What is chemiluminescence?

Glowing jellyfish, flickering fireflies, fun glow sticks; Emma Welsh introduces the beautiful and mysterious world of chemiluminescence.

Fireflies, jellyfish and glow sticks – one flies, one lives deep in the ocean and one provides entertainment in night clubs. What is the link? The answer is some intriguing chemical reactions that produce light.

Chemiluminescence is the production of light from a chemical reaction. Two chemicals react to form an excited (high-energy) intermediate, which breaks down releasing some of its energy as photons of light (see glossary for all terms in bold) to reach its ground state (see Figure 1 on page 64).

\[ A + B \rightarrow AB^* \rightarrow \text{Products} \rightarrow \text{Light} \]

Excited intermediate

Chemiluminescent reactions do not usually release much heat, because energy is released as light instead. Luminol produces a light when it reacts with an oxidising agent; the chemistry of this reaction is shown in Box 1 (page 66).
Chemiluminescence in forensics

Forensic scientists use the reaction of luminol to detect blood at crime scenes. A mixture of luminol in a dilute solution of hydrogen peroxide is sprayed onto the area where the forensic scientists suspect that there is blood. The iron present in the haem unit of haemoglobin (Figure 3, page 66) in the blood acts as a catalyst in the reaction described in Box 1.

The room must be dark and if blood is present, a blue glow, lasting for about 30 seconds, will be observed. The forensic investigators can record this glow by using photographic film, which can be used as evidence in court for the presence of blood at the scene. (For a teaching activity about forensic science, see Wallace-Müller, 2011.)

Because the iron acts as a catalyst, it is only required in trace amounts, therefore only a tiny amount of blood is required to produce a positive result. This means that blood can be detected even when it is not visible to the naked eye.

One of the drawbacks of using luminol is that the reaction can be catalysed by other chemicals that may be present at the crime scene, for example, copper-containing alloys, some cleaning fluids such as bleach, and even horseradish. Clever criminals can clean up the blood with bleach, which destroys the evidence of the blood, but bleaching the carpet may alert people to the crime sooner. Urine also contains small amounts of blood, which can be enough to catalyse the reaction of luminol. Once luminol has been applied to the area, it may prevent other tests from being performed there. However, despite these drawbacks, luminol is still used by forensic scientists as a tool to solve crime.

The article can be adapted for different age ranges and for different subjects and topics. For students aged 14-15, it could be used to teach chemistry (atomic structure and movement of electrons between shells, introduction to chemical reactions) or biology (bioluminescence). For this age group, the teacher would need to simplify the information in the article and omit the details of the reactions. For students aged 16-18, the article could be used to teach chemistry (redox reactions, catalysts, the influence of temperature on reaction speed, the effect of pH on a reaction, and covalent bonds), physics (the electromagnetic spectrum and photons) or genetics (genetic engineering). Suitable comprehension questions include:
• What is chemiluminescence?
• What do forensic scientists use chemiluminescence for?
• Explain some biological functions of bioluminescence.
• Why should you keep your glow stick in the freezer when you are not using it?
• How could you make a self-illuminated Christmas tree?

Ana Gil, Spain
Figure 1: Movement between electron shells
A hydrogen atom in its ground state. A single electron is in shell n=1. Each shell has its own energy level.
When the hydrogen atom absorbs a quantum (defined amount) of energy, it is promoted to a higher energy level (shell n = 2) and is now in an excited (high-energy) state. We draw an asterisk (*) next to the molecule to indicate this.

The electron falls back down to its original position in the ground state (shell n=1). In the process, energy (in the form of a photon of light) is emitted.

In the nightclub
When you snap a glow stick and it begins to glow, the light produced is an example of chemiluminescence (Figure 4, page 67). Glow sticks comprise a plastic tube containing a mixture including diphenyl oxalate and a dye (which gives the glow stick its colour). Inside the plastic tube is a smaller glass tube containing hydrogen peroxide. When the outer plastic tube is bent, the inner glass tube snaps, releasing the hydrogen peroxide and starting a chemical reaction that produces light (see Box 2, page 67). The colour of light that a glow stick produces is determined by the dye used (see Box 3, page 68).

Chemiluminescence reactions, such as those in glow sticks, are temperature-dependent. The reaction speeds up as the temperature rises – snapping your glow stick in hot water will produce a fantastic glow, but it will not last as long as it would at room temperature. Conversely, the reaction rate slows down at low temperature; this is why keeping your glow stick in the freezer for several hours can allow the stick to glow brightly again when
Science topics

Science in School
Issue 19 : Summer 2011

65

www.scienceinschool.org

light around your feet? Or been in the countryside at night and seen fireflies flitting about? These are examples of bioluminescence and around 90% of deep-sea life also exhibits this strange phenomenon. These organisms have evolved to produce light because it has many useful functions. Glowing can be used as a lure to catch prey, as camouflage, or to attract potential mates. Some bacteria even use bioluminescence to communicate. The term ‘glow worm’ describes the larvae of several species of insect, including fireflies; some of them glow to scare off predators, whereas other species use their glow to attract prey. There are species of squid and crustacean that can release clouds of bioluminescent liquid to confuse predators while...
they make their escape. Creatures living deep in the ocean have evolved to produce mainly blue or green light because it transmits well through seawater. This is because blue light has a shorter wavelength than red light, which means it is absorbed less readily by particles in the water.

