# 4 INSTRUMENTATION AND OPTIMISATION OF EXPERIMENTAL CONDITIONS

## Part I: GC×GC INSTRUMENTATION AND MODULATOR TYPES

### 4.1 GC×GC Instrumentation

Since the first publication in 1952 by James and Martin [1], gas chromatography has developed tremendously in its application, specialisation and productivity. This was largely due to developments in the general gas chromatographic system, improved column production techniques, variation in stationary phases and their coating techniques. The introduction of coupled chromatographic techniques such as GC-MS, heart-cutting GC (GC-GC), SFC-GC and comprehensive two-dimensional GC (GC×GC) has also added new dimensions to gas chromatography. Developments in data handling software and hardware to cope with the new techniques have been part of the overall progress.

The instrumentation in comprehensive two-dimensional gas chromatography is not fundamentally different from that in linear gas chromatography. However, GC×GC includes some additional hardware. The basic components of our GC×GC system are a 6890A gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a split/splitless sample inlet and a flame ionisation detector, two serially coupled columns, a modulating interface with all its accessories (Zoex, Lincoln, NE, USA) and a computer for instrument control and data handling.

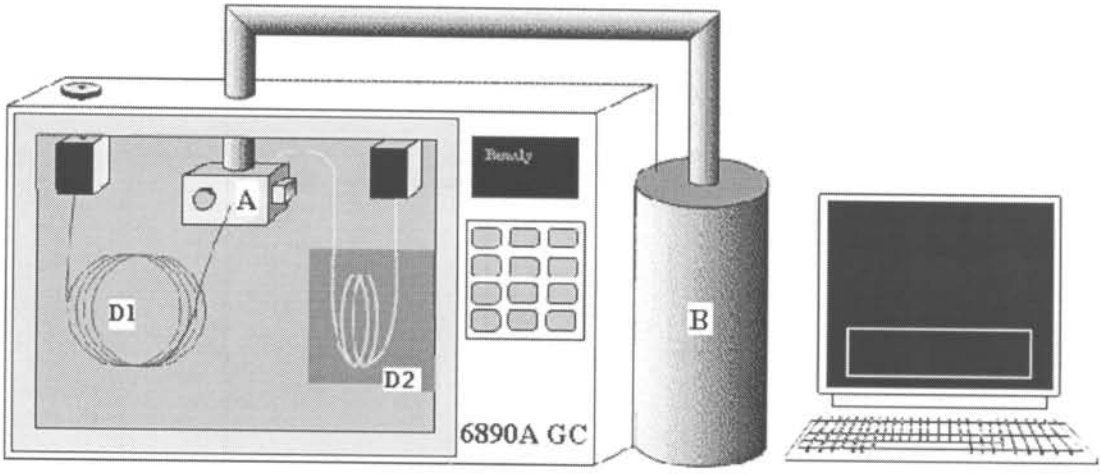


**Figure 4.1** The GC×GC system with the jet-cooled cryogenic modulator. (**A**) The jet modulator with no moving parts, (**B**) Auto-fill liquid nitrogen cooling unit (dewar), (**D1**) and (**D2**) are the first and second dimension columns, respectively.

### 4.1.1 The Sample Inlet

The sample inlet, as the part of the gas chromatograph responsible for the proper introduction of samples to the column, plays a major role in the overall quality of a gas chromatogram. The 6890A gas chromatograph used in this project is equipped with a split/splitless injector and all experiments were performed in the split mode.

In split injection a syringe is used to manually inject liquid samples into a heated chamber called the inlet liner. Then the injected sample is swept by a relatively large flow of gas and vaporised. The gaseous sample-carrier gas mixture is driven into the head of the analytical column and is split into two parts. The larger part of the mixture is vented out of the gas chromatograph *via* the split outlet and a small part is introduced into the analytical column. The split ratio, the flow through the column divided by the flow discharged through the vent, depends on the amount of sample the analyst wants to introduce to the system. Split ratios ranging from 1:5 to 1:1000 are possible [2].

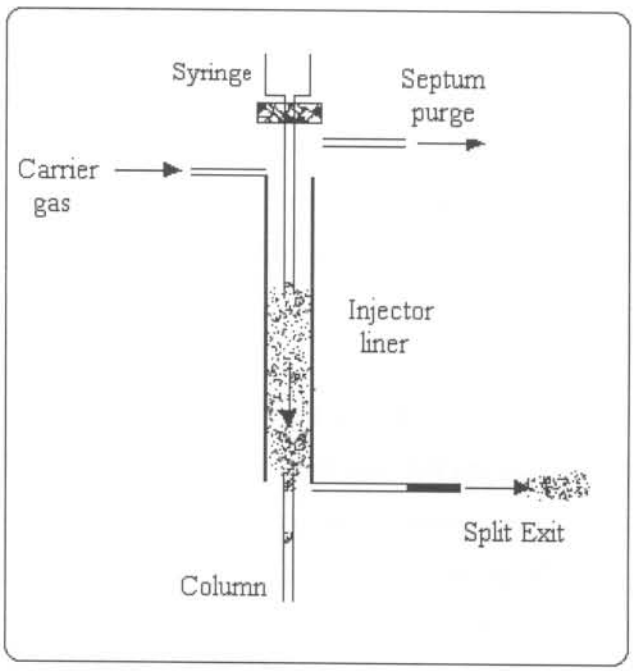


**Figure 4.2** The split injector: a small portion of the injected sample enters the capillary column and a major portion is vented through the split exit [2].

The split system is suitable for introducing samples in the micro and sub-microlitre range, and it is mostly used for relatively concentrated samples. The success of the split injection depends on some instrumental and personal factors. The split injector must vaporise the sample rapidly and completely and should mix it with the gas stream thoroughly [3]. Imperfections in injection, either from the instrument or the analyst, result in inaccuracies in the amount of sample introduced and distortions to the peak shapes. During manual injection into a hot injector liner the sample may start to evaporate inside the needle. This problem creates discrepancies between solutes injected due to losses on the needle wall. High boiling components, especially, are discriminated against. The success of manual injection depends on the analyst's good command of syringe techniques in rapidly executing the injection. To avoid such problems, either high boiling solvents or fast auto-samplers should be used. Properly executed split injections provide sharp initial bands [2].

### 4.1.2 The Analytical Columns

The analytical column, one of the main parts of a gas chromatographic system, is where all the sample interactions and separations take place. Fused silica capillary columns are produced in a wide variety of lengths and internal diameters. There are also a wide variety of stationary phases used with these columns, ranging from non-polar to highly polar substances.

The mechanism of separation in gas chromatographic columns depends on the type of stationary phase used. Depending on their chemical and physical properties (molecular mass, vapour pressure, polarity, solubility, stereochemistry, etc.) sample components are temporarily adsorbed on or dissolved in the stationary phase. The extent of stationary phase interactions affects the retention time of particular components. Effective separation is possible if sample components show different selectivity and interaction with the stationary phase [4].

Effective gas chromatographic analysis of samples of any type is influenced by parameters such as column length, column internal diameter, stationary phase film thickness, temperature programming rate, carrier gas linear velocity, etc. Good separation is possible when the above-mentioned parameters are successfully optimised and work in harmony according to the demands and requirements of the samples to be analysed.

This research was done under specific column parameters including column length, internal diameter, film thickness, and type of stationary phases. The GC×GC system consisted of two column sets. The first set consisted of a ~ 28.6 m length × 250 μm internal diameter (I.D.) × 0.250 μm film thickness ($d_f$) non-polar, 100% dimethylpolysiloxane stationary phase (HP-1, Hewlett Packard Corp., USA) first-dimension column and a 1 m × 100 μm I.D. × 0.100 μm $d_f$ medium polar, 14% cyanopropylphenyl 86% dimethylpolysiloxane stationary phase (Rtx-1701, Restek International, USA) second dimension column. The second set consisted of the same first dimension column and a polar carbowax stationary phase (Rtx-Wax, Restek International, USA) second dimension column with the same dimensions as above. The use of serially coupled columns of different dimensions and stationary phases is the main theme

exploited in comprehensive two-dimensional GC to effect superior sample resolution, as has already been dealt with in the previous chapter.

### 4.1.3 The Detector

The detector monitors analytes after the separation process depending on the different physical and chemical properties, which the eluted substances show towards it. In gas chromatography, the detector generates electrical signals, which are continuously recorded as a chromatogram. The intensity of this signal changes during the elution of a solute depending on the solute concentration or mass flow reaching the detector. When only the carrier gas reaches the detector, a 'straight' baseline is observed, but with increase in temperature, column bleed material is also measured, resulting in a continuous rise in the baseline [4].

