

CH 2252 Instrumental Methods of Analysis

Unit – V

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Chromatography Methods

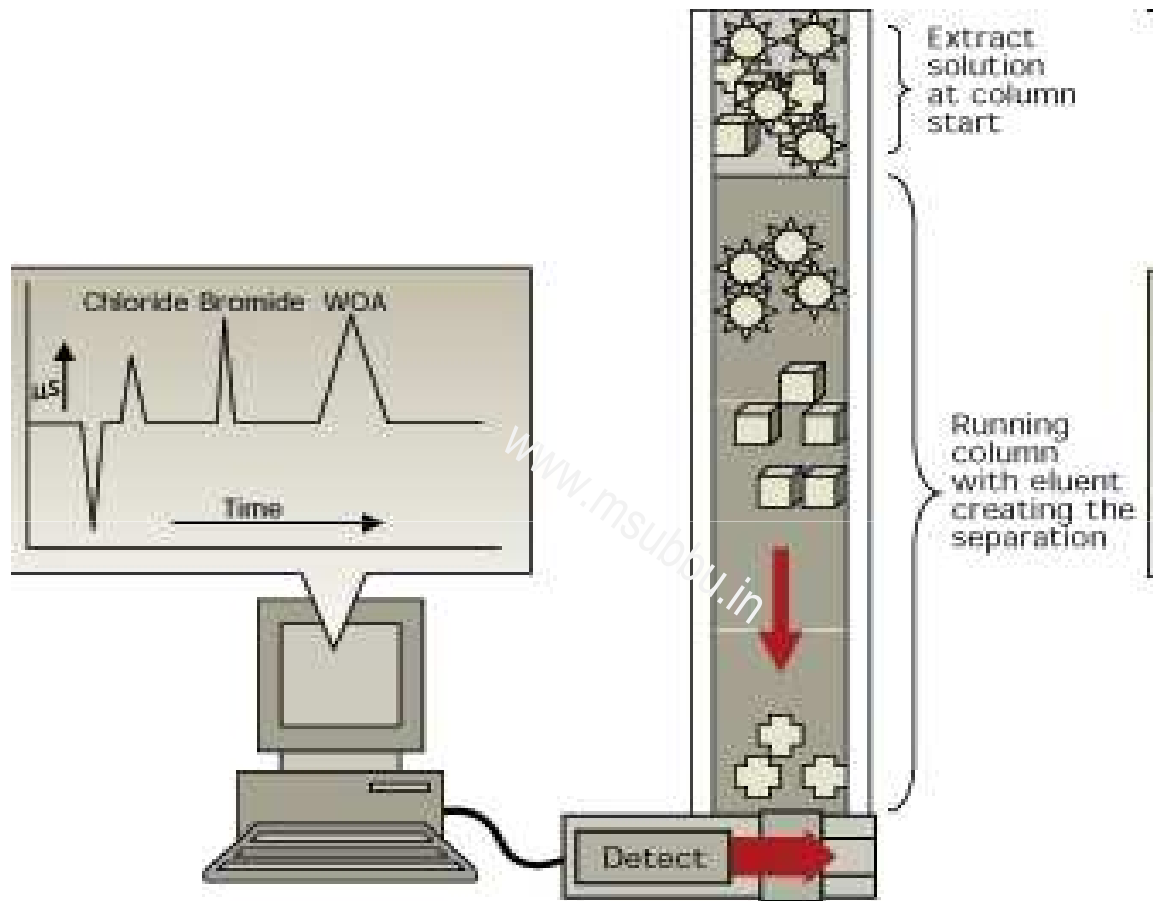
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Introduction

- Chromatography is a separations method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the components in a mixture
- A column holds the stationary phase and the mobile phase carries the sample through it.
- Sample components that partition strongly into the stationary phase spend a greater amount of time in the column and are separated from components that stay predominantly in the mobile phase and pass through the column faster.

