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Demonstration

# A Simple and Inexpensive Invisible Ink System Based on Red Cabbage Extracts

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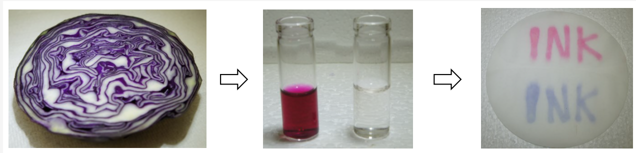


**ABSTRACT:** The use of red cabbage (*Brassica oleracea var. capitata F. rubra*) extract as a pH indicator is a versatile and popular demonstration that is employed by educators in many schools and universities. Previous variations of this demonstration have used red cabbage extracts to prepare paper that changes color when solutions of acid or base are applied as "inks". In this report, we highlight the ability of red cabbage extracts to function as invisible inks. Colorless solutions are prepared by bleaching anthocyanin extracts with aqueous sodium metabisulfite, and the resulting mixture can then be used to write hidden messages on paper. The invisible messages can be revealed by the addition of citric acid solution, or by using potassium aluminum sulfate (alum) as developers. The demonstration does not require specialist equipment or chemicals, and options to use additional plant sources to prepare the ink are described.

**KEYWORDS:** *High School/Introductory Chemistry, First-Year Undergraduate/General, Demonstrations, Acids/Bases, Aqueous Solution Chemistry, Plant Chemistry*

## ■ INTRODUCTION

Invisible inks are a popular feature in chemistry demonstrations and outreach activities, and as a result, there are many different recipes and variants available to educators.[1−5] Many invisible ink mixtures are based on metal ions, for example, $Fe^{3+}$ based inks produce strong colors when treated with solutions containing potassium thiocyanate or salicylic acid.[1,3] There are also many materials derived from plants[1,2] that can be utilized in making inks, or can be used to make the developing mixture for invisible inks. A popular approach is to use a colorless acidic or basic solution as the ink and then use a pH sensitive plant extract as a color changing developer. Red cabbage[1] leaf or rose petal[2] extracts have been found to be particularly useful pH-based developers since they are readily available and present a low hazard. It is also possible to soak different types of paper with red cabbage extract; the treated paper will then change color after application of acidic or basic solutions (using a brush or folding and then dipping the paper).[6,7] In this publication, we report a variation on the use of anthocyanin rich extracts for writing and revealing hidden messages. The activity described demonstrates the use of plant-derived material to make invisible inks that can be revealed using solutions of citric acid or potassium aluminum sulfate.

### Overview of the Activity

Our teaching laboratory is one of the hosts of an annual science summer school practical course for senior high school students (typically 16−17 years old). The chemistry component of the course includes activities focused on molecules isolated from flowers, fruit, and vegetables. We have found that working with colorful plant-derived anthocyanin extracts is very popular with the students and that this topic relates well to familiar materials. As part of our summer school program, we desired a simple, but engaging, demonstration of an aspect of anthocyanin chemistry that was relevant to the food and drink industry. In previous work, we have explored bleaching reactions of anthocyanins with solutions of sodium metabisulfite.[8,9] This type of bleaching process is well studied within the food industry; this is because sulfites are used as preservatives for fruit products, and in certain drinks.[10−13] The underlying chemistry also links to observations that may be familiar to students in everyday life. Useful examples of anthocyanin bleaching processes that involve sulfites include control of undesirable "pinking" of white wines,[11] and production of maraschino cherries.[12] A key step in the latter process requires decolorization of the fruit anthocyanins prior to addition of red food coloring. The mechanism of anthocyanin decolorization by bisulfite ions has been studied by NMR spectroscopy.[14] It has been shown that anthocyanin chromophores (in the flavylium ion form, Scheme 1) react with bisulfite ion to afford the corresponding flavene-4-sulfonate (often called a "bisulfite adduct", Scheme 1). In contrast to the red/pink flavylium ions, the corresponding

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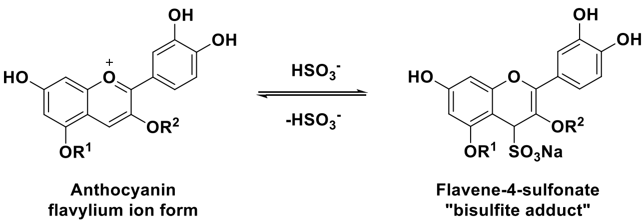
**Scheme 1. Equilibrium between Anthocyanin Flavylium Forms and Flavene-4-sulfonate "Bisulfite Adducts" in the Presence of Bisulfite Ion ($HSO_3^-$)[a]**




[a] $R^1$ = glycosyl substituent. $R^2$ = H or glycosyl substituent.

flavene-4-sulfonates are not fully conjugated, and these result in colorless solutions.

