Tested Demonstrations

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Greening the Blue Bottle

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The uses, studies, and impact of the Blue Bottle demonstration are legion. Those who have watched it and those who have done it are well beyond tabulation. It is likely that a large number of the readership of this *Journal* have viewed this demonstration, have performed this demonstration in front of a class or other group, or have directed a group of students to perform this demonstration as an investigative experimental exercise. From its inception (at least the 1950s; ref *1*) until the present day, it has served both as a visually impressive demonstration and as an academic tool for the study of rates and mechanisms.

The classical demonstration involves the redox cycling of methylene blue (MB^+) using glucose as the ultimate reductant and aerial O_2 as the ultimate oxidant in a strongly basic solution. Variations of dyes and sugars in the demonstration have been reported *(2–4)*. A typical preparation is shown in Table 1 as taken from a standard reference.[1] After initial agitation and dissolution, the solution is blue; as the solution stands undisturbed, glucose reduces MB^+ to its leuco-form, MBH_2^+, producing a colorless solution. The sample is shaken to mix air into the solution, resulting in oxidation of MBH_2^+ by O_2 back to blue MB^+. The sequence can be repeated through a number of cycles, although oxidized sugar products soon yellow the solution.

Given its widespread use, which includes direct student handling, two concerns can be identified for the classical formulation: (1) its extremely caustic nature, and (2) its large total mass consumption. Both of these are concerns toward growing interest in green chemistry, which promotes less harmful processes and processes that use less and wastes less *(5)*. For the 600-mL solution size shown in Table 1, 36 grams of solids are involved and the solution basicity is 0.48 M KOH. Here a totally different Blue Bottle formulation is described that uses less than one-tenth the total mass of solids and is conducted at pH 3.

Experimental Methods

Reactant quantities for the revised formulations are given in Table 1. The amounts in the table represent a good starting point for a 600-mL solution volume, but these can be modified to individuals' preferences for specific circumstances.

Sets of directly compared, parallel experiments were conducted. Comparisons were evaluated solely for visual effects and, as such, no quantitative evaluations of rate constants or orders are implied. The visual effects of primary importance were the depth of blue color upon shaking, the time required to achieve reasonable decoloration, and the faint coloration that remained following reduction. Reasonable decoloration was defined as nearly total color fade and a point at which the demonstration had sufficiently completed that cycle. Total decoloration required longer times and ended with a very slow final fading; thus, its timing could not be visibly judged. Some subtleties involved in color evaluation are also dependent on room lighting, which is another aspect for individual circumstances and preference.

Solutions were freshly prepared and all reactants were weighed as solids for each individual trial. This design was preferred in order to gauge reproducibility (which proved to be very good) and to allow for better comparisons that were not subject to variables such as solution stability. It is likely that the MB^+ and copper(II) solutions can be prepared from stock solutions of the reactants as long as they are reasonably fresh. This is questionable for the ascorbic acid solution, however, owing to its air sensitivity. When comparing runs with the classical Blue Bottle formulation, the classical's KOH solution was prepared in advance and allowed to cool to room temperature before use.

Typical experiments were conducted in 600 mL deionized water in a standard 1-L jar (approximately 9 cm outside diameter) with a screw-cap lid. The copper salt was added

Table 1. The Classical and the Revised Blue Bottle Formulations

Classical	Revised	Revised: Fast Modification	Revised: Consumer Products
20.0 g glucose	2.40 g ascorbic acid	4.80 g ascorbic acid	1/2 teaspoon vitamin C powder
16.0 g KOH	75 mg $NaHCO_3$	150 mg $NaHCO_3$	2.5 cups of water
1.0 mg methylene blue	3.0 mg methylene blue	3.6 mg methylene blue	1–2 drops MethyBlu (shake before dispensing)
600 mL H_2O	1.00 g NaCl	1.00 g NaCl	1/4 teaspoon table salt
	36 mg $CuSO_4{\cdot}5H_2O$	45 mg $CuSO_4{\cdot}5H_2O$	15 drops Had-A-Snail (~1/8 teaspoon)
	600 mL H_2O	600 mL H_2O	