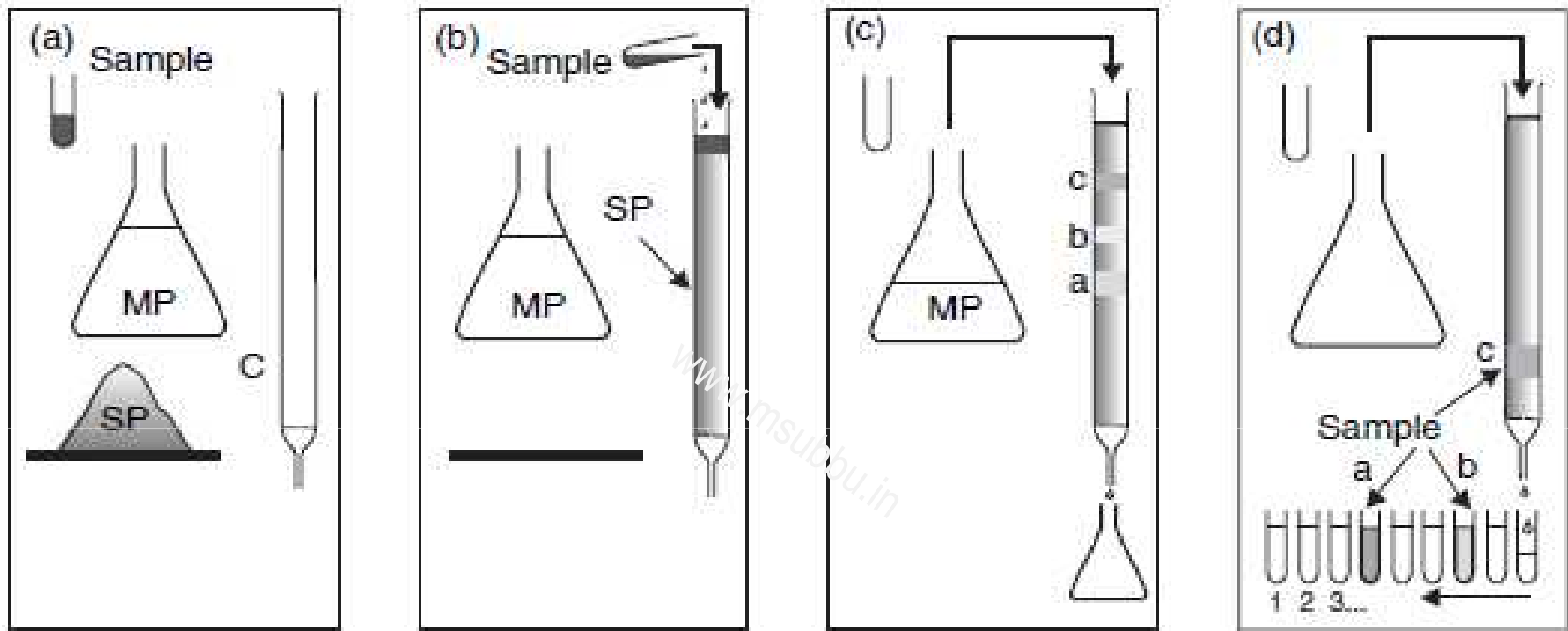
