Chapter TWO, Materials and Methods

Terminology

At its most basic, pyrolysis is defined as a chemical transformation that occurs at temperatures above ambient. (Șerban and Victord, 2002, page 869) Pyrolysis as an analytical technique was first described in 1862. In analytical chemistry, pyrolysis experiments monitor the changes to sample mass as pyrolysis products are released as gasses and/or capture these gasses and subject them to additional characterization. Even a cursory glance at the literature reveals that there is little standardization over the terminology used to describe different types of pyrolysis processes used in analytical chemistry. Variations exist in the choice of pyrolysis temperatures, the rate of sample heating, the use of matrix additions to catalyze decomposition reactions, or matrix additions to prevent decomposition. Different procedures vary the atmosphere under which the sample is pyrolysed, or use catalysts to enhance product decomposition after heating and before analysis. There are other chemical transformations, most commonly oxidation, that can alter also be used to alter pyrolysis products prior to analysis.

As a practical matter however, pyrolysis usually describes rapid heating in the absence of oxygen. (Vercammen et al. 2012)

The author prefers the term “analytical pyrolysis” to describe his protocols although it might also be described as “flash pyrolysis.” In flash pyrolysis sample heating can be as fast as 10,000 K / sec. (Șerban and Victord, 2002 page 869) There are two methods used to reach such temperatures quickly, resistance heating and Curie point pyrolysis. The latter use dedicated ferromagnetic alloys are exposed to a radiofrequency (RF) field. Heating is extremely rapid until the material reaches its Curie point temperature, when it becomes paramagnetic. The advantage of this is that temperature control can be very precise but not variable. Resistance heating provides much more control over heating rates and final temperatures. If a sample is subjected to a sequence of heating steps it may be referred to as "sequential flash pyrolysis. Early versions of resistance heating pyrolyzers placed the sample directly on a hot wire. While this was a very simple approach, it increased the risk of unwanted catalytic decomposition. (Vercammen et al. 2012)

The CDS Model 1500 used in this study employs a coiled heating element fabricated from platinum wrapping around a small quartz tube that holds the sample.

A variation of flash pyrolysis is non-discriminating flash pyrolysis where rapid heating is achieved using a Silcosteel capillary. (Poerschmann J, Parsi Z, Gorecki T., Non-discriminating flash pyrolysis and thermochemolysis of heavily contaminated sediments from the Hamilton Harbor (Canada), Chromatogr A. 2008 Apr 4; 1186(1-2):211-21) In a typical system, the sample is placed directly in a disposable capillary and held in place using two plugs of glass wool. The GC carrier gas flows directly through the capillary and into a column interface. This approach dramatically reduces discrimination against high molecular weight compounds as there is no cold spots for them to condense between the pyrolysis site and the GC column. (Z. Parsi,
Pyrolysis is usually thought of as involving thermal decomposition. But many non-polymeric organic compounds that are somewhat volatile at elevated temperatures do not necessarily fragment upon pyrolysis. Pyrolysis technology provides an effective way of removing them from complex sample matrices and sometimes this is referred to as flash evaporation pyrolysis or EV-Py. (Wampler, Page 157)

Flash evaporation pyrolysis combined with GC/MS can be used to detect sedimentary contaminants at low concentrations. It has been used to screen complex matrices for PAHs, halogenated organics, aliphatic hydrocarbons, heteroaromatics, elemental sulfur, and cyanides. In one demonstration, five-milligram samples of lake sediments were spiked with PCBs and heated to 1000°C. Even with the mass spectrometer operating in full scan mode, detection at the 10 ppb level was achieved. However with an electron capture detector (ECD), the signal from the PCBs was obscured by the many other electron-capturing compounds released by the sample. (Wampler, page 158)

In contrast to flash evaporation pyrolysis, thermal distillation pyrolysis (TD-Py) uses a slow temperature rise to separate low boiling point intact compounds (generally from 100 - 200°C) and thermal decomposition products created in the 350-600°C range. (Wampler, page 156)

EV-Py and TD-Py overlap somewhat with Thermal Extraction in which heating is used to release organic molecules from sample matrices. An official USEPA analytical method, Method 8275a, uses thermal extraction and capillary GC/MS procedure for rapid quantitative determination of selected PCBs and PAHs in soils, sludges and solid wastes. This method however stresses the release of compounds and not their destruction. Therefore the sample is only heated to a maximum of 340°C. (USEPA, Method 8275a, Revision 1, September 1996)


