IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Vol. 7, Issue 5, May 2020

Applications of HPLC and UPLC Techniques – A Short Review

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Abstract: In the present review two versatile analytical technique HPLC and UPLC discussed thoroughly for their unique properties, working and detectors which makes them very valuable in the drug and pharmaceutical industry. Here discussion starts from the origin of chromatography, types of chromatography techniques. It involves the intricate details of the functioning of HPLC and UPLC and how they are different from each other.

Keywords: Chromatography, HPLC, UPLC, Detectors, Separation techniques.

1.1 INTRODUCTION OF CHROMATOGRAPHY

Chromatography is discovered by Russian–Italian botanist Mikhail Semyonovich Tswett at the commencement of the 20th century, it is a physicochemical process for partition of composite mixtures [1]. In his paper 'On a new category of adsorption phenomena and its application to biochemical analysis' presented on 21st March, 1903 in frequent meeting of the biology section of the Warsaw Society of Natural Sciences, Tswett gave a very detailed report of the newly discovered phenomena of adsorption-based separation of composite mixtures, which he later called 'chromatography'. The word chromatography is a translation from Greek which means "color writing" [2]. Coincidentally, the Russian word "tswett" means color. He discussed in all his publications that he observed a colorful image of his first separation of plant pigments shown in Figure 1.1, so he has given that name for his new method. At that time of discovery, the chromatographic method was not appreciated among the scientists, additionally after almost 10 years when C. Dhere in Europe and L. S. Palmer [3] in the United States individually published the description of a similar separation process.

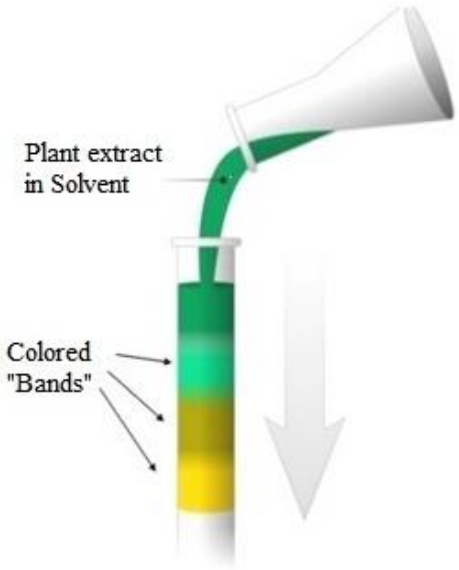

Figure 1.1: Tswett's Experiment

25 years later in 1931, Lederer read the book of Palmer and then found an original publication of Tswett, and in the same year he (along with R. Kuhn and A. Winterstein) published a paper [4] on purification of xanthophylls on $CaCO_3$ adsorption column following the process described by Tswett. In the year 1941, partition chromatography was discovered by R. L. M. Synge and A. J. P. Martin at Cambridge University in the UK, [5] for that in 1952 they were awarded the Noble Prize. In 1952, Martin and Synge published a seminal paper [6] which, along with the paper of A.T. James and A. J. P. Martin [7], laid a foundation for the quick growth of chromatographic techniques that shortly

IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Vol. 7, Issue 5, May 2020

followed. Prior to the 1970's, few good chromatographic methods were commercially obtainable to the laboratory scientist. During 1970's, most chemical separations were performed using different techniques including open-column chromatography, TLC (thin-layer chromatography) and paper chromatography. However, these chromatographic techniques were insufficient for resolution between similar compounds and quantification of compounds. During this time, to decrease flow-through time pressure liquid chromatography began to be used, thus reducing time taken for purification and separation of compounds being isolated by column chromatography. As flow rates were Inconsistent, the question if it was good to have a constant flow rate or constant pressure was debated [8]. In the mid-1970's, High-pressure liquid chromatography (HPLC) was developed and improved rapidly with the development of different column packing materials and the additional suitable detectors. Some new methods including reverse phase liquid chromatography allowed for better separation between very similar molecules. By 1980's, for the separation of chemical molecules, HPLC was widely used. New techniques enhance purification, separation, identification and quantification than the previous techniques [9]. For the convenience of HPLC, computers and automation were added for monitoring data. Enhancement in type of columns and thus reproducibility were made as such terms as affinity columns, micro-column.

1.2 CLASSIFICATION OF CHROMATOGRAPHY BASED ON SEPARATION TECHNIQUES

All the chromatographic separation methods need one static part called stationary phase and one moving part called mobile phase. Chromatography is a physical separation method that distributes components to be separated between two phases, one is stationary (stationary phase) and the other (mobile phase) moves in a definite direction. The mobile phase, leaving the column is eluate and the solvent (mobile phase) that carries the analyte is eluent.
The chromatographic technique depends on one of the following phenomena [10]:

- Adsorption
- Partition
- Ion exchange or
- Molecular exclusion

1.2.1 Adsorption

A physical attachment between the analyte and the particles of stationary phase is called Adsorption. Based on nature, non-polar analytes adsorb better to the non-polar stationary phase than polar analytes while polar analytes adsorb with greater or stronger intensity to the polar stationary phase than non-polar analytes.
Examples: Gas-solid chromatography, Liquid column chromatography, Thin layer chromatography (TLC) and High pressure liquid chromatography or High performance liquid chromatography (HPLC).