Bioluminescent reactions use ATP (adenosine triphosphate) as a source of energy. The structure of the light-producing molecules varies from species to species, but they are all given the generic name luciferin. The structure

Box 1: Luminol, a glow-in-the-dark chemical

The release of a photon of light from a molecule of luminol is a fairly complex, multi-stage process. In a basic (alkaline) solution, luminol exists in equilibrium with its anion, which bears a charge of -2. The anion can exist in two forms (or tautomers), with the two negative charges delocalised on either the oxygens (the enol-form) or on the nitrogens (the ketol-form; see Figure 5, below). Molecular oxygen (O₂) combines with the enol-form of the luminol anion, oxidising it to a cyclic peroxide. The required oxygen is produced in a redox reaction (i.e. one in which both reduction and oxidation occur) involving hydrogen peroxide (H₂O₂), potassium hydroxide and (for example) potassium hexacyanoferrate(III) (K₃[Fe(CN)₆]), also known as potassium ferricyanide. The hexacyanoferrate(III) ion ([Fe(CN)₆]³⁻) is reduced to the hexacyanoferrate(II) ion ([Fe(CN)₆]²⁻), giving potassium ferrocyanide, K₄[Fe(CN)₆], while the two oxygen atoms from the hydrogen peroxide are oxidised from oxidation state -1 to 0:

2K₃[Fe(CN)₆] + 2KOH + H₂O₂ → 2K₄[Fe(CN)₆] + 2H₂O + O₂

The cyclic peroxide then decomposes to give 3-aminophthalate (3-amino-1,2-benzenedicarboxylic acid) in an excited state, along with a molecule of nitrogen (N₂) – see Figure 5, below. This decomposition reaction is favoured because the cyclic peroxide molecule is highly unstable, and the reaction involves breaking some weak bonds. It is also favoured because of the increase in entropy (disorder) due to the liberation of a gas molecule. When the excited 3-aminophthalate drops down to the ground state, a photon of blue light is released.

Figure 5: Reactions leading to the emission of light from luminol

Tautomers are molecules with the same molecular formula, but different arrangements of atoms or bonds. The two tautomers can be interconverted; the curly arrows show the movement of electrons that brings about the change between the two forms.
Aequorin was first discovered in the jellyfish Aequorea victoria.

Box 2: Chemistry of glow sticks

When diphenyl oxalate reacts with hydrogen peroxide ($\text{H}_2\text{O}_2$), it is oxidised to give phenol and a cyclic peroxide. The peroxide reacts with a molecule of dye to give two molecules of carbon dioxide ($\text{CO}_2$) and in the process, an electron in the dye molecule is promoted to an excited state. When the excited (high-energy) dye molecule returns to its ground state, a photon of light is released. The reaction is pH-dependent. When the solution is slightly alkaline, the reaction produces a brighter light.

Safety note: phenol is toxic, so if your glow stick leaks, take care not to get the liquid on your hands; if you do, wash them with soapy water straight away.

See also the general safety note on page 73 and on the Science in School website: www.scienceinschool.org/safety

Luciferin + $\text{O}_2$ $\rightarrow$ Oxyluciferin + Light

Figure 6: The structure of firefly luciferin

There have been a number of experiments investigating aequorin, a protein found in certain jellyfish, which produces blue light in the presence of calcium (see Shaw, 2002, and...
Box 3: What makes glow sticks different colours?

The dyes used in glow sticks are **conjugated** aromatic compounds (arenes). The degree of conjugation is reflected in the different colour of the light emitted when an electron drops down from the excited state to the ground state.

![Image of glow sticks with chemical structures](image_url)

**BACKGROUND**

The dyes used in glow sticks are conjugated aromatic compounds (arenes). The degree of conjugation is reflected in the different colour of the light emitted when an electron drops down from the excited state to the ground state.

Furtado, 2009) and can thus be used in molecular biology to measure calcium levels in cells. Some scientists have come up with other ideas for utilising bioluminescence in the future, for example self-illuminated Christmas trees. Can you think of any other exciting potential uses for this amazing natural phenomenon?

**Acknowledgement**

The original version of this article was published in *Chemistry Review* and is reproduced with kind permission by the publisher, Philip Allan. To subscribe to *Chemistry Review*, a journal aimed at school chemistry students aged 16-19, visit: www.philipallan.co.uk/chemistryreview

**References**


**Resources**

For some experiments with luminol, see Declan Fleming’s website for older school students, all about the chemiluminescence of luminol: www.chm.bris.ac.uk/webprojects2002/fleming/experimental.htm

To learn about other types of light in chemistry, see:


Emma Welsh is a freelance science communicator with a PhD in synthetic organic chemistry and postdoctoral experience of medicinal chemistry, making drugs that inhibit enzymes which are involved in cancer biology.