RSC Sustainability

TUTORIAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *RSC Sustainability*, 2026, 4, 1760

Received 20th December 2025
Accepted 28th February 2026

DOI: 10.1039/d5su00933b

rsc.li/rscsus

Colourful chemistry of anthocyanins: a tutorial review of applications of anthocyanins in school and university teaching

Iain A. Smellie, *[a] Isobel Everest[b] and Iain L. J. Patterson[a]

The flowers, fruits and vegetables that we encounter in everyday life provide an opportunity for discussion of key chemistry concepts in school and university level chemistry. The rich variety of colours we see in the natural world are often due to anthocyanins present in flower petals, and the skins of fresh fruit and vegetables. The chemistry of anthocyanins has been studied for over 100 years, and throughout this period, chemistry educators have employed anthocyanin sources in a range of experiments and classroom activities. Anthocyanins are readily available from inexpensive renewable sources, such as flower petals, fruit peelings, foraged fruit and various vegetables. Anthocyanins are often accessible simply by soaking plant tissues in warm water, this allows simple equipment to be used and generation of large quantities of hazardous waste can often be avoided. In this tutorial review, a survey of a wide variety of example uses of anthocyanins in chemistry teaching is presented. Selected exemplar activities, developed by the authors, for use by school and university educators are also described.

Sustainability spotlight

An understanding the chemistry of plant-derived anthocyanins can be applied in classroom activities for students at various educational levels. This topic highlights the importance of organic chemistry in the natural world, the food industry and links to other scientific disciplines. A literature review of classroom activities is presented, all feature plant extracts that are available from renewable sources. Activities used by the authors in small group scenarios and as lecture demonstrations are also described. This review is primarily aimed at school and university educators—this aligns with UN sustainability development goal SDG 4. The ability to obtain extracts in water from renewable sources and food waste allows goals SDG12 and SDG15 to be highlighted to students.

Introduction

The chemistry of plant life has featured in chemistry courses for many years.[1,2] A diverse range of plant-based materials are available to educators, and these can be used to highlight a range of chemical concepts. These include: medicinal chemistry, environmental chemistry, production of food, production of dyes, colour chemistry, and aspects of analytical chemistry.[1–8] Materials derived from plants offer several advantages to chemistry educators, since they are often familiar to students, inexpensive, readily available, and environmentally benign.[4,5,8,9] Compounds isolated from plants are versatile since they can be used as chemical reagents, or as subjects for the demonstration of practical techniques.[4,5] One of the most familiar applications of plant-derived material in chemistry teaching is the application of flowers and other plants as colourful dyes or analytical reagents.[5–7] The use of flowers, fruit and vegetables in classrooms and teaching laboratories allows chemistry educators to incorporate some of the 12 principles of green chemistry in their teaching.[9,10] For example, plant-derived sources of pigments illustrate principle 7, the use of "renewable feedstocks".[9] Spinach and other green leaf plants have long been a popular source of carotenes and chlorophylls for teaching activities. However, a disadvantage with the extraction of these materials is often the need to use flammable and/or toxic organic solvents.[11,12] In contrast, anthocyanin extracts from a range of sources can often be obtained by extraction using water, and these pigments are available from a diverse range of plants.[13,14] The ability to extract many anthocyanins in aqueous conditions provides an opportunity for educators to illustrates principle 5, the use of safer solvents.[9] Furthermore, the waste products from these extraction protocols usually present a low hazard. The resulting aqueous solutions and plant-derived waste can be composted or disposed of with minimal impact on the environment. This latter point aligns with principle 10, "design for degradation", the plant tissue that has been extracted is biodegradable and avoids the formation of persistent degradation products in the environment.[9]

The vibrant red, purple, violet and blue colours of the flowers, fruit and vegetables we encounter in everyday life are

---

[a] EaStCHEM, School of Chemistry, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK. E-mail: ias10@st-andrews.ac.uk

[b] Bedford School, De Parys Ave, Bedford MK40 2TU, UK

often derived from anthocyanins. These plant pigments have been the subject of a wide variety of studies for over 100 years.[15–18] Early work was focused on determining their chemical structures, a major contribution in this area was made by Richard Willstätter.[19–21] In 1915, he was awarded the Nobel prize for chemistry, this accolade was partly in recognition of his key work on anthocyanins.[20,21] It is important to highlight that the term "anthocyanin" specifically refers to the glycosylated derivatives of anthocyanidins, of which, there are six commonly encountered in nature: cyanidin, pelargonidin, delphinidin, petunidin, malvidin and peonidin (Fig. 1). Willstätter's Nobel lecture described pelargonidin, cyanidin and delphinidin (as chloride salts) that had been isolated from flowers and berries that he and his co-workers collected and cultivated.

Within plant tissues, anthocyanidins are glycosylated at position 3, or positions 3 and 5 (Fig. 2).[15–17] Often D-glucose is found to be the carbohydrate substituent. However, there is a range of monosaccharides and disaccharides that can occupy the 3 or 5 positions. The presence of carbohydrates in anthocyanin-containing extracts was identified by Wilstätter, a point which he highlighted in his Nobel prize lecture.[21]

"The anthocyanins proved to be glucosides, in which the actual pigments, compounds with phenolic hydroxyl groups, are paired with one or two (or even more) molecules of sugars".[21]

Around the same time, Robert Robinson (later to be knighted and awarded the 1947 Nobel prize for chemistry) further extended the understanding of anthocyanins through the 1920s and 1930s.[22,23] Robinson and his co-workers were able to complete the total synthesis of a range of anthocyanins. Over the course of 22 years, Robinson and his co-workers developed methods to prepare anthocyanidins, and latterly, anthocyanins.[22,23] In this period, Robinson was not only able to demonstrate the ability to make naturally occurring pigments by artificial means, but he also gathered a large quantity of valuable data on the properties and reactivity of anthocyanins. Intriguingly, there is an early nod toward the relevance of anthocyanin chemistry in teaching, in a 1928 paper, as part of a synthesis of cyanidin chloride. Robinson wrote:[24]

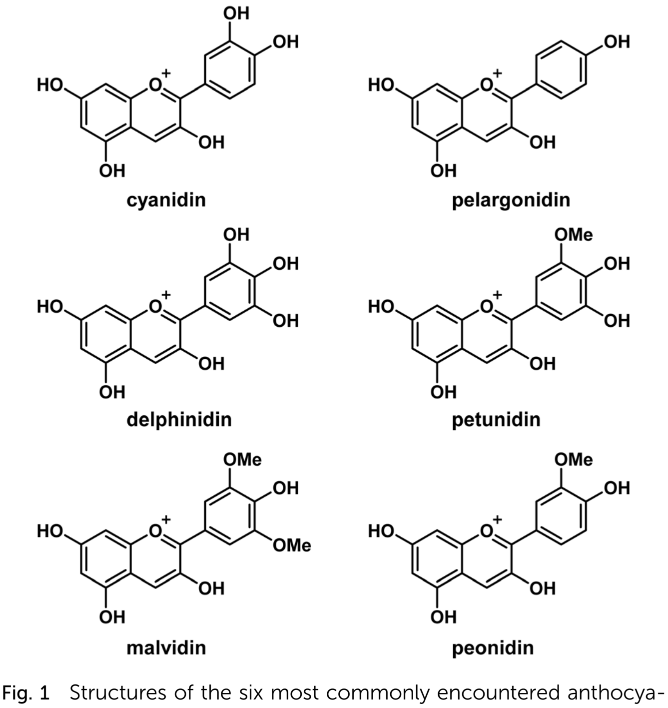

Fig. 1 Structures of the six most commonly encountered anthocyanidins in flowers, fruits and vegetables.

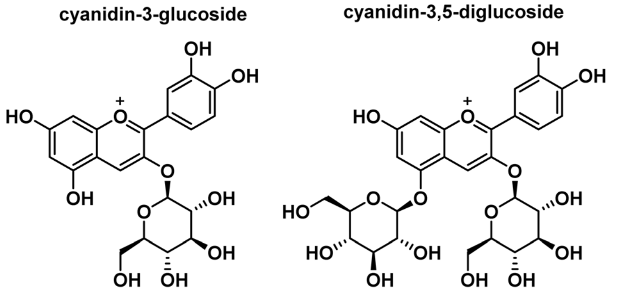

Fig. 2 Examples of glucosylated anthocyanidins.

"we have for some time realised the desirability of devising, if possible, a synthetic method of preparation of the pure anthocyanidins that could be used, say, as a laboratory exercise by students".[24]

The chemical education-focused literature was not slow to recognise the interest in anthocyanin chemistry. In 1928 and 1929 E. H. Huntress presented a three-part series of articles titled "The chemistry of the red and blue pigments of flowers and fruits" in the *Journal of Chemical Education*.[25–27] These papers provided a detailed overview of the developments in the understanding of anthocyanins that were taking place at the time. Further detail was provided by T. A. Geissman in two organic chemistry-focused articles on flower colouration in 1941 and 1949.[28,29]

Since the early work summarised above, anthocyanin extracts from plant sources have been widely adopted by chemistry educators. The range of applications in chemistry teaching, especially over the last 20 years, is wide, as evidenced by the more than 80 such publications cited herein. Agricultural sources of anthocyanins, such as red cabbage extract, are now in common use by school and university educators.[13,14] There is such a wide range of applications, that a section of this review is solely focused on the use of red cabbage extracts. Food-derived anthocyanins provide an opportunity to discuss aspects of "Kitchen chemistry", both in terms of the explanation of everyday observations, but also the potential to use anthocyanins in the development of methods to create sustainable food colourings, preserve foodstuffs and avoid waste.[30–32]

There are however many more anthocyanin sources that do not require the use of food crops, for example food waste, houseplants and sustainably foraged wild berries. Food waste includes out of date berries, discarded outer leaves of vegetables (such as red cabbage or red onion), and the soaking, canning or pickling liquors (from the preparation of pulses, canned berries or pickled vegetables). Flowers and leaves have the advantage that they are easily sustainably sourced through home cultivation. Berries and vegetables can also be grown in household or school gardens. Flowers featured frequently in Wilstätter's and

Robinson's work, as will be highlighted in this article, these plants are particularly useful, versatile and sustainable sources of anthocyanins for educational use.

In this article, we present a tutorial review that is divided into two parts: (1) a survey of diverse examples of the use of anthocyanins in chemistry teaching and (2) some selected examples of simple activities developed by the authors are described, these are provided for chemistry teachers (in schools and universities) to use and adapt for their courses. This tutorial review highlights many individual teaching activities focused on the chemistry of anthocyanins. As part of this, related theory (such as the explanation of anthocyanin halochromism) are signposted where appropriate. However, we would like to highlight some excellent review articles by Pina *et al.*,[15,17] Dangles *et al.*[16] and Yoshida *et al.*[18] These reviews offer very detailed surveys of key concepts that will prove useful to educators who use anthocyanin-based activities in their classes.

Literature survey

Use of anthocyanins as pH indicators: overview

The most familiar applications of anthocyanins in chemistry teaching are related to the concept of acidic and basic substances. Anthocyanins are halochromic, this colour response of anthocyanins to pH changes was studied in depth by Willstätter and other colleagues as part of their studies of plants in the early 20th century.[20,21] Tswett, described plant extracts containing anthocyanins as "vegetable chameleons" due to the wide range of colours observed when the pH of anthocyanin-containing solutions was varied.[33] Willstätter included the following anecdote in his Nobel speech:[21]

"I took a rose from my Zurich garden into the laboratory. The aqueous solution of the pigment prepared from this rose changed with sodium carbonate from red to emerald green; if, however, the anthocyanin solution was first acidified with a drop of mineral acid, then the colour changed with alkali suddenly from red to deep blue. Between the red of the acid solution and the blue of the alkali solution there appeared a violet neutral colour".[21]

The colour changes highlighted in Willstätter's lecture can be explained by the structures illustrated in Fig. 3.[15–17] The flavylium ion forms of anthocyanins are often red, magenta or pink at low pH. As the pH of a solution of anthocyanins is increased to 5–6, the colour can fade. This is due to addition of water to the flavylium ion and formation of a colourless hemiketal form, which is in equilibrium with the flavylium ion. At higher pHs, the violet quinoidal base or blue ionic quinoidal base forms are observed. Increasing the pH further will often lead to an initial green colour, slowly changing to yellow, or rapidly at very high pH. The latter observation is likely due to mixtures of quinoidal anthocyanins and the chalcone forms being present.[34] The blue and yellow colours associated with these species would be observed as green when mixed. However, in unpurified extracts, phenoxide ions derived from flavonoids will also contribute a yellow colour.