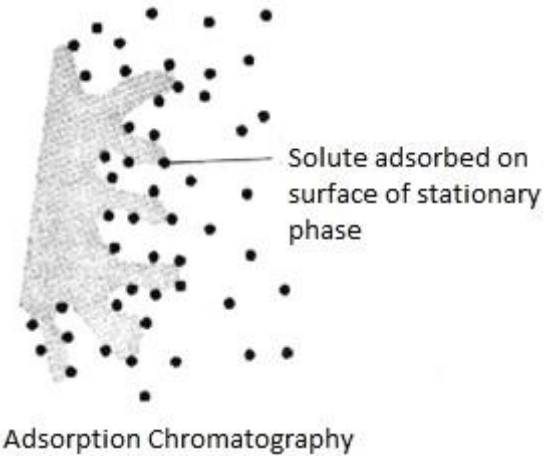

Figure 1.2: The schematic diagram of adsorption chromatography

1.2.2 Partition

Partition chromatography is a separation process based on partition coefficients whereby the component mixture gets distributed into two liquid phases during the flow of mobile phase in the chromatography column i.e, both phases mobile and stationary phases are liquid in nature. Non-polar molecules get partitioned into non-polar phases and Polar molecules get partitioned into polar phase.
Examples: Gas-liquid chromatography, liquid-liquid chromatography, paper chromatography, high-performance liquid chromatography and supercritical fluid chromatographyitical

IARJSET
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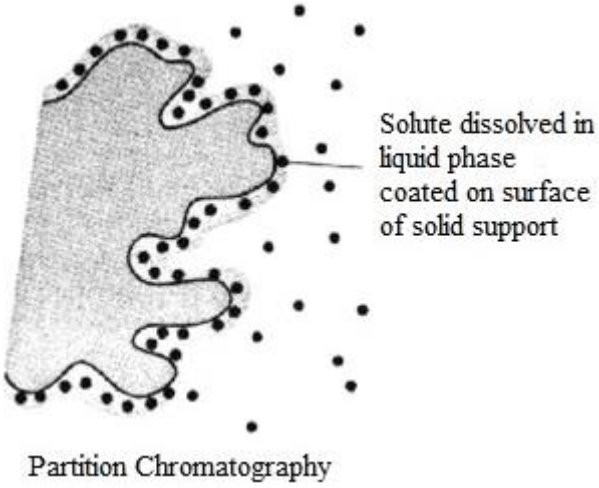

Fig. 1.3: The schematic diagram of Partition chromatography

1.2.3 Ion-Exchange

Ion Exchange Chromatography is a chromatographic technique which is used for the separation of charged molecules based on their total charge. Ion Exchange resins are insoluble granular compounds which have ions in their molecular structure like acidic or basic radicals that can be interchanged. The negatively charged ions and positively charged ions fixed on these radicals are replaced by ions of the same charge (positive to positive and negative to negative) in solution in the liquid in contact with them.
Example: Ion exchange chromatography.

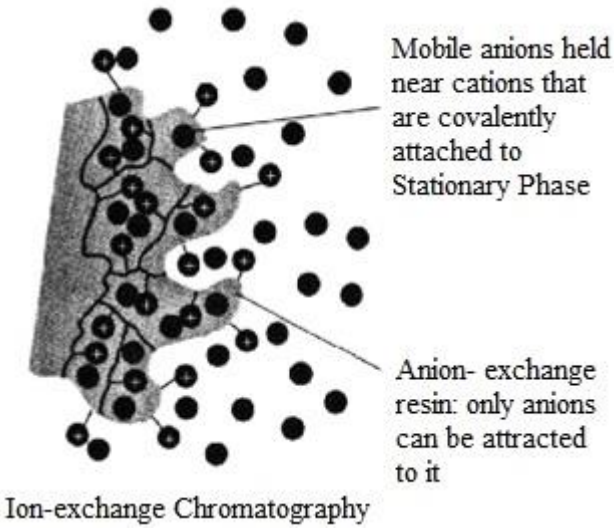

Figure 1.4: The schematic diagram of Ion Exchange chromatography

1.2.4 Molecular Exclusion

Molecular exclusion is one of the chromatographic technique, also called as Size exclusion chromatography (SEC) which separates molecules based on their molecular size by a filtration process through a gel. The gel consists of many pores of a particular size distribution. Separation occurs when molecules present in the mixture of various sizes are entered or removed from the pores within the matrix. Small molecules flow slowly through the column as they penetrate deep into the pores whereas large molecules flow rapidly through the column because they do not enter the pores. Therefore, separation of the molecules depends on their molecular size.
Example: Size exclusion chromatography.

