Beer's Law Experiments with Red #40 Food Dye
(Cherry Kool-Aid as a Laboratory Reagent)
By Professor David Cash
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This file contains the following sections:

Note that Part 1 and Part 2 were originally intended to be published separately. For this reason, the Reference Sources of the two parts are numbered and listed separately.

Part 1

Beer's Law Experiments with Red #40 Food Dye
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The food dye Red #40 is a safe and convenient subject for a Beer's Law exercise.
Cherry Kool-Aid® provides an inexpensive source of the dye for classroom use. This dye is often used alone to provide a pink or red colour in commercial products that also may be used as unknowns. Part 1 provides ideas and methods for a variety of experiments.
The Beer's Law equation including an authoritative value of the absorptivity of Red #40 is given. A question set resource is provided as Part 2.

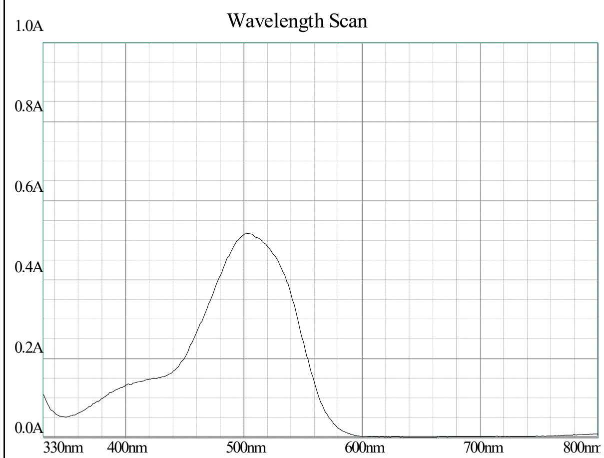
D. Cash 2005

1. Introduction

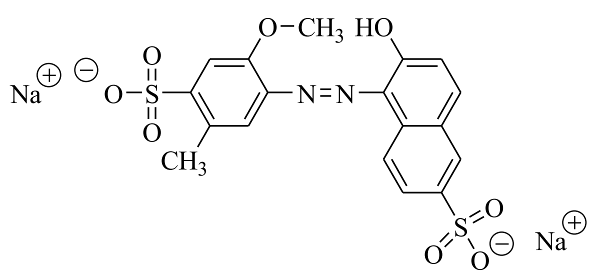
A visible wavelength spectrophotometer is a good choice for a first introduction to quantitative instrumental chemical analysis. Chemistry students can quickly learn to use this instrument. After doing such an experiment, they should be able to demonstrate an understanding of the basics of quantitative spectrophotometry: the percent transmission of light; the definition of absorbance; the spectrum convention that an absorbance band is displayed as peaking upwards from the baseline of zero absorbance; and the use of Beer's Law at a single wavelength for the estimation of the concentration of an analyte.

The azo dye FD&C Red #40 is an excellent choice of subject for a first experiment. First produced by Allied Chemical and known also as Allura Red AC, this water-soluble sodium salt absorbs intensely in the blue region of the visible spectrum. It is widely used as an FDA/Health Canada approved food dye additive. It is the only approved dye that is sometimes used alone in consumer products. Information about the water-soluble sodium salt form of this substance may be found in Wikipedia (1).

Visible Wavelength Spectrum of Food Grade Red #40 (Concentration = 8.5 mg/L)



Single wavelength spectrophotometry at the peak wavelength of absorbance (501 nm – 504 nm) is used in the food industries for quality control, process control, and analysis of unknowns containing Red #40. The spectral scans in this document were obtained using a NovaSpec Plus Diode Array Spectrophotometer, at visible wavelengths in a 1.0 cm cell. The venerable Spectronic 20 analog instrument in the image on page 2 and its comperes were retired from use in the first-year laboratory in 2005, replaced by Spectronic 20D digital instruments. See Part 2 “Information and Questions for Students” for background information about the commercial production and uses of food grade Red #40.

Name and Formula	Chemical Structure
Sodium Salt Form of Allura Red AC **AKA FD&C Red #40** **$C_{18}H_{14}N_2Na_2O_8S_2$** **496.4 g/mol**	 **D. Cash – ChemDraw Structure**

2. Why Choose FD&C Red #40 for Spectrophotometry Experiments

This substance is a very good choice for introductory spectrophotometry exercises:

1. **Safety**. This allowed food additive is non-toxic.
   The main hazard is staining of skin or clothing.
2. **Disposal**. It is safe to wash the small volumes of residues down any sink.
3. **Low Cost and Wide Application**. In many products Red #40 is the only dyestuff present. The amount present ranges from very low concentrations in some synthetic beverages and cough drops, to moderate amounts in products such as mouthwashes and cough syrups, to much higher amounts in a product such as Cherry Kool-Aid Jammers (2), and very much higher amounts in Cherry Kool-Aid Liquid Concentrate (3). The amount of dye required for a class experiment is extremely small.
4. **Options**. There are many types of experiments possible, from simple to complex.

3. Some Suggested Spectrophotometry Experiments with FD&C Red #40

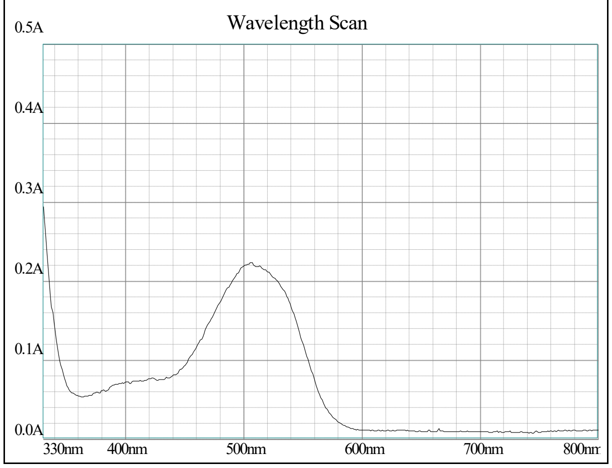
There are various types of experiments from which to choose depending on your needs. An authoritative source (4) states that wavelength of maximum absorbance (λ_{max}) is 501 nm and that the absorptivity at that wavelength is 54.0 L / g × cm. The analytical wavelength is 501 nm. The experimental methods here are brief summaries. Students will require more detailed instructions. For work using concentrations in mol / L and related units, a conversion of the value of the absorptivity is required.

$$\mathbf{54.0\ L / g \times cm} = (54.0\ L / g \times cm) \times (496.4\ g / mol) = \mathbf{26{,}800\ L / mol \times cm}$$

Measurement-Ready Samples

A very simple experiment employs a solution that can be placed directly into the spectrophotometer sample tube for an absorbance measurement.
Look in a grocery store for a synthetic drink product that is transparent and very pale pink in colour. It is ideal if the ingredient Red #40 (Allura Red) is listed alone as a colouring agent. Degas the drink if it is carbonated.