The chemistry of anthocyanins that was established over 100 years ago has now become a common feature in school and university classrooms. While red cabbage indicator solution continues to feature most prominently, many alternative anthocyanin extracts are readily accessible. In this section, a survey of the recent literature is provided. Applications as pH indicators are often focused on the associated colour changes, however, it is possible to measure the pH associated with each observed colourful solution.[13] The colour of these solutions also provides a useful scenario to introduce UV-visible spectroscopy to students.[14] This review will cover a range of teaching activities that go beyond the use of anthocyanins as pH indicators. Educators have incorporated anthocyanins into a variety of other topics, including electrochemistry, chromatography, metal-binding interactions and colour bleaching reactions.

Use of anthocyanins as pH indicators: red cabbage extracts

Extracts of red cabbage (*Brassica oleracea var. capitata F. rubra*) are often used by chemistry educators in teaching activities to

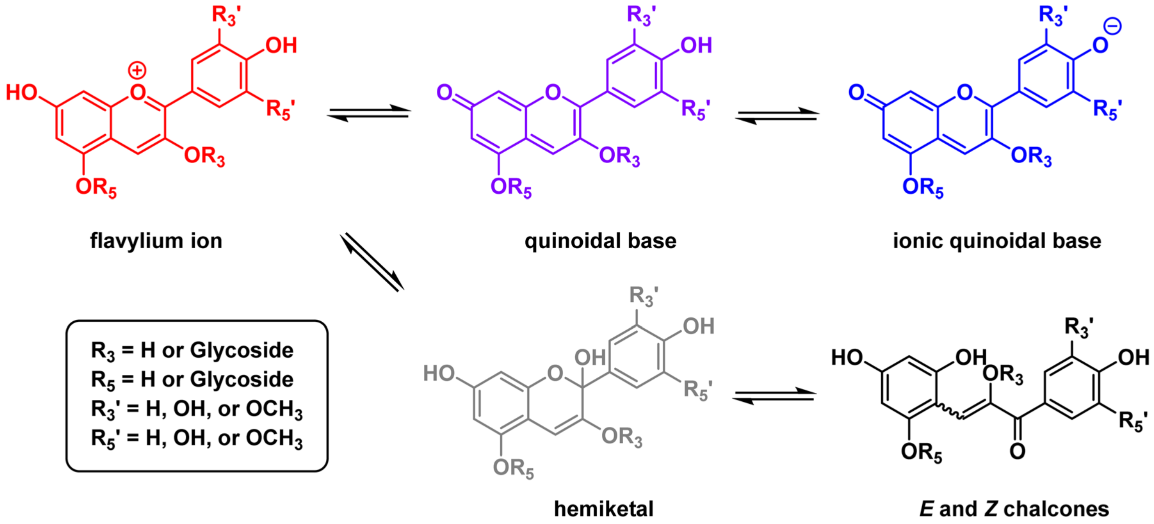

Fig. 3 Reactions of anthocyanins in aqueous solution controlled by pH.

illustrate colour changes in response to varying the pH of aqueous solutions.[13] There are various reasons for this popularity, red cabbage is widely available from grocery stores, and it is usually inexpensive. Furthermore, the extraction of the red cabbage anthocyanins in hot water is an easy and quick process.[13,35] The resulting extract also gives a pleasing range of colours when added to acids and bases.[13] There are however some disadvantages; the extracts can have an unpleasant odour, especially if attempts are made to store them at room temperature or in a domestic refrigerator. Anyone who works with red cabbage extracts will be very familiar with these issues but, fortunately, there are methods to ease storage problems, and these will be discussed in a later section.

Red cabbage extracts have proved to be versatile and have found a variety of applications in chemistry teaching. A common application is to use the extract as a pH indicator. A typical approach would involve preparing a series of dilute solutions of strong or weak acids and bases in suitable containers, followed by addition of red cabbage indicator to each solution. The colour response in each case can be noted, and if desired, the pH of each solution can also be measured.[13] There are many ways to adapt this type of activity further, for example, the colours of the solutions are easily projected onto a screen as a part of a classroom presentation.[35] An increasingly popular approach is to use microscale techniques; this type of activity uses laminated worksheets as a platform for droplets of reagents to be deposited on.[36] For example, a weakly basic solution can be mixed with red cabbage indicator and a droplet of this placed on the laminated sheet. An acidic solution can then be added carefully using a small pipette to initiate a colour change within the droplet. Some examples of this type of activity will be presented in a later section.

The pH responsive property of red cabbage indicator is often used in lessons that introduce students to the concepts of acidity and basicity. There are good examples of different contexts to present this information, examples include reactions with carbon dioxide gas,[35] an activity in an escape room themed task,[37] and as a sensor to identify unknown colourless solid materials.[38] It is also possible to employ red cabbage indicator in the context of electrolysis of water.[39,40] A lecture demonstration of this process has been developed for an introductory college course, where red cabbage indicator is added to a dilute solution of sodium nitrate in a large glass dish and placed on a projector.[39] Two metal electrodes attached to a DC power supply are then immersed in the solution, and the power switched on. As electrolysis proceeds, the oxidation reaction at the anode releases H_3O^+ ions and the reduction reaction at the cathode releases OH^- ions. The colour of the indicator solution in the vicinity of the electrode changes as the local pH increases or decreases. It is also possible to demonstrate that the solution returns to the original colour when the power is turned off and the solution is thoroughly mixed. More recently, an innovative version of this activity has been presented to senior level high school students; this features red cabbage indicator set in a seaweed-derived carrageenan hydrogel.[40] The gel is set in a glass dish, copper electrodes are then attached to a 9 volt battery, and the electrodes can then be applied directly to the gel surface to initiate colour changes. The gel is sufficiently firm that this allows a copper electrode to be used like a pen to write letters or numbers directly on to the gel surface. Furthermore, the gel surface will change colour if exposed to small quantities of hydrogen chloride or ammonia gas.

The pH-controlled colour changing reactions of red cabbage anthocyanins are not confined to classroom laboratories, they can also readily be presented in a "kitchen chemistry" context.[31] This approach proved to very useful to school and university educators during the COVID-19 pandemic in 2020, since acidic and basic household products could be purchased and used at home.[41] An engaging adaption of this idea was presented by McIndoe and Stoddard in 2006.[42] This paper described a lesson for high school level students where a colourful "sports drink" could be made by mixing solutions of dilute citric acid and sodium bicarbonate with red cabbage extract and observing the colour changes. Students were also directed to consider the other additives required to prepare a soft drink, such as sugar and electrolytes. Students may be familiar with household products that are used to treat symptoms of heartburn and indigestion. These products often contain sodium alginate; this material reacts with calcium ions in the digestive system to form a protective barrier or "raft" of calcium alginate gel.[43] This reaction has been adapted for use for pH demonstrations in high school classes, the objective being to make colour changing calcium alginate "chameleon" balls.[44] The procedure involves preparing a solution of red cabbage indicator that contains 2% sodium alginate, this is then added dropwise to a stirred 1% solution of calcium chloride. The result is the formation of small spheres of encapsulated indicator; the alginate balls can then be collected by filtration and are robust enough to be manipulated by hand. If the balls are immersed in dilute acid or basic solution, they will change colour in the same way as would be expected in solution. Since sodium alginate is derived from seaweed and is often used in the catering industry, food grade material is inexpensive and readily available. In most pH demonstrations involving red cabbage, the vegetable material is extracted using hot water. However, a kitchen-based example has been developed for use in school laboratories and public outreach events that avoids the need for the extraction step. In this case, the production of red cabbage sauerkraut is demonstrated, this allows the foodstuff to act as the indicator as well as being the intended product.[45] The initial purple colour of the salted cabbage (both the plant tissue and the liquid) is observed to progressively turn red as lactic acid is released during the 3 day fermentation period. This is a pleasing example where the system is self-indicating and serves as an example that is directly related to food production. Red cabbage indicator has been employed in a biochemistry context for high school students; in this example it can be used for the detection of ammonia released from an enzymatic process.[46] The associated protocol is simple and effective; it readily demonstrates that soya bean urease enzyme converts urea to carbon dioxide and ammonia. This is achieved when the students mix soya bean extract and urea in the presence of red cabbage indicator, they observe that the solution becomes green, this information then allows them to conclude that the colour change is due to the increased pH from the ammonia produced by the urease enzyme.

The examples described so far in this section all largely involve the indicator in aqueous solutions, however, red cabbage indicator can also be absorbed onto other substrates, such as silica gel beads, hydrogels and paper.[47–51] The use of red cabbage indicator on paper has been found to be particularly adaptable; the following examples show pH dependent colour changes associated with anthocyanins in the context of art and craft work.[50–54] An interesting example of this approach is illustrated by a Japanese artform that has been adapted for teaching purposes by S. Suzuki.[50] A piece of absorbent paper or napkin is allowed to soak in red cabbage extract, and the coloured paper is then allowed to dry. The paper is then folded into a pattern of the users choosing, and each end of the folded paper is dipped into dilute solutions of acids and bases. Once complete, the paper is unfolded, and intricate colourful patterns are obtained. A related activity, aimed at middle and high school students, involves soaking flat pieces of paper in red cabbage indicator and allowing them to dry.[51] Solutions of weak acids or bases can then be used as an ink or paint to write messages or paint pictures on the pre-treated paper. It is also possible to use red cabbage indicator to reveal hidden messages. This is achieved by writing a message on a piece of paper using a cotton swab dipped in vinegar or lemon juice. Once the paper has been allowed to dry, the message is revealed by spraying red cabbage indicator onto the paper using a water spray bottle.[52] A further variation on this theme is the use of an anthocyanin-derived ink with brushes, stamps or in screen printing. The colour of the ink applied by the chosen method can then be changed by treatment with a dilute acid or base solution.[53] The original report suggests that this activity has successfully been used with children younger than 11 years old in the context of drawing and painting. The activity has also been adapted for use by older school children. A very recent example of red cabbage extract being employed in the context of the arts, is a paper focused on making colourful Peking opera masks.[54] This activity for middle or high school students involves a paper mask being fashioned by intricate and precise cutting of the paper to give the desired mask shape. The paper masks are then soaked in mildly acidic or basic solutions and allowed to dry. Subsequent application of red cabbage indicator solution allows different colour masks to be prepared. This activity can be further extended in an innovative way to include electrochemical concepts. The masks are soaked in a mixture of red cabbage indicator and sodium chloride, to form a purple-coloured mask. Rather than apply an acidic or basic solution, graphite pencils are connected to the terminals of a small commercial battery and the pencil tips are applied to the paper mask. The pencil connected to the negative terminal of the battery results in a colour change from purple to green/yellow on the paper, this is due to a build-up of hydroxide ion from electrolysis of water. In contrast, the pencil connected to the positive terminal turns the paper red, associated with a decrease in pH. The lower pH at the positive terminal is believed to occur from initial oxidation of chloride ions to form chlorine, this in turn can react with water to form hydrochloric acid and hypochlorous acid.

