PART-II

CHROMATOGRAPHIC SEPERATION TECHNIQUES

CHAPTER-1

1.1. GENERAL INTRODUCTION OF COLUMN AND THIN LAYER CHROMATOGRAPHY

1.1.1. CHROMATOGRAPHY

Chromatography is one of the most powerful techniques for the separation of components from a mixture. There are different types of chromatography, such as paper, thin layer or column, Gas, High Performance Liquid chromatography etc. Chromatography systems have a stationary phase (which may be solid or liquid) and a mobile phase (usually liquid or gas). In column chromatography both phases are placed in a column container (figure 1.1). We have used liberally the techniques of TLC, Column and HPLC in our present work.

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass column and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). With the help of column chromatography, we can isolate or purify desired compounds from a mixture. The mixture to be analyzed by column chromatography is applied to the top of the column. In fact, it is the most frequently used method of purifying mixtures of products in research laboratories.

1.1.2. THE ADSORBENT

Silica gel (SiO_2) and alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. These adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label: "silica gel 60" or "silica gel 230-400" is a couple of example. This number refers to the mesh of the sieve used to size the silica

(particle size), specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. The relationship is: the larger the mesh size, the smaller the adsorbent particles. Adsorbent particle size affects how the solvent flows through the column. Smaller particles (higher mesh values) are used for flash chromatography; larger particles (lower mesh values) are used for gravity chromatography. For example, 70–230 silica gels are used for gravity columns and 230–400 mesh for flash columns.

Alumina is used more frequently in column chromatography than it is in TLC. Alumina is quite sensitive to the amount of water, which is bound to it, the higher its water content, the less polar sites it has to bind organic compounds, and thus the less "sticky" it is. This stickiness or activity is designated as I, II, or III, with I being the most active. Alumina is usually purchased as activity I and deactivated with water before use according to specific procedures. Alumina comes in three forms: acidic, neutral, and basic. The neutral form of activity II or III, 150 mesh, is most commonly employed.

1.1.3. THE SOLVENT

The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds are elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. TLC is generally used to determine

the system for a column chromatography separation. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound.

1.1.4. INTERACTIONS OF THE COMPOUND AND THE ADSORBENT

Compounds interact with the silica or alumina largely due to polar interactions. These interactions are discussed in the section on TLC.

1.1.5. ANALYSIS OF COLUMN ELUANTS

If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labeled tubes and the composition of each fraction is analyzed by thin layer chromatography.

1.2 CHROMATOGRAPHY THEORY

Chromatography is a separation method that exploits the differences in partitioning behavior between a mobile phase and a stationary phase to separate the components in a mixture. Components of a mixture may be interacting with the stationary phase based on charge, relative solubility or adsorption. There are two theories of chromatography, the plate and rate theories.

1.2.1.RETENTION

The retention is a measure of the speed at which a substance moves in a chromatographic system. In continuous development systems like HPLC or GLC, where

the compounds are eluted with the eluent, the retention is usually measured as the *retention time,* R_t or t_R, the time between injection and detection. In interrupted development systems like TLC the retention is measured as the *retention factor* R_f, the run length of the compound divided by the run length of the eluent front:

$$R_f = \frac{\text{distance moved by the compound}}{\text{distance moved by the eluent}}$$

The retention of a compound often differs considerably between experiments and laboratories due to variations of the eluent, the stationary phase, temperature, and the setup. It is therefore important to compare the retention of the test compound to that of one or more standard compounds under absolutely identical conditions.

Figure 1.1:
Column Chromatography

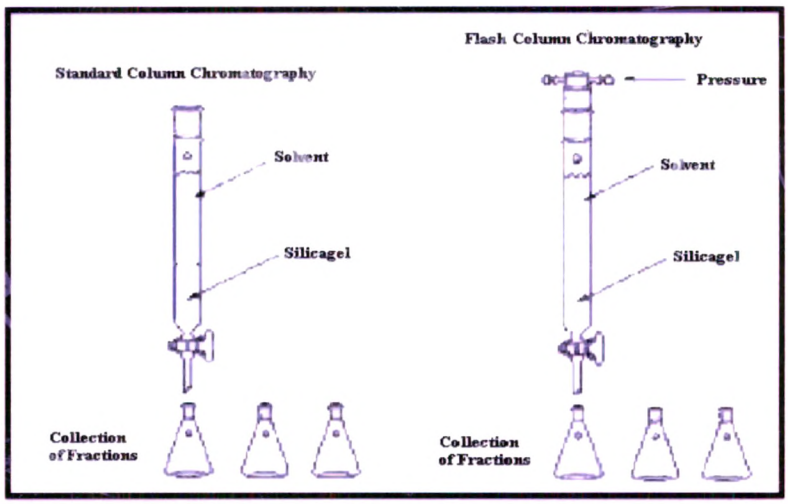


Figure 1. 2: Column Chromatography seperation system

Figure: 1.3 Thin Layer Chromatography

1.3 THIN LAYER CHROMATOGRAPHY (TLC)

TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate).

