Ministry of higher education and scientific research
AL-Mustaqbal University college
Department of medical physics

Analytical chemistry(practical)

Lecture 6

Chromatography ,Gas chromatographic analysis

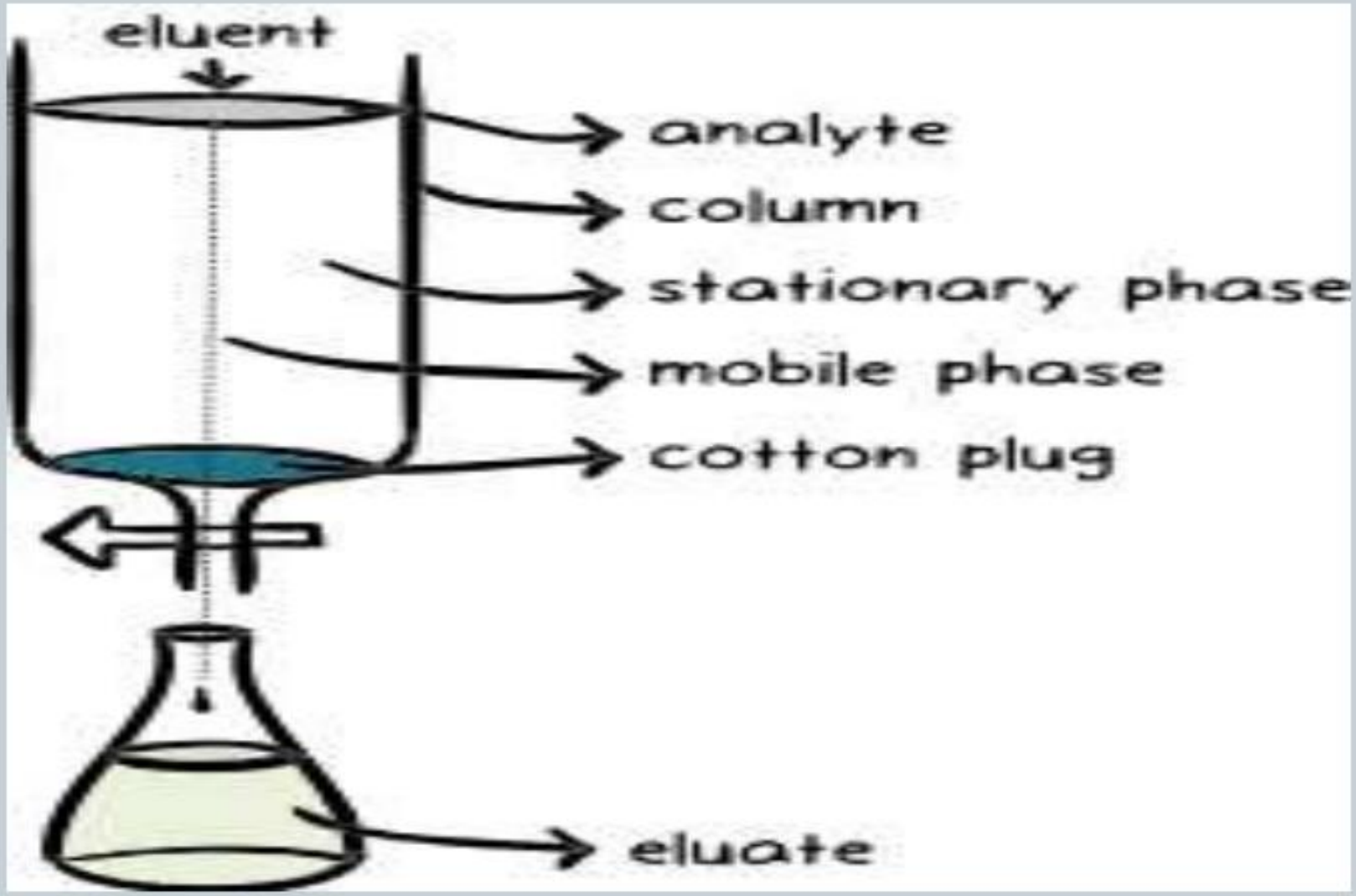
E. Learning by :

