Chemiluminescence

22

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22.1 Introduction

Luminescence is the production of light by organisms. Reactions occurring inside the bioluminescent organism release the energy and emit light. Bioluminescent organisms such as fireflies, glowworms, anglerfish, and those responsible for the phosphorescence of the sea are often familiar (Fig. 22.1). Still there are many marine organisms such as shrimp and squid which show more vivid effects. These chemical species are different from fluorescence.

Most bioluminescent reactions involve luciferin and luciferase. In the presence of oxygen enzyme, luciferase acts as an organic molecule luciferin to produce energy in terms of photon and oxyluciferin. Some bioluminescent organisms produce their own light, either making all of the ingredients themselves or making everything their own. Instead, some have a symbiotic relationship with bioluminescent bacteria that live inside their bodies which don't produce their own light. Various biochemical sequences are used to produce light. In the simplest case, luciferin is a small heterocyclic organic molecule whose enzyme-catalyzed oxidation leads to the formation of product, oxyluciferin. Luciferase involved uses common cofactors in the reaction shown in mechanism (Fig. 22.2). These cofactors are often central to light-emitting reaction and thus can be coupled to many reactions of biological significance, for example, bioluminescent reactions found in firefly (ATP as cofactor), *Renilla* (PAPS as cofactor), and luminous bacteria (NADH or NADPH as cofactor).

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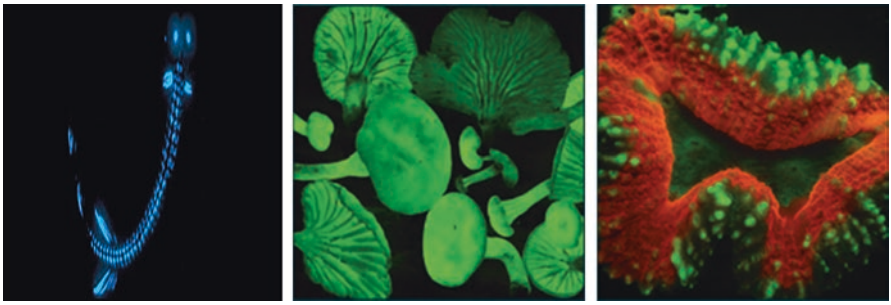
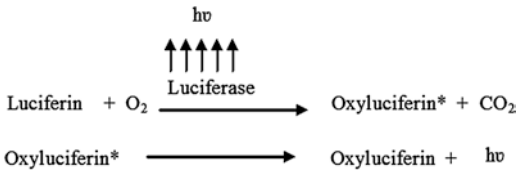


Fig. 22.1 Bioluminescent lucifer, mushrooms, and fluorescent corals. (Adapted from Google images)

Fig. 22.2 Bioluminescent reaction mechanism. (Adapted from Fraga et al. 2006)



22.2 Characteristics of the Light Emission

A large amount of energy is released when bioluminescence results from a chemical reaction which leads to the product molecule populated in excited electronic state instead of being dissipated in the form of heat as in a normal chemical reaction. Bands are found to be broad for the bioluminescence spectra with widths at half-height around 60–100 nm. Visible radiation appears in the wavelength range of 400–700 nm (Fig. 22.3). Terrestrial organisms have yellow-green bioluminescence color, whereas marine species show bioluminescence 450–510 nm. Maximum transmission luminescence has been achieved in ocean water for blue to green, whereas terrestrial group achieves the maximum transmission for yellow light.

22.3 Chemiluminescence

Light is emitted from the chemical reactions at ordinary room temperatures in chemiluminescence. The radiation produced may be in the UV, infrared, or visible region. The radiative process is similar to fluorescence when the excited state is a singlet and is similar to phosphorescence when the excited state is triplet. Several mechanisms have been described to explain the manner by which chemical energy is provided for chemiluminescent reactions. These include reactions involving peroxide decomposition, singlet oxygen, ion radicals, and chemically initiated electron exchange. This is a multistep process (Scheme 22.1); the final quantum yield is the product of the chemical and physical efficiencies of the processes involved.

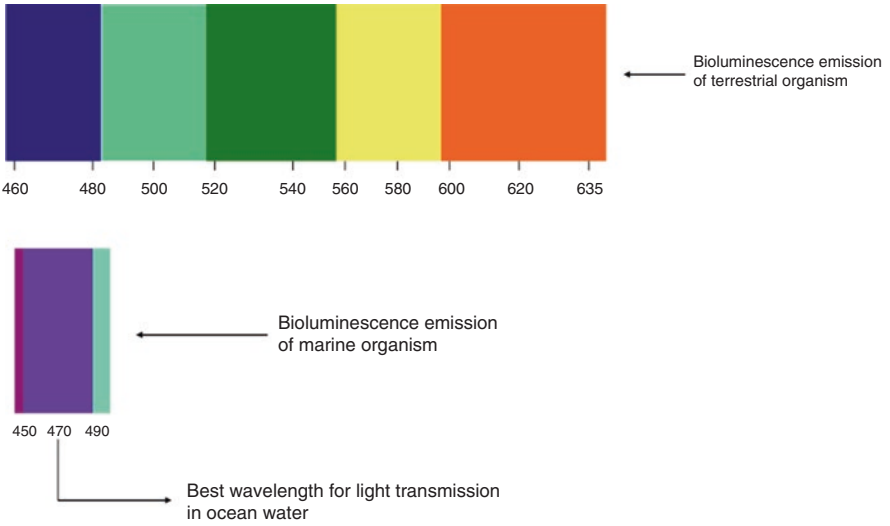
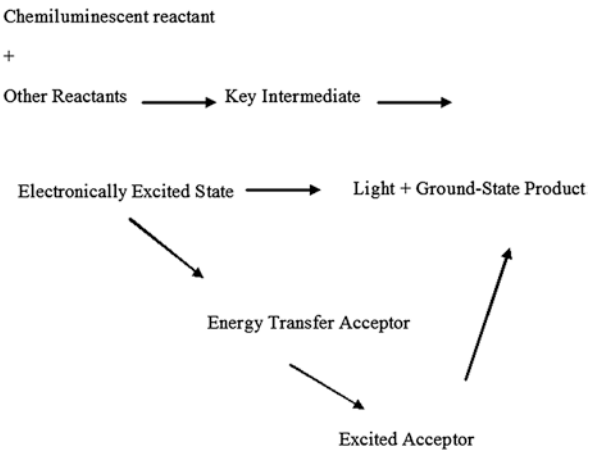


