6. Qualitative and quantitative analyses by gas chromatography

[Purpose]

You will learn the principles of gas chromatography through the separation and identification of xylene isomers in a commercial sample and the identification and quantification of an unknown mixed sample.

[Principle]

Figure 1 shows a schematic of a general gas chromatograph. A column filled with an adsorbent (stationary phase) is maintained in a constant temperature. A carrier gas, such as helium or nitrogen gas, flows in the column at a constant rate. A sample introduced into the injection port flows with the carrier gas in the column (mobile phase). Because the components of a sample have different affinities for the adsorbent, they elute from the column at different speeds, which enables the separation of the components. The technique for separating the components of mixtures based on this phenomenon is called chromatography. When the mobile phase is a gas, the technique is called gas chromatography.

There are various methods for detecting the components eluting from the column depending on the purpose. In this experiment, you will use a thermal conductivity detector (TCD), which does not have the highest detection sensitivity but can be used to detect almost all types of substances. When a sample is mixed in the carrier gas, the thermal conductivity of the carrier gas varies. On the basis of this phenomenon, the difference between the thermal conductivities of the gases from the reference column that contains the carrier gas alone and the main column is the output signal from the TCD.

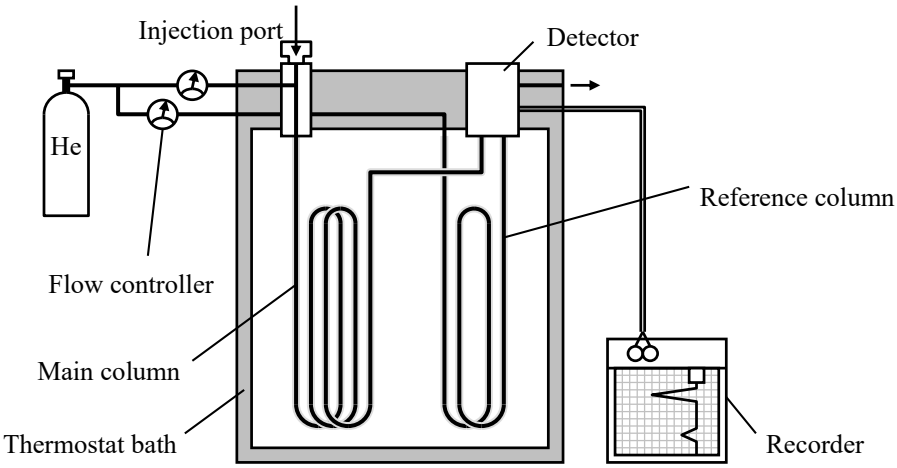


Fig. 1 Schematic of gas chromatograph.

Figure 2 shows examples of signal outputs from the detector. When the column temperature and the flow rate (FR) of the carrier gas are appropriate, a nearly triangular peak is observed for each component, as shown in Fig. 2 (a). The length of time from the injection of a sample to the appearance of each peak (retention time) is almost constant. Therefore, the components of an unknown mixed sample can be identified by comparing their retention times with those of known substances separately measured. Moreover, the area under the peak (peak area) is proportional to the amount of the corresponding component. Hence, clarifying the peak areas for the components of a sample of known concentration

(that is, developing a calibration curve) enables the determination of the concentrations of the components in an unknown sample.

