Chemiluminescence Chromatography Detectors

This article covers gas chromatography (GC) and liquid chromatography (LC) detectors based on chemiluminescence (CL), which is defined as the emission of radiation (light) from molecules caused by a chemical reaction. Like the evaporative light scattering detector (ELSD), LC with a CL detector can be used to determine analytes lacking a chromophore that provides visible (vis) or ultraviolet (UV) radiative absorption. GC–CL detectors are valuable because they have greater selectivity for compounds with particular atoms compared to the flame ionization detector (FID) and thermal conductivity detector.

An analyte to be detected by CL must chemiluminesce when mixed with a specific reagent, catalyze a CL reaction between other reagents, or suppress a background CL signal produced by reaction of other reagents. A classic example of a CL determination that appears in several analytical textbooks is based on the reaction of nitrogen monoxide (NO) with ozone (O₃) to produce nitrogen dioxide (NO₂) in an excited state and oxygen (O₂). The NO₂ emits photons of radiation around 1200 nm when it returns to ground state, which are measured with a detector such as a photomultiplier tube (PMT). This reaction can be used to determine O₃ (with an excess of NO) or NO (with excess O₂). In other CL analyses, the products of the reactions leading to the emitted radiation may not be totally defined.

Commercial Chemiluminescence Detectors

The following are brief descriptions of some CL detectors that are currently available commercially. The coverage is selective rather than exhaustive in terms of the number of companies and instruments described, as well as the features, options, and applications mentioned for each instrument. Readers are encouraged to contact the manufacturers for their complete CL detector lines and detailed instrument specifications.

Flame Photometric GC Detector

The gas chromatography flame photometric detector (FPD), which was invented over 30 years ago, uses CL reactions in a hydrogen/air flame to detect compounds containing sulfur or phosphorus atoms. The emitting species for sulfur compounds is excited S₂, with a wavelength of maximum emission at 394 nm. Excited HPO is formed from phosphorus compounds and emits at 526 nm. Selective detection is obtained by inserting an appropriate interference filter between the flame and the PMT, which converts the photons reaching it to a proportional electrical signal.

A schematic diagram of the SRI Instruments FPD (available from Alltech) is shown in Figure 1. The secondary hydrogen flow augments sensitivity by making the flame hydrogen rich, and the second air flow across the face of the PMT prevents hydrogen molecules from permeating its glass window and causing malfunction. Selectivity is 10⁶ compared to hydrocarbons, and detection limits are about 200 ppb for sulfur compounds and 10 ppb for phosphorus compounds. The linear range for phosphorus is 10⁻¹⁰⁻¹⁰, while sulfur response is exponential. The Dual FPD model with two PMTs is available for simultaneous detection of compounds containing S or P atoms. Either the single or dual GC–FPD instrument

![Figure 1. Simplified schematic diagram of the SRI FPD (courtesy of SRI Instruments).](image-url)
can be equipped with an FID for simultaneous detection of hydrocarbon peaks. However, the hydrogen-rich flame needed for optimum FPD detection of sulfur and phosphorus is cooler than the oxygen-rich flame usually used with an FID, so hydrocarbon detection levels will be lower.

The FPD has been used widely for determinations such as sulfur- and phosphorus-containing pesticides in environmental and food samples; sulfides, thiois, and methylthiophosphates in aqueous and other environmental samples; and sulfur odorants and impurities in petroleum products. The FPD with a tin-specific interference filter (SnH measured at 610 nm, or 394 nm for quartz surface-induced luminescence) has been applied to analysis of marine paint coatings, foods, tissues, and environmental samples for organotin (e.g., dibutyl and triphenyl) compounds. Thermo Finnigan offers an application sheet describing simultaneous determination of P- and Sn-containing organic compounds with a dual FPD.

**Pulsed Flame Photometric GC Detector**

The Model 5380 Pulsed FPD (PFPD) from OI Analytical (Figure 2) can be optimized for selective P and S detection as well as 28 other elements, including N, As, Sn, Se, Ge, Te, Sb, Br, Ga, In, and Cu. Stated limits of detection are <1 pg S/sec and <100 fg P/sec, and selectivities are >10^6 S/C and >10^7 P/C. Dual channel (dual gate) operation permits simultaneous detection of S+P, P+N, S+N, S+C, or other element pairs. For quantitative analysis, the detector is first order linear over four orders of magnitude for P and quadratic in response for S (linearity to ca. three orders of magnitude, which gives five orders of signal response). Response is equimolar +/-8% for S and P.

