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Introduction

Chemiluminescence, like atomic emission spectroscopy (AES), uses quantitative measurements of the optical emission from excited chemical species to determine analyte concentration; however, unlike AES, chemiluminescence is usually emission from energized molecules instead of simply excited atoms. The bands of light determined by this technique

emanate from molecular emissions and are therefore broader and more complex

then bands originating from atomic spectra. Furthermore, chemiluminescence

can take place in either the solution or gas phase, whereas AES is almost

strictly as gas phase phenomenon.

Though liquid phase chemiluminescence plays a significant role in

laboratories using this analytical technique (often in combination with

liquid chromatography), we will concentrate on gas phase chemiluminescence

reactions since the instrumental components are somewhat simpler. These

detectors are also often used as detectors for gas chromatography.

Like fluorescence spectroscopy, chemiluminescence’s strength lies in

the detection of electromagnetic radiation produced in a system with very

low background. And on top of this, because the energy necessary to excite

the analytes to higher electronic, vibrational, and rotational states (from

which they can decay be emission) does not come from an external light

source like a laser or lamp, the problem of excitation source scattering is

completely avoided. The major limitation to the detection limits achievable by chemiluminescence involves the dark current of the photomultiplier (PMT) necessary to detect the analyte light emissions. If the excitation energy for analytes in chemiluminescence doesn’t come from a source lamp or laser,

where does it come from? The energy is produced by a chemical reaction of the analyte and a reagent. An example of a reaction of this sort is shown below:

A chemiluminescence reaction

pic

In gas phase chemiluminescence, the light emission (represented as Planck’s constant times nu-the light’s frequency) is produced by the reaction of an analyte (dimethyl sulfide in the above example) and a strongly oxidizing reagent gas such as fluorine (in the example above) or ozone, for instance. The reaction occurs on a time scale such that the production of light is essentially instantaneous; therefore, most analytical systems simply mix analytes and the reagent in a small volume chamber directly in front of a PMT. If the analytes are eluting from a gas

chromatographic column then the end of the column is often fed directly into the reaction chamber itself. Since as much of the energy released by the reaction should (in the analyst's eye) be used to excite as many of the analyte molecules as possible, loss of energy via gas phase collisions is undesirable, and therefore a final consideration is that the gas pressure in the reaction chamber be maintained at a small pressure (~ 1 torr) by a vacuum pump in order to minimize the effects of little deactivation. It must be stated that the ambiguous specification of " products" in the above reaction is often necessary because of the nature and complexity of the reaction. In some reactions, the chemiluminescent emitters are relatively well known. In the above reaction the major emitter is electronically and vibrationally excited HF; however, in the same reaction, other emitters have been determined whose identities are not known and these also contribute to the total light detected by the PMT. To the analytical chemist the ambiguity about the actual products in the reaction is, in most case, not important. All the analyst cares about is the sensitivity of the instrument (read detection limits for target analytes), its selectivity-

that is, response for an analyte as compared to an interfering compound,

and the linear range of response.

Here is a schematic of the components necessary for a gas phase

chemiluminescence detector interfaced to a capillary gas chromatograph.

Schematic of a GC chemiluminescence detector

pic

HISTORY

The term “ chemiluminescence” was first thought up by Eilhardt Weidemann in

1888, and refers to the emission of light from a chemical reaction. In its

simplest form it can be represented by;

pic

Where I* is an excited state intermediate. This is termed “ direct

chemiluminescence”. In certain cases where the excited state is an

inefficient emitter, its energy may be passed on to another species (a

sensitiser, F) for light emission to be observed. This called “ indirect

chemiluminescence”;

pic

These reactions can occur in the gas, liquid and solid phases, the reactions studied at Deakin University for Chemical Analysis are all in the liquid phase. The light emitted from chemiluminescent reactions has differing degrees of intensity, lifetime and wavelength. The wavelength can extend across the spectrum from near ultraviolet, through the visible and into the near infrared. Solution phase chemiluminescent reactions which have found analytical application often produce light in the visible region.