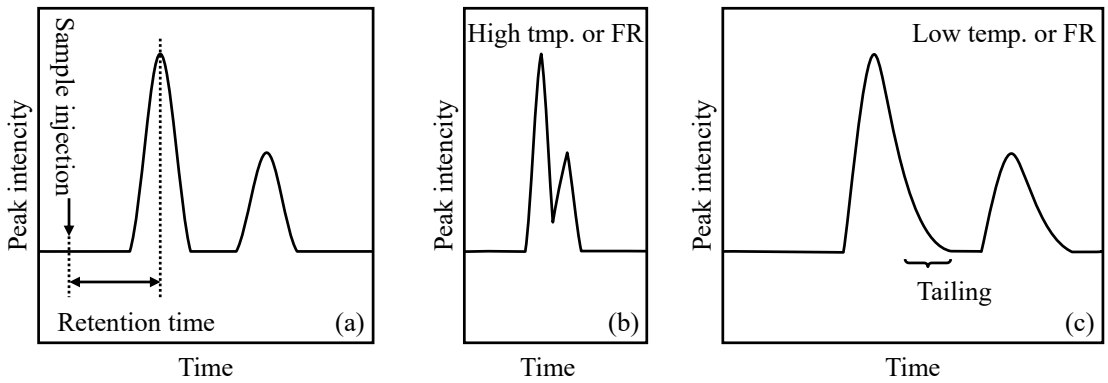


Fig. 2 Examples of gas chromatograms for different column temperatures.

The temperatures of the sample injection port, column, and detector of the chromatograph must be adjusted. A gaseous sample can be injected into the column as it is, whereas a liquid (or solid) sample must be vaporized by heating. Therefore, the sample injection port must be heated sufficiently to vaporize the sample. The detector temperature must be set equal to or 20~30ºC higher than the column temperature to prevent eluants from condensing. When the column temperature is set higher, the duration of the analysis is shortened but the resolution decreases [Fig. 2 (b)]. When the column temperature is set lower, the resolution increases but the measurement duration is prolonged and tailing of peaks occurs, making the accurate determination of the peak area difficult. Therefore, the column temperature must be carefully set depending on the measurement conditions.

[Preparation before experiments]

You will submit the report of four quizzes just before the experiment in Day 1. The quizzes are:

1. Summarize the general features of a chromatography.
2. List the experimental techniques of chromatography by means of the stationary and mobile phases
3. What is a retention time?

[Laboratory apparatus, instruments, and reagents]

《In container》			
Volumetric flasks (5 cm^3)	6,	Microliter syringe (10 μL)	1
Beaker (100 cm^3)	1,	Kimwipes	1
《On laboratory bench》			
Gas chromatograph	1,	Recorder	1
Volumetric pipettes (1 cm^3), (common use)	5,	Safety pipette fillers (common use)	5
Komagome pipettes (5 cm^3), (common use)	2,	PC	
2-propanol solution of Xylene (mixture)	,	*o*- Xylene, *p*- Xylene, *m*- Xylene	
Ethylbenzene	,	2- Propanol	

[Experimental procedures]

Carry out Experiments A on Day 1 and Experiment B on Day 2. Read the manuals of gas chromatograph and microliter syringe carefully before the experiment.

[Warming-up of the gas chromatograph]

The instructor or TA will turn on the gas chromatograph in the morning because signals from the detector require a long time to stabilize. For all experiments in this session, gas chromatography settings are as follows.

Injection temperature (INJ)	: Indicated on cover	He flowing rate	: 50 cm^3min^{-1}
Column temperature (COL)		Detector current	: 100 mA
Detector temperature (DET)		Attenuation	: 4
Column	: Bentone 34 5% + DIDP 5%, Uniport B 80/100		
	Stainless column 4m × ϕ_{ID}3mm		

Figure 3 is a picture of gas chromatograph (Shimadzu GC-8A). You don't need to touch this apparatus for warming-up. Basically, everything is ready for the chromatograph before the experiment. Therefore, you can observe chromatogram by checking the experimental conditions. Write down these conditions in your notebook.

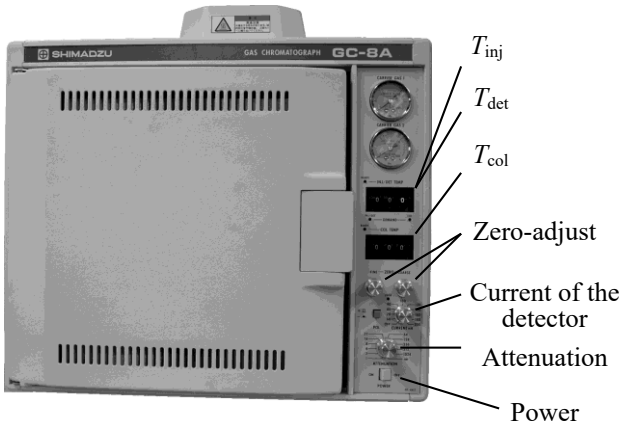


Fig. 3 Shimadzu GC-8A.

