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Greening the Traffic Light: Air Oxidation of Vitamin C Catalyzed by Indicators

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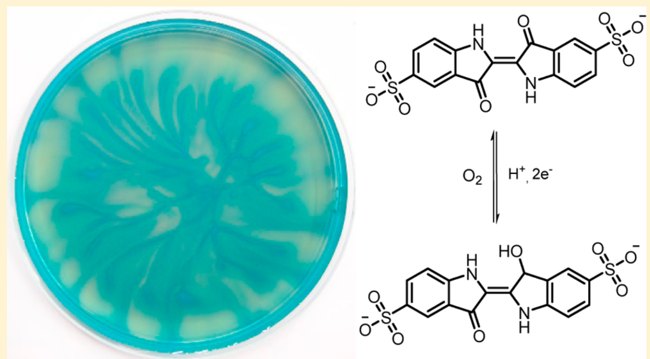
ABSTRACT: Modifications of the classical blue bottle experiment, air oxidation of glucose catalyzed by methylene blue under alkaline conditions, were investigated. Following the green chemistry formulation proposed by Wellman and Noble, we studied the visual behaviors of air oxidation of vitamin C catalyzed by redox indicators. New formulations for the chemical traffic light and vanishing valentine experiments are presented in this paper. We also report, for the first time, chemical pattern formation in this oxidation reaction of ascorbic acid. These results open many possibilities for laboratory practicals as well as demonstration activities.



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Campbell[1] was the first to report the blue bottle experiment in this *Journal* in 1963. The origin of this experiment was attributed to a 1954 demonstrations catalogue of the Chemistry Department of the University of Wisconsin—Madison,[1,2] but it may have been discovered at CalTech.[1] For more than six decades after the discovery, the experiment has proven very popular and versatile for both demonstration and practical activities. The pedagogical functions of this experiment are not only for kinetics and mechanism but also for a wide range of topics including scientific method, laboratory safety, and analytical, biological, and organic chemistry.[3]

The underlying reaction of the classical blue bottle experiment is air oxidation of glucose catalyzed by methylene blue. The solution is blue when it is shaken, and the blue solution fades to colorless when it is left to stand still. The blue color is due to methylene blue in its oxidized form. Glucose slowly reduces methylene blue to leucomethylene blue, and the solution becomes colorless upon standing. Shaking the bottle regenerates methylene blue since leucomethylene blue quickly reacts with dissolved oxygen. The coloration/decoloration cycle can be repeated many times, but after an hour or so the visual behavior deteriorates significantly. (The solution turns to yellow or even reddish brown.[2]) Many modifications of the blue bottle experiment have been reported in this *Journal*. The effect of the reducing sugar, the solvent, and the redox indicator have been investigated by Cook and his team at Valparaiso.[4,5] Two notable experiments using different redox-indicator dyes are the chemical traffic light[6] and the vanishing valentine experiments,[7,8] which use indigo carmine and resazurin as the catalyst, respectively. Though the experiment has been the subject of investigations by many groups, the details of the mechanism are not completely known.[2] A recent report by Anderson and co-workers established that the blue bottle experiment has enolization of the sugar as a key step in a two-stage process.[2]

Experiments involving reduction of methylene blue by vitamin C under acidic conditions were first reported in this *Journal* in 1997 as an alternative to the iodine clock.[9] The mechanism differs from that of the blue bottle experiment. It is, however, not a clock reaction in the usual sense and was suggested for uses under spectrophotometric measurements in 1999 by Mowry and Ogren.[10] Wellman and Noble proposed a further modification in 2003 that brings the vitamin C system on par with the alkaline-glucose system by adding Cu^{2+} to catalyze the reoxidation of leucomethylene blue to methylene blue.[11] The air oxidation of ascorbic acid catalyzed by methylene blue is considered to be an example of green chemistry since chemical waste is reduced and the conditions are less corrosive. The total mass of solid chemical dissolved in one liter of water is reduced from ∼60 g for the classical formulation to ∼6 g for the green chemistry version.[11] The pH is also changed from as high as ∼13 due to caustic soda to ∼3, which can be easily neutralized by half a teaspoon of baking soda before disposal.[11] There are, however, a number of drawbacks to this "green" version of the blue bottle experiment. First, the chemicals should be weighed carefully as the new reaction is quite sensitive to concentrations of reactants. Second, the rate of reaction is somewhat slower than that of the original formulation despite the effort to add more catalyst. Third, the blue color of the experiment is not as deep as that of the classical one. Lastly, the kinetics and mechanism of the ascorbic acid system is relatively more complicated than that of the reducing

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sugar system. Notwithstanding these disadvantages, this "green" version of the blue bottle experiment has plenty of room for further investigation.

