ANALYSIS OF MOUTHWASH

LAB

From Juniata College, Science in Motion

INTRODUCTION

This series of experiments is designed to show how a variety of instruments and techniques are used to solve a problem. Commercial mouthwashes are mixtures of water, alcohol, dyes, and other components. By combining several techniques, it is possible to separate and identify or quantify some of these components.

PURPOSE

The purpose of this experiment is to study a commercial product with a variety of techniques. In the course of the experiment, dyes will be extracted, separated, and identified. Additionally, the alcohol content of the mouthwash will be determined.

EQUIPMENT/MATERIALS

- mouthwash samples
- distilled water
- three-way stopcock
- test tubes
- 10 mL graduated cylinder
- 100 mL volumetric flasks
- pipet or buret
- syringe for gas chromatograph
- spec-20
- isopropyl alcohol (99%, 40%, 20%, 10%)
- C-18 tube (Prep-Sep)
- syringe
- test tube rack
- cuvettes
- ethanol standards (5%, 10%, 20%, 30%)
- gas chromatograph
- hot plate

SAFETY

- An apron and goggles should always be worn in the laboratory.

PROCEDURE

Part I: Separation of the Dyes

1. The sample should be heated gently on a hotplate to remove the alcohol and intensify the dyes. The volume should be reduced to about 1/3 of the original volume. For example, if a 5 mL sample is desired, you should begin with 15 mL of the selected mouthwash. The sample should cool before being used.

2. The C-18 tube should be conditioned with 5 mL of isopropyl alcohol followed by 5 mL of distilled water. These elutants may be discarded. Leave a small amount of water on top of the column if it will not be used immediately.

3. Place 5 test tubes in a test tube rack. As samples are drawn through the C-18 column, they will be placed in the test tubes.

   The following steps are used to place the sample on the column and complete the separation:

- Place a 2 mL sample of the concentrated mouthwash (prepared above) in the C-18 tube. Slowly draw the sample onto the column. Place the elutant in test tube number 1.
- Place 5 mL of water on the C-18 tube and draw through slowly. This elutant is placed in test tube number 2.
- Place 5 mL of 10% isopropyl alcohol in the C-18 tube. Slowly draw the sample onto the column. Place the elutant in test tube number 3.
- Place 5 mL of 20% isopropyl alcohol in the C-18 tube. Slowly draw the sample onto the column. Place the elutant in test tube number 4.

- Place 5 mL of 40% isopropyl alcohol in the C-18 tube. Slowly draw the sample onto the column. Place the elutant in test tube number 5.

4. If you see a distinct color change in the 3-way stopcock or syringe, switch test tubes. If all the color in a particular fraction is not removed from the column, a small amount of additional elutant may be used. Rinse the C-18 column with 99% isopropyl alcohol until the column is clean.

Part II: Dye Identification

1. Plug in the Spec 20 and allow it to warm up for 15 minutes.

2. Select two cuvettes. Fill one with distilled water to serve as a blank. Fill the other with a dye sample.

3. Obtain an absorbance value every 25 nm from 400 to 650 nm. Remember to rezero the Spec 20 at each wavelength. You may wish to go back and take additional readings every 5 to 10 nm in areas of greatest absorbance for a particular dye. Some dyes may have more than one absorbance peak.

4. Repeat for the other dyes extracted from the mouthwash.
   Plot absorbance values as a function of wavelength. Determine the wavelength of maximum absorbance and use the chart below to determine the identity of the dye(s).

This is a partial list of FD & C dyes from the *Handbook of U. S. Colorants, Foods, Drugs, Cosmetics and Medical Devices.*

Wavelength of Maximum Absorbance of Dyes

FD & C Dye	Wavelength, nm
Blue No. 1	630
Blue No. 2	610
Green No. 3	625
Red No. 3	527
Red No. 40	502
Yellow No. 5	428
Yellow No. 6	484
Yellow No. 10	413

Part III: Percent Alcohol Determination

1. Make sure that the gas chromatograph is ready for use.

2. Prepare standard dilutions of ethanol (95%) according to the following list. Each lab group should prepare solutions as assigned by the instructor. The alcohol is added to enough water to make 100 mL of diluted alcohol solution.

Solution	% vol. alcohol	vol. 95% alcohol
A	5 %	5.26 mL
B	10 %	10.53 mL
C	20 %	21.05 mL
D	30 %	31.58 mL

3. Inject 5 microliter samples of the standards and the original mouthwash sample into the gas chromatograph.

4. Determine the area under each peak by using an integrating plotter or by carefully cutting out each peak (be sure to label) and weighing.

5. For each sample, calculate the area due to water and alcohol. Also calculate the total area and the % area due to alcohol.
6. Plot percent area due to alcohol (y-axis) vs. percent alcohol (x-axis). Draw a best-fit line. Use this calibration graph to determine the percent alcohol in the original mouthwash sample.

ANALYSIS OF MOUTHWASH

DATA

Brand of Mouthwash Studied _____

Part II: Dye Identification

Wavelength	Absorbance	
	Dye 1	Dye 2
400		
425		
450		
475		
500		
525		
550		
575		
600		
625		
650		

Identity of Dyes: Dye 1 _____

Dye 2 _____

Part III: Percent Alcohol Determination

Solution	% Alcohol	Area - Water	Area - Alcohol	Total Area	% Area due to Alcohol
A	5				
B	10				
C	20				
D	30				
Mouthwash	-				

QUESTIONS

1. What information about your mouthwash is provided by each of the following?
   A. Spectrophotometer
   B. Gas Chromatograph
   C. Prep-Sep

2. Why were you instructed to switch test tubes if a distinct color change was observed in the stopcock or syringe?

3. Why were both a spectrophotometer and a prep-sep used in the analysis of the dyes?