We reasoned that the anthocyanin bleaching process would be a simple way to make an invisible ink for use in a demonstration (in a lecture or laboratory setting). Previous classroom demonstration protocols have used a mixture of sodium sulfite or sodium bisulfite with sulfuric acid to decolorize anthocyanin-containing solutions.[15] We have found that sodium metabisulfite is a more convenient reagent for the reaction, since it does not require mixing with sulfuric acid. Sodium metabisulfite is cheap and readily available from chemical suppliers; this material is also available in the UK in tablet form for domestic use (these are colloquially known as "Campden tablets").[13] To make the invisible ink, red cabbage extract is bleached with sodium metabisulfite solution and applied to absorbent paper, and the hidden message can then be revealed by treating the paper with an acidic solution. The message text is observed to be red (Figure 1, developer A); this is due to the reaction shown in Scheme 1 being reversed to form a flavylium ion species. The hidden message can also be revealed on treatment with an aqueous solution containing $Al^{3+}$ ions. The latter process involves a change from colorless to blue/purple, and this is due to binding of $Al^{3+}$ ions to anthocyanins (Figure 1, developer B). This reaction is of particular interest in the context of the color of certain flowers.[16] Kajiya has recently published very impressive examples derived from morning glory petals, red cabbage, red onion, and rhododendron.[17] Furthermore, work by Binder and Lämmle has demonstrated that $Al^{3+}$ can be used as a selective staining agent in reversed phase thin layer chromatography analysis of anthocyanin mixtures. This technique uses $AlCl_3$ solution to selectively form blue/purple-colored complexes with anthocyanins that contain two adjacent hydroxyl groups on the anthocyanin B-ring.[18]

The demonstration described has been designed to use inexpensive materials and to use basic equipment. The demonstration can be presented using a visualizer and projected on a screen in a lecture theater or teaching laboratory. Alternatively, it can be shown to smaller groups in a fumehood as part of a laboratory activity.

## ■ MATERIALS

The invisible ink demonstration requires the following materials and solutions:

- Red cabbage extract in 4% aqueous ethanoic acid (CAS 64-19-7, commercially available, household distilled vinegar can also be used) or propan-2-ol (CAS 67-63-0).
- An aqueous solution of sodium metabisulfite prepared from 1 g (5 mmol) of sodium metabisulfite (CAS 7681-57-4) and 10 mL of distilled water.
- An aqueous solution of citric acid prepared from 10 g (52 mmol) of citric acid (CAS 77-92-9) and 100 mL of distilled water.
- An aqueous solution of potassium aluminum sulfate prepared from 10 g (21 mmol) of potassium aluminum sulfate dodecahydrate (CAS 7784-24-9) and 100 mL of distilled water.

The red cabbage solutions can be prepared in advance and stored in a freezer for several months. The sodium metabisulfite solution is best prepared immediately before the demonstration is due to take place. In addition to the chemicals listed, cotton swabs, filter paper, and small paintbrushes are required.