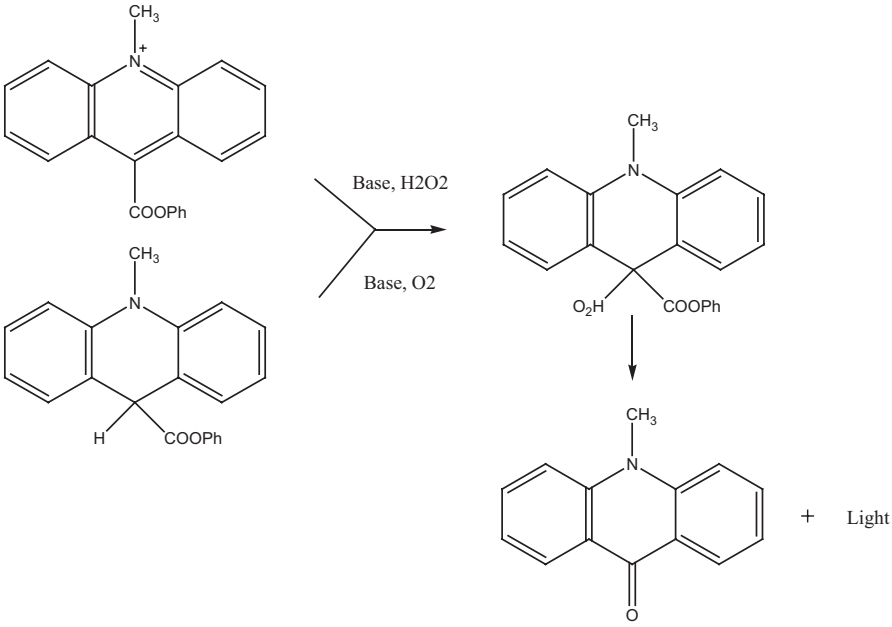
Fig. 22.3 Bioluminescence emission of various organisms

Scheme 22.1
Chemiluminescent reaction



22.3.1 Mechanism of Chemiluminescence

It is an interesting and important area; an understanding of this can be expected to improve the application of phenomenon. Compounds may react with oxygen, usually in the form of carbanion or electron-rich species to produce peroxide. A peroxide can also be produced if the structure of the compound invites attack by hydrogen peroxide, which is a powerful nucleophile in aqueous solution. The purpose is to produce an intermediate derived from luminol which will react with peroxide or its oxidation product superoxide ion. The fluorescent product is derived from the reactant.



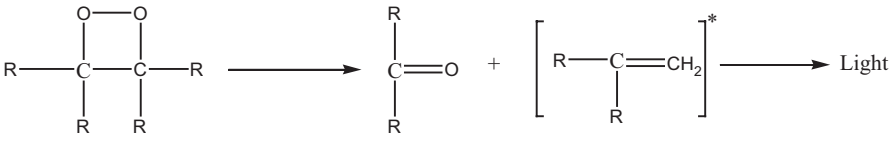
Chemiluminescence is classified on the basis of phase in which luminescence occurs, i.e., gas, liquid, or solid.

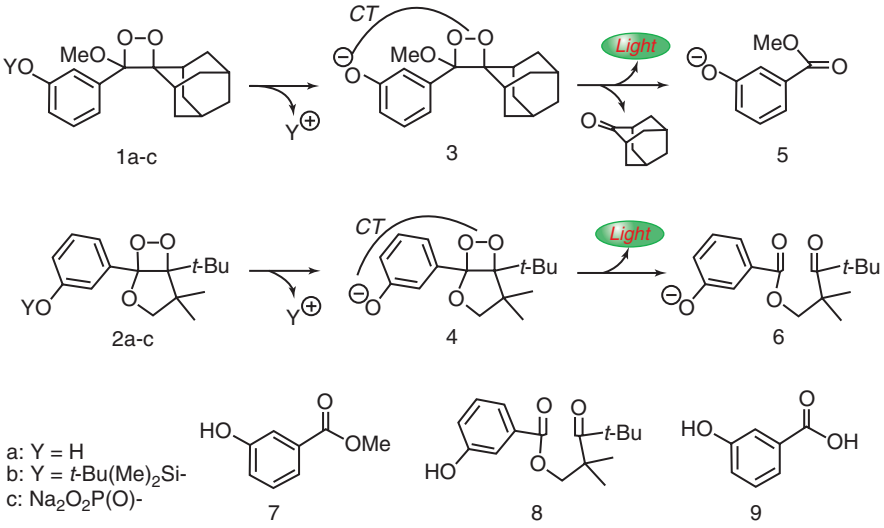
22.3.2 Liquid Phase Chemiluminescence

Liquid phase chemiluminescent reactions progress through the decomposition of intermediates with the instantaneous formation of two carbonyl groups. The concerted formation of these groups meets the energy requirements for the chemiluminescent reaction.

