Determination of Artificial Dyes in Mountain Dew Products

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Abstract: The concentrations of tartrazine (Yellow #5), Red #40, and Blue #1 were successfully determined for seven flavors of Mountain Dew using the method of constant volume standard addition. These data were used to determine the extent of possible health risks involved with drinking Mountain Dew by comparison to the acceptable daily intake (ADI) values and other research. The range of concentrations for the six varieties of Mountain Dew containing Yellow #5 ranged from 1.3 to 32 ppm. Of the four varieties containing Red #40, the concentrations ranged from 2.2 to 39 ppm. For two varieties containing Blue #1, the concentrations were both below 2.5 ppm. The experimental procedure was developed to teach students the method of standard addition and to illustrate an example of the matrix effect. After doing this experiment, students should also have a better appreciation of the concentrations and the potential health dangers of artificial dyes found in their diets.

Introduction

Colorants in various foods and drinks have been commonplace for hundreds of years but it was not until recently that synthetic color additives have been used extensively. Tartrazine, which is also known as Yellow #5, is a synthetic yellow azo dye used in many foods and drinks products [1]. There are a multitude of other dyes which are commonly used in food and drinks, the most common of which are Yellow #6, Red #40 and Blue #1. In recent years, it has been shown that consumption of these synthetic dyes can lead to multiple health risks [1].

One of the health effects associated with tartrazine is the disruption of biochemical markers in vital organs such as the liver and kidney. A study done by K.A. Amin *et al.* found that at low doses of tartrazine there were significant changes in the hepatic and renal parameters [2]. These effects were magnified when taken larger doses, where they induced oxidative stress by the formation of free radicals [2]. In addition, increased concentrations of aromatic amines, some of which are known carcinogens, were detected in the urine of test animals following the introduction of azo dyes [3].

Reproductive performance and sperm counts are have also been observed to be effected in laboratory rodents that consume substantial quantities of artificial dyes in their diet. In a study by Saidi *et al.* mice were orally treated with levels of tartrazine based on 0, 0.1, 1.0, and 2.5% of their body weight. The reproductive performance was shown to have been adversely affected in a group that received 2.5% tartrazine when compared to the control group ($p < 0.01$). In addition, the litter size was significantly reduced in all three experimental groups in comparison to the control. Furthermore, sperm counts were significantly reduced for the mice administrated 2.5% tartrazine ($p < 0.01$) and the sperm concentrations in the epididymis were reduced significantly in all three experimental groups [4].

Lastly, behavioral problems in children have also linked to the consumption of artificial dyes such as Red 40 and Yellow 5. In a blind study done by McCann *et al.*, parents and teachers recorded the behavior of children who were either being treated with mixtures of artificial dyes or part of a control group [5]. In addition, older children between 8 and 9 years old were given a computerized attention test. The findings clearly demonstrated a difference in behavior between the control and experimental groups. The children who were given the artificial dye mixture showed a significant increase in "hyperactivity" which included behaviors such as inattentiveness, impulsivity, and over activity [5].

From a pedagogical standpoint, Mountain Dew is an ideal candidate for teaching students about the uses of Beers' Law, standard curves, standard addition, and the concept of a sample matrix in spectrophotometric analyses. After obtaining an absorbance spectrum of the analyte, the analytical wavelength (λ_{max}) is determined for the analysis. At this wavelength, a standard curve is prepared by measuring the absorbance of samples of known concentrations. The plot of absorbance versus concentration (mol/L or ppm) yields a straight line that follows the equation $A = \varepsilon bc$, A is absorbance, ε is the (molar) absorptivity coefficient, b is the pathlength (in cm) and c is concentration. A solution of unknown concentration (of analyte studied) is determined by taking an absorbance measurement and subsequently calculating a concentration using the line equation determined by the standards. This procedure is followed by students to determine the amount of tartrazine in Mountain Dew by the standard curve method. However, this protocol assumes that the only analyte in Mountain Dew absorbing at the analytical wavelength is tartrazine in an aqueous medium. This is obviously not the case, and there are several ingredients in Mountain Dew that can be responsible for partial light absorption at λ_{max}. This effect is called the "matrix effect" and is defined as "a change in the analytical signal caused by anything in the sample other