MSc. Elham Faisal

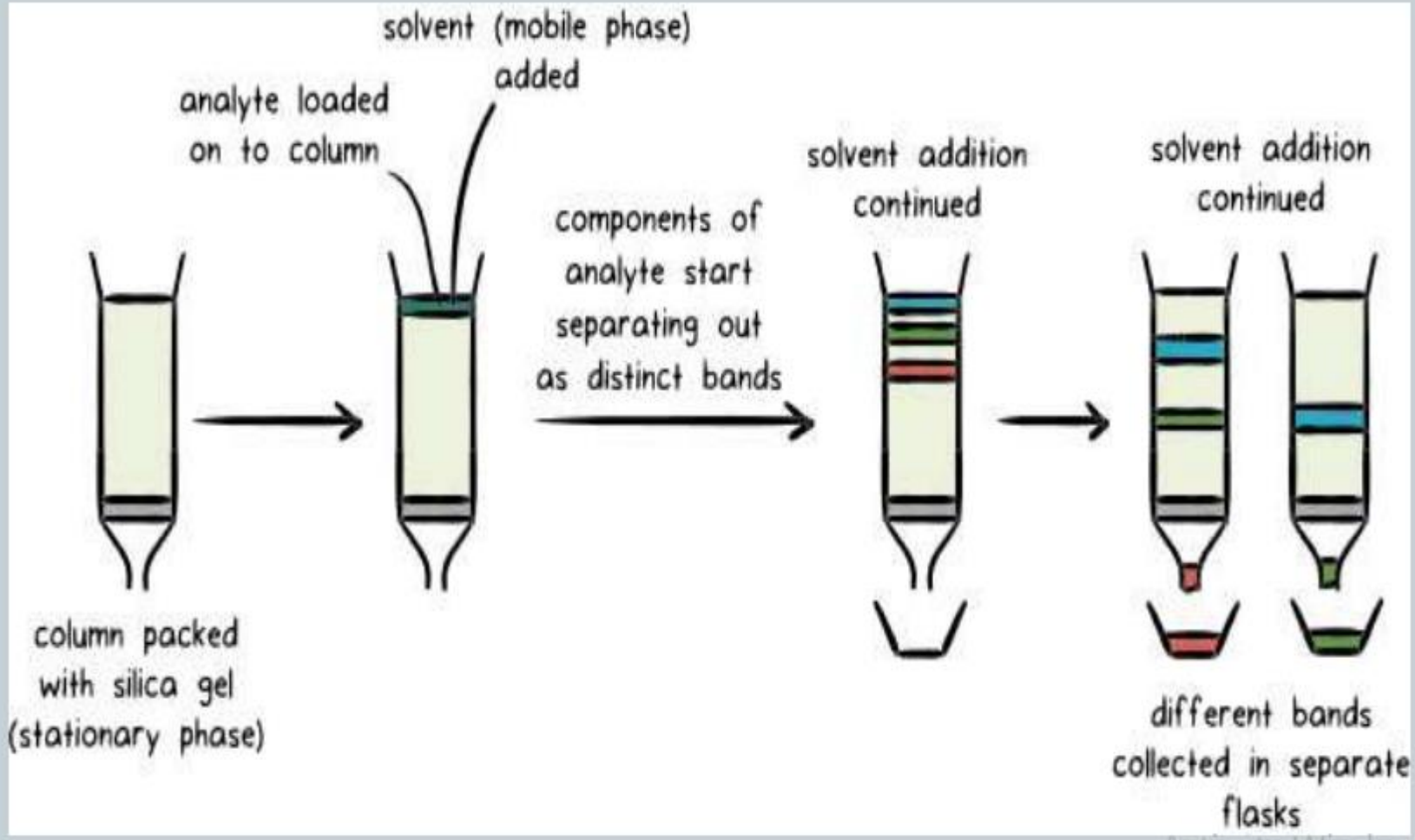
Introduction

Chromatography is the general name given to the methods by which two or more compounds in a mixture are physically separated by distributing between two phases: **a stationary phase** which can be a solid or liquid supported on a solid and a **mobile phase**, either a gas or a liquid which flows continuously around the stationary phase..

