http://www.globalfia.com/). (b) Exploded diagram of GloCel detector, comprising: A: flow-cell, B: main body to house the PMT module and position it against the flow-cell window, and C: nut and ferrule to light-seal the PMT compartment. The flow-cell consists of D: back plate (single-inlet configuration), which is fastened to the main detector body to seal the flow-cell; E: light-seal 'O' ring; F: Teflon disc with machined channels; G: sapphire window; and H: flat gasket. (Figure 7b reprinted with permission.[15] Copyright (2008) American Chemical Society).

of over 100 reversing turns into the spiral channel increased the intensity of the chemiluminescence by enhancing mixing efficiency.[15,16] Examples of chemiluminescence emanating from these 'serpentine' configuration flow-cells are shown in Figure 8.

Photodetector

The exceedingly low limits of chemiluminescence detection are derived from the measurement of very low levels of light against a dark background, which has most commonly been achieved with photomultiplier tubes.[7–9,52]

In our laboratory we have often used 29 mm diameter end-window photomultiplier tubes with enhanced green sensitive bialkali (model: 9924SB, wavelength range: 280–680 nm) or red sensitive (9828SB, 280–870 nm) photocathodes from ET Enterprises (formerly part of Electron Tubes Limited).[53] These photomultiplier tubes are generally operated at 900–1100 V, provided by a PM20S or PM20D power supply *via* an encapsulated voltage divider (e.g. E637–03 with B14B socket). Current output from the photomultiplier tube is converted to voltage with a transimpedance amplifier (model A1), or in the case of the photon counting approach, converted to an RS232 signal with an amplifier/discriminator and counter-timer module.

A simpler and more cost-effective (but less versatile) alternative is the use of photomultiplier modules from Sens-Tech (formerly Electron Tubes Limited, and Thorn EMI Electron Tubes before that), which contain high-voltage power supply, a photomultiplier tube, a voltage divider, and signal processing electronics, within a relatively compact housing (Figure 9).[54] The P30A-05 module, for example, contains an end-window photomultiplier with red sensitive (9828B) photocathode, negative high-voltage power supply and a high gain dc-coupled transimpedance amplifier, within a cylindrical mumetal case. An external 5 V power supply is required. The P30CWAD5-01 module is the photon-counting equivalent, containing a high-speed amplifier-discriminator. Other brands of photomultiplier tubes are also available; those from Hamamatsu Photonics (Japan) are a popular option.[55]

Detector Housing

Any suitably sized container that can be made light-tight can be used to house the detector. One example, constructed for an industrial process environment is shown in Figure 10. For our research, we have often used a small wooden or metal box (16 cm × 24 cm × 8 cm), into which we drilled holes for the solution inlets and outlet, and electrical cables. To prevent light entering the container, we have used black PTFE tubing (16T-030-BL; Global FIA) for the chemiluminescence reagent and waste-tubing lines. The PMT was mounted above the base of the container to protect the electronics in case of leaks due to blocked lines. A solution outlet at the base (a piece of black pump tubing) was used as an additional precaution. Commercially available photomultiplier tube housings such as those from ET Enterprises[56] could also be used to mount the photodetector against a chemiluminescence flow-cell. The GloCel detector housing shown in Figure 7 was designed to fit the Sens-Tech photomultiplier modules described in the previous section.