All of the examples of chemistry teaching presented so far that feature red cabbage indicator have largely been qualitative investigations. However, this indicator can be used in quantitative activities too.[55] Smartphones have become increasingly adopted by educators for a variety of purposes, the availability of these devices has allowed the development of experiments that can be done at home, as well as in the classroom.[56] Studies involving university students have recently been published which make use of red cabbage indicator in weak acid/weak base titrations, where a smartphone camera allows video or pictures of the titration to be captured and analysed.[57–59] These studies have focused on the use of low hazard chemicals that can be obtained from grocery or hardware stores for use at home. As well as manipulating the reagents, the students learn about the best way to light their experimental set up for optimum capture of video or pictures. The students also learn to use phone apps to detect colour changes and plot titration curves. This methodology is versatile, example applications include: the determination of the concentration of acetic acid in vinegar, investigation of the buffer capacity of sodium carbonate-sodium bicarbonate buffer and the effect of temperature on chemical equilibria.[57–59]

Use of anthocyanins as pH indicators: other flower, fruit and vegetable sources

Although red cabbage indicator is very popular, this is by no means the only source of anthocyanin plant pigments available. Fruit juices, berries, red onion and flowers can all serve as useful anthocyanin sources too. A paper by Garber, Odendaal and Carlson provides an excellent example of the application of flower, fruit and vegetable derived anthocyanins in an activity aimed at high school level students.[60] A series of pH tests are attempted in test tubes, however, unlike most other activities of this type, the observed colours from extracts are directly compared with commercial anthocyanidin samples. This process allows the students to try and match the botanical anthocyanin extracts to the anthocyanidin standard solutions, and hence identify which anthocyanidin is likely present in the plant extract.

Flowers can be very useful sources at the correct time of year, and they can provide a large quantity of plant material when they shed petals or are "deadheaded" by gardeners. Wild berries such as blackberries or elderberries can also be a useful source of anthocyanins if they are foraged responsibly. Berries are easily stored for future use in a domestic freezer, flowers on the other hand can be harder to store, they are often best dried prior to storage, or the aqueous extracts can be frozen. In 1985, R. C. Mebane and T. R. Rybolt published a helpful guide to extracting anthocyanins from familiar fruit and vegetables.[61] This work also features a very useful table that summarises the colours of each extract as the pH of the solutions is varied. A detailed list of anthocyanin sources has also been provided more recently in a book by Zhang, Celli and Brooks.[62] The range of anthocyanin sources available is undoubtedly large, and an exhaustive list is beyond the scope of this article. A selection of examples and applications of fruit and flower derived anthocyanins are described in this section.[63]

There is a range of flowers available from household gardens or from retailers. Red Poinsettias (*Euphorbia pulcherrima*) serve as a useful example of a readily available ornamental flower that can be used to make a pH indicator.[64] The anthocyanin extraction can be achieved by simply soaking the flowers in hot water, then filtering out the plant tissue and concentrating the liquid that is collected. The indicator can be used to test a selection of household chemicals to determine whether these materials are acidic or basic. An experiment that used petals from pansies was reported as part of a series of pH experiments that could be attempted at home.[65] In this activity, petals from purple pansies were treated directly with household vinegar to make the petals change to become red. Alternatively, the petals were treated with a solution of baking soda or washing soda, the latter resulted in the petals becoming blue.

Fresh flowers are not always available, especially during the wintertime, however, dried flowers can also be used as sources of anthocyanins. It may not be necessary to prepare a supply of dried flowers since some varieties are available in commercially available tea blends. A survey of the pH response of a selection of teas was published by D. N. Epp in 1993.[66] This work was conducted as part of a high school activity, it highlighted that spiced and fruit teas containing dried red hibiscus became red under acid conditions, and green when basic solutions were added. The vivid colour changes are due to glycosylated cyanidins present in the dried red hibiscus flowers (*Hibiscus rosa-sinensis*).[67] The extraction of hibiscus tea has also been shown to make an effective laboratory exercise for senior high school students.[68] The students use UV-vis spectroscopy to examine the extraction yield from sequential extractions of the plant material and the effect of varying the temperature of the extraction solvent.

In recent years, butterfly pea flower (*Clitoria ternatea*) extract has become a popular addition to the pH indicator repertoire available to chemistry educators in school and university settings.[69,70] Butterfly pea flower extracts impart a vivid blue colour to a variety of grocery items, such as dairy products and rice.[71,72] Butterfly pea flower extracts offer an alternative to blue dyes (such as indigo carmine) that are often used in food products.[73] The colour of butterfly pea flower extracts vary with pH in a similar way to other anthocyanin-containing extracts and the flowers are readily available in dried forms commercially as a tea. A school outreach activity demonstrating the pH response of butterfly pea flower tea to addition of household chemicals was presented in 2024.[69] This work elegantly shows how experiments that normally use red cabbage indicator can be accomplished using butterfly pea flower tea as the anthocyanin source. D. Kajiya has recently proposed a very innovative and engaging method for introducing the concept of the complimentary colour wheel to university students in the context of anthocyanin chemistry.[70] This teaching approach combines knowledge of anthocyanin provenance, structural relationships and UV-vis spectroscopy to not only understand anthocyanin structure and properties, but also to understand key principles of colour theory. In this activity a range of anthocyanin extracts is employed, largely derived from flowers, with butterfly pea featuring prominently.

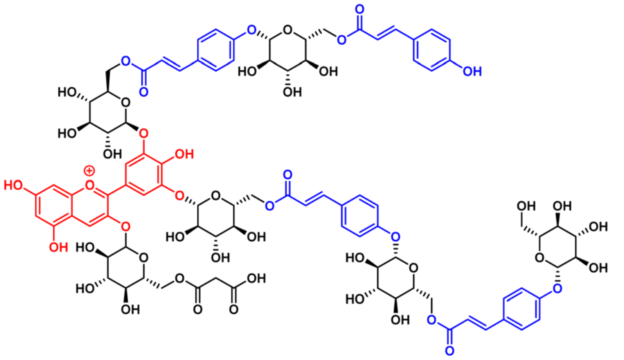

Fig. 4 Molecular structure of ternatin B1. Delphinidin is highlighted in red, acylating units are highlighted in blue and carbohydrates are shown in black.

The origin of the particularly rich colour palette available from butterfly pea flower indicator is due to the complex structure of the anthocyanins available in the flower petals. As was highlighted previously, anthocyanins in plant tissue are glycosylated, however, in many cases, the sugar units can be fuctionalised further. Ternatin B1 (Fig. 4), is one of the anthocyanins found in butterfly pea flowers, a key feature of this class of compounds is that they are acylated with phenolic acids.[74] Robinson was aware of the existence of acylated species and referred to them as "complex anthocyanins" in his 1935 review.[22]

The acyl units are significant, since they participate in intramolecular copigmentation, this interaction involves π–π stacking of the anthocyanidin rings and the alkene unit from the acylated phenolic acid (Fig. 5).[16] A full discussion of the precise nature of these interactions is beyond the scope of this article, however this type of effect can have a significant impact on the observed colour and stability the anthocyanin extracts. Highlighting the ability of anthocyanins to engage in intramolecular π–π interactions can be a useful example for students who are studying supramolecular chemistry.[75]

Flower petals often contain flavonoid compounds, and these can alter the observed colour of flower extracts during pH tests, especially in basic solutions. Recent work by Sampaio, Sousa and Dias has shown that extracting aqueous anthocyanin-containing solutions with ethyl acetate can help remove flavonoids from flower-derived anthocyanin extracts.[34] Flavonols

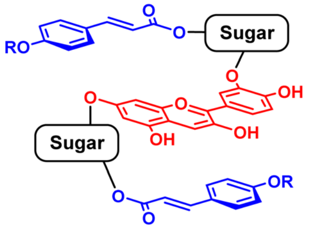

Fig. 5 π–π stacking interaction between anthocyanidin ring and acyl-linked side chains.

such as kaempferol, form yellow solutions in basic media due to phenoxide ion formation. This can contribute to basic solutions of anthocyanins appearing green rather than blue. The yellow colour of the flavonol phenoxide ions, mixed with the blue colour of the anthocyanin ionic quinoidal base forms, can appear green to the eye. The presence of flavonols in the colourless ethyl acetate extract can be demonstrated by adding dilute sodium hydroxide, this treatment results in yellow colouration of the aqueous sodium hydroxide layer.

A particularly striking variation on the demonstration of basic solutions of flower-derived anthocyanins has recently been published to highlight hazards posed by gaseous reagents.[76] Rather than mix anthocyanin-containing flower extracts with aqueous solutions of dilute bases, fresh flowers are suspended in a sealed glass chamber that contains ammonium chloride and sodium hydroxide. A small quantity of water is added and the subsequent reaction releases ammonia gas. Observers might expect that the anthocyanins would change colour in response to ammonia transfer into the flower petal tissue. However, the main observation is that the ammonia quickly and severely damages the plant tissues, often to the point that the flowers are observed to turn black.

Berries and grapes of various varieties have been used to prepare anthocyanin extracts as pH indicators. Appropriate berries are widely available from grocery stores, and many are available from the wild too. Some of the activities described in the previous section focused on red cabbage indicator, also included fruit as anthocyanin sources too. For example, in the study by Mebane and Rybolt, they highlighted that blueberries, cherries, grape juice, red apple skin, peach skin, pear skin and plum skin could all be used as pH indicators.[61] Further examples include the use of blueberries in the sports drink demonstration,[42] and the alginate "chameleon" balls activity can use red grape skin extract, as well as red cabbage indicator.[44] A recent report has described a high school activity that involves preparation of an agar gel.[77] The shaped pieces of agar gel can then be cut, and then soaked in acidic or basic solutions to initiate colour changes.

In 2020, a simple and thought-provoking series of kitchen-themed experiments with blueberries (*Vaccinium corymbosum*) was described.[78] It was noted that frozen blueberries left a red-violet residue in a breakfast bowl, however, when the bowl was rinsed with water, and the residue was diluted, the colour changed to blue-violet. It could be the case that the colour change was due to a change in pH, or the presence of a contaminant (such as a metal salt). However, testing of the blueberry extracts by varying the pH and addition of metal ions did not allow the colour change to be reproduced. It was concluded that the colour change was due to a pigmentation effect, the explanation being related to the π–π stacking interaction that has been identified in acylated anthocyanins. In this case it is likely that the colour change is due to "self-association" of anthocyanin molecules (Fig. 6). Studies of malvidin have shown that as the concentration of the anthocyanin in neutral solution becomes lower, the observed colour shifts from red, to become violet-blue.[79] This observation has been ascribed to a weakening of the intermolecular π–π stacking interaction. This example can serve as a simple and effective demonstration for students to illustrate copigmentation effects of anthocyanins.

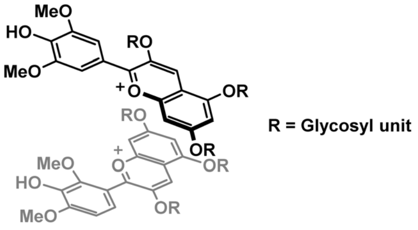

Fig. 6 Self-association of malvidin-containing anthocyanin molecules in solution.

Interactions of anthocyanins with metal ions: aluminium, iron and tin

The previous section focused on the halochromic properties of anthocyanin plant extracts and the diverse ways in which chemistry educators have used this chemistry in their classes. However, anthocyanin colour changes are not solely controlled by pH, many of these compounds can also bind to metal ions to initiate colour changes. This type of interaction is classed as a form of copigmentation,[16] and it is responsible for colour changes observed in the natural world, it is especially relevant to the colours of certain flowers.[18] In this section, the interactions with aluminium, iron and tin ions with anthocyanins will be highlighted.