Simply Kids Vitamin Enriched Water (Undiluted From Plastic Bottle)



A pale pink drink found in a Toronto grocery store in 2005 had a spectral scan consistent with Red #40 (shown at right). The absorbance in a 1.0 cm pathlength cell at 501 nm was about 0.220. The bottle (shown in the image on page 1) had no colour ingredient listed, nor was there any information on the listed web page or in response to a query.

Using the absorptivity of Red #40 and the Beer's Law equation given in the sections below the concentration of Red #40 may be estimated as about 4.1 mg/L in the drink. See Questions 1 and 2 in the Questions for Students in Part 2 for examples of the use of such experimental data. In the absence of a grocery-purchased sample, you can make a sample by appropriately diluting Cherry Kool-Aid Jammers. See Section 7 below for more information.

Samples Requiring Dilution
Samples containing higher concentrations of Red #40 require dilution before measurement of an absorbance value. These experiments will require more time, more equipment, and additional laboratory techniques and calculations. Examples would be Cherry Kool-Aid Jammers or a bright-red-coloured mouthwash (5) or cough syrup (6). Check that the ingredient list of your unknown includes Red #40 or Allura Red or that the spectrum is consistent with Red #40. You can choose between giving the students the dilution instructions or having them discover the required dilution as part of the experiment. You can adapt the instructions here to the available equipment.

Experimental Method for Cherry Kool-Aid Jammers
1. Open a Cherry Kool-Aid Jammers pouch. Pour 10 to 15 mL of the drink into a clean beaker. The beaker does not have to be dry. Rinse the walls of the beaker and discard the solution. Repeat. Pour about 50 mL of drink into the beaker.
2. Repeat the rinse and discard procedure with a 10 mL graduated cylinder and a dropper pipet.
3. Fill the cylinder to the 10.0 mL mark with the aid of the dropper pipet.
4. Transfer quantitatively to a clean 100 mL volumetric flask*. Fill to the mark with distilled water. Mix well by making 30 slow inversions.
5. Measure the absorbance of the diluted solution at 501 nm. An average of several repeats should be used and averaged.
6. Use the Beer's Law equation or plot to determine the concentration of the diluted solution in mg/L units.
7. Use the dilution equation to calculate the concentration of Red #40 in the original Kool-Aid Jammers solution in mg/L units. (Dilution Equation: $C_{dil}V_{dil} = C_{conc}V_{conc}$)

*The precision of a volumetric flask is not required. You can use small screw-cap bottles each calibrated with a fill line placed by adding 100 g of distilled water to the bottle. The important part of the method is to use 30 slow inversions to mix the diluted solution completely.

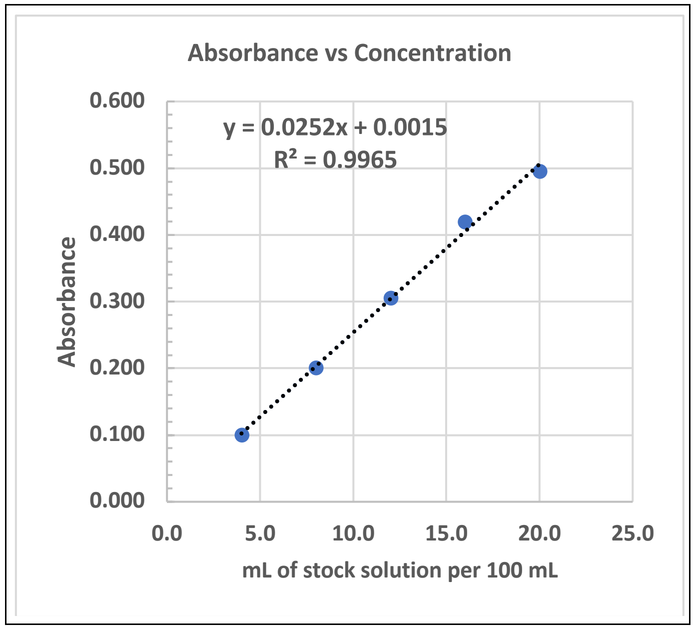
Solid State Samples
Cherry cough drops or hard candies can be used as samples. The analysis results can be used to calculate the mass of Red #40 in a single cough drop. By pre-weighing the cough drop it is possible to also calculate the Red #40 content in parts per million (mg/kg).
You can adapt the instructions here to the available equipment. If your volumetric flasks are larger than 100 mL, use multiple tablets, or find a larger cherry hard candy for the experiment.

Experimental Method for Halls® Menthol-Lyptus Cherry Cough Drops
1. Open a package of Halls® Menthol-Lyptus Cherry Cough Drops (7).
2. (Optional: Determine the mass of one cough drop to the nearest 0.01 g.)
3. Place the cough drop into a clean small beaker. Add about 25 mL of distilled water.
4. Warm gently and carefully swirl the beaker until the cough drop is fully dissolved.
5. Transfer quantitatively to a clean 100 mL volumetric flask. Fill to the mark with distilled water. Mix well by making 30 slow inversions.
6. Measure the absorbance of the diluted solution at 501 nm. An average of several repeats should be used and averaged.
7. Use the Beer's Law equation or plot to determine the concentration of the diluted solution in mg/L units
8. Use the solution volume to calculate the amount of Red #40 present in the cough drop.
9. If the mass of the cough drop has been measured, calculate the concentration of the Red #40 in the drop in mg/kg units (ppm*).