There are different types of detectors in gas chromatography, amongst others the thermal conductive detector (TCD), which works on the principle of changes in the thermal conductivity of the carrier gas caused by the presence of a solute. Another type of detector used in GC is the electron capture detector (ECD). This detector's measuring mechanism depends on the electronegativity of the eluted components, *i.e.* their ability to form negative ions by capturing electrons. A third type of GC detector is the photoionisation detector (PID), which measures the amount of ionisation current produced when the solutes are irradiated with vacuum UV photons. Other types of detectors include the nitrogen-phosphorous detector (NPD), the sulphur selective detectors and the flame ionisation detector (FID) [5] that has been used throughout this study.

#### 4.1.3.1 The Flame Ionisation Detector (FID)

The FID, which was introduced in 1958 is the most common mass flow type detector used for the detection of organic compounds [5]. The signal of the FID is generated by the ionisation of molecules of the eluted compounds in a hydrogen-oxygen (air) flame. When trace amounts of hydrocarbons or any carbonaceous material, including stationary phase bleed, enter the flame zone, the detector response increases due to the ionisation of these materials. The FID response is the sum of all the ionisation processes taking place in the

flame during the elution of organic substances. The response is highest for hydrocarbons, being proportional to the number of carbon atoms. The detection entirely depends on the mass flow of the solute into the detector and increase in carrier or make-up gas flow has no effect on detector signal. The area of the peak recorded during elution of a separated solute, is proportional to the amount of the relevant solute [4, 5].

The FID is a very sensitive detector, which has an ionisation efficiency of about $10^{-1}$ C/mole *i.e.*, a change of $10^{-6}$ mole per second solute in the detector leads to a change of $10^{-7}$ A in the current [5]. It has also a very broad dynamic range, the range of sample concentration for which the detector provides reproducible quantitation, covering a range of $10^{7}$ between the highest and lowest detectable mass flow.

## 4.2 Interfacing and Modulator Types in Comprehensive Two-Dimensional Gas Chromatography (GC×GC)

The proper performance of any multi-dimensional technique mainly depends on the quality and proper method of interfacing used. In comprehensive two-dimensional gas chromatography, the modulator plays this crucial role. In order to preserve the separation obtained and to further separate overlapping peaks from the first dimension column, the modulator collects peaks eluting from the first dimension column and re-injects each fraction into the second dimension column as sharply focused pulses. The modulator is the heart of a comprehensive two-dimensional GC system. The main functions of a GC×GC modulating interface include the following [6, 7]:

- Trapping and concentration of the effluents from the primary column during the trapping time, avoiding the possibility of remixing already separated components in the second dimension column, while the first dimension separation proceeds,
- Focusing of the trapped solutes into very narrow pulses, and
- Injecting the trapped solute plugs onto the head of the second column quantitatively, in as narrow a band as possible, for further separation and elution before the next batch of solutes is injected.

To perform these three functions properly for the whole chromatographic run, the modulator:

- Must start trapping again as soon as possible after each re-injection to prevent breakthrough of analytes eluting from the primary column at this time [6, 7],
- Should not only effectively retain substances diverted into it, but also should be of low thermal mass for rapid heating to allow rapid re-introduction of the trapped fraction into the second column [8].
- Should concentrate and re-inject solutes reproducibly and without discrimination across a range of anlaytes [7].

There are some factors that affect the above-mentioned interface (modulator) operations, including [9]:

1. The carrier gas velocity, which affects the width of the pulse leaving the modulator. High column head pressure with high carrier gas velocity produces sharper peaks mainly due to the shorter retention times in the second dimension column.
2. The heating and cooling efficiency of the modulator,
3. The thickness of the stationary phase. The modulated part of the column should not be exposed to higher temperatures, which may decompose its stationary phase. Hence, the use of thin film providing less analyte retentions is called for.
4. Heat capacity of the modulated column part. Insufficient heating of the modulated column part may result in irregular peak shapes.

### 4.2.1 Types of GC×GC Modulators

Since the publication of the first paper on comprehensive two-dimensional gas chromatography by Liu and Philips in 1991 [10] numerous authors on GC×GC have published work on new or modified modulators. Generally, all the modulators used so far can be considered to be of two types: thermal and valve flow modulators. The thermal modulators are the most widely used and constitute various designs and modes of operations. The first of the thermal modulators is the on-column thermal desorption modulator described by Liu and Philips [10], which was followed by the longitudinally modulated cryogenic

system (LMCS) used by Marriott *et al.* [11]. Later, a mechanical thermal sweeper modulator was described by Blomberg *et al.* [12] and recently jet cooled modulators were described by Ledford *et al.* [13] and then by Beens *et al.* [14]. For the purpose and convenience of this dissertation, GC×GC modulators are divided and described into five types, *viz.*:

1. The on-column thermal desorption modulator
2. The mechanical thermal sweeper modulator
3. The longitudinally modulated cryogenic system
4. The differential flow or switching valve modulator
5. The jet cooled non-moving cryogenic modulator

## 1. On-Column Thermal Desorption Modulator (TDM)

An on-column thermal desorption modulator is a simple trapping and focusing arrangement which constitutes part of the head of the second dimension column. It is prepared by spraying an electrically conductive paint onto the modulator portion of the second dimension column to obtain a uniform film by multiple coatings. The paint-coated part of the column is dried in the oven after each coating and its resistance measured until the target resistance is reached [6, 10]. The total length of the modulator part of the column depends on the length of the second dimension column (15 - 20 cm). The modulator part is divided into two stages and requires no moving parts. The two stages have different lengths, which help to improve the chromatograms. Making the first stage, the accumulator stage, longer prevents sample breakthrough. A shorter, focussing second stage accelerates the concentrated pulses to the velocity of the carrier gas in the rest of the second dimension column [6, 15, 16].

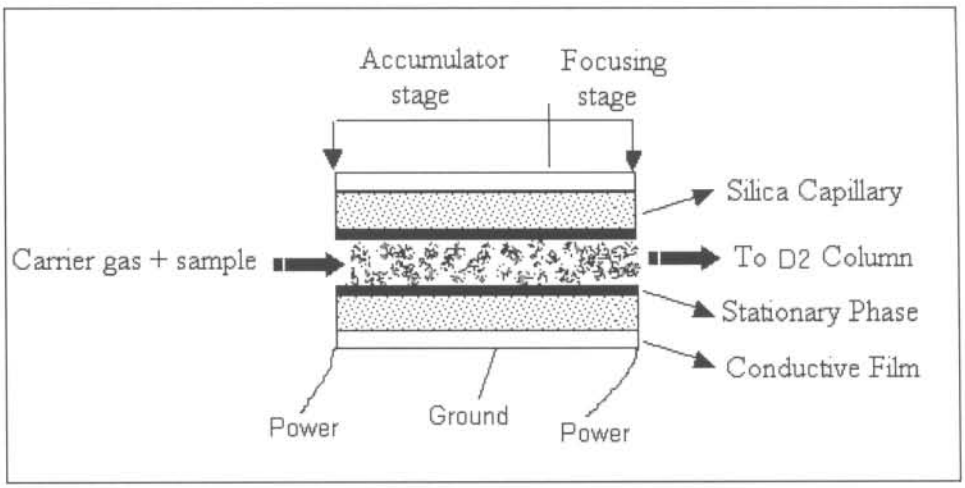


**Figure 4.3** An On-Column Thermal Desorption Modulator [17, 18]

After the first dimension column separation, the analytes are trapped for a certain period while the modulator is at room temperature in a separate temperature programmable box outside the GC oven. Next, they are quickly transferred to the second dimension column by applying a short current pulse to the two modulator stages, one after the other. The heat pulse releases components trapped in the stationary phase to be carried away by the mobile phase to the second dimension column. After each cycle the modulator is cooled by ambient air and the trapping and releasing process is repeated in the same manner. Such computer controlled current pulses are generated repetitively until the end of the run [6, 10, 16].

The initial modulator temperature should be low enough to retain solutes, the final temperature high enough to release them and the ramping rate must be fast enough so that the column rather than the modulator limits peak broadening. In this case, the fast second dimension column produces sharp secondary chromatograms while separation is in progress in the first dimension column. A comprehensive two-dimensional gas chromatogram is obtained by plotting all the second dimension chromatograms [15, 17].

The low thermal mass of fused silica columns and their ability to change temperatures rapidly are the two key features that affect the success of the TDM. The thin film of conductive paint affords superior thermal contact while inducing insignificant change in mass [16]. The TDM should not be heated for longer than the time required for the trapped

analytes to be pulsed out of it. The linear gas velocity and the length of the TDM determine the minimum heating required to release all trapped analytes from the TDM [19].