last, after total dissolution of the other solids. Solutions were typically shaken for at least 20 s for comparative trials. Ambient temperatures were 19–24 °C. Reactants were reagent-grade or suitably close; for example, ascorbic acid was 99%, but it was not reagent grade. Methylene blue was certified grade. Solution pH was determined using a pH meter calibrated at 4.00 and 7.00 using standards that were rated at ± 0.02; however, one-decimal consideration is sufficient. When runs were completed and neutralized, solution pH measurements were done simply with pH strips.

Results

For simplicity, MB^+ and $MBH_2{}^+$ shall refer to the blue (oxidized) and colorless (reduced) forms of the dye, respectively, regardless of state of protonation or association *(6–8).*

The revised Blue Bottle formulation still uses methylene blue as the dye and O_2 as the ultimate oxidant, but the ultimate reductant is now ascorbic acid, vitamin C. The dissolution of ascorbic acid gives a solution pH of 2.7–2.8, which is adjusted to 3.0 with $NaHCO_3$. The blue solutions decolor to a faint green tinge. The system is actually complex from a mechanistic viewpoint and the visual effects are strongly dependent on the amounts of all reactants. A number of observations are summarized as follows, derived from the many permutations that were conducted.

- By itself, ascorbic acid reduces MB^+ to $MBH_2{}^+$, but this solution does not conveniently reoxidize upon shaking with air. Copper catalyzes the oxidation of $MBH_2{}^+$ to MB^+ by O_2. The copper also catalyzes the reduction of the MB^+ by ascorbic acid. Thus, two reduction pathways of MB^+ (uncatalyzed and Cu-catalyzed) are operating.
- $CuSO_4 \cdot 5H_2O$ is not the only viable copper source; for example, $CuCl_2 \cdot 2H_2O$ behaves similarly at the same copper molarity. High copper concentrations shorten the reductive decoloration time, but can lead to a faint yellowing of the reduced solution. This faint yellowing is subtle and it is best determined observing directly compared runs. The $CuSO_4 \cdot 5H_2O$ range from 20 to 45 mg was studied, giving reasonable decoloration times of ~6 min to ~3 min, respectively.
- Higher MB^+ concentrations give a deeper blue upon shaking but require longer standing (reduction) times to decolor. The higher concentrations can also contribute to a faint yellowing in the reduced solutions.
- Upon shaking, full development of the blue color is not immediate. This necessitates that shaking be sustained for at least 20 s.
- Higher ascorbic acid levels (with correspondingly more $NaHCO_3$ for pH adjustment) accelerate the reduction of MB^+ and therefore the solutions decolor faster. Unfortunately, this has an adverse effect on the ease of oxidation of $MBH_2{}^+$ to MB^+ and the full development of the blue color is impeded.
- The chloride from NaCl improves the overall results by speeding the reduction of MB^+ and it also lessens the faint yellowing of the reduced solution. Curiously, too much chloride (4.00 g NaCl) has deleterious effects: it slows the reduction of MB^+, and impedes the development of the full blue color upon shaking.
- The initial pH adjustment using $NaHCO_3$ is not absolutely required, but it does give an overall improvement. Interestingly, higher pH (≥ 3.5) leads to poor visual results and some precipitation.

As can be seen by these observations, the overall situation is complex and several ingredients have limited windows of efficacy. The quantities in the table represent a reasonable optimization while leaving open the possibility of variations for further investigations and for personal preference. These quantities provide reasonable decoloration within ~3–4 min for cycles over several hours of total time.