IARJSET
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International Advanced Research Journal in Science, Engineering and Technology
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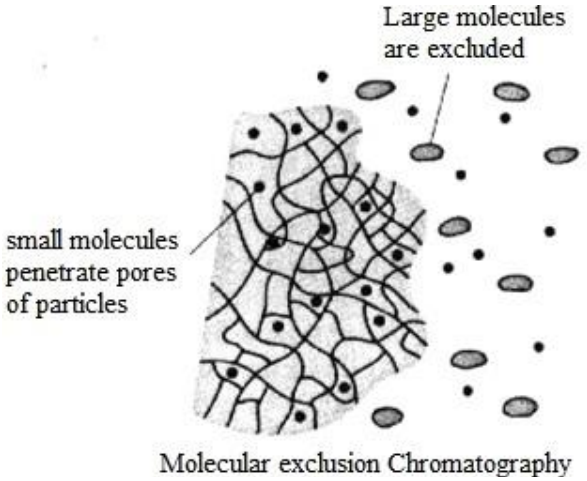

Figure 1.5: The schematic diagram of Molecular exclusion chromatography

1.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is abbreviated as High Performance Liquid Chromatography or High Pressure Liquid Chromatography [11]. In pharmaceutical and biomedical analysis HPLC has utmost feature that is for the development of the characteristic of the methodology since 25 years. During the process of discovery, development and manufacturing for the identification, qualification and quantification of drug analysis in active pharmaceutical Ingredient (API) or in the formulation, HPLC is the most important analytical tool. High Performance Liquid Chromatography (HPLC) is a one of the forms of column chromatography that pumps, solvent (called as mobile phase) and carried a sample mixture or analyte into the column have chromatographic packing material (known as stationary phase) with constant high pressure. The schematic diagram of HPLC is shown in Figure 1.6.

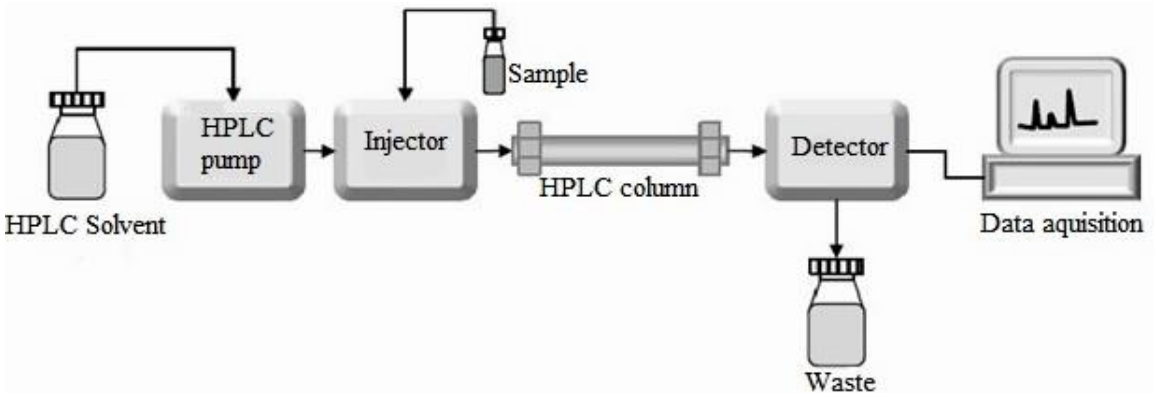

Figure 1.6: The schematic diagram of HPLC

The various components that are present in HPLC equipment are:

- HPLC Solvent
- Pump
- Injector
- Column
- Detector
- Data handling device and microprocessor control

1.3.1 HPLC Solvent

This part also called as the solvent reservoir. We store mobile phase here. For the preparation of mobile phase, we are using highly purified solvents like HPLC grade solvents and water, which have 18.2 MΩ·cm resistivity at 25 °C.

1.3.2 Pump

One of the most important components in HPLC is the pump, as its performance directly affects reproducibility and retention time. HPLC pump delivers mobile phase under pressure (up to 5000 psi or higher) from the solvent reservoirs to the column at a constant rate (up to about 10 mL/ min). The pump is equipped with a degasser, which is required to remove other gases and dissolved air from the solvent. An ideal pump should have desirable characteristics that are

IARJSET
ISSN (Online) 2393-8021
ISSN (Print) 2394-1588
International Advanced Research Journal in Science, Engineering and Technology
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constant flow delivery independent of back pressure, convenience of replacement of worn out parts, solvent compatibility, resistance to corrosion, low dead volume for minimizing problems on solvent changeover.

1.3.3 Injector

In HPLC, samples are injected through an injection port. Generally, an injection port consists of an injection valve and the sample loop. After the dissolution of the sample in mobile phase or suitable diluent, the sample is then drawn into a syringe and injected into the sample loop by using an injection valve. In order to inject the sample into the stream of the mobile phase, valve rotor is used to open the loop and close the valve. The range of loop volume is in between 10 µl to over 500 µl. The samples injected automatically in modern HPLC system [12].

