# 7. GAS CHROMATOGRAPHY

## Introduction

- Gas chromatography (GC) is a common type of chromatography extensively used in the field of pharmacy and chemistry for separating and analyzing compounds that can be vaporized without decomposition
- Gas chromatography (GC), also called gas-liquid chromatography
- Partition of molecules between gas (mobile phase) and liquid (stationary phase).
- GLC is the most widely used technique for separation of volatile species.

Criteria for compounds to be analyzed by G.C

**1.VOLATILITY**

**2.THERMOSTABILITY**

## Principles of GC analysis

- A sample is vaporized and injected into the chromatographic column
- As the carrier gas sweeps the analyte molecules through the column
- GLC is based on the partition of the analyte between a gaseous mobile phase and a liquid stationary phase
- Polar compounds interact strongly with a polar stationary phase, hence have a longer retention time than non-polar columns
- The mobile phase doesn't interact with the analyte ; its only function is to transport the analyte through column

# INSTRUMENTATION

A. Carrier gas

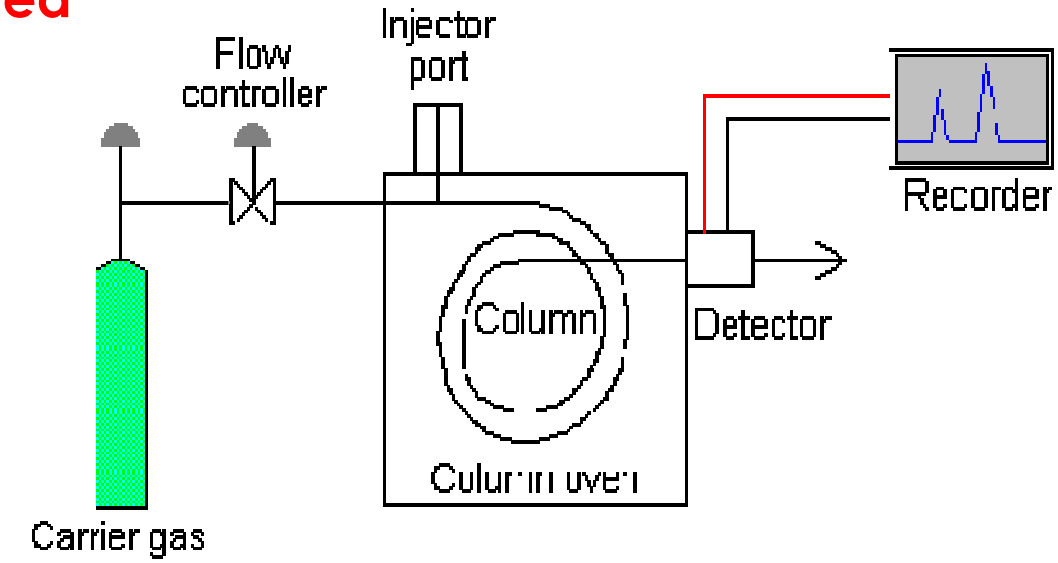
B. Injector

C. Column

D. Detector

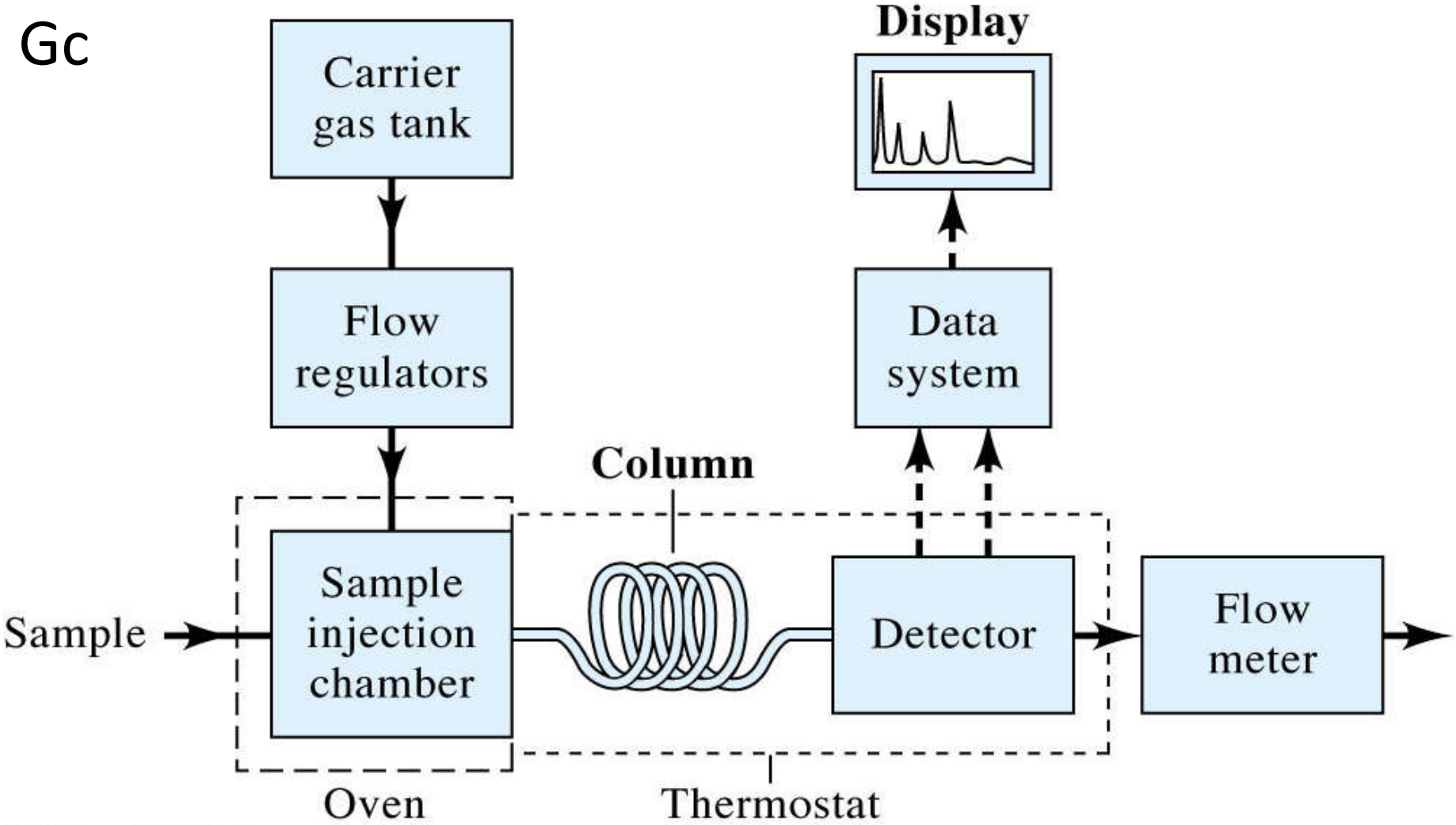
**Thermostated oven**

E. Display system - printer/monitor



# INSTRUMENTATION

Gc




© 2007 Thomson Higher Education

## 1.Carrier gas supply

- It is compressed gas cylinder which supplies carrier gas to the column of GC

**Carrier gas**

- It is the mobile phase in Gc
- It transports analytes through the column
- Most common carrier gas are Helium(He), Nitrogen gas($N_2$), Hydrogen gas($H_2$), Carbon dioxide gas ($CO_2$) and Argon
- GC requires a supply of carrier gas of sufficient quality and pressure to achieve the desired separations

# Carrier gas… cont'd

- The carrier gas should be
  - chemically inert and should not interact with sample and stationary phase
  - readily available, cheap, and of high purity

