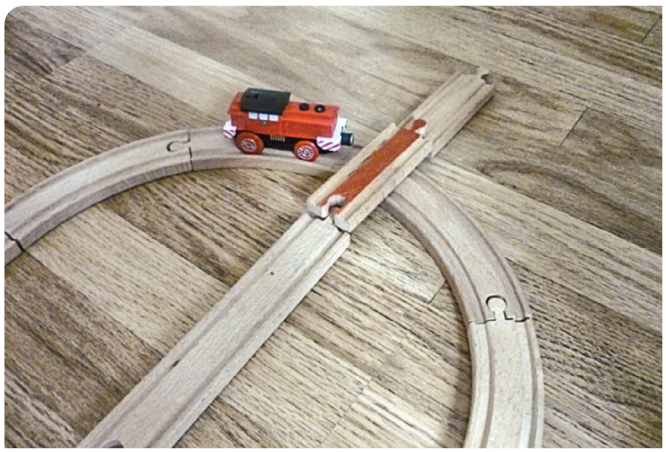

(a) Gene placed across plasmid

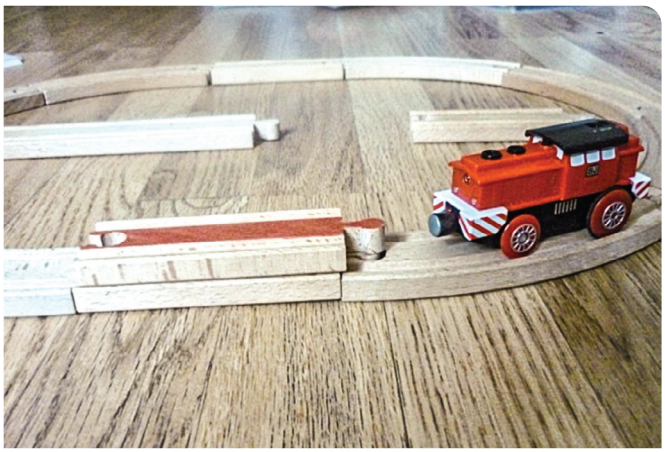

Figure 4 (b) Gene placed on top of plasmid

track model is flexible enough to allow the short 'gene' section to be inserted into the circuit with only one break – another point for discussion as many of the students will remove a section of the plasmid to allow insertion of the gene.

At this point, it is useful to point out that the ends of the pieces of track have been cut so that they fit into each other, and that this should also be true of the cut ends of the gene and the plasmid. If craft scissors have been used, the necessity to use the same scissors to cut out the gene and the plasmid is also identified.

Students have already studied the role of enzymes in digestion at key stage 3 and can identify them as 'biological scissors'. Therefore, the fact that they would use an enzyme, and furthermore the same enzyme, to cut both the chromosome and the plasmid comes as an easy discussion point.

The students were then sent back to amend their models and used the completed plasmid as a basis to produce a flow diagram illustrating the initial steps in the genetic manipulation of bacterial plasmids to produce human insulin.

Diet drinks and Mentos: a novel twist on an old favourite

Paul C. Beaumont, Andrew Boswell and Christopher Lloyd

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The phrase exocharmic was introduced into the literature to describe those (chemical) reactions and/or demonstrations which '*fascinate, allure or delight the observer*' (Ramette, 1980). In SSERC we actively pursue (see, for example, Adams *et al.*, 2012; Beaumont, 2011) opportunities to add to the variety and number of exocharmic systems available to educators, particularly those involved in teaching in schools and colleges.

A very popular and striking demonstration is the so-called Diet Coke™/Mentos™ eruption (Wikipedia – see *Websites*). A detailed explanation of the scientific principles involved has been published (Coffey, 2008a) together with a description of how the Diet Coke/Mentos experiment can be used as the basis for student investigations (Coffey, 2008b). A number of video clips of the experiment exist but, in our opinion, one of the most spectacular is available on the Eepybird website (see *Websites*). Virtually any carbonated drink can be substituted for the Diet Coke, including tonic water. Interestingly, tonic water contains quinine, which, when illuminated with ultraviolet (UV) light, emits a blue fluorescence. We thus wondered whether it might be possible to produce a fluorescent rainbow based on carbonated drinks/Mentos eruptions. Our results are summarised in this *Science note*.