## ■ PROCEDURE

### Overview of Procedure and General Method

The red cabbage "ink" in this case needs to be made from a strongly colored, concentrated extract. In testing, it was found that simply soaking red cabbage in freshly boiled water did not

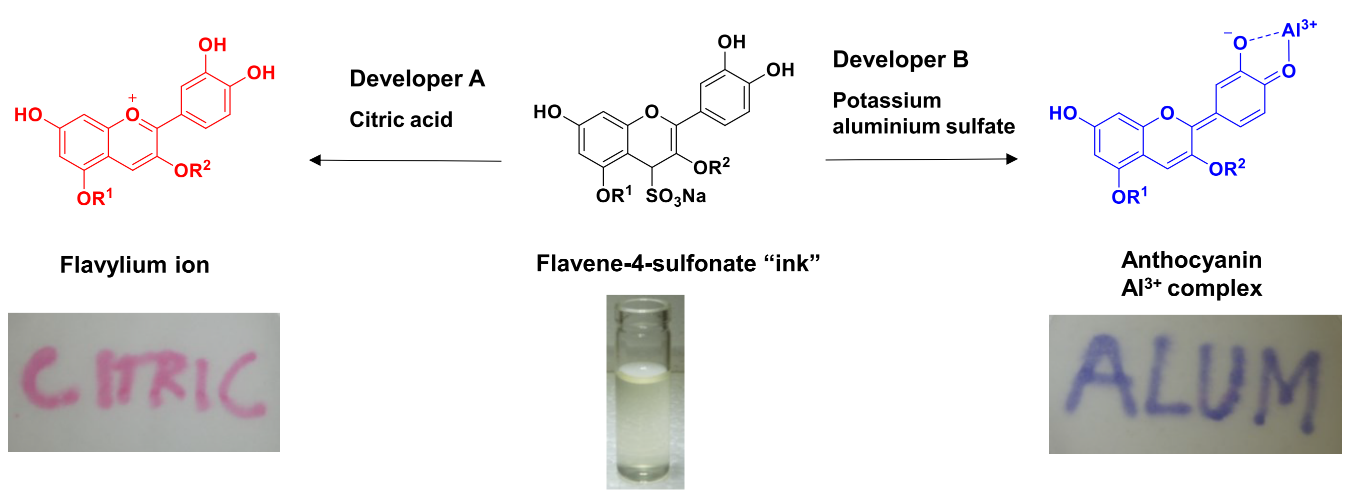



**Figure 1.** Example messages (and corresponding anthocyanin structures) revealed using developer: (A) 10% aqueous citric acid solution and developer and (B) 10% potassium aluminum sulfate. $R^1$ = glycosyl substituent. $R^2$ = H or glycosyl substituent.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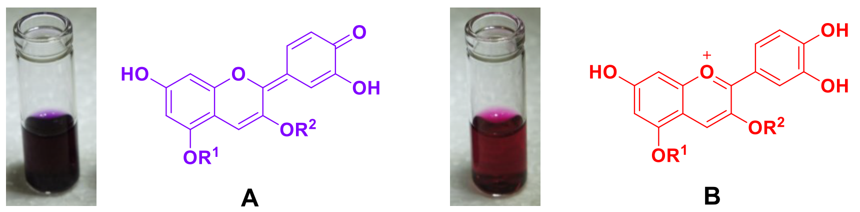


**Figure 2.** Example red cabbage extracts and corresponding anthocyanin structures in (A) propan-2-ol (pH 7) and (B) ethanoic acid (pH 2.5−3). $R^1$ = glycosyl substituent. $R^2$ = H or glycosyl substituent.

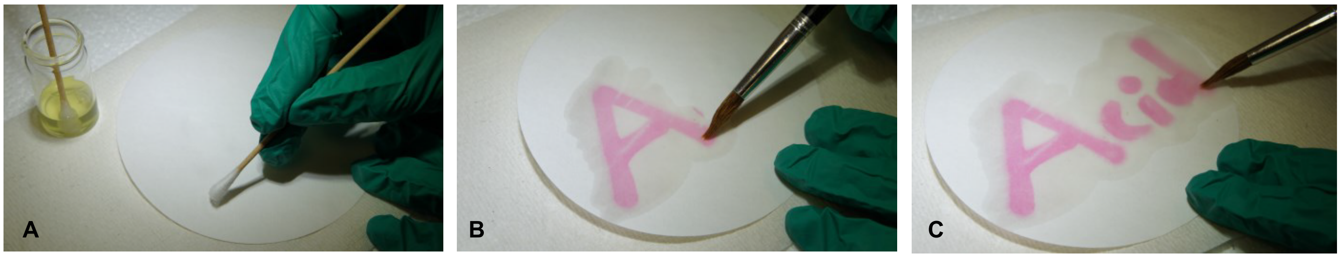


**Figure 3.** Revealing hidden messages written using bleached anthocyanin solution. Step A: Writing a message on filter paper using a cotton swab dipped in the bleached solution. Steps B and C: Application of 10% citric acid solution with a paint brush to reveal the hidden message.