22.3.3 Peroxide Decomposition

Some dioxetanes are stable at room temperature but most decompose below 80 °C and require careful synthesis and handling. Simple dioxetanes decompose to produce carbonyl compounds in excited states, including singlet states, and thus the decomposition is accompanied by the emission of light. Both photochemical and chemiluminescent methods have been used to determine the relative yields of the excited states.

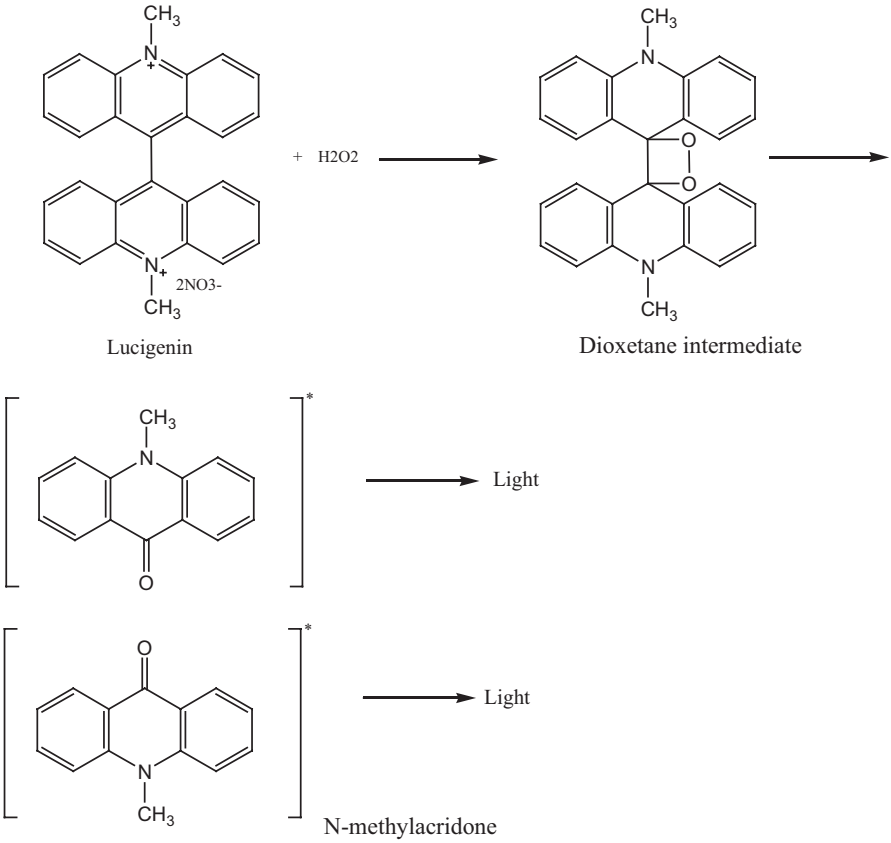




Scheme 22.2 Base-induced chemiluminescent decomposition of 3-oxyphenyl-substituted 1,2-dioxetanes. (Adapted from Watanabe et al. 2014)

The highest yield of excited product in dioxetane decomposition reaction is produced from the decay of tetramethyl-1,2-dioxetane, where 50% acetone produced is in the triplet form; the singlet state is formed only in the low yields. Watanabe et al. describe the chemiluminescent decomposition of 3-hydroxyphenyl-substituted dioxetanes in an aqueous system (Watanabe et al. 2014). Considerable attention has been received for the possible applications in modern biological and clinical analysis using chemiluminescence which includes the intramolecular charge-transfer-induced decomposition (CTID) of oxidophenyl-substituted dioxetanes. Typical examples are adamantylidene-substituted dioxetanes (1) and bicyclic dioxetanes (2), which undergo chemiluminescent CTID through unstable oxidophenyl-substituted dioxetane (3) or (4) produced by deprotonation (Scheme 22.2).

Gleu and Petsch reported the chemiluminescent substances acridine derivatives and lucigenin (Gleu and Petsch 1935). Its derivatives produce strong chemiluminescence (CL) properties with reducing agents like hydrogen peroxide. Veazey et al. have described the CL reaction oxidized by lucigenin in various reaction steps (Veazey et al. 1984). The reaction between lucigenin and hydrogen peroxide produces light which proceeds without a catalyst, although it is accelerated by metal ions leading to an intensification of chemiluminescence. Light emission occurs on the addition of numerous nucleophiles and reducing agents to solutions of lucigenin.



22.3.4 Electron Transfer Chemiluminescence

Electron transfer reactions are capable of producing excited products which may decay to the stable state with the release of energy, for example, resonance energy transfer. The oxidation of DBA which has been reported by Dong et al. (2016) arises via one-electron oxidation in nonaqueous solution. However, DBA oxidation is complicated at the GCE bare in an aqueous medium (Ahlberg et al. 1981). Dissolved oxygen can react with DBA-oxidized product (Luc^{*+}) to generate CL (Cui et al. 2007). Sun et al. (2001) have reported the transfer of energy during the chemiluminescence process. ECL emission was tested under the oxygen and inert atmosphere.

