Experiment. Alcohol analysis of Mouthwash by Gas Chromatograph

Objective: Mouthwash contains ethanol. The amount of alcohol in several commercial mouthwashes will be determine by gas chromatograph analysis. The result of these analyses will be used to determine the identity of two mouthwash unknowns.

Equipment		Chemicals	
100-mL Vol Flask	Micro pipets, misc. pipets	95% Ethanol	Mouthwash
GC sample vials	Varian 3900 GC	Deionized Water	

Safety and Waste Disposal

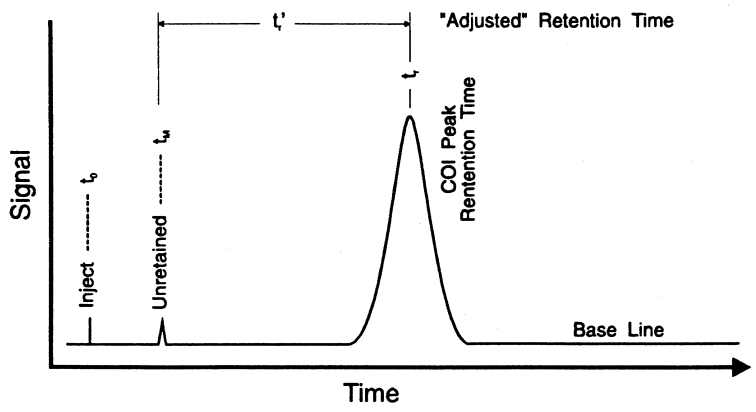
An apron and goggles should be worn in the laboratory at all times.
The chemicals used in this experiment should pose no significant safety hazards.
Good laboratory procedure should be followed at all times.

Discussion:

Gas chromatography is a technique used for separating volatile substances from one another in a gaseous mixture. A sample containing the materials to be separated is injected into the gas chromatograph. A carrier gas (helium) moves through a column that contains a wall coating or granular solid packed into it. The carrier gas is called the mobile phase – this contains the sample. The column is the stationary phase. As the mobile phase flows through the column, the components of the sample come in contact with the stationary phase. If the various components of the sample have different affinities for the stationary phase, separation will occur. The greater the attraction for the stationary phase, the longer a mixture component will take to exit (elute) from the column. A detector monitors the mobile phase exiting the column, and sends a signal to a recording device, which records the time that the sample components elute from the column.

GC analysis produces a chromatogram where each material is displayed by a peak. The figure below displays a typical chromatogram:

t_o = time of injection
t_r = retention time – time measured from injection to peak maximum
t_m = mobile phase time – time from injection to appearance of air peak; time needed for mobile phase to pass through the column
t_r' = adjusted retention time – t_r minus t_m; time component stays in the stationary phase



Chromatogram with Essential Elements Defined

The process of gas chromatography can be used for both qualitative and quantitative analysis. Comparison of retention times can be used to help identify materials in the sample (qualitative analysis). By comparing retention times of peaks in a sample to retention times for pure standards, it is possible to make tentative identifications – however, unless the detector is specific, identical retention times cannot confirm identities of materials in a sample. By measuring the areas under the individual peaks in a sample component and comparing each area to the total, it is possible to determine concentration (quantitative analysis).

Commercial mouthwashes are mixtures of water, alcohol, dyes, flavorings, and other compounds. This experiment is to determine the alcohol content of several commercially available mouthwashes. To complete this analysis, chromatograms will be recorded for a series of solution with known alcohol content. The concentration of the alcohol for the standards will be corrected because volumes of alcohol and water are not additive. Addition of 50-mL of water to 50-mL of alcohol leads to less than 100-mL of solution. This reduction in volume is due to the attractive forces between the alcohol and water. Since the volumes of alcohol and water are not additive when mixed, a calibration curve must first be prepared. The calibration curve will be prepared by placing a known amount of alcohol in an alcohol-water mixture. The observed percentage of alcohol will be plotted against the true percentage of alcohol.

Once the calibration data is collected and plotted, samples of mouthwashes will be analyzed using the gas chromatograph. The observed percentage of alcohol will be determined and the true percentage will be found using the calibration graph. These values may then be compared with the values reported by the manufacturer.

Procedure

Preparation of Standard Alcohol solutions

1. Prepare standard solutions of ethanol according to the following list using 95% ethanol ($C_1V_1 = C_2V_2$). Be careful to make accurate measurements! Pipette the alcohol, and add to a 100mL volumetric flask. Then, carefully fill the flask to the 100mL line with distilled water

% Volume Alcohol	95% alcohol (mL/100mL)
5%	___ mL
10%	___ mL
20%	___ mL
30%	___ mL

2. Take about 500-microL (0.500mL) aliquots of each of these samples and deliver to the 1-mL auto sampler vial for the GC using micro pipets. Note that these samples may have to be diluted by 10-folds if the ethanol signal overloads the detector limit. If this is the case, make the proper calculation and remember to correct for the dilution factor when you calculate your results.