* See Appendix 2 on page for explanations of the three possible meanings of the term ppm (parts per million).

4. Beer's Law Adherence of Red #40

Quantitative spectrophotometry is most appropriate for analytes that show good adherence to a linear Beer's Law correlation between absorbance and concentration.
Not having any literature information in 2005 when it was decided to use Red #40 in an experiment, it was necessary to demonstrate that the substance did exhibit such behaviour. This work was carried out by Emily Girard, a chemical engineering technology student employed by our department as a support staff technologist during one of her work terms.



We were working with a donated sample of food grade Red #40 and had limited information about the specifications: "food grade Red #40 is 80 % dye by mass". We did have the instructions for the quality control spectrophotometry procedure. From these instructions, Emily was able to devise an experimental method, the results of which are shown in the plot. This procedure became the basis for our Beer's Law experiment.

Experimental Method

1. One litre of stock solution of Red #40 of <u>nominal</u> concentration 980 mg/L (ppm) was prepared by dissolving 1.225 g of food grade Red #40 in a 1 L volumetric flask. This solution is essentially black in colour. This solution was used for many years to supply the dye needed for many classes performing the experiment.
2. The 980 mg/L stock solution was precisely diluted to a working solution of <u>nominal</u> concentration of 49 ppm (10 mL to 200 mL). The working solution was also almost black in colour.
3. The working solution was placed into a 50 mL buret.
4. Five solutions were prepared by placing respectively 4.00 mL, 8.00 mL, 12.00 mL, 16.00 mL, and 20.00 mL into each of five 100 mL volumetric flasks and mixing well (many inversions).
5. The absorbance of each solution was measured in a 1.0 cm pathlength cell at 501 nm. An average of several repeats was used for each solution.
6. The data was plotted as shown and a linear best-fit curve was added.
7. The equation of the trend-line and the R^2 value* were displayed.

* The closer R^2 is to 1.000..., the better the data points are to a straight line.

The mean absorbance value for each of the five solutions are given in Question 5 in the Student Questions in Part 2. The plotted values are shown. From the results, it was possible to conclude that Red #40 dye does adhere very well to Beer's law over the range of absorbance values from about 0.1 to 0.5. This is an excellent range for experimental determinations. It cannot be assumed that this adherence will continue above an absorbance value of 0.5.

The script of the Mohawk College Red #40 experiment may be downloaded (8).
The script of a similar experiment based on a red mouthwash may be downloaded (9).

5. Absorptivity of Red #40 and the Beer's Law Equation

The Beer's Law equation (9) has the form:

$$A = (\text{constant of proportionality}) \times (\text{cell path length}) \times (\text{concentration})$$

Absorbance (A) is a unitless quantity. The cell path length is by convention in cm units, and is usually given the symbol b. The path length is usually 1 cm for most analyses using solutions. The concentration of the absorbing substance will have whatever units are convenient (see below). The concentration is usually given the symbol c. The constant of proportionality may be given the symbol "a" called the absorptivity or given the symbol "ε" called the extinction coefficient. The ACS prefers the term absorptivity here (10).

$$\text{Beer's Law Equation: } A = abc \text{ or } A = \varepsilon bc$$

In 2005 it was not possible to obtains a value for the absorptivity of Red #40. In preparing this article an authoritative value of absorptivity was found in a 2016 document prepared under the auspices of the UN Food and Agricultural Organization and the World Health Organization (4). The absorptivity of Red #40 is given in the FAO/WHO document as 54.0 L / g × cm. The absorptivity value is found at the very end of the document, in a section titled 'Method of Assay'.

6. Units of Concentration and Units of Absorptivity

Units for the concentration of solutions and therefore the corresponding units for absorptivity are chosen according to the purpose of the analysis and the use of the resulting values. These uses fall into two general types. For use in research studies having to do with comparisons of molar absorptivity, equilibrium constants or kinetic rate laws, units based on moles per litre will be used. For all other applied uses, practical units based on mass per unit volume will be used. Examples are listed in the table.

Type of Use	Research	Applied
Examples	comparisons of molar absorptivity; solubility (K_{sp}); acid-base and buffers (K_a, K_b); complex formation (K_{stab}); redox and other equilibria (K_{eq}); Nernst equation and electrode potentials; kinetics and rate law equations	industrial process and quality control; food, drug and cosmetic; environment; agriculture; wastewater; pollution; drinking water; etc.
Base Unit of Concentration	mol / L	g / L
Base Unit of Absorptivity	L / mol × cm	L / g × cm

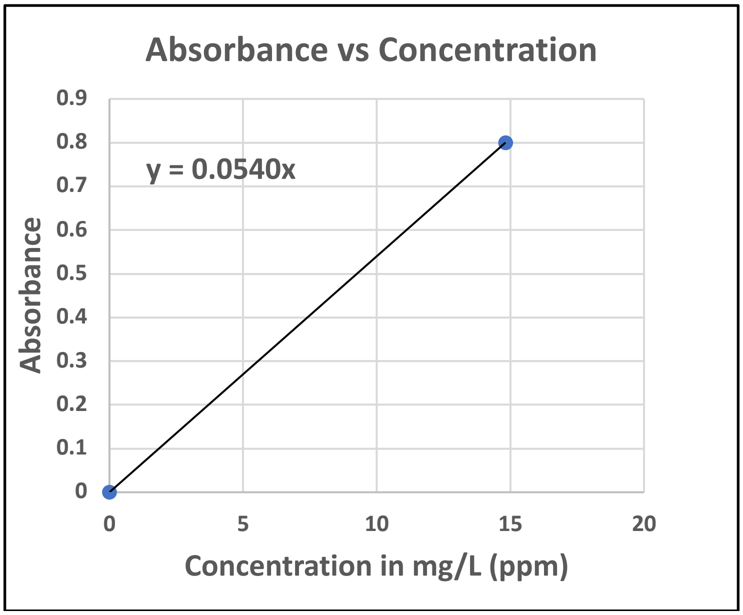
A good general rule is to tabulate values, prepare and display plots, and use plots and Beer's Law equations for numbers that are between at least 0.1 and at most 100. If the useful Beer's Law range of concentrations of the analyte is outside these limits using the base units (e.g. g/L or mol/L), then it is best to shift the values into the required range using SI prefixes. There are exceptions to this rule, such as spectral scans of visible wavelengths, where wavelengths are by conventions given in nm units (e.g. 501 nm) rather than in pm units (e.g. 0.501 pm).