Although many pyrolysis processes are carried out under inert atmospheres but it is possible to create additional reactions with a reactive atmosphere, the additions of another materials to the sample matrix, or to incorporate catalysts in the pyrolysis system. The most common added
reactants are oxygen, hydrogen, water, and quaternary N-alkyl ammonium hydroxides (tetramethylammonium hydroxide or TMAH). (Șerban and Victord, 2002 page 888) When molecular hydrogen is used, it can act like an inert gas and it is necessary to add a metal catalyst such as nickel or platinum. An advantage is that the number of pyrolysis products is reduced. For example two or more molecules with the same length carbon chains can have multiple double bonds in varying positions. Hydrogenation would eliminate the double bond in this situation and produce identical alkanes. (Șerban and Victord, 2002 page 889) The presence of water can create different pyrolysis products and for this reason samples are usually dried before analysis. However water can sometimes be added deliberately to create hydrolysis reactions. Chain scission through hydrolysis can be achieved in cellulose and starch. (Șerban and Victord, 2002 page 889)

TMAH is a widely used pyrolysis reagent. It is strongly basic and when added to a sample prior to pyrolysis, it can cause methylation of amides, esters, and ethers. These methylated products are often more volatile than the original molecules and have improved GC separation. When these reactions occur at low temperatures (250-300°C) the process is usually described as TMAH Thermochemolysis. Cleavage of the C-C bonds in macromolecules requires higher temperatures and is described as TMAH Pyrolysis. (Lehtonen, T., Peuravuori, J., Pihlaja, K., Degradative Analysis of Aquatic Fulvic Acid: CuO oxidation versus pyrolysis after tetramethylammonium hydroxide treatments in air and helium atmospheres, Analytica Chemica Acta, 511 (2004) 349-356)

The methylation occurring with TMAH it does not occur identically for all analytes. Lignin being somewhat acidic is very susceptible to TMAH methylation and acts predictably. Cellulose does not behave this way despite having many potentially reactive hydrogens. (Șerban and Victord, 2002 page 889)

Copper Oxide (CuO) or Alkaline Copper Oxide (CuO-NaOH) treatments are widely used to help break C-O bonds that hold phenols and aliphatic acids to humic matter. However breaking C-C bonds requires more energy and as a result the CuO pyrolysis products may only represent the more loosely held structural elements. (Lehtonen, et. al. 2004)

The combination of bond cleavage and improved chromatographic separation makes TMAH or CuO treatments a powerful tool for characterizing organic macromolecules found in humic substances. But using too much of these materials may destroy the desired analytes. There are other disadvantages such as the difficulty of distinguishing between pyrolysis products that are “naturally” occurring substances and those created with the chemical treatments. There may be a number of unwanted oxidative products if the TMAH treatment is not carried out under inert atmospheres. Another example of this phenomena is when CuO treatment causes aromatic rings to break apart and create low molecular weight aliphatic acids. The products of these chemical treatments may differ significantly from the original molecules. (Lehtonen, et. al. 2004)

Many of these approaches can be combined to create the optimal analytical system for a given analyte. There are also numerous possibilities for analyte detection and identification. Historically, most pyrolysis systems use a mass spectrometer or Flame Ionization Detector (FID) for peak detection. In recent years two-dimensional GC Pyrolysis and two-dimensional GC (GC
X GC) systems such as py-GC X GC/MS and py-GC X GC have been shown to have several advantages despite their complexity and costs. Rather than a point on a one-dimensional line (retention time), each compound is placed at an unique location on the separation plane. Distinct bands on the separation plane are often the result of compounds belonging to the same chemical class. This facilitates the identification of unknown compounds. This improved separating power is very useful when confronted with complex mixtures of pyrolysis products. ([Z. Parsi, T. Górecki, J. Poerschmann, Non-Discriminating Analytical Pyrolysis — A Novel Tool for Studying Environmental Samples, LCGC Europe, Nov 1, 2005, Volume 18, Issue 11]