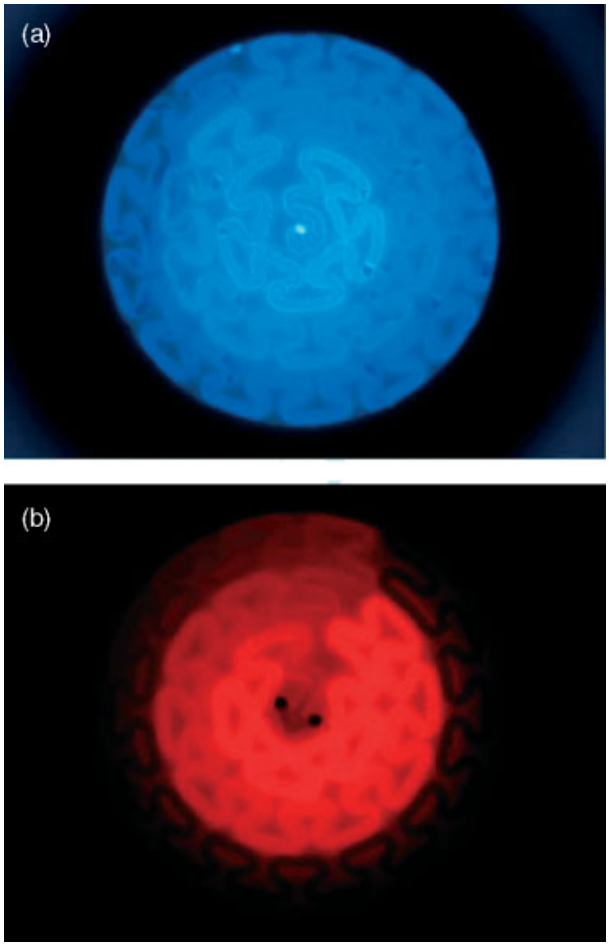

Figure 8. Chemiluminescence from the reaction of (a) luminol, hydrogen peroxide and potassium hexacyanoferrate(III) within the Global FIA 'GloCel' detector with single-inlet serpentine insert,[15] and (b) acidic potassium permanganate and morphine (enhanced with manganese(II)[51]) within the GloCel detector with dual-inlet serpentine insert.[15].

Figure 9. Photomultiplier modules available from Sens-Tech.[54].

Data Collection

The output from chemiluminescence detectors in flow analysis was originally documented using chart recorders and while this approach remains acceptable for some applications, there are now many options for digital data acquisition that provide convenient collection, manipulation and storage of information, and more accurate interpretation. Considering the vast quantities of information that can be derived from even a single chromatogram and the need for accurate comparison between samples, computer controlled data acquisition is essential to obtain the best results from modern liquid chromatography with chemiluminescence or any other mode of detection.

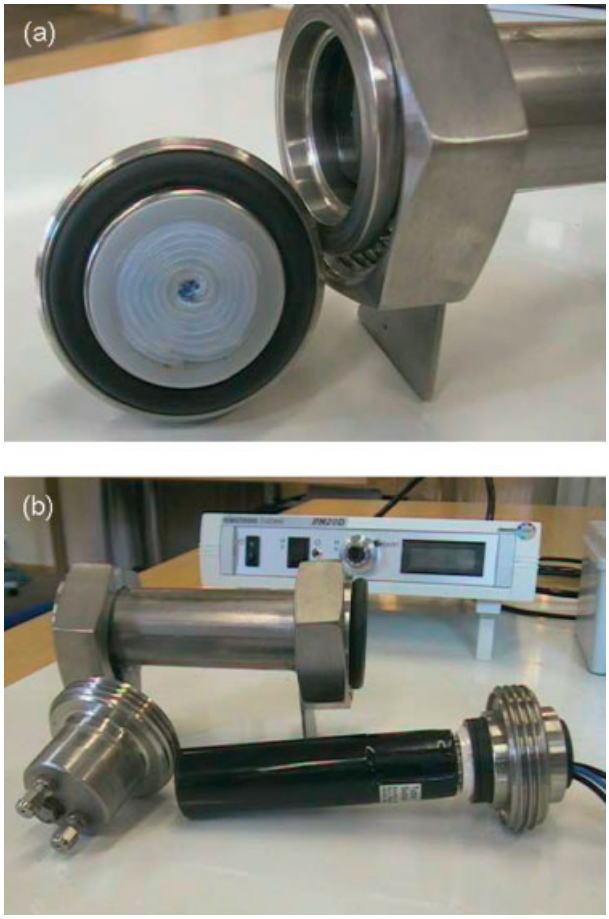

Figure 10. A chemiluminescence detector with (a) coiled-tubing flow cell, and(b) purpose-built housing. Photographs courtesy of GlaxoSmithKline Australia.

