VOLATILE COMPOUNDS IN THE BLOOD OF FIRE FATALITIES

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by

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For my parents.

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CONTENTS

SUMMARY

An attempt was made to evaluate the importance of smoke and toxic gas inhalation in fires by comparing the volatile constituents in blood taken from fire fatalities with those of normal healthy and post-mortem controls. At the initial stage of the project, particular attention was given to the measurement of carbonyl compounds which represent a series of toxic and strongly irritant thermal degradation products from many polymeric materials.

Three analytical methods, namely, i) gas chromatographic analysis of carbonyls after conversion to their corresponding 2,4-dinitrophenyl hydrazone derivatives, ii) direct static headspace gas chromatography, and iii) dynamic headspace gas chromatography, were compared for their applicability to the measurement of carbonyls in blood. Problems associated with binding of carbonyls to blood proteins were experienced in the first two methods which precluded their use for this application. The latter method was found to be most suitable in this respect since the bound carbonyls were released during the purging process. The method was also the most sensitive and when used in conjunction with a mass spectrometer, a detection limit in the nanogram per millilitre range was obtained.

Volatiles in blood were extracted by purging the samples (1 ml) with 0.6 litre of helium and collecting the components on a small Tenax-GC column. The volatiles were then thermally desorbed and analysed by gas chromato-

graphy-mass spectrometry using a 100 m x 0.5 mm i.d. Carbowax 20M SCOT column.

The sorption technique was found to extract a wide range of volatile components from blood enabling a comparison of volatile profiles to be made. Thus initially organic nitriles as well as the carbonyl compounds were quantified. Volatile components were identified by comparison of their retention indices and mass spectra with those of authentic standards. Where the latter were not available, tentative identifications were made on the basis of their mass spectral data only. Quantification of carbonyls and nitriles was achieved by comparing their response ratios to those from an external standard under identical conditions.

During the period from August 1981 to May 1982, thirty-one blood volatile profiles were studied. These included four normal healthy controls and six post-mortem controls. In general, more complex profiles were found in fire fatalities than those of the controls. Over 140 chemical species have been identified in these profiles and these include series of carbonyls, nitriles, alcohols, esters, aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, heterocyclic compounds and sulphur-containing compounds. A detailed examination of the profiles has indicated that those compounds which might be of significance fell into two main catagories: those which were strong sensory and respiratory irritants, and those which were depressants of the central nervous system.

Quantitative measurements of carbonyls in blood have

shown that the mean levels of 2-butanone, butandione, 2-pentanone, cyclopentanone, cyclohexanone and hexanal were higher in fire fatalities than those in the controls. Of particular concern were the very high levels of acetonitrile and the presence of acrolein (a highly toxic and strong sensory irritant) in the blood of some of the fire fatalities.

Although the toxicological significance of these gaseous toxicants in causing fire fatalities has yet to be established, the results have clearly demonstrated that most of the fire deaths included in this study had been exposed to a wide range of toxicants. It is likely that these compounds may have played a vital role in causing incapacitation during the fire.

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
CI	chemical ionisation
CO	carbon monoxide
DNPH	dinitrophenyl hydrazine/hydrazone
EI	electron impact
EPA	Environmental Protection Agency
GC	gas chromatography
HbCO	carboxyhaemoglobin
HCN	hydrogen cyanide
HPLC	high performance liquid chromatography
i.d.	internal diameter
LD50	lethal dose producing death in 50 per cent of test animals
MS	mass spectrometry
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
ppm	parts per million
RD50	concentration in the atmosphere required to decrease respiratory rate by 50 per cent
SCOT	support-coated open tubular
SIR	selected ion recording
TIC	total ion current/chromatogram
TLC	thin-layer chromatography
TLV	threshold limit value
UV	ultraviolet

SECTION I: GENERAL INTRODUCTION AND INSTRUMENTATION

CHAPTER 1 : INTRODUCTION

1.1) Fire Statistics in the United Kingdom

During the years 1955 - 1978, the number of fires in occupied buildings amounted to approximately one hundred thousand every year[1]. Although the number of fires per year remained more or less unchanged, there was a general increase in the total number of casualties during this period. More significantly, the percentage of casualties overcome by smoke and toxic gases increased five-fold over the same period[1,2]. (Figure 1.1).

Concern has been growing in recent years over the role of smoke and toxic gases in causing fire casualties[3-5]. The percentage of fatal casualties attributed to smoke and toxic gases has risen from 12 per cent of fire deaths in 1955 to 49.7 per cent in 1978, although this may arguably be in some part due to increased awareness by pathologists and better methods for carboxyhaemoglobin analysis. However, the absolute number of fire deaths has increased, regardless of cause. A recent study of fire deaths in the United Kingdom from 1976 - 1982 showed that over 50 per cent of the fire deaths were due to carbon monoxide poisoning[6]. In addition, most of the fatalities showed the presence of soot in the trachea and 72 per cent showed evidence of injury to the respiratory system as a result of inhaling smoke particles and fire gases.

It is generally believed that the increased risk of smoke and gas intoxication in fires in some ways reflects

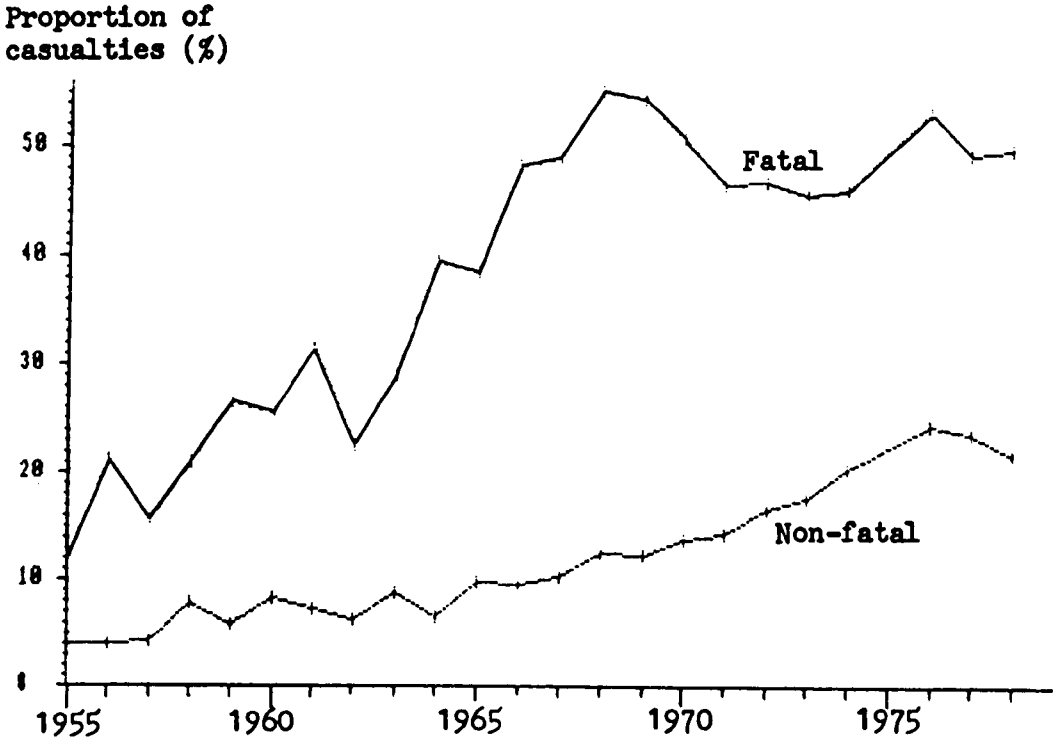


Figure 1.1 Casualties overcome by smoke and fire gases per annum.

the rise in the consumption of synthetic polymers in United Kingdom (Figure 1.2) as materials for construction, furniture, housewares and decoration[7,8]. Laboratory studies on many of the commonly used synthetic polymers have shown that large quantities of smoke and highly toxic decomposition products are formed during thermal degradation and combustion of these materials[9-13]. More importantly, the ease of ignition and rapid rate of burning of some of these materials may result in a more rapid incapacitation of the fire victims.

1.2) Fire Hazards

In order to assess the hazards involved in fires, it is essential to review some of the main life-threatening factors which operate in the fire situation.

1.2.A) Oxygen depletion

In the process of a fire, oxygen is consumed very rapidly to form an oxide, predominantly carbon dioxide. The level of oxygen in the fire atmosphere depends on the total quantity of combustible materials present, the rate of burning, the volume of the system, and the rate of ventilation. In general, a 10 per cent oxygen concentration is considered to be the minimum level to sustain life. Table 1.1 summarises the physiological effects of oxygen depletion.

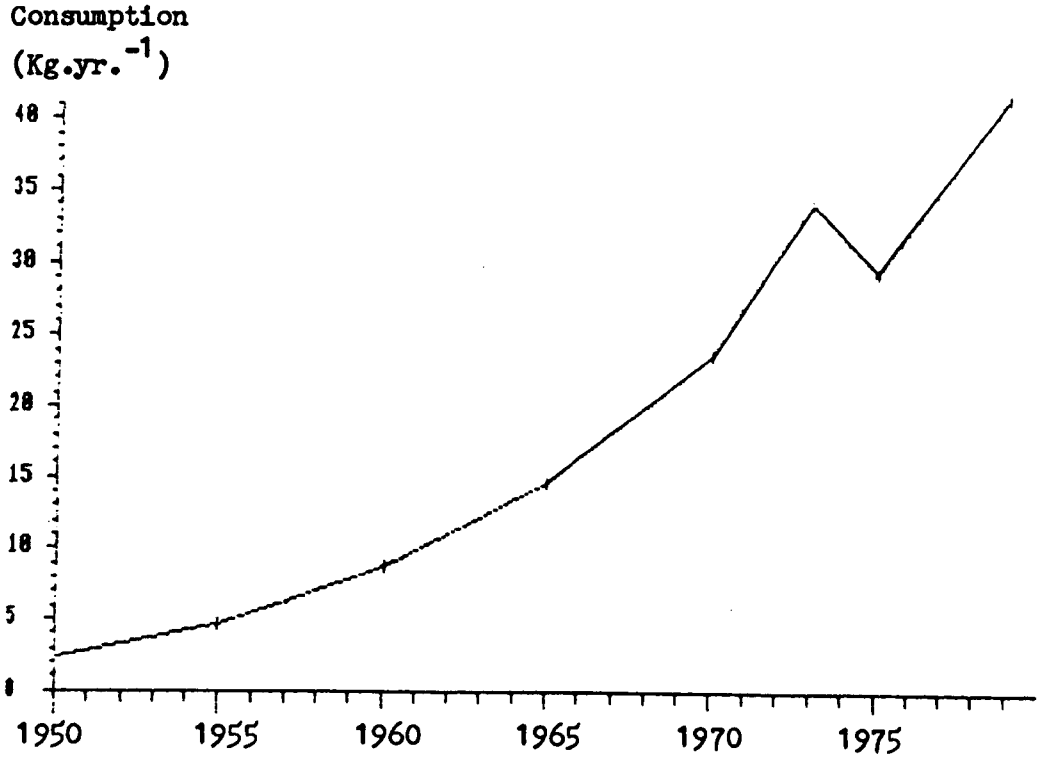


Figure 1.2 Per capita consumption of plastics in the United Kingdom (Ref. 7).

Table 1.1 The physiological response to different levels of oxygen in air[14,15].

Oxygen level in air (V/V%)	Response
21	Normal level in air
15 - 21	Muscle skill is diminished as a result of hypoxia
10 - 15	Consciousness maintained but faulty judgment exhibited
6 - 10	Collapse, revival through prompt treatment
3 - 6	Breathing ceases and death occurs in about 6 minutes
2 - 3	Death in 45 seconds

1.2.B) Flame and heat burns

Burns, heat stroke, burn shock, dehydration and oedema can be caused by direct contact with or radiant heat from flames[16,17]. Breathing in a very hot environment can also cause damage to the epithelial lining of the respiratory tract[18-21]. The damage sustained by tissues is proportional to the temperature and to the duration of exposure. In experiments carried out with animals, irreversible subcutaneous injury was produced after 12 minutes at 100°C or 30 seconds at 180°C[22]. In humans, second and third degree burns have been observed following exposure of the skin at 60°C for only 5 seconds[23]. Air temperatures as high as 100°C can be tolerated for a few minutes only and only when the air is still, and incapacitation can occur in some people breathing air at temperatures as low as 65°C[24,25]. The temperatures which are reached in real fires can be very high. Test-rig experiments in which fully furnished rooms were allowed to burn have shown that temperatures in the range 600 - 800°C are often achieved within 5 - 15 minutes and in some situations temperatures of 1000°C can be reached in only two minutes[26,27]. High temperatures may also discourage occupants of a burning building from entering passages leading to exits.

1.2.C) Smoke

Smoke is defined[28] as the airborne products evolved when a material is decomposed by heat or burning. It may

contain gases, liquids, solid particles, or any combination of these. Smoke not only reduces visibility but because of the adsorption of irritant and toxic species such as hydrogen chloride and aromatic compounds on the smoke particles, intense irritation to the mucous membranes of the respiratory tract and, in particular, of the eyes results[11,29]. Besides, it has been shown that smoke also contributes to panic[30]. On these counts smoke will hinder or even prevent the escape of the occupants and the entry of the firefighters.

Smoke is one of the major hazards at the early stages of a fire. It is often the case that smoke reaches unacceptable levels both in terms of toxicity and optical density in some areas before the temperature does so. The density of smoke depends on the rate of burning, the type of materials involved and the degree of ventilation.

other environmental factors.

1.2.D) Gaseous products

Fire fatality studies in the United Kingdom and United States of America over the last decade have shown that about half of the fatal casualties resulted from toxic gas and smoke inhalation[2,31-34]. The combustion of even a single relatively simple organic polymer may produce hundreds of decomposition products[35,36]. Some of the gaseous products emitted during the combustion and thermal decomposition of polymeric materials are shown in Table 1.2. Many of these compounds are extremely toxic and are strong irritants even at very low levels[39].

Carbon dioxide and carbon monoxide are produced in

Table 1.2 Combustion and thermal decomposition products that may be produced from polymeric materials[37,38].

Combustion products	Typical source materials
Carbon dioxide, carbon monoxide	All organic polymers
Nitrogen oxides	Cellulose nitrate, celluloid, polyurethanes
Ammonia	Wool, silk, nylon, melamine, urea-formaldehyde resins
Hydrogen cyanide, organic nitriles	Wool, silk, nitrogen-containing polymers such as polyacrylonitriles, polyurethanes, nylon, acrylonitrile-butadiene-styrene terpolymer etc.
Isocyanates	Urethane isocyanate polymers
Sulphur dioxide, hydrogen sulphide, carbon disulphide	Vulcanised rubber, thiokols, wool, sulphur-containing polymers
Halogen acids	Polyvinyl chloride, polytetrafluoro-ethylene, polymers containing halo-genated flame retardants
Alkanes, alkenes	Polyolefins and most other organic polymers
Carbonyls	Polyolefins, cellulose, polymethyl methacrylate, wood, paper, poly-vinyl alcohol
Alcohols	Polyvinyl alcohol, phenolic resins
Formic and acetic acids	Cellulosic fibres

large quantities in a fire. Carbon dioxide, although not generally considered as toxic except at very high levels, can lead to hazards such as oxygen depletion and hyperventilation[40] causing an abnormally high intake of other toxic gases. These include carbon monoxide, which is accepted as being the most important fire gas[2,11,29,38, 41-43]. Carbon monoxide binds to haemoglobin about 210 times more strongly than oxygen, resulting in the accumulation of carboxyhaemoglobin (HbCO) and in a reduction of the oxygen-carrying capacity of the blood. Carbon monoxide also has serious long-term effects such as memory impairment and psychiatric disturbances in non-fatal casualties[44]. Table 1.3 summarises the toxicity of carbon monoxide at different levels in air. Studies on simulated "real-fires" showed that the carbon monoxide level often reached several hundred parts per million (ppm) and even reached up to several per cent in a fully-developed fire[46].

Hydrogen cyanide and nitriles are a group of extremely toxic compounds found in the decomposition products of nitrogen-containing polymers[10-12]. Hydrogen cyanide reacts readily with the ferric ion of cytochrome oxidase in mitochondria to form cytochrome oxidase-CN complex which inhibits cellular respiration, an action referred to as cytotoxic hypoxia[47]. In low concentrations, cyanide stimulates respiration while it depresses the brain electrical activity. Considerable uncertainty still exists in interpreting cyanide levels in blood and in establishing the fatal threshold for cyanide

Table 1.3 Physiological response to different levels of carbon monoxide in air[45].

Carbon monoxide level (V/V%)	Response
0.005	EPA threshold limit value (TLV) for 8-hour shift
0.01	Allowable for an exposure of several hours
0.04 - 0.05	Can be inhaled for 1 hour without appreciable effect
0.06 - 0.07	Causes just-noticeable effects such as headache and nausea after a 1 hour exposure
0.1 - 0.12	Causes unpleasant but not dangerous effects after a 1 hour exposure
0.15 - 0.2	Dangerous for an exposure of 1 hour
0.4	Fatal in less than 1 hour
1.2	Unconsciousness results after two or three breaths of the gas and death may follow within 1 - 3 minutes

poisoning due to lack of toxicity data from the literature[34,48].

Inhalation of organic nitriles may cause dizziness, fatigue, nausea and unconsciousness[49], similar to the symptoms observed in cyanide poisoning. The toxicity of these compounds is due to *in vivo* liberation of cyanide following microsomal hydroxylation of the parent compounds to form an unstable cyanohydrin[50]. The role of nitriles in causing deaths in fires will be discussed in more detail in Chapter 7.

Other nitrogen-containing products include isocyanates, nitric oxide, nitrogen dioxide and ammonia. Isocyanates are potent respiratory irritants[51]. Although the presence of pyrolysis products, such as toluene-2,4-diisocyanate (TDI), has been reported in small-scale laboratory studies[52,53], their significance in actual fires is still undefined. The most toxic oxide of nitrogen, nitrogen dioxide, is an important pulmonary irritant since the gas on reaching the aveoli is converted to nitrous and nitric acids[54]. Inhalation of nitrogen dioxide at concentrations as low as 5 ppm for 10 - 15 minutes may cause an increase in both inspiratory and expiratory flow resistance in humans[55,56]. The toxicity of nitric oxide is primarily due to the oxidative reaction of the gas with oxygen to form nitrogen dioxide. Detailed information about the toxicity of nitrogen oxides may be found in reviews by Morrow[57] and Lee[58].

Among those carbonyl compounds found in fire gases [37,59], the aldehydes, especially the unsaturated ones,

are generally toxic and are strong irritants to the eyes and respiratory tract[60]. Acrolein, the most toxic and irritant aldehyde of all, has a threshold limit value (TLV) of 0.1 ppm, which is 100 times lower than that of hydrogen cyanide[61,62]. Air samples from 118 fires in dwellings have indicated that approximately 10 per cent had acrolein at concentrations which would cause immediate danger to life or health[63].

Hydrogen halides are frequently produced during the combustion of polymers which contain halogens. These compounds are very toxic as well as being corrosive. Sulphur-containing gases are formed from the combustion of polymers such as polysulphones[64]. Sulphur dioxide is an irritant even at very low levels and can produce harmful effects after 5 minutes exposure at a concentration of 500 ppm. This gas, as well as sulphates derived from it, increases human morbidity and mortality, especially in people who suffer from pulmonary or cardiac disease[65]. Hydrogen sulphide is rapidly fatal at 1000 ppm. Carbonyl sulphide is also extremely toxic, and has a recommended limiting concentration of 0.15 ppm[62].

Alkanes and alkenes are generally relatively non-toxic. However, certain simple aromatics like styrene, xylene etc., are toxic and are also irritant to eyes and the respiratory system[39]. Other aromatic hydrocarbons such as benzene and polycyclic aromatics are known to be carcinogenic[66] which may pose a long term problem, especially to fire fighters exposed to these compounds repeatedly.

In contrast to the attention which has so far been given to identifying the organic species produced in fires, the combustion and pyrolysis products from inorganic additives to synthetic polymers have often been overlooked[67]. The hazards associated with inorganic toxicants will depend on the chemical forms in which they are released in the fire atmosphere; for example, antimony in the form of gaseous stibine or antimony trichloride may exert an acute toxic effect[68] whereas particulate antimony metal or oxide may cause a long term hazard to survivors of the fire.

1.3) Fire Fatalities Study

Although the term "overcome by smoke and toxic gases" has been used more frequently nowadays, the exact reasons why people die in fires are not yet fully understood. A project was initiated in 1976 under contract to the Building Research Establishment, Fire Research Station, to evaluate the role of smoke and toxic gases in causing fire fatalities, particularly those occuring in domestic fires. The study was originally confined to the Strathclyde region of Scotland but was later extended to cover other areas in the United Kingdom. The project was subdivided into 4 sections: 1) pathology and histology, 2) routine toxicology, 3) fire toxicology, and 4) statistics. The work carried out in this project as a whole is briefly described below to provide the context in which the study of fire toxicology was carried out for the preparation of

this thesis.

1.3.A) Pathology and histology

In support of the project, all Procurators Fiscal were directed by the Crown Office that all fire fatalities in the Strathclyde region be submitted for autopsy by pathologists of the Department of Forensic Medicine and Science, Glasgow University. Each fatality was examined for physical injuries such as burns and broken bones, and for microscopic features such as bacterial infection or damage to the respiratory epithelium. Other pre-existing diseases such as heart disease or chronic bronchitis were also taken into account in establishing the cause of death since the condition of these vital organs might have affected the ability of the individual to survive in a fire situation.

1.3.B) Routine toxicology

Liver, blood and urine samples were collected at the autopsies and analysed for the presence of alcohol and drugs such as benzodiazepines and barbiturate which may have impaired escape ability.

1.3.C) Fire toxicology

The major areas covered in this aspect of the project were the analyses of carboxyhaemoglobin, blood cyanide (and its principal metabolite, thiocyanate), organic volatiles in blood and inorganic elements in the tracheae.

1.3.D) Statistics

As much information as possible about each case was gathered from the autopsy, police and fire brigade reports. This, together with the results from toxicological analyses were submitted to statistical analysis.

1.4) The Analysis of Carbonyls, Nitriles and Other Volatiles in Blood from Fire Fatalities

Blood samples from fire fatalities were analysed for volatile constituents and the results obtained were compared with those obtained from various control groups[Footnote a], in an attempt to recognise materials which had been inhaled from the fire environment. This study was aimed at evaluating the role of smoke and toxic gases in causing fire fatalities.

At the initial stage of the study, particular attention was given to the analysis of aldehydes and ketones in blood. Three different analytical methods (see Chapters 4 - 6) were used and their suitabilities for the assay were evaluated. The methods used were: (1) analysis

Note a) The control groups included the following:

i) normal subjects (smokers and non-smokers), ii) post-mortem controls which included those who died of natural or accidental causes but excluded those who died of drug overdose, alcohol intoxication, solvent abuse or those with known medical history of any metabolic disorder.

of carbonyls as their 2,4-dinitrophenyl hydrazone derivatives, (2) static headspace analysis and, (3) dynamic headspace analysis by gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). Chapters 8 and 9 describe how the dynamic headspace elution technique can be adapted to include the analysis of nitriles and other volatile components in blood. Most of these volatiles were identified and in some cases quantified by GC-MS.