**Historical Background**

Analytical pyrolysis combined with GC (py-GC) first became a common analytical technique in the mid 1950s for the study of polymers and related compounds. Although pyrolysis/mass spectrometry (py-MS) had been developed a few years earlier, GC offered the advantages of lower cost and less complicated equipment. There was an upsurge in interest in the technique during the early 1960s when it was seen as an effective way for space probes to detect biological molecules. This particular idea was not implemented for some years but it did lead to widespread use of py-GC and py-MS for the characterization of organic polymers, microbial materials, and other types of biological samples. ([Pyrolysis Mass Spectrometry of Recent and Fossil Biomaterials: Compendium and Atlas, Henk L. C. Meuzelaar, Johan Haverkamp, Fred D. Hileman, Elsevier, 1982, 293 pages, page 3]


An early example of py-GC/MS for the detection of phenolic and lignin pyrolysis products as a means of determining the origins of organic matter in estuary sediments was reported by Whelan et al. in 1986. (Jean K. Whelan, Martha E. Tarafa, and Evelyn B. Sherr, Phenolic and Lignin Pyrolysis Products of Plants, Seston, and Sediment in a Georgia Estuary, Organic Marine Geochemistry, Chapter 4, pp 62ñ75 ACS Symposium Series, Vol. 305, 1986) In the same year, automated py-GC/MS analysis combined with factor-discriminant analysis was used to map the geographical position and organic matter sources of the organic matter in the Rhine estuary and a nearby dredge-spoil disposal site. Among the findings were at that time at least, sewage sludge was a major source of particulate organic matter in densely populated regions of The Netherlands. ([Characterization of Particulate Organic Matter From Sediments in the Estuary of...])
the Rhine and from Offshore Dump Sites of Dredging Spoils, Jaap J. Boon, B. Brandt-de Boer, Wim Genuit, Jan Dallinga, and E. Turkstra, Organic Marine Geochemistry, Chapter 5, pp 76-90, ACS Symposium Series, Vol. 305, 1986) It should be noted that these two publications were the first time that pyrolysis as a means of sediment analysis appeared in Chemical Abstracts. Additional pyrolysis studies of the Rhine river sediments were published by Van de Meent et. al. in 1985. (Dik Van de Meent, J.W De Leeuw, P.A Schenck, W Salomons, Geochemistry of suspended particulate matter in two natural sedimentation basins of the river Rhine,Water Research, Volume 19, Issue 11, 1985, Pages 1333-1340)

The characterisation of humic substances originating from the decay of aquatic plants was reported by Gadel and Bruchet(1987). (F. Gadel, A. Bruchet, Application of pyrolysis-gas chromatography-mass spectrometry to the characterisation of humic substances resulting from decay of aquatic plants in sediments and waters, Water Research, Volume 21, Issue 10, October 1987, Pages 1195-1206) Fogel et.al. (1989) reported that organic matter preserved in sediments of St. Catherine's Island, Georgia, salt marshes originated in bacteria, plankton, and Spartina alterniflora. They used a combination of techniques including stable C and N isotopes and py-GC to examine muds up to 1400 years old. Isotopic mass balance allowed the tracing of how planktonic and higher plant debris inputs shifted over time and how microbial action resulted in the diagenesis of the organic material. The odd-to-even ratio of higher-plant derived n-hydrocarbons becomes less pronounced with age, as determined by depth. (Marilyn L. Fogel, E. Kent Sprague, Andrew P. Gize, Robert W. Frey, Diagenesis of organic matter in georgia salt marshes, Estuarine, Coastal and Shelf Science, Volume 28, Issue 2, February 1989, Pages 211-230)

Overview of the Procedures used in this Research

After sampling, the sediments were oven-dried overnight at 40°C.

The first step in the analysis was to hand-grind the dried sediments using a motor and pestle. Small pebbles, shells, and macroscopic plant materials such as blades of grass or leaves were removed at this time. Milligram quantities of the dried sediments were used for the analysis. Approximately 4 to 5 milligrams of fine, organic rich sediments were loaded into a quartz pyrolysis tube, but if the sediments were sandier, approximately 8 to 10 milligrams were used.

The dried sediments were spiked with 5 uL of an internal standard solution containing deuterated PAHs from Cambridge Isotopes, Andover, Ma.. The solution contained naphthalene (D-Naphthalene, cat. DLM-365-1), deuterated anthracene (D-anthracene, cat. DLM-102-1), and deuterated pyrene (D-Pyrene, cat. DLM-155) in hexane. The spike concentration was manipulated such that each injection introduced 24.50 ng of D-Naphthalene and 27.00 ng of D-Anthracene into the chromatographic system. D-Pyrene was not used for quantization.