Inspired by the work of Wellman and Noble,[11] we explored the extension of their work by adapting air oxidation of ascorbic acid reaction to the chemical traffic light and vanishing valentine experiments. The results are discussed in the next two sections.

Table 1. Formulations for Air Oxidation of Ascorbic Acid Catalyzed by Dyes in 100 mL of DI Water

Solution	Dye/ μmol	Ascorbic Acid/mg	$NaHCO_3$/ mg	NaCl/ mg	$CuSO_4\cdot 5H_2O$/ mg	NaOH/ mg
Methylene blue[11]	5.63	80	25	167	7.5	
Indigo carmine	6.75	93	25	167	7.5	260
Resazurin	2.81	80	25	167	7.5	
Safranin[a]	1.69	80	25	167	9.0	210
Safranin[a]	0.56	80	25	167	9.0	210

[a]For safranin, the reaction takes a very long time (~30 min) to return to reduced color. Two formulations are given. The one with less dye returns to reduced color faster than the other.

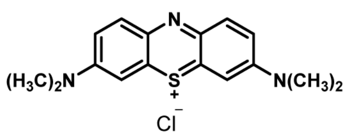
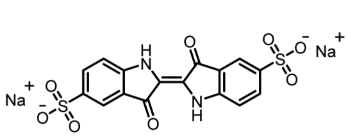
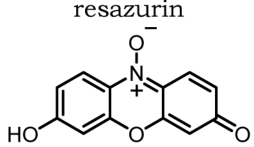
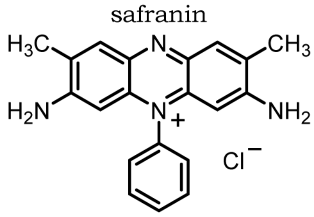
AIR OXIDATION OF ASCORBIC ACID CATALYZED BY DYES

There are four families of dyes, namely, thiazines, oxazines, azines, and indigo carmine, reported to work with glucose and caustic soda.[4,6] We successfully repeated the Wellman and Noble recipe[11] for methylene blue and developed similar formulations for the dyes from the other three families. We found that, in comparison to glucose oxidation, the reaction of ascorbic acid is viable in a wider pH range. We took advantage of this to adjust the pH to alkaline for the best visual effect and time for the color to change/return. The formulations for different dyes are shown in Table 1. Chemical structures and dye colors both in DI water and during the reaction are summarized in Table 2.

As the amounts of chemicals are extremely small, in practice, we prepared stock solutions of dyes (1.13 mM), ascorbic acid (2.67% w/v), $NaHCO_3$ (0.25% w/v), NaCl (0.835% w/v), $CuSO_4\cdot 5H_2O$ (0.075% w/v), and NaOH (42% w/v). Commercially available reagents were used without further purification. All experiments were conducted in an air conditioned laboratory set to ~25 °C (77 °F).

Our green versions of the chemical traffic light (indigo carmine) and the vanishing valentine experiment (resazurin) change color

Table 2. Chemical Structures and Dye Colors Both in DI Water and in the Reaction

dye structure	color in DI water	color in the reaction (reduced form $\underset{\text{leave to stand}}{\overset{\text{shake}}{\rightleftharpoons}}$ oxidized form)
methylene blue 		
indigo carmine 		
resazurin 		
safranin 		

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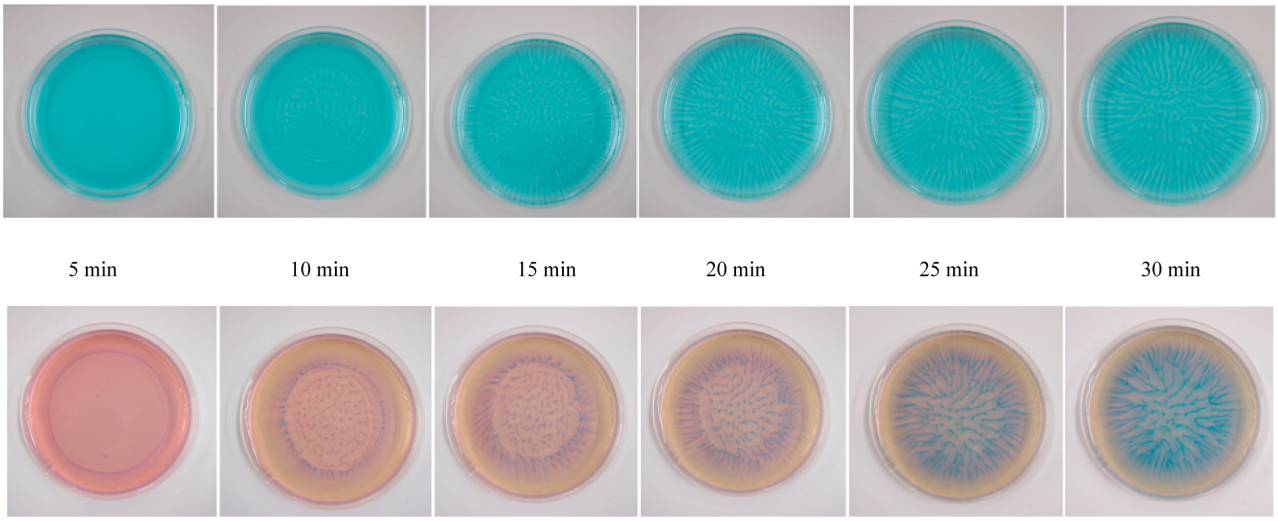