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than analyte” [6]. Using the method of constant volume standard addition, known amounts of a concentrated standard is added to a known volume of a solution that is unknown in analyte concentration. The “spiked” unknown solutions are then diluted to a constant volume creating a consistent matrix. The increased absorbance (at λ_{max}) over that of the unspiked unknown allows for the determination of analyte concentration in the original unknown. By having the students compare the results obtained using the standard curve method versus that of standard addition, a direct observation of the matrix effect is realized.

This research quantifies tartrazine, Red #40 and Blue #1 in several varieties of Mountain Dew by the determination the concentrations of each dye using the method of constant volume standard addition. The developed method is used in an analytical chemistry experiment dealing with standard addition. While it is not within the scope of the research project, potential health problems that could arise from consumption have been noted.

Experimental

Both standard curve and standard addition experiments used tartrazine standards prepared from tartrazine purchased from ACROS Chemicals (89% pure catalog number AC19189–1000). Degassed water was used to prepare a working solution of 6.1×10^{-3} M (this is a specific concentration, but any value close to this will work, so long as you know the concentration), which was then used to make a diluted stock solution by diluting the working solution 1:10. This stock solution was then used to prepare solutions for the standard curve analysis. The same stock solution was also used to prepare the standard addition solutions. Several types of Mountain Dew were purchased at a local retail store (Baja Blast was purchased from a local Taco Bell) and used in the analysis, including regular Mountain Dew, diet Mountain Dew, Mountain Dew Code Red, Game Fuel – Cherry-Citrus (Halo 4 Edition), Baja Blast, and Livewire. Each soda sample was degassed by placing the soda in a vacuum flask under reduced pressure for up to 60 minutes until the carbonation was removed. The “flat” soda samples were then used without further treatment. Absorbance measurements were performed on a Vernier Software & Technology SpectroVis Plus and data was collected using Logger Pro 3.8.4.

The procedure used for this work is very similar a wonderful procedure posted online by Dr. Lawrence McGahey at The College of St. Scholastica [7]. Changes to that procedure were as follows: For the standard addition analysis, flat soda was transferred by volumetric pipette to each of the six labeled 25 mL volumetric flasks. The volume of Mountain Dew added was adjusted so the blank absorbance was between 0.03 and 0.1 at the λ_{max} for the target dye, which was done so as to reduce the amount of observed scattering in an undiluted Mountain Dew sample – see Figure 1 and associated text). Then additions of 0 (sample blank), 1, 2, 3, 4 and 5 mL of standard at a concentration around 30 ppm (values can vary - see above), and fill each to volume with distilled water. The concentration of standard added raised the absorbance around 0.1 for each mL added.

A λ_{max} of 421.6 nm was used for the tartrazine analysis, 504.3 nm for red #40, and 620.5 nm for Blue #1. The same cuvette was used through the standard addition run, rinsing thoroughly between readings. The procedure was repeated to verify the results and evaluate the uncertainty in absorbance. Additionally, this work spawned a capstone laboratory experience, where students were asked to develop their own procedure to solve situational problems.

Results

It was alluded to previously that the concentration of Mountain Dew in a sample directly contributed to the amount of observed scattering in an absorption experiment, artificially enhancing the absorption signal at the analytical wavelength. This would lead an experimenter performing a standard curve analysis to think that the sample in question contained more Yellow #5 than it really does. To show the effect of this scattering, the absorption spectra of both diluted and undiluted Mountain Dew were taken and superimposed on the same wavelength scale, as can be seen in Figure 1. It is immediately evident that there is more than just Yellow #5 absorbing at 421.6 nm. It is this scattering, or the matrix effect, that makes standard curve analysis of a turbid solution like Mountain Dew difficult to perform.