22.3.4.1 Gas Phase Chemiluminescence

Air pollution can be monitored by it and has been studied in simple gases, flames, metal vapors, chemical lasers, molecular beams, etc. Ozone and atomic oxygen can react with other gases such as oxides of nitrogen, sulfur dioxide, olefins, and

hydrogen sulfide to emit light. NO is a relatively unstable molecule which forms NO_2 (especially) on oxidation in the presence of O_3. Photomultiplier tube or solid-state devices have been used to measure the quantity of light produced by the reaction of each NO molecule. Proposed mechanism is as follows:

$$NO + O_3 \rightarrow NO_2 + O_2 + hv$$

Total oxides of nitrogen (NOx) can be measured using chemiluminescence technique by passing the sample over a hot catalyst to reduce all oxides of nitrogen to NO. Just prior to the reaction chamber, this is done within instrument. The resulting signals may be compared indirectly to measure NO_2 in some instruments that can perform the automatic switching of the catalyst in and out of the sample path. Measurements of ammonia (NH_3) are done by variations of chemiluminescence:

$$4NH_3 + 5O_2 \rightarrow 4NO + 6H_2O$$
$$4NO + 4O_3 \rightarrow 4NO_2 + 4O_2 + 4hv$$

The base of CL reactions is the production of light in a flame in which high temperature stimulates the chemical reaction forming the intermediates (Stiles et al. 1994). This methodology has been used to detect the different elements like nitrogen, phosphorous, sulfur, boron, arsenic, antimony, and halogens.

Dunlea et al. (2007) have estimated nitro chemiluminescence (NO_x) in a polluted urban environment. They observed interference peaks in the ambient ozone concentration and found an important issue with CL screening regarding their inability to specifically detect nitrogen dioxide molecule. The CL NO_x monitor interference shows a reasonable association with the measured concentration of ozone given in Fig. 22.4.

22.3.5 Solid Phase Chemiluminescence

Light emission from solids, produced by processes such as electro luminescence, has been widely investigated (Jeon et al. 2015) and has found numerous applications in cathode-ray tube and x-ray screens. However, the number of solids which undergo chemical oxidation reactions leading to chemiluminescence is small. Best example illustrated the solid phase chemiluminescence produced from the oxidation of siloxene. The basic formula is $(Si_6H_6O_3)_n$ and structure is in Fig. 22.5. Oxidation of siloxene with hydrogen peroxide, permanganate, ceric sulfate, chromic acid, nitric acid, or other strong oxidants results in red chemiluminescence (Rauhut 1979). An energy transfer process after oxidation to hydrosiloxane present in the structure is believed to be responsible for the emission. Electron transfer reactions have been reported to produce chemiluminescence in solid phase reactions. Examples include the luminescence from anthracene crystals subjected to an alternating current in complexes of Nd(III), Er(III), and Yb(III) (Lazarides et al. 2007).

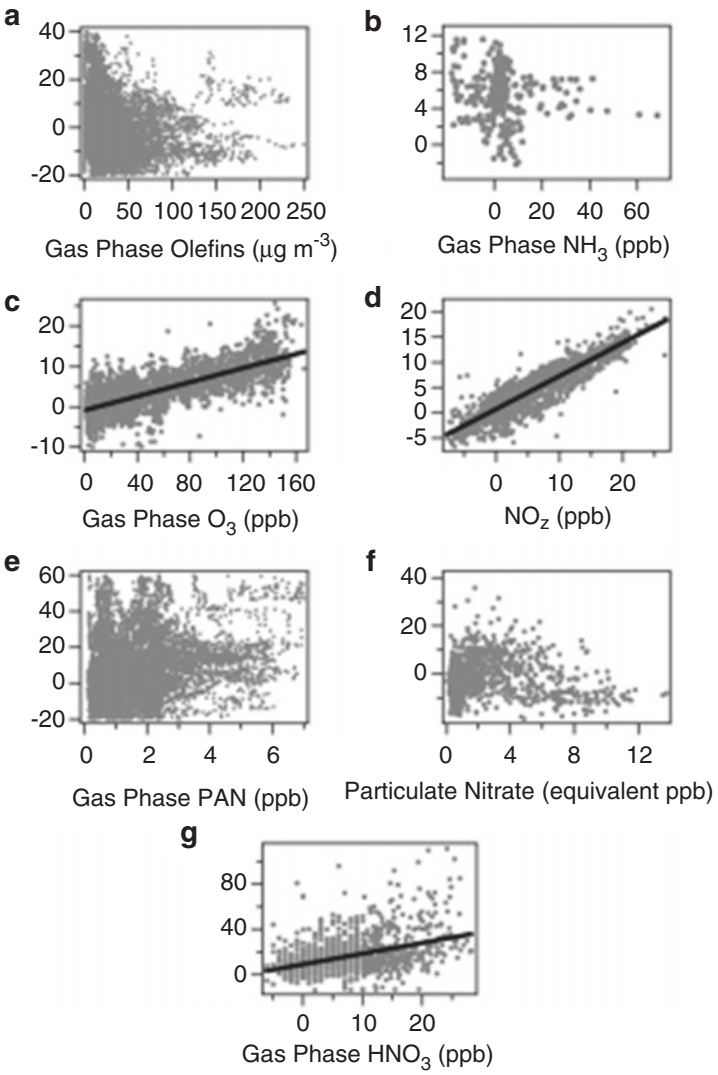


Fig. 22.4 CL NO_x monitor interference linear regression plots. (Adapted from Dunlea et al. 2007)