Using Red #40 as a concrete example: Solve the Beer's Law equation for an absorbance value of 0.5 in a cell of 1 cm path length. This will show what the upper limit of concentration will be for analytical measurement.

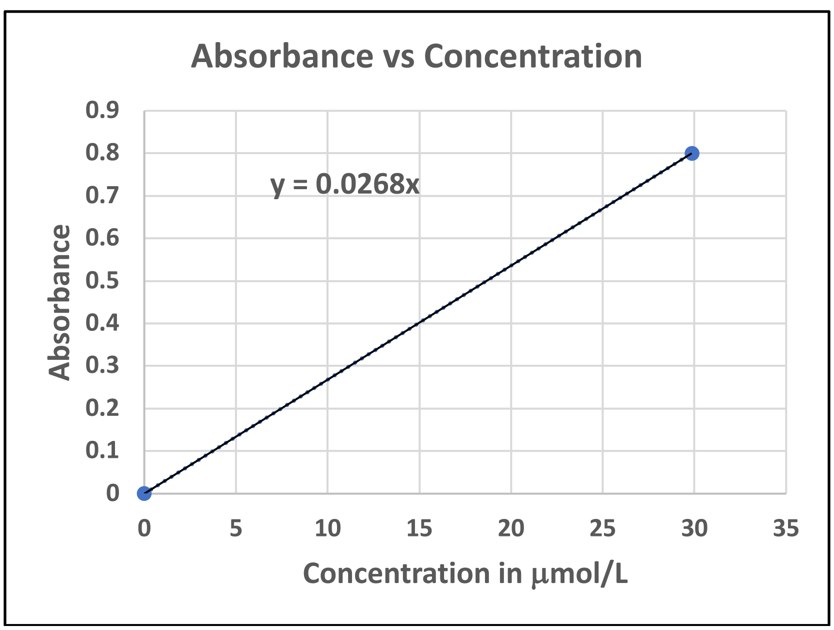
Base Unit mol/L	**Base Unit g/L**
Absorptivity = 26,800 L/mol × cm	Absorptivity = 54.0 L/g × cm
Beer's Law Equation A = (26,800 L/mol × cm) × (b cm) × (c mol/L)	Beer's Law Equation A = (54.0 L/g × cm) × (b cm) × (c g/L)
Value of c in a 1 cm cell for A = 0.5 concentration = 0.0000186 mol/L	Value of c in a 1 cm cell for A = 0.5 concentration = 0.00926 g/L
Working Unit Shift concentrations into μmol/L Absorptivity = 0.0268 L/μmol × cm concentration at A = 0.5 is 18.6 μmol/L	Working Unit Shift concentrations into mg/L Absorptivity = 0.0540 L/mg × cm concentration at A = 0.5 is 9.26 mg/L
Working Beer's Law Equation A = (0.0268 L/μmol × cm) × (1 cm) × (c μmol/L)	Working Beer's Law Equation A = (0.0540 L/mg × cm) × (1 cm) × (c mg/L)

Practical examples of this are shown in the two calibration plots for Red #40 given below.

Working Unit mg/L
Absorptivity = 0.0540 L/mg × cm
concentration at A = 0.5 is 9.26 mg/L



Working Unit μmol/L
Absorptivity = 0.0268 L/μmol × cm
concentration at A = 0.5 is 18.6 μmol/L



7. Using Cherry Kool-Aid as a Laboratory Reagent

Cherry Kool-Aid drink is coloured using a relatively high concentration of Red #40 dye. There are two available forms of this drink, ready-mixed Cherry Jammers or Cherry Liquid Concentrate. Either form may be used to provide Red #40 for class experiments at modest cost. However, there are two possible hazards associated with the use of the liquid concentrate: the very high concentration of Red #40* constitutes a staining hazard to skin and clothing; a very high concentration of citric acid** (or malic acid) constitutes a chemical burn hazard to skin or eyes and a hazard to clothing. Use of the liquid concentrate in class is not recommended.

*An experiment on Cherry Kool-Aid Jammers reported in Question 3 of the Information and Questions of Part 2 found that the concentration of Red #40 in this drink is just above 50 mg/L. If 50 mg/L is the target concentration of Red #40 in Cherry Kool-Aid drinks, then the Liquid Concentrate form of Cherry flavour Kool-Aid will contain about 300 mg per 48 mL container, intended to prepare 6 L of drink. This is 125 times 50 mg/L, or 6250 mg/L. This is well within the solubility limit of Red #40 (11), but at this concentration, the risk of severely staining the skin or clothing because of a mishap should be firmly in mind if you opt to handle the material.

**Experiments have shown that the concentration of citric acid in some flavours of Jammers can be about 0.01 M (12). This means that the concentration of citric acid in the Liquid Concentrate will be about 1.25 M. Many drinks are now using malic acid in addition to or in place of citric acid, but the concentration would be similar. This is well within the solubility limit of both acids (13, 14). Given that citric acid (or malic acid if present) is a relatively strong weak acid, this represents a serious corrosive hazard to skin or eyes. Ingestion of the concentrate would be dangerous.

