Chapter 7

The Modulator:

Background and literature survey

7.1 Introduction

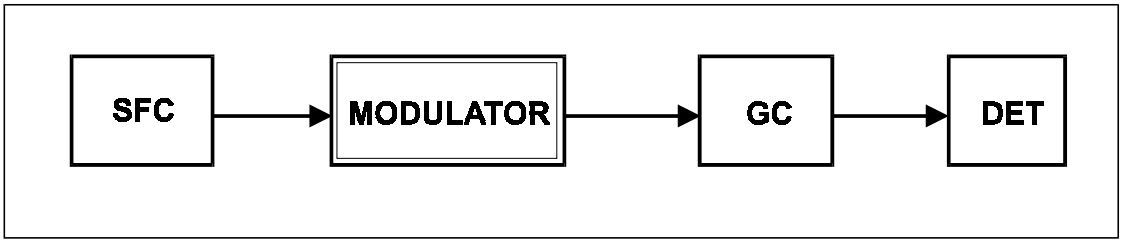
The fast GC and SFC instrumentation that were described in the previous chapters separate samples according to two different and independent types of selectivity. In order to combine these methods and harness the potential power of the comprehensive combination, a special interface is required. This interface must allow frequent sampling of the first analysis stream and provide fast, sharp injection bands for introduction into the second column. Furthermore, the physical state of the initial sample stream needs to be adjusted to make it compatible with the second analysis. These modifications include changing the state of the stream from being a supercritical fluid to a gas and exchange of the CO_2 gas with H_2. This chapter describes previous modulator designs available in the literature. In the next chapter, the necessary instrumentation for the coupling of the SFC and fast GC to produce a comprehensive two-dimensional chromatograph will be illustrated.

7.2. The modulator

The SFC and GC columns are connected together through a device called the modulator. The modulator is more than just a coupling between the two separation techniques. It is the heart of comprehensive multidimensional chromatography in that it provides a time base for each sequential second separation when it originates from the first dimension. It

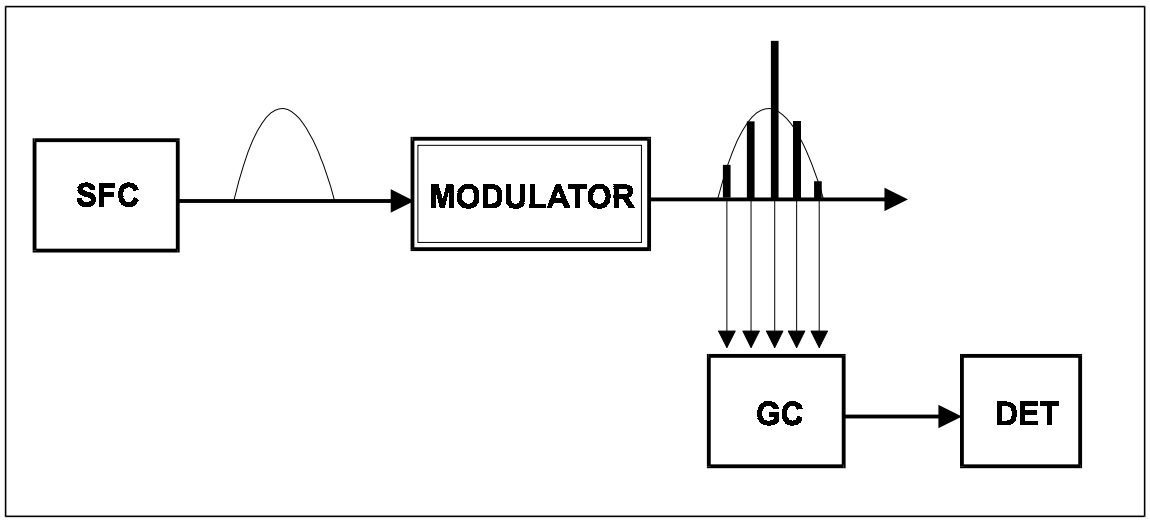
also re-concentrates peaks that elute from the first column (SFC) and injects them as very narrow peaks into the GC at fixed time intervals.

Figure 7-1: Schematics of the SFCxGC instrument



In other words, the injection time of each GC chromatogram can be traced back to a definitive retention time on the SFC chromatogram.

