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Chapter 1

Introduction

1.1 Project history

This thesis is a continuation of work that was submitted for a M.Sc. degree[1]. The purpose of that project was to find an analysis method that would substantially reduce the time required to characterize petrochemical samples in the Sasol laboratories. The supercritical fluid chromatographic (SFC) analysis that was described therein could successfully separate the different groups found in light to middle distillate petroleum samples. The groups could also be quantified because it was possible to use the flame ionization detector (FID)[2]. After SFC group separation, the groups were trapped on solid adsorbents and transferred off-line to a GC for subsequent boiling point analysis. The combination of SFC and GC analysis provided ample information for many applications.

Various problems were however encountered:

- The lengthy GC analysis times meant that some samples were only analyzed hours after sampling. This added additional uncertainties.
- Trapping times and duration was uncertain because of shifting in SFC retention times.
- The adsorbents used for the collection of SFC fractions produced background signals.
- Breakthrough of the more volatile components was encountered i.e. non-quantitative trapping of some compounds in the SFC fractions.

The study suggested that on-line transfer between the SFC and GC could alleviate many of these problems. Furthermore, if the two separation methods could be coupled comprehensively, a more complete picture of the sample properties would be obtained.

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1.2 Background

1.2.1 Chromatography in a nutshell

In general chromatography is a physical method of separation in which the components to be separated are repeatedly distributed between two phases. One phase is stationary and the other moves through it, carrying the components in a particular direction. The chromatographic process occurs due to repeated sorption and desorption as the sample components are washed through the stationary bed. Separation occurs due to chemical or physical differences of the individual sample components, which influence their affinity for the stationary or mobile phases.

The information obtained from a chromatographic experiment is contained in the chromatogram. The chromatogram is a record of the concentration or mass profile as a function of the movement of the mobile phase in time, volume or distance. Information readily extracted from the chromatogram includes an indication of sample complexity based on the number of observed peaks, qualitative identification of sample components based on the accurate determination of peak position, quantitative assessment of the relative concentration or amount of each peak, and an indication of column performance.

1.2.2 History

Chromatography and similar separation methods have been in use since ancient times[3]. Some authors find the first reference to ion exchange in the Old Testament (Exodus xv. 22-25) while others believed that Aristotle knew about the adsorptive properties of some soils for the purification of seawater. Since the principles of these phenomena were not known, no further development in this field could take place. Bacon again described the purification of seawater with soils in the seventeenth century. The systematic study of these separation processes was only initiated in 1850 when Runge and several other workers independently started to study the separation of dyes on filter paper[4]. Way[5] and Thompson[6] discovered the fundamental principles of adsorption of cations from salt

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solutions through different types of clay without realizing the significance of their observations.

The principals of adsorption chromatography was finally comprehended by the Russian scientist, Tswett, who correctly interpreted the previous observations and developed it into a reliable, sensitive and systematic method for separation of complex mixtures of even closely related chemical compounds[7]. Again Tswett's work was largely ignored until 1931 when it was 'rediscovered' by Kuhn et.al.[8]. Since then the art of chromatography has developed into the science we know today. From the first experiments using powdered calcium carbonate to separate green leaf extracts to the Nobel Prize awarded to Martin and Synge in 1952 for the invention of partition chromatography, samples of ever-increasing complexity could be analyzed.

New discoveries now occur at such a pace that there is hardly enough time to fully investigate and apply the existing technologies. However, some developments have really transformed the field. One of these giant leaps was the discovery of capillary columns in 1957 by Golay[9]. Despite the obvious advantages inherent to capillary columns it took many years before they were accepted for general use. This happened when fused silica capillary columns became readily available commercially in the early 1980's. Today, for newcomers to chromatography, capillary columns are synonymous with gas chromatography.

1.2.3 Modern Chromatography

A modern capillary column can easily produce 100 000 theoretical plates with a peak capacity of a 1000 or more. This means that if sample components would elute evenly, 1000 peaks could be observed side by side and resolved with unit resolution. Unfortunately this is not the case, as compounds tend to elute in a random fashion from the column and frequently overlap. Giddings and Davis showed by using the statistical model of overlap[10] that, to separate 98 out of 100 randomly eluting compounds, a system with 400 000 000 theoretical plates are required! As substantiated in Chapter 2, this translates into a peak capacity equal to 10 000 for non-programmed runs.

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Real samples frequently contain far more than 100 compounds. Petrochemical or biological fluids may easily contain thousands of different compounds. Table 1 shows the number of possible paraffinic isomers per carbon number and the boiling point of the n-alkane[11,12] for a limited carbon number range.