CHAPTER 2: INSTRUMENTATION

2.1) Gas Chromatography (GC)

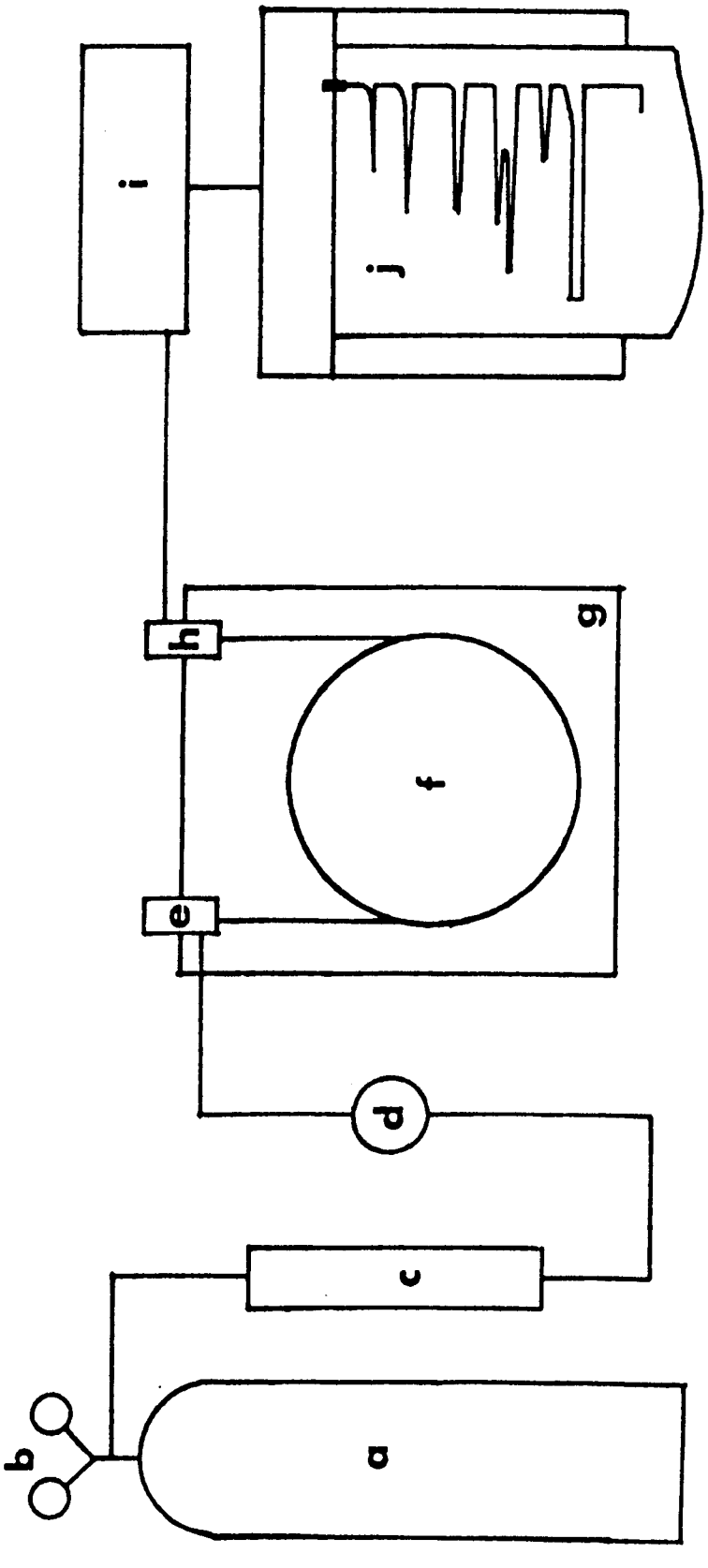
Chromatography is one of the most widely used techniques in chemistry for separating complex mixtures. It was invented and named by the Russian botanist Mikhail Tswett shortly after the turn of the century[69].

In gas chromatography, the components of a vaporised sample are fractionated as a consequence of partition between a mobile gaseous phase and a stationary phase held in a column. Gas-solid chromatography (GSC) employs a solid stationary phase whereas in gas-liquid chromatography (GLC), the stationary phase is a non-volatile liquid, supported on an inert solid matrix.

The concept of gas-liquid chromatography was first described in 1941 by Martin and Synge[70]. However it was more than a decade before the value of this method was demonstrated experimentally[71,72]. Since that time, the growth in applications of the procedure has been phenomenal.

2.1.A) Principles of gas chromatography.

A schematic diagram of a typical gas chromatograph is shown in Figure 2.1. The sample is introduced into the injector where it is vaporised at the head of the column in the narrowest possible band width in time and space. Those components having a finite solubility in the stationary phase distribute themselves between this phase and the



Key : a - Carrier gas supply
b - Pressure gauge and regulator
c - Gas purifier
d - Flow controller
e - Injector
f - Column
g - Oven
h - Detector
i - Signal amplifier
j - Chart recorder

Figure 2.1 Basic components of a gas chromatograph.

mobile gaseous phase according to their partition coefficients. Elution is accomplished by forcing an inert carrier gas through the column. The rates at which various components move along the column depend upon their vapour pressures in the stationary phase. The effluent from the column is monitored by various types of detectors which respond to the presence of sample components in the eluate as they leave the column. The response of the detector is plotted as a function of time. Such a plot, called a chromatogram, is useful for both qualitative and quantitative analysis. A recent review of this analytical technique is given by Risby et al.[73].

2.1.B) Basic definitions and nomenclature.

The number of theoretical plates (N) is a measure of the efficiency of a chromatographic column as a separation device. N relates to the band broadening of a component after a given time of migration through the column. Since the pathway of which the gaseous molecules migrate through the column is random in nature, the chromatographic peak has the shape of a typical Gaussian distribution (Figure 2.2). Hence it is best to measure the dispersion as standard deviation of time (dt) after a given time (tr),

$$N = (tr / dt)^2 \qquad \text{(Eq. 2.1)}$$

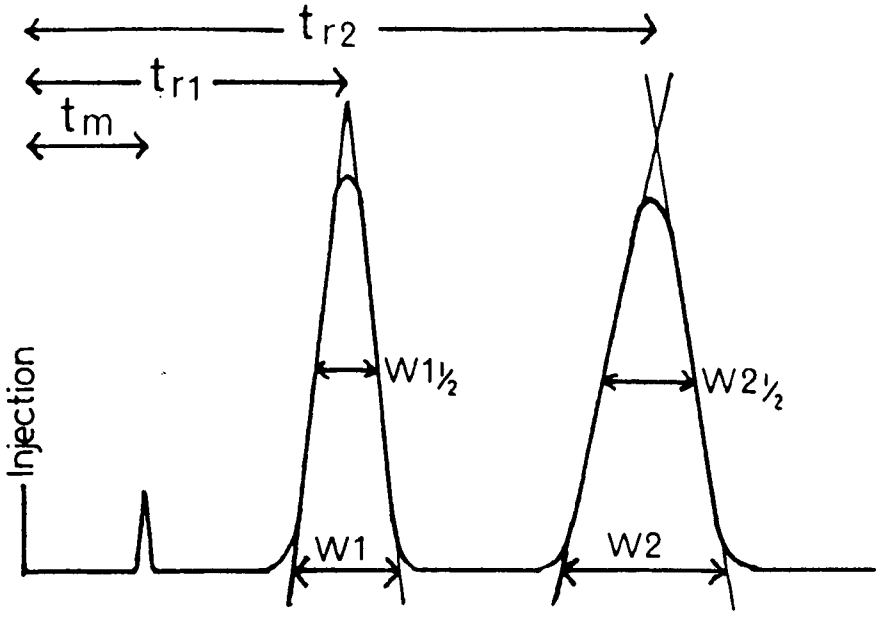
as a good approximation, the peak width (W) is equal to +/-2 standard deviations, therefore

$$N = 16 \times (tr / W)^2 \qquad \text{(Eq. 2.2)}$$

or

$$N = 5.54 \times (tr / W_{1/2})^2 \qquad \text{(Eq. 2.3)}$$

where $W_{1/2}$ is the peak width at half peak height. In



Key : tm - retention time of mobile phase (void time)
tr1 - ,, ,, ,, component 1
tr2 - ,, ,, ,, component 2
W - peak width at base line
$W_{\frac{1}{2}}$ - ,, ,, ,, half peak height

Figure 2.2 A typical two-component chromatogram.

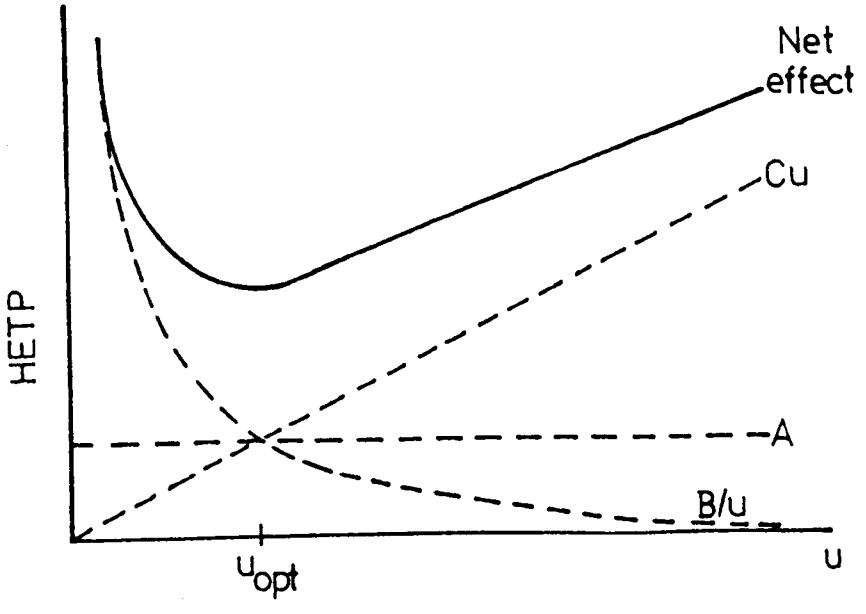


Figure 2.3 Relationship between the HETP and the linear velocity of the carrier gas (u) as described by the Van Deemter Equation.

the case of capillary columns, the void time (tm), may be large. Hence the effective number of theoretical plates (Neff) is more often used.

$$Neff = 5.54 \times ((tr - tm) / W_{1/2})^2 \qquad \text{(Eq. 2.4)}$$

where (tr-tm) is the adjusted retention time.

Another term commonly used for describing column efficiency is the height equivalent to a theoretical plate (HETP or H) which is given by

$$HETP = L / N \qquad \text{(Eq. 2.5)}$$

where L is the length of the column. When HETP is plotted against the linear carrier gas velocity (u), a minimum HETP is obtained at a certain value of u (Figure 2.3). The relationship between HETP and the linear carrier gas velocity is described by the Van Deemter Equation[74].

$$HEPT = A + B / u + C \times u \qquad \text{(Eq. 2.6)}$$

where: A = Eddy diffusion coefficient,

B = molecular diffusion coefficient,

C = resistance to mass transfer. A detailed explanation of the terms in the Van Deemter Equation and the experimental parameters which affect the efficiency of a column is given in Reference 75.

The resolution (R) of the two adjacent peaks is measured by

$$R = 2 \times (tr2 - tr1) / (W1 + W2) \qquad \text{(Eq. 2.7)}$$

where tr1, tr2 and W1, W2 are the retention times and peak widths of peak one and two respectively.

The capacity ratio (K') is a measure of the retention of a sample component relative to a component which is not retained on the column (i.e., void time).

$$k' = (tr - tm) / tm \qquad \text{(Eq. 2.8)}$$

The Kovats retention index system[76] is becoming the most widely accepted system for reporting retention data, normally for isothermal chromatography. This system expresses retention times relative to normal alkanes and the retention index is a logarithmic interpolation between two standards. The expression for the Kovats index is

$$\text{Kovats index} = \left(\frac{\log tr - \log tr(n)}{\log tr(n+1) - \log tr(n)} + n\right) \times 100 \qquad \text{(Eq. 2.9)}$$

where tr = retention time of unknown,

tr(n) = retention time of normal alkane having n carbon atoms,

tr(n+1) = retention time of normal alkane having (n+1) carbon atoms.

Another retention index system, which normally expressed in Methylene Units (M.U.), is the Arithmetic Index of Harbourn[77]. This index is used with temperature programming and is given by the expression:-

$$M.U. = \frac{tr - tr(n)}{tr(n+1) - tr(n)} + n \qquad \text{(Eq. 2.10)}$$

2.1.C) The choice of liquid phase

The single most important consideration in choosing a liquid phase is that it should give the best separation for the components of interest. A general rule is "like dissolves like", for example,

-for separating alcohols use polyglycol as the liquid phase,

-for separating hydrocarbons use hydrocarbon (e.g. Apiezon) as the liquid phase.

The temperature range of a liquid phase is also an important consideration. Operating at a temperature near the maximum limit may cause excessive "bleeding" of liquid phase from the column, resulting in an increased background interference and shortening the column's life.

Information on the polarity, temperature range and applications of liquid phases is widely available and is normally given in supplier's catalogues.

2.1.D) Types of column

Two main types of columns are used in gas chromatography: packed columns and open tubular columns. Packed columns contain an inert solid support of uniform size coated with a thin film of liquid phase. Open tubular (or capillary) columns originally contained a liquid film (approximately 0.5 μm thick) coated on the inside wall of a capillary tube. These are called wall-coated open tubular (WCOT) columns and are characterised by high efficiency (N may be as high as 100,000 - 200,000) and low sample capacity because of the low stationary phase loading. A sample splitter on the inlet system may be necessary. Later, support-coated open tubular (SCOT) columns emerged containing a layer of coated support on the inner wall but still having an open centre. These columns have a much higher sample capacity than WCOT columns and can be used without a sample splitter. Packed columns are the most common type due to their versatility, simplicity in preparation and operation, and above all, their much lower prices. Open tubular columns are becoming more popular

because of their separating power and the increasing number of types of columns available commercially. Some of the typical properties and characteristics of these two types of columns are compared in Table 2.1.

2.2) Mass Spectrometry (MS)

The basic principle of mass spectrometry is the production of ions from neutral molecules followed by the separation of these ions on the basis of their mass to charge (m/z) ratios. Ionisation of the molecules normally causes the ions to fragment in a characteristic manner and it is this fragmentation process which permits identification of the parent species on the basis of the number and relative intensities of the molecular and daughter ions.

Mass spectrometry evolved from studies of charged particles in magnetic and electrostatic fields at the turn of this century. In 1942, the first commerical mass spectrometer was produced by Washburn & Hoover (C.E.C.). Extensive use of mass spectrometry in organic chemistry only began around 1960. Since that time, it has become one of the most powerful analytical instruments for structural identification of organic compounds. Today, mass spectrometry has advanced to the point at which the detection of a single ion per second, resolutions of up to 100,000 and scan rates down to 0.1 s/decade can now be achieved.

	Open Tubular		Packed	
	WCOT	SCOT	micro-	conventional
Inside diameter (mm)	0.25 - 0.50	0.50	1	2 - 4
Column length (m)	10 - 100	10 - 100	1 - 6	1 - 4
Efficiency (plates/m)	1000 - 3000	600 - 1200	1000 - 3000	500 - 1000
Sample size	10 - 100 ng	10 ng - 1 ug	10 ng - 10 µg	10 ng - 1 mg
Optimum flow rate (ml/min)	1 - 5 (He) 0.5 - 4 (N_2)	2 - 8 (He) 1 - 4 (N_2)	2 - 6 (He) 1 - 3 (N_2)	20 - 60 (He) 15 - 50 (N_2)
Pressure drop	low	low	very high	high
Speed of analysis	fast	fast	medium	slow
Chemical inertness	best →			poorest
Permeability	high	high	low	low
Cost	high	high	moderate	low

WCOT = Wall Coated Open Tubular

SCOT = Support Coated Open Tubular

Table 2.1 Typical properties and characteristics of gas chromatographic columns.

The basic components of a mass spectrometer are shown schematically in Figure 2.4. The mass spectrometer used throughout this study was a VG Micromass 16F single magnetic focussing instrument. Therefore, it is more appropriate to describe each component based on this instrument.

2.2.A) Inlet system

There are four inlets in the VG 16F mass spectrometer, viz., the septum inlet, the direct probe inlet and two gas chromatographic inlets.

1) The septum inlet

This inlet consists of a 100 ml reservoir and a molecular leak which meters the sample to the ion chamber at a constant volume rate. The inlet is intended for pure volatile liquids or gaseous samples and is particularly useful for the calibration of the instrument using perfluorokerosene or heptacosafluorotributylamine. The reservoir is normally heated to a temperature of about 150°C.

2) The direct probe inlet

Non-volatile or thermally unstable materials are often introduced directly into the ion source by means of a sample probe, which is inserted through a vacuum lock. The probe consists of a holder for a small quartz capillary tube that contains the sample. The probe is equipped with a heater to volatilize the sample. However, at the low

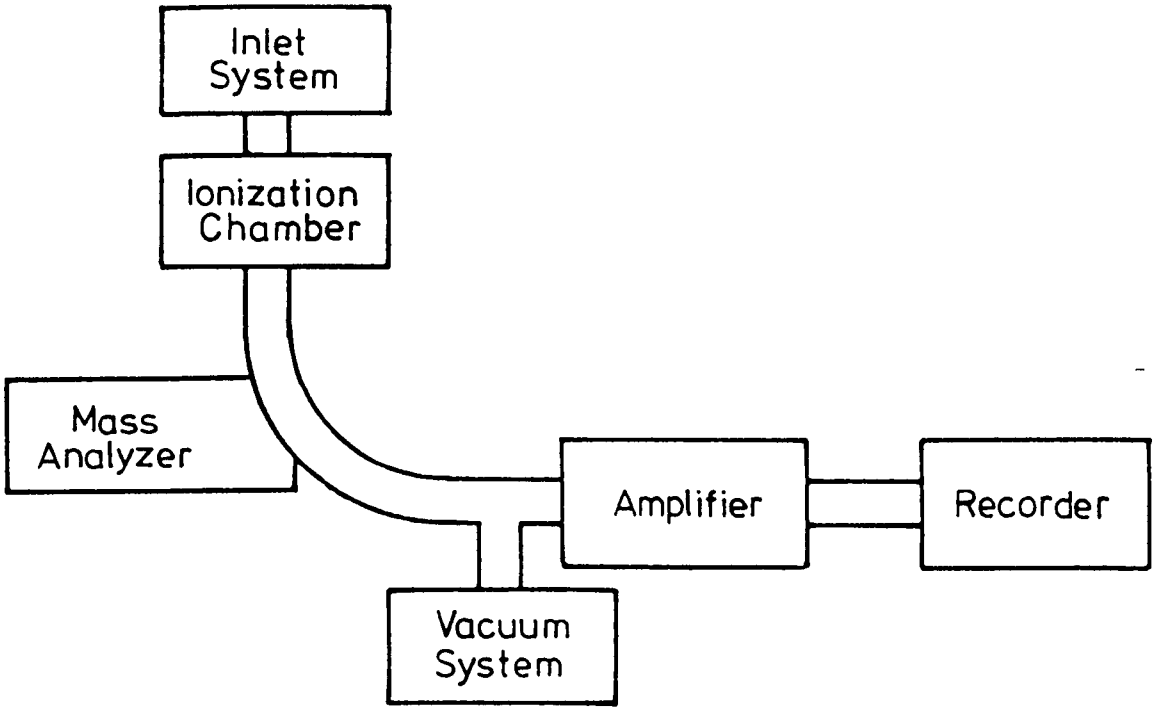


Figure 2.4 Basic components of a mass spectrometer.

pressure and high operating temperature of the ion source, most solid samples are vaporised without the use of the probe heater.

3) Gas chromatographic inlets

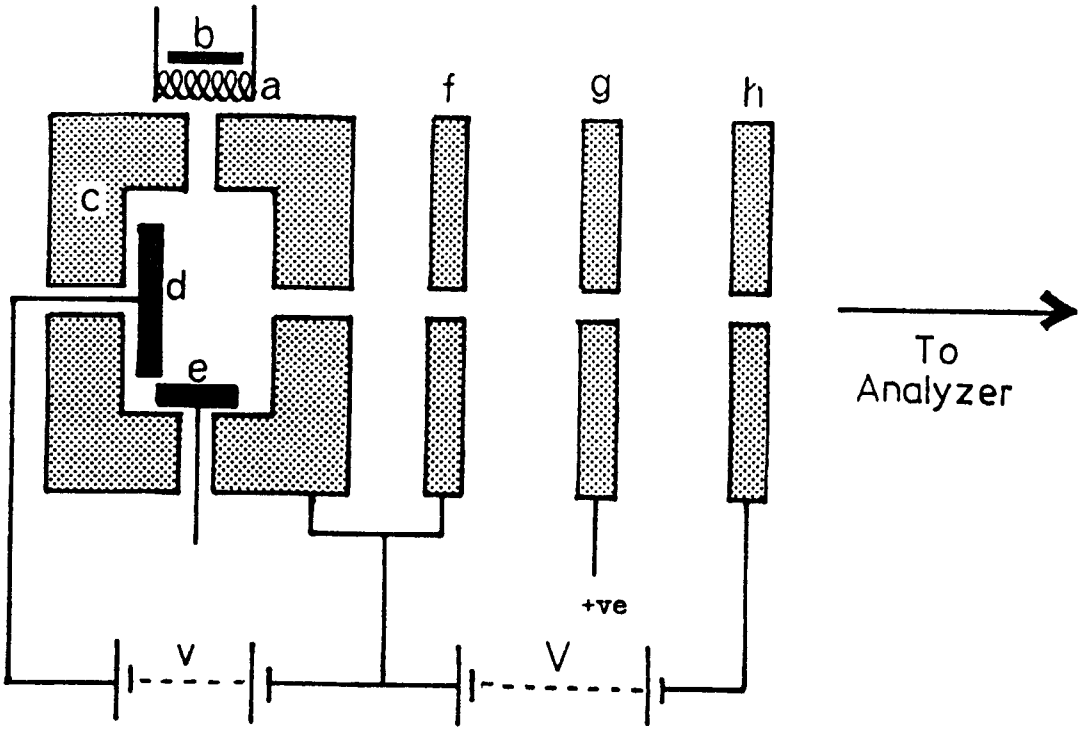
These two inlets are used to interface the mass spectrometer with a gas chromatograph. One inlet is used for capillary GC while the other, which is equipped with a separator, is used for packed column GC. Details of these two inlet systems will be discussed later in this chapter.

2.2.B) Ionization methods

Numerous methods are used in mass spectrometry for converting gaseous molecules into ions[78,79]. Among these methods are electron impact, chemical ionization, spark discharge, thermionic emission, field ionization and, more recently, atmospheric pressure ionization, electrohydrodynamic ionization, laser photoionisation, fast atom bombardment and radionuclide ionization. The two most widely used methods in organic mass spectrometry are electron impact and chemical ionization. A schematic illustration of the ion source is given in Figure 2.5.

1) Electron impact (EI)

Electrons are emitted from a hot filament and accelerated through the ion chamber towards an anode (the trap). The current flowing between the filament and the trap (emission current) provides a measure of the intensity of the electron beam. Emission currents in the range of 50



Key : a - Filament (e^- emitter) e - Trap (anode)
b - Filament shield f - First source slit
c - Source block g - Focussing plates
d - Repellor h - Accelerating slit

Figure 2.5 Schematic diagram of an ion source.

- 250 μA are commonly used. The energy of the emitted electrons (normally in the range of 22 - 100 eV) is varied by changing the potential between the filament and the ion chamber (sometimes called the "source block"). Interaction between the electrons and the organic molecules in the source causes ionization of the molecules and the energy exchange is often sufficient to cause fragmentation (Figure 2.6).

In general, more structural information may be obtained when there are more fragment ions in the mass spectrum. However, in quantitative mass spectrometry, extensive fragmentation may greatly reduce sensitivity and increase the chances of interference from substances other than the one of interest.

2) Chemical ionization (CI)

Under electron impact conditions, many compounds give molecular ions which are of low intensity or are completely absent. This normally makes the determination of the molecular weight very difficult. In chemical ionization, a reagent gas such as methane is leaked into the ion chamber to produce a pressure in the range of 0.1 - 1 torr. Interaction of the electrons and the reagent gas produces a host of ions. Some of these ions, such as CH_5^+, are strong Bronsted acids which normally react with the analyte molecules by proton transfer to produce quasi-molecular ions $(M+1)^+$.

$$CH_5^+ + M \longrightarrow MH^+ + CH_4$$

Since the process of chemical ionization is of lower

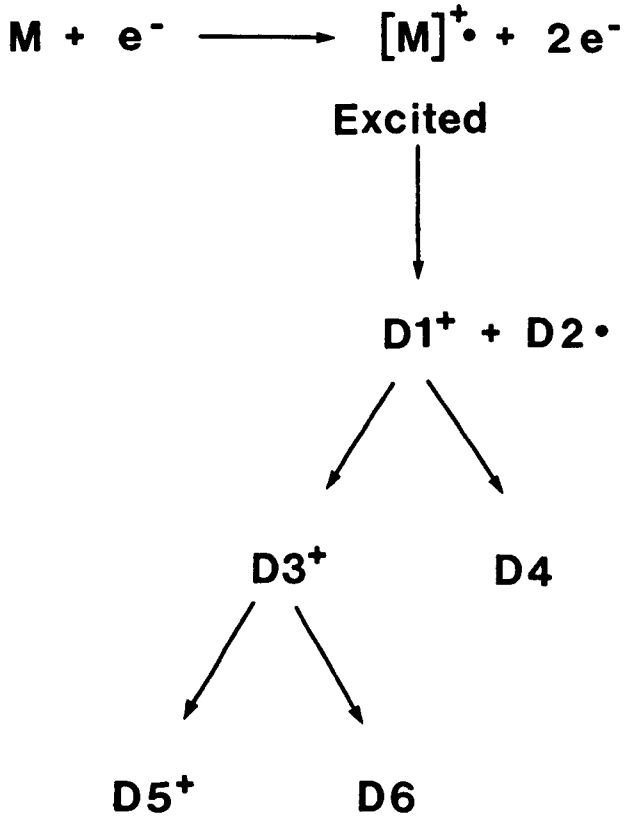


Figure 2.6 Ionisation by electron impact. The source pressure is kept sufficiently low that ion decompositions are unimolecular. M represents the gaseous molecule and D1 - D6 represent the daughter ions formed.

energy, therefore fragmentation of the parent molecules is less prevalent and an intense $(M+1)^+$ ion is normally observed. Figure 2.7 shows the comparison of the mass spectrum of acetone-DNPH acquired under EI and CI conditions.

2.2.C) Mass analyser

The positive ions formed in the ion source are repelled through the source slit by a small positive potential difference (v) between the slit and the repeller. These ions are then focussed to give a collimated beam and accelerated towards the mass analyser by a high potential difference (V, usually 2 - 4 KV) between the source block and the accelerating slit.