**2. Sample injector system**:

- mainly used to introduce the sample into the carrier gas flow
- The sample usually in the form of the solution (0.5 µl) is introduced into the chromatography with
  - a micro syringe
  - auto samplers in which the syringe and injection procedure are totally automated

**Functions**

- An inlet for the sample
- To vaporize and mix the sample with the carrier gas before the sample enters the head of the column

## 3. The column

- Part of Gc which contains the SP
- It is may made up of glass or metal
- is where the chromatographic separation of the sample occurs
- The heart of the system
- It is coated with a stationary phase which greatly influences the separation of the compounds.
- The column is enclosed in an oven which may be set at any temperature between ambient and 400 0C
- The columns are packed with particles of a solid support which are coated with the liquid stationary phase= packed column
- The wall of the column is coated with the liquid stationary phase = capillary column

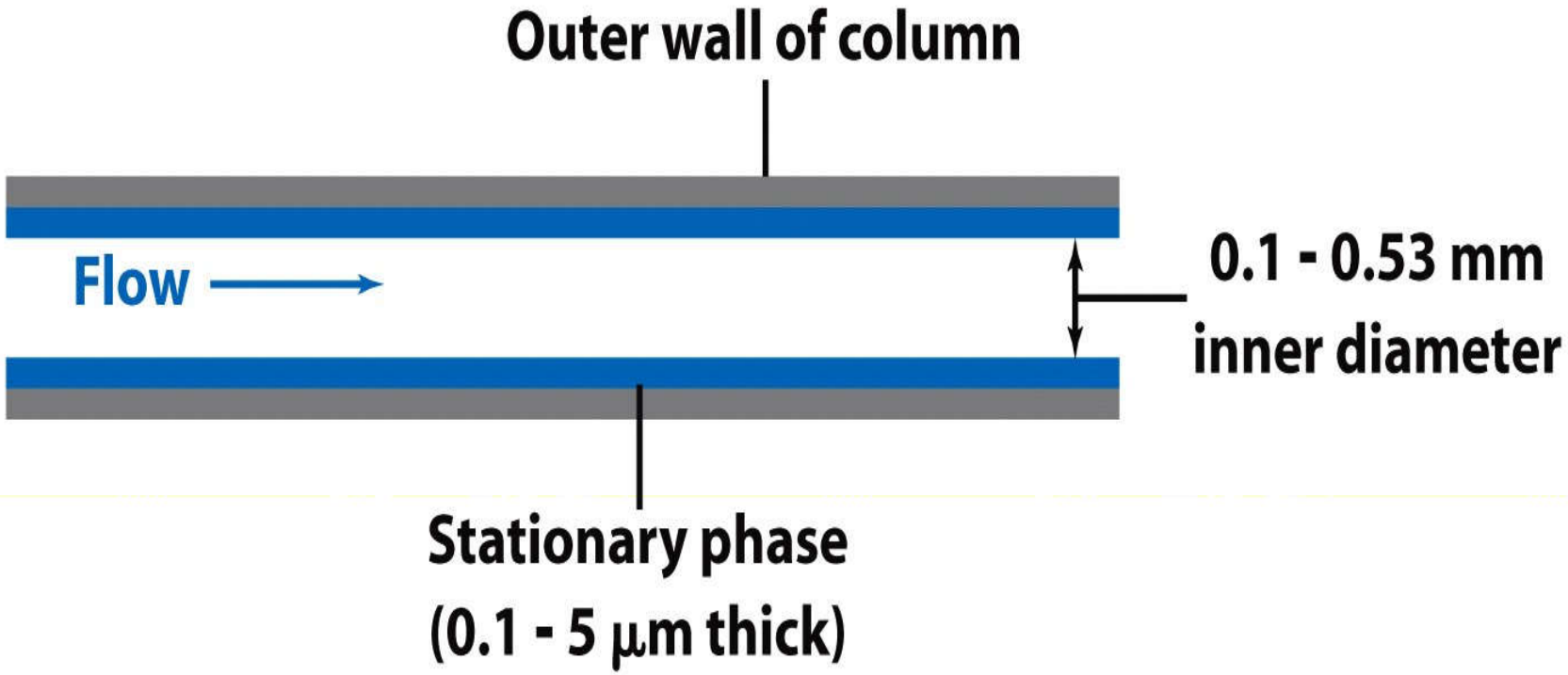
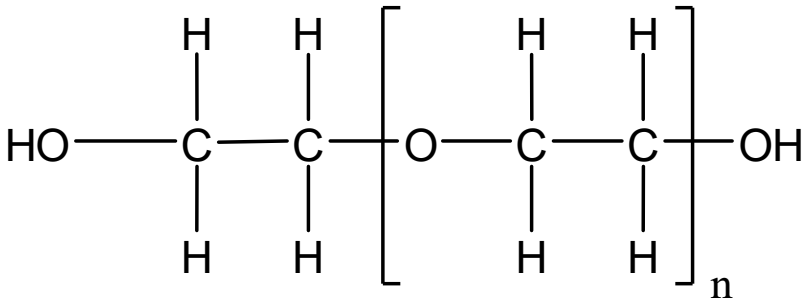