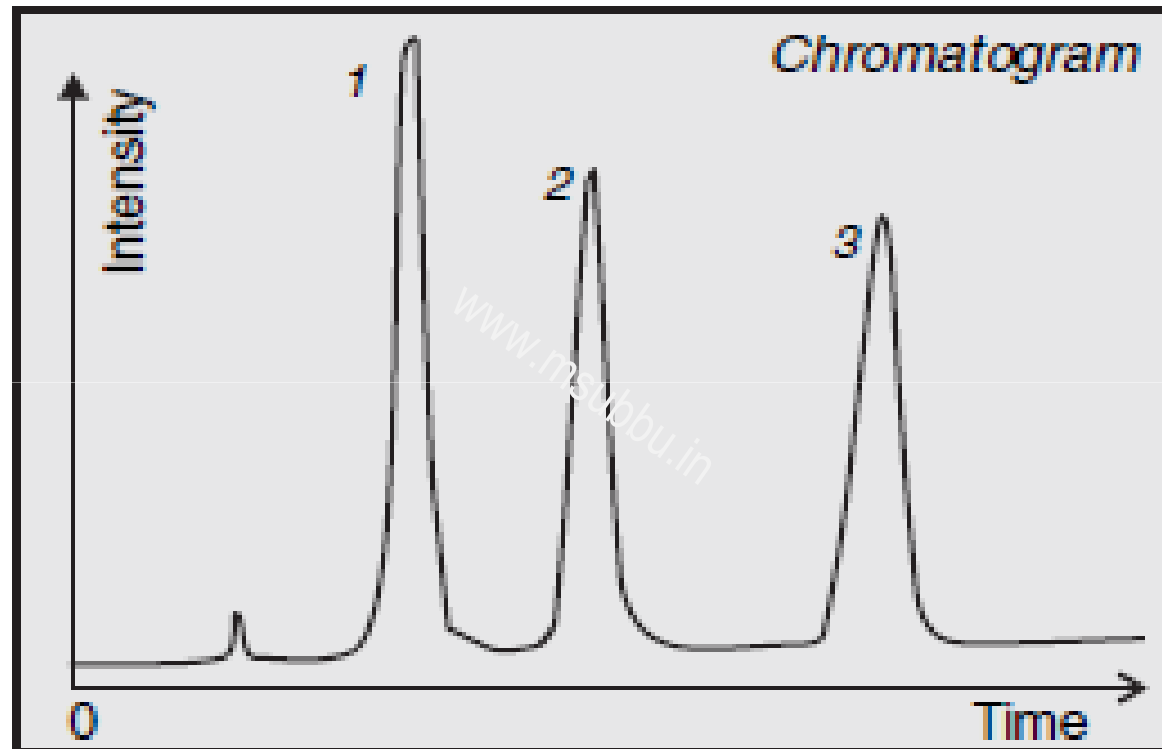
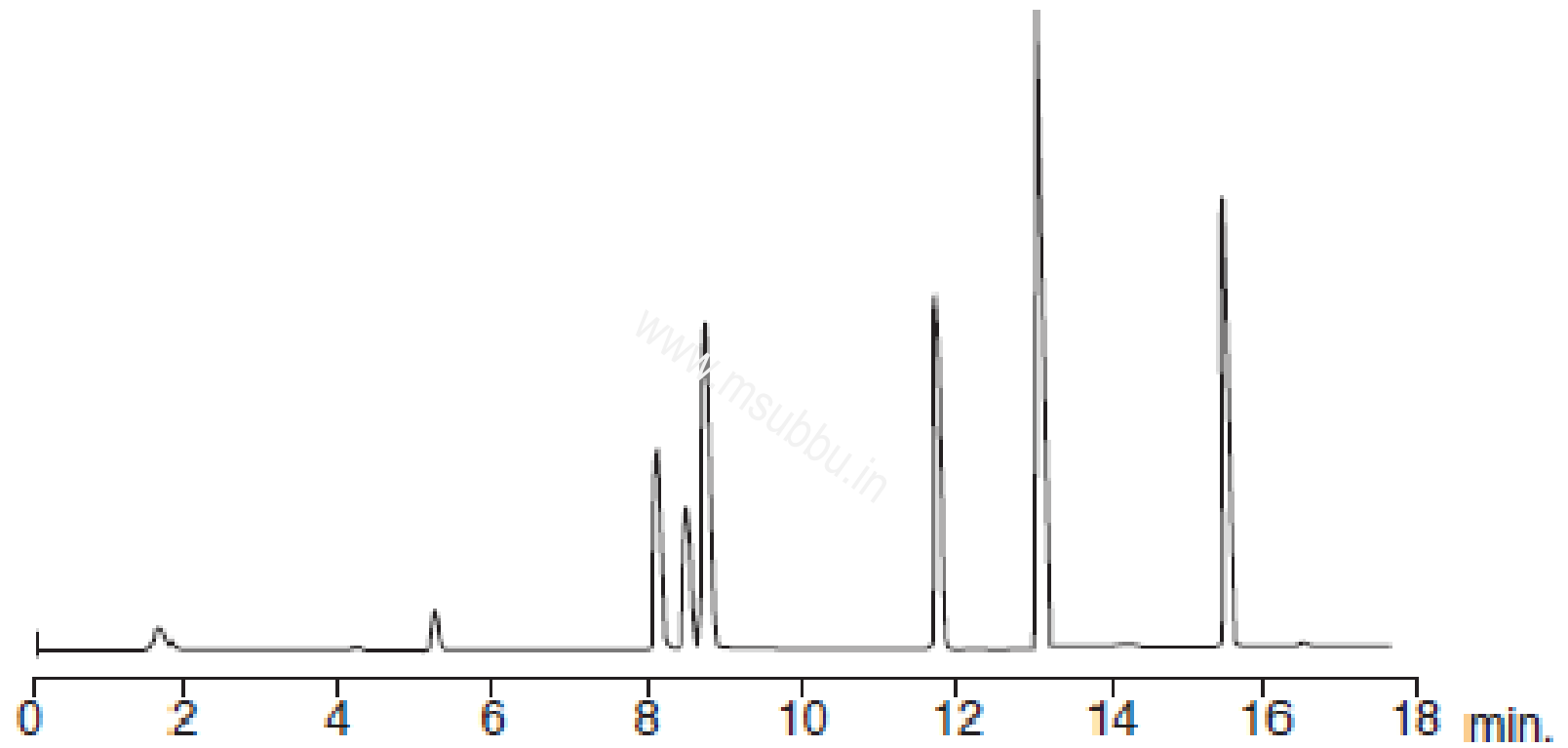