The chromatographic system used for the study was a Thermo Electron Focus GC and Thero Electron DSQ quadrupole type Mass Spectrometer. A CDS model 1500 pyrolysis system was used to heat the samples at 610°C for 20 seconds under a helium atmosphere. The extracted molecules and pyrolysis products are swept onto the GC column by a stream of helium gas. The GC column used for organic geochemical determinations was a 30m J&W Scientific DB-1MS
column, with a 0.25 mm i.d. and 0.25um film thickness. The PAH determinations were performed using a 60m J&W Scientific DB-1MS column, with a 0.25mm i.d. and 0.25um film thickness. All GC temperature programs began at 50c for 5 minutes and rose at a rate of 5c per minute until reaching 300c and holding for 25 minutes. Gas pressure at the column head was 33 psi with a split ratio of 1 to 25.

When operated in the full scan mode the mass spectrometer was set to 50-550 Da, 1.08 scans/sec., 70eV ionization voltage. In the SIM mode the MS was also set to 70eV ionization voltage and identification was based on a combination of molecular weight and retention time window.

Compound identification was through a combination of retention time window and or more characteristic ions. Concentrations of analyte molecules were estimated using the following formulas:

\[ \text{ng analyte} = \left( \frac{\text{ng internal std}}{\text{area counts internal standard peak}} \right) \times \text{(area counts analyte peak)} \]

\[ \text{ppm analyte} = \frac{\text{ng analyte}}{\text{mg dried sample}} \]

A correction factor was applied to each result. This factor was based on the difference in the peak area obtained in the total ion chromatogram and the peak area obtained from integrating only the ion used for quantitation.

The PAH analysis was conducted in the selected ion monitoring (SIM) mode. Compound identification was again based on a combination of molecular weight and retention time window. A correction factor was not applied for this class of compounds because both the internal standard and analytes were chemically very similar.

Detailed Discussion about Some Aspects of the Analytical Procedure

1. Drying, Grinding, and Culling:

Samples must be dried at the start of the pyrolysis process because as has already been discussed, water may introduce unwanted chemical reactions. At one time the author attempted to take wet samples directly from the field and dry them in the pyrolysis system before high-temperature heating. This did little more than create a hard crust on the exterior of the sample that could have trapped pyrolysis products. Grinding sediments prior to analysis creates homogenous samples where all particles have comparable surface areas. But the possibility that the heat and pressure even from hand-grinding might destroy low molecular weight compounds or allow them to volatalize is something that deserves additional investigation.

Removing macroscopic biomass such as insects, seeds, leaves, grass fragments, and other plant
materials is necessary to insure that large quantities of these materials do not skew the results.

2. Use of Dueterated internal standards

Dueterated internal standards are particularly useful in situations where it is necessary to mimic the behavior of analytes during clean up and extraction processes. They are also useful in situations where there may be significant matrix interferences. There is one critical caveat. The degree of dueteration must be high so that there can be no confusion between the standard and an analyte. Generally speaking dueterated compounds elute slightly earlier than the original materials and they can be readily identified through a combination of molecular weight and retention times. Dueterated standards also have an advantage in complex matrices, if added in excess, they can occupy potential sorption sites and enhance analyte recovery. (Handbook of GC/MS, Hans-Joachim Hübschmann, John Wiley & Sons, Jul 11, 2008 - 608 pages, page 318)

In this research the author has observed that the best results for internal standard quantitation are obtained when the dueterated internal standard and the analyte are of the same chemical class, PAH and d-PAH for example. This method of quantitation should not be confused with the use of dueterated standards as surrogates to measure analyte recovery during extraction and clean up procedures.