WHAT IS IT REALLY?

Chemiluminescence is the generation

electromagnetic radiation as light by the release of energy from a

chemical reaction. While e lig

the light, in principle, be emitted in the ultraviolet, visible

or in to those emitting visible light are

the most common. They are also most interesting and useful.

Chemiluminescent reactions can be grouped into three types:

1. Chemical reactions using synthetic compounds and usually involving a

highly oxidized species such as peroxide are commonly termed chemiluminescent reactions.

2. Light-emitting reactions arising from a living organism, such as the firefly or jellyfish, are commonly termed bioluminescent reactions.

3. Light-emitting reactions which take place by the use of electrical current are designated electrochemiluminescent reactions.

Chemiluminescent reaction usually involve the cleavage or fragmentation of the O-O bond an organic peroxide compound. Peroxides, especially cyclic peroxides, are prevalent in light emitting reactions because the relatively weak peroxide bond is easily cleaved and the resulting molecular reorganization liberates a large amount of energy. In order to achieve the highest levels of sensitivity, a chemiluminescent reaction must be as efficient as possible in generating photons of light. Each chemiluminescent compound or group can produce no more than one photon of light. A perfectly

efficient reaction would have a chemiluminescence quantum yield (pic) of one, i. e. one photon/molecule reacted according to the equation:

pic

The chemiexcitation quantum yield (pic) is the probability of generating an electronic excited state in a reaction and has a value between 0 and 1, with 0 being a completely dark reaction and, when 1, all product molecules are generated in the excited state. The most useful chemiluminescent reactions will have apicof about 10-3 or greater. The fluorescence quantum yield (pic) is the probability of the excited state emitting a photon by fluorescence rather than decaying by other processes. This property, which can have values between 0 and 1 is frequently at least 0. 1. The reaction quantum yield (pic) is the fraction of starting molecules which undergo the luminescent reaction rather than a side reaction. This value is usually about 1.

It is possible to increase the yield of chemiluminescence when the emitter is poorly fluorescent (lowpic). A highly fluorescent acceptor is used in these cases in order to transfer the excitation energy from the primary excited state compound to the fluorescent acceptor/emitter. The chemiluminescence quantum yield is then determined by the equation:

pic

Chemiluminescent Reactions and Liquid Chromatography

The applicability of chemiluminescence reactions as a means of detecting compounds in liquid chromatography (LC) is based to a large degree on post column reactions. A primer on liquid chromatography (and high performance LC) can be found here; however, a brief description follows. This describes, in the main, HPLC chromatographic systems.

Components of High Performance Liquid Chromatography

Liquid phase samples (mixtures) are injected onto an LC column usually using a syringe and specially devised injection valve. The sample is swept onto the chromatographic column by the flowing mobile phase and chromatographic separation occurs as the mixture travels down the column. Normal HPLC detectors detect the elution of a compound from the end of the column based on some physical characteristic such as ultraviolet light absorption, ability to fluoresce, or the difference in index of refraction between the analyst and the mobile phase itself. The majority of HPLC systems work this way.

An example diagram of an HPLC system is shown below:

pic

Need for HPLC Chemiluminescence Detection

The use of chemiluminescence detection for HPLC comes from the need to perceive compounds either very sensitively (at very low absorptions) or very selectively, that is, a target compound that must be determined in the presence of co-eluting compounds that just can not be successfully separated from the analyte. Since chemiluminescence derives from the generation of light cause by a chemical reaction, there is no source lamp light that must be filtered out (as in the case of fluorescence detection) in order to detect the analyte emission. This means that the photons coming from the de-exciting analyte molecule are detected against a black background, and this detection can be accomplished by a photomultiplier which can detect a large percentage of the emitted photons.

Methods of HPLC Post Column Chemiluminescence Detection

If a target analyte can be determined via HPLC chemiluminescence then it probably has one of three characteristics: 1) it either chemiluminesces when mixed with a specific reagent; 2) it catalyzes chemiluminescence

between other reagents; or 3) is suppresses chemiluminescence between other

reagents. Examples of all three will be given below using the well explored

luminol reaction.