The most important parameter of a mass analyser is the mass resolution, that is, it's ability to separate ions of different mass-to-charge ratio. The resolution (R) is expressed by

$$R = M / dM \qquad \text{(Eq. 2.11)}$$

where M is the mass of the first mass spectral peak and dM is the difference in masses of the two peaks. Two adjacent peaks of approximately equal intensity are commonly considered as being separated when the height of the valley between them is 10% of the peak height (Figure 2.8).

There are four different types of mass analyzers commonly used for organic mass spectrometry. They are the single focussing magnetic deflection mass analyzer, double focussing mass analyzer (which contains a magnetic sector

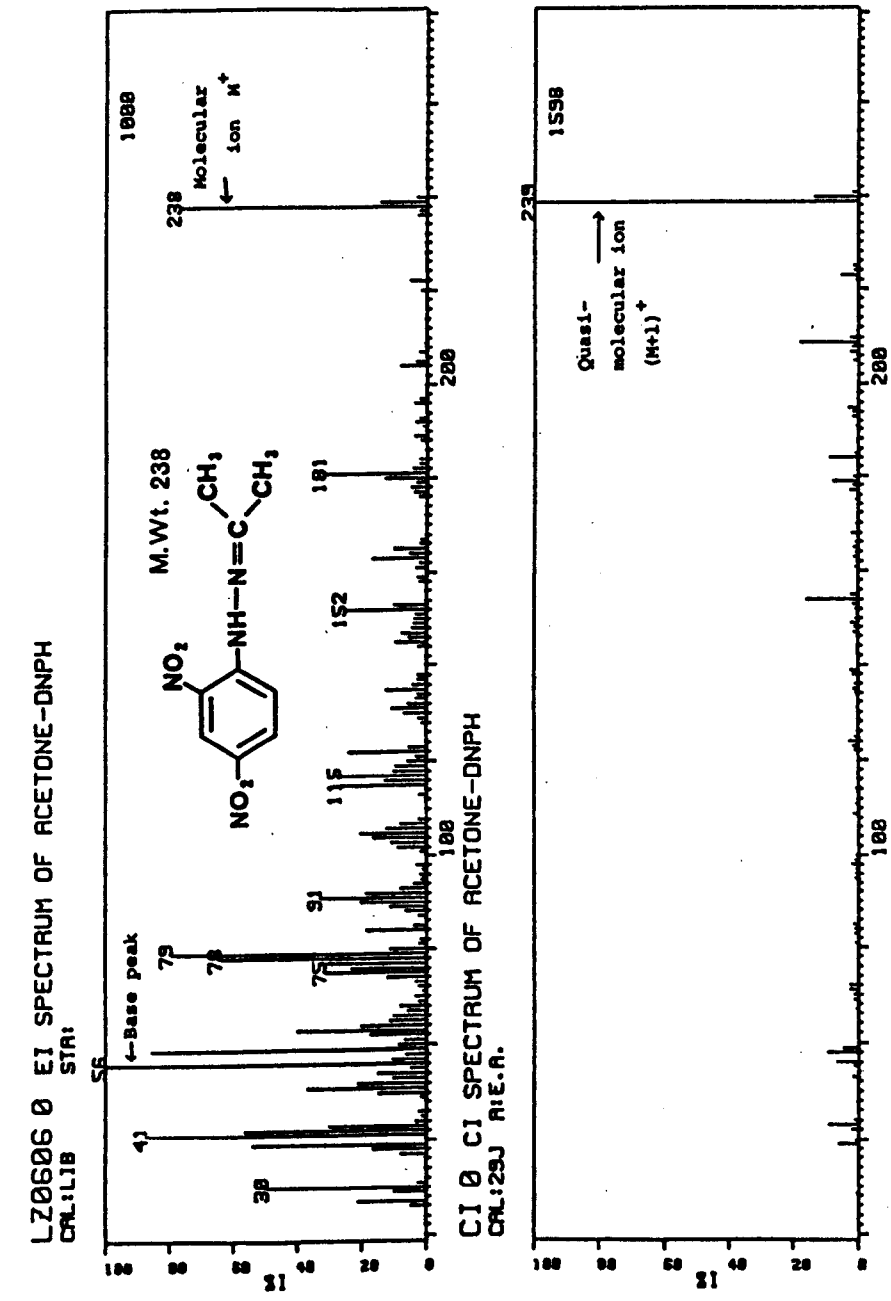


Figure 2.7 Mass spectrum of acetone-DNPH obtained under EI and CI conditions. Note the degree of fragmentation in EI.

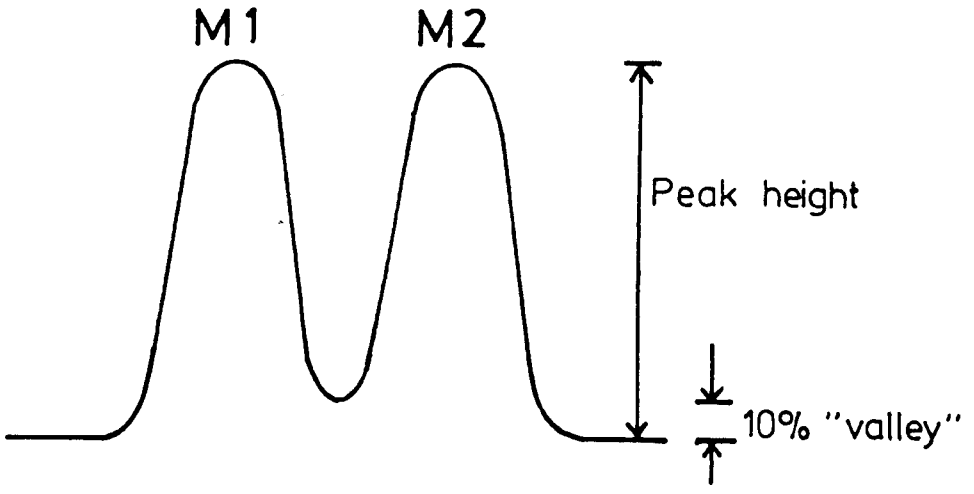


Figure 2.8 "10% valley" definition of resolution.

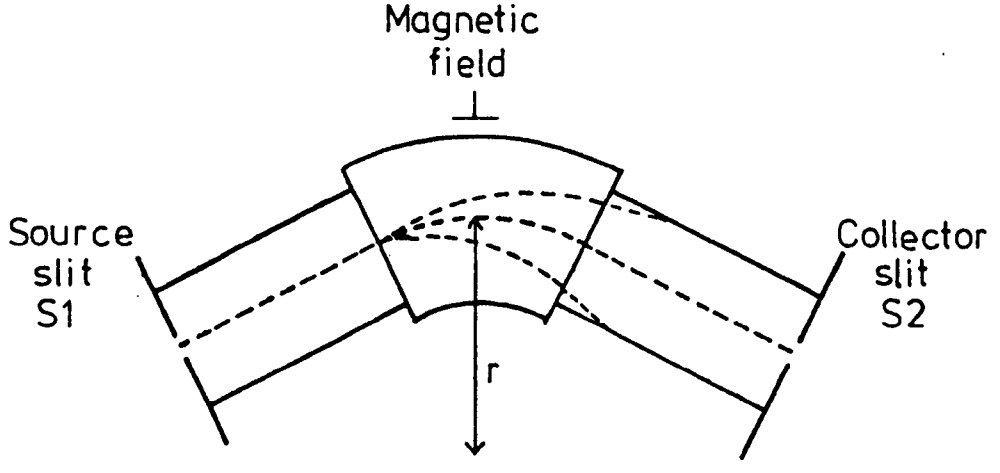


Figure 2.9 Schematic diagram of a magnetic deflection mass analyzer. 'r' is the radius of curvature of the magnetic sector.

and an electrostatic sector), the quadrupole analyzer and the time of flight analyzer. Only the single focussing magnetic deflection analyzer is discussed in this thesis. The single focussing magnetic deflection mass analyzer employs a curved ion path in which the accelerated ions travel through a homogeneous magnetic field which is perpendicular to the ion path (Figure 2.9). The ions experience a magnetic centripetal force (Fm) which is given by

$$Fm = H \times z \times v \qquad \text{(Eq. 2.12)}$$

where H is the magnetic field strength, z is the charge on the ion and v is the ion velocity. The centrifugal force (Fc) of the moving particle is given by

$$Fc = m \times v^2 / r \qquad \text{(Eq. 2.13)}$$

where m is the mass of ion and r is the radius of curvature. The ion is in focus when the two forces Fc and Fm, are exactly balanced, that is,

$$H \times z \times v = m \times v^2 / r \qquad \text{(Eq. 2.14)}$$

Since the kinetic energy (E) of the ion is given by

$$E = z \times V = m \times v^2 / 2 \qquad \text{(Eq. 2.15)}$$

where V is the accelerating voltage. Substituting Eq. 2.14 into Eq. 2.15 and rearranging gives

$$m/z = H^2 \times r^2 / 2V \qquad \text{(Eq. 2.16)}$$

Thus, at a fixed radius r, the ions can be brought into focus by varying either H or V. The resolution of a magnetic sector mass spectrometer is determined principally by the radius of curvature and by the width of the source and collector slits.

$$R = k \times r / (S1 + S2) \qquad \text{(Eq. 2.17)}$$

where k is a constant and S1 and S2 are widths of the source and collector slits. Decreasing the slit width increases the resolution but at the same time decreases the sensitivity of the instrument.

2.2.D) Amplification and recording

Typical ion currents measured in modern mass spectrometry range from 10^{-10} to 10^{-14} A. A current of 10^{-17} A corresponds to about 60 ions reaching the detector per second. Such a very low current can be amplified by an electron multiplier to produce a current high enough to be further amplified electronically and recorded.

1) Electron multiplier

The multiplier used in the MM16F is a 17-stage Venetian blind type with beryllium-copper dynodes. When the ions strike the first dynode, it produces electrons which are then accelerated toward the second dynode where their impact causes additional electron emission. The process is repeated down each stage of the multiplier. The gain of the multiplier (typically 10^6 - 10^{8}) is related to the accelerating voltage used which normally ranges from 1 to 2.5 KV.

2) Computer-aided data acquisition

Because of the amount of data generated from the mass spectrometer, especially during a long GC-MS run, it is essential that data acquisition and processing be rapid.

The computer is ideal for this purpose. It is also useful for controlling several instrumental variables during data acquisition. The role of the computer in mass spectrometry is well documented elsewhere[80]. The advances in computer technology have permitted it to become an integral part of a modern mass spectrometer. Details of the mass spectrometer-computer interface will be discussed later in this chapter.

2.3) Interface

There are two interfaces in a GC-MS-computer system, that is, the GC-MS interface and the MS-computer interface. A schematic diagram of these is shown in Figure 2.10.

2.3.A) The GC-MS interface

Although gas chromatography is an excellent technique for separating components in a mixture, its use in qualitative analysis is rather limited. Strictly speaking, retention data can only be used to exclude the possibilities of an unknown compound and it gives little or no information on the structure of the unknown. Therefore, coupling a gas chromatograph to a mass spectrometer, an excellent instrument for structural elucidation, became the dream of many chemists in the field of analytical organic chemistry during the 1950's. It is now twenty-seven years since the first successful attempt to couple gas chromatography with mass spectrometry[81]. Today, GC-MS

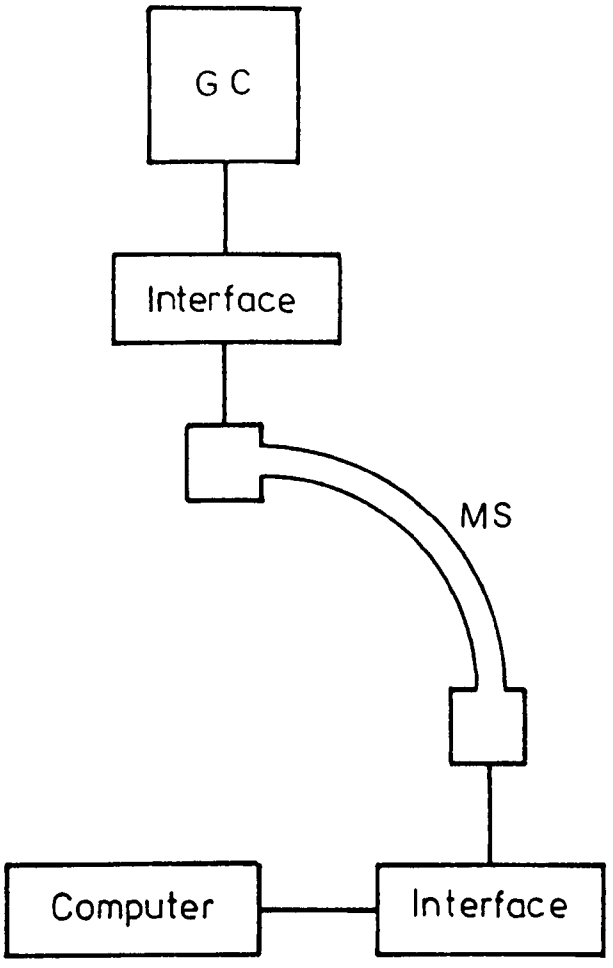


Figure 2.10 Schematic diagram showing the two interfaces in a typical GC-MS-computer system.

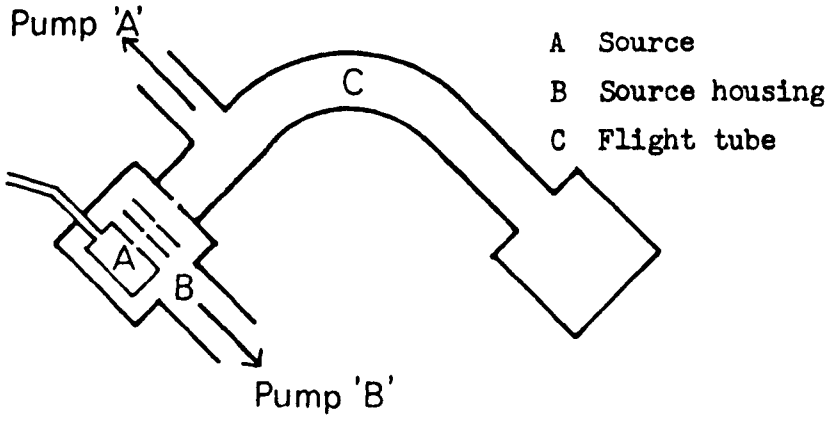


Figure 2.11 Schematic diagram of a differentially pumped mass spectrometer. A, B and C indicate regions of different pressure.

analysis is applied in every branch of organic chemistry[85].

1) Direct coupling

The most effective method of increasing effluent ultilization is with a differentially pumped mass spectrometer (Figure 2.11). This arrangement enables the instrument to be operated with a helium flow rate of up to 10 ml/min into the source, ie., ultilizing virtually all the effluent sample from a capillary column without the use of splitter or restrictor. The interface consists of a simple capillary tube between the column and the ion source. The main requirements of the interface are that column performance should not be lost between the GC and the ion source and care must be taken to avoid dead volume, surface activity and temperature variation.

The ratio of the quantity of carrier gas used under vacuum conditions (Qvac) to that used under standard conditions (Qstd) is given by the Poiseillne equation[83].

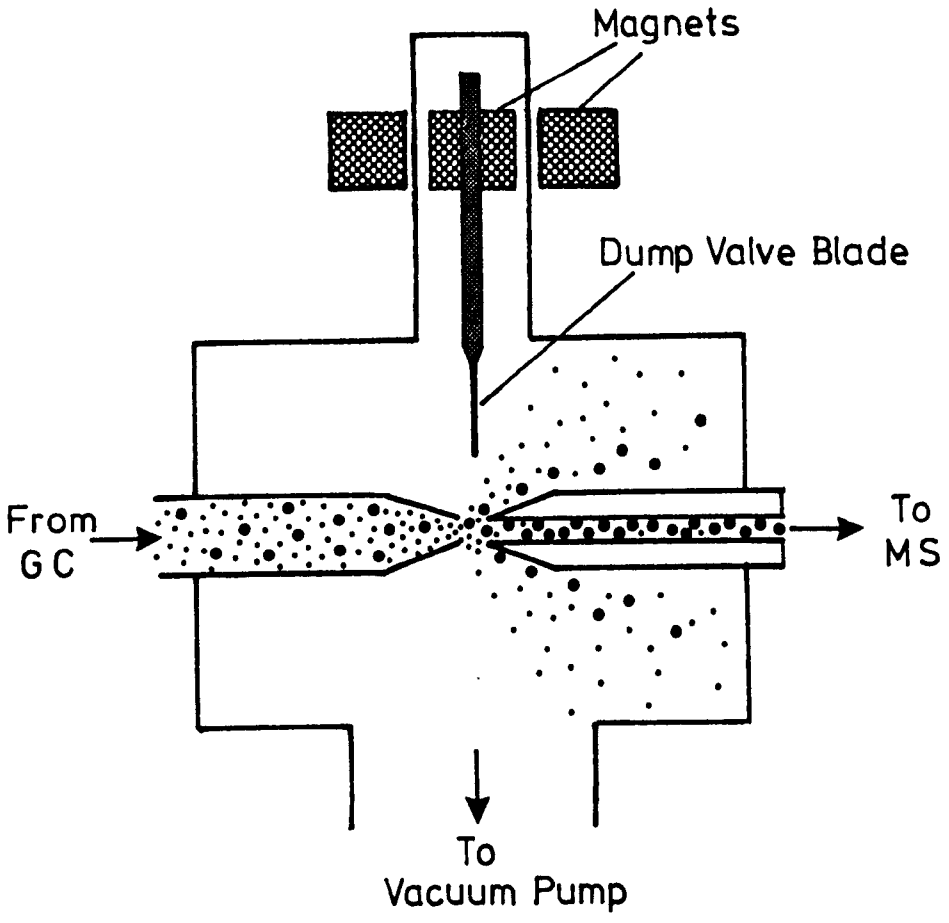
$$\frac{Qstd}{Qvac} = \frac{(Pi^2 - Po^2)std}{(Pi^2 - Po^2)vac} \qquad \text{(Eq. 2.18)}$$

where subscripts "std" and "vac" refer to standard and vacuum operation, Pi and Po are the inlet and outlet pressures of a capillary column. This means that for the same linear flow through the column, the vacuum operation would require much less carrier gas. As it is difficult to measure the flow rate of the carrier gas under vacuum operation, the void time is therefore often used to calculate the linear velocity of the carrier gas.

2) Molecular separators

The normal carrier gas flow rate through a packed column is about 40 ml/min. Using a splitter in the interface would mean only a very small fraction of the effluent is utilized. Therefore, it is essential to remove the carrier gas selectively prior to entry into the source. This enrichment process is achieved by the use of a molecular separator. Various designs of molecular separators have been used for different applications[84]. The operational parameters used to evaluate the performance of separators are the separation factor and the efficiency[85]. The separation factor, also known as the enrichment factor, is the ratio of sample concentration in the carrier gas entering the mass spectrometer to the sample concentration coming out of the chromatographic column. The efficiency is the percentage of sample transferred from the chromatographic column to the mass spectrometer. It is also important that the interface does not contribute to the band broadening of a chromatographic peak. The peak distortion factor indicates the ratio of the width of a chromatographic peak after and before entering the interface[86]. The distortion factor for a direct coupling interface often approaches 1 while that for the molecular separator ranges from 1 to 3.

A schematic diagram of a jet separator is shown in Figure 2.12. The characteristic rates of diffusion of different gases in an expanding supersonic jet stream can be used to accomplish fractionation of gaseous



Flow rate into separator	:	30 ml/min
Flow rate into source	:	1 - 2 ml/min
Efficiency (mass 300)	:	60 - 70 %
Operating temperature	:	200 °C

Figure 2.12 Schematic diagram of a single stage jet separator.

mixtures[87-89]. Effluent from the GC column passes through a restricted orifice (inlet jet, 0.1 mm i.d.) and expands rapidly in the evacuated area between the inlet and outlet jets (0.25 mm i.d., 0.3 mm apart), establishing a pressure gradient. The diffusion of gases in this region is a function of the molecular weight and is proportional to the diffusion coefficient (D). Thus the core of the jet stream is enriched in the heavier component while the lighter carrier gas in the peripheral portion is pumped away. The efficiency of this type of separator depends on the carrier gas flow rate and the molecular weight of the sample component. Figure 2.13 shows the relationship of the separation factor to the molecular weight of gaseous sample.

2.2.B) Mass spectrometer-computer interface

The primary function of any mass spectrometer-computer interface is to modify and digitize the analogue signal from the mass spectrometer and then pass it on to the computer. Through the interface, the computer may also be used to produce real-time mass marking for a U.V. chart recorder or control the start of a scan at preset time intervals during a GC run. A block diagram of the VG 2035 on-line data system is shown in Figure 2.14.

The analogue to digital converter (ADC) performs digital conversions of the input analogue voltage at precise time intervals set by the digitization rate. The computer thus receives a series of digital signals representing the incoming analogue signals (Figure 2.15).

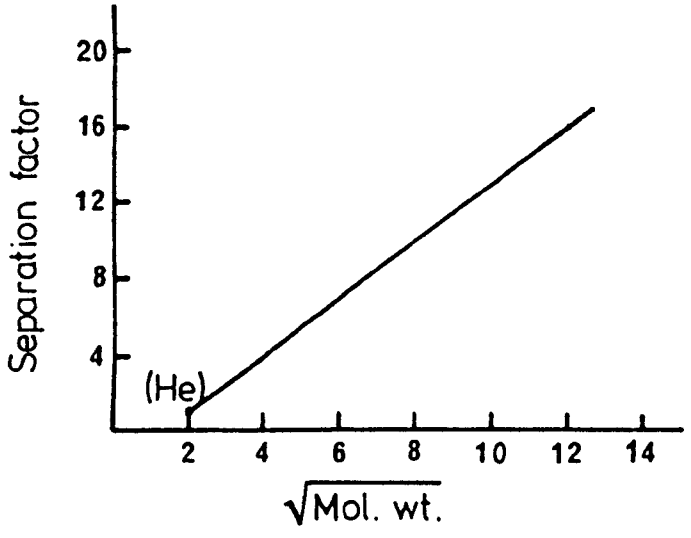


Figure 2.13 Relationship between the separation factor and the molecular weight of the gaseous sample (Ref. 90).

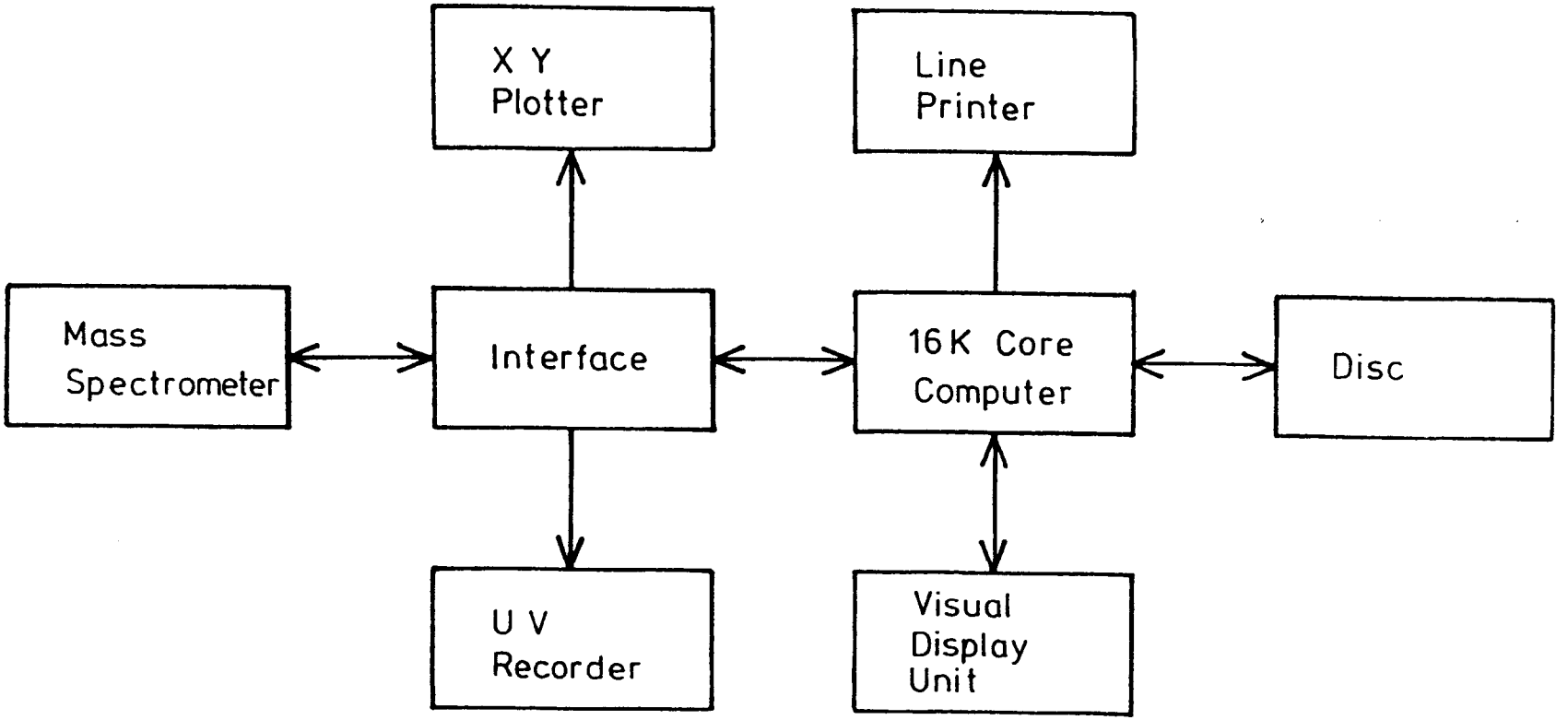


Figure 2.14 Block diagram of the VG2035 on-line data system.