The most problematic aspect of this type of modulator is the application and curing of the conductive film, attaching electrical leads of low thermal mass and avoiding cold spots. Moreover, thin film modulators may burn out at unpredictable times, limiting the modulator's life span [15]. In broad terms, the TDM lacks robustness for practical use [12].

## 2. Mechanically Driven Thermal Sweeper Modulator

A thermal sweeper modulator is a device that controls the temperature of a short length of a gas chromatographic column as a function of position and time. This modulator has two main components, *viz*. the modulator tube or modulator capillary used to serially connect the two capillary columns and the rotating slotted heater. The function of the slotted heater is to periodically sweep over the thick-film stationary phase section of the modulator tube to focus the solutes into very narrow bands and force them to evaporate from the stationary phase into the mobile phase. The thick film of the modulator tube helps to retard the solutes before they are swept away by the modulator. The narrow bands are then carried away by the higher linear velocity of the carrier gas into the second column for fast separation. The short second dimension column with high phase ratio (thin film) and small inner diameter allows all peaks to be eluted and detected before the subsequent thermal modulator injection [12, 20].

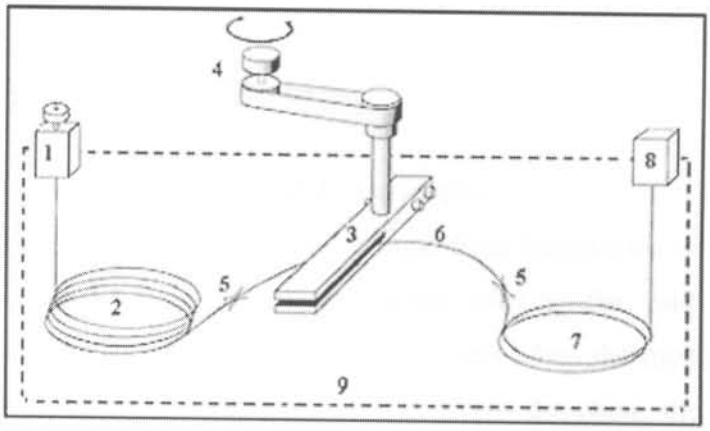


**Figure 4.4** Schematic diagram of a GC×GC system with the thermal sweeper modulator. (**1**) Injector, (**2**) 1st dimension column, (**3**) Heated slot, (**4**) Stepper motor, (**5**) Press-fit, (**6**) Modulation capillary, (**7**) 2nd dimension column, (**8**) Detector & (**9**) GC oven [12].

As noted above, during the transfer of components from the first dimension column to the second dimension column their bandwidths are greatly reduced. The reduction in bandwidth enhances the peak height and hence the limit of detection of GC×GC is much lower than in one-dimensional capillary gas chromatography [21].

When using the thermal sweeper modulator, the temperature of the modulation capillary should be kept at least 100 K lower than the temperature of the slit of the sweeper so that the modulator can effectively focus and produce sharp solute bands. Exposing the modulator capillary to temperatures above its maximum produces column bleed, hindering the analysis [21].

In addition to the temperature difference required between the sweeper and the modulation capillary, the overall design of the thermal sweeper modulator is complex. It has several moving parts, occupies large oven space and the GC has to be greatly modified to accommodate the modulator [22].

### 3. Longitudinally Modulated Cryogenic System (LMCS)

The longitudinally modulated cryogenic trap, as detailed in the works of Marriott and Kinghorn [11, 23, 24, 25, 26, 27 and 28] is constructed from a hypodermic-steel tube with three openings on the sides. One of the openings at the centre of the cryo-trap serves for cryogenic entrance and the other two holes on left and right ends of the sides of the trap are used for venting the coolant out (see details in Figure 4.5).

When the trap is in place, liquid cryogen ($CO_2$) is supplied through the trap inlet and expands to cool the body of the metal-trap. To prevent the trap from freezing the column, a small flow of nitrogen is allowed through the centre. Solutes entering the cold trap are stopped, accumulated and focused into sharp bands. This happens when the modulator is in the so-called *Trap* position. Moving the trap to the up-stream direction, the *Release* position, exposes the focused band to the oven temperature. When the trap is in the release position the cryogen supply is temporarily interrupted. The oven heat forces the band to be moved into the second dimension column and then continues its migration to the detector. The GC,

through a solenoid, controls cryogen supply and the trap movement is controlled by pneumatic drive [27] or by an electrically driven solenoid [26]. The modulator movement between the trap and release positions is repeated at a predetermined frequency for the duration of the chromatographic run [27].

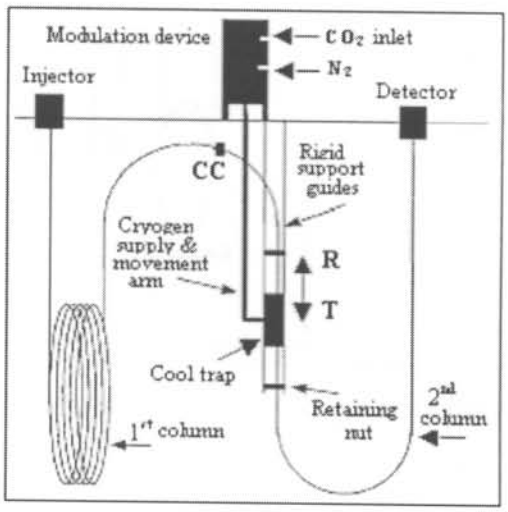


**Figure 4.5** Schematic diagram of the LMCS in a GC×GC system. (**CC**) Column coupling, (**T**) Trapping and (**R**) Release positions. The capillary is held by retaining nuts to the support. The cool trap is connected to a cryogenic supply and movement arm, and moves back and forth along the guides [28, 29].

A slightly different design of a moving cryogenic modulator from that of Marriott *et al.* is the one described by Beens and co-workers [30]. This cryogenic modulator has a cooling chamber and heating coil to help accelerate the heating of the trapped solutes. But, as reported by the authors, the heating coil was later removed, as it did not effectively perform the intended process of heating the trapped solutes for remobilisation. A pneumatic ram, connected to the cooling chamber with stainless-steel tubing from the top of the GC oven, performs the up and down movements of the modulator.

In this design the cryogen ($CO_2$) is directly sprayed on the capillary column inside the cooling chamber, instead of cooling a stainless steel modulator tube. Air or moisture is prevented from entering the cooling chamber by the escaping gas, avoiding any problem of freezing inside the chamber [30].

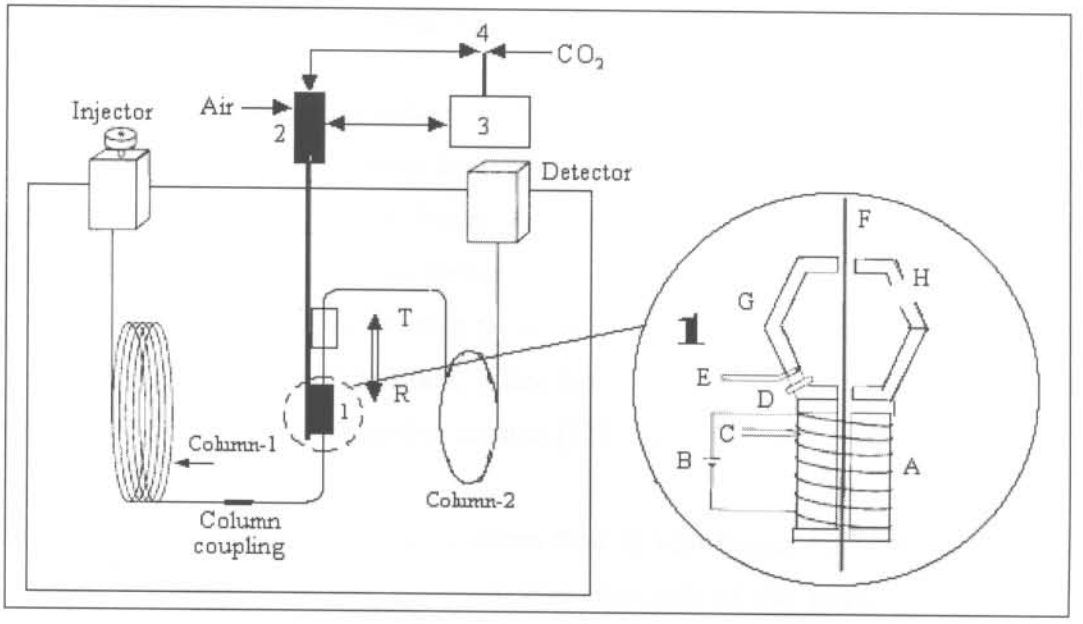


**Figure 4.6** Schematic diagram of a moving cryogenic modulator set-up. (**1**) Modulation chamber, (**2**) Pneumatic ram, (**3**) Modulation controller, (**4**) Two-way $CO_2$ valve, (**T**) Trapping position and (**R**) Release position. *Insert*: (**A**) Heating coil, (**B**) Electric power for heating coil, (**C**) Thermocouple, (**D**) Temperature sensor, (**E**) $CO_2$ inlet with orifice, (**F**) capillary column, (**G**) Cooling chamber and (**H**) $CO_2$ outlet [30].

