Hello all, so here we are looking

at course code CHD 103. Course

title selected Instrumentation

in Chemistry that is Section A.

In this module we are dealing with the

unit titled chromatographic techniques

which is Unit 2 and module name:

explanation of the factors affecting separation.

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Here we are going to deal with all

the factors that affect separation and

also the factors which include polarity,

column temperature,

column diameter etc.

Now at the end of this module students,

you will be able to understand how

these factors affect gas chromatography

and also which one of these

factors play a more significant

or dominant role in the same.

So separation of compounds in the case

of gas chromatography essentially,

we know depends on two factors.

That is the mobile phase

and the stationary phase

and of course their interaction.

Now the stronger the interaction is between

the mobile and the stationary phase,

the more time it will take for the

stationary phase to migrate to the column.

That is,

the retention time will be longer.

By retention time we mean the amount

of time lapse that it takes from

the time of sample introduction

to the time we get the signal.

Now,

the ability of the column to separate

mixture is measured essentially by

the number of theoretical plates,

which is also oftenly referred to as HETP.

That is,

the height equivalent to a theoretical plate.

Now for a given column length,

the separation efficiency increases

when you increase the number of height

equivalent to theoretical plates.

Moving on to the factors which

actually affect the separation,

the first one is the particle

size and surface area.

Now as we know,

once you decrease the size of the particles,

the surface area of the

particles is going to increase,

which subsequently leads to increase

in the number of theoretical plates.

Now an increase in the number of theoretical

plates we have already seen

helps to increase the separation.

Now in general,

mesh particle size of 60 by 80

is used in a .25 inch column.

The second factor is the volatility

of a compound.

Now the more volatile the compound is,

the lesser will be its boiling point.

It is seen that for the lower boiling

or volatile components,

the mobile phase will travel

faster through the column downwards.

The boiling point of a compound is

also often related to its polarity.

Now, the lower the boiling point is,

the higher will be the vapor

pressure of the compound.

We are aware of that and hence shorter,

will be the retention time.

This means, the time taken from the sample

introduction to the signal that we observe,

or the spot that we observe

on the chromatogram.

Now this is usually because the

compound will tend to spend

more time in the gaseous phase.

One of the reasons why we use

low boiling solvents

like dioctyl, ether and dichloromethane

are because they have a low boiling point

and so it is easy to dissolve the sample.

The third factor is the

polarity of the components.

That is the mobile phase versus the

polarity of the stationary phase.

Now, polar compounds will tend

to move more slowly, if at all.

The stationary phase is also polar.

We are all very aware of the

phrase ***'like dissolves like.'***

Similarly, polar compounds will

get interacted most strongly with

a more polar stationary phase.

Causing more retention time.

If the polarity of the stationary

phase and the compound are similar,

the retention time increases as

I have mentioned and the compound

interacts more strongly.

As a result,

the polar compounds have long

retention times on polar stationary

phases and shorter retention times.

Now,

in the case of chiral stationary phases,

which are amino acid based derivatives:

cyclodextrin,

ankyle, sellins,

they are capable of

separating the enantiomers.

Now this is because one enantiomer

interacts slightly stronger

than the other with the stationary

phase and this is often either due to

static factors or other interactions,

which of course includes the

polarity between the mobile

and the stationary phase.

Fourth factor is the column temperature.

Now column temperature is one

of the most important factors

actually affecting the separation,

and it is a factor that is actually in

the control of the experimentalist.

Column temperature further also

affects the efficiency of the separation.

Based on the temperature,

we can get high quality separation

or low quality separation.

So, temperature has a critical effect on

the partition ratios and consequently

on the retention volumes of the solutes.

Now,

the maximum operable temperature for the

column is determined by three factors:

which include the vapor pressure

of the liquid,

phase vapor pressure of the sample,

and efficiency of separation.

Now an excessively high column temperature

will result in very short attention time,

and this of course tends to give a

very poor separation because all the

components mainly will only stay in

the gaseous phase. In order for the

separation to actually be efficient,

the components need to interact

properly with the stationary phase.