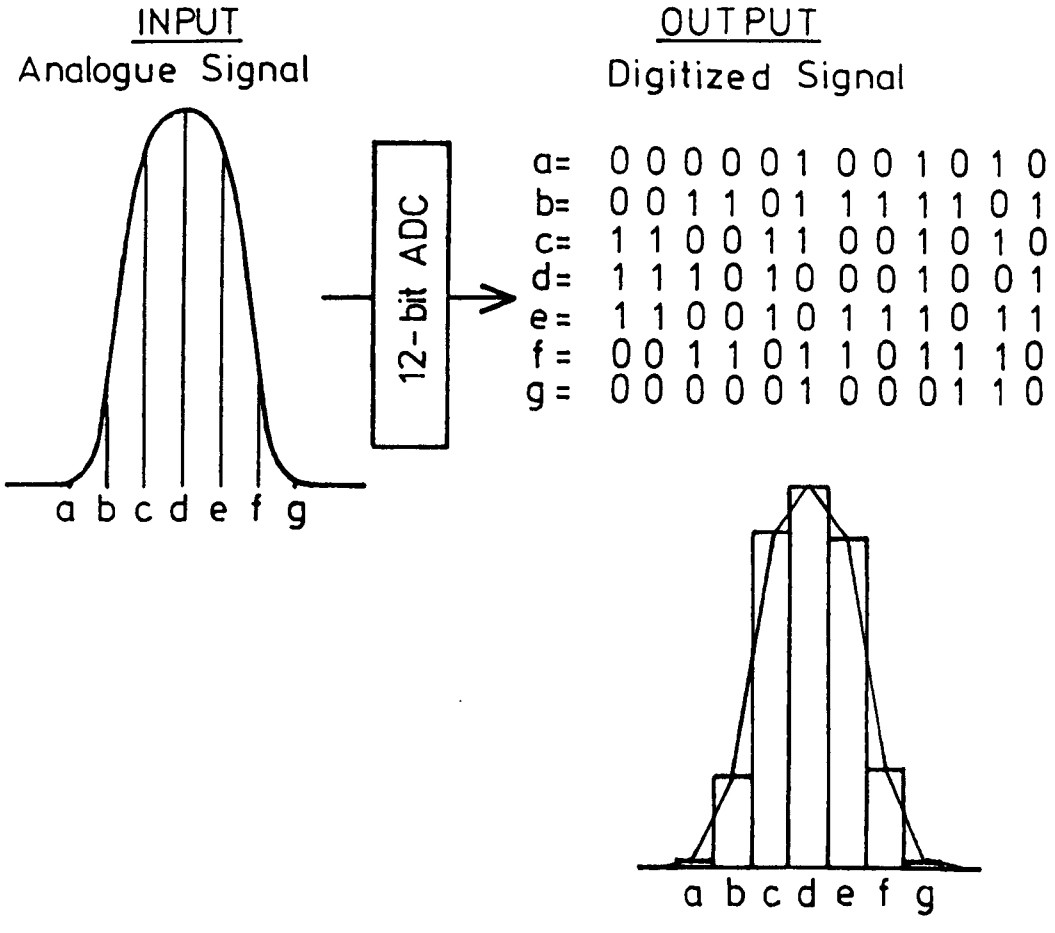


Figure 2.15 Diagram showing the function of an analogue to digital convertor and the reconstruction of the signal from the digitised output.

The number of unique digital levels obtainable from a converter is given by $2^n - 1$ where n is the number of bits in the converter. Thus a 12-bit converter has a normal dynamic range of 0 - 4095 of which each level represents 2.4 mV for a 10 V converter. The digitization rate is determined by the resolving power, the scan speed and the number of samplings required per mass spectral peak. For typical repetitive scan conditions (viz. an exponential magnet scan of 1 s/decade at 1000 resolution, seven samples per peak), a digitization rate of 13 KHz[91] is required. In general, digitization rates which are too high may result in loss of sensitivity while digitization rates which are too low may result in a less faithful reproduction of the peak shape[80,84].

2.4) Data Acquisition Techniques

Before the introduction of GC-MS, it was considered almost impossible in practice to identify each component in a complex mixture of organic compounds. Qualitative analysis using the retention index alone can be ambiguous unless the chemist knows before hand what he is looking for. For this reason, the mass spectrometer is still the most powerful detector for use with gas chromatography in terms of its specificity, flexibility and sensitivity.

2.4.A) Mass spectrometer as a GC detector

Chromatograms can be obtained from the output of the mass spectrometer by either recording the total ion current

(TIC), by recording the ion current of a preselected mass (Selected Ion Recording, SIR) or by computer-based methods in conjunction with repetitive scanning. In the former case, the mass spectrometer is used as a non-specific detector while in the two other cases, the mass spectrometer is used as a specific detector.

1) Total ion current monitoring

One way to measure the total ion current is the use of a small collector plate which is located in the ion beam between the ion source and the magnetic analyzer. The main disadvantage of this technique is the high standing current due to ionization of the carrier or reactant gas which limits the sensitivity of the instrument. An improved method is the use of an integrating ion monitor which integrates the output of the electron multiplier as the magnet or voltage scans across a preselected mass range. Thus the backgound ion current from the carrier gas can be excluded.

2) Selected ion recording

The mass spectrometer has an advantage over other specific detectors in that it can be set to monitor one or more specific ions which are characteristic of the compound or class of compounds under investigation. In other words, its specificity is not fixed as in the case of other specific detectors. Selected ion recording is two to three orders of magnitude more sensitive than total ion monitoring because:

i) the signal passes through the electron multiplier,

ii) the signal is not affected by the presence of other background ions,

iii) the proportion of time spent on collecting ions in the selected mass spectral peaks is increased.

Most modern mass spectrometers are equipped with a multiple ion monitor which enables several ion masses to be monitored simultaneously. Since the accelerating voltage can be reset much faster than the magnetic field, SIR is carried out by keeping the magnet current constant and switching the accelerating voltage between certain predetermined settings. The presence of several channels allows the measurement of several different compounds or the use of an internal standard in the sample for quantitative analysis. Another useful application of SIR is to resolve a GC peak which comprises several compounds. An example is shown in Figure 2.16 in which the components of fused and partially fused GC peaks can be distinguished by monitoring their specific ion masses.

3) Repetitive scanning

With a magnetic mass analyzer of fixed radius, the mass to charge ratio (m/z) is proportional to 1/V or to H^2 (Equation 2.16). When V or H is varied, different ion masses are successively passed through the analyzer to the collector whose output, when displayed graphically, constitutes a mass spectrum. Normally, magnet scanning is preferred because of the mass discrimination effect associated with the voltage scan mode[92]. The scan

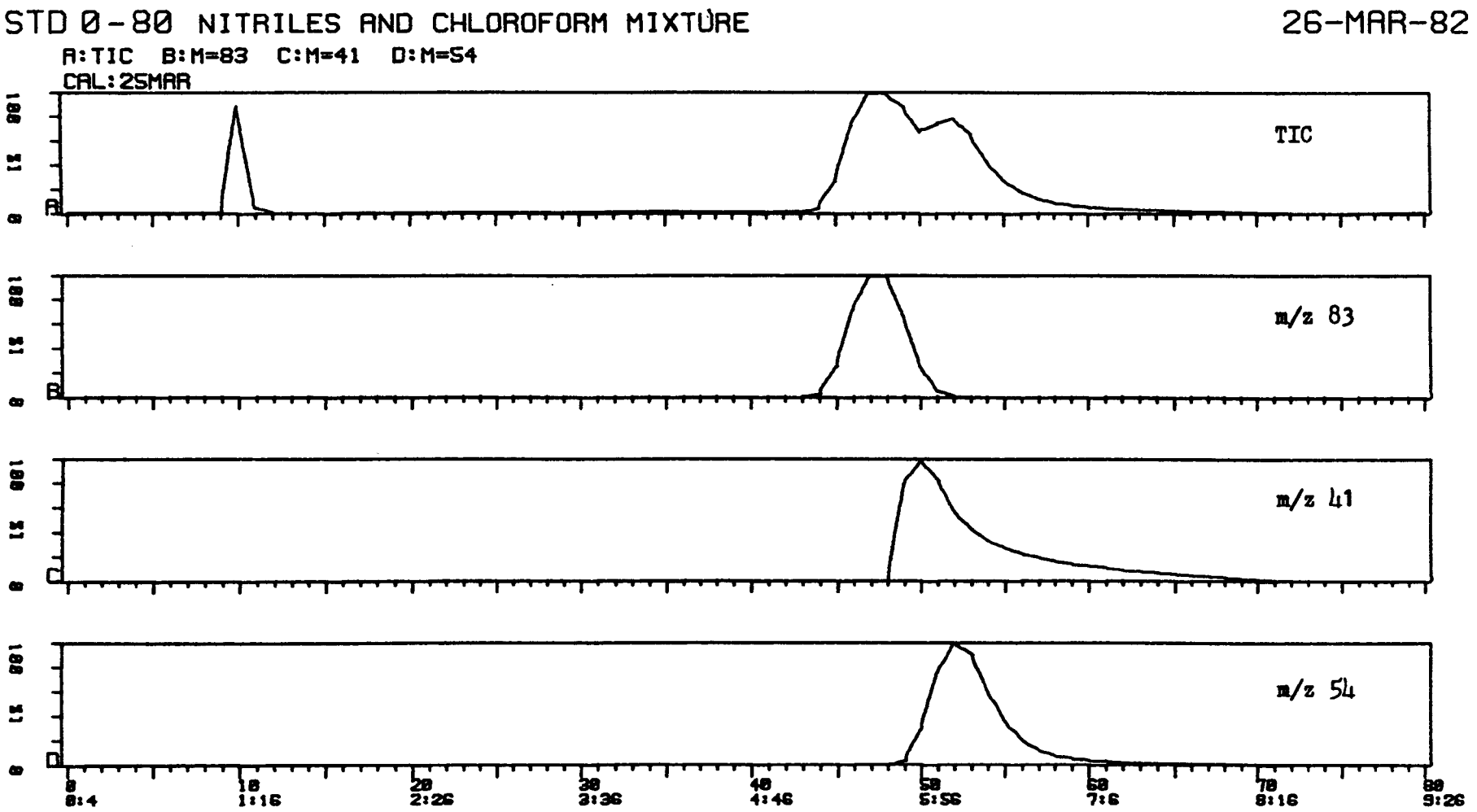


Figure 2.16 Selected ion chromatography as a way to resolve fused chromatographic peaks. m/z 83, 41 and 54 are the base peaks of chloroform, acetonitrile and propionitrile respectively.

function for magnet scan from high to low mass is given by

$$m = mo \times e^{-kt} \qquad \text{(Eq. 2.19)}$$

where m = mass at time t

mo = mass at the start of the scan

k = scan constant.

Thus, using an exponential scan law, the same time width is obtained for all mass peaks, which is ideal when using a computer for data acquisition. The scan can be initiated manually when a peak appears in the TIC chromatogram or initiated repetitively by the computer in order to monitor continuously the effluent from the GC column. The computer can also be used for a number of functions including mass calibration and calculation of relative intensity of mass spectral peaks before the data are deposited on other storage devices for later data processing. An unknown spectrum can either be interpretated manually or identified by the computer which matches the unknown spectrum to a library of spectra in the data-system. The closest fits based on ion masses and relative intensities are thus obtained, a process known as computer library search[93,94].

Repetitive scan data may also be used to produce software-generated TIC, partial ion current, selected ion and mass-max chromatograms. In the latter case, the computer compares every two consecutive scans for any changes in relative intensity of any mass within the scan range and uses only those masses which are maximising to construct the chromatogram, thus resolving any partially fused chromatographic peaks which may not be apparent in

the TIC chromatogram.

2.4.B) Sensitivity and detection limits of integrated GC-MS

It is often difficult to define the sensitivity of a mass spectrometer in terms of the amount of sample required since the spectrum intensity varies with parameters such as ionisation efficiency, scan speed, resolving power, bandwidth of the amplifier/recorder system and the nature of the inlet system[95]. On the GC side of an integrated GC-MS system, the detection limit will depend on various factors such as injection technique and column conditions. In general, the sensitivity of an integrated GC-MS system is comparable to, if not more sensitive than most other sensitive analytical techniques. In the repetitive scan mode, a full mass spectrum can be recorded with 10 - 100 ng. In SIR, the detection limit is normally in the picogram range although in certain favourable cases, femtogram sensitivity has been recorded[96].

2.4.C) Quantitative aspects of GC-MS

As in conventional gas chromatography, the response (measured as peak area or peak height) of the mass spectrometer in either scan or SIR mode is proportional to the amount of sample present. Calibration curves are used in the same way to determine the true concentration of analytes under investigation. The use of an internal standard will compensate or minimize errors in sample preparation, injection and instrumental fluctuation. Of greater importance is that mass spectrometry permits the

use of stable isotopically labelled analogues as internal standards, which give the greatest accuracy in quantitative analysis because of the similarity of their physical and chemical properties to those of the unlabelled analyte. Deuterium, carbon-13 and nitrogen-15 are the most common stable isotopes used for this purpose. D' Oyly-Watkins et al. determined the linear response range of the mass spectrometer in SIR mode for a variety of chemical compounds and reported a linear response range of around 10^4, which is comparable to the data obtained from an FID[97].

It is important that the correct cycle time is used when carrying out quantitative analysis either in scan or SIR mode. The optimum cycle time used will depend on the width of the chromatographic peak. Too long a cycle time may not give sufficient number of scans across the chromatographic peak resulting in mass spectral or chromatographic peak distortion. On the other hand, too short a cycle time may result in loss of sensitivity especially when performing repetitive scan on a magnetic instrument since the reset time for the magnet is relatively long. For example, to obtain 10 scans over a mass range of 200 - 20 amu across a GC peak 20 seconds wide, a maximum scan speed of 1 second per decade is required, which is about the limit that most mass spectrometers can achieve. In SIR mode, the accelerating voltage can be switched rapidly between certain pre-set values and cycle times of the order of milli-seconds can be achieved. This is particularly useful for high resolution

chromatography provided that the ion current is sufficient to maintain a suitable signal:noise ratio of at least 3:1.

As in most analytical techniques, the overall error in quantitative mass spectrometric analysis is a cumulative sum of errors in instrumentation and sample preparation. It is inappropiate to cover all the various sources of error in detail here. A good review of this subject is given by B.J. Millard[98]. With proper use of internal standards and in favourable analytical conditions, a coefficient of variation of 2 per cent or less can be achieved in SIR. The error is likely to be higher in repetitive scan, of the order of 5 - 10 per cent.

The choice of using scan mode or SIR in quantitative analysis depends very much on the requirements of a particular assay. Prior knowledge of the unknown is required in SIR for selecting ion masses to be monitored. Although repetitive scan is less commonly used for quantitative analysis, it permits both qualitative and quantitative determinations to be carried out simultaneously even in a sample of complex mixtures, especially when sample size is not a limiting factor.

SECTION II: ANALYSIS OF CARBONYLS IN BLOOD

CHAPTER 3: INTRODUCTION

Studies on the thermal degradation products of many polymeric materials such as polyethylene[99], polypropylene[99,46], polymethyl methacrylate[100], polyurethane[101], wood[41,102] and cellulose[103], have shown that a series of carbonyl compounds is present, many of which are extremely toxic and are strong irritants of the eyes and respiratory tract. These oxygenated species are formed during oxidative pyrolysis of polymers under medium temperature conditions (ie., 400 - 650°C)[46]. These compounds are unstable at high temperatures (eg. >700°C) at which they form simple combustion products. Potts et al.[104] reported that smoke produced from polyethylene-based materials under non-flaming conditions is more toxic than smoke produced by pyrolysis of wood on a sample weight basis. The increase in toxicity was due to the formation of a high level of 2-propenal (acrolein), one of the most toxic compounds and the strongest irritant in the carbonyl family. Analysis of air samples from 118 fires in dwellings indicated that approximately 10 per cent of them had acrolein at concentrations which would cause immediate danger to life or health[63]. Preliminary examination of the volatiles in blood samples from fire fatalities has shown the presence of these carbonyl compounds[105]. It is therefore of great interest to determine the role of these compounds in causing fire casualties.

3.1) Toxicity and Irritancy of Carbonyl Compounds

Carbonyl compounds are generally toxic and strong sensory irritants. For simple volatile carbonyl compounds (C2 - C8), their toxicities and irritancies are usually in the order :-

ketones < saturated aldehydes < unsaturated aldehydes.

Although the irritating effects of many aldehydes and ketones are well known, information on their toxicities in humans is still very limited. Acetaldehyde has received much attention because of its association with alcohol metabolism. Some other simple aliphatic aldehydes have also been studied recently because of increased concern about cigarette smoking. A summary of the reports on the effects of both long-term and short-term inhalational exposure to acrolein and formaldehyde is given in Reference 106. Table 3.1 shows the inhalation toxicity and the TLV of some carbonyl compounds commonly found in the pyrolytic products of both natural and synthetic polymers. Since information on the acute toxicity of these compounds in humans is rare, the TLV recommended by ACGIH[62] is used in this instance as a guide to their relative potency in the fire situation.

Inhalation studies of aldehydes in mice, guinea pigs and rabbits indicated that the toxicity of aldehydes decreased in the order: acrolein, crotonaldehyde, acetaldehyde, propionaldehyde, isobutyraldehyde and n-butyraldehyde[60]. After the initial irritation, the respiration became slow and deep, followed by convulsions

TABLE 3.1 Toxic effects and TLV of some carbonyl compounds commonly found in the pyrolytic products of polymeric materials[39,62].

Chemical name	Common name	TLV(ppm)	Toxic effects
Methanal	Formaldehyde	2	)Irritate eyes and all parts of the
Ethanal	Acetaldehyde	100	)respiratory system. Unsaturated aldehydes
Propanal	Propionaldehyde	N.E.	)cause burning sensation to nose and eyes
2-Propenal	Acrolein	0.1	)in addition to intense irritation.
2-Butenal	Crotonaldehyde	2	)
Propanone	Acetone	1000	)Cause dizziness, irritate eyes and
Butanone		200	)respiratory system. Narcotic at high
2-Pentanone		200	)concentrations. Hexacarbon ketones are
4-Methylpentan-2-one		100	)known to cause neuropathy.
2-Hexanone		25	)
Butenone		N.E.	)

N.E. = not established

and death. The acutely irritant aldehydes produced a bronchial constriction with a resulting distention of the alveoli and rupture of the alveolar septa. The less irritant aldehydes produced delayed death and increased lung damage due to irritation of the lung tissue, rather than of the bronchi. Apart from irritant effects, many aldehydes are known to affect blood pressure and heart rate in laboratory animals[107,108].

Experiments on the retention of inhaled formaldehyde, acetaldehyde, propionaldehyde and acrolein in the dog showed total respiratory tract uptake of formaldehyde was close to 100 %, uptake of acrolein was around 80 %, and uptake for acetaldehyde and propionaldehyde was in the range of 50 - 60 % and 74 - 82 % respectively. The retention of the latter two aldehydes was found to vary with ventilatory rate[109,110].

Laboratory studies on animals and in some cases on humans showed inhalational exposure to acrolein at a concentration as low as 1 ppm caused distinct eye and nasal irritation[106]. Egle and Hudgins reported that anaesthetised rats exposed to acrolein at 50 µg/l[Footnote a] of air showed a significant increase in heart rate and blood pressure[111]. Kane and Alarie found the RD50 in mice was 1.7 ppm[112]. Champlix and Catilina reported the

Note a) To convert µg/l to ppm at standard temperature pressure (STP) use:

$$X\ \mu g/l = Y\ ppm \times Mol.\ Wt. / 22.4\ l$$

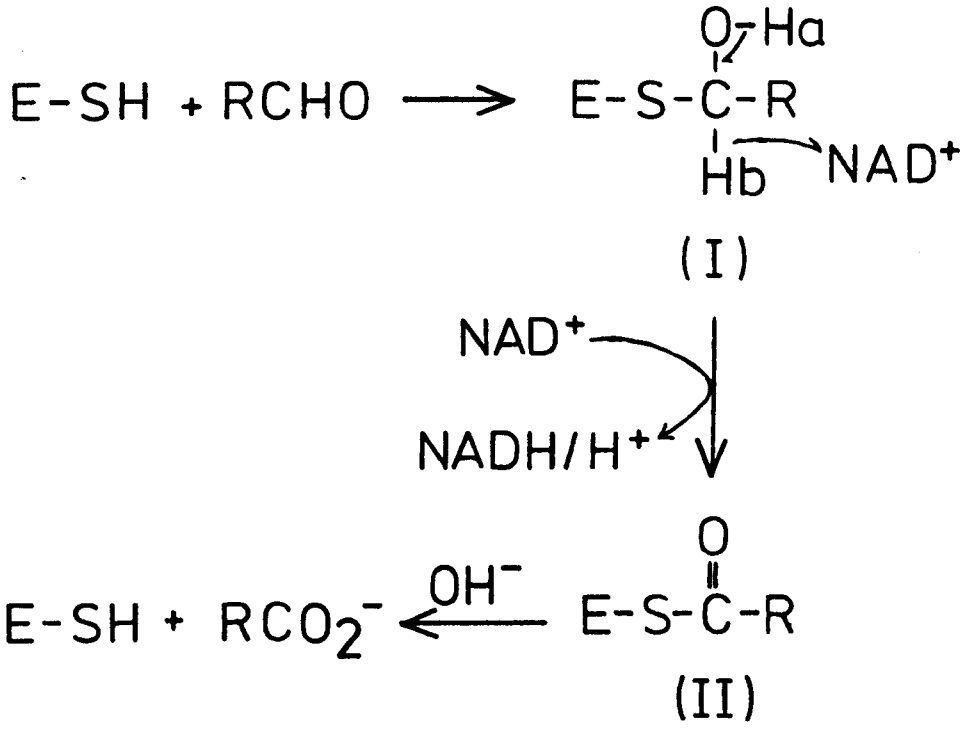
RD50 for rats was 750 μg/l for 10 minutes exposure (some died at 650 μg/l following cyanosis and dyspnoea)[113]. The LD50 decreased to 300 μg/l for 30 minutes exposure. Patty reported the lethal limit for man is 153 ppm for 10 minutes exposure[114].

Ketones have generally been considered innocuous, and thought to have little toxicity in humans. Inhalational exposure to the vapour, however, may cause irritation to mucous membranes and produce symptoms such as headache, drowsiness and perhaps nausea[115]. In recent years, some hexacarbon ketones such as 2-hexanone and 2,5-hexanedione, were discovered to be neurotoxic[116,117]. Consequently, the TLV for 2-hexanone was initially reduced from 100 ppm to 25 ppm and was further reduced to 5ppm in 1981[115].

3.2) Metabolism

Nicotinamide-adenine dinucleotide (NAD)-dependent aldehyde dehydrogenases exist in virtually every tissue where they are found in both cytosol and mitochondria. There are at least four enzymes that are capable of oxidizing aldehydes of both endogenous and exogenous origin[118]. The liver contains the greatest amount of total aldehyde-oxidizing capacity. The general metabolic pathway of aldehydes is shown in Scheme 1, in which E-SH represents an enzyme with a sulphydryl group.

In the case of the enzyme-aldehyde complex (I), the removal of the hydrogen (Hb) by NAD^+ is favoured by the loss of the hydroxyl hydrogen (Ha) to form the stable adduct(II). However, this is not possible with the



Scheme 1

enzyme-ketone complex. In fact, most ketones do not react with the sulphydryl group of the enzyme unless the ketone contains a strong electron-withdrawing group, such as CCl_3 or CF_3. Ketones which do react with the sulphydryl group are strong inhibitors of aldehyde dehydrogenase[119]. Ketones are, therefore, not readily metabolized in the body to any great extent. In fact, the major excretory routes for lower molecular weight ketones are via urinary excretion and expiration.

