

male rats. On the basis of that study, the FDA banned all uses of Red Lake No. 3 and several uses of Red Dye No. 3. You may be old enough to remember that FD&C Red No. 2 met a similar end several years ago, with the curious result that, for a time, there were no red M&Ms candies. As of today, Red Dye No. 3 remains certified for use in foods, drugs and cosmetics. However, food manufacturers in the U.S. have switched to FD&C Red No. 40, establishing it as the most widely used dye in the U.S.

Spectrophotometry

The Beer-Lambert law, shown in eq 1, states:

$$A = abc \quad (1)$$

In eq 1, A is absorbance (unitless), a is the absorptivity ($L \text{ mg}^{-1} \text{ cm}^{-1}$), b is pathlength (cm), and c is concentration (mg L^{-1}). Using this relationship, the concentration of an unknown may be determined by measuring its absorbance.

Note that when A is plotted versus c , a straight line passing through the origin and with a slope of (ab) is obtained. A standard, or calibration, curve relating absorbance to concentration is constructed using a number of standard solutions of known concentration. Such a calibration curve will be used in this experiment.

Procedure

Preparation of Stock Solution

Obtain a sample of Red Dye No. 40 from the storeroom. Weigh the entire sample of Red Dye No. 40 on an analytical balance and record the mass. Quantitatively transfer the sample to a 500 mL volumetric flask. Fill the flask to the line and mix well.

Preparation of Standard Solutions

Label four beakers (of at least 100 mL capacity) as 1, 5, 10, and 15.

Prepare the first of the standard solutions by pipetting 1.00 mL of the stock solution into a 100 mL volumetric flask. Dilute to the mark and mix well. Transfer this solution to the container marked "1", and save it for later.

Repeat this procedure using 5.00 mL, 10.00 mL and 15.00 mL samples of the stock solution to prepare the remaining three standard solutions. The standard solutions will be used to generate the calibration curve and to determine λ_{max} . For convenience, the standard solutions will be referred to as solution 1, solution 5, etc. The concentrations (in mg/L) of these stock solutions must be calculated.

Determination of λ_{max}

The wavelength at which a sample absorbs most strongly, i.e., at which the absorbance is largest, is defined as λ_{max} . Whatever their concentration, all samples of the same substance have the same value of λ_{max} . (The amount of light absorbed may vary, but the wavelength at which the maximum amount of light is absorbed remains the same.) In order to determine λ_{max} for FD&C Red No. 40, you will use solution 10, though any of the other standard solutions would also work.

Make sure that you use the same spectrophotometer for all measurements. Wipe the outside of the cuvette free of fingerprints and drops of liquid each time before inserting the cuvette into the instrument.