1.3.4 Column

One of the important components in HPLC is the column because the sample component separation takes place when those components pass through the column. Generally, columns are packed with silica gel because its pores structure, surface properties and particle shape help to get a good separation. Various chemical compounds are separated by using silica due to its chromatographic behaviour which is generally predictable and reproducible. Silica also has a higher surface activity which can be changed easily with water and other solvents. HPLC columns are classified into two, one is an analytical column and the other is guard column. Typically the lengths of analytical columns are 5, 10, 15 and 25 cm and are filled with 3, 5 or 10 µm particle diameter. Usually, the columns internal diameter is 4.6 mm. The guard column protects the analytical column and it is an essence a disposable top of the main analytical column. It increases the life of analytical column and protected from contaminants and particulate matter in solvents. The most popular material is octadecyl-silica means C18 column used in reverse phase HPLC and also C8, C6, C8, C4, cyano and amino columns are available [13].

1.3.5 Detectors

In HPLC, detector plays a vital role. The detector is one of the components of HPLC that emits a response and then signals a peak on the chromatogram when eluting the compound. To detect the compounds elute from the column, it is located behind the stationary phase. Generally, the coarse and fine-tuning controls were used to adjust the bandwidth and the height of the peaks.

In many cases, the detection and sensitivity parameters can also be adjusted by using the coarse and fine-tuning controls.

The desirable features of HPLC detectors are capable of detecting all compounds in the mixture; Low drift and noise level; High sensitivity and fast response; Low dead volume; should not respond to mobile phase; should be unaffected by variation in temperature and flow rate; Operational simplicity and reliability and should be non-destructive.

Many types of detectors can be used with HPLC.

1.3.5.1 Refractive index detectors

Refractive index Detector measures the difference in the refractive index between the eluent passes throws the flow cell and the pure mobile phase. It is called as a universal detector. It works in isocratic mode. It is very sensitive to pressure changes, flow-rate changes, changes in ambient temperature and cannot be used for gradient elution. This detector is intensely useful for the nonionic compound-detecting, these compounds do not absorb in the UV detector, and do not in fluorescence detector.

1.3.5.2 Ultra-violet (UV) detectors

The most commonly used HPLC detectors today is the UV-visible absorbance detector. In HPLC, UV detectors used to detect and identify components, showing an absorption spectrum in the UV or visible region (from 190–600nm). In UV detector, the deuterium discharge lamp is used as a light source with the wavelength from 190-380 nm. Tungsten lamp is used additionally when components are detected at the wavelength exceeds 380 nm.

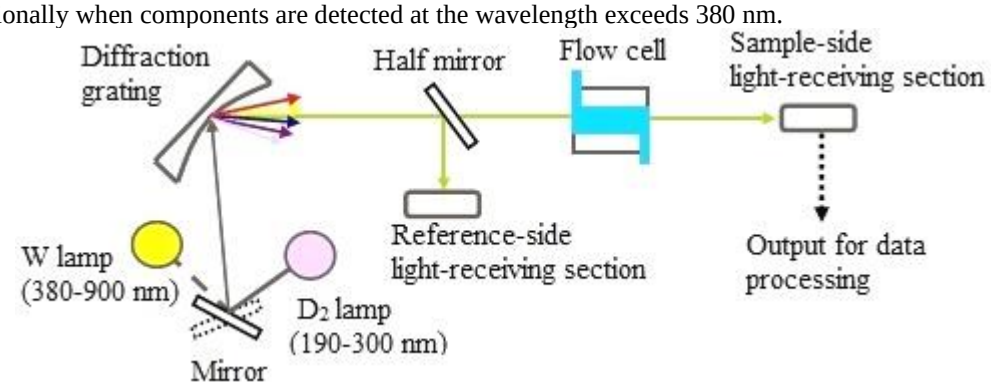

Figure 1.7: Diagrammatic illustration of a UV-VIS detector optical system

When the light emitted from the lamp is focused on the grating, it scattered according to its wavelength. The diffraction grating angle is adjusted according to the required wavelength. Then that light passes through the half mirror

IARJSET
ISSN (Online) 2393-8021
ISSN (Print) 2394-1588
International Advanced Research Journal in Science, Engineering and Technology
Vol. 7, Issue 5, May 2020

and it splits into two rays, one pass through reference-side light-receiving section and the other ray pass through the flow cell. The difference in intensity of light can be determined between light from reference cell and flow cell, the output obtained as absorbance. UV detector detects all the components with high sensitivity. The schematic diagram of UV detector is shown in Figure 1.7.

Fixed wavelength: detects only one wavelength, usually at 254 nm.
Variable wavelength: In this UV detector detects over a wide range of wavelengths but only one wavelength at a time.
Diode array: Here the light from the lamp collocated through the flow cell and then it is dispersed via a diffraction grating into individual wavelengths of light. The dispersed light is detected using PDA (Photodiode Array). A different narrow wavelength band is collected by each photodiode and is scanned by the microprocessor. The readings are shown on the monitor. The schematic diagram of the Diode array detector is shown in Figure 1.8.