Figure 1. Pattern development in air oxidation of vitamin C over 30 min for two catalysts, methylene blue (top row) and indigo carmine (bottom row). Small circular structures formed within the first 10 min gradually transformed into a network of radial streaks going to and from the center.

when shaken and return to their original color in a reasonable time when left to stand in a similar manner to Wellman and Noble's blue bottle experiment. Since indigo carmine is also a pH indicator, the inclusion of NaOH in our recipe in Table 1 helps us achieve the traffic light colors. Table 2 shows that the color in the ascorbic acid system is not exactly the same but is comparable to its glucose counterpart.

■ PATTERN FORMATION

Pattern formation has been reported in reactions involving methylene blue including the classical blue bottle experiments.[12−17] The dot and line pattern in the methylene blue−glucose−oxygen system was suggested to be due to chemoconvection where the denser layer of gluconic acid (a product of glucose oxidation) at the top of solution overturns.[15−17] The patterns are developed when the solution is poured into a Petri dish and eventually fade away after a period of time. There are many factors affecting pattern formation such as composition of the solution, thickness and surface area of solution, temperature, and time.[15−17]

Motivated by these reports of pattern formation,[12−17] we first explored the use of other dyes in the glucose system. We found that clear patterns form in a reasonable time (∼5 min) when more dye is added to the thin layer of the usual formulation of the experiment. Irrespective of the dye, the results were dots and lines similar to what was observed for methylene blue.

The same procedure was applied to the ascorbic acid system. We filled a plastic Petri dish (diameter = 85 mm) to a depth of ∼4 mm with a solution containing all of the reagents including the dye, added ∼2 mL of dye solution (concentration = 1.13 mM), and stirred. Without covering the Petri dish, a photo was taken every 5 min.

Figure 1 shows that the patterns formed initially were dot and line patterns, similar to what was reported in the methylene blue−glucose system.[13,15−17] However, when a larger amount of the dye was added, our patterns eventually developed into "networked lines" (Figure 2) resembling blood vessels or vascular bundles on plant leaves, which are markedly different from what has been reported for the blue bottle solution. This may help students better relate chemistry to pattern formation in biology beyond spots in leopards and stripes in zebras.[18]

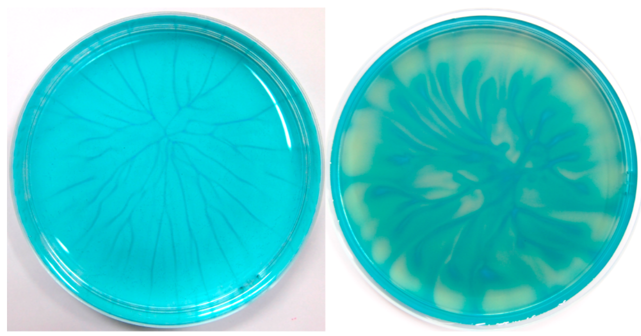

Figure 2. "Networked lines" patterns in air oxidation of vitamin C catalyzed by methylene blue (left) and indigo carmine (right) when a larger amount of the dye was added.

■ CONCLUSIONS

The results presented here demonstrate that the air oxidation of ascorbic acid can be catalyzed by various redox dyes. The visual results are somewhat similar to classical experiments that use a reducing sugar and caustic soda. Therefore, the entire set of demonstrations based on the system, the blue bottle, the chemical traffic light, and the vanishing valentine experiments, can be made more environmentally friendly or "green" by changing the reaction to air oxidation of ascorbic acid.

To the best of our knowledge, our preliminary investigation of pattern formation in ascorbic acid system is the first of its kind. This opens possibilities for future in-depth research into the mechanisms and kinetics of the process.

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Notes

The authors declare no competing financial interest.

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