Figure 7-2: Function of the modulator



A modulator can further be distinguished from other transfer setups such as heart cutting devices, in that it transfers hundreds of 'heart cuts' during a run and feeds them into a very fast second separation step. Preferably the resolution and peak shape of the first separation step should be maintained. In our case of the SFCXGC$_{ftp}$ it entails the transfer of at least 5-10 samples to the GC for every SFC peak. The SFC chromatogram is

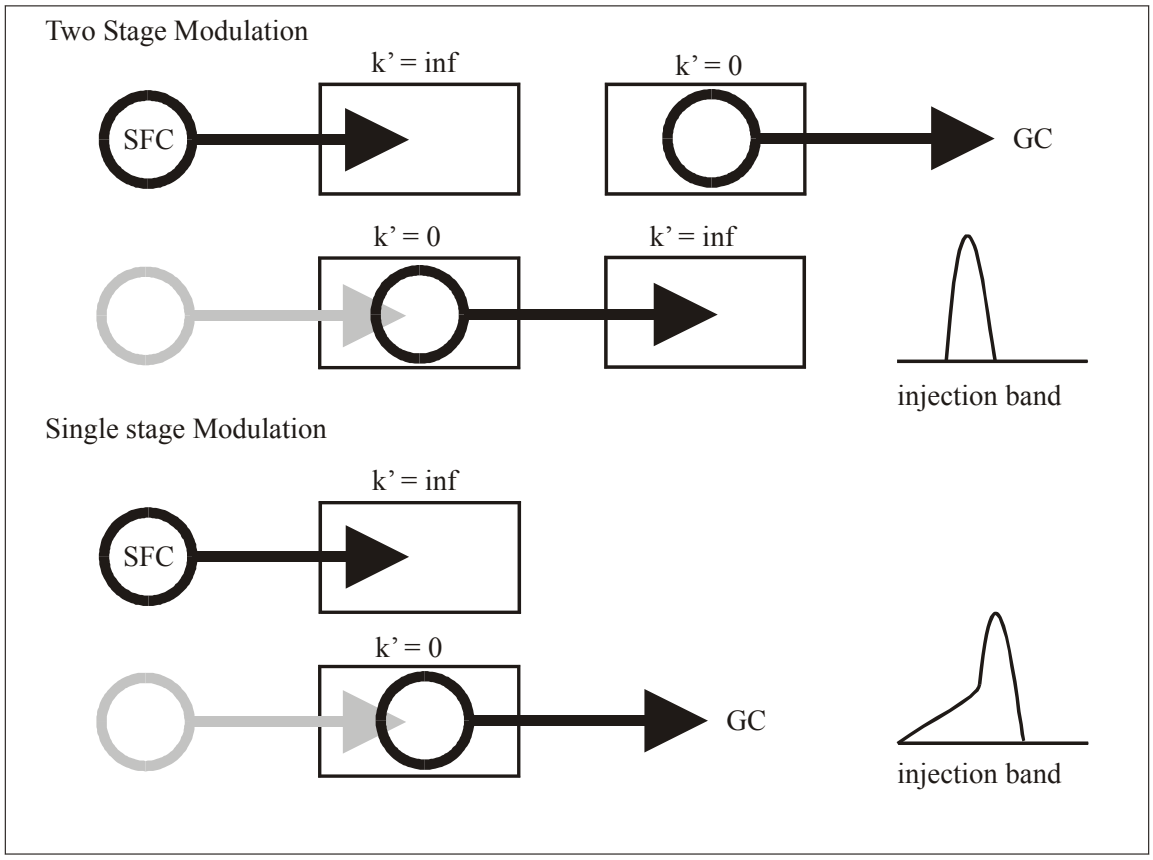
analogous to a total ion trace obtained in mass spectrometry with each GC chromatogram representing a mass scan. Since the entire SFC chromatogram is transferred for subsequent analysis, the system is potentially far less sensitive to shifting retention times in the first column than repetitive heart-cutting techniques where shifts give rise to major complications.

In the ideal case, modulation is achieved by repeatedly increasing the retention factors of all sample components to infinity as they leave the first column. The trapped fraction is then released into the second column by instantaneously decreasing their retention factors to zero. In order to provide a fixed time base for the second separation step and to ensure good input bandwidth, no sample components should reach the second column unmodulated. This necessitates at least two focusing stages. (See Figure 7-3.)