Reference Sources

1. https://en.wikipedia.org/wiki/Allura_Red_AC.
2. https://www.kraftheinz.com/en-CA/koolaid/products/00066188070815-jammers.
3. https://www.kraftheinz.com/en-CA/koolaid/products/00068100001273-cherry-liquid-drink-mix.
4. https://openknowledge.fao.org/server/api/core/bitstreams/1d90f15f-858c-49bb-b72f-b2789f6269e7/content or https://www.fao.org/3/br639e/br639e.pdf. (see page 4, Methods of Assay).
5. https://smartlabel.pg.com/en-us/00037000768661.html.
6. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=d1657403-b953-4db1-9a86-343e9e769a1a.
7. https://gethalls.ca/en/relief/halls-cherry-flavour-cough-drops.
8. https://www.uclmail.net/users/dn.cash/Spectroscopy1.pdf.
9. https://www.webassign.net/question_assets/ucscgencheml1/lab_9/manual.html.
10. Anal. Chem.,1990, 62, 91.
11. https://pubchem.ncbi.nlm.nih.gov/compound/Allura-Red-AC#section=Solubility.
12. https://www.chemedx.org/activity/koolaid-and-similar-drinks-convenient-laboratory-reagents-weak-acid-strong-base-titration.
13. https://en.wikipedia.org/wiki/Citric_acid.
14. https://en.wikipedia.org/wiki/Malic_acid.

Part 2

Beer's Law Experiments with Red #40 Food Dye
Information and Questions for Students

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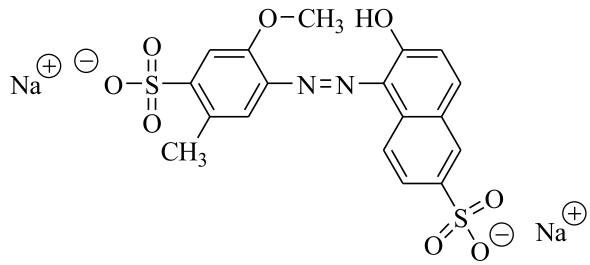
Background Information

According to Pavia, Lampman, and Kriz (1) there were more than 90 dyes regularly used in foods prior to 1906, many of them also used as textile dyes. As scientific knowledge of the hazards has become more precise and government safety regulation more stringent, the number of allowed food dyes has been gradually decreased. In 1938, the number of food dyes allowed in the U.S. was 15, and in 1950 it was 19. At the present time, there are seven FD&C (food, drug, and cosmetic) dyes allowed for food use in the U.S (2). The same seven and one other dye are allowed for food use in Canada. Some other dyes are also allowed in some other countries around the world.

FD&C Red #40 (Allura Red) is one of the FDA/Health Canada allowed food dyes. Information about the commercial production of Red #40 was obtained from Future Market Insights (3). The total worldwide food colours market in 2021 was about 185,000 metric tonnes, of which about 18,000 metric tonnes was Red #40. The production of Red #40 is projected to reach 34,000 metric tonnes in 2032. The US dollar value is expected to climb from $140 million to $245 million over that time. The markets are for the soluble dyes (e.g. – the sodium salt) used in aqueous products, and insoluble "lakes", (e.g. the aluminum salt) used in solid products. There are four grades: food; pharmaceutical; (animal) feed; and industrial.

Markets for Red #40:

- bakery
- snacks and cereals
- candy and confectionary
- dairy
- fruit preparations and fillings
- meat, poultry, fish and eggs
- potatoes, pasta and rice
- sauces, soups and dressings
- seasonings
- pet foods - 2% (4)
- others

Name and Formula	Chemical Structure
Sodium Salt Form of Allura Red AC **AKA FD&C Red #40** **$C_{18}H_{14}N_2Na_2O_8S_2$** **496.4 g/mol**	 **D. Cash – ChemDraw Structure**

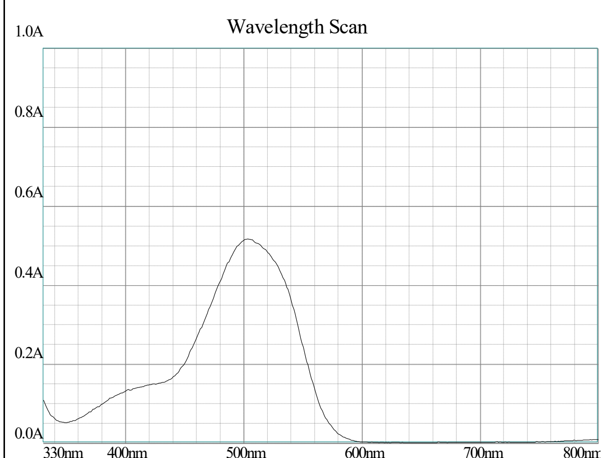
Spectrophotometry of Red #40

The azo dye FD&C* Red #40 was first produced by Allied Chemical and is known also as Allura Red AC (5). This water-soluble sodium salt absorbs intensely in the blue region of the visible spectrum. It is widely used as an FDA/Health Canada approved food dye additive.

* FD&C indicates a dye allowed for use in human foods, drugs and cosmetics.

Spectrophotometry at the peak wavelength of absorbance (501 nm – 504 nm) is used in the food industries for quality control, process control, and analysis of unknowns containing Red #40.

Visible Wavelength Spectrum of Food Grade Red #40 (Concentration = 8.5 mg/L)



The spectral scans in this document were obtained using a NovaSpec Plus Diode Array Spectrophotometer, at visible wavelengths in a 1.0 cm cell. Notice that as the dye absorbs light moving to an absorbance peak, the scan trace moves up from the baseline of zero absorbance.

Quantitative spectroscopy in the visible region applies the Beer's Law equation and usually also the dilution equation. For solutions of Red #40, the required equations are:

$$\mathbf{A = (0.0540\ L/mg) \times (1\ cm) \times (conc.\ mg/L)} \qquad \mathbf{C_{dil}V_{dil} = C_{conc}V_{conc}}$$

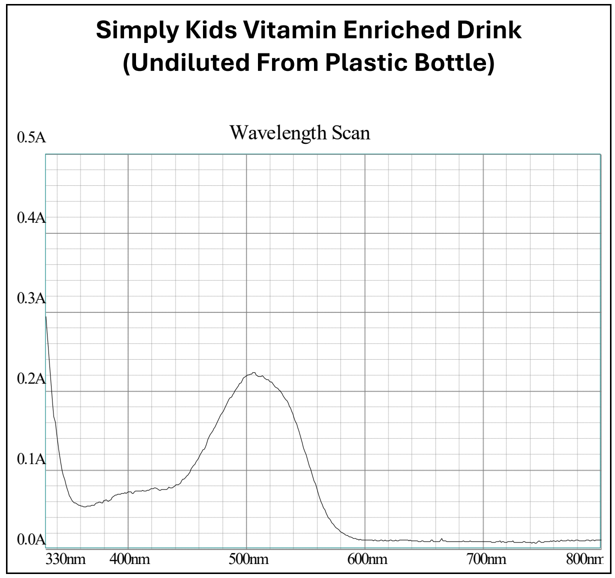
$$\textbf{Concentration (mg/L)} = \frac{\textbf{Absorbance}}{(\mathbf{0.0540\ L/mg \times cm}) \times (\mathbf{1\ cm})}$$

The value of the Beer's Law absorptivity constant used here was reported in a 2016 report to the WHO and FAO agencies of the United Nations (6). Prior to that date, there was no authoritative value of this constant generally available.