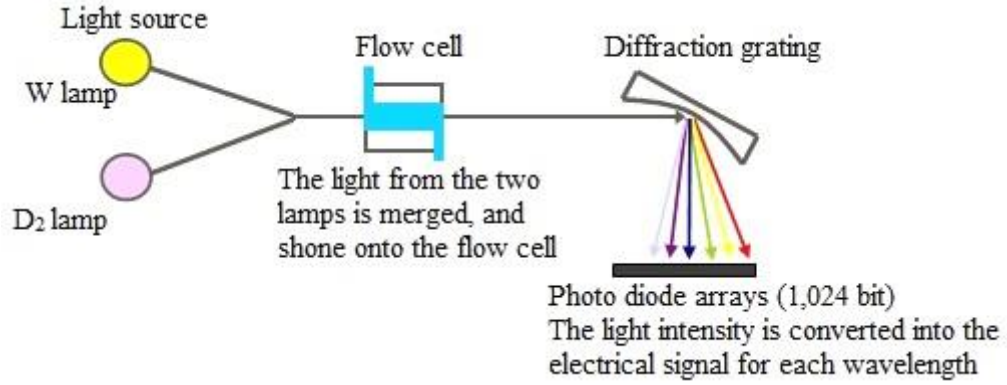

Figure 1.8: Diagrammatic illustration of a PDA optical System

1.3.5.3 Fluorescence detectors
Fluorescence detector is used to analyze the components which have fluorescence properties. The use of this detector is limited as only a few compounds possess the properties of fluorescence. It is most sensitive detector than the existing HPLC detectors. Even a single molecule in the flow cell can be identified by this detector. For strong UV absorbing materials, fluorescence sensitivity is 10 to 1000 times higher than that of UV detector. It is very selective for analyzing compounds from complex matrix due to the spectroscopic and chemical specificity. It is usually used to measure samples having specific fluorescent species.
1.3.5.4 Electrochemical detectors
Electrochemical detectors are used to detect the molecules that undergo oxidation or reduction reactions. When the sample passes between the electrodes, they measure the difference in electrical potential.
1.3.5.5 Evaporative Light Scattering Detector (ELSD)
ELSD detectors are used to analyze the components that do not absorb UV radiation. The mobile phase solvent passes from an HPLC, forced through a nebulizer. Then it passes into a heated funnel, where the mobile phase is evaporated to leave minute particles. These particles pass through a narrow light beam and scatter light which is measured by a photomultiplier. The response of the detector depends on the size and number of particles. Even with gradient elution, it provides a plane baseline.
1.3.6 Data handling device and microprocessor control
Once the signals detected, they are integrated using microcomputers. The retention time, peak area and peak height are reported using Integrator and also it makes the qualitative analysis and quantitative analysis easier. The collected data are interpreted and converted to a presentable format by using various chromatography software [14].

1.4 APPLICATIONS OF HPLC

1. Pharmaceutical
   HPLC is used for qualitative and quantitative analysis
   Separation of complex molecules
   Prepare huge quantities of pure materials
   To analyze the purified compounds for trace contaminants
   Self-life determinations of pharmaceutical products
2. Environmental
   It is used to test drinking water and to observe air quality.
   To identify very small quantities of contaminants such as PCBs in pesticides and waste oil.

IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

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3. Clinical diagnosis of diseases, disorders
4. Food industry

It is used for quality analyses and checker in the food industry, by identifying and separating, analyzing additives, preservatives, proteins, vitamins, and amino acids.

5. Forensic test
6. Determination of steroid in blood, sweat and urine
7. It is used in Bioinformatics and DNA fingerprinting.

1.5 ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

Since from thirty years, High performance liquid Chromatography (HPLC) is well accepted technique used in laboratories worldwide. It is one of the analytical techniques used to identify, separate and quantify each analyte in a mixture. One of the primary drivers for the development of this technique has been the progression of the packing materials (particle size, packing technology, chemistry) used to impact the separation. The fundamental principles of this progression are governed by the Van Deemter equation. It is a formula describing the relationship between plate height (H) and linear velocity (flow rate). The column efficiency is measured by the number of theoretical plates in a column, N, and normalized with the column length to give HEPT (Height Equivalent Theoretical Plate) or H. The Van Deemter equation defines the various factors influencing H and they are eddy diffusion (A-term), longitudinal diffusion (B-term) and mass transfer (C-term). The relative importance of these factors changes with the velocity of the mobile phase. Along with a variety of other factors, particle size and morphology also contribute to H. Understanding this equation allows to determine the maximum mobile phase velocity.

One of the most important factors in the Van Deemter equations is Stationary phase particle size. For a given column length, in relation to particle size, the plate number (Nth) is inversely proportional. Put simply, as smaller the particle size, the higher the plate number and the separation power. The theoretical plate number (Nth) also depends on the mobile phase.