Luminol based chemiluminescence detection

Luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) reacts with oxidants

like hydrogen peroxide (H2O2) in the presence of a base and a metal

catalyst to produce an excited state product (3-aminophthalate, 3-APA)

which gives off light at approximately 425 nm. If luminol is the target

analyte (seldom) then a schematic of a post column detector based on its

solution phase reaction would look like this:

In this case one reagent pump would send a solution containing a dissolved

metal ion like copper(II) or iron(III) to the mixer at the end of the LC

column, while the other reagent pump would send a solution containing the

oxidant such as H2O2 or hypochlorite (another oxidant) and a base.

Depending on the catalyst used (which basically controls the time necessary

for maximum light emission to develop AND the decay profile of that

emission) the distance from the mixer to the detection cell is carefully

determined to allow for the most sensitive detection-in this case the detection of luminol arriving from the LC column where it could have been separated from interfering compounds. More realistically, some important chemical species can be derivatized using luminol itself or luminol like reagents that can be detected in the same or similar ways.

Detection based on luminol suppression

What follows is a method of chemiluminescence detection in which the suppression of a background chemiluminescence signal could be used to determine a compound that elutes from the LC column. For instance, many organic molecules will complex metal cat-ions and thereby make them less available as catalysts in the luminol reaction. This is a nifty way to determine the concentration of the organic molecule: Mix a constant concentration of a metal cat-ion, luminol, base, and an oxidant. This will create a baseline light signal that is relatively constant. With the LC column output fed into the mixer, the amount of light detected will DECREASE when an organic analyte (which can complex with the metal ion) elutes from the column. The amount of light decrease depends directly on

the amount of the analyte. This is true as long as the amount of metal cation is not completely complexed. At this point the light decrease will no longer be linearly related to the amount of organic analyte. Basically the same schematic seen above is seen here with the metal catalyst coming from

the first reagent pump and feeding into a second mixer placed upstream of the first mixer. This is to allow the eluting organic molecules (e. g., analytes like amino acids) to have time to tie up the metal catalyst before they are mixed with the other reagents. The second reagent pump adds luminol, base and oxidant. When that metal/organic complex gets to the second mixer and ultimately to the detection cell, the baseline light intensity will drop off. An “ anti-signal”-proportional to the amount of the (analyte) organic molecules eluting from the column.

pic

The following liquid phase chemiluminescence reactions are currently being studied at Deakin University for their application to chemical analysis;

| | Ttion of tris(2, 2′-bipyridyl)ruthenium(III) by suitable|

| | substrates such as amines and the oxalate anion.|

| | The reduction of acidic potassium permanganate in the presence of|

| |” polyphosphates” by certain tertiary amines.|

| | The peroxyoxalate reaction; the reaction of certain oxalate|

| | esters and oxamides with hydrogen peroxide in the presence of a |

| | suitable fluorescent species (sensitiser).|

Schematic of a simple liquid chromatographic separation

Liquid phase chemiluminescence reactions have found applicability to the determination of a wide range of analytics from trace metals to pharmaceuticals. Analytically these reactions are attractive due to;

| | The potential for excellent limits of detection because of the|

| | absence of source noise and scatter.|

| | High selectivity due to the limited number of available reactions.|

| | Simple, robust and inexpensive instrumentation suitable to both|

| | batch and flow analytical techniques.|

To date the most successful analytical application area for solution phase chemiluminescence has been in the biomedical and clinical fields. And at Deakin, liquid phase chemiluminescence reactions have been used as the

basis for sensitive and selective discovery for a range of analytical practices including flow injection analysis (FIA), sequential injection analysis (SIA), high performance liquid chromat- ography (HPLC) and capillary electro- phoresis. Flow injection analysis (FIA) is a well established, powerful, sample handling technique for laboratory analysis and process analytical chemistry. It is an unsegmented flow technique where samples (10-200 micro L) are injected into a moving liquid carrier stream and are transported to a flow through detector via conduits constructed of teflon tubing (0. 3-0. 8 mm internal diameter). The sample is modified by reaction with reagents merging with the main carrier stream. The response at the detector is in the form of a peak, the dimensions of which are directly related to analyte concentration. Automated flow injection systems have been applied to on-line process analysis in industrial and environmental situations with a great deal of success. Flow injection analysis is also ideally suited to monitoring solution phase chemiluminescence’s reactions due to the capability to mix sample and reagent in close proximity to a detector.