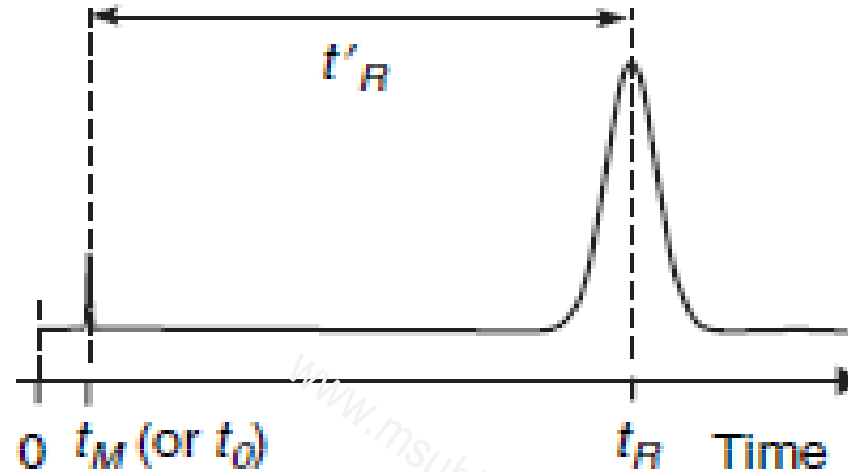