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate (Figure 1.3). The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that is applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components are differ in solubility and in the strength of their adsorption to the adsorbent and some components have been carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluor which fluoresces everywhere except where an organic compound is on the plate.) The procedure for TLC, explained in words in the above paragraphs, is illustrated with photographs on the TLC.

1.3.1. TLC ADSORBENT

Generally alumina (Al_2O_3) and silica gel (SiO_2) can be used as an adsorbent for TLC. In some circumstances, other visualization methods are used, such as the plate is held under a UV lamp. 5.3.2 TLC Solvents or Solvent Systems

Different types of solvents like hexane, ethyl acetate, methanol, benzene, chloroform etc can be used as a solvent in different ratios depending upon the nature of the polarity of the components present in the mixture.

1.3.2 INTERACTIONS OF THE COMPOUND AND THE ADSORBENT

The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar Si-O bonds of these adsorbents and tend to stick or adsorb onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules thus generally tend to move through the adsorbent more rapidly than the polar specie

1.4 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

1.4.1 INTRODUCTION

Nuclear Magnetic Resonance spectroscopy is a powerful and theoretically complex analytical tool. On this page, we cover the basic theory behind the technique. It is important to remember that, with NMR, we are performing experiments on the nuclei

of atoms, not the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei.

1.5 A FEW OF THE TECHNIQUES ADOPTED GENERALLY FOR STRUCTURE ELUCIDATION

1.5.1 NUCLEAR SPIN AND THE SPLITTING OF ENERGY LEVELS IN A MAGNETIC FIELD

Subatomic particles (electrons, protons and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C) these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in some atoms (such as ^{1}H and ^{13}C) the nucleus does possess an overall spin.

1.5.2 THE ABSORPTION OF RADIATION BY A NUCLEUS IN A MAGNETIC FIELD

The nucleus of an atom behaves as a "precessing top" when applied a magnetic field and the axis of rotation will precess around the applied magnetic field as shown in figure 1.4. If energy is absorbed by the nucleus, then the angle of precession, will change. For a nucleus of spin 1/2, absorption of radiation "flips" the magnetic moment so that it opposes the applied field (the higher energy state) as shown in figure 1.5.

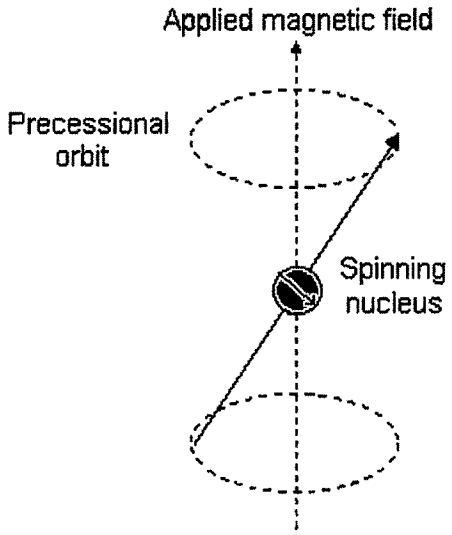


Figure 1.4: Spinning nucleus under applied magnetic field

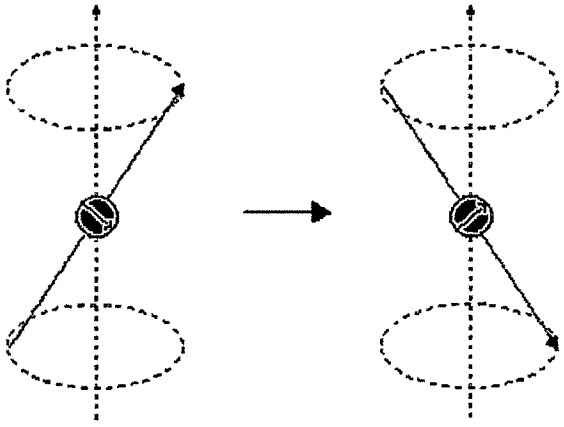
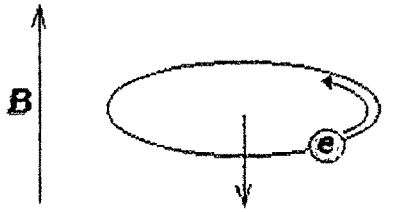


Figure 1.5: Spinning nucleus at higher energy state

1.5.3 CHEMICAL SHIFT

When a magnetic field is applied, electrons around the nucleus shield it from the applied field. The difference between the applied magnetic field and the field at the nucleus is termed the *nuclear shielding.*

Consider the s-electrons in a molecule. They have spherical symmetry and circulate in the applied field, producing a magnetic field which opposes the applied field. This means that the applied field strength must be increased for the nucleus to absorb at its transition frequency. This *upfield shift* is also termed *diamagnetic shift.*



Magnetic field produced by circulating electron

Electrons in p-orbitals have no spherical symmetry. They produce comparatively large magnetic fields at the nucleus, which give a *low field shift.* This "deshielding" is termed *paramagnetic shift.*

In proton (^{1}H) NMR, p-orbitals play no part (there aren't any!), which is why only a small range of chemical shift (10 ppm) is observed. We can easily see the effect of s-electrons on the chemical shift by looking at substituted methane, CH_3X. As X becomes increasingly electronegative, so the electron density around the protons decreases, and they resonate at lower field strengths (increasing δ_H values).