Hydrangeas (*Hydrangea macrophylla*) are probably the most familiar example of flower colouration that involves metal ion binding to anthocyanins. Hydrangeas that are grown in basic soil are often red or pink, in contrast, those grown in more acidic soil can display blue flowers (Fig. 7).[80] This observation is unlikely to be attributed to a pH induced anthocyanin colour change because red/pink colours would be expected under acidic conditions. It is currently believed that the soil pH controls the availability of Al^{3+} ions to the plant tissue *via* the soil. Under basic conditions, aluminium-containing species are less likely to be water soluble. It has been shown that complexes of Al^{3+} can form with delphinidin-derived anthocyanins,[80] a simplified structure that represents this type of interaction is shown in Fig. 8.[80,81]

The chemistry of hydrangeas has been studied in great detail, and a full discussion is beyond the scope of this article.[18,80,81] However, we highlight an engaging example of a virtual experiment relevant to this area.[82] Chen has developed

Fig. 7 Red and blue hydrangeas.

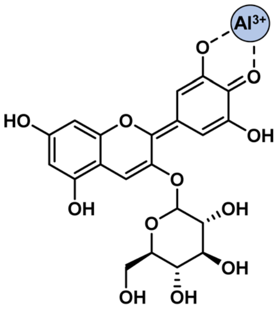

Fig. 8 A simplified representation of the binding mode of delphinidin-3-glucoside to Al^{3+} ions.

an activity for middle school students that allows them to investigate the relationship between pH, Al^{3+} availability and flower colour using a tablet computer or smartphone. The students are initially taught the underlying chemical principles; they are then directed to simulate what colour is likely to be observed under certain conditions. Once students are familiar with the background theory, they are then encouraged to develop their own virtual experiments and apply them.

D. Kajiya has developed a series of elegant practical tasks for high school and undergraduate students that illustrate the effect of Al^{3+} ions on the colours of solutions of cyanidin-3-*O*-glucoside and anthocyanin extracts from plant material.[83] The students investigate the effect on the solution colour when the pH and Al^{3+} content of the samples are varied. The students also include neochlorogenic acid as an additive. The effect of the latter compound is a useful addition to the study since it is believed that compounds of this type are bound to Al^{3+} ions in blue hydrangea sepals, in addition to delphinidin. In the presence of aluminium ions, the solutions become purple in colour, and the addition of neochlorogenic acid improves the stability of samples that are stored for long periods of time. This activity provides educators with the opportunity to discuss structure–activity relationships and lends itself well to the application of UV-vis spectroscopy as an analytical tool. The understanding of Al^{3+} binding to anthocyanins can be explored further in the context of dyeing fabric. Further work by Kajiya has demonstrated a practical activity for undergraduate students that uses red onion skin extract as a dye for silk and cotton fabric samples.[84] The anthocyanin content of the extract allows the expected pH response to be demonstrated, however, in addition, the extract can be mixed with aluminium potassium sulfate (alum) to initiate colour changes. Fabric samples dyed with red onion extract mixed with alum were found to appear green in colour, this observation is explained by the binding of both naturally occurring anthocyanins and quercetin (a flavonoid) to Al^{3+} ions. This activity allows educators to discuss the importance of mordants in the process of dyeing fabrics, in this case, the aluminium ions bind both to the fabric and to the dye molecules. The nature of these interactions can be discussed in addition to the chemistry that controls the observed colour changes.

These complex anthocyanin-containing pigment structures, known as metalloanthocyanins are found elsewhere in the natural world and are responsible for the colour of several varieties of blue flowers.[18] The complex pigment commelinin is a magnesium-containing metalloanthocyanin found in Asiatic day flowers (*Commelina communis*).[85] Another example present in flowers is protocyanin, this metallocyanin pigment is found in blue cornflowers (*Centaurea cyanus*).[86] The structure of the pigment features anthocyanin units bound to magnesium (Mg^{2+}) and iron (Fe^{3+}) ions, the presence of Fe^{3+} being particularly important for a blue colour.[18] Both commelinin and protocyanin result in beautiful and vivid flower colours, however, these pigments can be more difficult to extract than other anthocyanins from plant tissue. It is possible though to link these examples to education-focused activities, since simpler anthocyanins will also bind to iron ions. A recent example has been published that allows high school students to use anthocyanin-containing extracts from *Ruellia tuberosa* L. flowers to detect Fe^{3+} ions in solution.[87] The flowers used in these experiments are a particularly rich source of anthocyanins and the extraction works well when hot water is used. The presence of Fe^{3+} ions is detected by a change in colour, this allows UV-vis spectroscopy to be employed to determine the concentration of Fe^{3+}. Furthermore, a smartphone-based method is also reported, in this variant of the task, the students take pictures with their smartphones and use an RGB based method for the analysis.[87]

Complexation of Fe^{2+} ions by anthocyanins has been investigated in the context of teaching electrochemistry to senior high school students. R. Liu, Y. Song and W. Wang have developed an engaging activity, that uses an agar gel containing extracts from Chinese goldthread (*Coptis chinensis*) as an indicator for ion migration in an electrochemical cell.[88] The reported protocol used copper and iron electrodes connected to a DC power supply. A piece of copper sheet served as the cathode, and a piece of iron sheet was used as the anode. After immersion of the electrodes in the agar gel, a voltage was applied, and a purple colour was observed at the anode and a red to green colour change at the cathode within a few minutes. A highlight in this case is that the anthocyanins in the agar gel play a dual role, both as an acid-base indicator, and as chromogenic sensor for Fe^{2+} ions. The build-up of hydroxide ions at the cathode is detected by the pH dependent colour change, and the formation of Fe^{2+} ions at the anode is detected when they bind to the anthocyanins (the area around the electrode becomes purple).

The interaction of tin ions with anthocyanins provides educators with an opportunity to discuss chemical principles in the context of food production and storage. The ability to preserve fruit and vegetables is key to minimising wastage of valuable food crops after harvest. One of the main methods for the preservation of fruit and vegetables is the large-scale use of tinplated steel cans.[89,90] This technology is now well-established, however until the mid-20th century, canned food could become spoiled due to corrosion of the can and discolouration of the contents due to prolonged contact with the tin layer inside the can.[91–93] Studies in the 1920s demonstrated that Sn^{2+} ions, formed by reaction of acidic fruit juices with tin metal, can potentially complex with the anthocyanins in the fruit, and that these can lead to undesired colouration of the can contents. In 1927, a detailed investigation by C. W. Culpepper noted that certain canned fruits, especially peaches, became pink/purple when stored in tinplated cans.[93] After a series of trials, it was noted that peaches canned without removing the skin and pits, were particularly susceptible to discolouration. In contrast, peaches preserved in glass containers under the same conditions, did not show a significant degree of discolouration. These experiments, in addition to experiments with peach pits exposed to tin metal and tin(II) chloride, led to the conclusion that anthocyanin complexes with Sn^{2+} (and possibly Sn^{4+}) were responsible for fruit discolouration. Culpepper stated:[93]

"It is apparent that the purple discolouration observed in peaches canned in tin is due to a reaction between the tin of the can and the anthocyan pigment present".[93]

It is possible to replicate these studies with modern food cans; however, they must not have any protective polymer layer coating over the can interior. Experiments with various berries have been described, in each case the berries are frozen, transferred to a used food can, and then they are allowed to thaw overnight.[94] Acidic fruits such as raspberries (*Rubus idaeus*) and blackberries (*Rubus fruticosus*) work particularly well. The initial red colour of the fruit tissue and juice turns to a deep purple colour after storage (exposed to the tin surface) over a 16–24 hour period. These simple experiments allow educators to make a connection between the food we consume and the chemistry that is relevant to ensuring canned foodstuffs are safely preserved. There are additional advantages, since the food cans that are used are usually considered as household waste. The cans are inexpensive, safe to use and they can be recycled once the experiments are completed.

Anthocyanin bleaching reactions

Reactions involving anthocyanins can sometimes result in loss of colour, for example, the reversible bleaching of anthocyanins by bisulfite ions has been well-studied. It has been shown that anthocyanin flavylium ions can react quickly with bisulfite ions to form a colourless flavene-4-sulfonate (often known as a "bisulfite adduct", Fig. 9).[95–98] The reaction is in equilibrium, so on addition of source of bisulfite ions, the initial red solution will then decolourise. Subsequent addition of an oxidant (often hydrogen peroxide), or an acid, will consume bisulfite ions and allow the red flavylium ion to reform.[96–98]

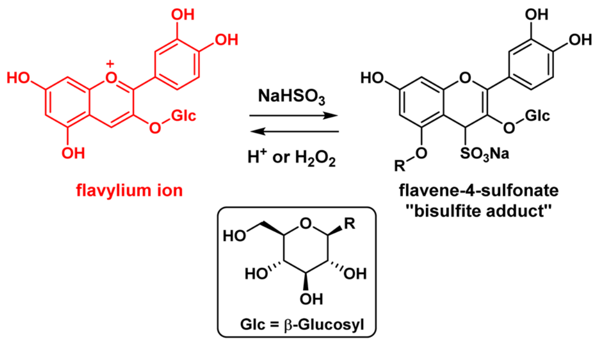

Fig. 9 The reaction of the flavylium ion form of cyanidin-3-glucoside with sodium bisulfite to form the corresponding flavene-4-sulfonate or "bisulfite adduct". Typical conditions for the reverse reaction are also shown.

There are various classroom examples that illustrate anthocyanin bleaching using bisulfite ions, these include: the use of sodium bisulfite to decolourise grape juice,[99] and sulfur dioxide gas or sodium metabisulfite to decolourise red cabbage extract.[96–98] More recent examples include the use of bleached red cabbage indicator as an invisible ink; the hidden messages in this case being revealed by exposing the dried bleached indicator "ink" to dilute acid or an aqueous solution of alum.[97] In a related example, an investigation of the kinetics of the decolourisation reaction of red cabbage indicator in the presence of bisulfite ions has been presented. The kinetics results from red cabbage extracts in different solvents in this study are found to be useful for the preparation of watercolour paints.[100]

The interaction of anthocyanins with bisulfite ions is well understood within the food industry, since sulfites are used as preservatives in fruit juices, wines and ciders.[99]

R. E. Wrolstad has highlighted anthocyanin bleaching reactions with bisulfite ions in the context of the production of Maraschino cherries.[101] In his 2009 education-focused article, a detailed description of the chemistry involved in bleaching, and subsequent dyeing fruit is provided. This resource also outlines practical activities and short research projects (featuring practical work and a literature study) that are directly linked to the processes involved in the commercial manufacture of Maraschino cherries. The reaction of bisulfite ions with anthocyanins accounts for an undesirable phenomenon sometimes observed in white wines known as "pinking".[102] In addition to serving as preservatives, the sulfites present in wine will react with anthocyanins to form the corresponding bisulfite adducts. However, under oxidising conditions, bisulfite ions are consumed, and a pink/red colour often appears due to the anthocyanins that form. Anthocyanin bleaching reactions of this type have featured in a practical activity for university students to determine the total sulfite content in white wines.[103] The samples of white wine were dosed with anthocyanin-containing flower extracts, this led to discolouration of the flower extract. The colour change was due to sulfite present in the wine reacting with the flower-derived anthocyanins. The students used UV-vis spectroscopy to prepare calibration curves from solutions of known sulfite concentrations mixed with flower extract; this information could then be used to determine the sulfite content of samples of wine. As expected, the students observe a decrease in the absorbance of samples as the concentration of sulfite increases. The students can also determine the sulfite content by conducting iodometric titrations, these results can then be compared to the results obtained using UV-vis spectroscopy.