PFPD operation is based on a propagating flame that terminates within a reactive volume (the combustor). As a result of gas phase reactions in the flame, molecular products emit chemiluminescent radiation with specific emission wavelengths and emission lifetimes. The differences in emission lifetimes combined with the propagation and termination properties of the flame permit both spectral and time information to be used to improve detection selectivity, decrease detector noise, and increase sensitivity. Use of a propagating flame lowers the needed combustible gas flow rates, and gated electronics permit rejection of noise outside the gate window.

Applications recommended by OI Analytical include total sulfur content in petrochemicals, process streams, and drugs; P and S pesticides in foods and water, soil, and sludges; organometallic detection; P, S, As, and Si in semiconductors; volatile sulfur compounds in beverages (headspace GC); sulfur compounds in beer (purge and trap GC); S and N in pharmaceuticals; SO_2 and NH_3 in beverage grade CO_2; arsenic–arsine in propylene; nitrate esters and other explosives; thiophene in benzene (ASTM standard); S, P, N, and As in chemical warfare agents; light sulfur compounds in pulp mill effluents; and organotin compounds in environmental samples. The PFPD can be coupled with mass spectrometry (MS) for simultaneous detection.

Details of PFPD operation as described by Chasteen (1) are as follow (Figure 3): Flows of two different combustible gas mixtures of hydrogen and air enter the bottom of the combustion chamber (or combustor tube) through narrow gas lines, the primary combustion gas plus analyte from the GC column, and the second gas mixture. The flow of the second gas helps to fill up the outer volume of the combustion chamber (and outside the combustion zone, which is the center of the combustor tube). This second flow also helps to optimize the analytic emission brightness in the combustion process. When the gases flowing into the combustor, including the column effluent, reach a flammable mixture, they are ignited by the red-hot ignition wire at the top of the PFPD, and the flame propagates back down the combustor. The flame front terminates (i.e., uses up all of the quickest burning flammable material in the combustor) in less than 10 msec, and the flame goes out. It is after this short flame pulse that
the slower burning analytes are excited and emit light characteristic of their elements, which is recorded by the PMT. After about 300 msec, the flame pulses again as new flammable material fills the combustion chamber from the inlet tubes and GC column and that mixture again constitutes a flammable mixture. In this way, about three flame pulses are recorded per second. The shape of the flame pulse profile (plot of the PMT signal representing light emission vs time in msec) depends on the particular elements in the analytes present in the flame. By using a gated amplifier, a specific part of each pulse is chosen to amplify and record. The ability to use different computer controlled amplifier gating schemes allows the PFPD to be configured to selectively and sensitively detect analytes containing a specific element but not to detect others that are coeluting.

Detailed information on the operation, advantages, and applications of PFPD detectors is available in the article by Amirav et al. (2). Varian is a second commercial source of a PFPD detector.

Nitrogen Chemiluminescence LC Detector

The Antek Model 8060 Chemiluminescence Nitrogen Detector (CLND; Figure 4) provides detection of compounds with at least one nitrogen atom that lack a UV-absorbing chromophore, without derivatization. The operation principles of the CLND are shown in Figure 5. N-containing compounds in the column effluent are nebulized and then react with oxygen at 1050°C to produce nitric oxide. Vaporized water in the effluent is removed in a membrane dryer, and the dried gas is reacted in a chamber with ozone to produce excited nitrogen dioxide. Upon decay to ground state, radiation is emitted at 600–900 nm, passes through an optical filter, and is measured by a PMT. The LC mobile phase cannot include solvents containing nitrogen (e.g., acetoniitride).

Sensitivity of the CLND is 0.1 ng of N, calibration curve linearity >10<sup>4</sup>, selectivity against compounds without N >10<sup>4</sup>, and response equimolar within 5–10% so that mixtures can be quantified with a single standard. Only chemically bound N is detected, not N<sub>2</sub>.