Compared to the thermal sweeper modulator, the longitudinally modulated cryogenic system has some practical advantages. When using the LMCS there is no need for using an extra thick film modulator capillary, only one ordinary column connector is used and there is no temperature restriction [26]. Therefore, the cryogenic modulator has a practical advantage, as heated modulators will put extra thermal stress on thermo-labile compounds during the few seconds of heating and they have limited potential for the remobilisation of high boiling compounds.

One of the problems in using the moving cryogenic modulator is the frequent breaking of the fragile second dimension column due to its contact with the moving modulator part [30].

## 4. Differential Flow Modulation

The differential flow modulator utilises a 6-port diaphragm valve fixed at the centre of the GC platform to collect effluents from the first dimension column and periodically inject them into the second dimension column. Fused silica unions connect the deactivated fused silica transfer tubes to the columns inside the GC oven. Solutes exiting the primary column enter the 6-port valve and they are collected in the sample loop, a deactivated stainless steel tubing, when the valve is in the collect position. When the valve is in the inject position the contents are transferred to the second dimension column [22]. (Refer to Figure 4.7b for details).

As the authors put it: "The secondary column flow is kept higher than the primary column flow. Thus, the volume of gas collected from the exit of the primary column can be transferred to the secondary column as a pulse with a width of approximately 5% of the collection time. In the absence of substantial axial mixing, the flux exiting the loop in the inject position should be a compressed mirror image of the flux that entered the loop during the previous collection cycle. The sample plug then passes through the secondary column where chromatographic retention and peak broadening occur. Assuming only moderate broadening, the peak flux exiting the secondary column is still several times higher than the flux that originally exited the primary column due to the higher secondary-flow. Detectors that have a response proportional to component flux, such as the flame ionisation detector, will have increased response when the differential flow method is used" [22].

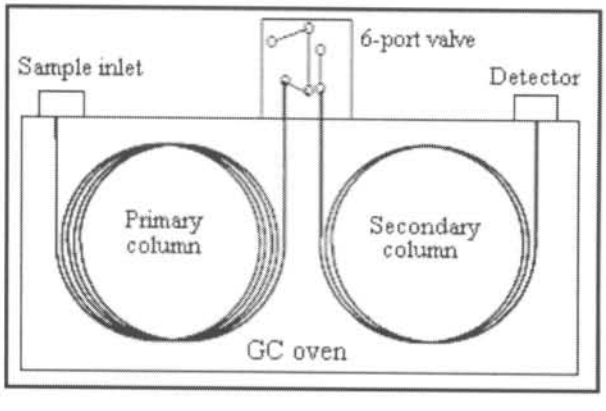


**Figure 4.7a** Schematic diagram of a differential flow modulated GC×GC System [22].

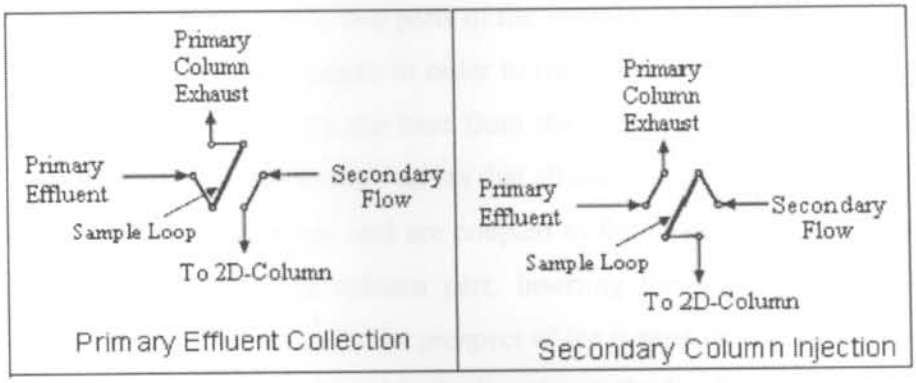


**Figure 4.7b** Details of the 6-port differential flow modulator [22].

The differential flow GC×GC system features a simple modulation system, which is cost effective as it can be constructed from easily available non-expensive materials, requires no great modification to commercial GCs and the system is quite durable [22]

The incomplete sample transfer from the first column to the second column in the valve based flow modulators restricts the use of these modulators to relatively concentrated samples. The other drawback of using this modulator is the limited high temperature tolerance of the diaphragm, which hinders the use of this modulator for the analysis of high boiling components [31].

## 5. Jet Cooled Non-Moving Dual Stage Cryogenic Modulator

The jet cooled, dual-stage, cryogenic modulator is another type of modulator used in comprehensive two-dimensional gas chromatography to enable the efficient trapping and re-injection of sample components eluting from the first dimension column to the second dimension column. There are basically two types of jet cooled, non-moving modulators. The one described by Beens *et al.* [14] uses two cold $CO_2$ jets to alternately trap analytes. Oven temperature is used to remobilise the focused spots while the cold jet is off. The other type, a prototype from Zoex corporation (Zoex, Lincoln, NE, USA), which has been employed in this project, has two cold and two warm nitrogen jets used to trap and re-inject the effluents from the first dimension column to the second dimension column.

In the dual stage $CO_2$ jet modulator, two parts of the modulated capillary column are directly and alternately cooled with the cryogen in order to trap and focus each subsequent fraction, which afterwards is remobilised by the heat from the surrounding oven air. The $CO_2$ jets consist of two electrical-driven two-way valves that alternately open and close the liquid-$CO_2$ line through two pieces of capillary and are coupled to the nozzles through which the cold gas is sprayed on the modulated column part. Inserting the jet tubes in a brass socket increases their heat capacity and curbs the prospect of ice formation on the outside of the jets at lower temperatures. Spraying the cold gas directly on the head of the second dimension column cools the column quickly down by about 100°C below the oven temperature. When the valves are closed and the cooling is interrupted, surrounding warm air from the GC oven heats up the cooled part of the modulated capillary to oven temperatures in a very short span (*ca.* 13 ms). A timing device controls the modulation process [14].

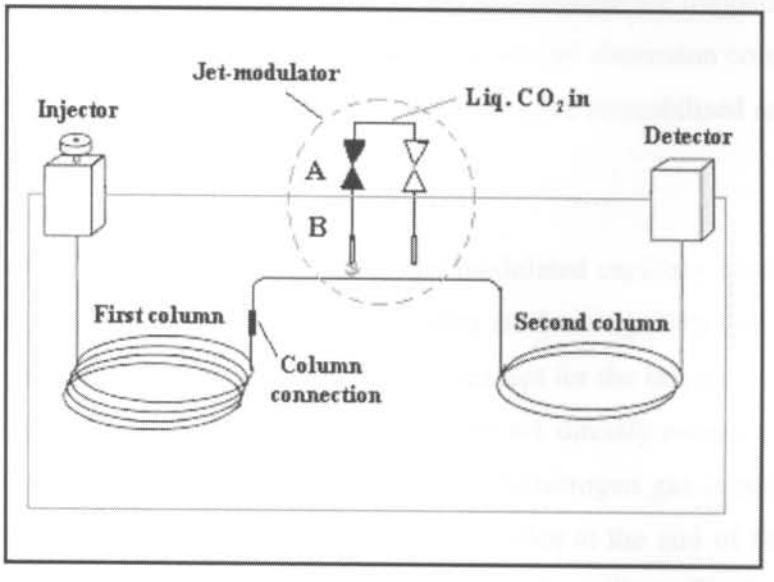


**Figure 4.8a** A GC×GC system with the dual-stage non-moving $CO_2$ jet modulator: (**A**) $CO_2$ valves and (**B**) $CO_2$ nozzles [14].