always give satisfactory results since the colored solutions were often too dilute. However, literature precedent[6] described optimized conditions for obtaining concentrated extracts. The report showed that efficient extraction could be achieved by placing red cabbage leaves in a plastic bag, adding propan-2-ol, and then rubbing the leaves to release the anthocyanins. We found that this method, or soaking red cabbage leaves in propan-2-ol for 3 h, worked well too (these treatments resulted in a dark purple extract, see Figure 2A for an example extract). Efficient extraction in aqueous conditions was also possible, and this was achieved by immersing red cabbage leaves in 4% aqueous ethanoic acid for an hour (this treatment resulted in a deep red extract, see Figure 2B for example extracts). Alternatively, immersing red cabbage leaves in a mixture of cold water and dish soap for 3−4 h was also found to be effective.[19]

The extracts described above can then be decolorized by dropwise addition of aqueous sodium metabisulfite solution; this addition is continued until the cabbage extract becomes colorless or a pale straw color (Supporting Information, p S4 and Video S1). The freshly prepared bleached solutions can then be applied to filter paper using a fine brush or cotton swab. The treated paper is allowed to dry and stored until needed, and this is best done an hour or so prior to the demonstration. Developing the paper results in red/pink colored text when an acidic solution is applied with a paint brush (see Figure 3 and Supporting Information, Video S2).

In testing, we found that 1 M hydrochloric acid, 1 M sulfamic acid, or 10% citric acid solutions were able to quickly reveal the hidden message after application. We also found that household vinegar could be used; however, this did not give satisfactory results in testing, as the resulting messages tended to be pale and harder to see (a picture of one of the tests is provided in the Supporting Information, p S6). Our preference has been to use citric acid solution since this is not as corrosive as mineral acids.

Furthermore, an aqueous solution of potassium aluminum sulfate ($KAl(SO_4)_2 \cdot 12H_2O$) can also be used as the developing solution. This gives a very pleasing purple colored text when applied to the filter paper with a paint brush. In contrast to the acidic developers, the alum solution can take a minute or two for the message to be fully revealed. However, the message becomes easy to read without having to wait too long (Supporting Information, Video S3).

A variety of papers have been evaluated for this demonstration, and the best results by far were obtained using filter paper (90 mm diameter). Printer paper and writing papers were found to be unsatisfactory since they were not absorbent enough for application and retention of the ink. It is also possible that the binders and fillers and other additives in many household or office paper products lead to undesirable interactions with the inks in this demonstration.[20]

## ■ HAZARDS

Wearing safety glasses and gloves is recommended for these activities. Do not allow any of the substances in use to come into contact with skin or eyes.

**CARE!** A small quantity of sulfur dioxide ($SO_2$) gas will be released when the metabisulfite solution is used and when the messages are developed using acid or alum solutions. Although the amount of gas released is small, it is strongly recommended that this activity should be performed in a fumehood or a well-ventilated lecture theater (many modern teaching laboratories are equipped with audio/visual equipment for presentations). Users of this demonstration must also be aware that people with asthma can be very sensitive to sulfur dioxide in solution or in the gas phase.[21] The bleaching reaction is the most likely point for sulfur dioxide release; therefore, it is best that this be completed an hour or two prior to starting the demonstration (when the audience is not present). The resulting "invisible ink" can be sealed in a suitable sample vial or flask until it is needed.

On completion of the demonstration, all the solutions should be diluted in water and disposed of according to local safety protocols.