To dilute the samples by 10-folds, take 60-microL of each of the alcohol solution using a micro pipet and add 540-microL of water. Prepare this solution using the 1-mL auto sampler vial.

Preparation of mouthwash samples

3. Your instructor will assign you to groups. Each group will have 90 min to work with the GC. The first group will begin at 5:00 PM to 6:45 PM. The second group will work from 6:45 PM to 8:15 PM. The third group will work from 8:15 PM to 9:45 PM. Each group will need to run the four ethanol standards, and six mouth wash. Each member in the group will also be will assign two unknowns.

4. Prepare a 1:10 dilution sample (unless instructed otherwise by your instructor) of each mouthwash in the 1-mL auto sampler vial.

5. Each known mouthwash will be analyze for its alcohol content and compared to the manufacturer label. The unknowns assigned will be among the six that are analyzed. Part of your grade is to identify the unknown based on alcohol content and chromatogram profile.

Preparation of Varian 3900 gas chromatograph

6. Prepare the Varian 3900 GC as instructed by your instructor. Use the following settings for the chromatograms.
   Initial Temperature: 40°C
   Final Temperature: 140°C
   Ramp 20°/min
   Hold time: 4 minutes
   Detector Limit: 10V
   If another programming sequence yield better separation, feel free to change these settings. The GC column spec will be provided upon request.

6. Place your sample in the auto sampler and program the GC to analyze the sample

7. If any of the solution overloads the detector limit, dilute the sample another 10-fold and repeat the GC analysis for the sample.

Analysis of Results

8. Record the retention time and identify the peaks in the chromatogram. Integrate the area under the peak for each of the sample and prepare a calibration curve using the alcoholic solution prepared in Part 1 described above. The graph will have the area of the alcoholic peak on the x - axis, and the percentage of alcohol on the y - axis. Using Excel LINST, apply regression on your data and interpolate the % alcohol of each mouthwash.

9. Analyze at the chromatogram profile and the % alcohol for your unknown and identify the mouthwash.

Calculations-

Raw Data of GC analysis

	Sample Description	Concentration (% by mass)	Retention time (min)	Area of Peak	Notes on chromatogram profile
1	5% Alcoholic Solution				
2	10% Alcoholic Solution				
3	20% Alcoholic Solution				
4	30% Alcoholic Solution				
5	Mouthwash 1				
6	Mouthwash 2				
7	Mouthwash 3				
8	Mouthwash 4				
9	Mouthwash 5				
10	Mouthwash 6				
11	Unknown #1				
12	Unknown #2				

Sample Result Table

Analysis

Obtain the chromatogram of each solution and analyze the alcohol signal for the retention time and peak area
Identify all signals on the chromatogram when possible.
Using excel, use LINEST to carry out a linear regression of the peak area versus concentration.
Calculate the % concentration of each mouthwash in the vial for the autosampler.
Calculate the corrected % concentration of the alcoholic solution in the mouthwash.
Look at the label of the mouthwash and calculate the % error of your result.
Calculate the molar concentration of the alcohol with the peak area
Analyze the chromatogram of your unknown and identify your unknown.
Calculate the % error of your unknown to the suspected mouthwash

Results –

Include in your summary table the items shown below -

Sample table of result summary:

Equation of Linear Regression				
R^2, correlation				
Identify your mouthwash below with the proper label	Alcohol conc. (M)	% alcohol in mouthwash	Manufacture % Alcohol	% error
Mouthwash 1				
Mouthwash 2				
Mouthwash 3				
Mouthwash 4				
Mouthwash 5				
Mouthwash 6				
Unknown #1				
Unknown #2				

Sample Result Table

Discussion-

The goal of this experiment was to determine the percentage of alcohol in mouthwash. In general, there is approximately 10% - 30% alcohol in mouthwash. Discuss the importance of the alcohol in the mouthwash. You may need to use the Internet to obtain this information. What other common chemicals are found in mouthwash. Are these chemicals observed in the chromatogram in the GC results? Is the result from your experiment consistent with the manufacturer label?

Questions –

1. Why did you prepare a graph using standards with known alcohol contents? What Law did this demonstrate?
2. When the auto sampler was preparing your sample for injection into the GC, what was the purpose of rinsing the syringe several times before injection?
3. How did the accepted values match the percentage of alcohol in your mouthwash samples? Explain your results?

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