1. Check the flowing rate of the carrier gas. The flowing rate is kept around 50 cm^3 min^{-1}. If you find the different value of the rate, contact to the supervisor.
2. Check the Power switch is ON.
3. Check INJ/DET TEMP (T_{inj} and T_{det} in Fig. 3) and COL TEMP (T_{col} in Fig. 3)
4. Check CURRENT is 100 mA.
5. Check ATTENUATION = 4.
6. Start your observation according to the pamphlet, "GC measurement manual", on the table.
7. Confirm the baseline of the output signal is flat.

A. Separation and identification of xylene isomers

1. Verify that the gas chromatograph is ready. (Never change the GC parameters.)
2. Read through "GC measurement manual" and [How to use microliter syringe], then carry out preliminary measurements for 2-propanol before the following experiments.
3. Pour a small amount of 2-propanol solution of xylene (a mixture of *o*-xylene, *m*-xylene, *p*-xylene, and ethylbenzene) into a volumetric flask and obtain a chromatogram of this solution. Ask the staff member to verify the chromatogram before proceeding to the measurement of the next solution.
4. Pour a 2 mL aliquot of *o*-xylene, *m*-xylene, *p*-xylene, or ethylbenzene into a volumetric flask and add 2-propanol to obtain a 5 mL solution.
5. Obtain chromatograms of the four solutions.
6. Referring to "GC measurement manual" and [How to analyze data], identify the components corresponding to the peaks in the chromatogram of the xylene solution (mixture).

B. Identification and quantification of components of unknown mixed sample

1. Verify that the gas chromatograph is ready. (Never change the GC parameters.)
2. Request an unknown sample from the staff member. This sample is a 2-propanol solution that contains two of the following four reagents: *o*-xylene, *m*-xylene, *p*-xylene, or ethylbenzene.
3. Obtain a chromatogram of the unknown sample.
4. Identify the reagents in the unknown sample by referring to the results of Experiment A.
5. Pour 2, 3, and 4 mL aliquots of each reagent identified in step 4 into volumetric flasks and add 2-propanol to obtain a total of six 5 mL solutions.
6. Obtain chromatograms of the six solutions. For the unknown sample, use the results obtained in step B-3.
7. If there is sufficient time, obtain chromatograms of the seven solutions, i.e., the above six solutions and the unknown sample (the second trial). If there is still time left, repeat this procedure (the third trial). Use the average of your results to quantify the components.
8. Develop calibration curves for the concentrations and peak areas of the two reagents and determine their concentrations in the unknown sample.

[How to use microliter syringe]

1. Draw a sample solution into the syringe to the maximum amount and drain it into a beaker. Repeat this operation at least three times to rinse the inside of the microliter syringe with the sample solution.
2. Simply drawing up a solution causes air bubbles to remain in the syringe. To remove the air bubbles, while keeping the needle inserted in the solution, slowly draw up the solution into the syringe and push it out rapidly. Repeating this operation will remove almost all air bubbles. Very small air bubbles can be removed simply by turning the needle tip upward.
3. After all the air bubbles are removed, draw up an aliquot of at least 5 μL of solution into the syringe.
4. At the side of the gas chromatograph, dab a Kimwipe near the root of the needle and drain the solution until the minimum necessary amount (1 μL) remains in the syringe.

5. Insert the syringe into the injection port completely while being careful not to bend the needle.
6. Quickly push the plunger (piston) to inject the solution. Then quickly remove the syringe from the injection port and simultaneously press the Feed button of the recorder to start recording.