A convenient replacement for chart recorders in any flow analysis system is the 'e-corder' from eDAQ,[57] which, when coupled with a computer (*via* USB) enables collection, display, and analysis of signals from a wide range of laboratory instruments. Data from different sources can be collected on independent channels, the number of which depends on the model (2 to 16). The e-corder is supplied with Chart and Scope software, which provide modes of operation analogous to a chart recorder and oscilloscope, but with many additional features.[58] Simple chromatographic analysis could be conducted using the Chart software, but more sophisticated PowerChrom software, designed specifically for chromatographic analysis, is also available.

Global FIA has developed FloZF software for the control of flow-based systems, which includes capabilities for acquisition and processing of data from the GloCel chemiluminescence detector.

The output signal from the laboratory-built or commercial chemiluminescence detectors described in the previous sections can also be acquired using an auxiliary channel of the instrument control and data acquisition software of commercial liquid chromatography systems. In our laboratory, for example, we have often used Agilent ChemStation software to simultaneously monitor the signals from the UV-Vis absorption detector and our laboratory-built chemiluminescence detectors (*via* an analogue to digital converter; Agilent Technologies).

Concluding Remarks

Chemiluminescence detectors have been used extensively in HPLC and they are instrumentally simpler than their more commonly employed UV-visible absorbance or fluorescence counterparts. Furthermore, they offer enhanced selectivity and sensitivity with no requirement for energy sources or spectrometers for wavelength discrimination. Several factors must be considered, however, including the rate of the light-producing chemical reaction, efficiency of solution mixing, volume of solution within the flow cell, and transfer of light to the photodetector, to obtain the best results from this mode of detection. The evolution of chemiluminescence from academic laboratories to routine HPLC analyses in industry, and in bioscience and biotechnology would be greatly enhanced if purpose-built detectors were available from the major chromatographic instrument companies.