One then can use constant volume standard addition to lessen the effect of the solution matrix. To illustrate the effect of scattering caused by the turbidity of the undiluted soda matrix, Figure 2 shows two standard addition plots on the same scale. The blue diamonds show the averaged data for an undiluted sample of Mountain Dew and the red boxes show averaged data for a 2:25 dilution of Mountain Dew in degassed water. The diluted sample data is linear, with an R^2 value of 0.99985, where the undiluted sample data is not linear. This nonlinearity can be directly attributed to the matrix effect. It is for this reason that the amount of Mountain Dew in the standard addition sample blank was adjusted accordingly so as to minimize the matrix effect, but still allow for quantifiable absorbance at the analytical wavelength.

The determination of concentration for Yellow #5, Red #40 and Blue #1 was successfully completed and shown to be quite precise. Of the seven varieties of Mountain Dew studied, six contained Yellow #5 in varying concentrations. The most concentrated (in Yellow #5) was found experimentally to be Game Fuel, which had a concentration of 32 ppm with a standard deviation of 2.1 ppm. Furthermore, concentrations of Livewire, Code Red, Regular Mountain Dew, Diet Mountain Dew and Baja Blast were found to be 13, 7.7, 4.1, 4, and 1.3 ppm with standard deviations of 0.89, 1.3, 0.39, 0.51 and 0.068 ppm, respectively. These data can be found in Table 1.

Red #40 dye was present in four of the seven varieties of Mountain Dew and was experimentally found to have the highest concentration in Game Fuel at 38 ppm with a standard deviation 2 ppm. The concentrations for Code Red, Livewire and Voltage were found to be 25, 39, and 2.2 with a standard deviation of 0.95, 1.8 and 0.14, respectively. These data can be found in Table 2.

Blue #1 was present in both Voltage and Baja Blast and found to have concentrations of 2.2 and 0.72 ppm with standard deviations of 0.11 and 0.02 ppm, respectively. These data can be found in Table 3.

Discussion

The data collected was observed to be precise as evidenced by the low standard deviation values; however, no

Table 1. Yellow-5 data for six varieties of Mountain Dew (MD)

	Reg. MD	Diet MD	Code Red	Game Fuel	Baja Blast	Livewire
n	36	10	10	18	10	10
Average Conc. (ppm)	4.1	3.1	7.7	32.	1.3	13
Std. dev. (ppm)	0.4	0.5	1.3	2.1	0.068	0.89

Table 2. Red #40 data for four varieties of Mountain Dew

	Code Red	Game Fuel	Voltage	Livewire
n	10	10	10	10
Average Conc. (ppm)	25	38	2.2	39
Std. dev. (ppm)	0.95	2	0.14	1.8

Table 3. Blue #1 data for two varieties of Mountain Dew

	Baja Blast	Voltage
n	10	10
Average Conc. (ppm)	0.72	2.2
Std. dev. (ppm)	0.020	0.11

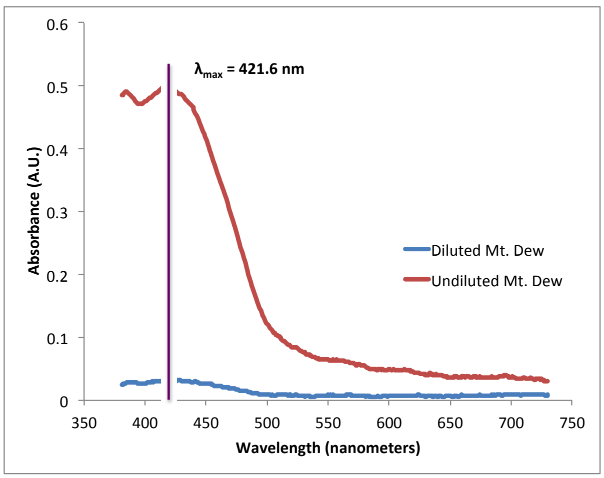

Figure 1. Plot of absorbance versus wavelength for diluted and undiluted Mountain Dew samples. Note the pronounced matrix effect at the analytical wavelength.

