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Solving Crimes with Chromatography

Introduction

Someone recently set a building on fire, burning it and the occupants to ashes. Because detectives are unable to find good suspects, in theory anyone in the world could have burned the building. What do we do? We use chromatography, a scientific process so sensitive that it is capable not only of identifying different molecules in a sample, but also in some cases, identifying the particular source of the sample. In that event, forensic scientists can use the evidence from chromatography to track down the source of the sample. They can test residues from the crime scene to know within hours the means and method of fire propagation; subsequently, other investigators can use the chromatographic information to check records of who purchased such materials, leading to the arrest of the suspect.

Although chromatography is indispensable in many different areas, it is particularly useful in forensic science, “the study and practice of applying science to the purposes of justice” (1). Other areas where chromatography is extensively used include the medical and pharmaceutical industry, environmental (air, water, and wildlife) monitoring, and the food, petroleum, and synthetic materials industries. Thus, it becomes clear why Andras Guttman of Diversa Corp said that the three most significant accomplishments of the 20^{th} century that fundamentally changed our lives were computers, cloning, and chromatography (Flavell-While).

Description of Chromatography

Chromatography is the name given to a set of separation techniques that are used for analyzing a variety of mixtures. It is an extraction process that proceeds continuously through the flow of a mobile phase that moves relative to a stationary phase. The solute species of interest enters the chromatograph in the mobile phase; it then partitions between the two phases. Partitioning solutes can be differentiated and identified based on the time they spend in the stationary phase relative to the mobile phase. Different types of chromatography use different types of mobile and stationary phases (Oxtoby 608). University of Tennessee's David Stafford writes that chromatography's "power is in separation; its weakness is identification" (3). Therefore, in many cases the identification of separated substances is enhanced by connecting the chromatograph to a detector or identifier (4). By far the most common additional apparatus is a mass spectrometer. Mass spectroscopy vaporizes the unknown compound and bombards it with electrons to separate it into small charged fragments. These fragments travel in a circular path when subjected to a magnetic field (Loudon 558-559). Whether by speed of movement or radius of path, the mass spectrometer detects the masses of the fragments, helping scientists to determine the identity of the previously unknown compound (Loudon 569).

Brief History

Perhaps the earliest example of chromatography is a story from the book of Exodus of the Pentateuch. When the people complained about the extremely bitter waters they were drinking, Moses, as commanded by God, took a tree and plunged it into the waters. The taste of the water became sweeter, the natural result of ion-exchange chromatography between the tree and the bitter water (Ettre 10).

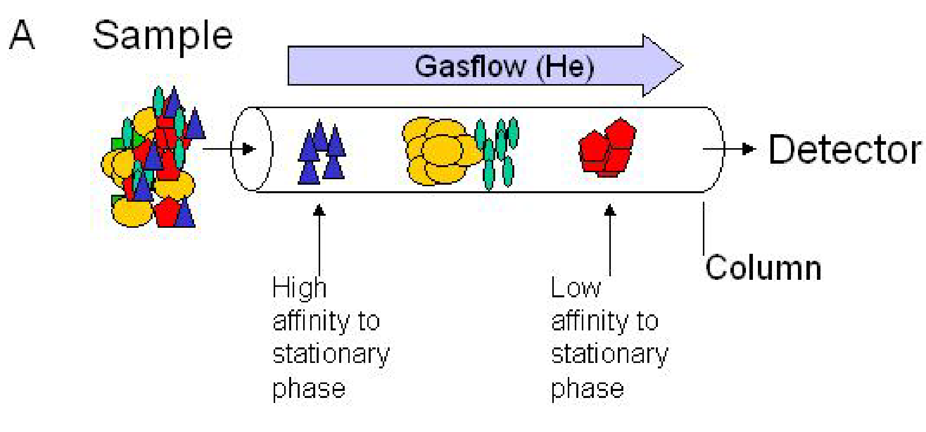
A later precursor of chromatography was Friedlieb Ferdinand Runge's experiments: he formed multicolored pictures by applying different dyes to the center of a filter paper (Ettre 20). However, because Runge did no analysis, but only goodhearted fun, his dye experiments cannot be considered true chromatography (Ettre 28).