The animation below shows simple two-line FIA manifold utilizing chemiluminescence’s detection. The reagent merges with the carrier stream at a T-piece (marked T on the diagram) just prior to a flow cell, typically a glass or Teflon coil.

Sequential injection analysis (SIA) employs a multi-position valve operated in synchronization with a pump, typically either a peristaltic or syringe type. The ports of the valve are connected to sample and reagent reservoirs, aliquots of sample and reagent are sequentially aspirated into a holding coil connected to the common port of the valve. The resulting stack of sample and reagent zones is then propelled towards the detector by the pump operating in the forward mode. The flow reversal leads to a mixing of the sample and reagent zones to create a zone of product whose properties are measured at the detector. The order in which the sample and reagent zones are stacked in the holding coil depends upon the type of chemistry being utilized.

Here are some reactions and their color of chemiluminescence:

| Reaction | Color (? max)| Quantum|

||| yield*|

| Oxidation of luminol in aqueous alkali | blue| 0. 01 |

||(425 nm) ||

| Oxidation of luminol in dimethyl | green-yellow| 0. 05 |

| sulphoxide|(500 nm) ||

| Oxidation of lucigenin in alkaline| blue-green| 0. 016 |

| hydrogen peroxide|(440 nm) ||

| Oxidation of lophine in alcoholic| yellow|-|

| sodium hydroxide|(525 nm) ||

| Peroxyoxalate reaction| sensitiser| 0. 05 – 0. 5 |

|| dependant||

| Reduction of| orange|-|

| tris(2, 2′-bipyridyl)ruthenium (III) by |(610 nm) ||

| certain amines, alkaloids and oxalate |||

| Oxidation of some alkaloids by acidic | red|-|

| potassium permaganate in the presence |(680 nm) ||

| of polyphosphates|||

| ATP-dependant oxidation of D-luciferin |||

| with firefly luciferase|||

| pH 8. 6| green-yellow| 0. 88 |

||(560 nm) ||

| pH 7. 0| red (615 nm)||

1The intensity of emission of a reaction is dependant on the quantum yield. The quantum yield is a measure of the efficiency of the chemiluminescence reaction. Quantum yields vary from 10-15 (ultra-weak chemiluminescence) to nearly 1 (bioluminescent processes).

Examples of Chemiluminescence

A buffered luminol solution with a copper(II) catalyst is added to the funnel on the left. A hydrogen peroxide solution is added to the funnel on the right. On releasing the rubber tubing the solutions mix. The chemical reaction generates energy which is seen as blue light. This process is chemiluminescence. When the reacting solution mixes with a dye, fluorescein, the light energy from the reaction causes the fluorescein to give off a greenish light in a process called fluorescence.

Commercially available light sticks contain a solution in a glass vial.

When the vial is broken, a second solution mixes with the first and light

is generated. Different dyes give off different colors when they are caused

to fluoresce by the light of the chemiluminescent reaction. Light sticks

give off light from 3 to 8 hours, depending on the concentrations of

reactants.

Chemiluminescence, or chemical light, is the production of light from

a non-heat generating chemical reaction. In nature, our model for

chemiluminescence has been the firefly, which uses a biochemical reaction

to produce light in an extremely efficient manner. Now with Inefficient

chemical reactions, coupled with short lifetimes of fluorescent molecules

(some can be as short as one billionth of a second), originally made the

commercial production of chemical light products highly impractical. In the

last two decades, chemical reactions using a fluorescent molecule, a key

intermediate and a catalyst have enabled OMNIGLOW to produce

sustainable,

instantaneous, highly visible light in many colors and much intensity.

