THE FASCINATING JOURNEY OF CHROMATOGRAPHY

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Chromatography has numerous applications in biological and chemical fields. It is widely used in biochemical research for the separation and identification of chemical compounds of biological origin. Chromatography is employed to analyse complex mixtures of hydrocarbons. As a separation method, chromatography has a number of advantages over other techniques such as crystallization, solvent extraction and distillation. It is capable of separating all the components of a multi-component chemical mixture without requiring an extensive fore knowledge of the identity, number or relative amounts of the substances present. It is versatile in that it can deal with molecular species ranging in size from viruses composed of millions of atoms to the smallest of all molecules – hydrogen– which contains only two atoms. Furthermore, it can be used even with large or small amounts of material. Some forms of chromatography can detect substances present at the pictogram (10^{-12} gram) level, making this method a superb trace analytical technique extensively. It is used in the detection of chlorinated pesticides in biological materials and the environment, in forensic science, and in the detection of both therapeutic and abused drugs. Its resolving power is unequalled among separation methods.

Chromatography is defined as a technique for separating the components or solutes of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase may be either a solid or a liquid or a gas, while the stationary phase may be either a solid or a liquid. The kinetic molecular motion continuously exchanges solute molecules between the two phases. If, for a particular solute, the distribution favours the moving fluid, the molecules will spend most of their time migrating with the stream and will be transported away from other species whose molecules are retained longer by the stationary phase. For a given species, the ratio of the time spent in the moving and stationary regions is equal to the ratio of its concentrations in these regions, known as the partition coefficient. A mixture of solutes is introduced into the system in a confined region or narrow zone (the origin), whereupon the different species are transported at different rates in the direction of fluid flow. The driving force for solute migration is the moving fluid, and the resistive force is the solute affinity for the stationary phase; the combination of these forces, on manipulation by

the analyst, produces the separation of various components.

The first purely pragmatic application of chromatography was with dyes. Various dye mixtures were tested by dipping strings or pieces of cloth or filter paper into a dye vat. The dye solution migrated up the inserted material by capillary action, and the dye components produced bands of different colours. In the 19th century, several German chemists carried out several experiments to explore the application of chromatography. They observed, for example, the development of concentric, coloured rings by dropping solutions of inorganic compounds onto the center of a piece of filter paper. A treatise was published in 1861 describing the method and giving it the name "capillary analysis".

The discovery of chromatography is generally attributed to the Russian Botanist Mikhail S. Tsvet, because he recognised the physicochemical basis of separation of plant pigments, particularly the carotenoids and the chlorophylls. Tsvet's book, published in 1910, described a technique that is used today in essentially the same form. He packed a vertical glass column with an absorptive material, such as alumina, silica or powdered sugar, added a solution of the plant pigments to the top of the column, and washed the pigments through the column with an organic solvent.

The pigments separated into a series of discrete coloured bands on the column, divided by regions entirely free of pigments. Because Tsvet worked with coloured substances, he called the method chromatography (from Greek word meaning colour writing). Tsvet's development of chromatographic procedures was generally unknown to chemists in the Western world because he published either in German botanical journals or in Russian works. In 1931, chromatography emerged from its relative obscurity when the German chemist Richard Kuhn and his student, the French chemist Edgar Lederer, reported the use of this method in the resolution of a number of biologically important materials. In 1941 two British chemists, Archer J.P. Martin and Richard L.M. Synge, began a study of the amino acid composition of wool.

Their initial efforts, in which they used a technique called liquid-liquid countercurrent distribution, failed to give them adequate separation. Therefore, they conceived of an alternative method in which one liquid was firmly bound to a finely granulated solid packed in a glass tube and a second liquid, immiscible with the first, was percolated through it. Silica gel served as the granular solid. Martin and Synge pictured silica gel as composed of water tightly bonded to the crystals of silica. The mobile phase was Chloroform. Their work with this technique was remarkably successful. Although their method was mechanically identical with Tsvet's approach, it was innovative as it involved the concept of a stationary liquid (water) supported on an inert solid (silica), with the result that the solute molecules partitioned between the stationary liquid and a separate mobile liquid phase (chloroform).