Chromatography began with a short lecture on March 21, 1903 by Dr. Mikhail Semenovich Tswett, a Russian plant scientist. Chromatography owes its name to Dr. Tswett. Although a popular belief is that "chromatography" means "color-writing" ("chroma-graphein," in connection with Runge's color separations), the word "Tswett" means "color" in Russian; thus perhaps, the meaning of "chromatography" is actually "Tswett's writing" (Ettre 66). Dr. Tswett's twin papers of 1906 formalized his procedure, an early form of modern liquid chromatography (Ettre 1). However, because Tswett's findings initially met the criticisms of many detractors, they did not garner widespread acceptance (Ettre 62). Later, scientists began to realize the superior separation capable with chromatography; the technique "took off" in the 1940's and 1950's and beyond, stimulated by new processes in petroleum refining and petrochemistry that required the analytical controls provided by gas chromatography (Ettre Chromatography). Indeed, although Tswett died before he could win the Nobel Prize, British scientists Archer John Porter Martin and Richard Laurence Millington Synge won the Prize in 1952 for improving chromatographic techniques, especially those with applications to biochemistry (10).

Different Types of Chromatography

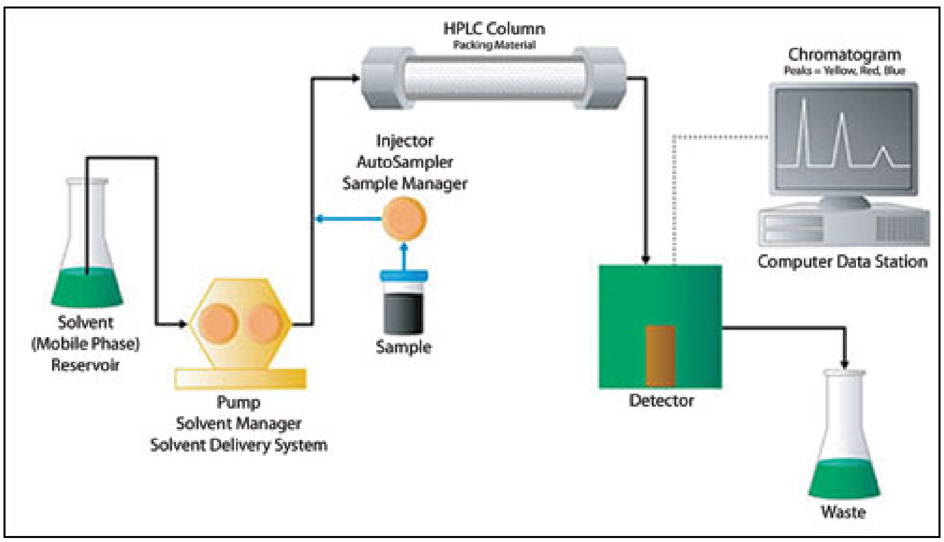
Gas chromatography means "gas-liquid partition chromatography." Developed in 1950, it uses an inert carrier gas (such as nitrogen or helium) as the mobile phase, and a liquid stationary phase as coating on the inner column walls. Before introduction into the column, the analytes are vaporized due to the application of heat (Niessen). One type of gas chromatography, glass capillary gas chromatography (more properly called open tube or open tubular gas chromatography) uses open columns instead of packed columns (Ettre Applications). Figure 1 shows a mixture of several substances represented by different symbols. In the tube, these substances become separated when helium gas moves through the tube. The substances with the least attraction to the stationary phase are the first to pass into the mass spectrometer for further analysis.