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References

[1] N. Kuroda, M. Kai, K. Nakashima, in *Chemiluminescence in Analytical Chemistry* (Eds: A. M. García-Campaña, W. R. G. Baeyens), Marcel Dekker: New York, **2001**, pp. 393.
[2] P. S. Francis, N. W. Barnett, S. W. Lewis, K. F. Lim, *Luminescence* **2004**, *19*, 94.
[3] N. W. Barnett, P. S. Francis, in *Encyclopedia of Analytical Science, 2nd edn, Vol. 1* (Eds: P. J. Worsfold, A. Townshend, C. F. Poole), Elsevier: Oxford, **2005**, pp. 511.
[4] M. Tsunoda, K. Imai, *Anal. Chim. Acta* **2005**, *541*, 13.
[5] B. A. Gorman, P. S. Francis, N. W. Barnett, *Analyst* **2006**, *131*, 616.
[6] J. L. Adcock, P. S. Francis, N. W. Barnett, *Anal. Chim. Acta* **2007**, *601*, 36.
[7] P. S. Francis, C. F. Hogan, in *Advances in Flow Injection Analysis and Related Techniques, Comprehensive Analytical Chemistry Series, Vol. 54* (Eds: I. D. McKelvie, S. D. Kolev), Elsevier: Oxford, **2008**, pp. 343.
[8] L. Gamiz-Gracia, A. M. Garcia-Campana, J. F. Huertas-Perez, F. J. Lara, *Anal. Chim. Acta* **2009**, *640*, 7.
[9] P. S. Francis, J. L. Adcock, in *Hyphenated and Alternative Methods of Detection in Chromatography* (Ed.: R. A. Shalliker), CRC Press: Boca Raton, Florida (in press).
[10] N. W. Barnett, P. S. Francis, in *Encyclopedia of Analytical Science, 2nd edn, Vol.1* (Eds: P. J. Worsfold, A. Townshend, C. F. Poole), Elsevier: Oxford, **2005**, pp. 506.
[11] D. Pérez-Bendito, M. Silva, in *Chemiluminescence in Analytical Chemistry* (Eds.: A. M. García-Campaña, W. R. G. Baeyens), Marcel Dekker: New York, **2001**, pp. 175.
[12] J. L. Adcock, P. S. Francis and N. W. Barnett, *J. Fluoresc.* **2009**, *19*, 867.
[13] J. L. Adcock, P. S. Francis and N.W. Barnett, *Anal. Chim. Acta*, **2009**, *652*, 303.
[14] P. S. Francis, N. W. Barnett, S. W. Lewis and K. F. Lim, *Talanta*, **2004**, *64*, 283.
[15] J. M. Terry, J. L. Adcock, D. C. Olson, D. K. Wolcott, C. Schwanger, L. A. Hill, N. W. Barnett, P. S. Francis, *Anal. Chem.* **2008**, *80*, 9817.
[16] S. Mohr, J. M. Terry, J. L. Adcock, P. R. Fielden, N. J. Goddard, N. W. Barnett, D. K. Wolcott, P. S. Francis, *Analyst*, **2009**, *134*, 2233.
[17] S. Stieg, T. A. Nieman, *Anal. Chem.* **1978**, *50*, 401.
[18] D. J. Tucker, B. Toivola, C. H. Pollema, J. Ruzicka, G. D. Christian, *Analyst*, **1994**, *119*, 975.
[19] P. S. Francis, S. W. Lewis, K. F. Lim, K. Carlsson, B. Karlberg, *Talanta*, **2002**, *58*, 1029.
[20] J. L. Adcock, P. S. Francis, K. M. Agg, G. D. Marshall, N. W. Barnett, *Anal. Chim. Acta*, **2007**, *600*, 136.
[21] M. Miró, J. M. Estela, V. Cerdà, *Anal. Chim. Acta*, **2005**, *541*, 57.
[22] H. Kodamatani, Y. Komatsu, S. Yamazaki, K. Saito, *J. Chromatogr. A*, **2007**, *1140*, 88.
[23] R. Nakao, K. Furutsuka, M. Yamaguchi, K. Suzuki, *Anal. Sci.* **2007**, *23*, 151.
[24] K. Takezawa, M. Tsunoda, K. Murayama, T. Santa, K. Imai, *Analyst*, **2000**, *125*, 293.
[25] S. Nakamura, M. Wada, B. L. Crabtree, P. M. Reeves, J. H. Montgomery, H. J. Byrd, S. Harada, N. Kuroda, K. Nakashima, *Anal. Bioanal. Chem.* **2007**, *387*, 1983.
[26] S. Ahmed, N. Kishikawa, K. Nakashima, N. Kuroda, *Anal. Chim. Acta*, **2007**, *591*, 148.