Bleaching of flower extracts using different reagents has also been reported. For example, a protocol to demonstrate flow injection analysis techniques to undergraduate students has been presented.[104] This activity makes use of anthocyanin-containing extracts obtained from azalea (*Rhododendron simsii*) or quaresmeira (*Tibouchina granulosa*) and is focused on the bleaching reaction observed when anthocyanins are exposed to sodium hypochlorite. The extracts are reacted with dilute sodium hypochlorite solutions and the bleaching response monitored by UV-vis spectroscopy. This technique was used to analyse samples of household bleach products, and the results were compared to those obtained by iodometric titrations.

Use of anthocyanins in teaching chromatography techniques

Plant extracts often contain more than one anthocyanin species, for example, red cabbage extract contains a range of glycosylated cyanidins. Furthermore, the rich colour of some flowers can often be due to the petals containing anthocyanins with varied anthocyanidin cores. As a result of this structural diversity, anthocyanins have found use in teaching activities focused on purification and analytical techniques, these range from simple thin-layer chromatography (tlc) to more advanced methods. Plant extracts, such as spinach and other green leaf species, are frequently employed in teaching thin-layer chromatography since the carotenes and chlorophylls from the leaves are readily separated and easily observed by eye.[11,12] These protocols often require organic solvents to extract the plant tissue and as the mobile phase for thin-layer chromatography or column chromatography on silica. Anthocyanins are more polar than carotenes and chlorophylls so they can be separated using paper,[105] cellulose,[106] or reverse phase silica as the stationary phase,[107] and more polar solvents as mobile phases. As is the case with carotenes and chlorophylls, the colours associated with anthocyanins allow the tlc spots to be viewed by eye. A selection of examples used to illustrate thin-layer chromatography techniques are described in the following section.

A detailed tlc analysis exercise focused on anthocyanin-containing extracts in a university teaching laboratory was published by Curtright, Ryanearson and Markwell in 1996.[106] This activity can be delivered as an open-ended task and it incorporates aspects of anthocyanin reactivity, in addition to analysis. Red cabbage, red apple peel, blueberries, strawberries and cranberries were extracted in 1% HCl in methanol or ethanol. Samples of the extracts were then spotted on glass or plastic backed cellulose tlc plates and a mixture of HCl, formic acid and water was used as the mobile phase. These conditions allowed results on plastic backed plates to be collected within 20 minutes. Glass backed plates were found to give superior resolution but use of these required up to 2 hours for the plate to be developed. Analysis of the tlc plates showed that the anthocyanins progressed further through the stationary phase than the anthocyanidins present in the mixture (anthocyanidin diglycosides move further than anthocyanidin monoglycosides). Furthermore, the paper reports that the composition of the mixtures could be altered by heating the plant extracts in aqueous HCl, these conditions facilitated hydrolysis of anthocyanins to form anthocyanidins. Further tlc analysis of the extracts after treatment with acid showed that the glycosylated anthocyanins had been consumed. The outcome of this process was evident since the associated spots became less visible and the slower moving anthocyanidin spots were easily visualised.

Later work by the same authors described experiments involving methanol extracts from *Coleus* leaf.[108] The tlc analyses were conducted using plastic backed silica gel tlc plates and a propanol-acetone-water mobile phase used was used as the mobile phase. A key objective was to allow students to investigate the effect of changing the composition of the mobile phase on the mobility of the anthocyanin samples through the stationary phase. Once familiar with the protocol, students were encouraged to locate other anthocyanin sources, obtain an extract, and then attempt tlc analysis.

A later procedure has been published for university teaching laboratory use where anthocyanin extracts were obtained from freeze-dried berries.[105] The fruit samples were extracted with acidic methanol and then heated with additional aqueous HCl to hydrolyse the anthocyanins to the corresponding anthocyanidins. Thin-layer chromatography in this case was conducted on paper, and a variety of aqueous acid mobile phases were employed. This activity also includes additional experimental techniques. The students acquired UV-visible spectra of the acid treated extracts and they conducted an antioxidant assay to evaluate how efficient the extracts were as radical scavengers.

Reverse-phase thin-layer chromatography can also be an effective analytical technique for investigating the anthocyanin content of plant extracts. This approach uses a hydrophobic stationary phase (often alkylated silica particles) instead of silica or cellulose.[107] The technique is particularly effective for polar anthocyanidins, since these hydrophilic molecules progress faster through the hydrophobic stationary phase than when they are applied to silica or cellulose. Binder and Lämmle have published an engaging teaching laboratory activity that introduces high performance thin-layer chromatography to university students.[107] Flower petals were selected for extraction, treatment with aqueous formic acid afforded an initial extract. Further treatment of the extract with trifluoroacetic acid hydrolysed the anthocyanins to form anthocyanidins. Careful application of the hydrolysed samples to tlc plates coated with C_{18}-silica, followed by elution with acidic methanol mobile phases, allowed excellent sample resolution. The acidic mobile phase helps clear observation of the sample spots by eye, since the flavylium ions are red or pink. The plates can be briefly exposed to ammonia to give blue spots. The authors have also demonstrated that treatment of the plates with $AlCl_3$ allows cyanidin and delphinidin to be readily distinguished from pelargonidin, malvidin and peonidin. The colour of the cyanidin and delphinidin spots change from red/pink to violet due to the ability of those anthocyanidins to complex to Al^{3+} ions.

Column chromatography of anthocyanins can also be investigated in laboratory teaching activities. A simple and inventive version for introductory work has been reported that uses a plastic syringe barrel as a small-scale chromatography column.[109] Commercially available baking soda or potato starch was used as stationary phases, although potato starch was found to work best with anthocyanin extracts. An example use featuring *Stromanthe sanguinea* leaves has been presented, where an acetone extract of the leaves was applied to the chromatography column. The leaves contain carotenoids and chlorophylls, as well as anthocyanins. The former could be eluted with petrol, or with mixtures of petrol and acetone. More polar solvents, such as ethanol, were needed to elute the more polar anthocyanins. Once collected, the ethanol fractions obtained from the column turned red when HCl was added, this observation was highlighted to students as evidence for the presence of anthocyanins.

A more advanced example of preparative chromatography for university students studying agriculture has recently been reported that involves purifying red cabbage extract.[110] The red cabbage was extracted using acidic ethanol, this was then purified by eluting the extract with water through a chromatography column that contained a hydrophobic resin as the stationary phase. This process allowed the red cabbage anthocyanins to be separated from water soluble impurities and then isolated by freeze-drying the fractions that were collected. This activity is extended further to demonstrate the encapsulation of the purified anthocyanins using maltodextrin. This process is valued in the food industry since it extends the shelf life and can enhance the colours and flavours of some foods. In the activity described, the students mixed the purified anthocyanins with maltodextrin, they then freeze-dried, or spray dried the mixture to afford encapsulated red cabbage anthocyanins.

A use of advanced chromatography and instrumentation for analysis of anthocyanin-containing extracts from plants has been presented. This report outlines a practical activity for high school and undergraduate chemistry students that allows mixtures containing carotenoids and anthocyanins to be separated using ultra-performance liquid chromatography (UPLC).[111] In this work, a wide range of plant samples were collected, freeze-dried, then extracted using an aqueous solution containing formic acid. The carotenoids in the aqueous phase could be extracted using a hydrocarbon solvent, while the anthocyanins were retained in the acidic solution. The aqueous extract was then diluted, and an instructor assisted the students in the use of the instrumentation required for operation of the UPLC equipment. The anthocyanins were detected by mass spectrometry as the analysis proceeded. This impressive activity provides a good balance between extraction tasks the students can complete without specialist equipment, and the demonstration of advanced instrumentation for the analysis of the extracts they obtained.

Further applications of anthocyanins in teaching

Anthocyanin extracts from various sources can also be used in more advanced undergraduate laboratory activities, some diverse examples are highlighted in this section.

Dye-sensitised solar cells (DSSCs) have increasingly featured in physical chemistry laboratory courses. These devices demonstrate harnessing of light to generate an electrical current. A dye is required to absorb the light required, and for this purpose students can select their own anthocyanin source.[112] Aqueous extracts from the following plant materials have been found to be effective as dyes for DSSCs, these include extracts from: raspberries, blackberries, pomegranate seeds and hibiscus tea. The dyes required for DSSCs are soaked onto a pre-prepared nanocrystalline titanium dioxide (TiO_2) film on a conductive glass surface. A very recent example focused on DSSCs has been published where postgraduate chemistry students are tasked to fabricate conductive glass slides, synthesise TiO_2 nanoparticles and prepare an electrolyte solution.[113] The students then use red cabbage extract as the dye for the DSSC and test the performance of the cell they have made. Nanocrystalline titanium dioxide can also be used to prepare titania on a paper surface which can then function as a photocatalyst.[114] When paper treated with TiO_2 is soaked in anthocyanin extracts from raspberries, the colour can be bleached when illuminated with different light sources. This has been used as a demonstration of a photocatalytic process and to highlight principles of semiconductor chemistry.

The colour changing properties of anthocyanins have found a novel use in the context of teaching chemical engineering principles. An ingenious series of experiments that demonstrate concepts related to mixing bulk quantities of a solid in a liquid medium have been developed.[115] The anthocyanins present in blue maize flour allow the mixing behaviour of suspensions of the flour to be examined. In an example experiment, a flour/water suspension was acidified so that the mixture appeared red/pink, the suspension was then stirred and sodium hydroxide solution added, this initiated a series of colour changes over a set period of time. The observations in the mixing tank were readily recorded visually and captured using a digital camera, the resulting images were then analysed by the students. The change in pH influences viscosity, as well as colour, therefore the observed colour can act as an indicator of viscosity differences within the flour/water mixtures.

The final example in this section highlights a classroom activity for high school and undergraduate students that is computer based, rather than a traditional "wet" laboratory task.[116] Bortoli and Orian have developed a computational approach to direct students to mechanistic concepts in the context of antioxidant properties of anthocyanins, in particular, radical scavenging reactions involving polyphenols. The students are provided with a selection of anthocyanins to evaluate; they are then guided in the use of density functional theory (DFT) calculations to investigate the radical scavenging ability of the compounds presented.

Example activities

Overview of selected activities

The preceding section highlights a wide variety of activities used by chemistry educators in their classes. In this section, we have selected some example short practical activities that have been used in our own work. The first example is perhaps unfamiliar to readers, we describe how a red cabbage indicator first described by James Watt in the 18th century can be advantageous in chemistry teaching. An adaption of Watt's method allows an easily stored red cabbage indicator to be prepared for use in teaching activities. We describe a simple and colourful method to use "droplet art" as a practical activity to introduce high school students to pH-controlled colour changing reactions. As part of a lecture for school teachers, demonstrations have been developed that highlight aspects of anthocyanin chemistry relevant to food production and preservation. We highlight how the interaction between anthocyanins in berries can be used to qualitatively detect tin ions that are present in canned fruit juices. The reaction of anthocyanins with bisulfite ions has also been identified as being important in food colour and preservation. We describe a simple demonstration of the underlying chemistry in the context of wine and cider production. In the final example, we summarise the often-overlooked chemistry that accounts for the colours observed in "Shinoda tests" for flavonoids in plant extracts. This test is often used in research and teaching laboratories, however the chemistry that accounts for the corresponding colour changes is frequently not discussed in detail.

A red cabbage indicator experiment inspired by James Watt

The halochromic properties of anthocyanins were known many years before the investigations conducted by Willstätter and Robinson. However, the structures of the compounds responsible were unknown. Robert Boyle had referred to "syrrup of violets",[117,118] this substance was a flower extract that changed colour under acidic or basic conditions. James Watt (1736–1819) was a famous Scottish engineer, well-known for his work on the development of steam engines, although some of his work was also directed toward studies in chemistry.

