Chemiluminescence Explained

What is Chemiluminescence?

Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. While the light can, in principle, be emitted in the ultraviolet, visible or infrared region, those emitting visible light are the most common. They are also the 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 a 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 and bioluminescent reactions 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 (Φ_{CL}) of one, i.e. one photon/molecule reacted according to the equation:

$$\Phi_{CL} = \Phi_{CE} \times \Phi_{F} \times \Phi_{R}$$

The chemiexcitation quantum yield (Φ_{CE}) 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 a Φ_{CE} of about 10^{-3} or greater. The fluorescence quantum yield (Φ_{F}) 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 (Φ_{R}) 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 (low Φ_F). 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:

$$\Phi_{CL} = \Phi_{CE} \text{ x } \Phi_R \text{ x } \Phi_{ET} \text{ x } \Phi_{F'}$$

The energy transfer quantum yield (Φ_{ET}) expresses the efficiency of converting the primary excited state formed in the reaction into the excited state of the acceptor. This value is often near 1. The fluorescence quantum yield of the acceptor/emitter ($\Phi_{F'}$) should be also be near 1.

An appreciation of some of these fundamental principles of chemiluminescent reactions will help in understanding how to design chemiluminescent assays.

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