Don’t Be Dyeing

QUESTION: What is the concentration of dye in different flavors of sports drinks?

HYPOTHESIS: If the different sports drinks have different concentrations of blue dye when stimulated with light, then the darker the shade of blue in the drink the higher the concentration will be, because 630nm is where blue dye absorbs light the strongest.

VARIABLES:

INDEPENDENT: The shade of blue of the different sports drinks.

DEPENDENT :Concentration of the Blue #1 dye in each flavor.

CONSTANTS : How much light is transmitted through each sample of sports drink to find the concentration.

Purpose of my Experiment

Purpose of my Experiment: The purpose of the experiment is to find the different dye concentration of the Blue #1 in various flavors of Gatorade.

Materials Required

-Sports drinks with various shades of blue coloring

–Filtered water

-Blue food coloring

-Cytation 5 Spectrophotometer with UV-Vis absorbance

-Clear 96-well plates

-Notebook

-Pen/Pencil

-Pipets and pipet tips

Procedures

1. 1.Mix blue dye standard dilutions
    1.1. Mix ⅛ teaspoon of blue food dye into 8oz of water. This will be Dilution 1, the most concentrated.
    1.2. Setup 8 tubes. Label 1-8. Put 500ul of water in tubes 2-8.
    1.3. Serial dilute Dilution 1 by placing 500ul into tube 2, mix, then place 500ul from tube 2 into tube 3, mix, and repeat for all 8 tubes. Each tube should be half the concentration of the previous tube.
2. Setup clear 96-well plate with Standards dilutions and sports drink samples.
    2.1. Pipet 100ul of each Standards dilution into the wells in the first column of the plate, repeat in duplicate in the second column of the plate.
    2.2. Pipet 100ul of each of the different sports drinks into 3 different wells to ensure repeated readings of each. Note which wells contain which samples on plate layout.
3. Read absorbance in Cytation5 plate reader.
    3.1. Open Cytation software and create an experiment protocol for reading absorbance at 620nM.
    3.2. In the software, label the wells according to the layout to include Standards, unknowns, and blanks so the software can calculate accordingly.
    3.3. Run the experiment in the software. Export data to an Excel file for calculations.
4. Open Excel file with raw data from plate reader to calculate the Standards curve and the sports drink samples.
    4.1. Calculate the standards curve by using the tool in Excel.
    4.2. Average the data from the wells of each of the different sports drinks.
    4.3. Plot the data of the different sports drinks to the standards curve to determine the concentration of blue dye within each drink.

Trial 1

Time/Date: 11:00 - 12:15 11/30/25

Variables:

- Independent – The shade of blue of the different sports drinks.
- Dependent –Concentration of the Blue #1 dye in each flavor.
- Controls – How much light is transmitted through each sample of sports drink to find the concentration.

Results of Trial 1 experiment were:

Concentration of blue dye in this flavor of drink(A) calculated to the standards curve using this calculation, $x=(y-b)/m$. Drink A's average was 0.75uM.

Observations/ thoughts from my experiment:

Some observations from my experiment are that the low uM of this drink is proving my hypothesis that the lighter drinks have a lower concentration of dye.

My expectation was that ….

My expectation was that this drink would have a uM closer to zero than the darker drinks.

Trial 2

Time/Date: 11:00 - 12:15 11/30/25

Variables:

- Independent – The shade of blue of the different sports drinks.
- Dependent –Concentration of the Blue #1 dye in each flavor.
- Controls – How much light is transmitted through each sample of sports drink to find the concentration.

Results of Trial 1 experiment were:

Concentration of blue dye in this flavor of drink(B) calculated to the standards curve using this calculation, $x=(y-b)/m$. Drink A's average was 0.97uM.

Observations/ thoughts from my experiment:

Some observations are that this was the darkest drink out of the four and it was very close to being a concentration of 1uM.

My expectation was that ….

My expectation was that this would have the highest concentration because of the dark and intense blue color of the drink.

Trial 3

Time/Date: 11:00 - 12:15 11/30/25

Variables:

- Independent – The shade of blue of the different sports drinks.
- Dependent –Concentration of the Blue #1 dye in each flavor.
- Controls – How much light is transmitted through each sample of sports drink to find the concentration.

Results of Trial 1 experiment were:

Concentration of blue dye in this flavor of drink(C) calculated to the standards curve using this calculation, $x=(y-b)/m$. Drink A's average was 0.74uM.

Observations/ thoughts from my experiment:

Some observations are that this was slightly lighter than drink A so it is surprising to see the lower average.

My expectation was that ….

My expectation was that this would be slightly more concentrated than drink A but less concentrated than drink D.

Trial 4

Time/Date: 11:00 - 12:15 11/30/25

Variables:

- Independent – The shade of blue of the different sports drinks.
- Dependent –Concentration of the Blue #1 dye in each flavor.
- Controls – How much light is transmitted through each sample of sports drink to find the concentration.