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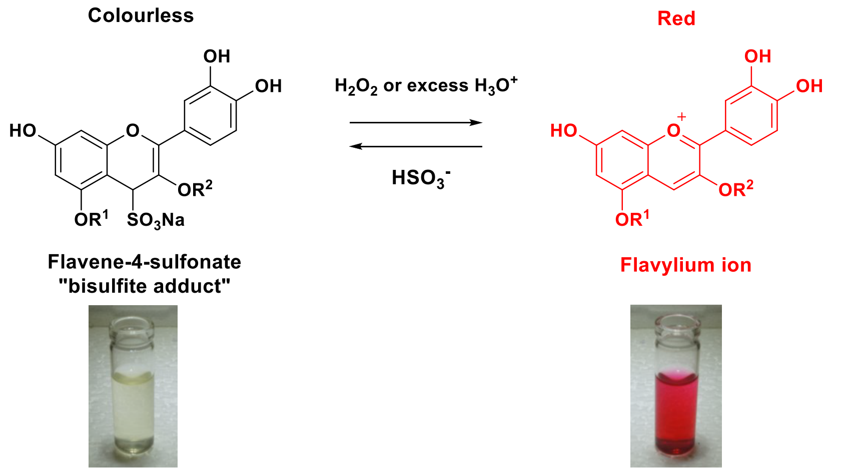
**Scheme 2. Equilibrium between Flavene-4-sulfonate "Bisulfite Adducts" and Anthocyanin Flavylium Forms**[a]




[a]$R^1$ = glycosyl substituent. $R^2$ = H or glycosyl substituent.

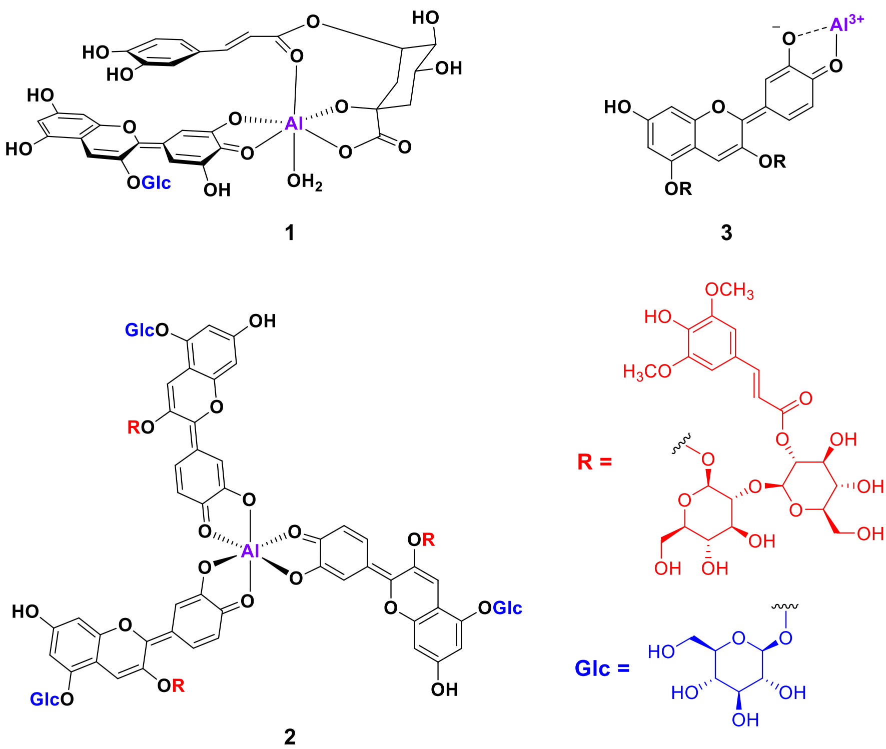



**Figure 4.** Example structures of anthocyanins bound to $Al^{3+}$ ions.

## DISCUSSION

### Chemistry of the Developing Process

The key reaction in this invisible ink process is the controlled bleaching of the anthocyanin solution. Red cabbage contains a variety of cyanidin-derived anthocyanins;[22,23] these compounds behave as shown in Scheme 2 in the presence of bisulfite ions. In our earlier work, dilute peroxide was used to consume bisulfite ion and shift the equilibrium from the colorless flavene-4-sulfonate form, to the red flavylium ion form (Scheme 2). In this demonstration, addition of acid can readily effect a color change on reaction with bisulfite adducts.