Basics of Chromatography



TERM	DEFINITION
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column using a suitable solvent
Analyte	mixture whose individual components have to be separated and analyzed



- ✓ The analyte is loaded over the silica bed (packed in the column) and allowed to adhere to the silica. Here, silica acts as the stationary phase.
- ✓ Solvent (mobile phase) is then made to flow through the silica bed(under gravity or pressure).
- ✓ The different components of the analyte exhibit varying degrees of adhesion to the silica and as a result they travel at different speeds through the stationary phase as the solvent flows through it, indicated by the separation of the different bands.
- ✓ The components that adhere more strongly to the stationary phase travel more slowly compared to those with a weaker adhesion.
- ✓ Analytical chromatography can be used to purify compounds ranging from milligram to gram scale..

Principle of separation of different components

- ✓ Differential affinities (strength of adhesion) of the various components of the analyte towards the stationary and mobile phase results in the differential separation of the components.
- ✓ Affinity, in turn, is dictated by two properties of the molecule: ‘Adsorption’ and ‘Solubility’.).
- ✓ We can define adsorption as the property of how well a component of the mixture sticks to the stationary phase, while solubility is the property of how well a component of the mixture dissolves in the mobile phase.

Principle of separation of different components

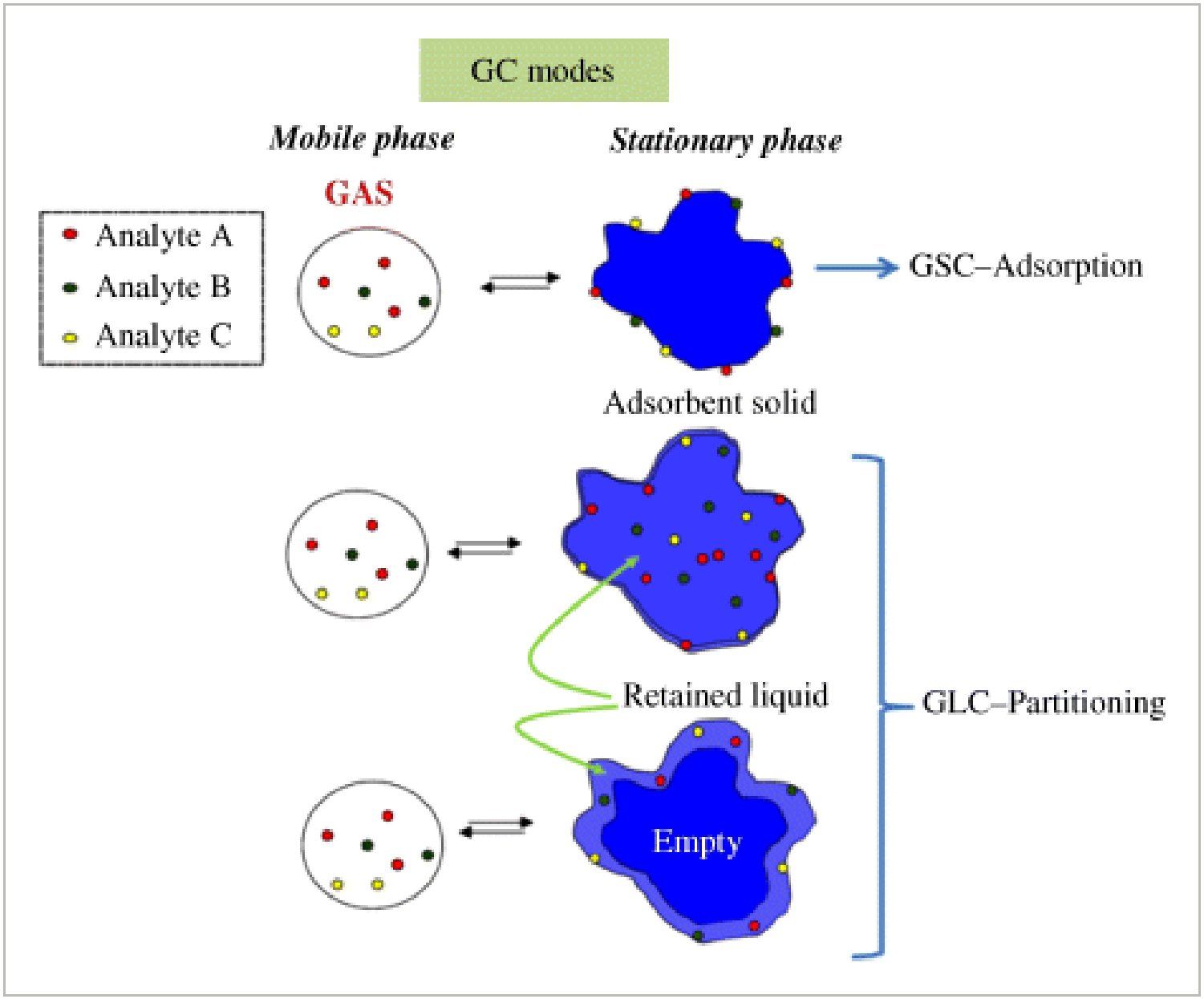
- ✓ Higher the adsorption to the stationary phase, the slower the molecule will move through the column.
- ✓ Higher the solubility in the mobile phase, the faster the molecule will move through the column.
- ✓ So, the interplay between the above two factors determines the differential rates at which the different components of the analyte will move through the column.
- ✓ Adsorption and solubility of a molecule can be manipulated by choosing the appropriate stationary phase and mobile phase