3.3) Literature Survey on Analytical Methods for Volatile Carbonyl Compounds

A number of analytical methods have been used for the analysis of carbonyl compounds. Direct analysis of plasma or serum by gas chromatography has a relatively high detection limit which renders the technique suitable only for major volatile components in blood, for example, acetone, ethanol or acetaldehyde in blood taken from individuals who have consumed alcohol[120]. Quash et al. reacted serum aldehyde with methyl benzolone thiazolone hydrazone hydrochloride followed by ferric chloride; the total serum aldehyde was estimated from the absorbance at 660nm of the resulting supernatant[121]. Stowell et al. used the enzyme aldehyde dehydrogenase and NAD^+ for the measurement of acetaldehyde in blood[122]. However, the method is non-specific and the experimental conditions have to be carefully controlled to optimise the enzymic activity. Liebich and Huesgen used cyclohexane to extract heptanone from urine followed by the analysis of the

solvent extract by GC-MS[123]. Although the authors claimed that the method was applicable to other ketones, it is unlikely to be suitable for lower molecular weight ketones or aldehydes which are extremely water-soluble and subsequent concentration by evaporation is not feasible.

One of the classical methods for analysing carbonyl compounds is to derivatize the carbonyl compounds to form the corresponding 2,4-dinitrophenyl hydrazones. The derivatives so formed can readily be extracted with organic solvent and concentrated for increased sensitivity, unlike the free carbonyls. More importantly, the reaction is specific to carbonyl compounds (aldehydes and ketones but not esters). Before the age of chromatography, the characterization of these derivatives was done by the measurement of their melting points after recrystallisation. This required a very large sample size by modern standards. With the development of chromatography, trace analysis of carbonyl compounds as their DNPH derivatives was possible using TLC[124], GC[125-130] and HPLC[131].

More recently, the technique of gas chromatographic headspace analysis has been used for volatiles such as acetaldehyde in blood[132-135] and glue sniffing[136]. In this method, the sample is placed in an enclosed vessel and the volatile components are allowed to equilibrate between the gaseous and the liquid (or solid) phase, usually at an elevated temperature in the region of 60 - 100°C. The method requires little or no sample pretreatment and avoids contaminating the column with non-volatile materials.

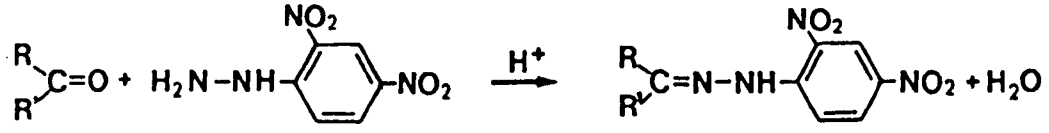
Recent developments of the direct sampling method include various headspace enrichment techniques such as condensation[137], circulation[138], and chromatographic adsorption[139] which have resulted in increased sensitivity.

Amongst the above-mentioned analytical methods, the DNPH derivatisation method and the headspace chromatographic method seemed suitable for both qualitative and quantitative analysis of carbonyl compounds in blood and were evaluated in this study.

CHAPTER 4: ANALYSIS OF CARBONYL COMPOUNDS AS THEIR 2,4-DINITROPHENYL HYDRAZONE DERIVATIVES BY GAS CHROMATOGRAPHY

4.1) Introduction

2,4-Dinitrophenyl hydrazine (DNPH) is one of most commomly used reagents for preparing derivatives from aldehydes and ketones. The reaction is catalysed by acid and the yield of the corresponding hydrazones is normally quantitative.



Before chromatographic methods were available, the reagent was solely used in qualitative organic analysis to test for the presence of carbonyl compounds which were characterised by the formation of a coloured precipitate. With the development of chromatography, the reagent is still being commonly used in various analyses such as flavour components[125], air pollutants[130] and auto exhaust[131]. Some of the main purposes of derivatisation are:

i) to isolate the carbonyl compounds from complex mixtures or from dilute solutions,

ii) to alter the volatility, and/or

iii) to convert the carbonyls to a more detectable form

for thin-layer chromatography or for some detectors such as the UV-photometer in high performance liquid chromatography and the electron capture detector in gas chromatography.

Mixtures of carbonyl-DNPH derivatives may be separated and detected by a variety of techniques, including paper chromatography[140], thin-layer chromatography[124], adsorption chromatography[141], high-performance liquid chromatography[131] and gas chromatography[125-130]. Of these techniques, the last two are also suitable for quantitative analysis. For reasons which included sensitivity, efficiency in separation and availability of equipment, gas chromatography was chosen in this study for the analysis of the carbonyl-DNPH derivatives.

Various liquid phases such as SE-30, SF-96, OV-17, F-60, OV-101, Dexsil 300 and FFAP have been used for the separation of DNPH derivatives[142] and amongst these, SE-30 is one of the most commonly used phases for these compounds. SE-30 is a non-polar phase consisting of methylsilicone rubber gum which can be used within a wide temperature range from 50 to 350°C. Because of its high upper temperature limit, it is particularly suitable for separating compounds of high molecular weight.

4.2) Experimental

4.2.A) Materials and reagents

Formaldehyde, acetaldehyde, propionaldehyde, acrolein, crotonaldehyde and acetone (reagent grade) were obtained from BDH (Dorset, U.K.). [$^2H_6$]-Acetone (99.8 atom%)

was obtained from Fluorochem Ltd. (Derbyshire, U.K.). 2,4-Dinitrophenyl hydrazine was obtained from BDH and was recrystallised from methanol (AnalaR grade) before use.

4.2.B) Preparation of carbonyl-hydrazone standards

The reagent was prepared by adding concentrated sulphuric acid (2 ml) to DNPH (0.4 g); water (3 ml) was then added dropwise followed by ethanol (95 %v/v, 10 ml). The carbonyl compound (0.5 ml in 20 ml of 95 %v/v ethanol) was added to the freshly prepared DNPH reagent and allowed to stand at room temperature until precipitation of the derivative was complete. The precipitate was filtered and washed with HCl (2 M) until the washings were clear. The derivative was finally rinsed with water (20 ml) and recrystallised from methanol.

4.2.C) Microgram-scale derivatisation of carbonyl compounds

An aliquot of the sample (2 ml) was shaken with saturated 2,4-dinitrophenyl hydrazine solution (in 2 M HCl, 2 ml). The mixture was allowed to react overnight at room temperature. The DNPH derivative formed was extracted with chloroform (3 x 2 ml). The combined chloroform extracts were washed with HCl (2 M, 3 x 2 ml) to remove excess unreacted DNPH reagent. The extract was then washed with reboiled distilled water (2 ml portions) until neutral, dried over anhydrous sodium sulphate and finally evaporated to dryness in a stream of nitrogen. The residue was redissolved in 500 µl of benzene (pesticide grade, distilled in glass) prior to analysis.

4.2.D) Preparation of packed GC columns

1) Preparation of support

The inert support material, Chromsorb W (Field Instruments, Surrey, U.K.) was sieved to obtain a uniform size (85 - 100 mesh). Care was taken in the subsequent stages of preparation to avoid breaking of the sieved particles. The support material was soaked in concentrated HCl overnight to remove traces of inorganic impurities. After washing with distilled water until the washings were neutral, the support was dried in an oven at 100°C overnight.

The support material (25 g) was placed in a round-bottom flask to which dimethyldichlorosilane in toluene (10 %v/v) was added to cover the support. The flask was connected to a vacuum pump to degas the support until effervescence ceased in order that the porous material should be completely filled with silylating agent. The slurry was left for 3 hours and then excess silylating agent was removed by washing with dry toluene. The silylation process was completed with the addition of sufficient methanol to cover the support. The slurry was then filtered under vacuum and dried in an oven at 70°C.

2) Coating of support with liquid phase

Silicone rubber gum, SE-30 (obtained from Wilkens Instrument and Research Inc., California, U.S.A.) was dissolved in toluene to make up the required concentration (2 %w/v). The inert support (25 g) together with the solution of liquid phase (100 ml) were gently mixed in a round-bottom flask which was then connected to a vacuum pump for 30 - 60 minutes. The slurry was filtered under gravity with smooth continuous stirring until it was almost dry. It was then filtered under suction and dried in an oven at a temperature slightly higher than the lower temperature limit for the liquid phase used (60°C). The exact percentage by weight of coating was calculated from the weight of the coated support.

3) Preparation of glass column

An empty glass column (4 mm. i.d. x 3 m) was filled with methanolic potassium hydroxide (0.2 %w/v) overnight to clean the inner surface of the column. Excess potassium hydroxide was washed away with methanol (100 ml). The column was then silylated overnight with dimethyldichlorosilane in toluene (10 %v/v) followed by flushing it with methanol (150 ml) and acetone (50 ml). The column was dried in a stream of nitrogen.

4) Column packing

A small piece of silanised glass wool was sucked into the detector end of the column by attaching it to a vacuum pump. The packing material was introduced via a filter

funnel connected to the top of the column with rubber tubing. The column was tapped gently to ensure uniform packing. Finally a wad of silanised glass wool was inserted at the top of the column to retain the packing material.

5) Column conditioning

The column was mounted in the chromatograph but was not connected to the detector during conditioning. The nitrogen carrier gas flow rate was adjusted to about 30 ml/min. The oven temperature was programmed at 1°C/min from the lower temperature limit of the liquid phase to within 10°C of its upper temperature limit (350°C) with initial and final isothermal periods of 1 and 12 hours respectively.

4.2.E) Analysis of carbonyl-hydrazone derivatives by gas chromatography

The gas chromatograph used was either a Pye Unicam 204 fitted with a flame ionisation detector or a Pye Unicam GCD fitted with a ^{63}Ni electron capture detector. Each chromatograph was connected to a Servoscribe chart recorder in series with a Laboratory Data Control LDC308 digital integrator. The column used was either the packed column prepared in section 4.2.D or an SE-30 SCOT column (0.5 mm i.d. x 30 m) run either isothermally at 200°C or temperature programmed at 4°C/min from 120°C to 245°C with an initial isothermal period of 3 min. The carrier gas (nitrogen for FID and argon/methane for ECD)

flow rate was 40 ml/min for the packed column and 2 ml/min for the capillary column. An all-glass moving-needle solid injector (Chrompack, London) was used with the capillary column. The injector and detector temperature were 250°C and 275°C respectively.

4.2.F) Quantification of carbonyl-hydrazone derivatives by selected ion recording GC-MS

The analysis was performed on a VG MM16F mass spectrometer interfaced to a Pye Unicam 104 gas chromatograph. The column used was either an SE-30 SCOT column (0.5 mm i.d. x 30 m) with an all-glass moving needle solid injector or a glass column (4 mm i.d. x 1.5 m) packed with 3 % OV-1 on Gas Chrom Q (80 - 100 mesh). The chromatographic conditions used were as described in section 4.2.E except that helium was used as the carrier gas. The mass spectrometric conditions used were as follows:

Source temperature	: 220°C
Source pressure	: 4 x 10^{-4} torr
Accelerating potential	: 4 KV
Cycle time	: 1 s
Electron energy	: 30 eV
Emission current	: 200 uA
Multiplier voltage	: 2.5 KV

Standard solutions containing acetaldehyde, acrolein, crotonaldehyde and acetone at concentrations ranging from 10 ng/ml to 100 µg/ml were used for the purpose of calibration. After the formation of derivatives, $[^2H_6]$-acetone

2,4-dinitrophenyl hydrazone (150 µg/ml, 200µl) was added as the internal standard in the first solvent extraction procedure. A blank sample was prepared in the same way except that 2 ml of reboiled distilled water was used instead of the sample solution. The molecular ions of the carbonyl-DNPH derivatives were monitored, for example, m/z 224 for acetaldehyde-DNPH, m/z 238 for acetone-DNPH and m/z 244 for [$^2H_6$]-acetone-DNPH. The ion at m/z 207 from the column background was used as the reference mass.

4.2.G) Application to biological fluids

As a preliminary test of the analytical method for application to biological fluids, the levels of acetaldehyde and acetone were measured in the urine samples from two healthy volunteers who had not consumed alcohol during the preceding 24 hours. The samples were analysed in duplicate: one was treated normally as described in section 4.2.C while the other was heated to 80°C for 30 minutes in a sealed culture tube prior to derivatisation. This was to check if the level of acetone would increase due to its formation from thermal decarboxylation of acetoacetate[143].

Time-expired human blood was obtained from the Blood Transfusion Unit of the Haematology Department, Western Infirmary, Glasgow, to which acrolein was added at a concentration of 100 µg/ml. Various sample pretreatments prior to derivatisation were examined:

Method 1: 3 ml of blood were haemolysed with 6 ml of distilled water for 2 hours at room temperature; the

haemolysate was then reacted with the DNPH reagent as described previously.

Method 2: 3 ml of blood were centrifuged at 3000 rpm (1500 g) for 10 minutes after which the plasma (1 ml) was reacted with the DNPH reagent.

Method 3: as for method 2 except that the plasma proteins were precipitated by the addition of trichloroacetic acid (10 %w/v, 1 ml) and the supernatant was reacted with DNPH reagent.

Method 4: 3 ml of whole blood were reacted with the DNPH reagent without any pretreatment. This served as a control for comparision with the other 3 methods.

The samples were then analysed by selected ion recording GC-MS as described in section 4.2.F.

4.3) Results and Discussion

The procedure described by Shriner et al.[144] was suitable for the large-scale preparation of standard carbonyl-DNPH derivatives. The reaction was complete within 5 - 10 minutes and the yield was high (approximately 90%) even after the recrystallisation process. The physical properties of the carbonyl-DNPH derivatives are shown in Table 4.1. The sharp melting points and the U.V./visible spectra of the products suggested that these crystalline derviatives were free from impurities. This was later confirmed by gas chromatography and thin-layer chromatography on silica gel using chloroform:methanol (3:1) as the solvent system.

Table 4.1 Physical properties of the carbonyl-2,4-dinitrophenyl hydrazones.

DNPH derivative of	Melting pt. (°C) a	λmax in MeOH (nm) b	Ret. indices SE-30 c	Principal ions in mass spectrum (m/z) d
Formaldehyde	167	346	1950	210(M^+), 63, 79, 51, 78, 30, 64, 39
Acetaldehyde	155	359	2050	224(M^+), 79, 78, 77, 63, 51, 122, 75
Propionaldehyde	155	368	2160	79, 238(M^+), 152, 30, 78, 41, 29, 63
Acrolein	168	370	2130	236(M^+), 189, 69, 63, 89 141, 142, 116
Crotonaldehyde	189	376	2300	39, 41, 203, 63, 202, 250(M^+), 30, 53
Acetone	114	364	2130	56, 41, 59, 79, 238(M^+), 78, 43, 42

Notes:
a) The melting points were measured using a Gallenkamp melting point apparatus.
b) The UV-visible absorption spectra were recorded on a Pye Unicam SP1700 spectrophotometer.
c) See experimental section for chromatographic conditions.
d) The mass spectra were obtained with a VG MM16F mass spectrometer in E.I. mode. The samples were introduced with a direct insertion probe. The electron energy, source temperature and source pressure were 70 eV, 220°C and 2×10^{-7} torr respectively.

During the entire column preparation procedure, care was taken to prevent fracturing of the support material since a large variation in particle size would seriously affect the column performance. When dissolving the liquid phase for coating, high boiling solvents are preferred because a slower rate of evaporation gives a more uniform coating. Smooth continuous stirring of the slurry during the filtration process was required which otherwise would result in an uneven distribution of liquid phase. For liquid phase loadings greater than 10%, a much simpler evaporation technique may be used which involves evaporating the solvent in a shallow evaporating dish, without the need for filtration. When packing the coated material in a column, it is important to ensure that the packing is as close and uniform as possible. This minimizes the coefficient of solute diffusion in the gas phase, as considered by the Van Deemter equation and hence increases the efficiency of the column. Finally, the freshly prepared column was conditioned before use to flush out any traces of solvents and other impurities which might interfere with an analysis.

The performance of the column was tested using normal hydrocarbons (C14 - C24). The peak shapes of the test chromatogram were all symmetrical and the total number of theoretical plates of the column was 7200 which was high even compared with commercial columns. The procedure described here provides a simple method, though time-consuming, for preparing packed columns. It provides a significant cost saving over commercially prepared

columns and yet does not require a great deal of equipment.

Several organic solvents including chloroform, ethyl acetate, toluene, methanol and benzene were tried for the preparation of standard carbonyl-DNPH solutions. Of these, benzene was found to be most satisfactory with respect to solubility of the derivatives and solvent peak tailing on the SE-30 column. In addition, pesticide grade benzene (distilled in glass) was found to give the least background interference when used with an electron capture detector.

Two phthalates were found in the chromatogram which interfered with the analysis. These were later identified by mass spectrometry to be butyl-carbobutoxymethyl phthalate and di-isobutyl phthalate. These interferences were effectively removed when all the apparatus used was washed with methanol and dried in an oven at 90°C overnight before use.

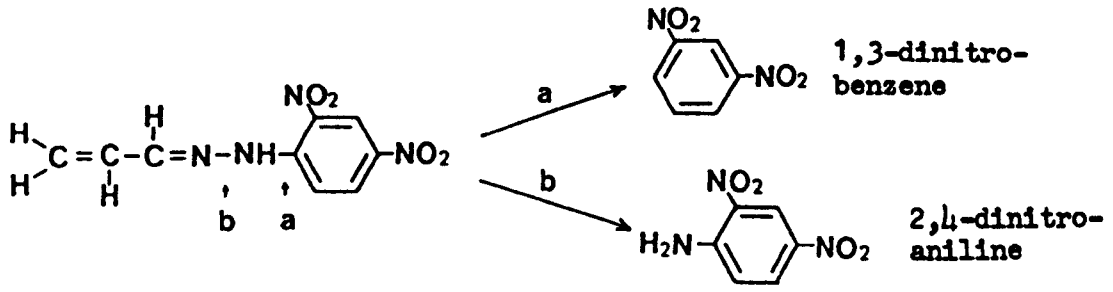
The retention indices of carbonyl-DNPHs on the SE-30 column are shown in Table 4.1. Although the column used was efficient by packed column standards, it could not separate acetone-DNPH and acrolein-DNPH which differ in molecular weight by only two mass units. Propionaldehyde-DNPH which has the same number of carbon atoms also overlapped with these two peaks. The effect of altering the GC conditions (initial time, ramp rate and final temperature) was tried but separation still could not be obtained. The problem of separating compounds of similar structure and molecular weight is common in chromatography. One solution may be to use a more polar column such as OV-17. However, this would require higher

operating temperatures which might result in higher background due to column bleed. The best solution to this problem of separation is to use a more efficient non-polar column, ie., capillary column or to use selected ion recording GC-MS to distinguish the components in the overlapping peaks.

The use of an electron capture detector gave a sensitivity one hundred times better than that from a flame ionisation detector. This was in good agreement with that reported by Kallio et al.[125]. The detection limits (signal to noise ratio > 3) for these carbonyl-DNPHs were of the order of 10^{-7} g and 10^{-9} g for flame ionisation detector and electron capture detector respectively. The detection limit for acetone-DNPH was about ten times lower. When the same quantity (10 ng) of the DNPH derivatives was injected separately onto the column, it was found that both the electron capture detector and the flame ionisation detector gave a ten-fold higher response to acetone-DNPH than acetaldehyde-DNPH, acrolein-DNPH or crotonaldehyde-DNPH. It is unusual for these two types of detector to give large differences in the response factor for compounds of the same family. One possible explanation is that the DNPH derivative of these compounds might have different thermal stabilities. In fact, decomposition of some DNPH derivatives during the chromatographic process has been mentioned by many other workers[126,130,145].

An experiment was carried out in which the above-mentioned carbonyl-DNPHs were heated separately from room temperature to 250°C at a rate of 20°C/min in a

Gallenkamp melting point apparatus after which the samples were analysed by GC-MS. It was found that acrolein-DNPH had undergone decomposition to form 1,3-dinitrobenzene and 2,4-dinitroaniline whereas the sample of acetone-DNPH was chemically unchanged. In the former case, cleavage of the molecule occurred at two positions (a) and (b) as shown below:



Analysis of the heated crotonaldehyde-DNPH sample also showed traces of the decomposition products mentioned above but to a much lesser extent. Chromatographing 2,4-dinitrophenyl hydrazine on the packed column under the same conditions also resulted in complete decomposition of the compound to form 1,3-dinitrobenzene. Although heating the carbonyl-DNPHs in the presence of air might not represent the true conditions in the gas chromatograph, nevertheless, it indicated the thermal instability of some carbonyl-DNPH compounds. Because of the time limitations, it was not possible to carry out a detailed study on the thermal stability of the rest of the carbonyl-DNPH

derivatives.

A solid sample injector was used for the analysis of carbonyl-DNPHs on the capillary column. It is useful for injecting substances which are solid at room temperature. It eliminates the problem of solvent shock to the capillary column which normally accompanies the use of a splitless injector. An injector incorporating a sample splitter will result in a loss of sample through the vent whereas a solid sample injector may be used to concentrate dilute samples before injection. Silanisation of the injector was found to improve sample evaporation from the needle tip and reduce sample adsorption.

A typical chromatogram of DNPH derivatives is shown in Figure 4.1. The SE-30 SCOT column gave excellent separation of the derivatives of acetaldehyde, acetone and crotonaldehyde. It also gave partial separation of the derivatives of acetone and acrolein which completely overlapped in the packed column.

Bachmann et al.[127] and Smith et al.[130] reported the presence of double chromatographic peaks for certain carbonyl compounds due to the existence of syn- and anti-isomeric forms of the DNPH derivatives. Double peaks were not observed in this study which might be due to insufficient resolving power of the columns being used.

The principal ions in the mass spectra of carbonyl-DNPHs using electron impact ionisation at 70 eV are shown in Table 4.1. Although extensive fragmentation of these compounds occurred under electron impact conditions, their molecular ions were still amongst the major ions in the

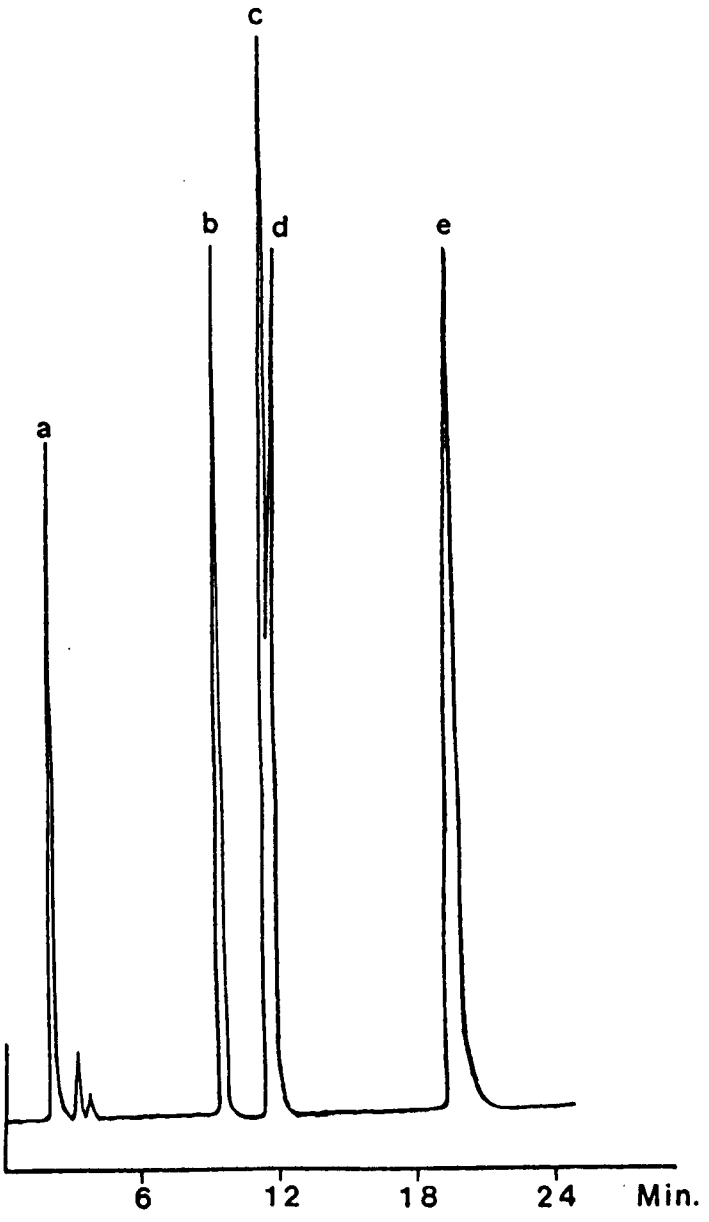


Figure 4.1 Chromatographic separation of carbonyl-DNPHs on an SE-30 SCOT column (0.5mm i.d. x 30m) at 200°C. The identities of the peaks were: a) solvent, b) acetaldehyde-DNPH, c) acetone-DNPH, d) acrolein-DNPH, e) crotonaldehyde-DNPH.

spectra. Selected ion recording of these ions had few, or no, cross-channel contribution problems and could therefore be used to quantify unresolved carbonyl-DNPHs. Chemical ionisation using isobutane as the reactant gas was evaluated briefly for these compounds, giving quasi-molecular ions with little fragmentation. However, for reasons which included ionisation efficiency and geometry of the ion source, the sensitivity obtained was unsatisfactory.