22.4 Analytical Applications of Chemiluminescence

The CL agents mainly utilized for the investigative purposes are luminol (Khan et al. 2014), hydrogen peroxide, and fluorescein. Luminol chemiluminescence (LCL) is commonly used for the qualitative and quantitative analysis of macromolecules. Biosensors for environmental monitoring and cellular localization in pharmaceutical industry and as biological tracers and several other immunoassays use luminol-based techniques. LCL has a lot of merits due to its selective nature, ease

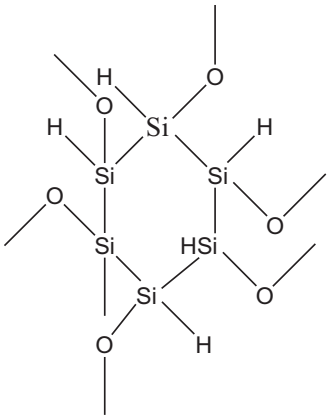


Fig. 22.5 Basic structure of siloxene

of use, being economical, and high sensitivity. Luminol is a crystalline, diprotic with pKa values of 6.74 and 15.1 soluble in polar solvents (Barni et al. 2007). An adverse analytical application of CL has been there including the detection of inorganic and trace metal analysis (Zhang et al. 2007). The toxicity has not been completely explored, although some part of it is described in the cataract, skin, breathing, and gastrointestinal tract (Rose and Waite 2001). Some luminol derivatives have been developed by researchers to maximize its intensity and increase the emission wavelength range in the visible region due to its great application part (Jiao et al. 2011). Many substances are known to stimulate or hinder the chemiluminescence of luminol, and good reviews of their use in analytical chemistry have been presented by Cui et al. (2004). There are a number of compounds which provide stronger inhibition for LCL, whereas benzoic acid and sulfosalicylic acid showed weak signals. Hydroxyl group proves to be an essential element for the CL inhibition, whereas the acidic group proves to be favorable for the CL enhancement shown in Figs. 22.6 and 22.7.

Since a number of metal ions may activate a particular chemiluminescent material under similar conditions, some form of prior treatment is essential to gain a degree of analyte selectivity. This lack of specificity has probably hindered attempts to apply chemiluminescent trace metal analysis in the past. Seitz et al. give the best example of trace metal analysis that applies to biological samples, in which chromium is determined by adding excess ethylenediaminetetraacetic acid (EDTA) to the sample (Seitz et al. 1972).

Over the past few years, chemist has been attracted by the analytical applications of carbon nanostructure chemiluminescence detection (CNS-CL). Recently CNSs revealed to be chemiluminescent (Amjadi et al. 2014; Lin et al. 2011; Bulgakov et al. 2009; Amjadi et al. 2014) can usefully address selectivity and sensitivity requirements. Furthermore CNSs have been used as reagents as well as catalysts which emit light upon direct oxidation, (Amjadi et al. 2014; Wang et al. 2013; Chen et al. 2011), quenchers (He et al. 2013), biomolecule (Wu et al. 2015; Bi et al. 2009), background reducer (Gao and Li 2013), catalyzer support (Zhang and Cui

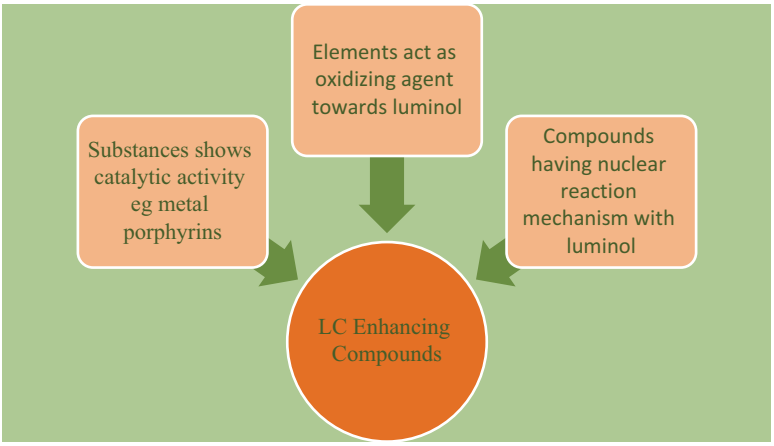


Fig. 22.6 LC enhancing compounds

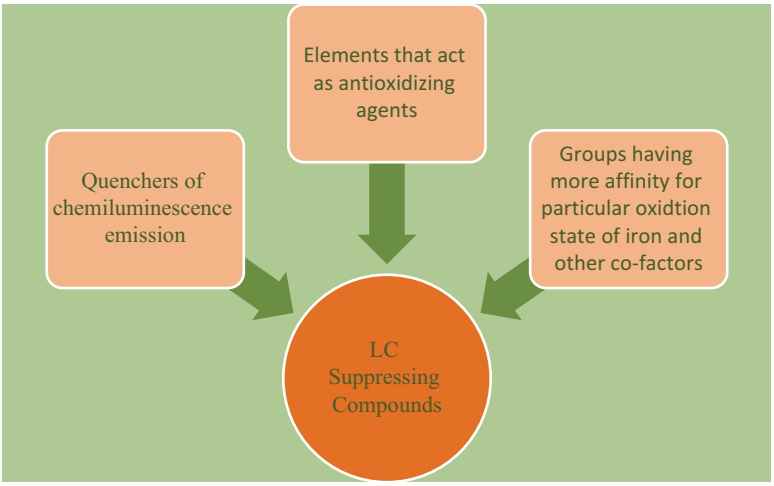


Fig. 22.7 LC suppressing compounds

2014; Safavi et al. 2009), chemiluminescence resonance energy transfer (CRET) (Lee et al. 2012; Gao et al. 2014), and even recognition elements (Huamin et al. 2013; Qiu et al. 2012a, b). Due to the great potential of CNS analytical applications, it has gained the attention of many researchers.