In 1784, he published a paper titled "On a new method of preparing a test liquor to shew the presence of acids and alkalies in chemical mixtures".[117] In this work, Watt outlined a detailed a set of observations on the behaviour of red cabbage extracts when treated with acids and bases. Watt's descriptions will be very familiar to educators today who use red cabbage indicator in teaching. For example, he stated:[117]

"I found the red cabbage to furnish the best test and in a fresh state to have more sensitivity both to acids and alkalies than litmus, and to afford a more decisive test from its being naturally blue, turning green with alkalies, and red with acids".[117]

Furthermore, Watt noted that although freshly prepared indicator was very useful, it quickly degraded and released unpleasant odours. He was successful in retaining samples for a period of a few days, but stated:[117]

"None of these means will preserve the liquor long, without requiring to be neutralised afresh, just before it is used; and as the putrid and acid fermentation which it undergoes".[117]

Watt was persistent, and he was able to identify a method to prepare red cabbage extract that could easily be stored without the problem of decomposition, he described the following method:[117]

"In order to preserve its virtues while it is kept in a liquid state, some fresh leaves of the cabbage, minced as has been directed, may be infused in a mixture of vitriolic acid and water".[117]

In our own work, we have attempted to replicate James Watt's findings to make more efficient use of the red cabbages we use for laboratory activities. We have employed this version of red cabbage indicator in lecture demonstrations and outreach events. We have found that extraction of chopped red cabbage can readily be achieved using 0.4 M sulfuric acid. The resulting deep red solution is remarkably stable, even if it is not stored in a refrigerator. We have samples of indicator that are still in good condition 12 months after being prepared. These observations are in stark contrast to samples of red cabbage indicator prepared by extraction with hot water. Neutral aqueous red cabbage indicator samples are unstable over long periods of time, especially when they are not stored in a freezer or refrigerator. Surprisingly, the odour of the extract prepared using Watt's method is not too disagreeable, certainly not as unpleasant as simple aqueous red cabbage extracts can be! A description of the extraction procedure and pH tests are provided in the SI document. We have found that the process described allows 2–3 litres of strongly coloured extract to be prepared from a single red cabbage. Prior to use, the indicator can be diluted further if required. Overall, this approach has allowed us to minimise the number of cabbages required for the associated activities compared to using the more familiar extraction method that uses hot water.[13]

In Watt's method, he treated the indicator with calcium carbonate to afford a blue coloured solution. This observation is consistent with a modern understanding of the reaction, the red flavylium form of the anthocyanidin from the cabbage being converted to the corresponding ionic quinoidal base form. In this case it is likely that the carbohydrate substituents are cleaved from the anthocyanins during storage under acidic conditions.[107] Fig. 10 shows a portion of the extract treated with calcium carbonate, followed by filtration of the gypsum (calcium sulfate dihydrate) that is formed during the reaction with the acidic extract. This simple test shows that Watt's indicator can be used in demonstrations as a pH indicator in a similar way to the red cabbage indicator extracted in hot water. The acid–base reaction between the dilute sulfuric acid and calcium carbonate is a notable additional feature of the test. The immediate colour change and formation of precipitate (calcium sulfate) can be used as a prompt for a discussion about the reactions taking place.

Colourful pH and metal ion binding experiments for droplet art

In recent years, microscale versions (sometimes known as "droplet" versions) of well-known school chemistry practicals and demonstrations have become popular.[119] This approach

Fig. 10 Samples of red cabbage extract prepared according to the method described by James Watt. Sample 1 shows cabbage extract in dilute H_2SO_4, sample 2 shows cabbage extract mixed with $CaCO_3$ (unfiltered) and sample 3 shows extract mixed with $CaCO_3$ and resulting $CaSO_4$ has been filtered off.

provides several advantages: it reduces the amount of reagents needed, requires only low-cost equipment, enables rapid evaluation of a range of colour-changing reactions, and is readily adaptable. Microscale versions of acid/base reactions of anthocyanin extracts have been featured in the literature and this approach has been found to be effective.[36]

In this article, we provide an overview of a microscale link between chemistry and art that has been used in high school teaching (as a science classroom activity, or as part of an extra-curricular science club for 11–13 year old students).[120] Connections between STEM subjects and the arts have been found to be a helpful way to engage school and university students in science and art.[8,49] Several examples are highlighted in an earlier section of this article in the context of red cabbage indicator. The microscale version of pH testing with anthocyanin extracts involves applying droplets of the chemicals to be tested onto a plastic or laminated sheet containing the template design (Fig. 11).[36,120] The droplets are applied using a pipette or plastic dropper bottle. Single drops of weak acids or bases, such as household vinegar or sodium hydrogen carbonate solution are added onto the template, then droplets of anthocyanin-derived indicators are added to these droplets to bring about a change in colour in response to the pH.

Alternatively, the indicators can be added to the design first, and then reagents added to change the colours. The plastic sheet can simply be wiped clean at the end of the practical. It is also possible to add a drop of potassium alum solution to affect a colour change by binding aluminium ions to particular anthocyanins. The students can select and experiment with different extracts and additives to adjust the pH, and therefore the colour of the droplets. This approach allows students to apply newly acquired knowledge in a creative and expressive way. A range of anthocyanin extracts can be used in this activity; the most vivid colours are often observed when extracts containing acylated anthocyanins are used. Good examples of these include solutions obtained from red cabbage, butterfly pea flower tea, and oxalis leaves. All of these can be readily obtained; we find oxalis (*Oxalis triangularis*) especially useful since the plants can be grown and kept as a renewable source of anthocyanin extracts in our teaching laboratories. In spring and summertime, flowers are dead-headed by gardeners and disposed of (often to make compost), we find that discarded petals from various varieties are another useful source of indicators for droplet art.

Fig. 11 An example of droplet art, the different colours are made by adjusting the pH of various anthocyanin-containing solutions.

Detection of tin(II) ions in fruit juices

In the earlier section that was focused on the interaction of metal ions, it was highlighted that anthocyanins can complex with tin ions. This reaction is often undesirable since acidic canned fruits that are rich in anthocyanins will become pink or purple. Previous work that illustrates this reaction to students has used food cans to demonstrate discolouration of berries. This is an engaging activity that makes good use of discarded food cans, but the reaction is slow since the colour changes require several hours. However, it is possible to show the interaction of tin ions with anthocyanins in a related-context, anthocyanin-containing fruit juices can be used to detect tin ions in the fruit juice or syrup of canned pineapple, pears and peaches. There are numerous studies on the metal ion content of canned goods, these have found that there are a variety of factors that control metal ion concentrations.[121,122] Values of 50–150 mg tin per kilogram of canned fruit are not uncommon. The maximum permissible levels of tin in food depend on the country where the products are sold, the values are typically in the 150–250 mg kg^{-1} range for foods and fruit drinks.[121,122] The Tayside region of Scotland is a major producer of soft fruit so commercially supplied or foraged berries are readily available to us. Fruit that is not considered fit for sale or consumption can easily be frozen and the juice extracted. We have attempted to use raspberry and blackberry juices to qualitatively detect tin ions. These experiments have been successful, and can be demonstrated easily, in our case as part of a lecture for school teachers about anthocyanins. The juice or syrups from canned pineapple, pears and peaches all show a visible colour change from red to purple when a portion of berry juice is added to a juice sample taken from a tinplate can. Fig. 12 shows an example series of tests on pineapple juice. The samples from fresh pineapple or pineapple juice from a carton did not cause a colour change when berry juice was added, however, a purple colour formed quickly when berry extract was added to a sample of canned pineapple juice. The results from this short series of

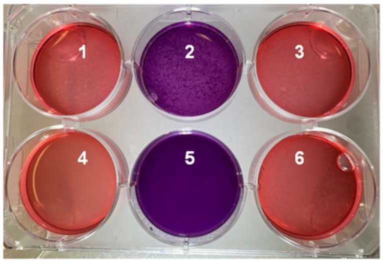

Fig. 12 Tests with pineapple juices and blackberry extracts. Sample 1 contains blackberry extract mixed with dilute HCl. Sample 2 contains blackberry extract mixed with $SnCl_2$. Sample 3 contains blackberry extract. Sample 4 contains blackberry extract mixed with pineapple juice from a carton. (e) Sample 5 contains blackberry extract mixed with pineapple juice from a tin can (f) Sample 6 contains blackberry extract mixed with fresh pineapple juice.

tests demonstrate that berry juice can be used as an indicator for tin ions that are formed on storage of acidic fruit products in tinplated cans. The purple-coloured tin/anthocyanin complex is not formed when berry juice is mixed with fresh pineapple juice or juice that has been stored in a carton. In these cases, the fruit juice has not been exposed to a source of tin ions during storage. The absence of detectable tin shows the fruit itself did not contain measurable quantities of tin originally. The conclusion is that the tin detected in the first experiment must have come from the tinplate rather than from the fruit itself. These tests are quick and easy to do, and allow aspects of food production to be highlighted. The associated lecture emphasises the point that different preservation methods for fruit products are available to avoid waste, but that there are some limitations on acceptable storage time.

A simple and quick demonstration of wine "pinking"

As described previously, the reaction of bisulfite ions with anthocyanins has received much attention in the literature, especially in the context of food production and preservation. In a recent lecture for school teachers, we have incorporated a demonstration of this chemistry as a variation on the classic "water into wine trick", where a basic solution is poured into a wine class containing phenolphthalein pH indicator.[123] Part of the presentation is focused on the chemistry of wine "pinking", although initially the audience are unaware of this. A solution of blackberry extract (raspberry or red begonia flower extract could also be used) is diluted with water to look like rosé wine, then bleached using sodium metabisulfite. The bleached solution is then transferred to a white wine bottle and placed next to 2 empty wine glasses. One of the wine classes contains a few drops of 5 M H_2SO_4, this is not visible to audience, even when viewed using a well-lit visualiser. The presenter then pours a glass of "white wine" from the bottle into the untreated wine glass, the audience don't see anything unusual at this point. However, when some of the "wine" from the bottle is poured into the treated glass, the colour immediately becomes pink and is referred to as "rosé wine". The presenter then explains to the audience how bisulfite ions react with anthocyanins and briefly discuss the "pinking" of wine and the relevance of this reaction to wine and cider production. The associated lecture featuring the demonstration highlights that the presence of bisulfite ions in wines and ciders is important in preservation of such products, as well as controlling the colour. Pictures of the bleaching reaction in a wine glass are shown in Fig. 13. All the flower and fruit sources used in this demonstration are readily available, either as garden waste or discarded during fruit picking and selection.

Fig. 13 Wine "pinking" demonstration. The left panel shows anthocyanin solution bleached with sodium metabisulfite. The right panel shows a bleached solution treated with dilute H_2SO_4.

Anthocyanins and the Shinoda test

Scientists investigating the composition of plant tissues often use a "Shinoda test" to detect the presence of flavonoids. This rather simple test involves mixing an ethanol extract from the selected plant tissue with dilute hydrochloric acid and a small piece of magnesium metal. If flavonoid compounds are present, the expectation is that a range of pink/red/violet colours can be observed. This qualitative test is often used in research and university teaching laboratories, although, a detailed outline of the origin of the observed colours is often not presented. The test is likely named after Junzo Shinoda who published an article titled "Ueber die bei der Reduktion von Pflanzenfarbstoffen entstehenden Farbenreaktion",[124] this study reported the results from testing 22 compounds (flavones and flavanols). Shinoda identified that the flavone or flavanols being tested needed to have a hydroxyl or methoxy group on the phenyl substituents to show a red or violet colour. The origin of the observed colours has been the subject of debate since the 1940s,[125] a full discussion of this is beyond the scope of the article. However, a brief summary of the key features is outlined herein to provide school or university educators, with some background information that is not readily available in current textbooks.