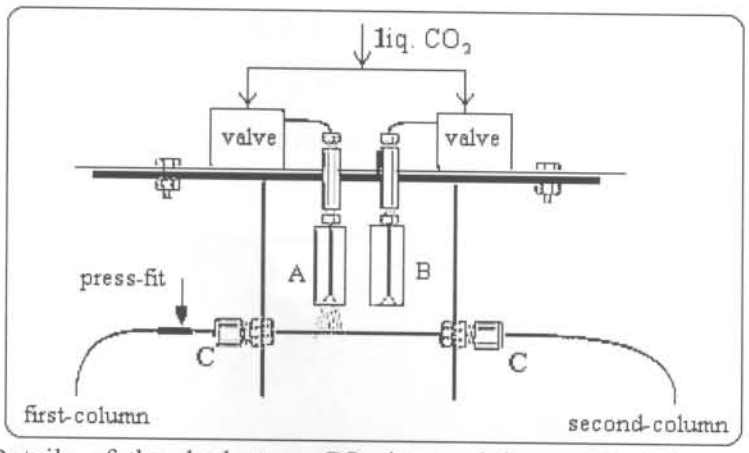


**Figure 4.8b** Details of the dual stage $CO_2$ jet modulator: (**A**) $CO_2$ nozzle on, (**B**) $CO_2$ nozzle off and (**C**) Tight column unions [14].

In the dual-stage nitrogen cooled and warmed modulator, cool and warm nitrogen gas pulses are used to rapidly alter the temperatures of part of the head of the second dimension column for two-stage modulation. Analytes eluting from the first column are trapped and focused by a continuous stream of cold nitrogen at the head of the second dimension column. Alternately operating hot nitrogen gas jets heat the trapped analytes to be re-mobilised as sharp pulses to the rest of the second dimension column [14].

The cold jet tubes are mounted directly above the modulated capillary from the roof of the GC oven and are insulated inside a vacuum-stainless steel tubing from the top of the liquid nitrogen container unit (dewar) until inside the oven except for the last part, which is exposed to the oven temperatures. Inside the dewar these tubes are directly connected to two copper coils immersed in liquid nitrogen. A continuous flow of nitrogen gas is passed through the cooled coils and is expelled continuously from the nozzles at the end of the stainless steel tubes inside the GC, forming cold spots on the modulated capillary. The hot jet tubes, also mounted from the roof of the GC oven at right angles to the cold jet tubes, are fed with nitrogen gas by two Teflon tubes from the top of the GC. The hot jet tubes have wider diameter than the cold jet tubes to enable them to efficiently heat the cold spot after each cold trapping. To facilitate the heating of the gas, the wider diameter tubes are passed through a drilled-in heating block mounted before the exit of the gas inside the GC oven. All the operations of the modulator, including heating of the heating block are facilitated and monitored by a KT-2001 (Zoex, Lincoln, NE, USA) control board. The level of the liquid

nitrogen in the dewar is controlled by TERAGON liquid nitrogen level controller (TERAGON Research, Sanfrancisco, USA). The GC×GC system is interfaced with the computer *via* an SCB-68 (National Instruments, Austin, Texas, USA) interface modem.

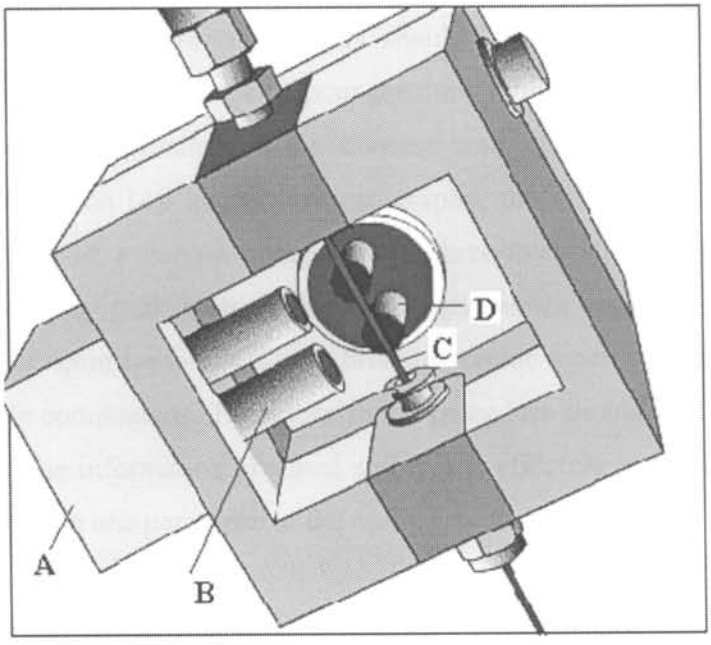


**Figure 4.9** The cryogenic jet cooled and warmed dual-stage non-moving modulator. (**A**) Heating block, (**B**) Hot gas jets, (**C**) Modulated capillary and (**D**) Cold gas jets.

The part of the second dimension column in which the modulation takes place is stretched and secured between unions mounted in stainless steel bracket. The tightly stretched modulated capillary is secure from being vibrated by the gas continuously sprayed onto it [14]. Contact of the column with the metallic unions is prevented by passing the column through graphite-vespel ferrules placed on both ends of the tightening Swagelocks.

The dual-stage, non-moving jet modulator is very robust and modulation is performed satisfactorily. Very low temperatures are reached, enabling the modulation of compounds with very low retention factors. As it lacks any moving parts, column breaking during modulation is unlikely [13]. A point of concern is that the extra heating needed to remobilise trapped fractions puts the second dimension column under extra stress and shortens its life span. Moreover liquid nitrogen, although a very effective coolant, is expensive and needs bulky insulation when transported through tubing [14].

# Part II: OPTIMISATION OF EXPERIMENTAL CONDITIONS

## 4.3 Optimisation of Gas Chromatographic Parameters

The purpose of optimising any analytical system is to get the maximum possible information from it. Optimisation in gas chromatography should be done with the ultimate goal of effectively separating the widest range (vapour pressure, molecular mass, polarity, functional group, etc.) of substances possible, in the shortest time, at the lowest cost and with the highest possible resolution [4]. In gas chromatography, the information one gets from the detector output signal of a certain component is three-fold, *viz*. retention time used for qualitative identification, peak width and peak height which together give a measure of quantity. The proper optimisation of a gas chromatographic system involves improving the resolution of sample components. The optimisation procedure should only be carried out if it generally improves the information obtained and if it is effective in terms of cost and time. One should not optimise one parameter at the cost of another [8].

Optimisation in chromatography revolves around the resolution between two peaks called the critical pair and encompasses the optimisation of different variables, which are interdependent. The number of variables which need to be optimised and their interdependency becomes much more complicated and even more difficult in two-dimensional than in linear GC, since two serially coupled columns of different dimensions are employed. However, as we were working under some fixed variables such as column length, internal diameter, carrier gas type, stationary phase composition and film thickness, the number of variables that needed to be optimised around these conditions was brought down to the minimum. Therefore, the linear gas velocity in both columns and the temperature programming rate(s) were the two main parameters that were optimised.

A chromatographic peak is characterised by the retention time ($t_R$) and the peak width. The complete resolution of two components depends on their having base-line separated Gaussian shaped peaks with an average peak width smaller than their retention time difference. The retention time difference is affected by the type of stationary phase used, the column length, the vapour pressures of the components, column temperature and activity coefficient of the

two species. Mathematically, the resolution of two peaks can be defined by equation ((4.1a) and (4.1b)) [4, 32]:

$$R = \frac{2\Delta t_R}{w_{b1} + w_{b2}} \qquad (4.1a)$$

$$R = \frac{2\Delta t_R}{4(\sigma_1 + \sigma_2)} \qquad (4.1b)$$

Where $\Delta t_R$ is retention time difference between two peaks

$w_{b1}$ and $w_{b2}$ are the peak widths at base

$w_h$ is width at half height and

$\sigma$ is the standard deviation

For a Gaussian peak $w_b = 4\sigma$ and $\sigma = (\frac{w_h}{2.355})$

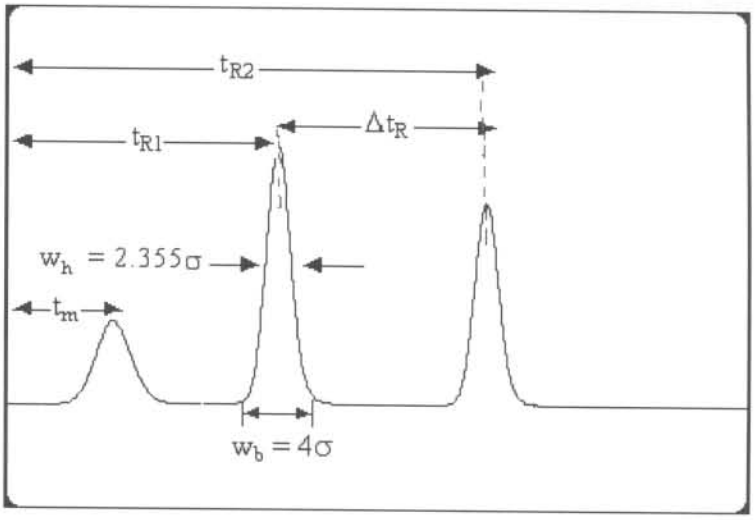


**Figure 4.10** Resolution of two peaks: $t_m$ is the dead time, $t_{r1}$ and $t_{r2}$ represent the retention times of peaks one and two.