A slice from a peak is collected by the first stage where the eluting components experience strong retention. The retention factors of the sample components are then decreased and sample components move to the second stage where they are once again focussed. At the same time as the first modulation stage collects sample components that are continually eluting from the SFC, the second stage launches the trapped analytes as a narrow band into the GC column for a very fast second separation.

Figure 7-3 demonstrates what happens when only one modulation stage is used: While the modulator desorbs the trapped components into the GC, more sample is bleeding unfocused onto the GC column leading to a bad injection profile. Unfocused volatile sample components that elute from the SFC during this time may overtake heavier molecules that are still being analyzed by the GC and cause 'cross-over'. This significantly complicates data analysis.

Figure 7-3: Two stages are required for modulation.



There are a number of ways to achieve the modulation of retention factors. Diaphragm valves and stop flow conditions have been used as alternatives to methods that use modulation by retention factors. A wide peak eluting from the SFC can be sharpened into a narrow peak or a series of narrow peaks. Because of the law of conservation of mass the narrow peaks must have higher amplitude. This results in lower detection limits and improved quantitation[1].

Both stationary phase and cryogenic focussing techniques have been used for modulation.

7.3 Stationary Phase Focusing

Stationary phase focusing uses capillaries with a thick stationary phase film to decrease retention factors of volatile components at the ambient oven temperature. Additional heat is supplied at the modulation frequency to re-volatilize analytes in a focussed band. These analytes are then displaced into the second separation step by the carrier gas. This technique is generally known as thermal modulation.

The thermal modulator was originally developed as a sample injector for a technique called multiplex chromatography[2]. With this technique the same sample was injected repeatedly and the resultant chromatograms were averaged to improve signal to noise ratios. The inventive step from multiplex to comprehensive multidimensional gas chromatography was to repeatedly sample the *changing* sample stream of an initial chromatographic separation developed by the first column.

The modulator was built from a thick film fused silica capillary tube that was coated with a resistive coating to make it electrically conductive. The tube was then alternatively heated and cooled down to produce a series of chromatograms. It was soon realized that a single stage was not adequate and a two-stage modulator was developed[3,4]. This design ensured that no sample components reached the second column unmodulated (see Figure 7-3) and implied that a fixed starting time was realized for every injection into the second column.

The initial design, comprising of conductive fused silica tubes, had a low thermal mass and could be heated up and cooled down at high rates. However, these devices were not very stable or reliable and would suffer from unpredictable burnout[5]. This was due to the small thermal mass of the metal coating, which was hundreds of times smaller than the mass of the fused silica capillary that it needed to heat up. The power dissipation needed by the thin resistive layer to heat the capillary caused more thermal stress than what the materials could handle. Any irregularity in the coating would result in burn out. The modulator was modified in an attempt to improve reliability by inserting the capillary into a metal sleeve or by coiling heating elements around the capillary[6]. However these

modifications increased the thermal mass and resulted in slow cooling, effectively slowing down the process of GCxGC. It also proved difficult to attach low thermal mass electrical leads to the capillary without causing cold spots. Electrical connections need to have negligible thermal mass, and should not cause an increase or decrease of resistance at the site of attachment.

7.4 The sweeping arm thermal modulator

All these problems added to the movement away from resistively heated modulators to mechanically operated versions. A more robust thermal modulator was created without adding additional thermal mass when Philips and Ledford decided to apply an external, moving heated element to the modulator capillary. In the sweeping arm modulator, solutes are retained by stationary phase focusing and then volatilized by movement of a slotted heater over the stretched capillary. At the end of the sweep path, the stationary phase ended abruptly and solutes were launched into the second column for further analysis. The large thermal mass ensured stable and well-controlled temperatures. The sweeping movement of the element segregated a section of the first chromatogram, refocused and launched it as a narrow band into the second column. Typically 60ms injection peak widths into the second column were obtained[7].

Close thermal contact between the heater and capillary is essential for fast local heating of the capillary but is also the downfall of this approach. Heating the capillary caused expansion and subsequent breakage as the slotted heater could no longer travel freely over the capillary. Difficulties were also encountered with connection of the modulator capillary to the two columns. Later configurations used the beginning of the 2nd dimension column as the modulator tube. Alignment between the stretched capillary and slotted heater was difficult and while some installations worked fine, others failed to produce acceptable results[5].