22.5 Graphene Molecularly Imprinted Polymer

The synthetic materials with selective cavities of different form and functional groups of the target analyte form the molecularly imprinted polymers. Magnetic molecularly imprinted polymers (MMIPs) are generally formed by encapsulating a

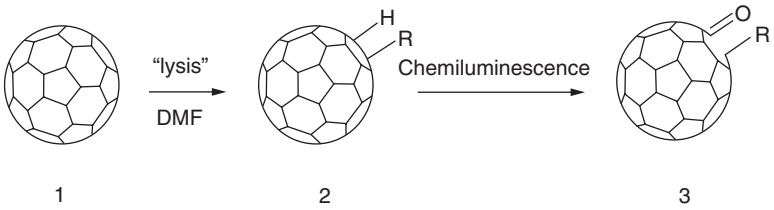


Fig. 22.8 Chemiluminescence of C_{60} fullerene in DMF. (Adapted from Papadopoulos et al. 2001)

magnetic particle with an organic polymer in order to facilitate the separation of MIPs from the reaction solution. These are readily used in crude samples containing a lot of suspended solid materials.

22.5.1 Graphene Oxide (GO)-Catalyzed CL Reactions

The synthesized graphene oxide-catalyzed CL reactions have possessed more catalytic activities with more binding sites than graphene, for example, luminol-H_2O_2 and luminol-O_2 (Hao et al. 2013; Wang et al. 2012; Song et al. 2013; He and Cui 2012; Yang et al. 2014a, b). Wang et al. (2012) have illustrated six times enhancement in CL intensity due to graphene oxide. It is concluded from the spectral analysis that the graphene oxides improved the electron transfer reaction provided with the more yield of oxygen on GO surface.

22.6 Chemiluminescent Reaction of Fullerenes

A radiation dosimetry method was developed using the CL emission of fullerene C_{60}. As a dosimeter, it has described well the relation between CL fullerene intensity and an irradiation dose. Papadopoulos et al. found the stable products with CL reaction of fullerenes with Fenton's reagent (Papadopoulos et al. 2001) (Fig. 22.8).

22.7 Chemiluminescent Reactions for Cancer Detection Therapy

Researchers are currently fascinated in oncology for perfect diagnosis with an efficient tool for timely detection by the methods provided by CL. For monitoring the treatment of cancer, patients have undergone chemotherapy and radiotherapy. CL techniques have been widely applied nowadays. Yao et al. recently reported the determination of anticancer drug mitoxantrone (MTX), having high efficiency in the treatment of breast cancer by the copper-based CL method (Yao et al. 2014). The complex has provided lower level of concentration in CL reaction as compared to schedule emission of CL luminol. Indeed, when 10^{-7} molL^{-1} luminol reacted with

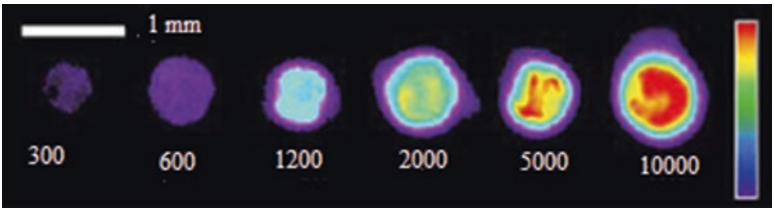


Fig. 22.9 Imaging CL microscopy (Creton and Jaffe 2001)

other oxidants, CL was hardly observed. Probably this fact is the main reason for the reduction in interference of other substances. In modern existence, considerable attention was received by the new oxidant reagents developed for the CL reaction, which are used to extend the application part.

22.7.1 Analytical Technique CL As Detection Technique

22.7.1.1 Imaging Microscopy

To accomplish chemiluminescence and bio imaging microscopy analysis, optical microscope can be easily connected to similar imaging devices for the process of execution. To target the maximum analytical detectability, the light gathering system should be modified as small-sized sample leads to a very weak emission of light (Christenson et al. 2002). Contact of external light can be avoided by enclosing the whole microscope or sample area in a dark box. Specific substrates or reagent solutions are used to accomplish its detection. The localization and quantification of biomolecules in single cell as well as tissue sections is best represented by ultrasensitive analytical figure (Fig. 22.9). Bio- and chemiluminescence can detect inorganic and organic molecules and enzymes by the use of appropriate reagents coupled with enzymatic reactions.

The study of metabolites in living cells and tissues has been done by using chemiluminescence imaging. The methods for the real-time image formation have been developed successfully by exploiting the specific advantages of CL detection such as, for example, high sensitivity and rapidity. The release of nitric oxide is followed by proper stimulation which can be measured in different cell cultures and tissues by the chemiluminescence imaging on the sample of a hydrogen peroxide/luminol solution which undergoes a chemiluminescent reaction (Wiklund et al. 1997).