Figure 1.1 A basic experiment in chromatography. (a) The necessary ingredients (C, column; SP, stationary phase; MP, mobile phase; and S, sample); (b) introduction of the sample; (c) start of elution; (d) recovery of the products following separation.





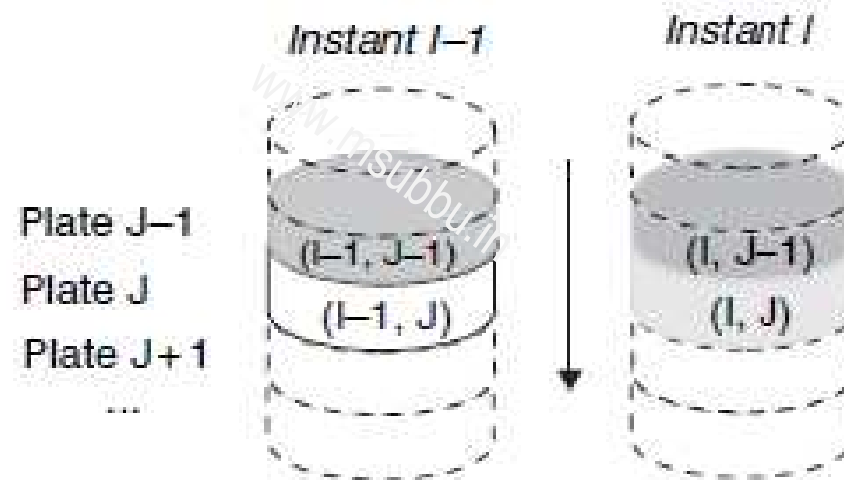


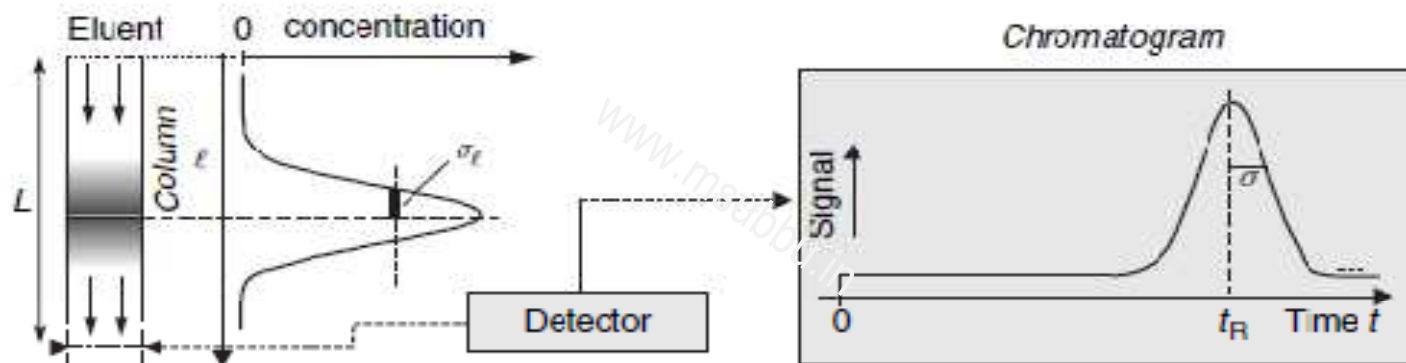
Retention time t_R , represents the time elapsed from the sample introduction to the detection of the peak maximum on the chromatogram.

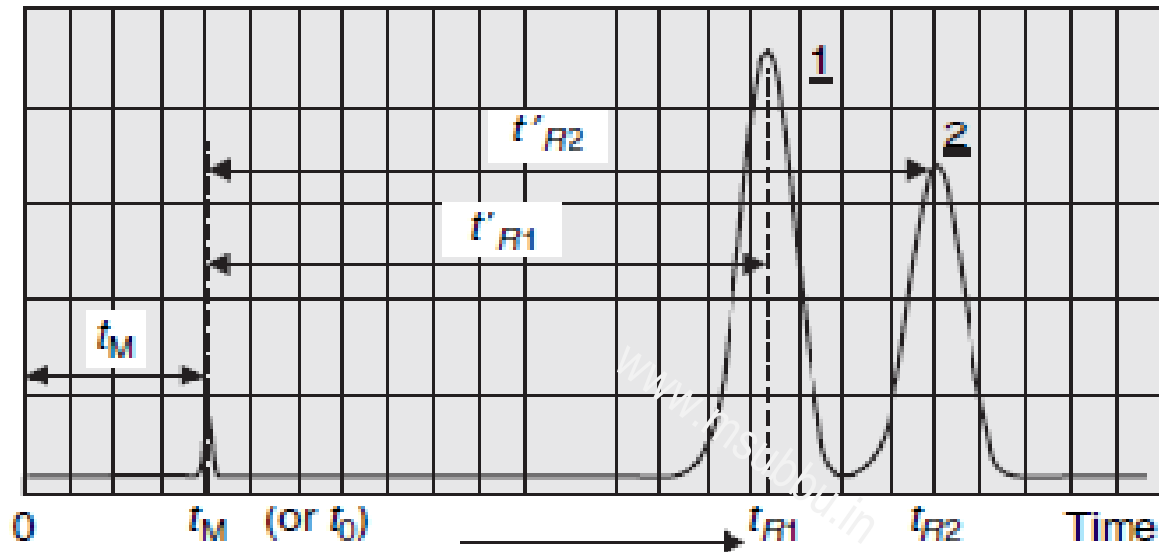
Holdup time or dead time t_0 : is the time required for the mobile phase to pass through the column.