Summary of the report and how it is used

Analytical methods based on chemiluminescence have taken their strong position among the more mundane analytical techniques because of a triumvirate of strengths: sensitivity, selectivity, and in many cases, a wide linear detection range. This is true even though chemiluminescence is not as widely applicable as absorption, emission, or even fluorescence methods of detection since so few molecules undergo chemiluminescent reactions. Because chemiluminescence, light emission generated from a chemical reaction, requires no light source for excitation, the analytical signal appears out of an essentially black background, and the only background signal is that of the photomultiplier tube’s (PMT) dark current. Therefore light source warm-up and drift and interference from light scattering are absent. In the case of systems where red and near infrared light are observed in analytical detection, red sensitive PMT’s dark current can be minimized by cooling; with blue light emission detection, cooling is not required. Detection limits routinely orders of magnitude lower than fluorescence methods are achievable. In addition, interfering molecules are often less of a problem too since chemiluminescence reactions

can be so selective.

The initial reports in the scientific literature of lophine and then lucigenin chemiluminescence in the last quarter of the nineteenth century blossomed in this century into reports involving many different chemiluminescent reagents. The most common or well known solution phase systems involve luminol (or its derivatives), oxalate esters, lucigenin (or its derivatives), ruthenium tris-bipyridine, and luciferin. Gas phase examples include the ozone- and fluorine-induced, sodium vapor, and chlorine dioxide chemiluminescence detectors for gas chromatography. Because many of the solution phase systems use hydrogen peroxide or organic

peroxides as oxidant and these can be generated many ways in liquid systems

and because many of the solution phase reagents mentioned above can be tagged onto a large variety of analytes, high performance liquid chromatographic (HPLC) solution phase chemiluminescence is more common and

variously applied than gas phase chemiluminescence reactions.

In a very general way, the requirements for the analytically useful production of light from a chemical reaction are:

1) Excess chemical energy produced by the reaction must be relatively efficiently used to populate the excited state of the emitter

2) The excited species must have few mechanism of deactivation except light

emission. In many systems, the initially excited state molecule is used as a conduit of energy to excite a second or third molecule which, instead, is the actually emitting species. In solution-phase systems, pH and catalysts must also be considered; however, in gas phase systems reaction cell pressure and temperature can be important factors. A more detailed description of these phenomenon and their applications can be found in the literature in a number of places.

The use of chemiluminescence as a detection method following analytical separation makes up a significant share of its application. For example, liquid phase chemiluminescence has been applied to high performance liquid chromatography and very recently to capillary electrophoresis.

Gas phase analytical chemiluminescence reactions have in the main been employed with gas chromatography to detect trace chemical species or target analytes in complex matrices . Other workers have recently employed “ separationless” chemiluminescence methods to determine total sulfur content in gasoline and coal and nitrate and nitrite in flow injection analysis . A very recent supercritical fluid chromatographic interface to a chemiluminescent nitrogen detector has also been reported for the examination of polymers and pharmaceuticals although SFC/chemiluminescence techniques have appeared before.

This glancing survey of liquid and gas phase chemiluminescence is not meant to imply that these are the only roles for this method. Researchers have, for instance, recently used in vivochemiluminescence initiated by UV-A irradiation of mouse skin as a means of determining skin oxidative stress processes. Others have used chemiluminescence as a means of following antioxidant evaluation in mouse kidney and brain and plasma, as a means of DNA detection and sequencing, detection of polymerase chain reaction-derived nucleic acids, and alkaline phosphatase determination.

Finally, chemiluminescence has long been used as a means of measuring concentrations of short lived species in gas mixtures and in the atmosphere and to that end chemiluminescent techniques have been used to determine ozone and hydrogen peroxide in the atmosphere, to detect the possible emitter in the reaction of tetrakis ethylene with oxygen, and to map out the “ hot bands” of HNO produced in the reaction of NO with HCO among many

other gas phase applications. The Journal of Bioluminescence and Chemiluminescence is obviously an excellent source in this field and periodically publishes literature searches sorted by year and author.

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