The effect of electron energy on the absolute molecular ion intensities of three carbonyl-DNPHs is shown in Figure 4.2. At 10 eV, the energy was insufficient to cause ionisation of these compounds. The absolute molecular ion intensities rose rapidly as the electron energy was increased to 20 eV and reached a maximum at 30 eV (except for crotonaldehyde-DNPH which had a maximum at 50 eV). The intensities then fell steadily as the electron energy was increased above 30 eV. This was because at high electron energies, more fragmentation occurred and hence resulted in a decrease in the intensity of molecular ion.

Although the molecular ions of these derivatives were amongst the most abundant ions in their spectra, the detection limit of these compounds by SIR was rather poor (of the order of ng) and was no more sensitive than ECD-GC. The advantage of GC-MS is obviously the specificity obtained, allowing the overlapping peaks to be distinguished.

In gas chromatographic analysis, the detection limit can normally be improved by raising the column temperature,

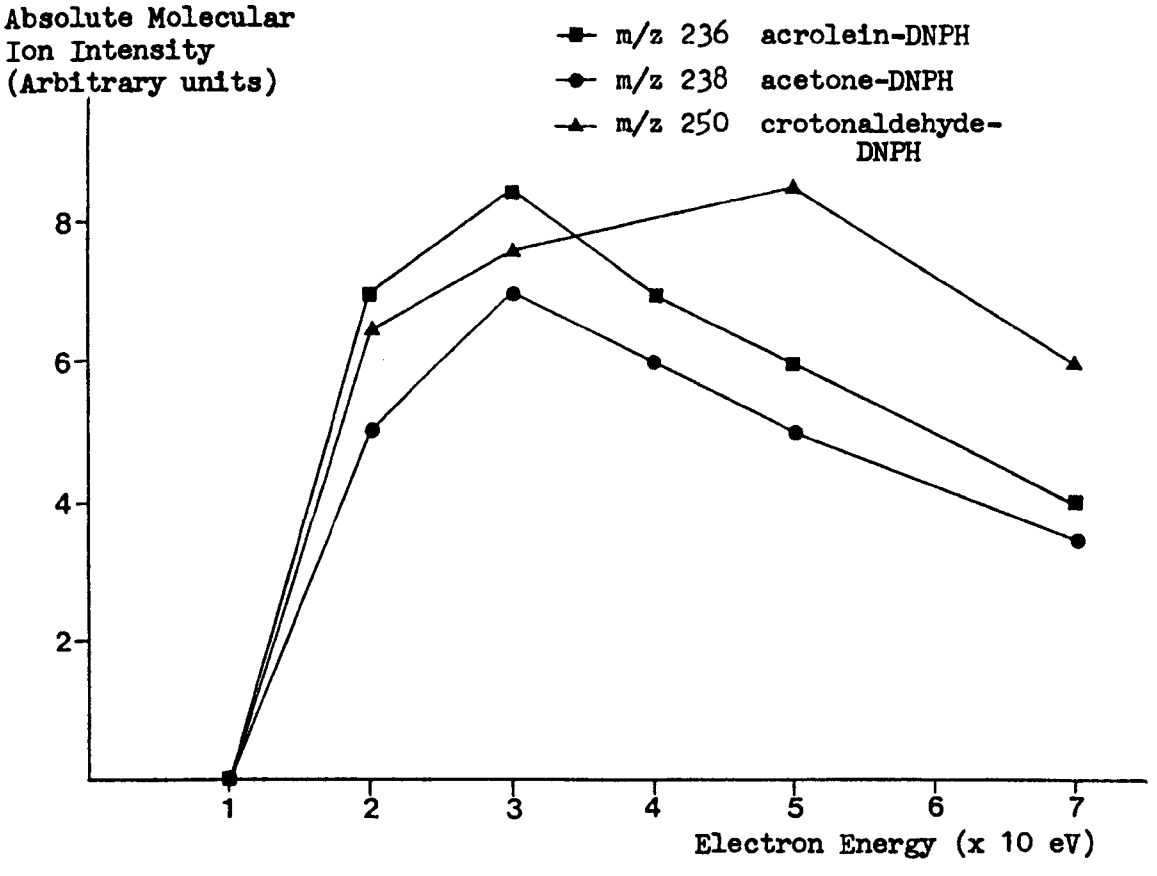


Figure 4.2 Effect of electron energy on the molecular ion intensity of carbonyl-DNPHs.

provided that column bleed is low and separation is adequate. Lower column temperatures normally result in longer retention times and peak broadening, hence in a decrease in peak height and higher detection limit. The effect of column temperature on the signal height at m/z 238 and m/z 236 from acetone-DNPH and acrolein-DNPH respectively is shown in Figure 4.3. At 200°C or 220°C, the chromatographic peaks were sharp and symmetrical. Below these temperatures, the peaks began to broaden. There was only a slight increase in peak height when the column temperature was increased from 200°C to 220°C. The shape of the graph suggests decomposition has started to occur above 200°C otherwise a straight line is predicted.

The addition of [$^2H_6$]-acetone to the sample as internal standard prior to derivatisation was not successful. This was probably due to deuterium loss through enolisation under the acidic conditions required for the reaction. The problem was solved by adding [$^2H_6$]-acetone-DNPH in the first extraction procedure. This would not, however, compensate for losses in the derivatisation step. A typical calibration curve is shown in Figure 4.4. The calibration curves were found to be linear with correlation coefficients of 0.9985, 0.9992, 0.9919 and 0.9899 for acetaldehyde, acrolein, crotonaldehyde and acetone respectively. The quantitation limits for acetaldehyde, acrolein and crotonaldehyde were 0.1 μg/ml while that for acetone was 1 μg/ml. These values

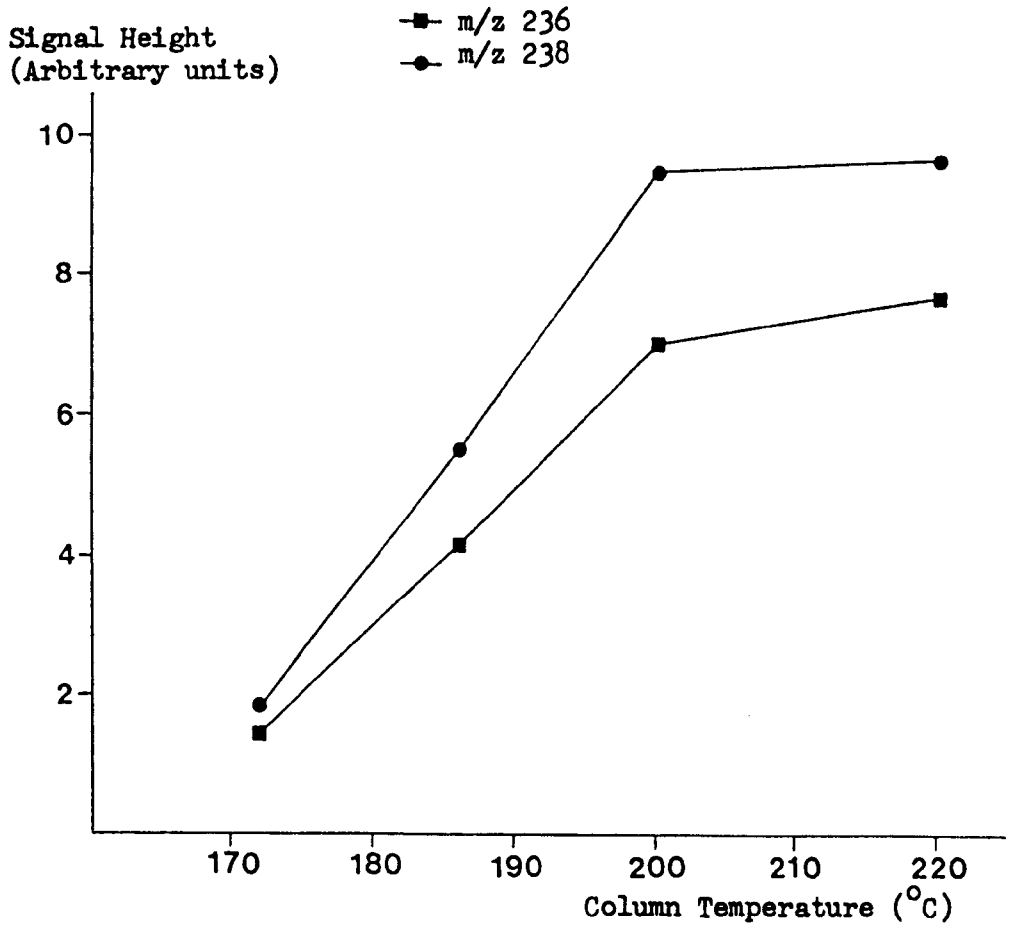


Figure 4.3 The improvement in signal height obtained for m/z 236 from acrolein-DNPH and for m/z 238 from acetone-DNPH by increasing the column temperature. The column used was a glass column (4 mm i.d. x 1.5 m) packed with 3% OV-1 on Gas Chrom Q and the sample size was 50 ng.

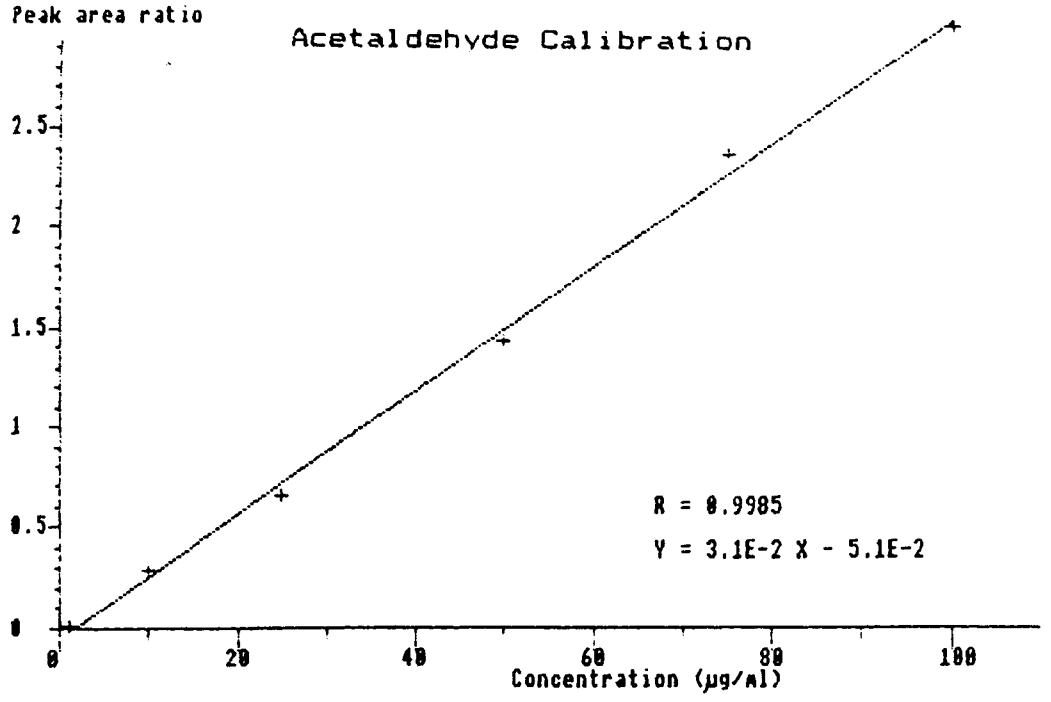


Figure 4.4 Calibration curve for acetaldehyde in aqueous solutions constructed by plotting the peak area ratio of m/z 224 from acetaldehyde-DNPH to m/z 244 from $^{2}H_{6}$-acetone-DNPH (internal standard) against the acetaldehyde concentration.

were by no means the absolute detection limits of the mass spectrometer but were practical limits as a result of column background and recovery of hydrazones at low concentrations. The high quantitation limit for acetone was due to the high level of acetone in the procedure blank, coming from two major sources; the ion contribution from the internal standard and traces of acetone present in water which was used for sample preparation. At a concentration of 2.5 µg/ml, the coefficient of variation for ten successive analyses of acetaldehyde-DNPH and acetone-DNPH were 2.3 % and 1.8 % respectively. At lower carbonyl concentrations, two factors contributed to a loss of sensitivity in the method. Firstly, the extent of the conversion of carbonyl to the corresponding DNPH derivative was reduced and secondly, the recovery of the derivative during the sample clean-up step was low. The degree of recovery of carbonyl-DNPHs at various carbonyl concentrations is shown in Table 4.2.

The concentrations of acetaldehyde and acetone were measured in urine samples from two healthy volunteers as a preliminary test of the application of the method to biological fluids. The subjects had not consumed alcohol during the preceding 24 hours, to avoid a rise in the level of acetaldehyde due to the metabolism of ethanol[118,119]. The results of the analyses are shown in Table 4.3. The results indicate that the levels of acetaldehyde and acetone in these two urine samples were within the normal ranges[146]. There was no significant difference in the acetone level between the heat-treated and untreated

Table 4.2 Degree of recovery of carbonyl-DNPHs at various carbonyl concentrations calculated by comparision with standard carbonyl-DNPH solutions.

Carbonyl conc. (μg/ml)	Percentage recovery of acetone-DNPH	acetaldehyde-DNPH
100	96	95
75	75	58
50	45	38.5
25	20	12.9
1	2.2	4.8

Table 4.3 Levels of acetaldehyde and acetone in urine samples from two healthy human subjects.

Subject	Acetaldehyde (μg/ml)	Acetone (μg/ml)
1, untreated *	0.3	8.3
1, heat-treated **	0.3	7.9
2, untreated *	0.4	7.7
2, heat-treated **	0.4	8.5

Note: * Urine samples were reacted normally with DNPH reagent at room temperature. ** Urine samples were heated at 80°C for 30 minutes prior to derivatisation.

samples.

Although the conversion of carbonyl compounds to their corresponding DNPH derivatives has been widely used for analysing gaseous and aqueous samples, the method has never been applied to blood samples. The prime purpose of this study was to adapt the analytical method for the measurement of carbonyls in blood samples obtained from fire fatalities.

Amongst the four methods of sample pretreatment tried (Paragraph 4.2.6), methods 2 and 4 were found to be unsuitable in practice because the addition of the DNPH reagent resulted in coagulation of the sample which rendered the derivatisation and extraction processes extremely difficult. The problem of protein coagulation was solved in method 3. However, added acrolein and endogenous acetaldehyde were not detected.

The absence of these two carbonyl-DNPHs could be due to the binding of these aldehydes to the plasma proteins which were subsequently removed by centrifugation after the protein precipitation procedure. It is known that aldehydes react with amines and sulphydryl groups of proteins to form adducts[133]. In method 1, when the acidic DNPH reagent was added to the haemolysed blood, protein precipitation also occurred but in the form of a suspension. Analysis of the derivatised sample showed the presence of DNPH derivatives of acetaldehyde and acetone but not acrolein (Figure 4.5). Some of the possible explanations are: irreversible protein binding, rapid metabolism in blood or, chemical reactions other than those

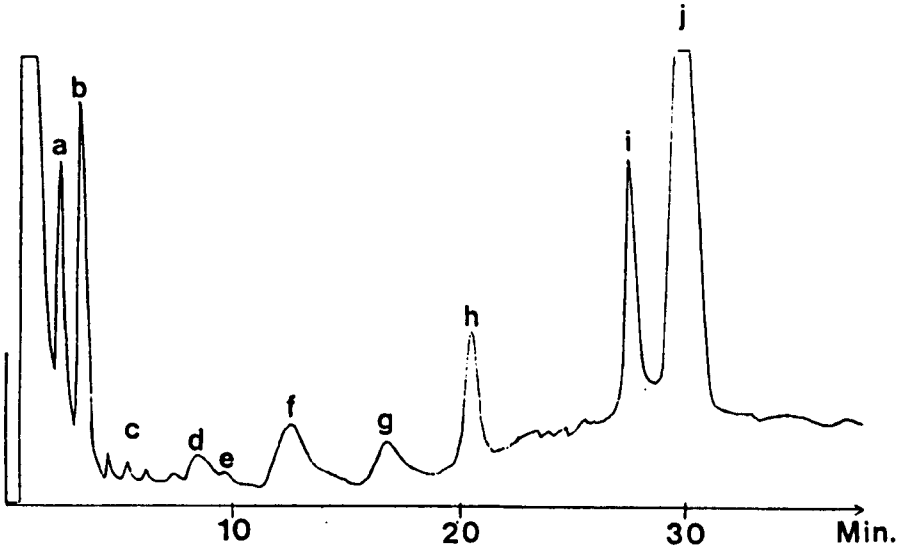


Figure 4.5 Total ion chromatogram of carbonyl-DNPHs from haemolysed blood separated on a glass column (4 mm i.d. x 1.5 m) packed with 3% OV-1 on Gas Chrom Q at 200°C. The identities of the peaks were: a) 1,3-dinitrobenzene, b) 2,4-dinitrochlorobenzene, c) hydrocarbons, d) 2,4-dinitroaniline, e) di-nor-butylphthalate, f) acetaldehyde-DNPH, g) acetone-DNPH, h) acetyl tributyl citrate, i) phthalate ester, and j) unknown.

mentioned above, since acrolein is the most reactive compound in the carbonyl family. DNPH derivatives of other carbonyls which are normally present in human blood at trace quantities were not detected. The sensitivity of the method thus presented another major problem if the carbonyl profiles in normal blood and in blood from fire deaths were to be compared.

The chromatogram also showed the presence of some contaminant peaks, which were extracted from the blood together with the carbonyl-DNPHs. Two of these components were identified as di-nor-butylphthalate and acetyl tributyl citrate. Several hydrocarbons and one other phthalate ester were also present but their structures were not identified. Elution of the DNPH derivatives of higher molecular weight carbonyl compounds would require higher column temperatures which would lead in turn to higher column background and, subsequently, higher detection limits for these compounds.

4.4) Conclusions

As the developmental work proceeded, it became clear that the method was not suitable for use in this study. Some of the major problems associated with this analytical method can be summarised as follows:

The carbonyl compounds of interest were mainly C2 - C7. It was difficult to separate carbonyl-DNPH derivatives which had the same number of carbon atoms even with the use of a capillary column although a more efficient column than

the one available might have effected complete separation. The peaks of DNPH derivatives of acetone, acrolein and propionaldehyde all overlapped in the chromatogram. Although it is possible to use selective ion recording to resolve some of these overlapped peaks, isomers having the same molecular weight still could not be distinguished.

Derivatisation of the carbonyl compounds in blood required complicated solvent extraction and clean-up procedures following conversion, and the recovery of the derivatives was often low at micromolar sample concentrations. Since the lower aliphatic carbonyls were more soluble in aqueous media than in organic solvents, therefore, solvent extraction and concentration techniques could not be applied to the blood samples prior to derivatisation. In addition, polar organic solvents usually have relatively high concentrations of carbonyl impurities which preclude their use.

Chromatographic separation of carbonyl-DNPH derivatives required high column temperatures. However, some of these compounds had poor thermal stability which resulted in decomposition during the flash vapourisation and subsequent chromatographic process. On-column injection using capillary columns has recently been shown to be useful for thermally unstable compounds such as underivatised steroids[147].

The quantitation limit of this method was in the region of 0.1 μg/ml which might not be sensitive enough to measure trace amounts of carbonyl compounds in blood resulting from exposure to smoke and toxic gases in fires.

Because of these reasons, it was decided to seek an alternative method for the analysis of carbonyls in blood. The technique of gas chromatographic headspace analysis, both static and dynamic, was then studied separately.

CHAPTER 5: ANALYSIS OF CARBONYLS IN STATIC HEADSPACE SAMPLES BY GAS CHROMATOGRAPHY

5.1) Introduction

Gas chromatographic headspace analysis is a relatively new technique used for both trace analysis and for the measurement of physicochemical data. It is an indirect method for the determination of volatile constituents in liquids or solids by gas chromatographic analysis of the vapour phase in thermodynamic equilbrium with the sample in a closed system.

It is particularly useful in some applications in which samples such as soil, sewage, blood etc., are difficult to analyse by direct methods. The technique is relatively simple, accurate and reproducible. Above all, it can easily separate the volatiles in blood from the non-volatile components, for example proteins and inorganic salts, without the need for lengthy sample preparation procedures such as derivatisation, extraction and purification. The technique can be used for both qualitative and quantitative analyses. Other examples of its applications can be found in Reference 148.

This chapter presents the results of a feasibility study of static headspace analysis by gas chromatography for acrolein and other carbonyls in blood. The binding of aldehydes to proteins and the use of arsenic trioxide and perchloric acid to liberate bound aldehydes in blood were also examined.

5.1.A) Basic principles of gas chromatographic headspace analysis

In headspace analysis, the gas chromatographic peak area (Fi), of a volatile component (i) is proportional to the partial vapour pressure of the component (Pi) in the headspace above the sample. Therefore,

$$Fi \propto Pi \qquad \text{Equation 5.1}$$

or $$Fi = Ci \times Pi \qquad \text{Equation 5.2}$$

where Ci is a constant which depends on the characteristics of the detector being used. The sample to be analysed, in equilibrium within the closed system, is normally a solution of the volatile component of interest (i) in a liquid solvent. In an "ideal" solution, the partial vapour pressure Pi can be expressed by Raoult's Law as

$$Pi = Poi \times Xi \qquad \text{Equation 5.3}$$

where Poi is the vapour pressure of pure component i and Xi is the mole fraction of component i in the solution. In most cases, however, molecular interactions exist in solution which leads to the expression:-

$$Pi = Poi \times Xi \times Ai \qquad \text{Equation 5.4}$$

where Ai is the activity coefficient of component i. The activity coefficient Ai depends on the nature of the component i and other components in the mixture, the mole fractions of all the components, the temperature and to a small extent on the pressure. For dilute solutions as used in trace analysis, the vapour pressure of the solute tends to vary linearly with its concentration, hence, the activity coefficient becomes a constant. Such behavior is

known as Henry's Law. Combining Equations 5.2 and 5.4, the following relation is produced:-

$$Fi = Ci \times Poi \times Xi \times Ai \qquad \text{Equation 5.5}$$

or $$Xi = Fi / (Ci \times Poi \times Ai) \qquad \text{Equation 5.6}$$

where $1 / (Ci \times Poi \times Ai)$ is a constant called the calibration factor which has to be determined experimentally. The concentration of the component can thus be calculated from the chromatographic peak area.

5.2) Experimental

5.2.A) Materials and apparatus

$[^2H_6]$-acetone and the carbonyl compounds used were as described in section 4.2.A. Hypo-vials (6 ml) with butyl rubber septa and aluminium caps were purchased from Alltech (Lancashire, U.K.). A Hamilton Series 1000 gas-tight syringe (500 µl) was used for headspace sampling. Arsenic trioxide (As_2O_3) was obtained from BDH (Poole, U.K.). Perchloric acid (AnalaR, 72 %) was obtained from BDH and diluted to 0.6 N with distilled water. Time-expired blood was provided by the Blood Transfusion Unit, Haematology Department, Western Infirmary, Glasgow.

5.2.B) Standard solutions

Standard solutions containing acetaldehyde, propionaldehyde, acrolein, crotonaldehyde and acetone at concentrations ranging from 0.01 - 100 µg/ml were prepared by dissolving each compound in reboiled distilled water. Calibration standards in the same concentration range were

also prepared by adding carbonyl compounds to time-expired blood. [$^2H_6$]-acetone (10 µl/ml, 100 µl) was added to the sample (1 ml) as the internal standard. The peak height ratios of the carbonyl compounds relative to the internal standard were used for the construction of calibration curves.

5.2.C) Headspace equilibration

The sample (1 ml) was pipetted into a 6-ml hypo-vial, sealed with a butyl rubber septum and aluminium cap, and then equilibrated in a Tecam heating block at 60°C for at least 20 minutes. Samples of the headspace (50 - 100 µl) were removed for analysis using a Hamilton gas-tight syringe which was preheated to the same equilibration temperature to avoid condensation of sample on the inner surface of the syringe barrel.

5.2.D) Gas chromatography and selected ion recording

Analysis of headspace was performed on a Pye Unicam 204 gas chromatograph equipped with a flame ionisation detector. Separation of the carbonyl compounds was carried out using a glass column (4 mm i.d. x 2.5 m) packed with 3 % Carbowax 20M on 80 - 100 mesh Chrom. W. at 60°C with nitrogen flowing at 40 ml/min. The injector and detector temperatures were 200°C and 250°C respectively.

Selected ion recording was performed on a VG MM16F mass spectrometer interfaced to a Perkin Elmer Sigma 3B gas chromatograph fitted with a glass column (4 mm i.d. x 3.5 m) packed with 10 % Carbowax 20M on 60 - 80 mesh Gas Chrom

Q at 80°C. Selected ion recording used a VG PP2 four-channel peak selector unit monitoring m/z 43 or 58 for acetone, m/z 44 for acetaldehyde, m/z 56 for acrolein, m/z 58 for propionaldehyde, m/z 70 for crotonaldehyde and m/z 46 or 64 for [$^2H_6$]-acetone. The MS conditions used were: ionisation method, EI at 70 eV; source temperature, 200 - 220°C; source pressure, 1 x 10^{-4} torr; cycle time, 1 second.