There are many examples of the quantitation using this approach. The use of dueterated n-alkane internal standards for py-GC/MS analysis of kerogen was reported by Eglinton, et. al. 1991. The standards were only used to quantify compounds most like themselves, i.e. alkanes. (Eglinton, Tim. Fry, Brian. Freeman, Katherine. and Hayes, J.M., Carbon-isotopic compositions of products from flash pyrolysos of kerogens, in Organic Geochemistry: Advances and Applications in Energy and the Natural Environment: 15th Meeting of the European Association of Organic Geochemists, edited by D. A. C. Manning, Manchester University Press ND, 1991 - 662 pages. Page 413, ) Wagener et. al. (2010) used deuterated internal standards for PAH quantitation in addition to conventional calibration. (Wagener, Hamacher, etc etc, Evaluation of tools to identify hydrocarbon sources in recent and historical sediments of a tropical bay, Marine Chemistry, 121 (2010) 67-79) The extraction efficiency for fecal and plant sterols was measured by Benfenati et a. (1994) was quantified through the use of internal standards. (Emilio Benfenati, Etienne Cools, Elena Fattore, Roberto Fanelli, A GC-MS method for the analysis of fecal and plant sterols in sediment samples, Chemosphere, Volume 29, Issue 7, October 1994, Pages 1393–1405) The characterisation of organotin compounds in natural waters and sediments by Arnold et. al.(1998) used accelerated solvent extraction and perdeuterated organitins as internal standards. They achieved method detection limits between 0.4 to 2 ng/g for the sediment samples. (Cédric G. Arnold, Michael Berg, Stephan R. Müller, Urs Dommann, and René P. Schwarzenbach, Determination of Organotin Compounds in Water, Sediments, and Sewage Sludge Using Perdeuterated Internal Standards, Accelerated Solvent Extraction, and Large-Volume-Injection GC/MS, Anal. Chem., 1998, 70 (14), pp 3094–3101)

Dueterated PAH internal standards were used to quantify the PAH concentrations in the Gulf of Trieste, Northern Adriatic Sea (M Notar, H Leskovšek, J Faganeli, Composition, Distribution and Sources of Polycyclic Aromatic Hydrocarbons in Sediments of the Gulf of Trieste, Northern
Adriatic Sea, Marine Pollution Bulletin, Volume 42, Issue 1, January 2001, Pages 36–44) The performance of ion-trap MS for PAH detection was optimized using a strategy of selected ion storage (SIS) and deuterated internal standards. Method sensitivity reached 0.02–11.0 ng/g with 77% recovery was achieved on a reference sediment sample from Lake Ontario. (Natalicio Ferreira Leite, Patricio Peralta-Zamora, Marco Tadeu Grassi, Multifactorial optimization approach for the determination of polycyclic aromatic hydrocarbons in river sediments by gas chromatography–quadrupole ion trap selected ion storage mass spectrometry, Journal of Chromatography, Volume 1192, Issue 2, 30 May 2008, Pages 273–281)

3. Selection of a pyrolysis temperature

Selection of a pyrolysis temperature is often a balance between maximum desorption of intact molecules and the desired degree of thermal degradation in the macromolecules. Generally speaking, as pyrolysis temperature increases the chromatogram becomes dominated by smaller fragments with less diagnostic value. (Șerban and Victord, 2002 page 869)

Temperatures in the range 300 to 350°C are widely used to desorb lighter materials without creating breakdown products. (Medina-Vera 1996, Faure and Lanadais 2000). A temperature of 300 was found adequate to thermally desorb saturated hydrocarbons greater than C27. (Faure and Lanadais 2000)

Pyrolysis is particularly useful for large biological molecules that are not well suited for chromatographic analysis. While the pyrolysis products of the these materials are often well-suited for GC analysis they are not all created at the same temperature.

In "typical" dry weight biomass, cellulose is the most abundant material 40-60%, hemicellulose concentrations are slightly lower at 20-40%, and lignin only comprises 10-25%. Of these, hemicellulose being amorphous and easily hydrolized is the most prone to decomposition. Cellulose is harder to decompose but lignin as it is made from benzene-propane units and heavily cross-linked is most resistant to decomposition. (Yang, Yan, Chen, Energy & Fuels, 2006, 20, 388-393, In Depth Investigation of biomass pyrolysis)