Figure 1. Gas-Liquid Chromatography



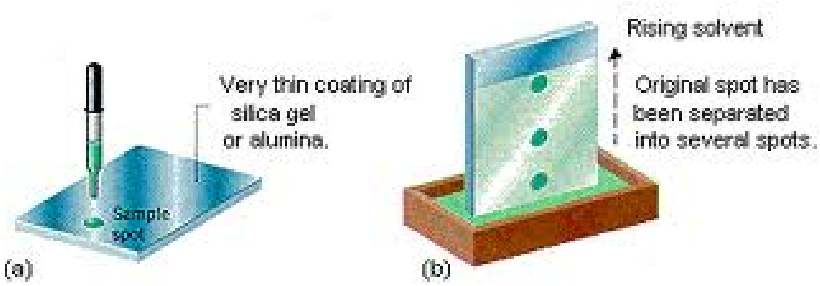
Liquid-liquid chromatography began with Tswett, who dissolved chlorophyll in polar and nonpolar solvents to resolve components in his experiments investigating plant pigments (Ettre Chromatography, Ettre Milestones). However, gas-liquid chromatography became dominant in the 1950's and the most widely used scientific technique because the slow diffusion in the liquid mobile phase limited the efficiency of liquid-liquid chromatography. This changed when Yale chemical engineering professor Csaba Horvath revolutionized liquid chromatography by introducing "high pressure, sample injections at [high] pressure[], accurate flow controls, and continuously monitoring detectors without band spreading" (Ettre Chromatography). These developments improved traditional liquid-liquid chromatography so much that this new type acquired a different name: HPLC (high pressure liquid chromatography or high power/performance liquid chromatography). An advanced form of HPLC is multidimensional liquid chromatography, which is easier for the mind to process because it shows the data in two or three dimensions. (Cohen Foreword, Introduction). Today, HPLC is the most commonly used form of chromatography (Ettre Chromatography). Figure 2 shows a general HPLC system. The mobile liquid phase containing the sample moves through a column, and a pump applies pressure, making the solvent flow faster. At the end, a secondary apparatus (the detector) detects the residence time of the sample and sends the information to a computer.

Figure 2. HPLC



Another widespread chromatography technique is thin-layer chromatography (TLC). This is a form of planar chromatography that proceeds on a sheet of glass or plastic containing a smooth fixed sorbent such as silica gel (the stationary phase) placed in an organic or aqueous solvent (the mobile phase). The sample is applied as a small dot to the plate and rises with the movement of the solvent up the plate by capillary action (Fried 3, (2)) as shown in Figure 3, where the sample spot added to the plate in part a) becomes three different spots in part b) due to the solvent rising up the plate after a given amount of time. These spots represent different substances present in the original sample.

Figure 3. TLC



Drugs and Toxicology

Samples obtained from most crime scenes consist of complex mixtures, requiring techniques for separation of these mixtures into their pure components (5). Refinement for this detective work continues today. A recent example is provided by a new Thermo Scientific solution invented by Thermo Fisher Scientific Inc. In recent years, this invention has used liquid chromatography and mass spectroscopy to screen and confirm forensic toxicology samples (6). While gas-liquid chromatography is a common tool for analyzing poisons and drugs in the human body, multidimensional separation has become a leading technique in peptidomics because of its finer scale and increased resolution. Peptidomics studies peptides that indicate biological processes and protein degradomes. Indeed, liquid chromatography has been an effective method for body-sample analysis because physiological and pathological characteristics are evident in the metabolism of proteins and peptides; these products can be isolated with chromatography (Machtejevas). Dr. Marquet Hoja asserts that “nearly 70% of everyday samples in the toxicological laboratory can be handled by liquid chromatography” (1).

When exploring illegal steroid use, thin-layer chromatography often aids the investigator (Fried 373). Glass capillary gas chromatography can also examine steroid samples. Dr. Carl Pfaffenberger notes that glass capillary gas chromatography has achieved satisfactory separations for steroids and steroidal metabolites, tobacco smoke, marijuana constituents, and all types of volatile constituents of physiological fluids, including serum and urine. Thousands of components in tobacco smoke can be separated using glass capillary gas chromatography (Pfaffenberger). As early as 1973, glass capillary gas chromatography unequivocally determined that the accidental death of a child was the result of poisoning with a pine oil preparation (Pfaffenberger). Chromatography allows these tests to proceed efficiently because it is capable of providing exact drug concentrations in the body from easily and quickly attainable bodily components, such as hair, nails, sweat, and saliva (Brettell).

