CHAPTER 1

# Chromatography

## 1.1 Introduction

The Russian-Italian botanist M. S. Tswett devised chromatography, a physicochemical technique for separating complicated mixtures, at the very beginning of the 20th century. Few reliable chromatographic techniques were commercially accessible for laboratory scientists until the 1970s. In the 1970s, a number of methods, such as open-column chromatography, paper chromatography, and thin-layer chromatography, were used to perform the majority of chemical separations. These chromatographic methods, however, could not provide sufficient resolution between similar compounds or for compound quantification.

Now-a-days, chromatography is a very flexible technology that can separate gases, volatile substances, and in-volatile chemicals from materials with extremely high molecular weights (including biopolymers) using GC, in-volatile chemicals, and materials using LC, and if necessary, very cheaply using TLC.

## 1.2 Various Types of Chromatography

Chromatography can be categorized in several ways. (I) Techniques based on solute- stationary phase interaction, (II) Techniques based on chromatographic bed shape, and (III) Techniques based on mobile phase physical state.

## 1.3 Techniques based on Solute-stationary Phase Interaction

### 1.3.1 Adsorption Chromatography

One of the most traditional forms of chromatography is likely the adsorption chromatography. It makes use of a mobile liquid or gaseous phase that has been adsorbed onto a surface of stationary solid phase. The

separation of various solutes is caused by the equilibrium between the mobile and stationary phases.

### 1.3.2 Partition Chromatography

This chromatography technique is based on the formation of a thin film by a liquid stationary phase on the surface of a solid support. Between the stationary liquid and the mobile phase, the solute reaches equilibrium.

### 1.3.3 Ion Exchange Chromatography

Anions or cations are covalently attached to a resin (the stationary solid phase) in this form of chromatography. Electrostatic forces cause solute ions with the opposite charge in the mobile liquid phase to be drawn to the resin.

## 1.4 On the basis of Chromatographic Bed Shape

### 1.4.1 Column Chromatography

In the separation method known as column chromatography, the stationary bed is contained inside a tube. The mobile phase may have an open, unconstrained passage through the center of the tube while the solid stationary phase or support covered with a liquid stationary phase fills the whole interior volume of the tube (packed column) or is centered or along the interior tube wall (open tubular column). Different retention durations for the sample are determined based on variations in sample movement speeds through the medium.

### 1.4.2 Paper Chromatography

A little dot or line of sample solution is applied to a strip of chromatography paper as part of the paper chromatography procedure. The paper is put inside a sealed jar with a thin coating of solvent. The sample mixture begins to ascend the paper with the solvent as it climbs through the paper and meets it. Because the cellulose in this paper is polar, non- polar compounds within the mixture move more slowly. More polar chemicals easily bind with the cellulose paper and hence do not travel quite far.

### 1.4.3 Thin Layer Chromatography

Similar to paper chromatography, thin layer chromatography (TLC) is a common laboratory method. However, it uses a stationary phase comprising a thin layer of adsorbent like silica gel, alumina, or cellulose over a flat, inert substrate, in contrast to employing paper as the stationary phase. Faster runs, better separations, and the option of multiple adsorbents are advantages over paper. High-performance TLC can be employed for even better resolution and for enabling quantification.