Chemical shift is defined as *nuclear shielding / applied magnetic field.* Chemical shift is a function of the nucleus and its environment. It is measured relative to a reference compound. For ^{1}H NMR, the reference is usually tetramethylsilane, $Si(CH_3)_4$.

1.5.4 SPIN - SPIN COUPLING

Another important experiment of NMR Spectroscopy is Spin-Spin Coupling. This occurs because there is a small interaction (*coupling*) between the two groups of neighbouring protons. The spacings between the peaks is measured in Hertz and is called the *coupling constant, J.*

1.6 MASS SPECTROMETRY

Mass spectrometry (also known as mass spectroscopy (deprecated) or informally, "mass-spec" and MS) is an analytical technique used to measure the mass-to-charge ratio of ions (M/Z). It is most generally used to find the composition of a physical sample by generating a mass spectrum representing the masses of sample components.

All mass spectrometers consist of three basic parts: an ion source, a mass analyzer, and a detector system. The stages within the mass spectrometer are:

- Producing ions from the sample
- Separating ions of differing masses
- Detecting the number of ions of each mass produced
- Collating the data and generating the mass spectrum
- The technique has several applications, including: identifying unknown compounds by the mass of the compound molecules or their fragments determining the isotopic composition of elements in a compound determining the structure of a compound by observing its fragmentation.
- Quantifying the amount of a compound in a sample using carefully designed methods (mass spectrometry is not inherently quantitative)
- Studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in vacuum)

The isotopes of each of the chemical elements have different masses. This fact is used in a mass spectrometer to determine a) what elements are present in a sample and b) the isotopic compositions of those elements. Chemical compounds are fransfered to ions with various techniques and are accelerated in a controlled electric magnetic field. The path of the charged particles can be controlled with an electric or magnetic field. This force deflects the ions (makes them curve instead of traveling straight) to differing degrees depending on their mass to charge ratio. The lighter ions are deflected more than the heavier ions. Thus the magnetic field deflects the lighter ions more, and the heavier ions less. The detector measures the deflection of each resulting ion beam. From this measurement, the mass-to-charge ratios of all the ions produced in the source can be determined. From this information it is possible in principle to determine the chemical composition of the original sample (i.e., in principle, that both sodium and chlorine are present in the sample) and the isotopic compositions of its constituents.

1.7 IR-SPECTROSCOPY

Infra red (IR) absorption spectroscopy is a power full tool in the study of the basic structure of chemical properties of molecules or the functional groups present in the organic compound and the effect of different chemical treatments on the behavior of these groups have been studied with the help of Infrared spectroscopy. The analysis of I.R. absorption spectra is greatly facilitated by the fact that certain grouping in the molecules gives rise to characteristic group frequencies which in most cases are not strongly affected by neighboring atoms & IR-Spectroscopy gives us the information about the vibration of the atoms in a particular compound like stretching, bending, twisting etc. From the characteristic frequencies the nature of the functionalities can be determine. Surface and fine structural characteristic of cotton fibres are investigated by

Bora and Talukdar[5] with the help of Infrared spectroscope and found that the structural behaviours with set up of chemical bond of the cellulosic cotton fibre remain unaltered in caustic and treatments structural properties of cellulose materials are investigated by several workers [4-9]. Nadiger et.al [10-11] studied the structural and chemical properties of natural Indian silks with the help of Infra-red spectroscopy. Baruah et.al [12] studies these properties of muga, eri and pat fibres. For estimation of linier in Jute fibre, B.N. Bandyopadhyay et.al. used the I.R. spectroscopic method [13] Rowen and Plyler [14] describe the effects of deuteratian , oxidation and hydrogen banding of the Infra –red spectrum of Cellulose. Mann and Marrinan [15] have carried out detailed studies of deuterium exchange with cellulose and shown that the exchange takes place first in the amorphous takes place first in the amorphous regions. The crystalline regions are affected only after considerable time. In all the forms of cellulose, there is no free O-H stretching frequency in the crystalline region and all the hydroxyl groups are hydrogen bonded[16] for detailed information on the structural properties of cellulose materials several workers,[17,18] have used the potassium bromide disc technique to record the Infra-red spectra. The Infra-red absorption spectrum of cellulose shown many bands date are poorly resolved or of lower intensity. But second derivative infra red spectra may provide more detailed on structural information. Panday[19, 20] studies the derivative infra red spectra of cotton and ramie cellulose. Talukdar et.al.[21] studied the functional groups present in some plant fibres and effect of chemicals and temperature on the behaviour of these groups with the help of Infra red spectroscopic method.

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