Causes of Death

Television shows such as CSI (Crime Scene Investigation) have brought attention to perhaps a more exciting application of chromatography in toxicology: dead bodies. Scientists use toxicological analysis to clarify when the cause of death is known, when it is certain that drugs directly resulted in death, and when it is believed that disease was the primary factor responsible for death (5). Dr. Robert Middleberg, the Laboratory Director at National Medical Services, says that many of the current practices of toxicologists can seem archaic: “When we want to confirm carbon monoxide poisoning, for example, we use a little white disk where we put chemicals, mix, and come back later to look for a color change. It's easy and it works” (5).

Dr. Jan Bocxlaer writes that liquid chromatography – mass spectroscopy systems are useful for "measuring drug concentrations in body fluids, identifying the nature of evidence materials, and investigating the implication of toxins in suicide or…homicide cases." Further, he points to the plethora of legal cases where scientific evidence was the determining factor (1). Such cases display the usefulness and reliability of chromatographic methods. Often, small pieces of evidence are left at crime scenes, including paint, fibers, and various polymers. These fragments can go a long way towards assisting investigators in solving crimes. In one example, gas chromatography helped to characterize rubber from a truck tire and a hammer handle. In another case, gas chromatography discovered a link between a rubber bumper guard of a suspect car to one that had been obtained at a hit-and-run scene (Brettell).

Arson and Explosives

Forensic scientists employ various types of chromatography in cases of arson or in cases where damage was done by explosives. Dr. Krull says that gas chromatography and high-power liquid chromatography are common tools for investigation of explosives or arson residues (Krull). The U.S. Fire Administration's statistics show that arson is the leading cause of fire and the second leading cause of deaths and injuries (7). In these cases, chromatography provides an essential technique because the fires result from freely available fuels that contain hundreds of components; a thorough study of these fuels requires the separation of the residues into their constituent compounds. Gas chromatography can separate these compounds easily, with temperature programming and fused-silica columns separating the wide-boiling-point range of petroleum distillates (Brettell). A type of gas chromatography, called pyrolysis gas chromatography, is often the method of choice for establishing the identity of accelerants in arson cases (8). The advantage of this method is its ability to "convert a nonvolatile organic material into a number of volatile organic compounds that can be separated by gas chromatography” and then identified (Brettell).

Thin-layer chromatography can be used to detect chemical weapons, explosives, and illicit drugs. Advances in TLC technology have been driven by the threat of terrorism. The Forensic Service Center of Lawrence Livermore National Laboratory recently constructed a computerized and portable TLC machine that can function on-site and analyze 20 samples simultaneously with results emerging in just 30 minutes (4). For explosives, the main complication comes from interfering compounds in the post-blast debris. Gas chromatography separates the explosives from interfering substances and unambiguously identifies the explosive when used in conjunction with a sensitive detector (Brettell).

Determining a Forged Document

Ink analysis is a concrete method where chromatography advances forensic science and benefits society. Ink has been around for about 5,000 years, but today, when important matters are settled by documents, the study of the ink used to sign or write these documents aids legal investigation. Using chromatography, scientists can establish the manufacturer, brand, composition of ink. The leading technique for this analysis has been TLC. Previously, the United States Secret Service’s International Ink Library contained analyses of over 10,000 inks, but many of the ink samples and chromatography results have faded. Since 2009, The U.S. Secret Service, with the Department of Homeland Security and Technology Directorate, has been working to digitize the information in the International Ink Library. Robert Ramotwoski, a chief research scientist with the Secret Service Forensic Services Division, explains the advantages of this database: “With this digitized search system, search times through the repository are reduced to minutes, resulting in a list of potential candidates available for comparison” (9). Already, over

9,000 ink samples have been put into the digital system, with the oldest coming from the 1920's. More than half of the library's use is for law enforcement investigations at the local, state, federal, and international level, most often to determine a document's authenticity. This repository of information has led crime examiners to determine that documents had been forged, solve threats against government officials, hunt down suspected terrorists, cut down on child pornography, and expose medical fraud (9).

Chromatography provides one of the most useful methods for obtaining pertinent information that can lead to identifying and apprehending criminals.

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