The plate number is higher and H is lowest at a certain velocity, so called optimum flow. The higher or lower flow rate (F) provides less plate count and H is higher.

The **Van Deemter equation** gives the relation between mobile phase velocity and plate height (H) is shown below (Figure 1.9)

$$H = A + \frac{B}{u} + C \times u$$

Where,

H = HETP (Plate Height)

A = eddy diffusion term

B = longitudinal diffusion term

u = linear velocity

C = Resistance to the mass transfer coefficient

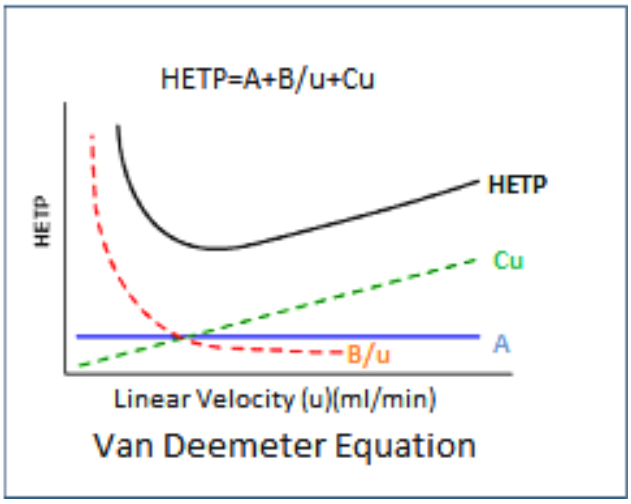

Figure 1.9: Plot showing the relationship between the height of a theoretical plate, H, and the linear velocity (u) **based on the van Deemter equation.**

The three terms that contribute to band broadening described in the Van Deemter equation are:

IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

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A-term: Eddy diffusion: Eddy diffusion is caused by a disturbance in the solute flow path as solute molecules will take different paths through the stationary phase at random. These multiple paths arise due to the difference in column packing and small variations in particle size of the packing material. As different paths are of different lengths, this causes broadening of the solute band.

B-term: Longitudinal diffusion: Longitudinal diffusion is the movement of an analyte molecule outside from the center to the edges of its band as the analyte concentration is lower at the edges than at the center of the band. Analyte diffuses from the center to the edges. Thus band broadening occurs. If the mobile phase velocity is high, then the analyte spends less time on the column, this limit outward distribution, keeping the band tighter.

C-term: Resistance to Mass transfer coefficient: To equilibrate between the mobile phase and stationary phase the component takes a certain amount of time. If the component has a strong affinity with the stationary phase and the mobile phase velocity is high, then the component in the mobile phase will travel ahead of the component in the stationary phase, then the component band is broadened. The higher the mobile phase velocity, the worse the broadening becomes.

By using particles of size < 2.5 µm, speed and peak capacity, i.e., number of peaks resolved per unit time in gradient separations can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC [15-17].

1.5.1 Theory of separations using smaller particles

The chemistry of the particles used in this course of the method provides the increased efficiency and potential to work at amplified linear velocity, by that providing the resolution and the speed. Efficiency is one of the important separation technique which plays an important role in UPLC science it depends on the same retentivity and selectivity as HPLC. By the following basic resolution (Rs) equation this may be understood (18)

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

Where N is efficiency,
α represent to Selectivity factor and
k is mean proportionality constant

From the above equation, the resolution increase with the increase in the efficiency. We know that efficiency (N) is inversely proportional to particle size (dp).

$$N \propto \frac{1}{dp}$$

As the particle size decreases by a factor of three, here the efficiency and resolution increases three times, there is an increase of the square root of three (nine times). Thus, there is an increasing in efficiency with resulting increase in the sensitivity and the resolution as the particle size decrease. And also efficiency (N) is directly proportional to column length (or) length of the column. The above equation is written as the following equation.

$$N \propto \frac{L}{dp}$$

Hence, the length of the column (or) column length (L) may be reduced by the same ratio of the particle size without losing the resolution.

The relationship between the efficiency (N) and the peak width (w) is inversely proportional, the equation is

$$N \propto \frac{1}{w^2}$$

This illustrates that the separation of the narrower peaks is easier for each other. Peak height (H) also the relationship between the height (H) and the peak width (w) is inversely proportional, equation is shown below

$$H \propto \frac{1}{w}$$

According to the above equation in UPLC, when the size of the particle is reduced ($1/3^{rd}$) then the column length is reduced ($1/3^{rd}$), increased (3 times) the flow rate and the separation is done faster ($1/9^{th}$) with maintaining the good resolution. Based on the resolution, short columns are packed with small size particle (about 2µm), to accelerate the separation with higher efficiency, while maintaining a tolerable loss of load. The effect of linear velocity and particle size on HETP has been illustrated in Figure 1.9. By using van Deemeter plot, shows that the particles with a smaller diameter are contributing less to band broadening compared to particles with a larger diameter. Smaller diameter particles are less affected by higher column flow rate.