There is considerable literature on the components of the revised solution that provide mechanistic clues; representative works are found in refs *6–16* and include studies of MB^+–ascorbic acid interactions, MB^+–$MBH_2{}^+$ protonation, MB^+ monomer–dimer–trimer equilibria, Cl^- effects on MB^+ or its aggregates, Cu^{2+}–ascorbic acid interactions, Cu^+–Cu^{2+}–O_2 redox, and Cl^- ligation to Cu^+. Other metal ions were also tested, by themselves and with copper. Of these, only iron with copper gave positive kinetic results but the overall color was less satisfactory. Studies of iron in related systems have also been reported *(11, 16, 17).*

Discussion

Direct comparison of the revised Blue Bottle formulation to the classical Blue Bottle has been done, using the amounts listed in Table 1. After standing undisturbed, both the classical and the revised versions show some blue at the solution–air interface as a result of the O_2 surface absorption and reaction. The overall appearance for the classical formula degrades owing to glucose oxidation products: the solutions yellow considerably and this is a significant visual detraction. Solutions of the revised formula have comparatively excellent stability over time and can still be used after several hours. Even after 24 h, the solutions will cycle fully, although they are slowed and faded a bit. Some of this appears to be the result of irreversible events, but some is the result of simple depletion of reactants: refreshing the air space restores the depth of blue.

Direct comparisons also show that the revised formulation gives a somewhat bluer solution but initially slower reduction when compared to the classical formulation. Both of these points require elaboration. The "blueness" is a difficult comparison, since the two methods have distinctly different shades of blue. Relative to each other, the classical mixture is on the purple side of blue while the revised mixture is on the green side of blue. In terms of the speed of reduction, the classical formula starts fast but varies considerably during later cycles. The revised formula is much more uniform. For example, the revised formula is approximately one minute slower than the classical formula to achieve reasonable discoloration in the first cycle; however, by 30 min total time, the classical formula has slowed and the two are of comparable speeds. At much longer times, the color degradation within the classical formulation renders the timing difficult to evaluate.

When speed of the reaction is a priority, the revised fast modification formulation (Table 1) can be used. This formulation doubles the amount of ascorbic acid and $NaHCO_3$.

The quantity of copper is elevated slightly and the quantity of methylene blue is increased to compensate for the poorer blue color at high ascorbic acid quantities, as noted earlier. This modification is faster than the classical formulation at all times, even in the early cycles. Furthermore, it stays more uniform and cleaner throughout. Such speed is not always desired, however, so this modification may or may not be favorable for a particular application. Again, this is subject to an individual's preference. This fast modification illustrates some of the options available for consideration.

Experiment Using Consumer Products

All ingredients of the revised formulation are obtainable from retail stores. A recipe has been developed using the commercially available reactants and kitchen tools. It is important to note that commercial or retail products can have a much greater variation in quality. Furthermore, kitchen measuring devices are highly imprecise; for example, simple spoon measures can vary considerably depending on manufacturer. Thus, the recipe presented is intended as an operational starting point.

The selection of the individual ingredients is described:

- Vitamin C is widely available as a source of ascorbic acid, but results were poor when working with common tablets. It took several hours with occasional shaking to disintegrate the tablets in water, although it only took 20–30 s using a kitchen blender. Additives and binders clouded the solution and some added a bit of froth. Even after filtering (using a filter paper and holder from a drip coffeemaker), the final solutions had a poor appearance. Superior results were realized using vitamin C powder, which was obtained in a health food store. Numerous versions of vitamin C powder (also called vitamin C crystals on some product labels) are available and some versions appear to be fairly pure; however, others still contain additives. For the present work, "Vitamin C Powder" by Solaray, Inc. was used; the label stated that no other ingredients were present.
- MB^+ is available in pet stores for use with aquarium fish. It is commonly used to treat "ick" (or "ich", short for Ichthyophthirius) and to protect eggs from fungus. It is available as tablets containing additional ingredients (which could potentially cause interference) or as a solution. The product used was "MethyBlu" by Aquatronics; it is an aqueous suspension that is stated to contain 5% methylene blue and came in a dropper bottle. The sample was not homogeneous: along with the dissolved dye, it contained very small dye particles that slowly dissolved while preparing the demonstration solution. It was absolutely necessary to freshly shake the bottle for each use.
- The source of sodium chloride was common table salt.
- $CuSO_4 \cdot 5H_2O$ is available from hardware stores, but another source is pet stores, again in the aquarium section. The product "Had-A-Snail" by Aquarium Products, used for snail control, is a simple aqueous solution that is stated to contain 1.61% copper from $CuSO_4 \cdot 5H_2O$. This product came in a dropper bottle. Given the small quantities of copper used for the revised Blue Bottle, this proved to be a very convenient means of working with this ingredient.
- Tap water was used for this recipe. Tap water varies significantly, depending on the source. Primary factors of concern are solute contents and pH. The local municipal water supply is rated at pH 8.0 *(18)*. Making the present recipe with tap water gave a pH of 3.0 without any bicarbonate addition. On the other hand, making this recipe with deionized water gave a pH of 2.7 and a weaker blue color. Adding $NaHCO_3$ to raise the pH to 3.0 darkened the blue color. Under circumstances of low pH, one could add a pinch of baking soda, although overshooting is also deleterious. It may simply be easier to use more MB^+.