Adjusted retention time t'_R : the difference between the retention time and the hold-up time

$$K = \frac{C_S}{C_M} = \frac{\text{Molar concentration of the solute in the stationary phase}}{\text{Molar concentration of the solute in the mobile phase}}$$







here,

$$k_1 = \frac{t'_{R1}}{t_M} \quad k_1 = 3.07$$

$$k_2 = \frac{t'_{R2}}{t_M} \quad k_2 = 3.92$$

$$\alpha = \frac{t'_{R2}}{t'_{R1}} \quad \alpha = 1.27$$

Retention factor: k

Capacity factor: α

Resolution factor: R

$$R = 2 \frac{t_{R(2)} - t_{R(1)}}{w_1 + w_2}$$

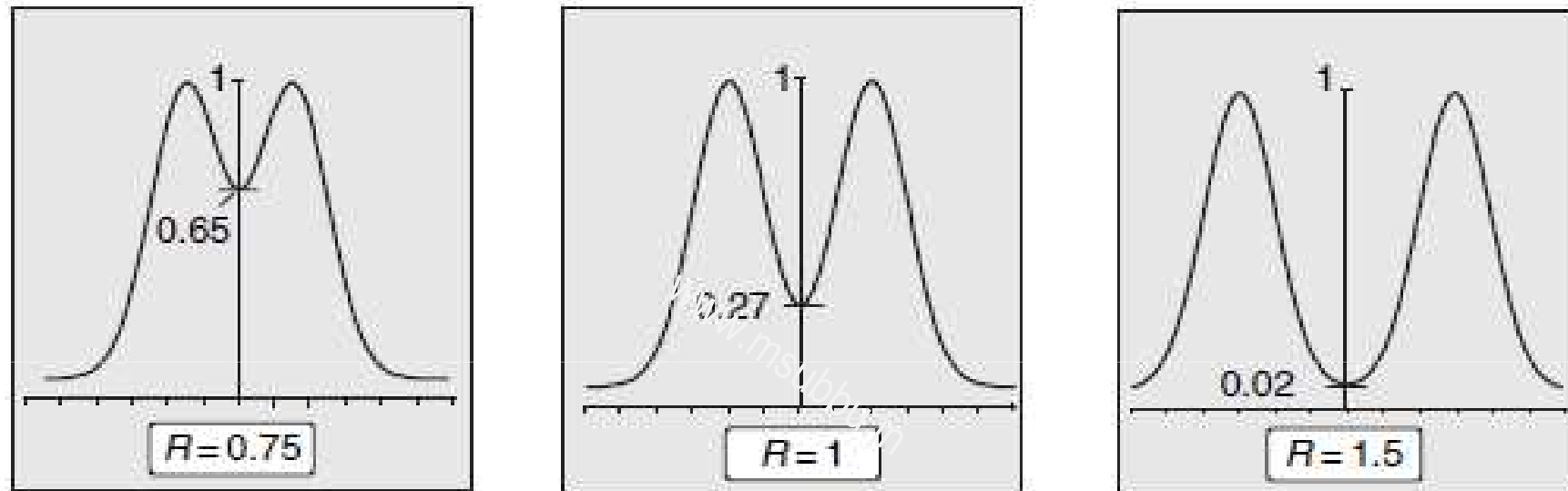
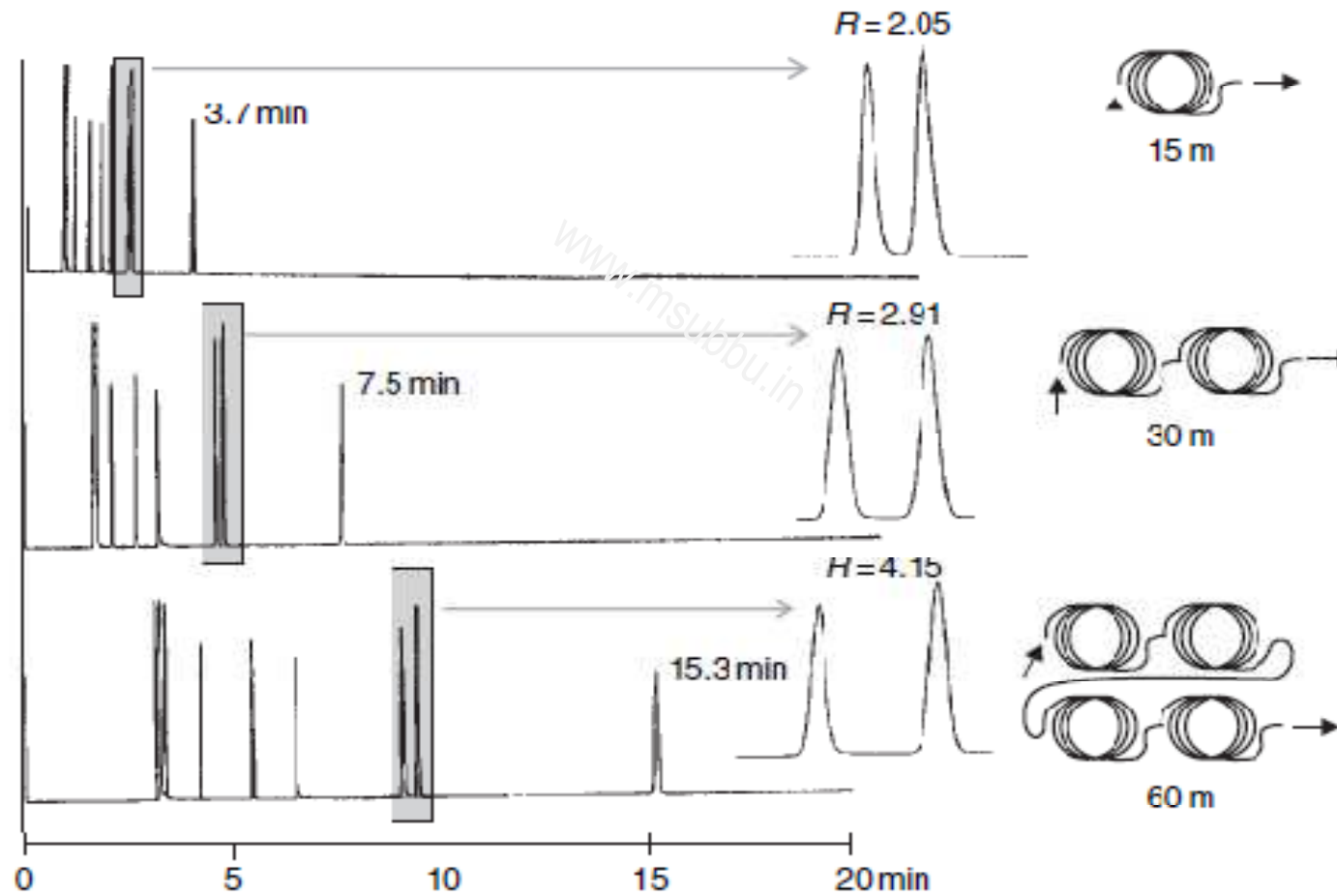