5.2.E) Evidence for acrolein binding to haemoglobin

Whole blood (4 ml) was divided into four equal aliquots. Sodium dithionite (2 - 3 mg) was added to the first aliquot to reduce the haemoglobin. The other three aliquots of blood were pipetted into three 10-ml syringes labelled "O_2", "CO" and "N_2". Oxygen (10 ml) was introduced into the syringe labelled "O_2" and the contents were mixed gently for 3 to 5 minutes. The gas was then expelled and the exchange process repeated two more times to give saturated oxyhaemoglobin. The same procedures were carried out for the other two samples of blood using carbon monoxide and nitrogen. The use of nitrogen was to remove the bound oxygen to give free unbound haemoglobin. Acrolein (50 mg/ml, 10 µl) was then added to the fourth aliquot of blood.

Portions of each blood sample (3 µl) were diluted with distilled water (3 ml) and their absorption spectra from 290 - 610 nm were measured using a Pye Unicam SP1700 spectrophotometer.

5.2.F) Liberation of the bound carbonyls in blood

1) The use of As_2O_3

The method described by Egyud et al.[149] was slightly modified for the present study. Acrolein and crotonaldehyde were added to blood at concentrations ranging from 100 µg/ml to 10 µg/ml. [$^2H_6$]-acetone (1 µg/ml) was used as the internal standard. As_2O_3 (0.2 g) was added to the blood sample (5 ml) which was then mixed and incubated at 37°C for 30 minutes. The blood was then centrifuged at 1400 g for 10 minutes. An aliquot of plasma (2 ml) was removed and added to a tube containing trichloroacetic acid (10 %w/v, 2 ml). The contents of the tube were mixed on a vortex mixer and then centrifuged at 600 g for 10 minutes. A portion of the supernatant (1 ml) was removed and sealed in a 6-ml hypo-vial for gas chromatographic headspace analysis using SIR to monitor the molecular ions of acrolein, crotonaldehyde and [$^2H_6$]-acetone. The GC-MS conditions used were as described in section 5.2.D.

2) Deproteination with perchloric acid (PCA)

Method 1: Immediately after the addition of acrolein (100 µl) to blood (10 ml) containing [$^2H_6$]-acetone (1 µl/ml, 100 µl), an aliquot of blood (5 ml) was centrifuged at 1400 g for 10 minutes. The plasma (1 ml) was then mixed immediately with PCA (0.6 N, 4 ml), and centrifuged at 1400 g for 10 minutes. The supernatant (1 ml) was pipetted into a 6-ml hypo-vial for headspace analysis. The conditions used were as described previously.

Method 2: As in Method 1 except that PCA was added directly to the whole blood sample.

The time between addition of acrolein and the precipitation of protein by PCA was kept as short as possible to minimise any possibility of metabolism[150] and time-dependent binding[133]. Unlike acetaldehyde, acrolein is known to polymerize under acidic or basic conditions. Therefore PCA might not be suitable for this application. To determine whether this was an important factor, an experiment was carried out in which an aqueous acrolein solution (50 µg/ml) containing [$^2H_6$]-acetone (10 µg/ml) was diluted with either 4 parts of distilled water or 4 parts of perchloric acid (0.6 N). The diluted solution (1 ml) was then pipetted into a 6-ml hypo-vial for headspace analysis. Headspace samples (100 µl) were removed at certain time intervals and the peak areas of acrolein and [$^2H_6$]-acetone were measured.

5.3) Results and Discussion

Polar liquid phases such as Carbowax 20M or Carbowax 400 were found to be suitable for the separation of lower aliphatic carbonyl compounds. Although Carbowax 400 gave slightly better separation, the high column bleed from the liquid phase precluded its use in GC-MS. The retention indices of some lower aliphatic carbonyl compounds on Carbowax 20M are shown in Table 5.1.

Analysis of the headspace from aqueous standards equilibrated for various lengths of time has indicated that

Table 5.1 Retention indices of lower aliphatic carbonyl compounds on 3 % Carbowax 20M at 60°C.

Name	Retention index
Formaldehyde	650
Acetaldehyde	694
Propionaldehyde	776
Acetone	807
Acrolein	829
Crotonaldehyde	1064

equilibrium was accomplished in less than 20 minutes. Repeated sampling from the same hypo-vial was possible provided that sufficient time was allowed for the system to re-equilibrate. The maximum number of samples which could be taken from each vial depended on the sample size and the headspace volume in the hypo-vial. While butyl rubber septa were used to seal the headspace samples of acetaldehyde, acetone and propionaldehyde, teflon-lined butyl septa were used for the analysis of acrolein and crotonaldehyde samples to avoid the absorption of samples by the septa. The absorption of various organic vapours by rubber septa during the equilibration process has been noticed by some workers as a principal source of error in gas chromatographic headspace analysis[151 - 153]. In an analysis of an aqueous standard solution containing acrolein at 10 μg/ml, a three-fold increase in the chromatographic peak height was observed when teflon-lined septum was used instead of butyl rubber septum.

When 100 μl of headspace was injected onto the column, the quantitation limit of this method using a flame ionisation detector was in the region of 5 μg/ml for carbonyls in aqueous solution. The detection limit could be increased by either injecting a larger headspace sample onto the column or by raising the equilibration temperature. The relationship between the equilibration temperature and the vapour pressure (measured by the peak height) of propionaldehyde and acetone is shown in Figure 5.1. The results showed that there was, more or less, a linear increase in the peak height (ie. the vapour

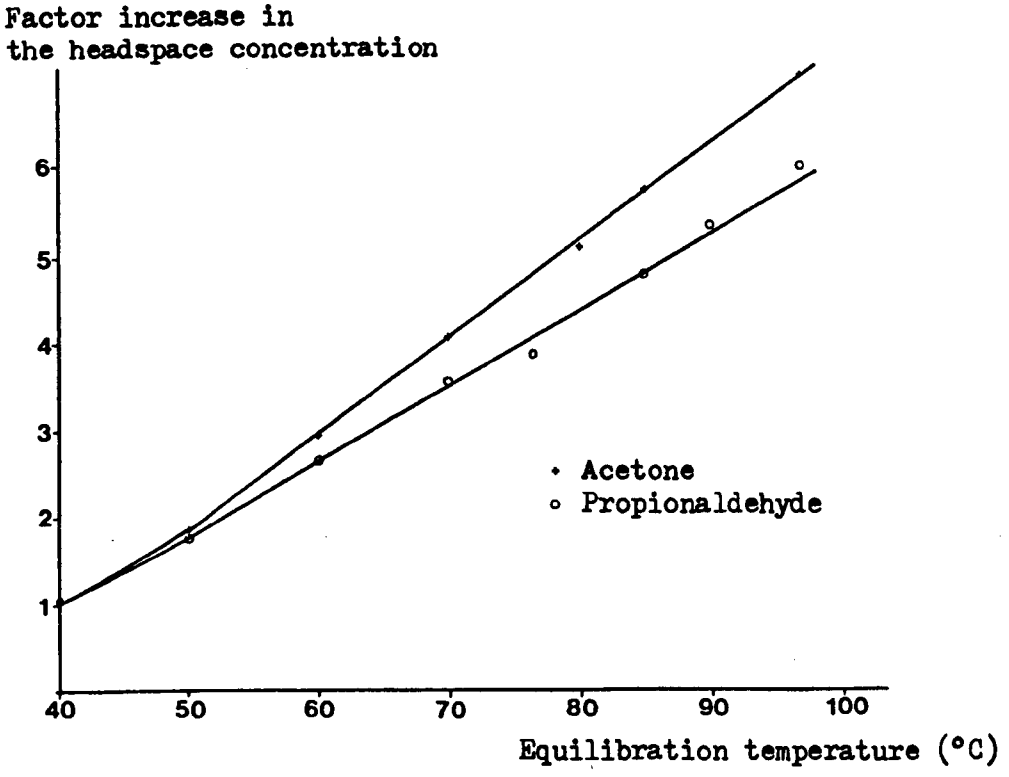


Figure 5.1 The effect of equilibration temperature on the partition of acetone and propionaldehyde in the headspace.

pressure) as the equilibration temperature increased. The expression for the temperature-vapour pressure relation known as the Clausius-Clapeyron equation is

$$d (\ln P) / d T = \Delta Hvap / RT^2 \qquad \text{Equation 5.7}$$

where P is the vapour pressure, T is the absolute temperature, $\Delta Hvap$ is the change of heat of vaporisation and R is Boltzmann's gas constant. The integrated form, with the assumption of a constant value of $\Delta Hvap$ over the temperature range considered, written in terms of logarithms to the base 10, is

$$\log P = -\Delta Hvap/(2.3RT) + \text{constant} \qquad \text{Equation 5.8}$$

The preceding derivation indicates that a plot of log P versus 1/T should give a straight line with the slope equal to $-(\Delta Hvap/2.3R)$. However, in practice, the curve shows deviations from linearity (Figure 5.2) because of several reasons: the build up in the pressure in the closed system as the equilibration temperature increases; non-ideal nature of the gas; $\Delta Hvap$ is not a constant.

The use of a mass spectrometer as the detector resulted in higher sensitivity and specificity compared to the flame ionisation detector. At 80°C, the quantitation limit for the lower aliphatic carbonyls was in the region of 0.01 - 0.1 µg/ml in water. Typical ion chromatograms and calibration curves for these carbonyl compounds are shown in Figures 5.3 and 5.4. At a concentration of 1 µg/ml, the coefficients of variation for ten successive analyses of acetone and propionaldehyde were 1.7 % and 4.3 % respectively.

The mass spectra of lower aliphatic carbonyl compounds

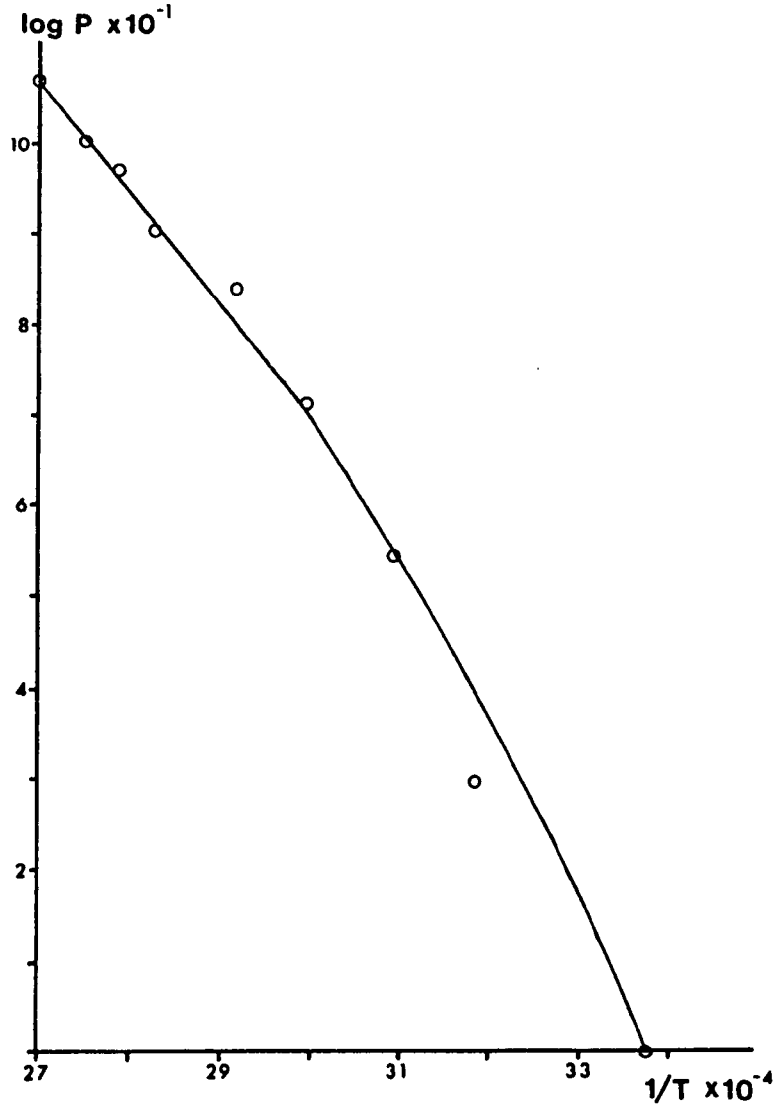


Figure 5.2 Relationship between log P and 1/T for the partition of propionaldehyde in the headspace. Note that P was measured from the chromatographic peak area, hence P was only proportional, but not equal to the vapour pressure of propionaldehyde (see Equation 5.2).

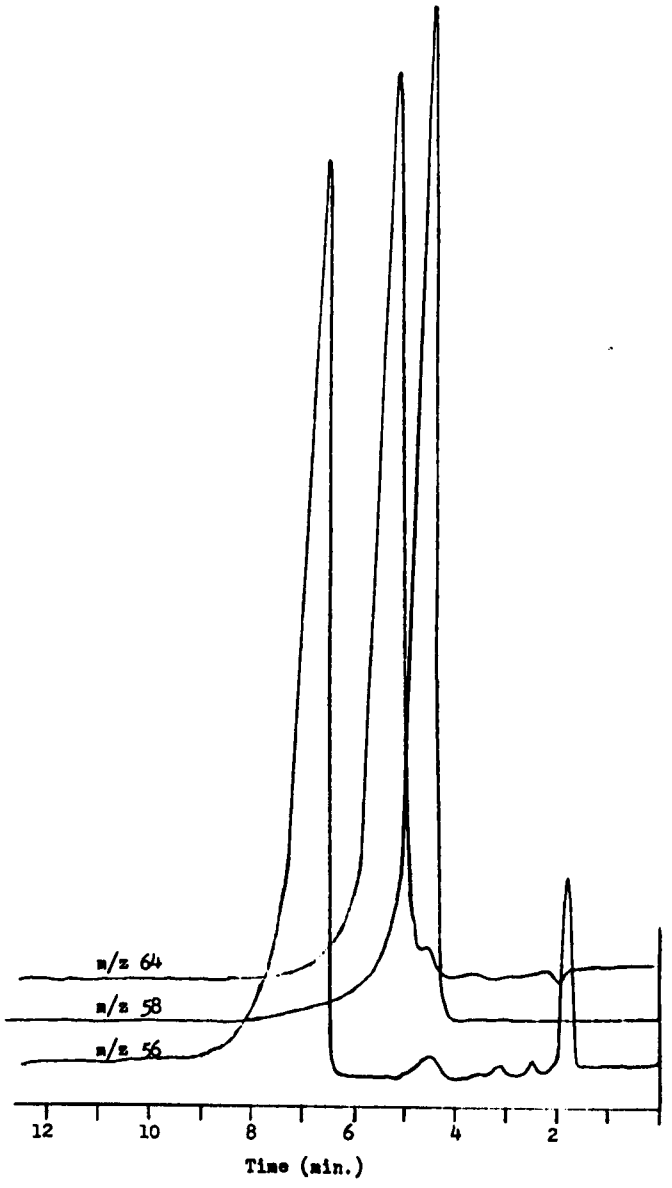


Figure 5.3 Typical ion chromatogram of $[^2H_6]$-acetone (m/z 64), propionaldehyde (m/z 58) and acrolein (m/z 56) separated on a 3.5 m x 4 mm i.d. column packed with 10% Carbowax 20M on 60 - 80 mesh Gas Chrom. Q at 80°C. Helium flow rate was 40 ml/min.

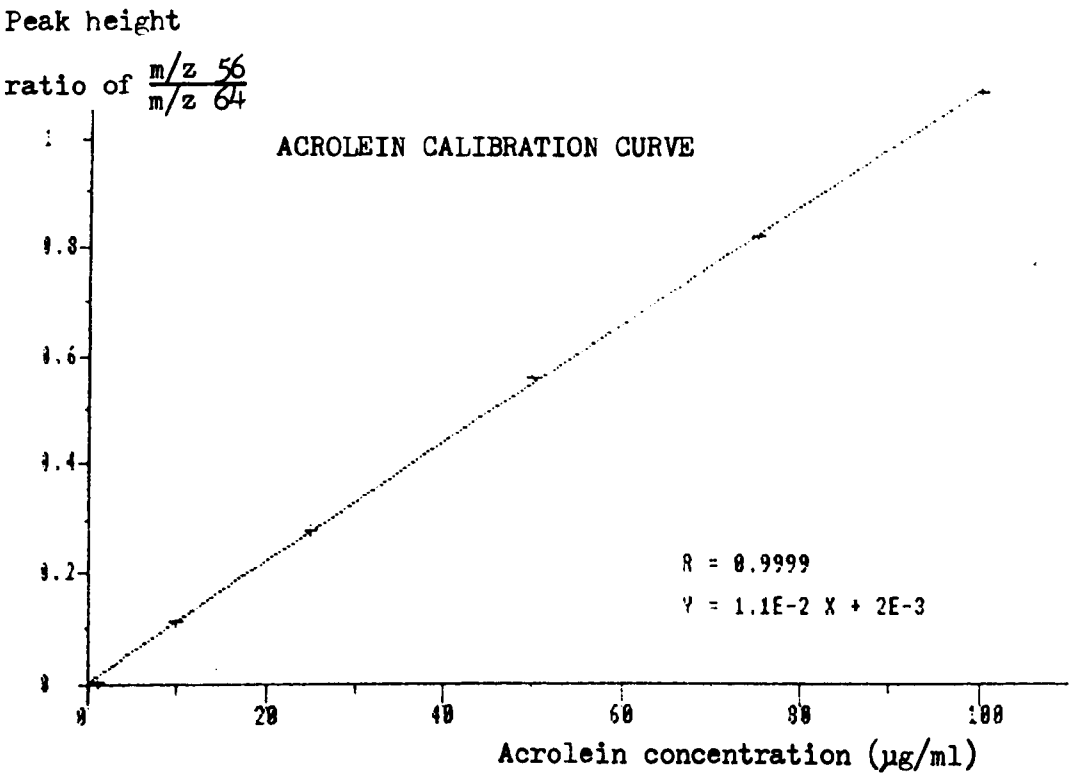


Figure 5.4 A typical calibration curve for the headspace analysis of acrolein in water using [$^2H_6$]-acetone as the internal standard.

frequently show prominent molecular ions and it was therefore convenient to monitor these ions in selected ion recording. The effect of varying the electron impact energy on the absolute molecular ion intensity of several carbonyl compounds including acetaldehyde, propionaldehyde, acetone, acrolein and crotonaldehyde was studied. For each of these compounds, the intensity of the molecular ion maximised at two electron energies, one in the region of 20 eV and the other at about 40 eV (Figure 5.5). Both of these were approximately twice the absolute molecular ion intensity at 70 eV.

In contrast to the derivatisation technique which involved complicated sample preparation procedures, the headspace technique was simple and fast. The reproducibilities of both techniques were similar but the sensitivity of the headspace technique was slightly better, both being in the parts per million region. It was important to control the equilibration conditions as precisely as possible, particularly the temperature, to obtain good reproducibility from the headspace technique. Another potentially major source of error in headspace analysis was derived from differences in composition between the calibration standard and the sample. For example, analysis of volatiles in blood using aqueous calibration standards would give rise to some degree of inaccuracy since the constituents in blood would affect the activity coefficient of the volatile component being studied and hence affect its vapour pressure in the headspace.

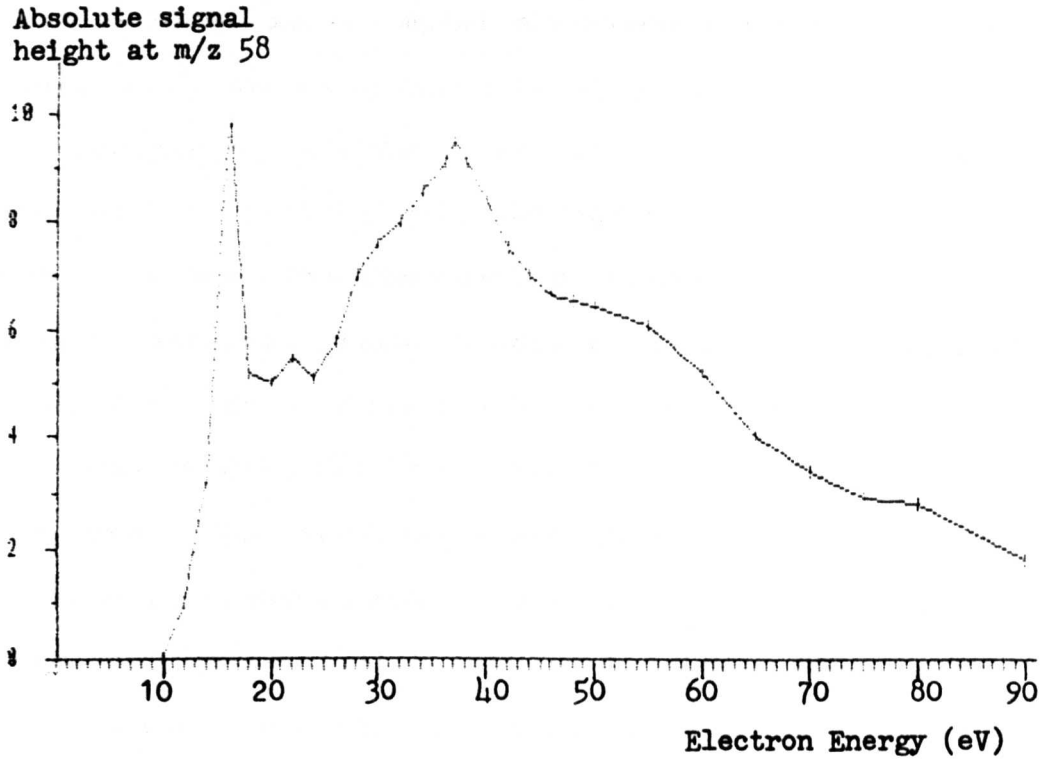


Figure 5.5 The effect of electron energy on the absolute molecular ion intensity of acetone. The source temperature and pressure were 220°C and 1 x 10^{-6}torr.

Blood samples containing added acrolein at concentrations ranging from 1 - 100 µg/ml were prepared and analysed for the purpose of calibration. However, acrolein was not detected in the headspace of any of these samples. Analysis of the headspace from a blood sample containing acrolein as high as 1 mg/ml also gave a negative result. The addition of ammonium sulphate (0.5 g/ml) to the blood sample containing acrolein at 100 µg/ml was tried, a process known as salting out, but again no improvement was obtained. Since the time-expired blood obtained from the blood bank contained lithium heparin as an anti-coagulant, the possibility of a reaction between acrolein and lithium heparin was studied but the results showed no reaction between them. The exact cause for this rapid disappearance of acrolein in blood is not known. It is believed that its disappearance is associated with protein binding.

It has been reported by Egyud et al. that the detection of ketone aldehydes in tissues is masked by the sulphydryl group of an unidentified substance which they called the "carrier" and that the carbonyls can be unmasked with As_2O_3 which interacts with the sulphydryl group of the carrier[149]. Quash and Maharaj also reported the use of As_2O_3 for the liberation of bound aldehydes in serum for quantitative analysis[121]. The method described by Egyud et al.[149] was used in the present study in an attempt to liberate bound acrolein and crotonaldehyde in blood. Analysis of the headspace from samples containing added acrolein and crotonaldehyde at concentrations ranging from 10 - 100 µg/ml showed an absence of these two

compounds in the headspace.

The addition of perchloric acid for deproteination of plasma and whole blood proteins has been used in the headspace analysis of blood acetaldehyde to increase its recovery[132, 133, 150]. An experiment was carried out in which the blood samples containing added acrolein and [$^2H_4$]-acetone were analysed immediately after the addition of perchloric acid to the plasma or whole blood samples (see section 5.2.F). Again, only [$^2H_4$]-acetone but not acrolein was detected in the headspace of these two samples.

Wartburg et al. reported that apparently there is a fast metabolism of acetaldehyde to acetate by aldehyde dehydrogenase in human erythrocytes[150]. They reported a 30 % loss after 90 s and a 50 % loss in just 5 min with an initial acetaldehyde concentration of 100 µM. The disappearance of acetaldehyde could be prevented by chloral hydrate. Eriksson et al. reported that there occurred a time dependent binding of acetaldehyde in the blood prior to PCA precipitation, so that bound acetaldehyde was centrifuged away during the PCA precipitation procedure[133]. They also reported that 10 % of acetaldehyde was lost in blood within 15 seconds at 0°C and therefore a rapid thermolabile binding of acetaldehyde in blood was a more probable explanation than its enzymic oxidation. The binding of acetaldehyde to blood proteins and possibly also its metabolism create many problems which may explain why measurements of whole blood acetaldehyde have been so difficult[154]. Some or all these

observations may also be true for acrolein. Besides, acrolein is known to polymerise under acidic or basic conditions and therefore PCA might not be suitable for this application.