### Message Reveal Using Acidic Developing Solution

To understand the behavior of the anthocyanin species involved in the developing process, it is necessary to consider the behavior of the bisulfite ion in aqueous solution.[24,25] It is known that when the solution pH is lowered to 2 or below, the quantity of available bisulfite ion is rapidly diminished. Equation 1 can be proposed to account for this observation. However, this is not the best representation since undissociated sulfurous acid ($H_2SO_3$) is not a detectable species in solution, although it may exist as an intermediate with a very short lifetime.[24,25] Equation 2 provides a better description; at low pH, bisulfite ion forms hydrated (undissociated) sulfur dioxide under aqueous conditions. It should be noted that eq 2 is still a simplified representation; the solution chemistry of sulfur dioxide is complicated and controlled by numerous factors.[24,25]



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Bisulfite equilibrium in the presence of acid (featuring short-lived sulfurous acid)

$$H_2SO_3(aq) + H_2O \rightleftharpoons HSO_3^-(aq) + H_3O^+(aq) \quad (1)$$

Bisulfite equilibrium in the presence of acid (featuring undissociated, but hydrated sulfur dioxide).

$$SO_2(aq) + 2H_2O \rightleftharpoons HSO_3^-(aq) + H_3O^+(aq) \quad (2)$$

The instability of anthocyanin bisulfite adducts in acidic solutions can now be understood, since a reduction of available bisulfite ion shifts the equilibrium from the colorless bisulfite adduct toward the corresponding red flavylium ion (Scheme 2).

### Messages Revealed Using an "Alum" ($KAl(SO_4)_2 \cdot 12H_2O$) Developing Solution

As highlighted in the Introduction, the blue/purple colored text observed when bleached anthocyanin "inks" are treated with potassium aluminum sulfate is most likely due to complexation of anthocyanin molecules to $Al^{3+}$ ions. Recent literature reports provide some useful suggestions, and it is known that $Al^{3+}$ complexes of anthocyanins can afford blue/purple solutions.[8,12,18] However, a precise description of the process that takes place in the presence of potassium alum is harder to elucidate. Perhaps the most well-known example of an anthocyanin complex with aluminum ions is the delphinidin/$Al^{3+}$ complex[16] (structure **1**, Figure 4) that is responsible for the color of blue hydrangea sepals. More recently, a blue food dye has been reported that is an aluminum complex formed from red cabbage-derived anthocyanins[26] (structure **2**, Figure 4). The structures of the species formed in the developing process reported here are unknown, although it is not unreasonable to expect that these compounds could be similar to structure **2**. The key binding anthocyanin $Al^{3+}$ binding motif is shown in Figure 4 (structure **3**).

## SUMMARY

We have developed a simple method to prepare an invisible ink activity based on the chemistry of red cabbage anthocyanins. Aqueous or alcohol extracts of red cabbage leaves can be decolorized by addition of aqueous sodium metabisulfite, and the resulting solution can be used as an "ink" to write a message on absorbent paper. The hidden message can be revealed by treatment with aqueous solutions of citric acid or potassium aluminum sulfate; the text appears pink or purple, respectively. The procedures described are inexpensive to conduct, and they do not require specialist equipment or chemicals. Furthermore, many of the materials needed are available from grocery stores, hardware stores, or online retailers. Vinegar is readily available in grocery stores, citric acid[27] is a component of some household cleaning products, and potassium aluminum sulfate "alum" is commercially available for domestic use (for example, it is often used as a mordant[28] in home dyeing of fibers). Red cabbage extract is not unique in having the ability to react with bisulfite ions in solution, and many other anthocyanin sources exhibit very similar behavior.[12] In the Supporting Information (pp S6 and S7), we identify additional sources of anthocyanins that can also be used to make anthocyanin-derived invisible inks. We hope that colleagues will be encouraged to try other sources of plant-derived anthocyanins. The experiment described has successfully been employed as part of a science summer school laboratory course that typically involves 20−40 senior high school students. We have found that students find the activity engaging, either as a demonstration as part of a lecture or as a demonstration in a laboratory setting.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c01032.

Technical notes and procedures for red cabbage dye extraction, bleaching, and developing invisible inks (PDF, DOCX)

Video S1, bleaching reactions of red cabbage extracts (MP4)

Video S2, developing invisible ink with citric acid (MP4)

Video S3, developing invisible ink with alum (MP4)

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### Notes

The authors declare no competing financial interest.

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