[Notes]

1. Carry out preliminary measurements for 2-propanol to familiarize yourself with the operation of a microliter syringe.
2. The needle tip of the microliter syringe is sharp; be careful not to prick your finger.
3. The needle of the microliter syringe is easily bent. Take great care when inserting the needle into the injection port of the gas chromatograph.
4. If the needle of the microliter syringe is bent, ask your instructor or TA to replace the needle.

[Shutdown the chromatograph]

1. When all the observations are finished, set CURRENT = 0.
2. Turn the INJ/DET TEMP and COL TEMP from the initial values to 000. Open the front door of the apparatus. Beware of the high-temperature inside the apparatus.
3. Turn off the POWER switch.
4. Stop the carrier gas.
5. If you find the leaking of the injection port, change the adapter.

[How to analyze data]

A. Retention time

To determine the retention time, measure the length of time from the injection of the sample to the appearance of a peak. However, the time of sample injection into the column cannot accurately be measured. Therefore, assume the time at the start of recording (i.e., when you inject the sample) to be the time of sample injection. With this assumption, the retention time of Component A, τ_A, in Fig. 4 is given by $\tau_A = L_A/v$, where L_A is the length of time from the start of recording to the peak appearance and v is the feed speed.

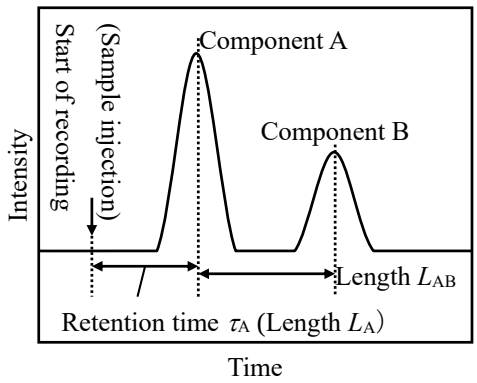


Fig. 4 Determination of retention time.

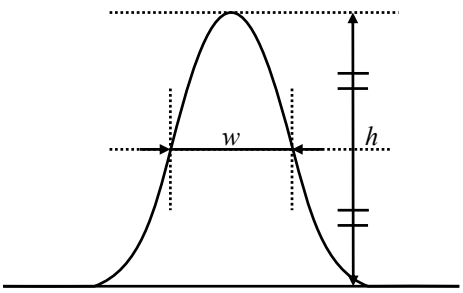
The retention time calculated in this manner may vary depending on the injection conditions (*e.g.*, the length of the needle inserted, sample injection speed, and feed timing). In this case, examine the relative retention time by referring to the position of the peak corresponding to the identified component. For example, when Component A in Fig. 4 has been identified, Component B can be identified as follows. First, obtain a chromatogram of a mixture of Component A and a known substance. Then, compare the interval between their peak positions with L_{AB} in the figure. If the interval is equal to L_{AB}, Component B is considered to be successfully identified.

Although the components of an unknown sample are basically identified by comparing their retention times with those of known substances, different substances do not always have different retention times. Therefore, the identification of the components of an unknown sample may be difficult unless their identities can be roughly determined.

B. Peak area

The peak area for a solute is usually proportional to its concentration in the solution. By clarifying the relationship between the concentration and the peak area (that is, developing a calibration curve), you can calculate the concentration of a component in a solution from the peak area.

Various methods for determining the peak area in chromatograms have been proposed. An automatic calculation using an integrator (or computational integration) has recently become available. In this session, you will use the half-width method because the calculations are simple. First, draw lines on your chromatogram, as shown in Fig. 5, and measure the peak height h and the full width at half maximum (the width of the peak at half of the height) w. The product of h and w is assumed to be the peak area.



(h : peak height, w : FWHM)

Fig. 5 Determination of peak area.

[Checklist]

1. What kind of technique is chromatography?
2. Do you understand the operating principle of a TCD?
3. Why is the relative retention time more suitable for accurate identification than the retention time?
4. Have you determined what interactions occur between the reagents (*o*-xylene, *m*-xylene, *p*-xylene, and ethylbenzene) and the adsorbent used in this experiment during their separation?