Materials and methods

The light source used was a 365 nm XX-40BLB lamp from Ultra-Violet Products Ltd (Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG; see *Websites*). Because the fluorescence from the samples is best observed under reduced lighting levels (preferably when one's eyes have become dark-adapted), it is important that precautions to reduce exposure to UV light, especially of the eyes, are taken.

In all experiments reported here, we used 'diet' carbonated drinks because any residues that are produced are less 'sticky' and are more easily removed. Where indicated, proprietary brands of drinks were used without special preparations being made; for example, Schweppes Diet Tonic Water was used. In most of the

experiments reported, we used Premium Diet Lemonade from Tesco – this choice was based on two key factors:

- both container and lemonade were non-fluorescent when viewed under UV light
- the drink was on 'special offer' on the day of purchase.

Rhodamine B and rhodamine 6G dyes were drawn from laboratory stock (both had been originally purchased from Sigma-Aldrich; see *Websites*) and used without further purification. Aqueous stock solutions of the dyes were prepared at a concentration of 5×10^{-4} mol dm^{-3} (the molecular mass of both rhodamine dyes is 479 g mol^{-1}) and appropriate aliquots (typically 30–40 cm^3) of these solutions were added to bottles (1 dm^3) of diet lemonade at room temperature. To produce green fluorescence, we used Tesco Lemon All Purpose Cleaner and, in this case, approximately 40 cm^3 of undiluted cleaner was added to a bottle (1 dm^3) of diet lemonade.

Mentos were placed in a 'geyser tube', which allows for a controlled release of Mentos into the bottle containing the carbonated drink. Geyser tubes are available from a number of sources including Amazon (see *Websites*) and a typical experimental setup is shown in Figure 1.

Those familiar with the Diet Coke/Mentos experiment and its variants will know that significant volumes of liquid can be released and this activity is not normally performed indoors. The experiments described here require the environment to be blacked out or, at the very least, lighting levels should be kept to a minimum and for this reason it is convenient to perform the experiment indoors. Consequently, you will need to consider how best to reduce the effects of spillages. We place our carbonated drinks bottles (1 dm^3) in the centre of a large paddling pool and we find that, of the 500 cm^3 of liquid typically released (the actual volume released depends on a number of factors including the number of Mentos used and temperature of the carbonated drink), at least 90% of this volume falls back into the paddling pool.

Safety note

None of the experiments here present significant health and safety risks provided standard laboratory practices are observed. Eye protection to reduce exposure to UV light should be worn by those carrying out the experiment. We recommend that the experiments, as described, should not be carried out by students.

At the final concentrations used, the fluorescent dyes do not pose significant health risks although care should be taken when handling pure rhodamine dyes and undiluted Tesco Lemon All Purpose Cleaner. When preparing stock solutions of rhodamine dyes, appropriate care should be taken to avoid skin and eye contact.

Figure 1 Geyser tube arrangement with three Mentos in place; removal of the pin (located just underneath the Mentos) releases the Mentos into the bottle

In line with good laboratory practice, we recommend that the demonstrator wears eye protection. Clearly, it is important to avoid directing the liquid at any electrical (for example, ceiling lights) or sensitive equipment and we recommend that the UV lamp should be kept at a minimum of 2 m from the drinks bottle.

Results and discussion

We purchased a variety of carbonated drinks from our local supermarket and tested them for fluorescence under UV illumination. Tonic water, because of its intrinsic ability to fluoresce (owing to the presence of quinine, which has a high quantum yield (Φ_F) of fluorescence), can be used in combination with Mentos to produce a visually stunning demonstration when using a UV lamp as the light source (Figure 2).

Figure 2 Diet tonic water + Mentos eruption; the illumination was from a 365 nm UV lamp located just out of the camera's view

A B C D

Figure 3 The Mentos eruption in the presence of fluorescent dyes and illuminated with 365 nm UV light. In each case, two Mentos were added via a geyser tube. A: tonic water; B: lemonade + Tesco Lemon All Purpose Cleaner (40 cm^3 cleaner added to 1 dm^3 of lemonade); C: lemonade + rhodamine 6G (1.5×10^{-5} mol dm^{-3}); D: lemonade + rhodamine B (1.5×10^{-5} mol dm^{-3}).

Other than tonic water, the only other possible candidate for inclusion in our 'commercially available' rainbow was Red Bull, although the level of fluorescence was low under UV illumination compared with that obtained from tonic water. We did get quite excited after buying a bottle of Mountain Dew Energy, only to find that the yellow-green fluorescence we observed was from the container rather than the contents!

