Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third year)

Subject: Chemistry

Course Code: CHD 103

Course Title: Selected Instrumentation in Chemistry

Unit: 02

Unit Title: Chromatographic Techniques

Module Name: Explanation of Factors affecting Separation

Name of the Presenter: Ms. Priyanka R. Fernandes

Notes

Separation of compounds in Gas Chromatography (GC), is based on the different strengths of interaction of the compounds with the stationary phase.

The stronger the interaction is, the longer the compound interacts with the stationary phase, and the more time it takes to migrate through the column which means longer retention time.

Factors affecting Separation:

The efficiency of a column to resolve components of a mixture is dependent upon the following factors:

1) **Particle size and surface area**:

- The number of theoretical plates increases with a decrease in particle size or increase in surface area.
- In general, a 60/80 mesh particle size is used in a 0.25inch column.

2) Volatility of Compound:

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

- The boiling point of a compound is often related to its polarity.
- The lower the boiling point is, the higher the vapor pressure of the compound and the shorter retention time.

3) Polarity of components versus the polarity of stationary phase on column:

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

- If the polarity of the stationary phase and compound are similar, the retention time increases because the compound interacts strongly with the stationary phase.
- As a result, polar compounds have long retention times on polar stationary phases and shorter retention times on non-polar columns using the same temperature.

4) Column temperature:

In GC, ***temperature is the most important variable*** *under the control of the experimentalist.*

- It has a critical effect on the partition ratios and consequently on the retention volumes of solutes.
- The maximum operable temperature for the column is determined by the following factors:

i. Vapour pressure of the liquid phase

ii. Vapour pressure of the sample

iii. Efficiency of separation

iv. An excessively high column temperature results in very short retention time and in very poor separation because all components mainly stay in the gaseous phase.

v. In order for the separation to occur, the components need to be able to interact with the stationary phase.

vi. If the compound does not interact with the stationary phase, the retention time will decrease.

vii. At the same time, the quality of the separation deteriorates because the differences in retention times are not as pronounced anymore.

5) Carrier gas flow rate through the column:

*Speeding up the carrier gas **(Helium, Argon or Nitrogen)** flow increases the speed with which all compounds move through the column.*

- Maximum efficiency can be obtained with an optimum flow rate i.e. the rate of flow should neither be too low nor too high.
- The elute peaks will tend to be broad if the gas flow rate is too low and the peaks will not be resolved if the rate of flow of the gas is too fast.

6) Length of the Column:

*The longer the column, the longer it will take all compounds to elute. However, **longer columns generally improve the separation.***

- Retention time increases proportionally to the column length and a significant peak broadening will be observed as well, because of increased **longitudinal diffusion** inside the column.

7) Amount of material injected:

*Ideally, the peaks in the chromatogram display a symmetric shape **(Gaussian curve).***

- If too much of the sample is injected, the peaks show a significant **tailing**, which causes a poorer separation.
- Most detectors are relatively sensitive and do not need a lot of material in order to produce a detectable signal.
- Ideally, only 1-2 % of the compound injected into the injection port passes through the column because most GC instruments are operated in **split-mode** to prevent overloading of the column and the detector.

- Split-less mode will only be used if the sample is extremely low in concentration in terms of the analyte.

Generally, the number one factor to consider in separation of compounds on GCs in laboratories is the **boiling points of the different components.** Differences in polarity of the compounds is only important if you are separating a mixture of compounds which have widely different polarities. Column temperature, flow rate of carrier gas and column length are usually kept constant in the Gas Chromatograms run in Organic Chemistry laboratories.