In comprehensive two-dimensional GC the overall resolution of two neighbouring components is given by the contribution of the resolutions in both dimensions according to the relationship mathematically expressed in equation (4.2) [33]:

$$R \cong \sqrt{(R_1^{\,2} + R_2^{\,2})} \qquad (4.2)$$

If either of the resolutions is greater than one, the final resolution will always be greater than one. Accordingly, the probability of resolving two neighbouring components is more certain in two-dimensional systems than in linear techniques, because two components are much less likely to show similar displacements in the two-dimensional separation space [33].

### 4.3.1 Optimising the Inlet Pressure for Optimum Linear Gas Velocity[1]

In comprehensive two-dimensional gas chromatography, the first dimension column is chosen to be long and the second dimension column to be short to achieve the desired relative speeds of analysis. The first and second dimension columns normally operate at two different linear gas velocities, as the latter, by virtue of its smaller internal diameter, has a faster linear gas velocity. So, the optimisation of the linear gas velocity (inlet pressure) is to achieve the optimum speed at which the two columns can operate in harmony, that is, the best overall resolution is achieved.

The first column was 28.6 m in length with 250 μm I.D. and 0.250 μm $d_f$. The second column was 1 m × 100 μm × 0.100 μm $d_f$. To find the pressure at which the average linear gas velocity is optimum, a series of runs were performed at different pressures. Pairs of peaks were selected and their resolution was calculated using equation (4.1b) for each run. The resolution is preferably calculated from the standard deviation (σ) value, which is easily calculated from the peak width at half-height. It is inconvenient to accurately determine the width at base due to the frequent absence of a smooth baseline. Then the results were plotted against pressure for both columns (figures (4.12) and (4.14)) and the best linear velocity value was selected. In choosing the optimum pressure, the widths of the peaks were also considered. Refer to tables ((4.1) and (4.2)).

[1] The optimisation procedure was conducted using diesel samples. Diesel was chosen for its wide variety of components, which cover almost all the available GC×GC separation space. [*W. Welthagen, A.Z. Zellelow and E. Rohwer, Poster Presentation at the International Symposium on Analytical Science, Analytica 2002, Stellenbosch, South Africa]*

**Table 4.1** Peak measurements for the first dimension column

| Pressure kPa | $t_{m1}$ sec | D1 L cm | u cm/sec | $t_{r1}$ msec | $t_{r2}$ msec | $\Delta t_r$ msec | $w_{h1}$ msec | $w_{h2}$ msec | $4\sigma_1$ msec | $4\sigma_2$ msec | $R_1$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 60.00 | 224 | 2857 | 12.75 | 74686 | 81582 | 6896 | 704 | 727 | 1195.75 | 1234.82 | 5.67 |
| 80.00 | 173 | 2857 | 16.51 | 59889 | 65575 | 5686 | 483 | 496 | 820.38 | 842.46 | 6.84 |
| 90.00 | 156 | 2857 | 18.31 | 52333 | 57402 | 5069 | 401 | 419 | 681.10 | 711.68 | 7.28 |
| 100.00 | 143 | 2857 | 19.98 | 48408 | 53114 | 4706 | 365 | 383 | 619.96 | 650.53 | 7.41 |
| 110.00 | 130 | 2857 | 21.98 | 45955 | 50441 | 4486 | 320 | 358 | 543.52 | 608.07 | 7.79 |
| 120.00 | 125 | 2857 | 22.86 | 42281 | 46433 | 4152 | 291 | 308 | 494.27 | 523.14 | 8.16 |
| 130.00 | 121 | 2857 | 23.61 | 39972 | 43920 | 3948 | 283 | 296 | 480.68 | 502.76 | 8.03 |
| 150.00 | 100 | 2857 | 28.57 | 35254 | 38763 | 3509 | 228 | 249 | 387.26 | 422.93 | 8.66 |
| 190.00 | 82 | 2857 | 34.84 | 28783 | 31675 | 2892 | 185 | 203 | 314.23 | 344.80 | 8.78 |
| 220.00 | 80 | 2857 | 35.71 | 25365 | 27922 | 2557 | 160 | 174 | 271.76 | 295.54 | 9.01 |
| 280.00 | 68 | 2857 | 42.01 | 20502 | 22582 | 2080 | 140 | 150 | 237.79 | 254.78 | 8.45 |
| 320.00 | 60 | 2857 | 47.62 | 18065 | 19889 | 1824 | 125 | 138 | 212.31 | 234.39 | 8.17 |

*For the meaning of symbols in the table, see text.

$$u = \frac{L}{t_m} \tag{4.3}$$

Where u is the average linear gas velocity in cm/sec

L is the column length in cm and

$t_m$ is the dead time in seconds.

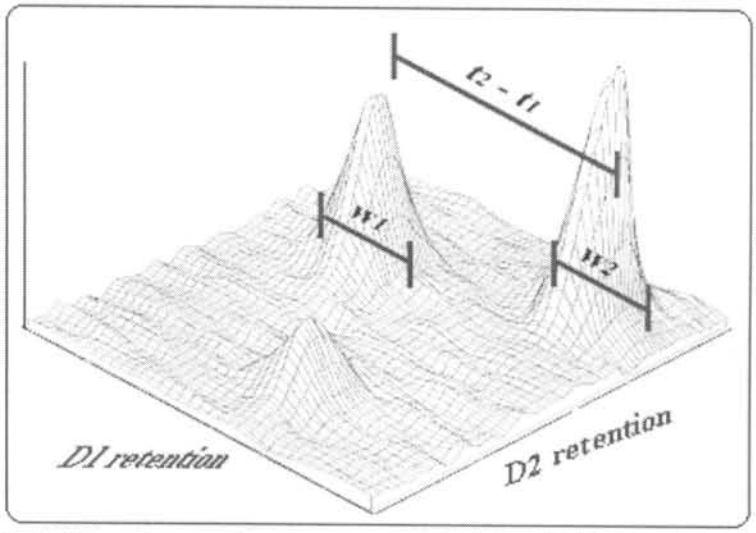


**Figure 4.11** First dimension peak measurements: ($t_1$) and ($t_2$) are the retention times and ($W_1$) and ($W_2$) are the peak widths at half height for peaks one and two in the first dimension, respectively

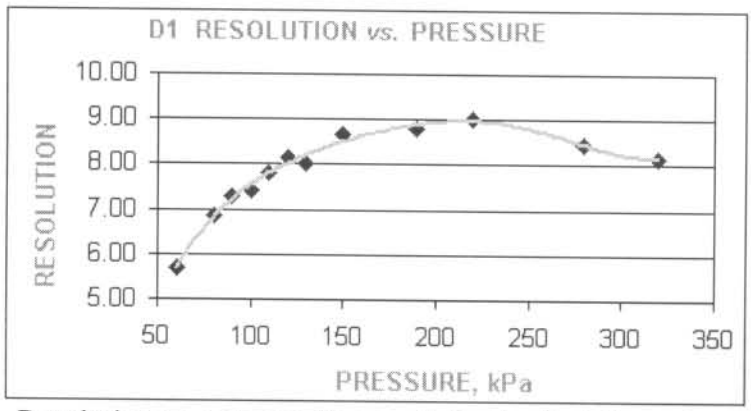


**Figure 4.12** Resolution *vs.* pressure line graph for the first dimension column

**Table 4.2** Peak measurements for the second dimension column

| Pressure kPa | $t_{m2}$ sec | D2 L cm | u cm/sec | $t_{r1}$ msec | $t_{r2}$ msec | $\Delta t_r$ msec | $w_{h1}$ msec | $w_{h2}$ msec | $4\sigma_1$ msec | $4\sigma_2$ msec | $R_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 60 | 751 | 105 | 139.81 | 2626 | 2926 | 300.00 | 72 | 90 | 122.29 | 152.87 | 2.18 |
| 80 | 561 | 105 | 187.17 | 2151 | 2401 | 250.00 | 57 | 65 | 96.82 | 110.40 | 2.41 |
| 90 | 500 | 105 | 210.00 | 2025 | 2270 | 245.00 | 55 | 61 | 93.42 | 103.61 | 2.49 |
| 100 | 447 | 105 | 234.90 | 1872 | 2107 | 235.00 | 55 | 60 | 93.42 | 101.91 | 2.41 |
| 110 | 410 | 105 | 256.10 | 1744 | 1964 | 220.00 | 55 | 60 | 93.42 | 101.91 | 2.25 |
| 120 | 374 | 105 | 280.75 | 1654 | 1864 | 210.00 | 55 | 60 | 93.42 | 101.91 | 2.15 |
| 130 | 344 | 105 | 305.23 | 1594 | 1903 | 309.00 | 55 | 60 | 93.42 | 101.91 | 3.16 |
| 150 | 300 | 105 | 350.00 | 1600 | 1800 | 200.00 | 70 | 75 | 118.90 | 127.39 | 1.62 |
| 190 | 237 | 105 | 443.04 | 1574 | 1754 | 180.00 | 90 | 90 | 152.87 | 152.87 | 1.18 |