The technique used by Martin and Synge came to be called partitioned chromatography. At that time, they suggested that the moving phase could well be a gas. It is a historical oddity that this idea was overlooked for nearly a decade, possible because of the war, until Martin in collaboration with British chemist Anthony T. James initiated

studies of gas-liquid partition chromatography. In 1952, Martin and Synge were awarded the Nobel Prize for their work, perhaps not so much for the newness of the technique but for a model that suggested other systems, a mathematical theory, and applicability to amino acids and peptide separations with far reaching impact on biochemical studies.

The initial partition-chromatography system presented difficulties because of lack of reproducibility in the properties of the silica gel and lack of uniformity in the packing of columns. Partly for this reason, Martin and his co-workers worked out a new procedure in which the stationary medium was a sheet of filter paper. The paper was thought of as water bonded to cellulose, providing another partition method. The technique gave the desired reproducibility, and from the beginning of 1940s, paper chromatography found wide application in the analysis of biologically important compounds, such as amino acids, steroids, carbohydrates and bile pigments. In this field, to a large extent it replaced the column technique initiated by Tsvet.

Motivated probably by the same drawbacks to column chromatography, two soviet pharmacists, Nikolay A. Izmaylov and Maria S. Shrayber, distributed the support material as a thin film on a glass plate. The plate and support material could then be manipulated in a fashion similar to that of paper chromatography. The results of the Soviet studies were reported in 1938, but the potential of the method was not widely realised until 1956, when the German chemist Egon Stahl began intensive research on its application. This system became known as Thin Layer Chromatography (TLC).

Yet another chromatographic technique, gas chromatography, was first carried out in Austria in 1944 by the chemist Erika Cremer, who used a solid stationary phase. The first extensive exploitation of the method was made by Martin and James in 1952, when they reported the elution gas chromatography of organic acids and amines. In this work, small particles of support material were coated with a non-volatile liquid and packed into a heated glass tube. Mixtures injected into the inlet of the tube and driven through by compressed gas appeared in well separated zones. This development was immediately recognised by petroleum chemists as a simple and rapid method of analysis of the complex hydrocarbon mixtures encountered in petroleum products. British Petroleum Co. and Shell Oil Co. immediately began basic research in their own laboratories. Various instrument manufacturing companies envisaging an extensive market, also made major contributions.

In 1957, while doing a theoretical study of gas chromatographic columns, Marcel J.E. Golay, as a consultant for the Perkin-Elmer Corporation, concluded that a very long column (90 to 180 metres or 300 to 600 feet) of narrow–diameter tubing (internal diameter of 0.25 millimetres) with its wall coated with a thin film of liquid would yield superior separations. Fortunately, at about the same time, detectors with extremely low limits of detection, which could sense the small sample sizes required by these new columns became available. These capillaries, or Golay columns, now called open tubular columns and characterised by their open design and an internal diameter of less than one millimetre, had an explosive impact on chromatographic methodology. It is now possible

to separate hundreds of components of a mixture in a single chromatographic experiment.

Molecular sieves are porous substances that trap a mobile-phase gas. Large molecules cannot enter the pores. Therefore they flow unimpeded through the system. Small molecules are interrupted in their migration as they meander in and out of the pores by diffusion. Molecules of intermediate sizes show different rates of migration, depending on their size. In 1959 Per Flodin and Jerker Porath in Sweden developed cellulose polymeric materials that acted as molecular sieves for substances dispersed in liquids. This extended the molecular weight range of chromatography to polypeptides, proteins and high molecular weight polymers. The generic term for such separations is Size-Exclusion Chromatography (SEC).

