Introduction

If one is involved in the large-scale buying of almost any consumable material, the determination of water becomes extremely important. One does not want to pay for a constituent which is available from a faucet! This is particularly true if the product is high-priced even if some water is part of the natural makeup. Methods such as oven-drying or distillation are obviously not very accurate (and may be completely inapplicable) when the sample has a very low water content. "Nonaqueous" solvents either have to be carefully dried, or at least the water content needs to be known. One should remember that, because the formula weight of water is only 18, a solvent containing only 0.02% of water is really an approximately 0.01 M solution of water in this solvent.

This lab involves the measurement of water in a solvent using gas chromatography. To obtain a quantitative value, the method of standard additions is used. This method is a general procedure that is often useful in the determination of a substance present in low concentration. A suitable response, e.g., chromatographic peak area, polarographic wave height, light absorbance, etc. given by the substance of interest in the sample, is measured. The measurements are repeated after the additions of known amounts of the substance to a given volume of the sample. A plot is then made of the signal versus amount of substance added. The plot will cross the y-axis at a non-zero value which represents the signal arising from the substance, alone, before any additions have been made. The (hopefully) linear plot is extended back until it intersects the x-axis. The distance on the x-axis from the x-intercept to the y-axis represents the amount of material in the "neat sample" before any additions have been made (see Figure 1 below).
The method of standard additions is especially useful when the matrix of the sample affects the signal. Essentially a calibration curve is made in the same matrix that the substance finds itself. Separately, an internal standard is also used in this experiment since it is difficult to inject a reproducible volume of sample for each run. Precision of manual injections is usually reported as 2-3 parts per hundred. To get around this problem, an internal standard, in this case methanol, is used. The internal standard is added to the original acetonitrile sample. As water is added to the solution in the method of standard addition, the peak due to water will increase. But, the peak due to methanol will remain the same. Since the injections are not as reproducible as might be desired, the exact size of the water and methanol peaks will not be exactly the same for a given solution, but the ratio of the areas (or heights) of the water to methanol peak should remain constant for the same sample.

Note: Reminder! On the last (in order not to throw off your replications)-remember to increase the integrator chart speed to 4.0 cm/min so as to obtain a longer chromatogram on which to measure the retention distance and peak widths to calculate the number of theoretical plates.

Procedure

Chemicals Required: Bottle of reagent acetonitrile
Bottle of anhydrous methanol

Special Equipment: Gas Chromatograph with thermal conductivity detector (Gow-Mac 69-350) containing two matched packed columns of Chromosorb 101, 6 ft. in length, 0.25 in. outside diameter.
Recorder, 10-inch. Beckman
Integrator, Hewlett-Packard 3390A
Cylinder of Helium with regulator

Available in a kit to be checked out from the stockroom:
1-5-ml volumetric flask with polypropylene stopper
3-Small beakers
1-Pasteur pipet
1-5 µl Hamilton Syringe with Chaney adapter set at 2.5 µl
1-10 µl Hamilton Syringe with Chaney adapter set at 10.0 µl
1-Adjustable Eppendorf pipet to add designated volume of methanol
1-Reusable Eppendorf pipet tip

Always have about 7 psi of Helium carrier gas pressure showing on the regulator and passing through the gas chromatograph. It takes about 3 hours for the gas chromatograph to heat up and equilibrate. Customarily, during the active lab program, the heaters of the gas chromatograph are left on with about 10 psi of helium carrier gas flowing through the instrument but the thermal conductivity bridge current is turned off.
When actually ready to work on this laboratory experiment, increase the helium gas regulator pressure to 40 psi which corresponds to a column flow rate of 20 ml/min. **Please do not touch the secondary flow control knobs on the gas chromatograph since it took some time to precisely adjust these flow rates.** Before turning on the thermal conductivity bridge current, it is absolutely essential that a flow of about 20 ml/min of helium carrier gas is flowing over the detector’s sensing wires. If that is not the case they can be easily burned out!

Check that the following conditions exist (any revised updating conditions will be posted by the instrument): Gas chromatograph: Injector temperature- 150 °C, Column temperature- 120 °C, Detector temperature- 180 °C. Recorder settings: on 10-mv. range. Set the recorder pen on the mid-scale, or a setting of 50 units. Chart speed to 0.5 in/min. Integrator settings: Zero 10, Attenuation -3 (or 0.1 mv. full scale), Chart speed 1.0 cm/in, Peak width 0.16, Threshold -3 and Area reject of 0. (Do a “List List” to verify that the integrator parameters are entered successfully).

The chromatogram of this laboratory experiment will consist of the following peaks in increasing order of time of elution: air, water, methanol (internal standard) and acetonitrile peaks. The water and methanol peaks provide the data needed, and the experimental conditions are adjusted to optimize these data. The acetonitrile comes through at the end as a large tailing peak; it is of no experimental interest except to indicate that everything has passed through the column. The air peak is not retained and occurs because air is introduced during the puncture of the injector’s septa and is an indicator of the dead space of the column. The air peak often is so small that it may not always be detected.