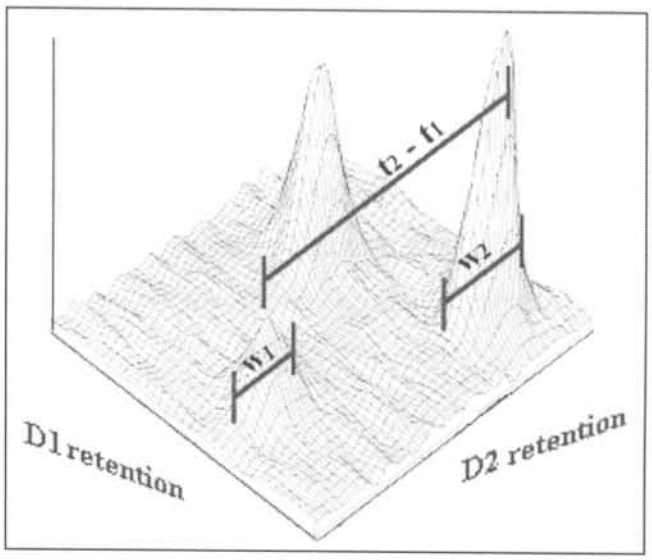


**Figure 4.13** Second dimension peak measurements: ($t_1$) and ($t_2$) are the retention times and ($W_1$) and ($W_2$) are the peak widths at half height for peaks one and two in the second dimension, respectively.

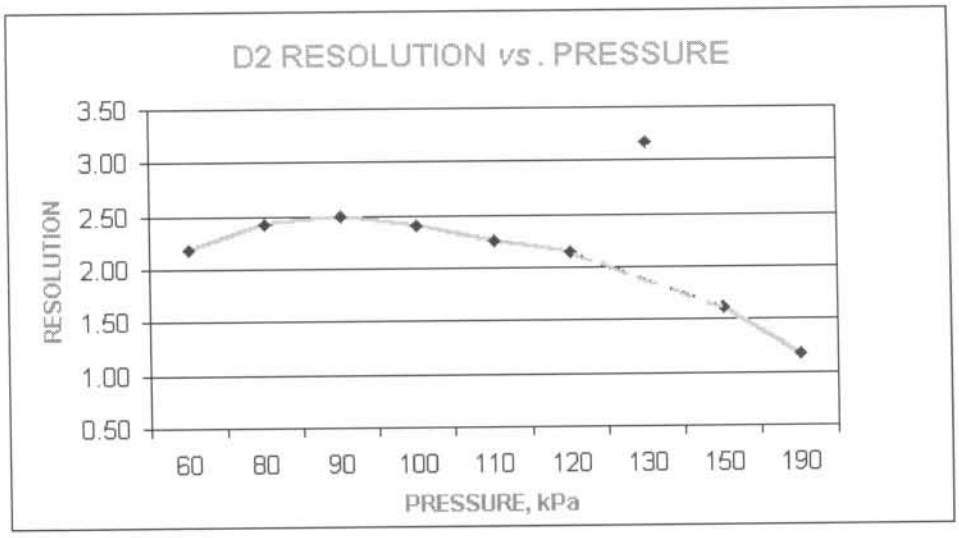


**Figure 4.14** Resolution *vs.* pressure line graph for the second dimension column

As can be seen from table (4.1), at low pressures the first dimension column has very broad peaks with relatively low resolution. With increased pressure both the peak widths and resolution between the two peaks improve. On the other hand, if we see the situation in the second dimension column from table (4.2), the peak widths and resolution are at their best between pressures 100 - 130 kPa. The value at 130 kPa is treated as an experimental error.

After considering the above results, a compromise was taken between the conditions in the two columns and the working pressure was set at 110 kPa for all subsequent runs. At this pressure, the overall peak resolution is satisfactory and the peak widths in the first dimension are slightly wider than those achieved at optimum D1 resolution, however, allowing more time for the second dimension run. From a fundamental point of view, the first dimension column is run slightly slower and the second dimension column slightly faster than required for optimum resolution.

### 4.3.2 Optimising the Column Temperatures

The GC×GC system utilised in this research is equipped with an auxiliary oven (Aux-2) for housing the second dimension column. Therefore, the two columns could be individually temperature programmed. In optimising the temperature, two aspects were considered:

1. **The ramping rate:** The ramping rate affects the retention times of components in general. At faster ramping rates, the retention times of the components are decreased, eluting with increasingly lower retention factors, k. Ramping rates 5°C per dead times ($t_m$) or lower are required for optimum resolution. The lower rates are not normally, used in linear GC, as it only slows down the analysis. However, in GC×GC, slower first dimension analysis is required in order to have wider peaks and thus more time for the second dimension run. After trying several runs at $1^0$C/min, $2^0$C/min and $3^0$C/min, a $1^0$C/min ramping rate was found to be most appropriate for analysing the essential oil samples at hand.

2. **The temperature difference between the two columns**: Operating the two columns at different temperatures has a distinctive advantage in manipulating the retention time of components in the second dimension column. Increasing the temperature difference between the two columns (running the second column at higher temperatures) reduces the second dimension retention times of more polar components. However, in this case, the second dimension separation between less polar analytes is decreased (too low k values). Reducing the temperature difference towards the end of the run increases the second dimension retention time but improves the separation of less-polar high boiling components [34]. Accordingly, the second auxiliary oven was operated at $30^0$C higher than the main GC oven for most of the run time. The difference is decreased slowly, and eventually the main oven temperature catches-up with the auxiliary oven temperature towards the end of the run. This temperature programme also ensures that the second, less stable, polar column, does not exceed its maximum allowable temperature at the end of the run. The final temperature programming rate used for the first column was from 30°C (2 min. hold) – 160°C at 1°C/min, then ramped to 250°C at 5°C/min (5 min hold). The second column was temperature programmed from 60°C (2 min hold) – 190°C at 1°C/min, and ramped to 250 at 5°C/min.

## 4.4 Optimising the Modulator for Better Performance

The prime task of a modulator is to produce narrow solute injection peaks. Pulsed peak widths of <100 ms have been reported [35]. The required final second dimension peak width

is a function of the second dimension column length and other operating conditions including temperature and carrier gas velocity. To obtain maximum resolution from the second dimension column, the modulator injection bandwidths should be much narrower than the widths produced by the column itself. This is often difficult to achieve in the case of the fast, almost unretained peaks in isothermal GC. The shapes of the second dimension injection profiles are also important. Although peak asymmetry could be the result of column performance and activity of solutes in the column, it might also indicate the inability of the modulator in delivering sharp symmetrical bands to the second dimension column.

The dual-stage modulator controls two trapping-remobilisation steps. In the first step a fraction of the analytes is trapped by the cold jet and remobilised when the first hot pulse blows on the trapped spot. Then the analytes are re-trapped by the second cold jet and after a while the second hot pulse remobilises the spot to be re-injected into the rest of the second dimension column. The accuracy of the duration of the warm pulses and the pulse delay between the two jets affects the re-injection and the final appearance of the chromatograms. Short pulses may help to introduce sharp injections, but too short a pulse may also not effectively and completely release and transfer trapped solutes. If the pulse is too long the injection band broadens and the second dimension peaks become asymmetrically distorted and tail. On the other hand a proper setting of the pulse delay, the time between the first pair of pulses and the next, is also equally important. Pulsing with too short delay reduces the efficiency of the cold jets to effectively trap and a long delay may induce freezing of the modulated capillary which will make it difficult for the hot pulses to release the trapped components. The up-stream and down-stream warm jet pulses were pulsed with 2 seconds break between them to effectively release the cold-trapped solutes from the first and then the second stages of the modulated capillary.