Table 1-1. Carbon number, boiling point and number of possible paraffin isomers.

carbon number	Boiling point n-alkane (°C)	number of isomers
5	36	3
8	126	18
10	174	75
15	271	4347
20	344	3.66×10^5
25	402	3.67×10^7
30	450	4.11×10^9
35	490	$4.93. \times 10^{11}$

Even for simple aliphatic hydrocarbons as illustrated in Table 1-1, the numbers are intimidating. For carbon numbers up to C12, more than 10 000 alkane, naphtene and aromatic structures are possible[13]. Most of these are likely to be present in a petroleum oil sample and may need to be analyzed for.

1.2.4 Multidimensional Chromatography

Unfortunately, single column gas chromatography fails even to analyze samples that contains as few as 150 to 250 relevant compounds[14]. In such cases the scientist has to apply to multidimensional chromatographic techniques. Often target analysis of only a handful of analytes is required. 'Sample clean up' is then used to remove relevant components from an interfering matrix or samples are pre-separated into fractions that can be analyzed with available separation capabilities. Sometimes a selected part of a chromatogram is cut from the column exit and subjected to a different kind of chromatographic separation. This can be done on-line or off-line and the technique is

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referred to as heart cutting. Only a few cuts can generally be analyzed for every injection. When detailed analysis of complex samples such as petrochemical samples are required, these techniques become extremely time-consuming due to the number of injections required for each sample.

It is sometimes possible to analyze for target compounds in complex samples by using selective detection. Spectroscopic detectors can be set to detect the absorbance of UV or IR radiation at predetermined wavelengths where only specific compounds absorb electromagnetic energy. Mass spectrometers are also frequently used for selective detection. Here the mass analyzer is set to only transmit ions that have a particular mass-to-charge ratio. This is called single ion monitoring (SIM) mass spectrometry.
It is also possible to repeatedly scan through a range of electromagnetic frequencies or mass-to-charge ratios at every data point on the chromatogram. In the process a two dimensional analysis is obtained. Generally, when two analytical techniques are applied simultaneously to the analysis of a sample, it is referred to as a *hyphenated* technique.

1.2.5 Comprehensive multidimensional chromatography

A special case of multidimensional chromatography, called comprehensive multidimensional chromatography, has recently been developed.
Comprehensive multidimensional chromatography is a hyphenated technique where two or more chromatographic separations with different selectivity are coupled together. However, the analysis is far more complete than for heart cutting techniques, since the entire sample eluting from the first separation is analyzed by the second separation and because the resolution achieved in the first analysis is conserved throughout subsequent analysis steps[10].

The total amount of information that can be obtained from a comprehensive multidimensional method is surprisingly high if the combination consists of completely independent techniques. Any correlation between the selectivities of the two separations, however, leads to the wasteful production of separation space that cannot be used[15]. Ultimately this synentropy or cross-information[16] leads to an increase in total analysis

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time without an increase in information production. Separation mechanisms that are free from synentropy are said to be orthogonal to each other. In such a case the two separation dimensions produce a rectangular separation space with compounds distributed evenly across the plane.

The fundamental principles of comprehensive multidimensional chromatography have historically been applied to thin layer chromatography, electrophoretic techniques[17] and high performance liquid chromatography (HPLCxHPLC)[18]. But comprehensive multidimensional chromatography caused relatively little excitement until the recent development of comprehensive two-dimensional gas chromatography (GCxGC)[19].

A GCxGC instrument generally uses a non-polar column in the first dimension to separate samples according to volatility. Consecutive small sections of this chromatogram are refocused and introduced into a short polar column where each fraction is separated by differences in polarity. By using the same temperature ramp rate for the two columns, orthogonality is achieved by effectively removing the volatility aspect of the retention mechanism in the second column, leaving only the resultant polar interactions.

Selectivity in GC is always primarily a volatility separation. The number of GC stationary phases with additional selectivity is limited and most of these secondary interactions are weaker at high temperatures. This restrains the scope of GCxGC type analysis. Even so, this technique has proved to be very powerful for detailed analysis of complex samples. More than 6000 peaks have been observed for a kerosene sample[20]. As was the case for capillary columns, it is once again the petrochemical industry that is the main driving force behind the advancement of GCxGC.

Attempts at combining HPLC with GC are hampered by the large amount of solvent that needs to be removed. This requires time and can lead to a loss of volatile sample components. Despite refinements in technology, even simple online HPLC-GC is seldom

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accepted as a method of choice [21]. However, commercial instruments are available and the technique is used in practice. An example is the SRI LC-GC Combo system[22]. Conventional temperature programmed GC typically runs for about an hour and the oven requires time to return to the starting temperature. This is not fast enough to allow a potential an HPLCxGC analysis to be completed in a reasonable time.