22.7.1.2 Liquid Chromatography

The widespread applicability of CL reaction is its detective approach in liquid chromatography. As far as the trace analysis is concerned, fluorescence serves as the most efficient detection technique where the elimination of excitation source in CL mode reduces stray light, background emission, and unsteadiness of light source. Analysis of FICs with luminol-H_2O_2 system via post column method for the detection of CL has been developed by Ariga et al. for high-performance liquid

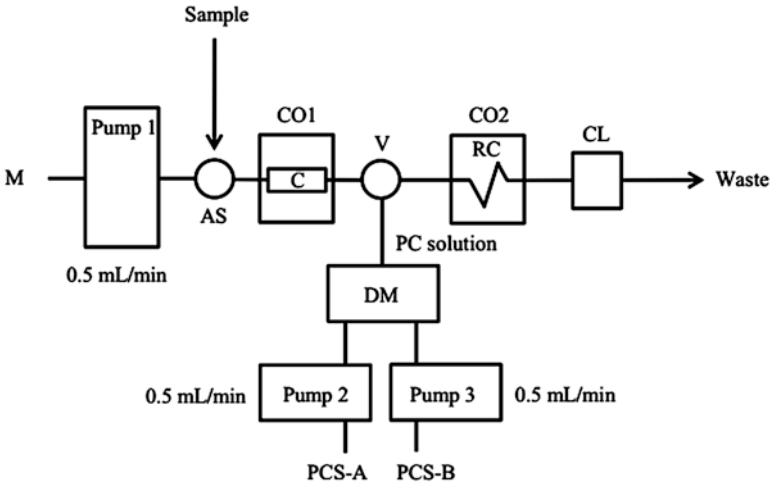


Fig. 22.10 Scheme of HPLC system. (Adapted from Ariga et al. 2016)

chromatography (HPLC). Delivery of the solutions has been done separately using two pump systems; thereafter thorough mixing of luminol with H_2O_2 was feasible in post column reaction (Ariga et al. 2016). Figure 22.10 shows the analytical approach of HPLC can be employed for selective detection of FIC due to its capacity to bind Fe(III), irrespective of its structural feature. The limits of detection were quite low.

Yong Xie et al. have developed an online analysis method by HPLC-DAD coupled with chemiluminescence (CL) for simultaneous detection and identification of antioxidants in three natural plants of traditional Chinese medicine "she gan" (Xie et al. 2014).

22.7.1.3 Capillary Electrophoresis

Chemiluminescence detection with capillary electrophoresis mode as a prior separation analytical technique has started is being explored. Research area in this field serves as a strong analytical device to resolve and quantify biomedical analytes. CL reactions are employed for postcapillary detection of various groups like luminol, firefly, acridinium esters, luciferase peroxyoxalates, acidic potassium permanganate, etc. and speed up the CL reaction after the separation of analytes in electrophoretic capillary. Application of different interfaces is used to set up the channel depending upon the type of analytes targeted with the combination of CL reagents in Fig. 22.11.

Su et al. have designed the microchip based on the principle of flow injection chemiluminescence system and capillary electrophoresis (Su et al. 2004). It has three main channels, five reservoirs, and a detection cell. By using permanganate chemiluminescent system, dopamine and catechol were separated and detected on the prepared microchip. Acridinium esters can also be successfully employed in CE as CL detection system. CE analysis of amino acids, peptides, and proteins can be achieved by their derivatization with acridinium esters. Cao et al. characterized a

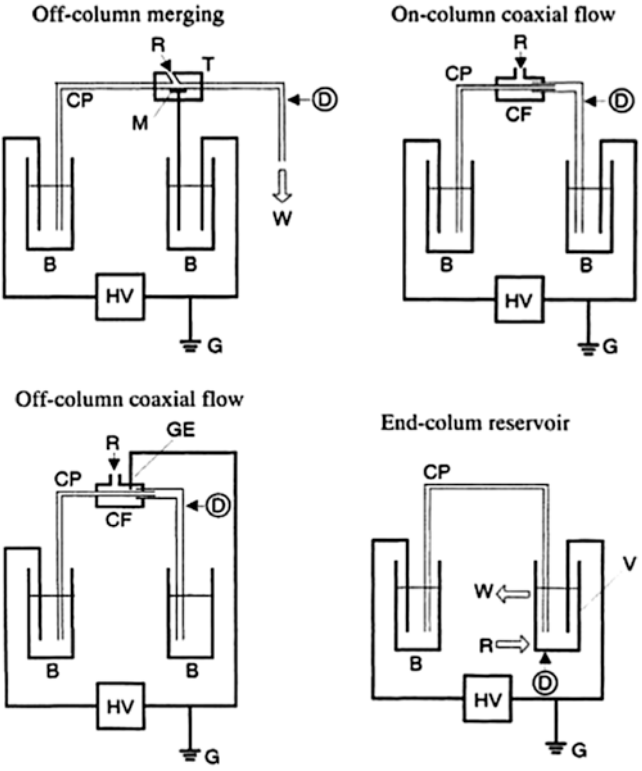


Fig. 22.11 Devices using capillary electrophoresis with chemiluminescence with different interfaces. (Adapted from Cao et al. 2002)

new end-column electro-chemiluminescence (ECL) detection technique coupling to capillary electrophoresis (Cao et al. 2002). Exclusive to the use of decoupler, a platinum electrode was used to directly connect to an inner diameter of capillary. During the optimization of various detection parameters, the distance between the capillary and an electrode was an important one for the determination of actual concentration of $Ru(bpy)_3^{2+}$ in the suitable detection region.