Gas chromatography

Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: **a stationary phase and a mobile phase**.

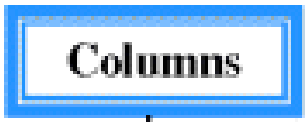
The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column. Gas chromatography is one of the sole forms of chromatography that does not utilize the mobile phase for interacting with the analyte.

The stationary phase is either a solid adsorbant, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC).


GC modes
Mobile phase
Stationary phase
GAS
Analyte A
Analyte B
Analyte C
GSC–Adsorption
Adsorbent solid
Retained liquid
Empty
GLC–Partitioning

How many types of GC columns are there?

Two types of columns are used in gas chromatography: packed columns and capillary columns:



Packed columns	Capillary columns
Stationary phase is coated directly in the column	Stationary phase is coated with the inner wall of the column
Applicable for both GSC and GLC	Applicable only for GLC
Liquid phase is adsorbed onto the surface of the beads in a thin layer or onto the solid inert packing	Liquid stationary phase is immobilized on the capillary tubing walls

What are the types of gas chromatography?

*Two types of gas chromatography are encountered
1. Gas-solid chromatography (G**S**C) .
2. Gas-liquid chromatography (G**L**C).

- **Gas-solid chromatography**

  Is based upon a solid stationary phase on which retention of analytes is the consequence of physical adsorption.

- **Gas-liquid chromatography**

  Is useful for separating ions or molecules that are dissolved in a solvent.

How does gas chromatography work?

1. The sample is first introduced into the gas chromatograph (GC), either with a syringe or transferred from an autosampler (Figure 1 (2)) that may also extract the chemical components from solid or liquid sample matrices.
2. The sample is injected into the GC inlet (Figure 1 (3)) through a septum which enables the injection of the sample mixture without losing the mobile phase.
3. Connected to the inlet is the analytical column (Figure 1 (4)), a long (10 – 150 m), narrow (0.1 – 0.53 mm internal diameter) fused silica or metal tube which contains the stationary phase coated on the inside walls.
4. The analytical column is held in the column oven which is heated during the analysis to elute the less volatile components.
5. The outlet of the column is inserted into the detector (Figure 1 (5)) which responds to the chemical components eluting from the column to produce a signal.
6. The signal is recorded by the acquisition software on a computer to produce a chromatogram (Figure 1 (6)).