**Figure 24-2a**
***Quantitative Chemical Analysis, Seventh Edition***
© 2007 W. H. Freeman and Company

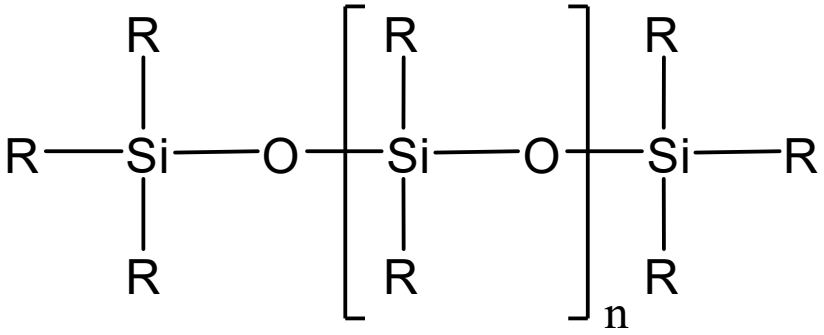
# Stationary Phase

- Liquid which is coated on to a solid support is used as stationary phase
- Many statationary phase are based on Polysiloxanes or polyethylene glycol (PEG)




Polyethylene glycol (PEG) (Polar )

- Polar compounds interact strongly with a polar stationary phase, hence have a longer retention time than non-polar cpds




Polydimethyl siloxane, the R groups are all $CH_3$ (Non-polar)

## *Factors which influence the GC separation*

**Volatility of compound**:

Low boiling (volatile)components will travel faster through the column than high boiling point components.

**Polarity of compounds**:

Polar compounds will move more slowly, especially if the column is polar.

**Column temperature**:

Raising the column temperature speeds up all the compounds in a mixture.

**Column packing polarity**:

Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.