If the compound does not interact,

then the retention time will

naturally decrease at the same time.

We also need to take care of

the quality of the separation,

which might tend to detoriate because

the differences in the retention time

are not pronounced anymore.

And for this,

the temperature needs to be optimum.

Now fifth we have the column packing factor.

Now there are two distinct types

of columns in common use,

which is the packed column and the open

tubular column also known as capillary.

Now the packed columns are easier

to fabricate, less expensive,

last longer and of course they

have higher capacity to separate.

The bent columns are usually

1 to 20 meters long and three

to 10 millimetre in diameter,

and they can be bent in a U shape or

a W shape in order to save space.

The open tubular columns on the other hand,

have less pressure drop and therefore

they can be made much longer.

Six, we have the gas that is the carrier

gas flow rate through the column.

Now carry gases used are helium,

argon or nitrogen and the speed of

this carrier gas flow increases the

speed with which all the compounds

will move through the column.

Maximum efficiency is only obtained when you

have an optimal flow rate of the carrier gas.

That is, it should neither be too fast,

not too slow.

Now the elute peaks that is the final

piece that we observe on the chromatogram, will tend to be broad if

the gas flow rate is too low.

In that case, the peaks will not

be properly resolved.

If the rate of the flow gas is also too fast,

we need to maintain an optimum flow rate.

Now the flow through the column is

caused by the difference in pressure

between the inlet and the outlet.

And how do we maintain this constant

pressure or constant flow rate?

It is by having a certain component known as

pressure regulating valves for the column.

Seventh factor is the length of the column.

Now, the longer the column is,

ofcourse longer will the mobile phase taken

to travel or elute through the column.

However,

it is known that the longer columns

generally improve separation.

Now 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.

One has to keep in mind that

the gas molecules are not only

traveling in one direction,

but also sideways and backwards

to achieve the elute peaks.

This broadening now is inversely

proportional to the flow rate and the

number of theoretical plates increases

with an increase in the length of the column

which means that the efficiency of

separation will also be better.

But there is of course a practical

limit to the length of the column.

You cannot have a very long column.

The optimum length of the column

is usually maintained

to be around 1 to 10 meter long.

Eighth factor is the column diameter.

Now the number of theoretical

plates increases with a decrease

in the diameter of the column.

The usual diameter for columns

taken is 0.25 inch.

The 9th factor is the type and

amount of stationary phase.

This is of course a very,

very important factor in determining

efficiency of the column.

The best separation is only obtained

when the stationary phase is also

structurally and chemically similar to

the compounds that are being separated.

That is,

to the mobile phase.

Now the amount of stationary phase

also will affect the efficiency of

the column as the concentration

of the liquid phase increases.

The number of theoretical plates

for column also increases.

Now if you have excessive amount of

liquid support it can cause ***tailing***

of the peaks in the chromatograms.

You won't be able to achieve a proper peak.

In general light loading is

preferred with most columns

containing only 1% to 15% of liquid.

amount of material injected.

So, ideally the peaks in the chromatogram

displays symmetrical shape,

known as the Gaussian curve.

If you have too much of sample loaded,

then the peaks will 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.

So ideally only around 1% to 2% of

compound is injected into the

injection port which passes through

the column because most of the GC

instruments nowadays are operated

on a split mode to prevent automatic

overloading of the column and the detector.

So, the split mode will only be

used if the sample is extremely low in

concentration in terms of the analyte.

Now let us look at a conclusion that

we can draw from this entire module.

Generally what is observed is

that the boiling points of the

different compounds play a very,

very important role in the separation

in the GC instrument. Differences

in polarity of the compounds is

also another very essential factor,

which helps to separate the compounds.

Other factors include column temperature,

flow rate of carrier gas,

and column length

which are usually kept constant,

in a gas chromatogram that is run in

the Organic Chemistry laboratories.

These are my references,

the first one being an online reference.

The remaining 3 offline references.

Thank you.