The modulation period was set at 6 seconds. This modulation period was long enough to allow most components to be eluted from the second dimension column. There was no serious wrap-around effect of polar compounds, especially when using the medium polar second dimension column (Rtx-1701). The wrap-around effect increases when a polar second column (Rtx-Wax) is used because of the tendency of the polar stationary phase to hold polar

components longer. The components spread out, and the more polar components show retention times longer than the set 6 seconds modulation period.

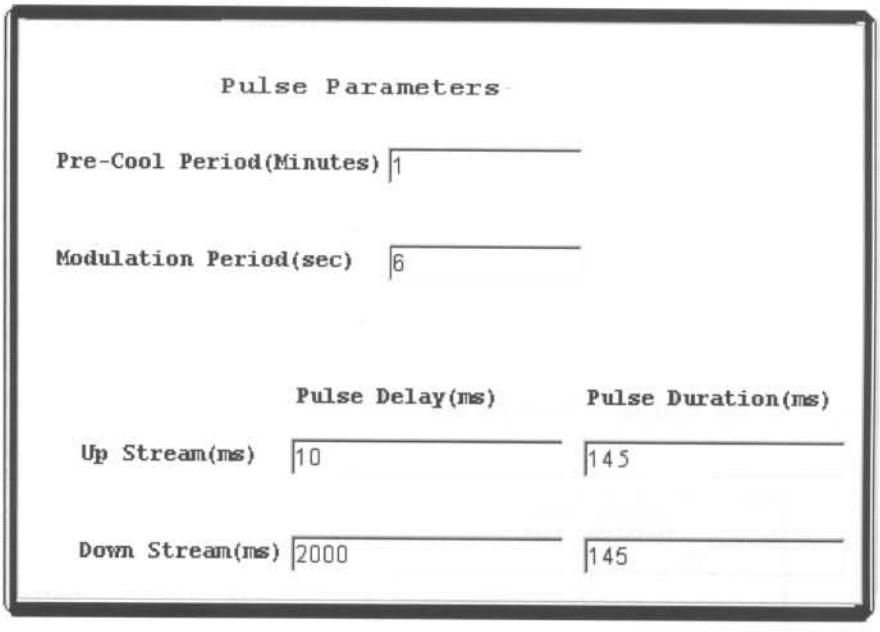


**Figure 4.15** The hot pulse parameters.

The temperature of the heating block responsible for heating the hot jet tubes inside the oven is set at a constant temperature of about 250°C. Therefore, the hot jets are hot enough when they exit the nozzle so that they can effectively release the trapped solutes.

A further optimisation of the modulator involves the flow rates of the cold jets. The cold jet flow rate is optimised for two reasons: to maximise its solute trapping efficiency and to reduce liquid and gaseous nitrogen consumption and thus the associated cost. The temperature of the cold gas stream increases with the distance from the nozzle depending on the gas flow rate and the temperature of its surrounding. The higher the gas flow rate or the lower the temperature of the surrounding air, the lower the temperature of the cold gas jets [13]. The use of appropriate cold jet flow rates guarantees that solute fractions are properly trapped before being released by the hot pulses. The cold nitrogen flow was kept between 11 – 12 l/min, depending on the oven temperature. At this flow the cost of nitrogen (liquid + gas) was about R20.00 per analysis.

Excessive cooling was observed not only to prevent effective re-injection but it also induces changes in retention times, especially in the first dimension. In such cases a cold spot apparently occurs in the column just before the first modulation stage, giving rise to additional retention as shown in table (4.3). This type of problem is very hard to detect as it does not show up in the shape of either the first or second dimension peaks. It only becomes apparent when accurate and reproducible first dimension data is required for peak identiffication. The surface temperature of ferrules and Swagelocks in the modulator bracket was monitored during runs, by attaching thermocouples, to make sure that their temperature is above the oven temperature.

**Table 4.3** Variation in first dimension retention times of *n*-alkanes due to improper modulator optimisation.

| Compound | D1 retention times, minutes | | |
|---|---|---|---|
| | Optimised modulator | Cold modulator | $\Delta t_r$, minutes |
| Nonane (C-9) | 20.2 | 20.9 | 0.7 |
| Decane (C-10) | 32.5 | 33.7 | 1.2 |
| Undecane (C-11) | 46.6 | 47.7 | 1.1 |
| Dodecane (C-12) | 60.7 | 61.9 | 1.2 |
| Tridecane (C-13) | 74.7 | 76.1 | 1.4 |
| Tetradecane (C-14) | 87.8 | 89.1 | 1.3 |
| Hexadecane (C-16) | 112.1 | 113.8 | 1.7 |

Another point that needs mentioning in using this modulator is the humidity and moisture level inside the laboratory or inside the GC oven. At higher humidity levels, water vapour might condense and create frost/ice on the modulated capillary, especially at lower oven temperatures. This reduces the effective release of the trapped solutes by the hot jets. In such cases it is essential to dry the oven by pumping in dry nitrogen. Keeping silica pebbles in the oven floor also helps to ward off moisture. More discussions on the performance of the modulator will follow in the section on discussion of results (refer to (5.4), pages 5-12 to 5-15).

## 4.5 Stationary Phase Selection for Essential Oil Analysis

The selection of appropriate stationary phases in gas chromatography is a crucial issue as the efficiency and precision of a chromatographic column depends on the general characteristics and coating efficiency[2] of the stationary phases. Marriott *et al.* [36] have outlined the following as important points to be looked into when developing and choosing appropriate stationary phases for essential oils analyses:

- The phases should be thermally and chemically stable.
- They should give greater selectivity in the separation of components by different phase chemistry. For example, as in the two different phase columns in GC×GC.
- They should allow better efficiency by making a more regular surface coating, or producing a thinner film coating.

In developing such phases:

- Specific components should be incorporated to allow new interactions such as those available with chiral selectors.
- Different technologies could also be used to optimise the available phases to the specific regions of the analysis that require better resolution.

Although a thermally stable column indicates its reliability over extended use, most essential oils only require column temperatures of about 200°C for complete elution of components. Still this problem is more evident in the case of polar stationary phases, which show more susceptibility to high temperatures and hence have a shorter life span when compared to non-polar phases that show more efficiency and thermal stability over an extended period of time. The proper analysis of any sample depends heavily on how efficient and selective the stationary phase is [36].

The availability of a wide range of stationary phases gives an analyst the freedom of choosing the appropriate phase(s), which can properly handle the sample matrix at hand.

[2] **Coating Efficiency** (CE%) is a measure of how smoothly and homogeneously the stationary film has been deposited on the column's internal wall.

Chiral phases are essential when enantiomer separation is required, such as in authentication of the purity and quality of an essential oil or in the complete characterisation of essential oil profiles [36].

Due to their ability to fulfil the above-mentioned characteristics, silicones are the most widely used stationary phases in gas chromatography. They exist in three phases: silicone oils, which are linear polysiloxanes; silicone gums, which are linear high molecular weight polysiloxanes and silicone rubbers, which are the cross-linked gums [37].

Gum phases are preferred to liquid phases due to their ability to efficiently and easily coat on capillary walls and give higher theoretical plate numbers [37]. Silicone gum apolar stationary phases, including SE-30, SE-52-, SE-54 and OV-73, are the most frequently used phases in perfumes and essential oils analysis. In addition to the above-mentioned qualities and characteristics, these phases exhibit high inertness, low bleeding and excellent column-to-column reproducibility. The purity of the stationary phases is high and the structure well defined. The drawback of these phases comes from their low polarity [1], which prevents them from separating non-oxygenate essential oil components from oxygenated counterparts of the same volatility.

The use of the medium polar pure polypropylene glycol (PPG) or more polar polyethylene glycol (PEG) phases, especially in two-dimensional gas chromatography coupled with apolar phases, is taken as an alternative. Another alternative is to use high molecular weight (HMW) PEG and its nitroterephthalic esters (FFAP phases), but these phases show increased activity compared to pure polyethylene glycol coatings. Aldehydes, for example, are very sensitive to acid-base effects and show reversible or irreversible adsorption on immobilised films and therefore tail or disappear completely. Therefore, for the analysis of essential oils and perfumes a normal PEG column is preferred [1]. In comprehensive two-dimensional gas chromatography, the coupling of gum phase capillary columns (*e.g.* HP-1) with medium (*e.g.* RTX-1701) or high polar (*e.g.* Rtx-Wax) phases is the solution adopted in the analysis of essential oils. The performances and results of the medium polar (Rtx-1701) and polar (Rtx-Wax) second dimension columns are given in more detail in the next chapter.

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