D. Cash 2005

Questions

1. a. A pale pink drink (image on previous page) purchased in a Toronto grocery store in 2005 had a spectral scan consistent with Red #40 (right). There was a web site for this product in 2005, but it is no longer available. The bottle had no colour ingredient listed, nor was there any information on the web page or in response to a query. The absorbance at 501 nm in a 1 cm pathlength cell was 0.220. Use the absorptivity of Red #40 and the Beer's Law equation given above to estimate the concentration of Red #40 in the drink. Answer in mg/L units or ppm. (See Appendix 2 for information about the three meanings of ppm.)

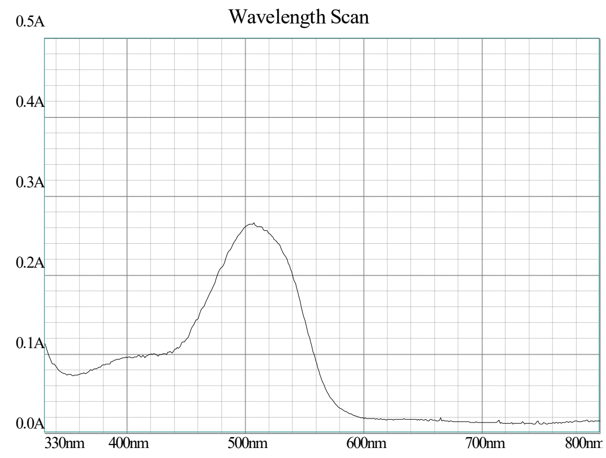


   b. The volume of the drink was listed on the bottle as 250 mL. Calculate the mass of Red #40 contained in the solution in the bottle. Answer in mg units.

   c. A process technologist is preparing a batch of the drink. The tank volume is 15,000 L (7). Calculate the required mass of food grade Red #40. Food grade Red #40 is about 80 % by mass dye. The remainder is inert non-toxic sodium chloride. Calculate the mass of Food Grade dye material required for the batch. Answer in kg units.

   d. How would this amount of dye material be measured out and added to the tank?

2. a. Halls Menthol-Lyptus® Cherry Cough Drops are coloured using Red #40 dye (8). One cough drop was fully dissolved by warming in 30 mL of distilled water. The solution was completely transferred to a 100 mL volumetric flask. The flask was filled to the mark and completely mixed by inverting slowly 30 times. The absorbance of the resulting solution in a 1 cm pathlength cell at 501 nm was 0.262. Use the Beer's Law equation to estimate the concentration of Red #40 in the 100 mL of solution. Answer in mg/L units (ppm).



Halls Menthol-Lyptus Cherry Cough Drop
One Drop Dissolved in 100 mL Solution

b. Calculate the mass of Rd #40 contained in the 100 mL of solution and thus in the cough drop. Answer in mg units.

c. The mass of the cough drop was measured before dissolving as 3.75 g.
Calculate the concentration of the dye in the drop.
Answer in mg/kg units (ppm).

Introduction to Question 3

D. Cash 2024

Kool-Aid Jammers are ready-to-drink pouches of Kool-Aid sold in boxes, each of which contains 10 × 180 mL pouches. The Cherry Jammers list Allura Red (Red #40) as the only food dye ingredient (9). This drink is very dark red, almost black in colour. A dilution by a factor of about 10 lightens the colour to a pale pink.

An experiment was performed in 2013 to determine the concentration of Red #40 in Cherry Jammers. Five boxes of the Jammers were purchased in several Toronto area stores, such that each box had a different lot number and expiry date. Each drink was diluted 10.0 mL to 100.0 mL before measuring the absorbance. Two pouches from each box were tested using a Spectronic 20D instrument set to wavelength 501 nm. Each absorbance was measured a second time with a new sample of the diluted solution. The sample cell had a 1 cm path length. The experimental values are listed in the table on the next page.

Red #40 in Diluted Cherry Kool-Aid Jammers				
Pouch	A_1	A_2	Mean A	Conc (mg/L)
Box A #1	0.294	0.293	0.293_5	
Box A #2	0.294	0.290	0.292	
Box B #1	0.273	0.272	0.271_5	
Box B #2	0.296	0.293	0.294_5	
Box C #1	0.291	0.291	0.291	
Box C #2	0.286	0.288	0.287	
Box D #1	0.279	0.277	0.278	
Box D #2	0.274	0.273	0.273_5	
Box E #1	0.288	0.289	0.289_5	
Box E #2	0.291	0.290	0.291_5	

3. a. Use the Beer's Law equation to calculate the concentration of Red #40 in each of the 10 pouches. Use the mean absorbance given for each pouch.

   b. Calculate the mean (average) value of the concentration. Determine the highest and lowest two values. Calculate the range (highest value minus lowest value). Calculate the percentage value of the range (the range as a percentage of the mean value). This last value gives a rough indication of the precision of the experimental value.

   c. Use the mean value from 3 b and the dilution equation to calculate the concentration of Red #40 in undiluted Cherry Kool-Aid Jammers.

Introduction to Question 4

Kool-Aid is also sold as a liquid concentrate called Kool-Aid Liquid (10). This is packaged in a small squeeze battle containing 48 mL of product, intended to be diluted to a total volume of 6 L (16 portions@375 mL each). The instructions to the user are "take one quick squeeze of liquid, add to a 375 mL volume of water, adjust to taste, mix well and drink".