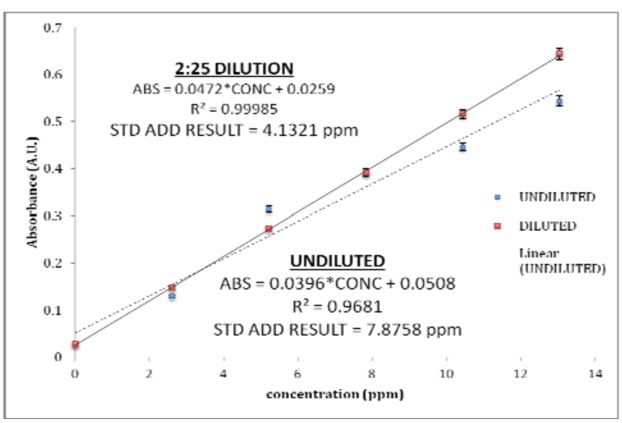

Figure 2. Standard addition plot for averaged diluted and undiluted Mountain Dew samples (five runs averaged. Standard error is reported in the error bars). The marked nonlinearity for the undiluted sample is a direct result of a matrix effect at the analytical wavelength.

manufacturer or literature data could be found to confirm accuracy. An experimental value from a lab experiment in Microspectral Analysis i-LAB Academic Package where 10 mL of Mountain Dew was spiked with a Tartrazine standard [8] resulted in a concentration of tartrazine in regular Mountain Dew of 1.3 ppm but no standard deviation was given. The discrepancy between our experimental value of 4.10 ppm and this value is likely due to the matrix effect stemming from their larger amount of Mountain Dew used (10 mL instead of the 1 mL per 25 mL used in this work). We also performed a constant volume standard addition experiment instead of their singularly spiked standard addition. This allowed for more sample runs and let us compare our trials and determine the effectiveness of our data more efficiently.

The concentrations of artificial dyes in each Mt. Dew variety, along with the total artificial dye concentration, was determined and plotted in Figure 3. These data can be used in further investigation into the potential health implications of the consumption of Mountain Dew. The highest total concentration of dyes in was found in Game Fuel, totaling 70.0 ppm. However, this is far lower than the acceptable daily intake (ADI) of Yellow #5 (5 mg dye/kg body weight/day) and Red #40 (7 mg/kg bw/day).

Conclusions

This experiment may serve as a laboratory experiment for General or Analytical Chemistry effectively illustrating the method of standard addition and provides an example of a matrix effect. In addition, the procedure incorporates basic laboratory techniques including performing dilutions, preparing standards, UV-Vis spectrometry, along with various safety precautions. Analytical calculations such as unit conversion, concentration determination, and linear regression analysis are also reinforced.

Although all of the total dye concentrations found in our work are well below the ADI for adults, more research needs to be conducted to look into the long-term potential health complications associated with the consumption of artificial dyes. In addition, particular attention needs to be made about children consuming large quantities and Mountain Dew and potential developmental complications involved. Further work needs to be done to explore the concentration of artificial dyes

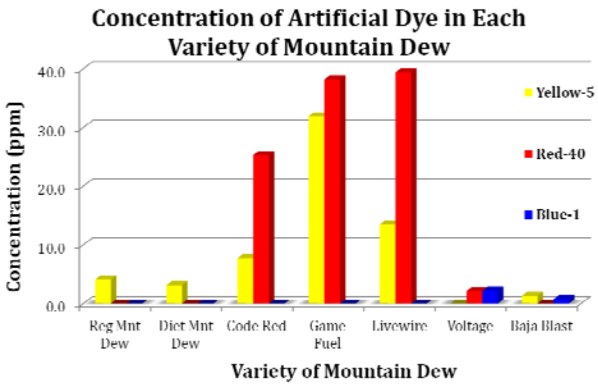

Figure 3. Concentration of Yellow #5, Red #40 and Blue #1 in seven varieties of Mountain Dew.

in other food sources. These findings could lead to stricter regulations regarding the use of artificial dyes in food and beverages and improve the health of millions of people.

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Supporting Materials. One supporting file is available. The Capstone Laboratory as used at Baker University (http://dx.doi.org/10.1333/s00897132512a).

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