1.5.2 Instrumentation

The UPLC (Ultra performance liquid chromatography) System has been specially designed to resist higher system pressures during the analysis of chromatographic methods and to match the performance needs of innovative column chemistries with easy-to-use software, robust hardware and specific support services.

The advancements in Chromatographic separations involve

- Small, pressure-tolerant particles

IARJSET
ISSN (Online) 2393-8021
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International Advanced Research Journal in Science, Engineering and Technology
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- Minimized system volume
- High-pressure fluidic modules (up to 100 MPa or 15000 psi)
- Reduced cycle time
- Negligible carryover
- High-speed detectors
- Integrated system software (novel communication protocols and advanced diagnostics)

The ACQUITY UPLC system is the only UPLC system that is available commercially from Waters. The ACQUITY UPLC System consists of high-pressure fluidic modules. To make the most of the small particle technology, these modules optimize flow rates. The sample-handling architecture of the UPLC system is designed to ensure especially reduced cycle times and low carryover. The unattended sample capacity is increased up to ten times when it is interfaced with the Sample Organizer.
In UPLC High-speed detectors (both optical and mass) provides the speed of analysis, good resolution and increased sensitivity.

The components in UPLC are:
- Pumping System
- Sample injection
- UPLC columns
- Column manager and heater/cooler
- Detectors

1.5.2.1 Pumping System
To obtain a small particle, high peak capacity separations needs a higher pressure range than that achievable by present-day HPLC instrumentation. The calculated pressure drop is approximately 15,000 psi for the length of the column 15 cm packed with 1.7 µm particle sizes at the optimum flow rate. Hence, at these pressures, the pump was suitable for delivering solvent reproducibly and smoothly. These pressures can recompense for solvent compressibility and can operate in both the gradient separation modes and an isocratic separation mode was required.
1.5.2.2 Sample injection
In UPLC, the introduction of a sample is critical. The injector is used to inject an accurately measured volume of solution containing the sample into the mobile phase. Conventional injection valves, either programmed or manual, are not designed and hardened to work under extreme pressure. To guard the column from extreme pressure variations, the injection process must be comparably pulse-free and to reduce potential band spreading, the swept volume of the device also needs to be minimal. A rapid injection cycle time is essential to fully avail the speed afforded by UPLC, which requires a high sample capacity. To increase sensitivity, low volume injections with minimum carryover are needed. Generally, in UPLC, the sample volume is 2-5 µl but nowadays for biological samples, direct injection approaches are utilized.
1.5.2.3 UPLC columns
Columns play a vital role in UPLC. Without smaller particles, the vows of the van Deemter equation cannot be satisfied. Researchers have been very active in the design and development of sub 2µm particles to capitalize on advantages of smaller size particles (Jerkovich *et al*., **2003**; Wu *et al*., 2001). Nonporous 1.5µm particles with high efficiency columns are commercially available, but they have some disadvantages of low surface area, leading to poor loading capacity and retention. To withstand high pressures, UPLC must use a novel porous particle to maintain retention and capacity similar to HPLC. Silica based particles used in columns have good mechanical strength, but it has some disadvantages, which include tailing of basic components and a limited pH range. Another alternative is polymeric columns, which can overcome pH limitations, but these columns have their own issues containing limited capacities and low efficiencies.

A first-generation hybrid chemistry introduced by Waters in 2000 is called XTerra that combines advantageous properties of polymeric and silica columns. These XTerra columns can operate over an extended pH range and are mechanically strong with high efficiency. XTerra columns manufactured using a classical sol-gel synthesis that includes carbon in the form of methyl groups. Still, in order to provide the kind of improved mechanical stability that UPLC required, a second generation bridged ethane hybrid technology (BEH) was developed. This technology is called ACQUITY UPLC. ACQUITY 1.7µm particles enhance their mechanical stability by bridging the methyl groups in the silica matrix as shown in Figure 1.11.

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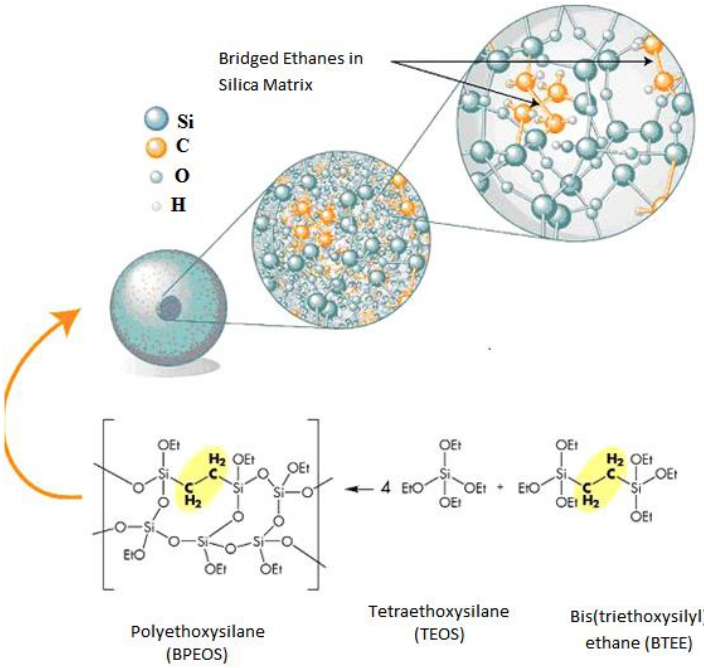