As well as food dyes, artificial sweetener, and flavourings, Kool-Aid contains citric acid and/or malic. Previous experiments have shown by acid-base titration using sodium hydroxide and phenolphthalein indicator that the equivalent citric acid concentration of several flavours of Kool-Aid Jammers was in the range of 0.0075 to 0.011 M (11).
Cherry Kool-Aid was not titrated due to its intense red colour.


D. Cash 2024

Cherry Kool-Aid Liquid Concentrate Package Information
(One Serving) Give one quick squeeze into 375 mL of water. Adjust to taste. Must dilute before consuming. Contains natural and artificial flavouring and colours, malic and citric acids, sucralose. Volume of contents 48 mL. Makes 16* servings of 375 mL each. (*Previously labelled as 24 servings of 250 mL each.)

4. a. Calculate the total volume of 16 servings of 375 mL.
   If Cherry Kool-Aid contains about 50 mg/L of Red #40, calculate the total amount of Red #40 contained in the 48 mL of liquid concentrate.

   b. Calculate the required concentration of Red #40 in the liquid concentrate.
   Answer in g/L units. Is this high a concentration possible?
   Check the solubility of Red #40 in water (12).

   c. Assume that each 375 mL portion Cherry Kool-Aid is intended to be 0.010 M in citric acid when diluted for drinking. This portion was made from a 3 mL portion of the Liquid Concentrate. Calculate the molarity of citric acid in the Liquid Concentrate.

   d. The molar mass of citric acid is 192.1 g/mol. Calculate the required concentration of citric acid in the concentrate in g/L units. Is this high a concentration possible? Check the solubility of citric acid in water (13).

Introduction to Questions 5 and 6
Before using spectrophotometry as an analytical method for a substance it is necessary to prove that the substance does have an absorbance maximum wavelength and that the absorbance at that wavelength does obey Beer's Law over a suitable range of absorbance values. This task was assigned to Emily Gerard, a student in our chemical engineering technology program at Mohawk College, while she spent a 4-month co-op work term in our teaching laboratories as a support staff technologist.

The absorbance spectrum of Red #40 shown earlier demonstrates an absorbance maximum at about 501 – 504 nm in the blue region of the visible spectrum. We were working with a donated sample of food grade Red #40 and had limited information about the specifications: "food grade Red #40 is 80 % dye by mass". We did have the instructions for the quality control spectrophotometry procedure. From these instructions, Emily was able to devise an experimental method to check for Beer's Law adherance. This procedure became the basis for our Beer's Law experiment.

Emily Girard's Experimental Method

1. **Dissolve 1.225 g of Food Grade Red #40 in 1000.0 mL distilled water**	→	**2.** **Precisely dilute 10.00 mL sample to 200.0 mL volume**	→	**3.** **Place diluted solution in 50 mL buret**
4. **Deliver 5 samples in turn: 4.00 mL; 8.00 mL; 12.00 mL; 16.00 mL; 20.00 mL**	→	**5.** **Precisely dilute each sample in turn to 100.0 mL volume**	→	**6.** **Measure absorbance of each solution in turn at 501 nm wavelength (1 cm cell pathlength)**

The data and results are given in the table.

<table>
<tr><th>Volume (mL)</th><th>A value</th><th>Conc. (mg/L)</th><th colspan="2">Conc. buret solution (mg/L)</th></tr>
<tr><td>4.0</td><td>0.100</td><td></td><td></td><td rowspan="5">Mean = _____ mg/L
Range = _____ to _____ mg/L</td></tr>
<tr><td>8.0</td><td>0.201</td><td></td><td></td></tr>
<tr><td>12.0</td><td>0.305</td><td></td><td></td></tr>
<tr><td>16.0</td><td>0.420</td><td></td><td></td></tr>
<tr><td>20.0</td><td>0.495</td><td></td><td></td></tr>
</table>

5. a. Use the Beer's Law equation to calculate the concentration of Red #40 in each of the 5 diluted solutions (Step 5 above).

   b. For each of the five results of Part 5 a use the dilution equation to calculate a value for the concentration of Red #40 in the solution delivered from the buret. (Step 4 above)

   c. Calculate the mean (average) experimental value of the concentration of the solution in the buret. Determine the highest and lowest two values. Calculate the range (highest value minus lowest value). Calculate the percentage value of the range (the range as a percentage of the mean value). This last value gives a rough indication of the precision of the experimental value.

   d. Use the mean value from 5 c and the dilution equation to calculate the concentration of Red #40 in the undiluted original stock solution (Step 1 above).

   e. Use your answer to 5 d to calculate the percent by mass of Red #40 dye in the sample of food grade dye used for the experiment.

6. Does Red #40 dye obey Beer's Law? Plot your data to find out. There are five data points. Use the absorbance value as the y-coordinate of each point and the concentration value in mg/L as the x-coordinate of each point.

   Can you use a computer app to plot?
   Here are the steps if using Microsoft Excel for iMac:

   a. On a work sheet page: place the five values of concentration in five cells of a vertical column.

   b. Place the corresponding five values of absorbance in a column directly to the right of the concentration values.

   c. Use the cursor to select the block of 10 cells.

   d. Go to the Insert menu – scroll down to Chart – select X-Y Scatter Plot.
      Your plot will appear on the worksheet.

   e. Make sure your chart is selected: Use the 'add chart element' menu at the far upper left – choose trendline – choose linear. A best-fit least-squares straight line is added to the chart. How does it look?