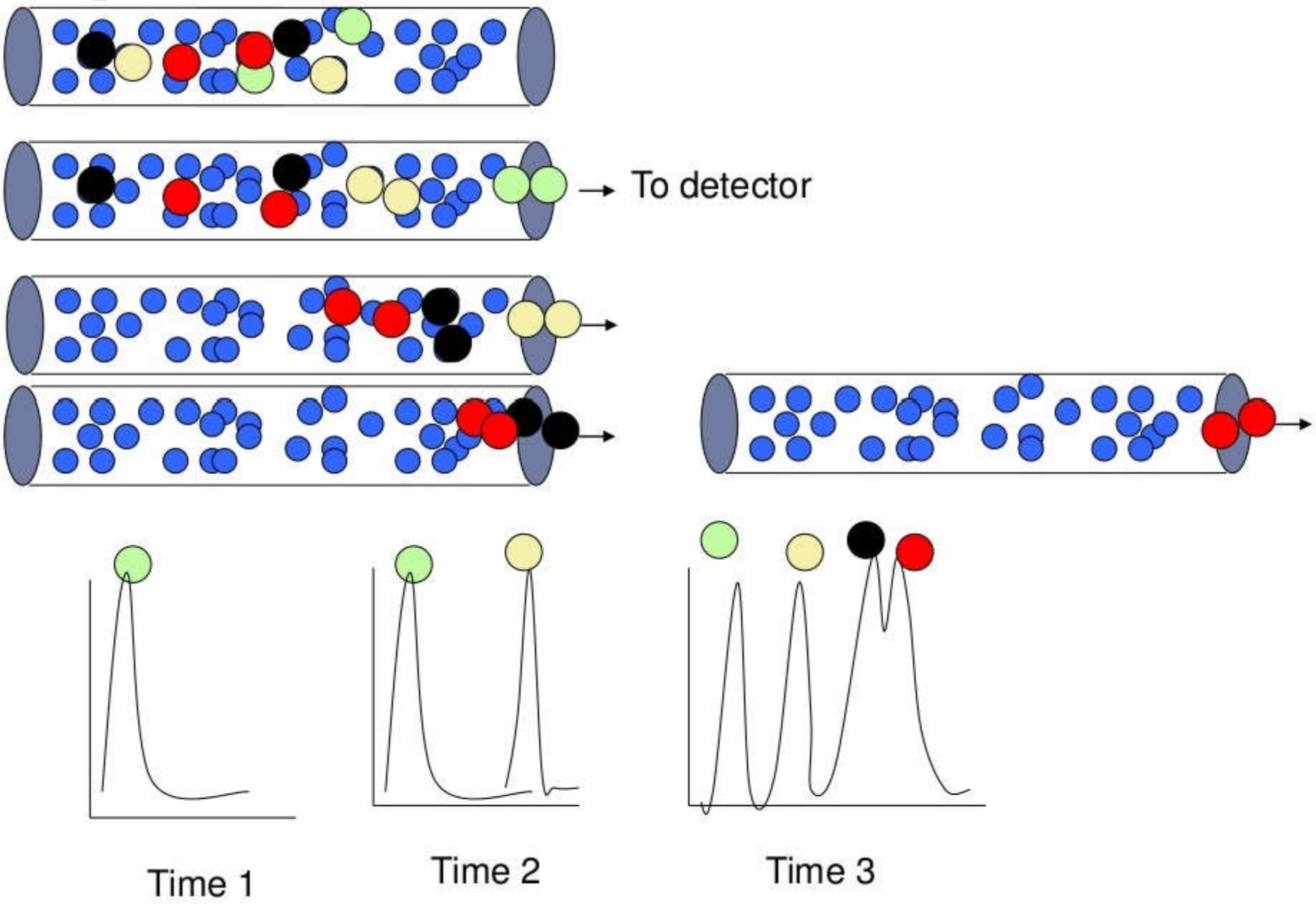
**Flow rate of the gas through the column**:

Speeding up the carrier gas flow increases the speed with which all compounds move through the column.

**Length of the column**:

The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

# Separations



# Temperature Programming in GC

- A technique in which the **column temperature** is **increased** either continuously or in steps as the separation proceeds
- Column temperature is an important variable that must be controlled for precise work
- The optimum column temperature depends upon the boiling point of the sample and the degree of separation required
- Roughly a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time 2 -30 min
- For samples with a broad boiling range, it is often desirable to employe temperature programming

## Temperature Programming in GC

- In general, optimum resolution is associated with minimal temperature
- Low temperature, result in longer elution times hence slower analysis

There are two methods :

**1. Isothermal elution**

- A technique in which the column temperature is constantly maintained throughout the separation
- Temperature of oven remains constant
- Not very useful