[27] M. Fukumoto, M. Saitoh, H. Kubo, *Anal. Sci.* **2000**, *16*, 97.
[28] G. Rule, W. R. Seitz, *Clin. Chem.* **1979**, *25*, 1635.
[29] W. R. Seitz, K. Van Dyke, *Crit. Rev. Anal. Chem.* **1981**, *13*, 1.
[30] A. Townshend, *Analyst*, **1990**, *115*, 495.
[31] R. W. Abbott, A. Townshend, R. Gill, *Analyst*, **1986**, *111*, 635.
[32] K. Honda, K. Miyaguchi, H. Nishino, H. Tanaka, T. Yao, K. Imai, *Anal. Biochem.* **1986**, *153*, 50.
[33] M. Maeda, A. Tsuji, *J. Chromatogr.* **1986**, *352*, 213.
[34] R. L. Veazey, T. A. Nieman, *J. Chromatogr.* **1980**, *200*, 153.
[35] M. S. Gandelman, J. W. Birks, *J. Chromatogr.* **1982**, *242*, 21.
[36] E. A. Boyle, B. Handy, A. Van Geen, *Anal. Chem.* **1987**, *59*, 1499.
[37] K. M. Scudder, C. H. Pollema, J. Ruzicka, *Anal. Chem.* **1992**, *64*, 2657.
[38] J. Cepas, M. Silva, D. Perez-Bendito, *Anal. Chem.* **1995**, *67*, 4376.
[39] H. Nakamura, Y. Murakami, K. Yokoyama, E. Tamiya, I. Karube, M. Suda, S. Uchiyama, *Anal. Chem.* **2001**, *73*, 373.
[40] T. Pérez-Ruiz, C. Martínez-Lozano, M. D. García, *J. Chromatogr. A*, **2007**, *1169*, 151.
[41] S. N. Brune, D. R. Bobbitt, *Anal. Chem.* **1992**, *64*, 166.
[42] A. Safavi, M. A. Karimi, M. R. H. Nezhad, *Luminescence*, **2005**, *20*, 170.
[43] Y. Wei, Z.-J. Zhang, Y.-T. Zhang, Y.-H. Sun, *J. Chromatogr. B*, **2007**, *854*, 239.
[44] H. Shi, X. Xu, Y. Ding, S. Liu, L. Li, W. Kang, *Anal. Biochem.* **2009**, *387*, 178.
[45] N. Li, J. Guo, B. Liu, Y. Yu, H. Cui, L. Mao, Y. Lin, *Anal. Chim. Acta*, **2009**, *645*, 48.
[46] P. S. Francis, J. L. Adcock, N. W. Barnett, *Spectrochim. Acta, Part A*, **2006**, *65*, 708.
[47] Optical Detectors (Jasco Corporation), http://www.jascoinc.com/Products/Chromatography/HPLC-Systems/HPLC-Components/Optical-Detectors.aspx [January 2010].
[48] Model 660 HPLC Chemiluminescence Detector (McPherson Inc.), http://www.mcphersoninc.com/hplcdetectors/model660description.htm [January 2010].
[49] M. Martínez Galera, M. D. Gil García, R. Santiago Valverde, *J. Chromatogr. A*, **2006**, *1113*, 191.
[50] GLO-CEL-1 Chemiluminescence flow cell (Global FIA Inc.), http://174.132.81.251/~globalfi/index.php?page=shop.product_details&product_id=71&flypage=flypage-ask.tpl&pop=0&option=com_virtuemart&Itemid=54 [January 2010].
[51] T. Slezak, J. M. Terry, P. S. Francis, C. M. Hindson, D. C. Olson, D. K. Wolcott, N. W. Barnett, *Anal. Chem.* **2010**, *82*, 2580.
[52] A. C. Calokerinos, L. P. Palilis, in *Chemiluminescence in Analytical Chemistry* (Eds: A. M. García-Campaña, W. R. G. Baeyens), Marcel Dekker: New York, **2001**, pp. 321.
[53] Photomultipliers (ET Enterprises Ltd), http://www.electrontubes.com/ [January 2010].
[54] Photodetector modules (Sens-Tech Inc.), http://www.sens-tech.com/modules/modules.html [January 2010].
[55] Photomultiplier Tubes (Hamamatsu Photonics), http://jp.hamamatsu.com/products/sensor-etd/pd002/index_en.html [February 2010].
[56] Ambient Temperature Housings (ET Enterprises Ltd), http://www.electrontubes.com/ambient-housings/ [January 2010].
[57] eDAQ Hardware: e-corder (eDAQ), http://www.edaq.com/ecorder.html [February 2010].
[58] Software: Chart™ and Scope™ (eDAQ), http://www.edaq.com/chartandscope.html [February 2010].