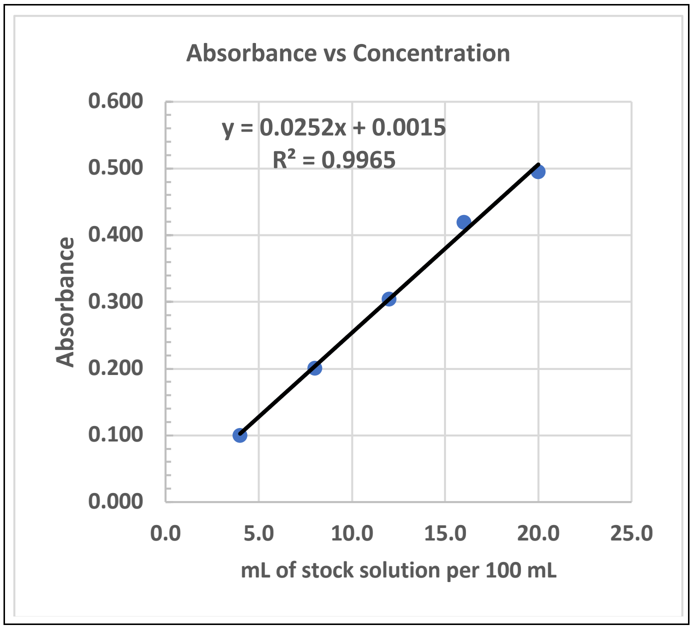
   f. Go once again to the Add Chart Element Trendline and now choose 'more trendline options'.

   g. The Format Trendline panel opens – choose the third option – format trendline. Tick the boxes for display equation and display R^2 value. A value of R^2 close to 1 indicates a linear plot.

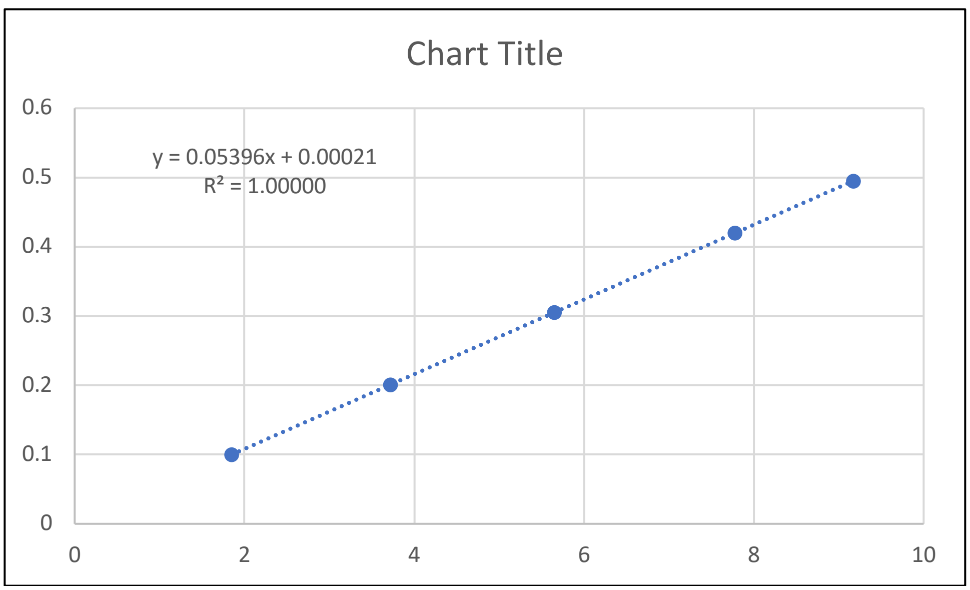
See Appendix 1 on the next page to check how this plot should look.

Appendix 1. Plots

Emily Girard's Plot



Your Plot Should Look Like This



Appendix 2. Concentration in ppm Units

The use of the unit parts per million (ppm) as an expression of concentration may lead to error because this is an ambiguous term. There are three common uses of the term ppm which may be confused by the unwary. A similar difficulty occurs with percent as a unit of concentration.

It is not always easy to be sure which meaning is intended, but usually the context will make this clear to the audience.

Parts per Million

There are at least three common uses of this concentration unit:

- parts per million mass to mass for solid state mixtures or in general.
- parts per million mass to volume for dilute aqueous solutions.
- parts per million volume to volume for gas phase mixtures.

The three analogous uses of percent which may cause confusion are:

- percent mass / mass (% w / w).
- percent mass / volume (% w / v).
- percent volume / volume (% v / v).

Parts per Million Mass to Mass

This is the most general use of the term. This concentration unit refers to any mixture in which the amount of one component is conveniently expressed as a ratio of “x” parts by mass of a million total parts by mass. A typical example would be gold (solid) in an ore.

Examples: $\frac{\mu g}{g}$ $\frac{mg}{kg}$ $\frac{g}{tonne}$ **All are referred to as ppm**

Parts per Million Mass to Volume for Dilute Aqueous Solutions

This is a commonly used adaptation of the previous usage, applicable only to dilute solutions in water. The rationale for this usage is that a dilute solution in water has a density close to 1.0 g / mL or 1.0 kg / L or 1.0 tonne / m^3. A typical example would be gold, dissolved as a soluble salt in dilute aqueous solution.

Examples: $\frac{\mu g}{mL}$ $\frac{mg}{L}$ $\frac{g}{m^3}$ **All are referred to as ppm**

As the above two definitions and uses are very similar, no great harm can arise from any confusion.

Parts per Million Volume to Volume

This usage is reserved almost exclusively for gas phase mixtures or perhaps dusts and aerosols. It has most often been used in occupational hygiene and safety.

This concentration unit refers to any mixture in which the amount of one component is conveniently expressed as a ratio of "x" parts by volume of a million total parts by volume. A typical example would be a contaminant in air.

Examples: $\frac{\mu L}{L}$ $\frac{mL}{m^3}$ **All are referred to as ppm**

This third definition is very different from the previous two.

Any error or confusion will result in estimated values which are wrong by a factor of approximately three orders of magnitude (10^3).

An error of this magnitude could be extremely serious in industrial health and safety applications.

Since the number of molecules in equal volumes of gases are ideally equal, ppm for a mixture of gases means that the ratio of molecules present is "x" per million total molecules. An example of ppm in a gas mixture is carbon dioxide in air. A concentration of 400 ppm CO_2 in air means that there are 400 molecules of CO_2 in a million total molecules of air.

Reference Sources

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8. https://gethalls.ca/en/relief/halls-cherry-flavour-cough-drops.
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