Figure 1.11: Synthesis and Chemistry of ACQUITY 1.7µm particles for UPLC

Compared to silica columns, BEH columns have enhanced mechanical and chemical stability and also reduce peak tailing significantly for basic components, due to the reduced acidity of the four unreacted bonded phases are available on the surface of the silanol group.

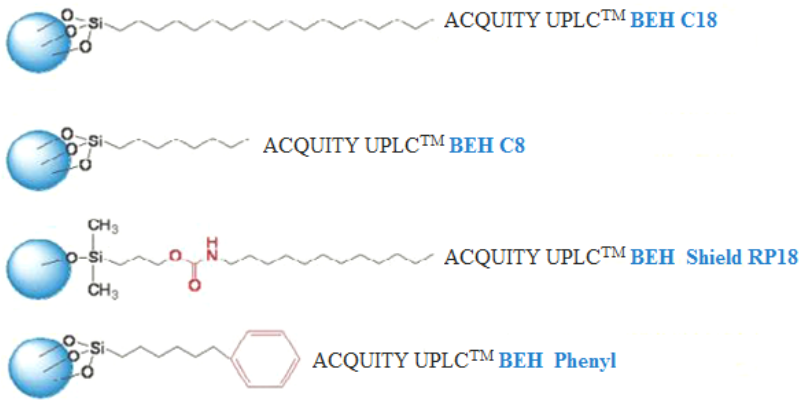

Figure 1.12: Bonded phases available for UPLC

Each column chemistry provide a different combination of silanol activity, hydrolytic stability, hydrophobicity and chemical interaction with analytes.

1.5.2.4 Column Manager and heater/cooler:

The ACQUITY UPLC Column Manager is intended for high productivity UPLC sample processing and also it offers a bypass channel and automated column switching for up to 4 columns with dimensions of 20 to 150 mm length and 1.0 to 2.1 mm internal diameter (I.D). The Column heater/cooler is intended for ultra performance where the temperature is used as a method parameter for high-resolution separations. The column manager regulates the temperature from 10 °C to 90 °C (50 °F to 194 °F).

1.5.2.5 Detectors

The detectors used with UPLC should be able to handle very fast scanning methods because half-height peak widths of less than one second are usually obtained with columns packed with 1.7 µm particles. The detector must be able to give high sampling rate adequate to capture enough data points across the peak for an accurate and reproducible integration analyte peak. The dispersion (volume) of the detector flow cell must be minimal to maintain separation efficiency. Conceptually, the increase in sensitivity for UPLC detection should be 2-3 times more than previous HPLC separations,

which is due to the detection technique. ACQUITY Photodiode Array (PDA) and Tunable UV (TUV) are the detectors employed in the UPLC. In these detectors, Teflon AF is used which provides an internal reflective surface and improves the light transmission efficiency by eliminating the internal absorptions. These have 10 mm flow cell path lengths, acquisition rates 40 (TUV) and 20 (PDA) points, and a total internal volume of 500 nL. Mass Spectrometric (MS) detection is also considerably enhanced by UPLC.

1.5.3 Advantages of UPLC

UPLC is more sensitive and selective with rapid resolving power and high-resolution performance. It decreases process cycle time and persuade quality of end-product with reduced run time and decreased the cost of operation. Through the use of a novel column material of very small particle size, it provides quick analysis and increases sensitivity. It reduces the solvent consumption and raises sample throughput and also supports real-time analysis in step with manufacturing processes.

Tabular Conclusion between HPLC and UPLC

Comparison	**HPLC**	**UPLC**
Particle size	3-5 µm	Less than 2 µm
Maximum back pressure	35 to 40 MPa	103.5 MPa
Analytical Column	Alltima C18	ACQUITY UPLC BEH C18
Column dimensions	150 ×3.2 mm	150×2.1 mm
Column temperature	30 °C	65 °C
Injection Volume	5 µL	2 µL
Analysis time	More	Less
Sensitivity	Less	Higher

ACKNOWLEDGEMENT

The authors likes to thank Head of Department Chemistry, Deen Dayal Upadhaya Gorakhpur University, Gorakhpur 273009, Uttar Pradesh and Head of Department Chemistry, Gitam Institute of Science, Gitam University (Deemed to be University), Visakhapatnam 530045, Andhra Pradesh, India for providing necessary facility in writing this short review.

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