## 2. Gradient elution

- Increase the column temperature during the analysis
- Rate of temperature increase
- It allows adequate separation for the analyte that elute early in the analysis, and
- shortening the time it takes for late-eluting analyte to pass through the column

# 4. detectors

- The detector is placed at the exit of the column
- Gc detectors senses the solute vapor in the mobile phase as they emerge from the column
- The solutes emerging from the column interact with the detector and the detector converts this interaction into an electronic signal
- Detectors give response that is dependent on the conc. Of the analyte in the carrier gas
- The magnitude of the signal is plotted versus time and a chromatogram is generated

# Detectors…cont'd

- The following are the ideal requirements of the GC detectors:
  - It should have high sensitivity
  - It should have high stability
  - It should have high reproducibility
  - It should be feasible with wide range of temperature
  - It should be easy to handle

# 4. Detectors

Several types of detectors

- detectors include
  - Thermal conductivity detector (TCD)
  - Flame ionization detector (FID)
  - Nitrogen phosphorus detectors (NPD)
  - Electron capture detector (ECD)

# Sample preparation

1. The prerequisite in GC separation is that all solutes being separated must be: (a) fairly volatile, and (b) thermally stable.
   (c) Usually, the solute should be dissolved in a non-aqueous matrix ($H_2O$ changes column behavior).

2. Lack of volatility prevents the direct use of GC for many solute. One way to overcome this difficulty is to *derivative* the solutes into more volatile forms.

## GC chemical derivatization

- There are many compounds, which cannot be readily analyzed by GC because they are
  - not sufficiently volatile
  - are too strongly attracted to the stationary phase,
  - thermally unstable
- Derivatization converts polar functional groups into less polar derivatives, which are then volatile
- Chemical derivatization prior to analysis is generally done to:
  - increase the volatility and decrease the polarity of compounds
  - reduce thermal degradation of samples by increasing their thermal stability;
  - increase detector response
  - To improve the separation of the solute from other sample components (i.e., changing the structure of a solute will also affect its retention on the column)

- Common derivatization methods can be classified into 4 groups
  - Silylation
  - Acylation
  - Alkylation
  - Esterification

## Silylation

- active H atoms are replaced by a trimetylsilyl group
- The most common reagent used in silylation is trimethylchlorosilane (TMS).
- Chemical groups such as OH, COOH, NH2 are well suited for silylation
- Derivatives are thermally stable, volatile and suitable for GC

## Alkylation

- hydrogen is replaced by an alkyl group

## Acylation

- replaces an active hydrogen atom with an acyl group

# Applications of Gc

- Separation of mixed components in a sample
- Qualitative identification of components
- Quantitative determinations of components in a sample

# Qualitative analysis

- chromatographic data is presented as a graph of detector response against retention time, which is called a chromatogram
- Retention time can be used to identify analytes if the method conditions are constant.
- Gc is widely used as criteria of purity for organic compounds
    - Contaminants, if present, are revealed by the appearance of additional peaks. The areas under these peaks provide rough estimates of contamination

## Quantitative Analysis

- The area under a GC peak is proportional to the amount of analyte present in the chromatogram
- Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analyte, or by determining the relative response factor of an analyte

# Exercise

An analysis was carried out on hyoscine hydrobromide using GC. A one-point calibration was carried out against a calibration standard containing 0.03 mg/100ml hyoscine hydrobromide. Calculate the percentage of stated content in hyoscine hydrobromide tablets using one point calibration curve given and the following data:

- Weight of 20 tablets = 2.15 g
- Weight of tablet powder taken = 0.95 g
- Stated content per tablet =0.6 mg
- Initial extraction volume=200 ml

## Dilution steps

- 10 ml into 100 ml
- 10 ml into 100 ml
- Percentage of w/v of hyoscine in calibration standard=0.03 mg/100ml
- Peak Area of hyoscine sample solution=147881
- Peak Area of hyoscine standard solution=167799