A plausible explanation for Shinoda test results is that a flavonoid compound can be reduced to the corresponding flavylium ion. For example, quercetin would be converted to the flavylium ion form of cyanidin. This type of reaction has been reported before, Willstätter claimed in 1914 to have converted quercetin to cyanidin (Fig. 14).[126] However, the yield was low and there were difficulties isolating the product. In 1927, Robinson reported that attempts to directly convert rhamnetin to rhamnetidin were unsuccessful (Fig. 14).[127]

Later work in 1946 by Geissman and Clinton cast further doubt on the likelihood of anthocyanidins being major products when flavonoids are reduced under Shinoda conditions, especially if the substrate was a flavanol.[125] Their 1946 paper

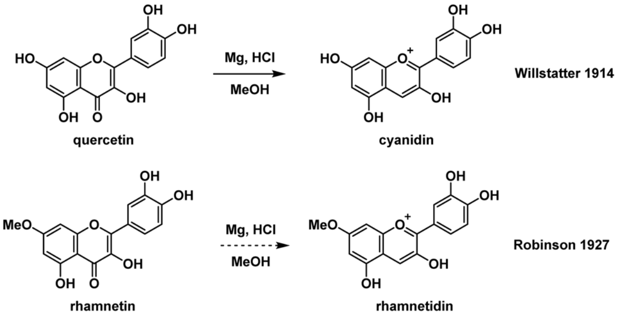

Fig. 14 Reactions of quercetin and rhamnetin with magnesium metal in methanolic HCl (this reaction was not successful).

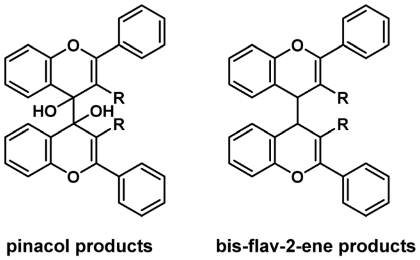

Fig. 15 Potential products formed from anthocyanins under Shinoda test conditions.

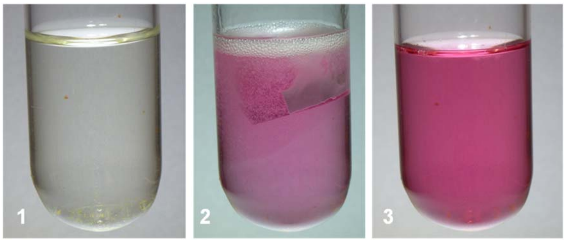

Fig. 16 Shinoda test using rutin. (1) Rutin mixed in ethanol and dilute HCl, (2) magnesium metal added to the mixture (3) mixture after completion of the reaction.

outlines several reaction pathways to a range of products that can form resonance stabilised carbocations.[125,128] Geissman and Clinton were able to isolate some of the reaction products, for example, pinacol compounds (Fig. 15) were isolated.[125,128] Under acidic conditions, these species would be expected to show red or violet solutions.[129] Later work in the 1960s demonstrated that flavylium ions can also dimerise to form colourless bis-flav-2-ene products (Fig. 15) under reducing conditions.[130]

In summary, the presence of colourful flavylium ions in a positive Shinoda test solution is plausible, but it is unlikely that these species are the sole origin of the observed colour. An example Shinoda test is shown for rutin which is isolated from various fruits (Fig. 16).[131] A solution of the flavonoid in ethanol is mixed with dilute HCl, then magnesium metal added. The solution becomes warm as the reaction between magnesium and HCl takes place, any colour is usually observed at this stage of the test.

Conclusions

In this tutorial review we have surveyed the educational literature to highlight the wide variety of teaching activities that involve anthocyanin-containing extracts from plants. There are many sustainable and inexpensive sources of anthocyanins so these are widely accessible to chemistry teachers in schools and universities. The most familiar use of anthocyanins in chemistry teaching is as acid/base indicators. The halochromic property of anthocyanins has been demonstrated in solution, and when plant extracts have been incorporated into gels and solid materials. However, anthocyanins can also change colour when exposed to other stimuli. Colour changes of anthocyanin-containing plant extracts can be observed in the presence of aluminium, iron and tin ions. This type of interaction is important in the understanding of flower colours and is significant within the food industry.

Anthocyanin bleaching processes have been highlighted, this type of reaction is useful for analysis and is also used to control the colours of certain foods and wines. Anthocyanins have found to be useful in teaching chromatography techniques, examples from high school level to advanced level undergraduate work are presented.

In the second part of the review, we have identified simple examples of anthocyanin-based demonstrations and practical activities for use by chemistry educators in schools or universities. These activities use anthocyanin extracts and chemicals that are readily obtained from household products, and they do not generate hazardous waste. The examples of anthocyanin interactions with tin ions and bleaching of fruit extracts are relevant to food production and preservation and could be incorporated into relevant classroom teaching. We also attempt to provide educators with an understanding of the reactions that account for the colour changes observed in a commonly used chemical test for flavonoids.

Author contributions

Iain. A. Smellie – conceptualization, investigation, writing – original draft. Isobel Everest – conceptualization, investigation, writing – review and editing. Iain L. J. Patterson – conceptualization, investigation, writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Supplementary information (SI): experimental protocols for example activities. See DOI: https://doi.org/10.1039/d5su00933b.

Acknowledgements

The authors express gratitude to the University of St Andrews, School of Chemistry and Bedford School, for the use of their laboratory facilities and provision of materials.

Notes and references

1 M. Séquin, *J. Chem. Educ.*, 2005, **82**, 1787–1790.
2 K. Andreoli, F. Calasscibetta, L. Campanella, G. Favero and F. Occhionero, *J. Chem. Educ.*, 2002, **79**, 976–979.
3 S. K. Hartwell, *Chem. Educ. Res. Pract.*, 2012, **13**, 135–146.
4 K. Grudpan, S. K. Hartwell, S. Lapanantnoppakhun and I. McKelvie, *Anal. Methods*, 2010, **2**, 1651–1661.

5 M. Sequin-Frey, *J. Chem. Educ.*, 1981, **58**, 301.
6 J. R. Silverman, *J. Chem. Educ.*, 2024, **101**, 3904–3911.
7 C. Machado, A. O. Oliynyk and J. R. Silverman, *J. Chem. Educ.*, 2022, **99**, 2431–2437.
8 J. L. Blatti, *J. Chem. Educ.*, 2025, **102**, 4747–4759.
9 P. Anastas and N. Eghbali, *Chem. Soc. Rev.*, 2010, **39**, 301–312.
10 M. A. Benvenuto and G. M. Bodner, *Phys. Sci. Rev.*, 2017, **2**, 20170003.
11 S. M. Diehl-Jones, *J. Chem. Educ.*, 1984, **61**, 454–456.
12 W. Mewaldt, D. Rodolph and M. Sady, *J. Chem. Educ.*, 1985, **62**, 530.
13 M. Forster, *J. Chem. Educ.*, 1978, **55**, 107–108.
14 R. D. Curtright, J. A. Rynearson and J. Markwell, *J. Chem. Educ.*, 1994, **71**, 682–684.
15 F. Pina, M. J. Melo, C. A. T. Laia, A. J. Parola and J. C. Lima, *Chem. Soc. Rev.*, 2012, **41**, 869–908.
16 (*a*) O. Dangles, *J. Agric. Food Chem.*, 2024, **72**, 12536–12372; (*b*) P. Trouillas, N. J. C. Sancho-García, V. de Freitas, J. Gierschner, M. Otyeka and O. Dangles, *Chem. Rev.*, 2016, **116**, 4937–4982.
17 L. Cruz, N. Basilio, N. Mateus, V. de Freitas and F. Pina, *Chem. Rev.*, 2022, **122**, 1416–1481.
18 K. Yoshida, M. Mori and T. Kondo, *Nat. Prod. Rep.*, 2009, **26**, 884–915.
19 R. Willstätter and R. A. Everest, *Adv. Cycloaddit.*, 1913, **401**, 189–232.
20 R. Huisgen, *J. Chem. Educ.*, 1961, **38**, 10–15.
21 R. Willstäter, *Nobel prize speech*, (1920), https://www.nobelprize.org/prizes/chemistry/1915/willstatter/lecture, accessed December 12 2025.
22 R. Robinson, *Nature*, 1935, **135**, 732–736.
23 A. Siegel, *Ambix*, 2008, **55**, 62–82.
24 A. Robertson and R. Robinson, *J. Chem. Soc.*, 1928, 1526–1532.
25 E. H. Huntress, *J. Chem. Educ.*, 1928, **5**, 1392–1398.
26 E. H. Huntress, *J. Chem. Educ.*, 1928, **5**, 1615–1620.
27 E. H. Huntress, *J. Chem. Educ.*, 1929, **6**, 52–58.
28 T. A. Geissman, *J. Chem. Educ.*, 1941, **18**, 108–110.
29 T. A. Geissman, *J. Chem. Educ.*, 1949, **26**, 657–665.
30 E. Chambers IV and C. S. Setser, *J. Chem. Educ.*, 1980, **57**, 312–313.
31 A. M. Munro, *J. Chem. Educ.*, 2023, **100**, 3347–3354.
32 J. M. Kolekar, C. S. Reddy, S. Sharma and S. Roy, *Curr. Food Sci. Technol. Rep.*, 2024, **2**, 157–167.
33 V. Heines, *J. Chem. Educ.*, 1972, **49**, 605–606.
34 C. I. Sampaio, L. F. Sousa and A. M. Dias, *J. Chem. Educ.*, 2020, **97**, 4533–4539.
35 J. J. Fortman and K. M. Stubbs, *J. Chem. Educ.*, 1992, **69**, 66.
36 K. Davilla-Diaz, *J. Chem. Educ.*, 2024, **101**, 2149–2155.
37 R. T. M. P. de Souza and A. C. Kasselboemher, *J. Chem. Educ.*, 2022, **99**, 1132–1139.
38 S. Solomon, B. Brook, J. Ciraolo, S. Daly, L. Jackson, M. Oliver-Hoyo and D. Allen, *J. Chem. Educ.*, 2001, **78**, 1475–1478.
39 J. F. Skinner, *J. Chem. Educ.*, 1981, **58**, 1017.
40 A. Fogde, B. Kurtén, T. Sandberg and T.-P. Huynh, *J. Chem. Educ.*, 2020, **97**, 3702–3706.
41 J. L. Radzikowski, L. Delmas, E. Cohen, J. Viola, J. Youssef, A. C. Spivey and R. Kneebone, *J. Chem. Educ.*, 2024, **101**, 535–5360.
42 R. L. Stoddard and J. S. McIndoe, *J. Chem. Educ.*, 2013, **90**, 1032–1034.
43 K. G. Mandel, B. P. Daggy, D. A. Brodie and H. I. Jacoby, *Aliment. Pharmacol. Ther.*, 2000, **14**, 669–690.
44 N. Kanda, T. Asano, T. Itoh and M. Onoda, *J. Chem. Educ.*, 1995, **72**, 1131–1132.
45 J. L. Linder, S. Aljic, H. M. Shroof, Z. B. Di Gusto, J. M. Franklin, S. Keaney, C. P. Le, O. K. George, A. M. Castaneda, L. S. Fisher, V. A. Young and A. M. Kiefer, *J. Chem. Educ.*, 2019, **96**, 304–307.
46 Science in School (Investigating the action of urease), https://scienceinschool.org/article/2008/urease, December 12 2025.
47 A. F. K. Endo and A. Yamasaki, *J. Chem. Educ.*, 1995, **72**, 832–833.
48 C. Y. Park and S. H. Yang, *J. Chem. Educ.*, 2025, **102**, 3685–3690.
49 B. McCall, L. Diener and J. A. Gimm, *J. Chem. Educ.*, 2009, **86**, 464A–464B.
50 C. Suzuki, *J. Chem. Educ.*, 1991, **68**, 588–589.
51 J. Lech and V. Dounin, *J. Chem. Educ.*, 2011, **88**, 1684–1686.
52 J. S. Copes, *J. Chem. Educ.*, 2006, **83**, 1479–1483.
53 Science in School (An artistic introduction to anthocyanin inks), https://scienceinschool.org/article/2015/artistic-introduction-anthocyanin-inks, December 12 2025.
54 W.-Q. Xu, D.-E. Huang, J. Li, Y.-L. Dai, X. Feng, A.-Q. Pang, L. Lin, M.-C. Chen, W.-T. Liu and J.-X. Zhong, *J. Chem. Educ.*, 2025, **102**, 372–378.
55 M. S. H. Hussain, A. Dildar, M. N. Akhtar, R. Rahman, R. Repon, M. M. R. Khan, M. M. Rahman and A. Siddique, *Microchem. J.*, 2025, **213**, 113613.
56 J. C. Ramos, R. S. de Souza Júnior and E. M. Borges, *ACS Omega*, 2025, **10**, 32651–32672.
57 Y. Wu, C. Hu, T. Hu, X. Cao, J. Zhang, M. Wang, J. Cao and R. Que, *J. Chem. Educ.*, 2024, **101**, 4967–4974.
58 W. Maqsood, T. A. Sheikh, S. M. Al-Baqami, A. U. Rehman, S. M. S. Jillani, R. M. Caraballo, L. M. Saleh Medina, S. G. J. Gomez, P. Vansaus and M. Hamer, *J. Chem. Educ.*, 2021, **98**, 958–965.
59 J. L. Andrews, J. P. de Los Rios, M. Rayaluru, S. Lee, L. Mai, A. Schusser and C. H. Mak, *J. Chem. Educ.*, 2020, **97**, 1887–1894.
60 K. C. A. Garber, A. Y. Odendaal and T.-P. E. E. Carlson, *J. Chem. Educ.*, 2013, **90**, 755–759.
61 R. C. Mebane and T. R. Rybolt, *J. Chem. Educ.*, 1985, **62**, 285.
62 J. Zhang, G. B. Celli, and M. S. Brooks, in *Anthocyanins from Natural Sources: Exploiting Targeted Delivery for Improved Health*, ed. M. S. Brooks and G. B. Celli, The Royal Society of Chemistry, 2019, ch. 1, pp. 1–33.
63 Chemical Education Xchange (Cabbage, colours and cleaning products), https://www.chemedx.org/article/