Applications reported by Antek for the CLND have included drug compounds synthesized by standard and combinatorial methods and N-containing vitamin formulations and dietary supplements. The CLND and ELSD have been compared (3) for analysis of caffeine and three other pharmaceutical compounds that lacked sufficient UV chromophores and had different numbers and bonding of nitrogen atoms. The use of LC–CLND combined with UV and MS detection has been shown to provide a very powerful analytical system for identification, quantification, and purity determination of small N-compounds and peptides.

Nitrogen and Sulfur Chemiluminescence GC Detector

Antek offers three GC detectors based on CL, the Model 7090N for nitrogen compounds, the 7090S for sulfur compounds, and the 7090NS for nitrogen and sulfur detection (Figure 6). The chemistry behind nitrogen detection is the same as described for the Model 8060 above. Antek gives the following
three reactions as the basis of sulfur CL detection:

\[
\text{R-S + R-H + O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{SO}_2 + \text{MO}_x
\]

\[
\text{SO}_2 + \text{H}_2 \rightarrow \text{H}_2\text{S} + \text{other reduced sulfur species}
\]

\[
\text{H}_2\text{S} + \text{other reduced sulfur species} + \text{O}_3 \rightarrow \text{SO}_2^*
\]

in which \( \text{MO}_x \) represents various other oxides and \( \text{SO}_2^* \) is excited sulfur dioxide, which returns to ground state with emission of CL and detection by a PMT at 200–400 nm. The reaction of eluting sulfur compounds with oxygen takes place at >1000°C. Response is equimolar (+/-10%) for all S- and P-compounds, and sensitivity limits are in the ppb range (<1 pg S/sec). An FID can be added to the 7090NS so that results for N, S, and hydrocarbons can be obtained with one sample injection. Application areas suggested by Antek include agriculture, biotechnology, biochemical, foods and flavors, solid phase synthesis, organic synthesis, petrochemical, petroleum, pharmaceutical, and polymers/plastics. The Model 7090N can be used to selectively detect nitrosamines at ppm–ppb levels without any hardware changes. Reaction of R-N-NO with oxygen is carried out at 350–550°C, causing cleavage of the N-NO bond to produce NO. The NO is subsequently reacted with ozone to form excited nitrogen dioxide and CL emission.

The Ionics Sievers Models 355 SCD and 255 NCD are other commercial CL S- and N-selective GC detectors, respectively. The Model 355 (Figure 7) has a sensitivity of 0.5 pg S/sec and equimolar response to S-compounds. The reaction mechanism given for it is shown in the following equations; the combustion process occurs at >1800°C:

\[\text{S-compound} + \text{O}_3 \rightarrow \text{SO} + \text{other products}\]

\[\text{SO} + \text{O}_3 \rightarrow \text{SO}_2 + \text{O}_2 + \text{hv}\]

The light (hv) is detected by a PMT after passing through an optical filter. Accessories available for the Model 335 are an FID adapter that allows direct simultaneous determination of S-compounds and hydrocarbons without column effluent splitting and a decocking valve to prevent accumulation of carbon in the stainless steel burner when samples have high hydrocarbon content. General application areas stated by Ionics are gases, petroleum and petrochemical products, polymers, chemicals, foods, beverages, fragrances, pesticides, and environmental samples. Figure 8 shows a chromatogram resulting from the GC analysis of sulfur compounds in air at <1 ppm without preconcentration. A method for analysis of sulfur compounds in beverage grade carbon dioxide using the Ionics Sievers Model 355 as well as atomic emission and flame photometric detectors is available from Agilent Technologies (4).

The Ionics Sievers Model 255, like the Antek Model 8060, is based on high temperature combustion of nitrogen-containing compounds to form NO, followed by detection by a PMT of light produced by a subsequent CL reaction of NO with O₃. Response is linear and equimolar, and organonitrogen com-
pounds, ammonia, hydrazine, hydrogen cyanide, and NOx are detected.

**Sulfur Chemiluminescence LC Detector**

The Antek web site contains information on the Model 8040 sulfur-selective chemiluminescence LC detector. CL emission results from the same three reactions described above for the GC CL detector. Applications cited include pharmaceutical, biomedical, biotechnical, agricultural, foods, and petrochemicals. According to discussions with a company representative, this detector is under development and not being sold at this time.