In 1964, an American chemist, J. Calvin Giddings, referring to a theory largely worked out for gas chromatography, summarised the necessary conditions that would give liquid chromatography the resolving power achievable in gas chromatography, that is, very small particles with a thin film of stationary phase in small-diameter columns. The development of the technique now termed High-Performance Liquid Chromatography (HPLC) depended on (i) the development of pumps that would deliver a steady stream of liquid at high pressure to the column to force the liquid through the narrow interstitial channels of the packed columns at reasonable rates, and (ii) detectors that would sense the small sample sizes mandated. At first, only adsorptive solids were used as the stationary phase, because liquid coatings were swept away by the mobile phase.

Previously gas chromatography had employed chemical bonding of an organic stationary phase to solids to reduce adsorptive activity; Istvan Halasz of Germany exploited these reactions to cause a separation based on liquid solution effects in the bonded molecular layers. These and similar reactions were employed to give firmly attached molecules that acted as a thin film of solvent in liquid systems. These bonded phases gave high-performance liquid chromatography such scope and versatility that the technique is now a dominant method for separations.

Ion exchangers are natural substances. For example, certain clays or deliberately that synthesised resins, containing positive ions (cation exchangers) or negative ions (anion exchangers), that exchange with those ions in solution that have a greater affinity for the exchangers. This selective affinity of the solid is called Ion, or cation Exchange Chromatography (IEC). The first such chromatographic separations were reported in 1938 by T.I. Taylor and Harold C. Urey, who used a zeolite. The method received much attention in 1942 during the Manhattan Project as a means of separating the rare earths and trans-uranium elements, fission products of uranium and other elements produced by thermonuclear explosions. Ion-exchange chromatography can be applied to organic ion separations and is of special importance for the separation of amino and nucleic acids.

The solubility of solids in gases at high pressure had been observed as early as 1879. In 1958, the British scientist James Lovelock suggested that gases above their critical temperature (i.e., the temperature above which a liquid phase cannot be produced by increasing the pressure) might be

used at high pressure as mobile phases. A substance in this state is termed a supercritical-fluid. At very high pressure, the density of the fluid can be 90 per cent or more of the liquid density. The German chemist Ernst Klesper and his colleagues, working at the Johns Hopkins University, were the first to report the separation of the porphyrins with dense gases in 1962. Carbon dioxide at 400 atmospheres (one atmosphere equals 760 millimetres or 29.92 inches of mercury; standard sea-level pressure is one atmosphere) is a typical supercritical fluid mobile phase. In an extreme case, Giddings and his groups used gases at pressures of up to 2,000 atmospheres to chromatograph carotenoids, sugars, nucleosides, amino acids and polymers. Supercritical fluid chromatography brings a gap between gas chromatography and liquid chromatography. In a gas chromatography concentration of solutes in the gas phase is achieved with increased temprature. Supercritical-fluid chromatography achieves this result with increased pressure so that thermally unstable compounds may be analysed. Additional advantages include increased speed and resolution.

A technique exhibiting great selectivity, affinity chromatography, was first described by Pedro Cuatrecasas and his co-workers in 1968. In these separations, a biomolecule such as an enzyme binds to a substrate attached to the solid phase while other components are eluted. The retained molecule can subsequently be eluted by changing the chemical conditions of the separation.

Another separation technique is based on the fact that the velocity of a fluid through a tube is not uniform. In the region immediately adjacent to the wall the fluid is nearly stationary. At distances farther from the wall, velocity increases, reaching the maximum value at the centre of the channel. In 1966, Giddings conceived the idea that a field, electrical or gravitational, might be used to selectively attract particles to the wall, where they will move slowly through the system. Diffusion, away from the high concentrations at the wall, into faster inner streams would enhance migration. The net effect would yield differential migration. A thermal gradient between two walls has also been used. This recently developed technique is called field-flow fractionation. It has been termed one-phase chromatography because there is no stationary phase. Its main applications are to polymers and particulate matter. One phase chromatography has been used to separate biological cells, sub-cellular particles, viruses, liposomes, protein aggregates, fly ash, colloids and pigments.