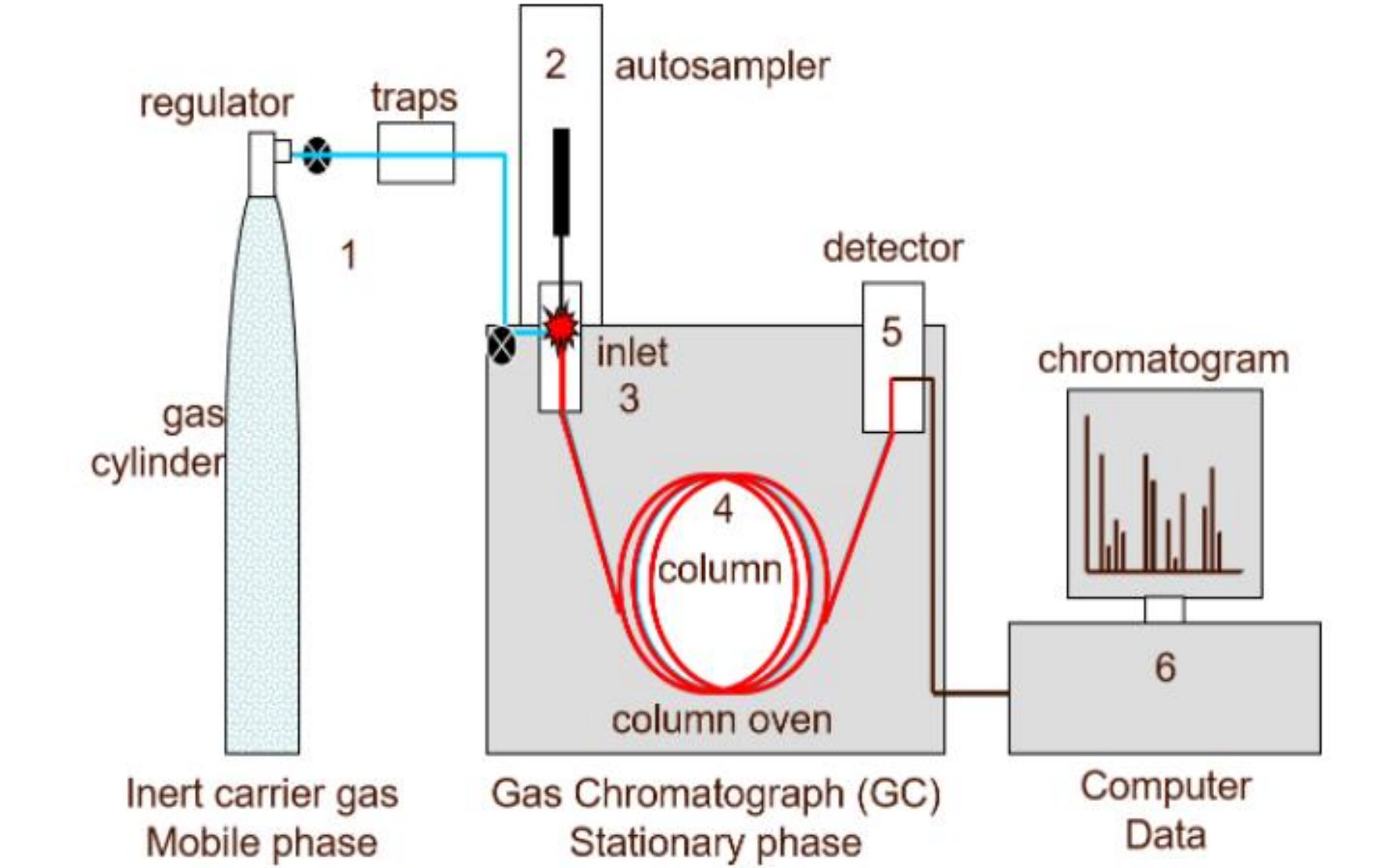
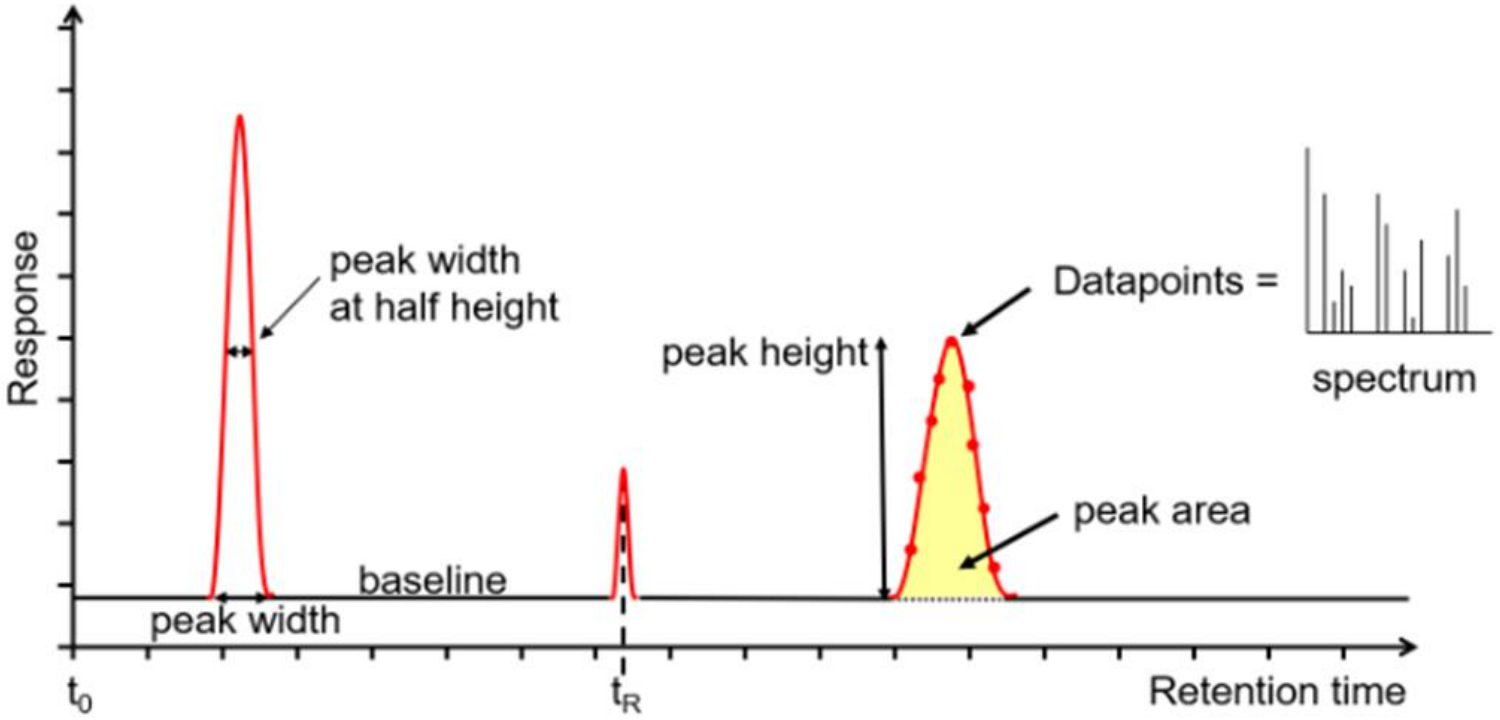