Because there is little or no interaction between hemicellulose, cellulose, and lignin, the pyrolysis process can be considered a simple superimposition of temperature profiles. Yang et al (2006) measured pyrolysis temperatures for synthetic biomass. Below 230°C moisture was evolved, hemicellulose decomposed from 230-315°C, and cellulose decomposed from 315-400°C. Lignin decomposed above 400°C. However these were not rigid boundaries as there was some overlap. And while there was little chemical interaction between these three materials particle formation and interjection into the sample matrix could physically interfere with the process. There are also examples of particular types of lignins that decompose at low temperatures. Of particular relevance to complex sample matrices is that metals salts (NaOH, Na2CO3, NaCl, NiCl2, CuSO4) lower the temperature of cellulose pyrolysis. (Yang, Yan, Chen, Energy & Fuels, 2006, 20, 388-393, In Depth Investigation of biomass pyrolysis)

One of the more common thermal degradation products encountered in pyrolysis are PAHs which can be formed at temperatures between 300 and 600°C (Del Rio and Philip 1992) The
most common chemical reaction during pyrolysis is the B (beta) elimination in which two adjacent atoms lose attached groups of an form a double bond. Six membered rings can be formed as an intermediate in this situation. (Șerban and Victord, 2002, page 877) In the author’s experience naphthalene is readily formed and for this reason will not be reported in subsequent discussions.

Higher temperatures are required to desorb heavier molecules. Pyrolysis temperatures in the range of 600 to 750°C have been used for sediment studies. (Faure and Lanadais 2000, Poerschmann 2008) However studies of sewage sludge pyrolysis have shown that most biodegradable organic matter volitalizes in the temperature range 150 to 400°C and non-biodegradable organic matter volitalizes between 400 to 550°C. (Barneto et al. 2009)

4. Use of correction factors

Every GC detector requires the use of a response factor for quantitation. The area of a GC peak is proportional to the amount of analyte that reached the GC detector. But no detector responds equally to different compounds and so the peak area is a combination of the amount of analyte and the response of the detector to that particular type of molecule. (response factor) An FID will not produce a large signal for a compound that does not burn. An ECD will not produce a strong signal from a weakly halogenated compound.

This problem is especially acute in mass spectrometry. The size of the peak is proportional to the number of ions that are generated by the molecule when it is fragmented in the ion source. Consider group of benzene molecules and an equal number of hexane molecules. Because the benzene does not fragment readily it will only generate one ion. The hexane will fragment into C5, C4, and C3 chain-length ions in addition to C6 ions. The resulting hexane peak will have a much greater area than the benzene peak even though the number of molecules was the same.

The correction factors used in this research were developed by Michael Kruege at Montclair State and builds on the principle of the response factor. A dueterated PAH will generate a mass spectrum dominated by a single molecular ion because aromatic molecules tend not to fragment. There will of course be smaller fragments but there are only a comparatively small number of them. Most of the other analytes will generate a larger number of fragments.

The large number of fragments can be used to advantage in a complex sample matrix. For example, two compounds may co-elute but they may also have characteristic fragments. Using the GC/MS software we can isolate these unique fragments thus deconvolute the peaks. To quantitate the peak, one ion is selected and the area under the GC trace for that ion is integrated. The advantage of this approach is that only the ions from a particular compound are used to quantify that compound but the disadvantage is that this one ion represents only a small fraction of the total peak area. Thus the correction factor compensates for this difference.

An example of a similar approach comes from biomedical science. Isotopic metabolic tracer studies employ labeled variants (isotopomers) of the desired analytes. Raw GC/MS data must be corrected using three conversions. The background must be corrected for. Ion abundances in the isotopomers will be different than those from the original molecule, and the resulting "skew" is
corrected. Lastly, any overlapping spectra must be convoluted. (Rosenblatt J, Chinkes D, Wolfe M, Wolfe RR, Stable isotope tracer analysis by GC-MS, including quantification of isotopomer effects. Am J Physiol. 1992 Sep;263(3 Pt 1):E584-96.)

NOTES FOR CHAPTER TWO:

Sample Preparation in Chromatography
Şerban Moldoveanu, Victor Dr David
Elsevier, 2002 - 930 pages

Joeri Vercammen, ValÈrie Winne and Myriam Madani, How to analyse unknown samples by pyrolysis GC/MS?, Separation science, volume 4 issue 3, 2012, Page 11,

Thomas P. Wampler
CRC Press, 2007 - 288 pages

Chapter THREE, Pyrolysis Across Space: Gateway NRA

Chapter Four, Pyrolysis Over Time? Passaic River Dundee Lake Core

Chapter Five, Sediment Management in Urban Areas
PART TWO  The Environmental Management and Mismanagement of Jamaica Bay