Given the absence of suitable commercially available fluorescent drinks, we decided to add fluorescent materials to diet lemonade to provide us with a range of colours of the rainbow. Our choice of fluorescent dye was based on a number of criteria:

- water solubility
- availability
- high yield of fluorescence
- UV absorption properties
- low toxicological concerns at the concentrations used.

Shortly into our investigations we encountered difficulties, yet to be overcome, in identifying water-soluble dyes which when illuminated with the UV lamp available to us gave rise to suitably intense fluorescence in the violet and far-red portions of the spectrum. However, we have made good progress with other parts of the spectrum and the results we obtained are shown in Figure 3.

The amount of fluorescence emitted by a sample is related to: (i) the fluorescence efficiency (that is, the ratio of emitted photons to absorbed photons); and (ii) the extent to which excitation light (in this case 365 nm) is absorbed by the sample (Haugland, 2002). The fact that the fluorescence fountains observed are not all of the same light intensity can be explained by consideration of these two factors. We would be keen to hear from anyone who can suggest dyes that display fluorescence in the violet or red portions of the spectrum and that additionally meet the criteria stated above.

It had been our original intention to use fluorescein as one of the chosen dyes but the observed yield of fluorescence was rather low under the conditions used. In part, the explanation for this is that Φ_F for fluorescein is pH dependent. At pH 3 (the approximate pH of the lemonade used), Φ_F is reduced by about 90% compared

Update note (March 2022): You can still buy the 365 nm XX-40BLB UV lamp, although the company has changed its name and the lamp appears expensive. However, since this *Science note* was written, LED UVA lamps have become widely available and less expensive than fluorescent devices and these would now be preferred. The excitation wavelength of quinine is 350 nm, but it will become excited at 365 nm, so a 365 nm LED torch, say 10 W, should work OK. Note that 395 nm torches are likely to be ineffective, and UVC LED lamps should ***not*** be used. The torch should be clamped, and it is important to make sure that there are no mirror-like surfaces to cause reflections towards the observers. The demonstrator and audience should be ***behind*** the torch so it can't be viewed directly.

with that at pH 7 (Haugland, 2002). Tesco Lemon All Purpose Cleaner contains the trisodium salt of 8-hydroxypyrene-1,3,6-trisulfonic acid (also known as pyranine or Solvent Green 7) as the fluorophore (information from www.detergentinfo.com) and the yield of fluorescence is appreciable and apparently not affected by changes in pH.

We recognise that the costs of the rhodamine dyes used in these experiments may be beyond the scope of many school budgets in the current economic climate but we expect that access to diet tonic water and Tesco Lemon All Purpose Cleaner should not be too problematic. If you have a UV lamp and a room that can be blacked out, we thoroughly recommend that you try one or more of the fluorescent combinations described here – in our view, they are indeed exocharmic.

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Titration curve of ethanedioic acid (oxalic acid) with sodium hydroxide

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This *Science note* arose out of class practical work involving recording (via dataloggers) the titration curve (Figure 1) when ethanedioic acid (oxalic acid) is neutralised by excess sodium hydroxide solution:

$(COOH)_2(aq) + 2NaOH(aq) \rightleftharpoons (COO^-Na^+)_2(aq) + 2H_2O(l)$

Figure 1 shows one data set obtained by one group of students, but other groups of students in the same class and other classes obtained similar data sets using the same pH probes and meters.

The titration curve did not fully match the idealised titration curve (Figure 2) found in many textbooks and websites. The curve around the second equivalence point closely resembled that in Figure 2 but the first equivalence point was surprisingly not clearly defined and did not have a well-defined vertical region.

This *note* aims to provide the necessary theory to explain these observations. Since the second half of the titration curve is correct, we assumed that the pH probe and meter were working correctly and that we were seeing a real effect that needs an explanation.

We expected the two protons to be removed in a stepwise manner:

$(COOH)_2(aq) + OH^-(aq) \rightarrow HOOC–COO^-(aq) + H_2O(l)$,

then

$HOOC–COO^-(aq) + OH^-(aq) \rightarrow {}^-OOC–COO^-(aq) + H_2O(l)$

Databooks give two clear pK_a values for ethanedioic acid at, for example, 1.2 and 4.2, suggesting two clear