Figure 1.9 *Resolution factor.* A simulation of chromatographic peaks using two identical Gaussian curves, slowly separating. The visual aspects corresponding to the values of R are indicated on the diagrams. From a value of $R = 1.5$ the peaks can be considered to be baseline resolved, the valley between them being around 2 per cent.

Effect of column length on resolution



Effect of mobile phase composition

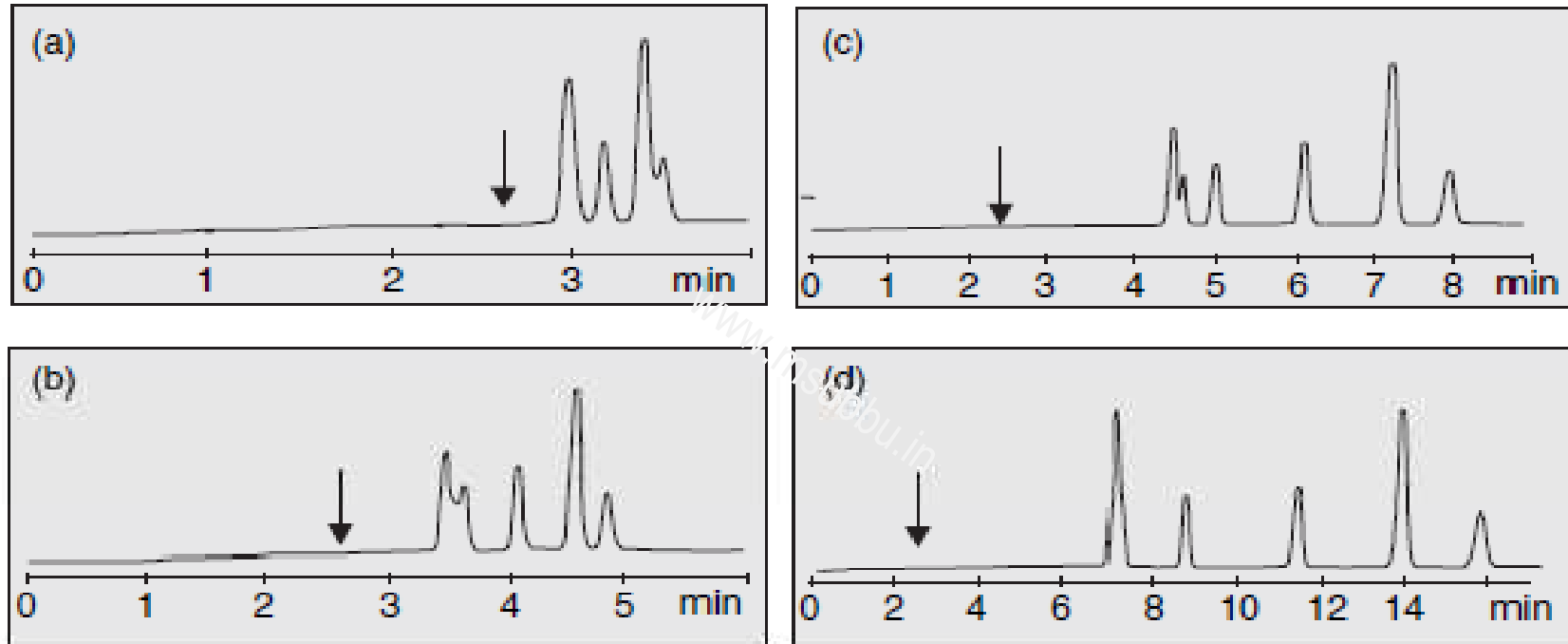


Figure 1.12 Chromatograms of a separation. The mobile phase in each trace is a binary mixture water/acetonitrile: (a) 50/50; (b) 55/45; (c) 60/40; (d) 65/35. The arrow indicates the dead time t_M (min) (*J.W. Dolan, LC-GC Int., 1994 7(6), 333*).

Invention of Chromatography

- 1900s - Michael S Tswett – separation of chlorophyll and other pigments from plants

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Phases for separation techniques

Technique	Mobile Phase	Stationary Phase
Gas chromatography	Gas (helium, nitrogen, or hydrogen).	Viscous liquid such as squalane, polyethylene glycol, or polymethyl siloxane. Adsorbent solid such as silica, molecular sieves, alumina, and porous polymers. Phase is housed in a column of glass or metal.
Liquid chromatography	Liquid water or organic solvents such as methanol, acetonitrile, propanol, or hexane.	Solid silica or a polymer such as polysaccharide or polystyrene housed in a column made of stainless steel.
Ion chromatography	Aqueous solutions of acids, bases, or salts. These solutions are sometimes modified with water-miscible organic solvents such as acetonitrile or methanol.	Ion exchange resins, alkyl-bonded porous silica resins, and styrene–divinylbenzene polymers.
Planar chromatography (thin-layer chromatography)	Solvent mixtures such as hexane/acetone, chloroform/ethyl acetate, ethyl acetate–methanol, butanol/acetic acid/water, or methanol/acetonitrile.	Precoated layers of silica gel, aluminum oxide, cellulose, polyamide, or ion exchange material supported by glass, plastic sheets, or aluminum foil.

Sample Types and Sensitivity Ranges

Technique	Type of Sample Analyzed	Sensitivity Range
Gas chromatography: very versatile, economic, and widely used.	Solid, liquid, or gaseous volatile organics and inorganic permanent gases	Parts per trillion or nanogram/liter to percent or grams/liter levels
Liquid chromatography: worldwide, a leading analytical technique for components not suitable for volatilization.	Liquid volatile and nonvolatile organic, inorganic, and biological compounds, polymers, chiral compounds, thermally labile compounds, small ions, and macromolecules	Parts per billion or micrograms/liter to percent or grams/liter levels
Ion chromatography: provides separation of inorganic and organic ions and ionizable species.	Liquid inorganic anions or cations, organic acids, amines, amino acids, carbohydrates, and nucleic acids	Parts per billion or micrograms/liter to percent or grams/liter levels
Planar chromatography: simple, economic alternative to LC with simultaneous analysis of a number of samples.	Same as for liquid chromatography	Parts per billion or micrograms/liter to preparative scale