Principles of Chromatography

- In all chromatography there is a **mobile phase** and a **stationary phase**. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to be tested. Chromatography Separation is based on **differential migration**. The solutes in a mobile phase go through a stationary phase. Solutes with a greater affinity for the mobile phase will spend more time in this phase than the solutes that prefer the stationary phase. As the solutes move through the stationary phase they separate. This is called **chromatographic development**.

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distanc travelled by the solvent}}$$

Example 1; If one component of a mixture travelled 9.6 cm from the base line while the solvent had travelled 12.0 cm, then what is the R_f value for that component?

Solution;

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distanc travelled by the solvent}}$$

$$\frac{9.6\ \text{cm}}{12.0\ \text{cm}} = 0.80\ \text{cm}$$

Example 2; Compound X has R_f value of 0.25. How far will compound X have travelled from the origin when the eluent front has traveled 4 cm?

Solution;

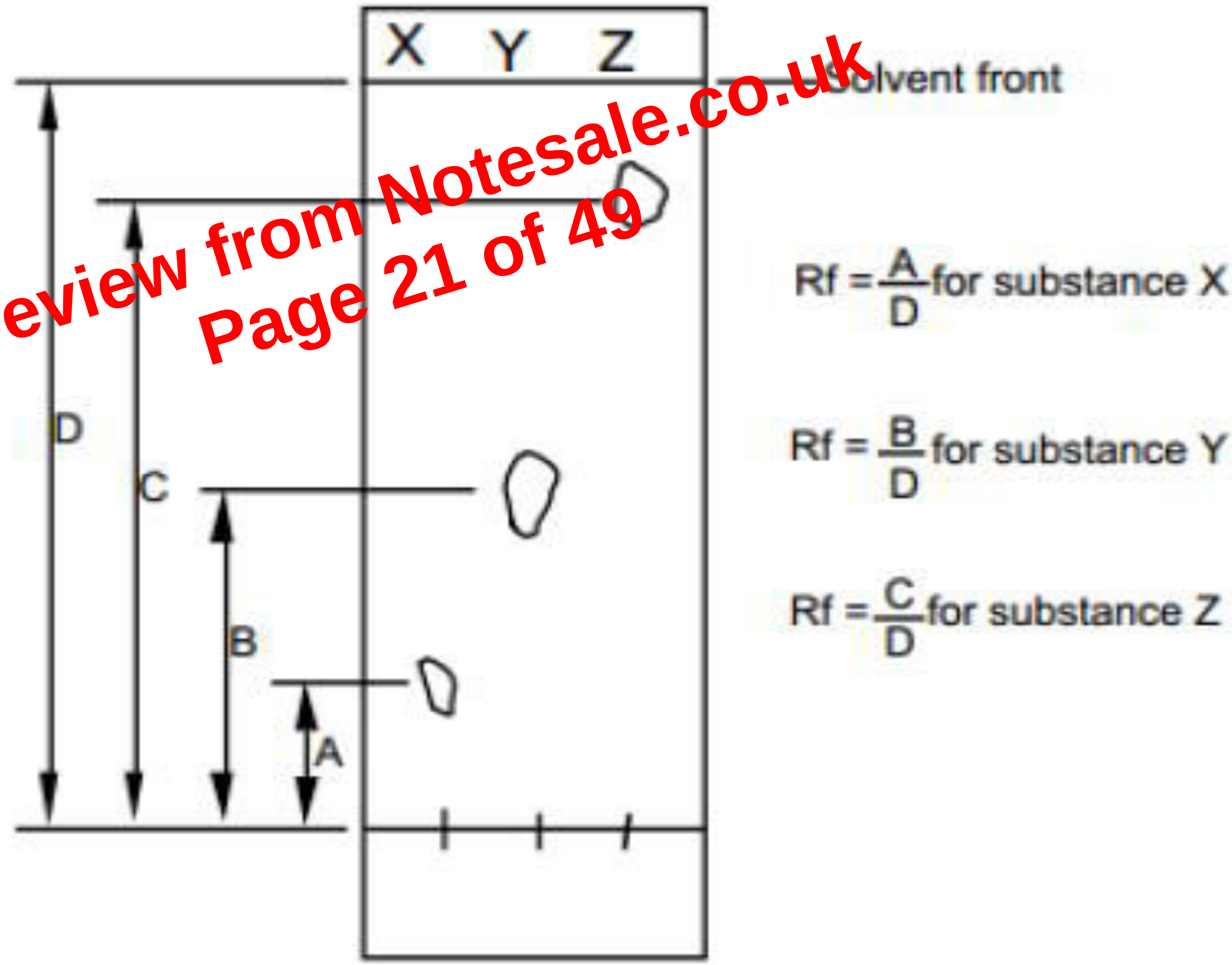

Figure 1.2: Elution process in thin layer chromatography.

Column Chromatography

✪ Of the two methods for bringing the stationary and mobile phases into contact, the most important is column chromatography. In this section we develop a general theory that we may apply to any form of column chromatography. With appropriate modifications, this theory also can be applied to planar chromatography.

✪ A typical column chromatography experiment is outlined in Figure below. Although the figure depicts a liquid–solid chromatographic experiment similar to that first used by Tswett, the design of the column and the physical state of the stationary and mobile phases may vary.

$$(\text{moles S})_{tot} = (\text{moles S})_m + (\text{moles S})_s$$

$$D = \frac{\{(\text{moles } S)_{tot} - (\text{moles } S)_m\}/V_s}{(\text{moles } S)_m/V_m}$$

Where V_m and V_s are the volumes of the mobile and stationary phases. Rearranging and solving for the fraction of solute in the mobile phase, f_m, gives;

$$f_m = \frac{(\text{moles } S)_m}{(\text{moles } S)_{tot}} = \frac{V_m}{V_m + DV_s}$$

Since the volumes of the stationary and mobile phase may not be known, equation above is simplified by dividing both the numerator and denominator by V_m; thus

✪ The solute can only move through the column when it is in the mobile phase. Its average linear velocity, therefore, is simply the product of the mobile phase's average linear velocity and the fraction of solute present in the mobile phase.

$v = uf_m$

$$\frac{L}{t_r} = \frac{L}{t_m}\left(\frac{1}{1+k'}\right)$$

Finally, solving this equation for *k'* gives;

$$K' = \frac{t_r - t_m}{t_m}$$

Example; In a chromatographic analysis of low-molecular-weight acids, butyric acid elutes with a retention time of 7.63 min. The column's void time is 0.31 min. Calculate the capacity factor for butyric acid.

<u>Answer;</u> 23.6

NB: Summary of plate theory

- ✓ Successfully accounts for the peak shape and rate movement.
- ✓ Does not account for the mechanism causing peak broadening
- ✓ No indication of other parameters effects
- ✓ No indication for adjusting experimental parameters

Rate theory

Band broadening

A part from specific characteristics of solute that cause differential migration, average migration rates for molecules of the same solute are not identical. Three main factors contribute to this behavior. These are;

$$H = A + \frac{B}{u} + (C_s + C_M)\,u$$

Longitudinal diffusion (B/u)

✪ Molecules tend to diffuse in all directions because these are always present in concentration zone as compared to other parts of the column. Longitudinal diffusion term, *B/u*: Solute molecules diffuse from the concentration center of a zone to the more dilute region (ahead of and behind the zone center). The longitudinal diffusion effect on *H* is inversely proportional to *u* because the solute residence time is shorter at high *u* and the extent of diffusion is less.

$$\frac{B}{u} = \frac{2\gamma D_m}{u}$$

where D_M is diffusion of solute in mobile phase,

γ is constant called the obstructive factor