cabbage-colours-and-cleaning-products-citizen-science-inspired-review-anthocyanin, December 12 2025.
64 A. García-Zavala, *J. Chem. Educ.*, 2023, **100**, 3731–3738.
65 J. I. Selco, *J. Chem. Educ.*, 2020, **97**, 2617–2623.
66 D. N. Epp, *J. Chem. Educ.*, 1993, **70**, 326.
67 C. Grajeda-Iglesias, M. C. Figueroa-Espinoza, N. Barouh, B. Baréa, A. Fernandes, V. de Freitas and E. Salas, *J. Chem. Educ.*, 2016, **79**, 1709–1718.
68 E. Bergamin, G. Fabris, M. Neffat, P. B. Toaldo, D. De Luchi and R. Bonomi, *J. Chem. Educ.*, 2023, **100**, 875–879.
69 W. Naimi, G. A. Vinnacombe-Willson, S. Saldana, L. Ronduen, H. Domjan and N. Chiang, *J. Chem. Educ.*, 2024, **101**, 1373–1378.
70 D. Kajiya, *J. Chem. Educ.*, 2025, **102**, 3671–3678.
71 O. Maneechot, W. Hahor, K. Thongprajukaew, N. Nuntapong and S. Bubaka, *J. Food Sci. Technol.*, 2023, **60**, 2255–2264.
72 G. Chandrajith, V. Gamage, J. K. Goh and W. S. Choo, *Int. J. Gastron. Food Sci.*, 2024, **37**, 100957.
73 B. Olas, J. Białecki, K. Urbańska and M. Bryś, *Adv. Nutr.*, 2021, **12**, 2301–2311.
74 T. Kondo, M. Ueda and T. Goto, *Tetrahedron*, 1990, **46**, 4749–4756.
75 B. Jacobus, J. Timmer and T. J. Mooibroek, *J. Chem. Educ.*, 2021, **98**, 540–545.
76 A. Sienkiewicz, I. Rusinek, A. Siatecka and S. Losada-Barreiro, *J. Chem. Educ.*, 2019, **96**, 1982–1987.
77 R. Im, T. Iwayama and M. Osa, *J. Chem. Educ.*, 2023, **100**, 4707–4713.
78 Chemical Education Xchange (Berries are red, berries are blue…I've got a berry surprise for you!), https://www.chemedx.org/blog/berries-are-red-berries-are-blueE280A6iE28099ve-got-berry-surprise-you, December 12 2025.
79 T. Hoshino, U. Matsumoto and T. Goto, *Phytochemistry*, 1981, **20**, 1971–1976.
80 T. Ito, D. Aoki, K. Fukushima and K. Yoshida, *Sci. Rep.*, 2019, **9**, 5450.
81 H. D. Schreiber, A. M. Swink and T. D. Godsey, *J. Inorg. Biochem.*, 2010, **104**, 732–739.
82 B. Chen, *J. Chem. Educ.*, 2025, **102**, 3734–3742.
83 D. Kajiya, *J. Chem. Educ.*, 2020, **97**, 4084–4090.
84 D. Kajiya, *J. Chem. Educ.*, 2024, **101**, 1241–1247.
85 M. Shiono, N. Matsugaki and K. Takeda, *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.*, 2008, **84**, 452–456.
86 M. Shiono, N. Matsugaki and K. Takeda, *Nature*, 2005, **436**, 791.
87 R. Meelapsom, W. Rattanakaroonjit, A. Prakobkij, N. Malahom, S. Supasorn, S. Ruangchai and P. Jarujamrus, *J. Chem. Educ.*, 2022, **99**, 1660–1671.
88 Y. Song, W. Wang and R. Lui, *J. Chem. Educ.*, 2025, **102**, 2243–2251.
89 L. V. Taylor, *J. Chem. Educ.*, 1947, **24**, 558–560.
90 K. G. Deshwal and N. R. Panjagari, *J. Food Sci. Technol.*, 2020, **57**, 2377–2392.
91 F. A. Norton, *J. Am. Chem. Soc.*, 1906, **28**, 1503–1508.
92 F. W. Salt and J. G. N. Thomas, *J. Appl. Chem.*, 1957, **7**, 231–238.
93 C. W. Culpepper, *J. Agric. Res.*, 1927, **35**, 107–132.
94 Chemical Education Xchange (Colourful Chemistry of Canning), https://www.chemedx.org/article/colourful-chemistry-canning, December 12 2025.
95 B. Berké, C. Chèze, J. Vercauteren and G. Deffieux, *Tetrahedron Lett.*, 1998, **39**, 5771–5774.
96 S. Solomon, M. Oliver-Hoyo and C. Hur, *J. Chem. Educ.*, 1998, **75**, 1581–1582.
97 L. A. Keiller, I. L. J. Patterson, D. M. Stewart and I. A. Smellie, *J. Chem. Educ.*, 2023, **100**, 1398–1403.
98 Chemical Education Xchange (The Disappearing Rainbow Demonstration-A colourful variant using red cabbage extracts), https://www.chemedx.org/article/disappearing-rainbow-demonstration-colourful-variant-using-red-cabbage-extracts, December 12 2025.
99 J. F. Castka, *J. Chem. Educ.*, 1962, **39**, A431.
100 M. A. Cutrín, V. Carro, B. Rocha, J. B. Solano and A. Ciolino, *Educ. Quím.*, 2025, **36**, 191–211.
101 R. E. Wrolstad, *J. Food Sci. Educ.*, 2009, **8**, 20–28.
102 J. Andrea-Silva, F. Cosme, L. F. Ribiero, A. S. P. Moreiira, A. C. Malheiro, M. a. Coimbra, M. R. M. Domingues and F. M. Nunes, *J. Agric. Food Chem.*, 2014, **62**, 5651–5659.
103 M. H. F. B. Soares, L. A. Ramos and E. T. G. Cavalheiro, *J. Chem. Educ.*, 2002, **79**, 1111–1114.
104 L. A. Ramos, K. R. Prieto, É. T. G. Cavalheiro and C. C. S. Cavalheiro, *J. Chem. Educ.*, 2005, **82**, 1815–1819.
105 K. R. Galloway, S. Lowery Bretz and M. Novak, *J. Chem. Educ.*, 2015, **92**, 183–188.
106 J. Markwell, R. D. Curtright and J. A. Rynearson, *J. Chem. Educ.*, 1996, **73**, 306–309.
107 R. Binder and C. Lämmle, *J. Chem. Educ.*, 2019, **96**, 1922–1927.
108 R. D. Curtright, R. Emry and J. Markwell, *J. Chem. Educ.*, 1999, **76**, 249–252.
109 A. M. Dias and M. L. S. Ferreira, *J. Chem. Educ.*, 2015, **92**, 189–192.
110 A. Paul, A. Dutta, A. Kundu and S. Saha, *J. Chem. Educ.*, 2023, **100**, 885–892.
111 M. Manninen, V.-M. Vesterinen and J.-P. Salminen, *J. Chem. Educ.*, 2020, **97**, 772–777.
112 G. P. Smestad and M. Gratzel, *J. Chem. Educ.*, 1998, **75**, 752–756.
113 V. S. Watwe, S. D. Kulkarni and P. S. Kulkarni, *J. Chem. Educ.*, 2025, **102**, 4622–4630.
114 J. G. Ibanez, M. W. Tausch, C. Bohrmann-Linde, I. Fernandez-Gallardo, A. Robles-Leyzaola, S. Krees, N. Meuter and M. Tennior, *J. Chem. Educ.*, 2011, **88**, 1116–1118.
115 G. Trujillo-de Santiago, C. Rojas-de Gante, S. García-Lara, A. Ballescá-Estrada and M. M. Alvarez, *J. Chem. Educ.*, 2014, **91**, 1729–1735.
116 M. Bortoli and L. Orian, *J. Chem. Educ.*, 2023, **100**, 2591–2600.
117 J. Watt, *Philos. Trans. R. Soc.*, 1784, **74**, 419–422.
118 S. Silver and J. Silverman, *Postmedieval*, 2025, 2040–5979.

119 B. Worley, E. M. Villa, J. M. Gunn and B. Mattson, *J. Chem. Educ.*, 2019, **96**, 951–954.

120 https://uwaterloo.ca/chem13-news-magazine/september-2019/feature/indicator-droplet-art December 12 2025.

121 S. Blunden and T. Wallace, *Food Chem. Toxicol.*, 2003, **41**, 1651–1662.

122 C. C. Seow, Z. Abdul Rahman and N. A. Abdul Aziz, *Food Chem.*, 1984, **14**, 125–134.

123 B. Z. Shakhashiri, *Chemical Demonstrations: A Handbook for Teachers of Chemistry*, University of Wisconsin Press, Madison, 1983.

124 J. Shinoda, *J. Pharm. Soc. Jpn.*, 1928, **48**, 214.

125 T. A. Geissman and R. O. Clinton, *J. Am. Chem. Soc.*, 1946, **68**, 700–706.

126 G. M. Robinson and R. Robinson, *Biochem. J.*, 1931, **25**, 206–212.

127 A. Robertson and R. A. Robinson, *J. Chem. Soc.*, 1927, 2196–2206.

128 K. Freudenberg and L. Orthner, *Ber. Dtsch. Chem. Ges.*, 1922, **55**, 1748–1751.

129 H. G. Krishnamurty and T. R. Seshadri, *Curr. Sci.*, 1965, **34**, 681–685.

130 L. Jurd and A. C. Waiss, *Tetrahedron*, 1968, **24**, 2801–2808.

131 C. F. Krewson and J. F. Crouch, *J. Am. Chem. Soc.*, 1948, **70**, 257–258.