Figure 1: A simplified diagram of a gas chromatograph showing: (1) carrier gas, (2) autosampler, (3) inlet, (4) analytical column, (5) detector and (6) PC.

How do you read a chromatogram and what does it tell you?

- The x-axis is the retention time, taken from the time the sample was injected into the GC (t0) to the end of the GC run.
- Each analyte peak has a retention time measured from the apex of the peak, for example tR.
- The y-axis is the measured response of the analyte peak in the detector. The baseline shows the signal from the detector when no analyte is eluting from the column, or it is below the detection limit.
- The baseline response is a mix of electrical noise (usually low) and chemical noise, such as impurities in the carrier gas, column stationary phase bleed and system contamination.
- Hence, if the baseline is higher than it should be, it is an indication of a problem or that maintenance is required.
- Various measurements can be taken from the peak, such as width at the baseline, width at half height, total height and area.



The latter two are proportional to the concentration, however it is the area that is used for quantitation as it is less affected by band broadening.

The measurements can be used to calculate the extent of band broadening, the spread of the analyte molecules on the column.

Narrower, sharper peaks give better sensitivity (signal to noise ratio) and better resolution (peak separation).


THE KEY TO SUCCESS IS
TO FOCUS ON GOALS, NOT
OBSTACLES.
مفتاح النجاح هو التركيز على
الأهداف لا العقبات.