A. Preparation of the Solution.

1. Locate the bottles of acetonitrile and the methanol usually kept near the instrument.

**NOTE: ACETONITRILE IS TOXIC. DISPOSE OF PROPERLY IN THE ACETONITRILE WASTE BOTTLE.**

2. Rinse the 5 ml volumetric flask and its stopper with several small portions of commercial (neat) acetonitrile and then fill about halfway. First check for any posted note if a revised volume of methanol has been specified. The exact, suggested volume may vary depending on the column and acetonitrile sample used. If there is no revision, add 50 µl of methanol, the internal standard, to the 5 ml flask using the Eppendorf pipet provided. Carefully fill the 5 ml volumetric flask to its calibration mark with commercial acetonitrile using the Pasteur pipet provided. Mix well. This solution contains air, water, methanol, and acetonitrile. Keep the volumetric flask stoppered with the polyethylene stopper as much as possible to avoid losses by evaporation!

1. The instructor will demonstrate how to fill the syringe and inject. Rinse the 2.5 µl microsyringe only once. Wipe the exterior of the needle with a Kimwipe® to remove excess sample, being careful not to draw sample from inside the tip by capillary action.

2. Inject and have your laboratory partner simultaneously push the start
button on the integrator.

B. Analysis of Water in Acetonitrile

In subsequent steps, pure sample and then samples to which standard additions have been added will be injected. Note all samples will contain the internal standard, methanol. It is convenient for calculations if the total volume of each standard additions solution (sample plus water addition) be the same; namely 5.00 ml which is the size of the volumetric flask in which the solution is contained. The sample size injected is 2.5 µl and each standard addition of water is 10.0 µl. This means that 10.0 µl (or 4 X 2.5 µl) may be removed from the 5 ml volumetric flask before the next 10.0 µl addition of water. The most successful scheme is to use the first 2.5 µl portion to rinse the syringe and then inject and obtain the chromatograms and the data for the next three 2.5 µl portions. Thus each new solution containing a standard addition of water should start out on the calibration mark of the 5 ml volumetric flask.

1. Solution without the addition of water

When the recorder output shows a steady baseline on the recorder, rinse the syringe once (2.5 µl) and inject the sample (2.5 µl). Do not forget to label the integrator output. This is best done with a felt-tip pen. You need to do two more runs with this solution so 10 µl of the solution has been removed from the volumetric flask.

2. Solutions Containing Standard Additions of Water

After working with the "neat acetonitrile", carefully make at least three 10 µl standards additions of water to the acetonitrile solution. It is suggested that you do this as follows:

a. Rinse and fill the 10 µl syringe (WATER ONLY syringe) with distilled water. Use a Kimwipe® to blot the exterior of the needle to remove excess water. Insert the tip of the needle into the liquid in the 5 ml volumetric flask and deliver the water by completely depressing the plunger. Remove the syringe immediately without moving the plunger.

b. Stopper the flask and mix well by shaking. The volume should now be at the 5.00 ml calibration mark since 10 µl of liquid has been returned to the flask.

c. Using this new solution, rinse the 2.5 µl syringe once with the new solution and then obtain three repetitive chromatograms with the next three 2.5 µl samples. Stop after 3-4 standard additions have been made. Note: Reminder! On the last (in order not to throw off your replications)- remember to increase the integrator chart speed to 4.0 cm/min so as to obtain a longer chromatogram on which to measure the retention distance and peak widths to calculate the number of theoretical plates.

Hopefully a good method of standard addition plot (such as Fig. 1) has been obtained. Since you are still in the laboratory, the solutions are still available and the gas chromatograph is still on, make a rough plot to be sure! (Remember you are plotting the ratio of peak areas of water to methanol versus µl of water added).
Summary of runs:

1) Runs 1-3: neat acetonitrile/methanol
2) Runs 4-6: 10 µl of water added
3) Runs 7-9: 20 µl of water added
4) Runs 10-12: 30 µl of water added
5) Runs 13-15: 40 µl of water added

In your laboratory notebook, record the area of each water and methanol peak. Calculate the ratio of the peak area of water to that of the methanol for each individual run. (See the Calculation Section).

C. Shut Down Procedure

Once that you have decided that your group has a satisfactory standard addition plot, turn the switch to the current of the detector to "off". Then slowly lower the helium regulator pressure from 40 to 10 psi. Please leave all the temperature settings on and notify the instructor that you have finished this experiment.
CALCULATIONS

1. Tabulate for each trial the peak area for each water and methanol peak in the "neat acetonitrile" and in each standard addition.

2. Tabulate the average ratio of peak areas of water to methanol in the "neat acetonitrile" and in each standard addition. If from the group of three values, one value does not agree, while two values are close, it is suggested that you average the close values. In your report, explain your data treatment.

3. Using linear regression, find the regression equation that relates the ratio of the areas of peaks to the volume of water added (on the X-axis). Refer to Figure 1. Report the linear correlation coefficient.

4. As depicted in Figure 1, use the X-intercept to find the volume of water present in the "neat acetonitrile". Report this value on the basis of a volume/volume per cent of water to acetonitrile.

5. Draw a standard additions plot based on your data following the format of Figure 1.

6. Calculate the number of theoretical plates, n, for one representative methanol peak and one representative water peak according to the following two formulas:

   Formula 1  \[ n = 16 \left( \frac{d}{w_1} \right)^2 \]
   where \( d \) = distance between the point of injection and the apex of the peak.
   \( w_1 \) = width of peak at baseline.

   Formula 2  \[ n = 5.54 \left( \frac{d}{w_2} \right)^2 \]
   where \( w_2 \) = width of peak at half height.

   Note, in these equations, the distance and width must all be in the same units, for example millimeters.

7. Which formula would you expect to give the most reliable number of theoretical plates? Explain!