Other sources could be used for the ingredients. The manner of dispensing would simply need testing by the individual.

The directions to make the revised formulation using consumer products is as follows: Into a 1-qt jar, combine the first four reactants listed in Table 1 under Consumer Products. Close and shake the jar until all ingredients are dissolved. Then add the Had-A-Snail. Close and shake the jar for at least 20 s. The color will fade over 2–3 min (one drop MethyBlu) or 3–4 min (two drops MethyBlu). Repeat the color–decolor cycle as desired. This recipe provides excellent results and long-term stability. Prior to disposal, the addition of ½ teaspoon baking soda gives a pH of ~7.

As with the reagent grade formulation, all quantities are subject to experimentation by the individual for personal preference. Again, trade-offs abound. The recipe can be easily doubled and conducted in a 2-L soft drink bottle (PETE). The advantage of PETE over glass (besides less breakability) is that the PETE bottle will indent as a result of the decreasing oxygen levels. This can be used as a clue for students when studying the demonstration. The indentation is noticeable within three cycles done over 10 min. After 24 h, the indentation is fairly drastic.

Hazards

Although fairly mild, the ingredients are not totally safe. In particular, the methylene blue and the copper products are not intended for human contact and the cited retail products are labeled to that effect. The jar or bottle used for the demonstration should not be used later for drinks or foods. Methylene blue can be absorbed by some polymers; contact with plasticware may render that plastic unfit for later use for food or drink applications. Although used in medical applications, methylene blue has caused some seriously harmful effects thereby *(19)*.

Summary

The revised Blue Bottle formulation represents a vast improvement in safety and a vast reduction in total mass consumed relative to the classical formulation. It achieves this while utilizing routine reactants that are environmentally mild. While technically not perfect, the solution is comparatively safe; it is even less acidic than common carbonated beverages.[2] These factors greatly improve the overall safety concerns for direct handling by students, especially by younger students. When completed, the solutions can be easily neutralized for disposal: the addition of more (beyond the starting amount) $NaHCO_3$ until the total $NaHCO_3$ mass equals the initial ascorbic acid mass typically gives a solution pH 6.5–7.0. In essence, one obtains a reasonably neutral, salty solution of vitamin C that contains 6 ppm MB^+–MBH_2^+ and 15 ppm Cu.

Certainly, variations remain, as does the potential for more detailed, quantitative aspects. It is hoped that this report will spark interest and inquiry, much as the classical Blue Bottle has done over the years.

Additional Comments

A number of widely-used demonstrations employ strong oxidants. Ascorbic acid is a versatile, mild, fairly inexpensive reductant that is suitable for those demonstrations as a treatment method prior to disposal. Redox neutralization, along with pH neutralization, should be considered for disposal of all such demonstrations.

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Notes

1. The quantities for the classical formula are derived from those in ref *2*, scaled to 600 mL. The quantity of methylene blue is estimated from the information therein and rounded for convenient weighing.

2. The pH of room temperature, freshly opened cans of Coca-Cola and Sprite were measured to be 2.4 and 2.8, respectively.

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