Another disadvantage of this technique is that the modulator tube needs to be heated 100°C higher than the ambient oven temperature to ensure proper re-launching of focussed analytes into the second column. The polyethyleneglycol or carbowax columns typically used for the second dimension have relatively low maximum allowable temperatures. This reduces the final boiling point of the samples that can be analyzed.

7.5 The Cryogenic Modulator

An alternative way to effect stationary phase focusing was developed by Marriot and Kinghorn[8,9,10,11,12]. They used a moving cryogenic trap to focus analytes on the second column. When the trap moves away from the zone where the analytes were collected the exposed capillary heats up to oven temperature within a few ten's of a millisecond. The small thermal mass of the capillary column ensures that this temperature is reached almost instantaneously. Since the analytes can migrate at the temperature used to bring the analytes into the trap, the heated part does not need to be warmer than the ambient temperature of the oven and no additional heating was required. The cooled section of the modulator tube was shielded from the oven fan by the cooling device, which facilitated rapid temperature reduction. To prevent ice build up and subsequent breakage of the capillary column, dry nitrogen was flushed between the cryogenic vessel and the trapping tube.

The inventors claim that the cryogenic modulator should function over a larger volatility range than the thermal modulators. The cryogenic trap should also focus lighter molecules more effectively. Using CO_2 as cryogen, analytes as volatile as hexane can successfully be immobilized. The maximum temperature is not limited by having to heat the capillary above ambient oven temperature. However, the injection bandwidth is determined by how quickly the trapping capillary heats up to oven temperature.

7.6 Diaphragm Valve Modulator

A gas-actuated diaphragm valve was used to sample the effluent from a wide bore column into a second, narrow-bore, column. Sampling was performed twice per second. To prevent immoderately fast linear flow rates in the second column, a portion of the flow after the diaphragm valve was diverted through a restrictor to waist. Splitting together with incomplete sampling of the sample stream impedes quantitative work with this system. Nevertheless chemometric methods were successfully applied to GCxGC with such instrumentation[13,14].

7.7 Non-mechanical modulators

Both types of modulators with moving parts have problems with mechanical failure, often resulting in breakage of columns. Future improvements will move away from modulators with moving mechanical parts.

7.7.1 Thermal modulation with hot and cold gas jets

In the latest commercial GCxGC version, streams of hot and cold gas are pulsed through jets to alternately heat and cool two short sections at the beginning of the second dimension column. This two-stage modulator uses high flow rates of nitrogen gas heated with a heating block close to the column for mobilization and cold air that passes through a liquid nitrogen dewar for trapping of sample components. Gases generally have a very low heat capacity and high flow rates are required. We found the total consumption of N_2 to be in excess of 450 L/hour.
Commercial prototypes are available operated with software written for the moving heater and have, with some effort, been successfully used in our laboratory to produce GCxGC chromatograms.

7.7.2 Resistive multi-segmented thermal-gradient modulator

A recent design again attempted to use resistive heating of up to 10 segments[15] for modulation. The trapping tube is placed into a metal sleeve that is designed to have a close fit. Connections are made of the same material as the heating sleeve and silver-soldiered in place. Successful multidimensional chromatograms have been produced with this modulator. Six stages are considered to be required for good modulation with this system. An advantage of many stages is that a thermal gradient can be created. (See the discussion on thermal gradients in Chapter 3.1). In a thermal gradient the front of a peak is at a lower temperature than the back. This allows peak compression to occur since the back of a peak catches up to the front and can potentially lead to very narrow injection bands. A major advantage of this arrangement is that large volumes of cryogens are not required for successful operation.

However, thermal gradients are generally not required when adequate focusing is attained in the trap and when it is ensured that remobilization occurs fast enough to not significantly contribute to the elution peak width.

Resistive heating of the metal sleeve does not suffer from the instabilities reported earlier for the painted capillaries since great care is taken to produce a homogenous sleeve. The separate sleeve is not affected by differences in expansion of fused silica and the metal. However, a close fit between the capillary and heating sleeve has to be ensured for good thermal contact.

7.8 Conclusion

While the different modulator designs produced results of varying success with GCxGC, none could be directly used for SFCxGC$_{ftp}$. The modulator in SFCxGC$_{ftp}$ requires a different design because the two columns are operated at very different conditions. The modulator design is further complicated by the need for mobile phase exchange. The design of a modulator for SFCxGC$_{ftp}$ will be demonstrated in the following chapter.

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