22.7.1.4 Gas Chromatography

A lot of chemiluminescence detectors have been developed along with the gas chromatography detection technique. The various detectors involve the flame photometric detector (FPD), sulfur chemiluminescence detector (SCD), thermal energy analysis (TEA) detector for reaction analysis of nitrosamines, and redox chemiluminescence detector (RCD) for the specific detection of compounds containing heteroatoms. FCLD for S, Se, Te, and P compounds are promisingly accepted (Ramírez et al. 2015). Figure 22.12 captured a conventional device for the detection of CL in gas chromatography.

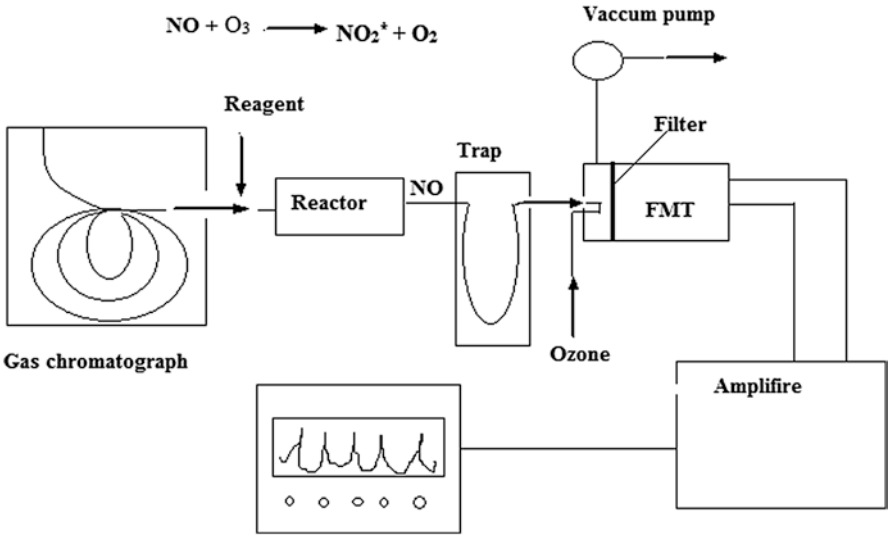


Fig. 22.12 Schematic diagram for CL detection in gas chromatography

The methods of flowing stream involve the mixing of chemiluminescent reagent along with analyte stream to carry out the analysis of CL emission after its incorporation. The examination of CL reactions in liquid phase seems to be simple for FIA because of its feasibility, robustness, precision, and quick response (García-Campaña and Baeyens 2000). The injection of a sample along with CL reagent into the flowing stream is in close proximity to detector emission that occurs in a cell placed in front of the detector. Various experimental variables like temperature, pH, flow rates, dimension of mixing, and detector coils needed to be optimized for increasing the sensitivity of detection. There are widespread applications in quality control in pharmaceutical analysis using flow injection method. The emergence of the immobilization technique has provided a preface of enzymatic reactors, which can be positioned before the CL reaction takes place. The substrate and one of the products of enzymatic reaction are actively involved in CL reaction. The substrates detected by this method are amino acids, glucose, choline, aldehydes, cholesterol, and lactate that generate peroxide during a movement through a selective column reactor with immobilized oxidase enzyme in the presence of oxidant, oxygen, present in the samples. For the post column determination of peroxide, luminol is used along with peroxide catalyst. The determination of glucose by CL emission which is produced when hydrogen peroxide is formed by the reaction of luminol with immobilized glucose oxidase in the presence of potassium hexacyanoferrate is shown in Fig. 22.13.

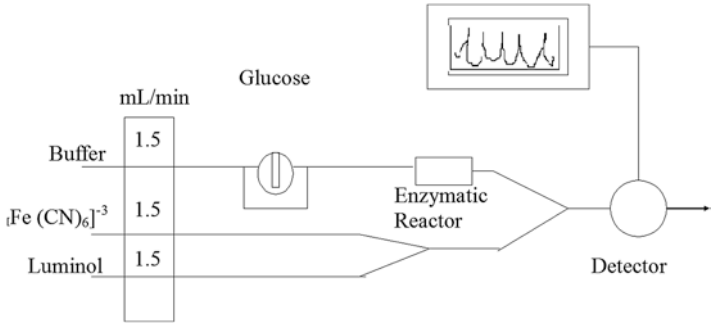


Fig. 22.13 Scheme of FIA. (Adapted from García-Campaña and Baeyens 2000)

22.8 Conclusion

Chemiluminescent reactions occur in solid, liquid, and gas phase and involve a diversity of organic and inorganic molecules. The analytical potential of most chemiluminescent reactions is unexplored, and only liquid phase reactions have found widespread application. In the last decade, recent advancement has been achieved with respect to the CL reagents as labels to derivatize and selectively find out the analytes. A variety of analytical applications have been found in CL reactions in various scientific fields such as pharmaceutical, environmental, and food analysis and in clinical laboratories for diagnosis of disease, prognosis, and monitoring of patient treatments. The widespread applications of CL techniques can be attributed to their sensitivity, simplicity, cost-effectiveness, and wide linear range.

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