1.3 Comprehensive supercritical fluid and gas chromatography

1.3.1 Comments on previous SFCxGC attempts

SFCxGC has been attempted using the same interface between the two columns that was used for GCxGC with essentially the same experimental conditions[23]. These include the simultaneous temperature programming of both columns and approximately isothermal operation of the second column for the duration of each consecutive 2nd dimension chromatogram. This approach to SFCxGC does not utilize the full potential of supercritical fluid chromatography. Many of the available SFC selectivities such as polar, enantiomeric and size or shape separation are temperature sensitive. It would make sense to operate the SFC at low temperatures to increase these different modes of selectivity. However, to make full use of the GC and to circumvent the *general elution problem* it is required that the GC be operated in a temperature programmed mode.

Recent advances in fast programmable GC have brought turnaround times down to minutes or less, even for samples exhibiting relatively high complexity. This opens up a new avenue towards comprehensive multidimensional SFCxGC where a low temperature SFC separation is followed by a fast temperature programmed GC (GC_{ftp}) run for volatility analysis.

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1.3.2. Advantages of $SFCxGC_{ftp}$

As demonstrated in the following chapters, there are many potential advantages to $SFCxGC_{ftp}$ over GCxGC and existing SFCxGC approaches. These include:

1. There are a large variety of stationary phases available for SFC separation, which allow for improved selectivity for a variety of sample dimensionalities especially at low temperatures.
2. Only the temperature stable non-polar siloxane stationary phases are heated, allowing for samples with higher final boiling points to be analyzed than in GCxGC.
3. Due to the very fast heating rates, the GC column is at the higher temperatures for a very short time. Not only will the stationary phase last longer, but also thermally labile compounds that would usually not be observed with GC may be analyzed for[24], as most of the analysis time is spent in the low temperature SFC column.
4. Another potential advantage is that, for the first time, it would be possible to use the FID together with modifiers in SFC. The modifier can continuously be added to the SFC mobile phase as it will be separated form the analytes by the GC column before detection.

With these advantages in mind, a project was started to construct and evaluate a comprehensive two-dimensional supercritical fluid and fast temperature programmed gas chromatograph ($SFCxGC_{ftp}$).

1.4 Approach followed

For the first dimension a silica gel column with supercritical CO_2 as mobile phase was used for separation of sample components according to polarity.

For the second dimension, an in-house designed, resistively heated, temperature programmable gas chromatograph was used for volatility-based separation. The simplest case where the decompressed CO_2 from the SFC eluent stream was used as GC mobile phase was theoretically and experimentally investigated.

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An interface between the SFC and GC was designed that allowed for the exchange of mobile phase for improved GC performance. As an example, flow modulation (stop-flow chromatography) was demonstrated to digitize the SFC separation, where loss in solvation strength due to depressurization of the SFC mobile phase, combined with a low starting temperature, served to focus the fractions on the GC column. The stopped flow version was easy to construct form a standard GC injector and a stop flow valve and excellent results were obtained with it. The continuous flow version was not constructed as part of this study, however the operation and principles were outlined shortly for future investigation. The stop-flow modulator was used for all $SFCxGC_{ftp}$ experiments presented in this thesis.

1.5 Presentation and arrangement

In Chapter 2 the basic principles and ideas behind comprehensive multidimensional chromatography are explained. This chapter includes topics such as the current state of development of comprehensive multidimensional chromatography, the statistical model of overlap, sample dimensionality, orthogonality, modulation etc.

In Chapter 3 theoretical aspects pertaining to fast GC analysis and resistive heating are explored for use as the second dimension volatility separation. In Chapter 4, the construction of a novel fast temperature programmable gas chromatograph is described. The different electronic circuits that were constructed for controlling the resistively heated GC column are compared. Although it represents a large portion of the effort that went into the project, the reader can skip this part (section 4.2 and 4.3 up to 4.3.6) without losing track of the bigger theme. This section also demonstrates that a micro-thermocouple can be used with great success for temperature control of resistive GC's.

The basic principles of supercritical fluid chromatographic analysis are discussed in Chapter 5. Chapter 6 demonstrates group separation on normal phase SFC columns. A silica gel packed column was used for separation of petrochemical mixtures into the aliphatic, mono-, di-, and tri- aromatic compounds classes.

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Chapter 6 also contains the novel application of a Silica PLOT column for the SFC group separation of various oxygenated compound classes in petrochemical mixtures and herbaceous essential oils.

Chapter 7 reviews some of the modulator designs found in the literature, while in Chapter 8, the design of a novel interface that allowed for the connection of SFC separation methods to fast resistive GC for comprehensive two-dimensional $SFCxGC_{ftp}$ is described.

Demonstration of comprehensive two-dimensional $SFCxGC_{ftp}$ is presented in Chapter 9. Chapter 9 also includes a comparison between chromatograms obtained with the $SFCxGC_{ftp}$ and a commercial GCxGC instrument.

A summary of results together with recommendations for further research is presented in the concluding Chapter 10.

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Chapter 1

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