Results of Trial 1 experiment were:

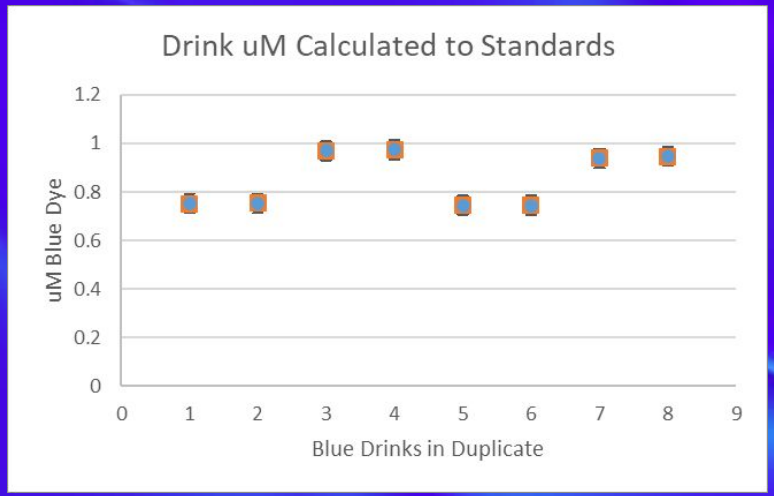
Concentration of blue dye in this flavor of drink(D) calculated to the standards curve using this calculation, $x=(y-b)/m$. Drink A's average was 0.94uM.

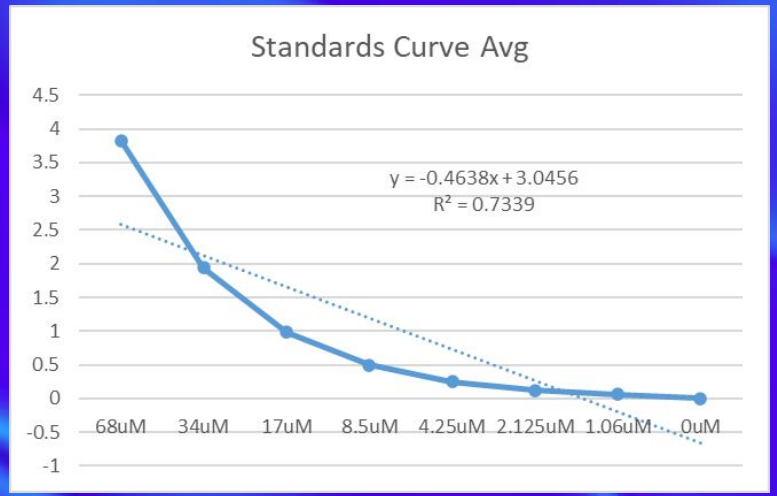
Observations/ thoughts from my experiment:

Some observations are that it was slightly lighter than drink B so the slightly lower concentration proves my theory.

My expectation was that ….

My expectation was that this drink would be second to drink B in concentration but just less than it.


Drink uM Calculated to Standards
uM Blue Dye
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
9
Blue Drinks in Duplicate


Standards Curve Avg
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
y = -0.4638x + 3.0456
R² = 0.7339
68uM
34uM
17uM
8.5uM
4.25uM
2.125uM
1.06uM
0uM

Concentration	Stds Avg		Drinks Avg		Drinks in uM
68uM	3.824		0.088	A1	0.751874
34uM	1.9365		0.08866666667	A2	0.752782
17uM	0.981		0.2473333333	B1	0.968979
8.5uM	0.494		0.251	B2	0.973975
4.25uM	0.2475		0.08266666667	C1	0.744606
2.125uM	0.1245		0.083	C2	0.745061
1.06uM	0.0615		0.2243333333	D1	0.937639
0uM	0.0005		0.231	D2	0.946723

Reflections

I think my experiment turned out to be very interesting and exciting, in that the equipment and techniques that I got the privilege to use to conduct my experiment were what scientists get to use in their labs. My experiment went well but the results were very hard to calculate into the correct numbers to show the concentration of the drinks. If I were to do this experiment again I would include different colored drinks because it would let me explore the concentration differences, or evidence of higher concentrations of dye in different colors.

My project benefits the world by telling us how much blue dye is in various sports drinks. Allowing us to know what different colors or types of drinks are truly “safer” and healthier to drink, which can be important information when deciding which drinks to buy.

Closing

In my experiment I measured four different sports drinks for the concentration of blue dye using a Biotech spectrophotometer. My hypothesis was, “If the different sports drinks have different concentrations of blue dye when stimulated with light, then the darker the shade of blue in the drink the higher the concentration will be, because 630nm is where blue dye absorbs light the strongest”. My data and observations told me that my hypothesis was valid in that sports drinks that have a darker shade of blue have a higher concentration of dye than lighter drinks. From this project I learned that lighter drinks have less dye so they are “healthier” compared to darker sports drinks.

Some things that I would like to find out relating to this experiment are the other colors I can use to test my hypothesis because it would be interesting to see if it is still valid with different colors of drinks.