**LC Chemiluminescence Detector for Fluorescent Compounds or Compounds that Suppress Chemiluminescence**

These detectors are based upon combination of the analyte with reagents in a postcolumn reactor to initiate a CL reaction, followed by measurement of the intensity of the emitted light with a detector such as a PMT. The sensitivity and/or selectivity of detection by CL can be greater than that obtained by fluorescence detection for some analytes under certain conditions of temperature, ionic strength, pH, and solution composition. One reason for increased sensitivity is that a UV light source for excitation of fluorescence is not needed, thereby reducing detector noise from stray or scattered light and source instability. Neither is a filter needed to isolate a discrete emission wavelength.

A common reaction that is used involves hydrogen peroxide oxidation of an aryl oxalate ester [such as bis (2,4,6-trichlorophenyl) oxalate (TCPO)] in a mixed organic-aqueous solution, which produces the high energy intermediate 1,2-dioxethane-dione. This product reacts with a fluorescent analyte, resulting in decomposition with energy transfer to the analyte. The excited analyte returns to ground state with emission of CL. The analyte can be hydrogen peroxide, but it is usually a naturally fluorescent compound or fluorescent derivative. Specific fluorophores that have been determined by TCPO CL include 9,10-diphenylanthracene and derivatized propentofylline (detection limit 31 fg/injection).

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) can be oxidized by hydrogen peroxide in basic aqueous solution in the presence of a metal cation catalyst [e.g., cobalt (II)], forming excited 3-aminophthalate, which emits CL at 425–445 nm. An analyte that suppresses this reaction, e.g., because it complexes with the metal ions, can be quantified based on the degree of reduction of a baseline signal of emitted light. Amino acids can complex metals and be determined in this manner. The luminol system has been used to determine the following other analytes: luminol, luminol-like derivatives, hydrogen peroxide or compounds that can be converted into peroxides, and metal cations.

The chemiluminescence reactions of luminol and TCPO were described by Chasteen (5).

McPherson sells the Model 660 CL detector (Figure 9) as a part of a complete LC system or as a modular detector head for use with a laboratory’s existing LC pumps, plumbing, and PC or Mac.
computer. The detector has a spiral flow design that places the reagent mixing point directly in front of the PMT, leading to picomolar detection sensitivity for compounds such as dansyl amino acids. McPherson lists common CL reagents for use with the Model 660 as luminol, lucigenin, and lophine; the oxidants hydrogen peroxide, hypochlorite, and ferricyanide; and imidazole catalyst.

The Shodex CL-2 CL detector (distributed by JM Science Inc. among other companies) uses a spiral-type flow cell and single photon counting system for detection of some compounds at levels as low as atto moles. The measuring wavelength range is 300–650 nm. Applications listed for the detector include easily oxidizable fluorescent compounds; hydrogen peroxide and compounds related to the generation of hydrogen peroxide (i.e., glucose, choline, acetylcholine, urea, creatine, and bile acids); pharmaceutical and environmental compounds that easily undergo CL reaction (i.e., amino acids, amines, organic acids, immunoproteins, and polycyclic aromatic hydrocarbons); and cobalt ions. Figure 10 shows the higher detection sensitivity of femtomole levels of dansyl amino acids by CL compared to fluorescence.

The Cam spec CL detector (Figure 11) is useful for LC and flow injection analysis (FIA). Two versions, the CL-1 and CL-2, have a three-port flow cell design that allows integral flow mixing of the reagents and sample in the cell. The CL-1 flowcell has a 120 mL volume and 5 mm working path length, solid state detector, and 320–900 nm wave-
metal ions, inorganic ions, biomolecules, carcinogens, and drugs have been reported.

The Jasco Model CL-2027 (Figure 12) is a CL detector equipped with a coiled PTFE tube flow cell placed immediately next to an end-on PMT and digital filtering for analysis of femtomole levels of compounds including lipids, nucleotides, nitrogen oxides, and catecholamines. The wavelength range is 300-600 nm.

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Bibliography