To find out whether this was an important factor, an experiment was carried out in which aqueous acrolein solution (50 μg/ml) containing [$^2H_6$]-acetone (10 μg/ml) was diluted with either 4 parts of distilled water or 4 parts of perchloric acid (0.6 N). Headspace from the diluted samples was then analysed by selected ion recording at successive equilibration time intervals, monitoring at m/z 46 for [$^2H_6$]-acetone and m/z 56 for acrolein. The results are shown in Figure 5.6. They clearly indicated that in the absence of PCA, the level of acrolein and [$^2H_6$]-acetone remained constant with time, whereas in the presence of PCA, the level of these two compounds were found to decrease rapidly. The level of acrolein in the headspace decreased to less than 50 % of the initial concentration in 30 min compared to the sample without PCA. Analysis of the ethyl acetate extract of this sample after equilibration did in fact show the presence of 2-formyl-3,4-dihydropyran, a dimer of acrolein. In the presence of PCA, [$^2H_6$]-acetone also showed a steady decline in the headspace concentration. This was probably due to the increased rate of deuterium and protium exchange through enolisation under acidic condition.

Another problem which may also exist in the headspace analysis of acrolein in blood is that acrolein, having

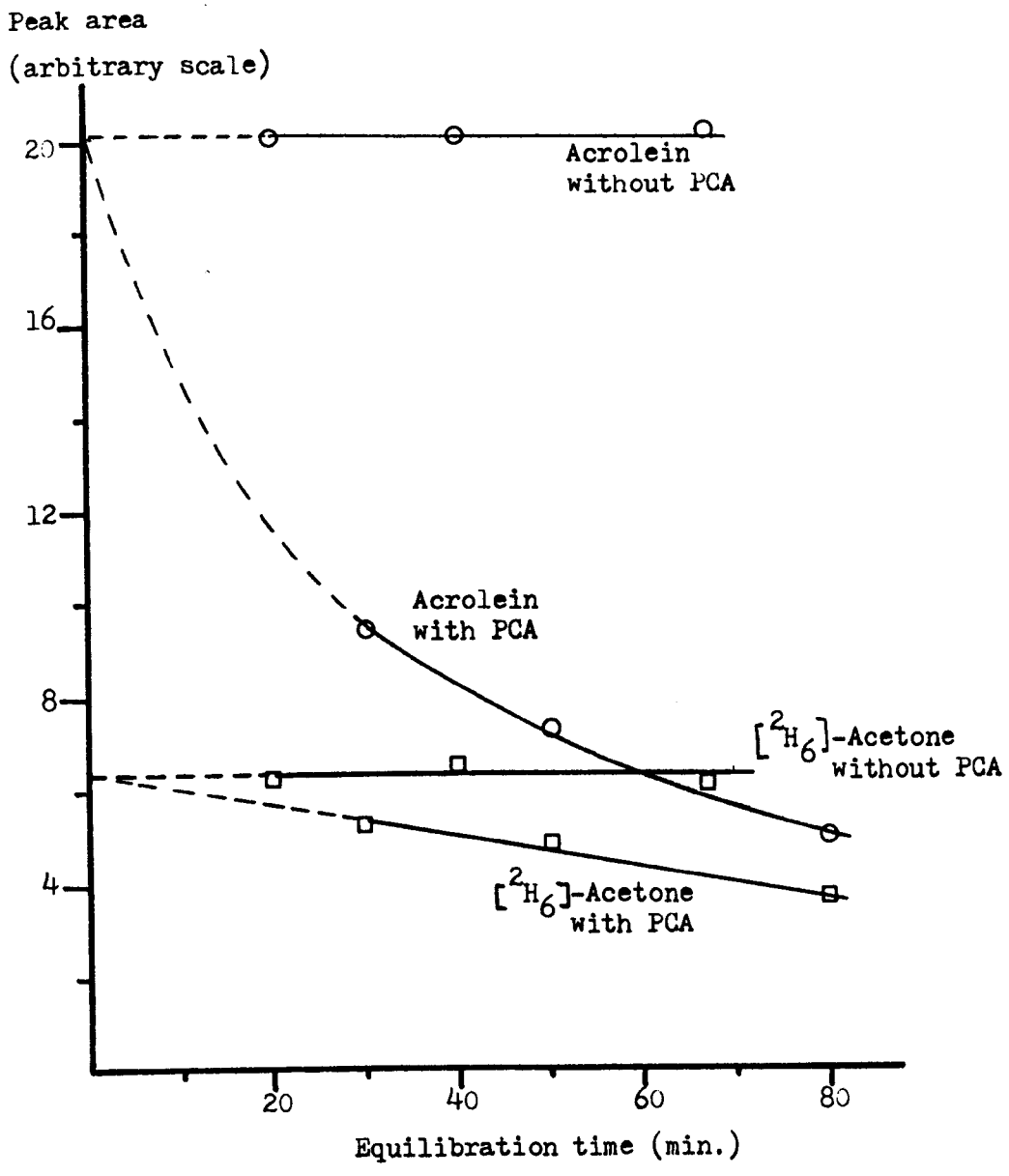


Figure 5.6 The effect of PCA on the headspace concentrations of acrolein and [$^2H_6$]-acetone.

conjugated double bonds, may act as a ligand to haemoglobin. The possibility of ligand binding was studied by comparing the absorption spectrum of haemoglobin before and after the addition of acrolein (Figure 5.7). The spectrum showed a significant "distortion" of the soret band at around 428 nm, indicating an interaction between acrolein and the metallo-porphyrin of haemoglobin. There was also a small increase in the absorbance at 590 nm, similiar to that resulting from the binding of oxygen to haemoglobin. The change in the absorbance at 426 nm with time after the addition of acrolein (50 μg) to blood (1 ml) is shown in Figure 5.8. Initially, there was a decrease in the absorbance but after about 6 minutes, the absorbance increased and then leveled off after about 10 minutes. The cause of these changes is unknown. Presumably there occurred two or more equilibria with different rate kinetics.

In a recent paper, Steven et al. reported the formation of acetaldehyde adducts with haemoglobin[155]. The adducts involve at least three different amino acid residues valine, lysine and tyrosine, in addition to two modified residues glucosyl-valine and glucosyl-lysine. Unlike acrolein, acetaldehyde has no conjugated double bonds, hence cannot act as a ligand to metallo-porphyrin. On the other hand, acrolein may behave similarly to acetaldehyde in which it forms adducts with certain amino acid residues of the haemoglobin molecule.

The binding of acrolein to metallo-porphyrin was probably not the prime cause which procluded its detection

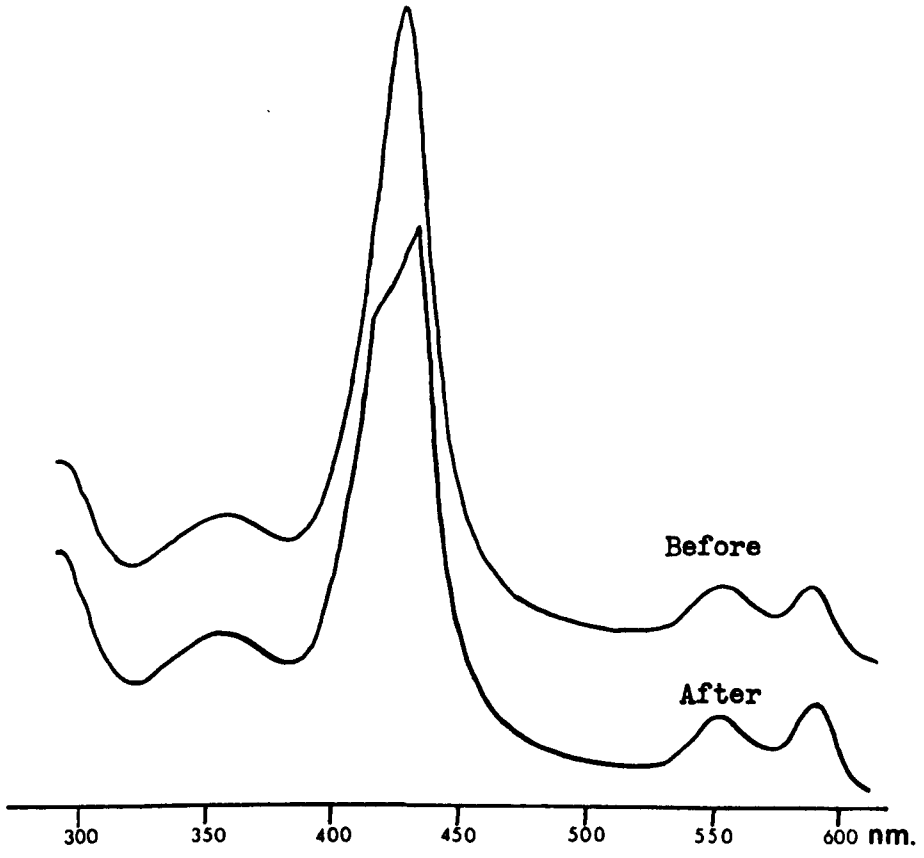


Figure 5.7 The change in the absorption spectrum of haemoglobin after the addition of acrolein (50 µg) to blood (1 ml). The spectrum before the addition of acrolein has been offset for clarity.

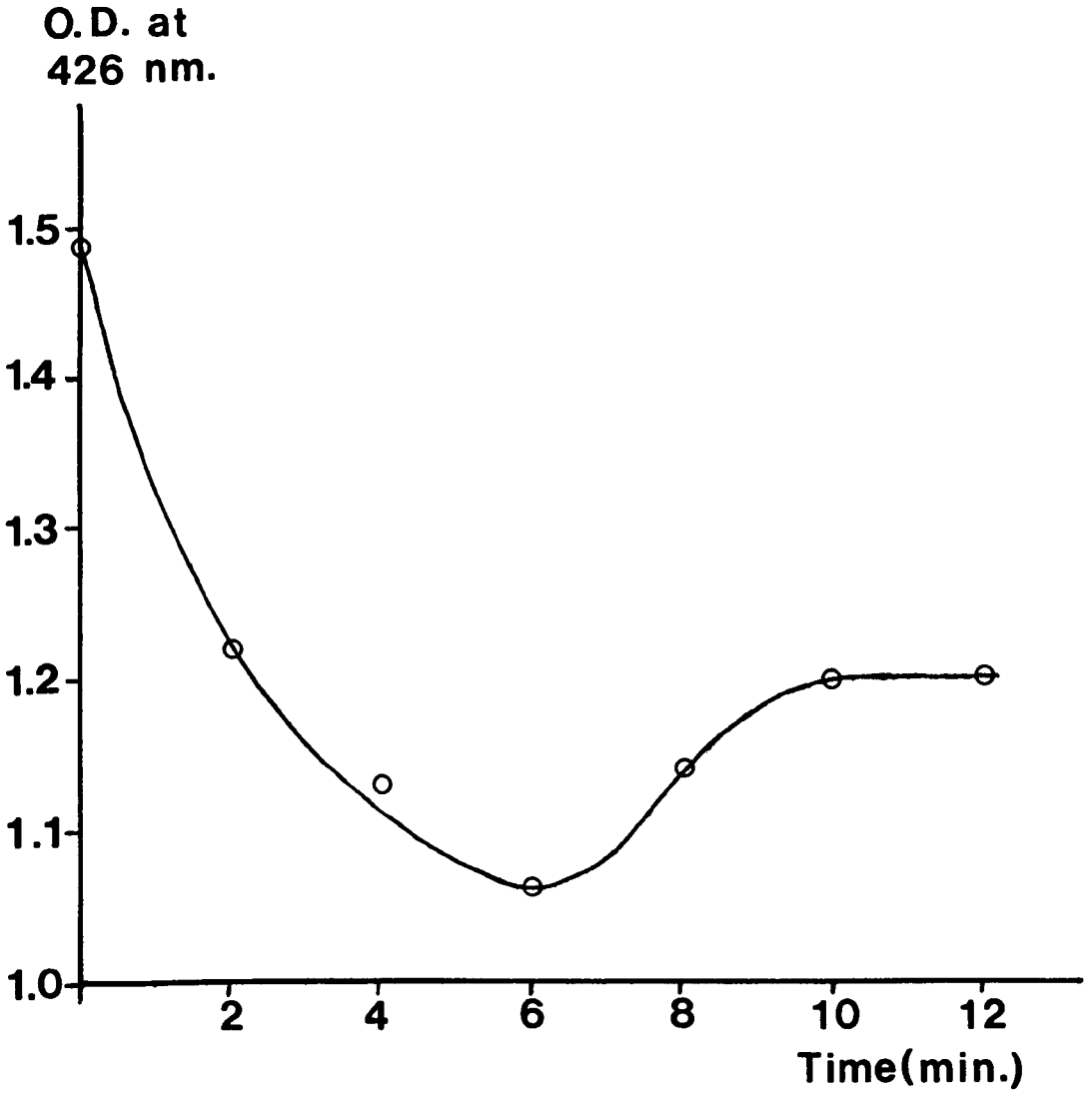


Figure 5.8 The change in the optical density of haemoglobin at 426 nm after the addition of acrolein (50 µg) to blood (1 ml).

in headspace analysis, otherwise, it would be possible to displace it with a stronger ligand such as cyanide. However, addition of alkaline potassium ferricyanide, which converted the haemoglobin to cyanomethaemoglobin[156] still did not make the assay possible.

So far, attention has been focussed on the analysis of acrolein in blood. However, the headspace analysis of other lower aliphatic carbonyl compounds also resulted in the same degree of difficulty. Table 5.2 shows the comparision of the results from headspace analysis of some lower aliphatic carbonyls after the same amount of each compound (50 µg/ml) were added to blood and to water. The equilibration times for each of the samples were kept identical to minimise changes in the headspace concentration due to any time-dependent binding or metabolism. [$^{2}H_{6}$]-acetone was used in this case to represent [$^{1}H_{6}$]-acetone rather than as an internal standard to avoid confusion between acetone and propionaldehyde since both compounds had the same molecular ion (m/z 58) in the mass spectrum and similar retention times in the chromatogram. The headspace concentration of each of the carbonyl compounds was found to be lower in the blood sample than in the aqueous sample suggesting that the binding of aldehydes, possibly also including their metabolism in blood, occurred not only to acetaldehyde and acrolein but also to other lower aliphatic carbonyl compounds. While acrolein was not detected in the headspace of the blood sample, crotonaldehyde was only barely detectable even with a very large sample size of

Table 5.2 Comparision of the vapour pressure of carbonyl compounds (measured by their peak areas) in blood and water.

Name	Peak area from aqueous sample (A)	Peak area from blood sample (B)	Ratio of A/B
Acetaldehyde	20900	2540	0.122
Propionaldehyde	36200	4390	0.121
[$^2H_4$]-Acetone	22400	3250	0.145
Acrolein	16500	N.D.	-
Crotonaldehyde	27800	542	0.019

N.D. = not detected

headspace (2.5 ml) indicating the significance of conjugated double bonds in these two compounds.

The binding of carbonyl compounds in blood and the relatively high detection limits remained the two major problems in the static headspace technique. However, if the binding was a reversible process, both of these problems might simply be solved by the use of dynamic headspace technique in which the headspace is continuously eluted with an inert carrier gas and the volatiles collected by a "trap" such as a Tenax-GC adsorption column. The feasibility of such a technique will be discussed in Chapter 6.

5.4) CONCLUSIONS

Static headspace analysed by gas chromatography is a very simple and moderately sensitive technique for volatiles such as lower aliphatic carbonyl compounds in aqueous solutons. When used together with selected ion recording GC-MS, the quantitation limit of the technique can be lowered to approximately 0.1 µg/ml. However, the use of this technique for the analysis of carbonyl compounds in blood has resulted in some difficulties including the interaction of these compounds with blood proteins and certain amino acids, possibly including their metabolism in blood. Aldehydes that contained conjugated double bonds also appeared to bind to the metallo-prophyrin of haemoglobin. The existence of all these interactions has rendered this analytical technique unsuitable for use in this study.

CHAPTER 6: ANALYSIS OF CARBONYLS IN BLOOD BY DYNAMIC HEADSPACE GAS CHROMATOGRAPHY

6.1) Introduction

In dynamic headspace analysis, the headspace is continuously purged with inert carrier gas followed by collection of the vapour by condensation or chromatographic sorption. It is an enrichment process used in cases when better sensitivity is required than can be achieved by the static method. In recent years, various designs of enrichment techniques have been used for the study of volatile metabolites in urine[157,158], volatile constituents in body fluids[159,160], and volatile environmental pollutants in biological matrices[161]. In general, an adsorbent such as Tenax-GC or activated charcoal is used to "trap" the volatiles eluted from the headspace after which the volatiles are released by thermal desorption onto the GC column for analysis or by solvent extraction (charcoal).

This chapter describes the application of dynamic headspace gas chromatography using Tenax-GC as the trapping material to the analysis of carbonyls in blood. Samples from 26 fire fatalities and 10 control subjects were analysed and the results were compared. Since the enrichment technique used was essentially a non-specific volatiles extraction process, it could therefore be used to measure volatiles other than the carbonyls in a single analysis. Such an analysis, often known as the volatiles profile analysis, has received an increasing amount of

interest in recent years. This includes the study of volatile metabolites in blood, urine and serum[157-160]. However, most of these studies were only concerned with profile comparision or profile recognition. Qualitative identifications of the components in these profiles are few in number, and quantitative data are even rarer.

In this study, profiles of volatile constituents in blood samples from fire fatalities were compared with normal control groups. Most of these volatile components were identified by capillary gas chromatography-mass spectrometry. Certain carbonyls and nitriles were also quantified. Only the results on carbonyl measurements are discussed in this chapter. The results on nitriles and profiles comparision will be discussed later in the next two chapters.

6.1.A) Tenax-GC

Tenax-GC (sometimes simply called Tenax) is a porous polymer column packing material that is based on 2,6-diphenyl-p-phenylene oxide. It strongly adsorbs volatile organic compounds on its surface and is currently used extensively as a sorbent material for volatiles analysis. The volatile compounds can even be stored on Tenax-GC for several days before being thermally desorbed for analysis. Particles of diameter 35-60 mesh are most popular for trapping devices because of the lower back pressure resulting from coarser materials. Other special features of Tenax-GC include excellent thermal stability, extremely low column bleed and low water retention.

6.2) Experimental

6.2.A) Materials and apparatus

Tenax-GC (35 - 60 mesh) was purchased from Alltech (Lancashire, U.K.). The apparatus used for headspace elution is illustrated in Figure 6.1. It consisted of a purge gas supply, a perfusion flask, a constant temperature water bath, a water-cooled condenser, a Tenax trap and a gas burette. The desorption port consisted of a heating jacket, a sample loop and a 6-port valve as shown in Figure 6.2. All the glassware used was cleaned with chromic acid followed by detergent and distilled water and stored in an oven at 97°C until used.

6.2.B) Preparation of Tenax-GC traps

Straight stainless steel columns (23 cm x 4 mm i.d.) were soaked in detergent for two hours. The columns were then rinsed with water followed by acetone and dried in an oven at 100°C for one hour. Each column was then packed with Tenax-GC (500 mg) and conditioned at 300°C for two hours in a flow of nitrogen or helium (20 ml/min).

6.2.C) Volatiles extraction and thermal desorption

The sampling procedure used was similar to that described by Anderson et al.[162]. Helium was used as the purge gas because it was found that it contained a lower level of impurities than nitrogen (BOC oxygen-free nitrogen). The purge gas was purified with a charcoal

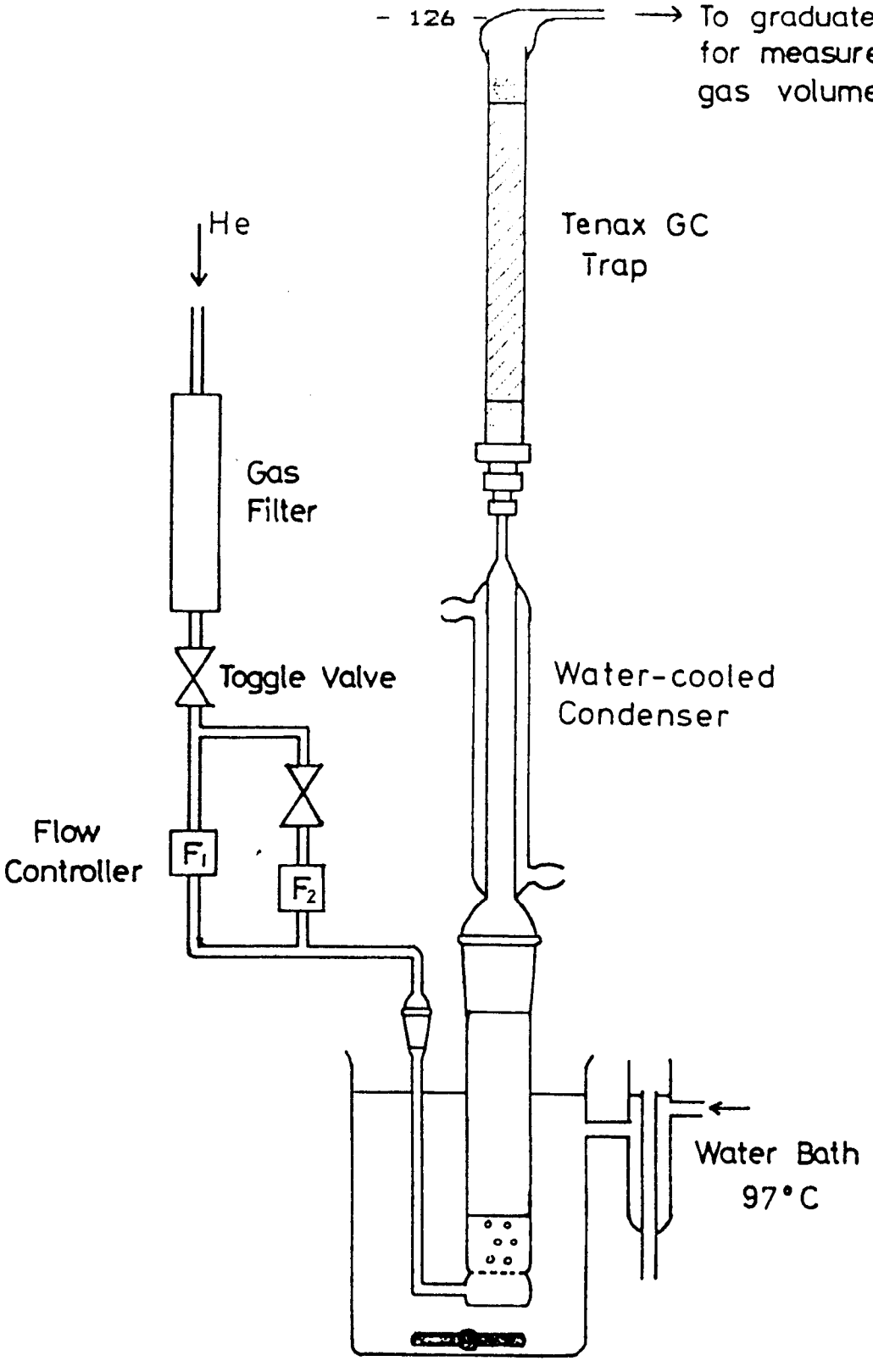


Figure 6.1 Apparatus for the gas phase extraction of volatiles from blood.

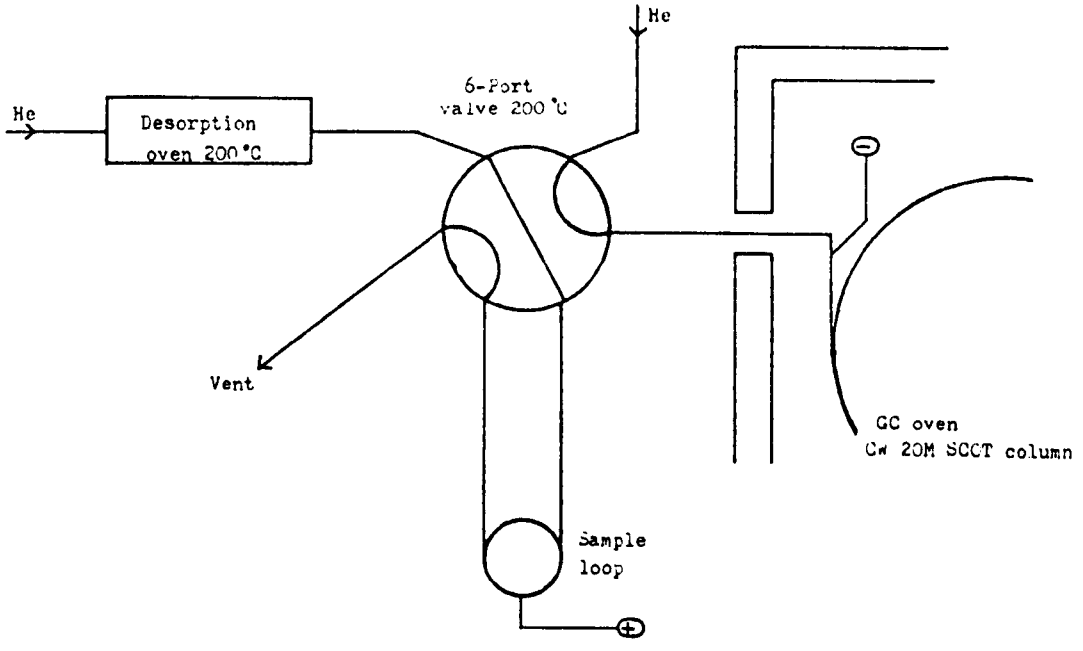


Figure 6.2 Inlet system for introducing volatiles to the gas chromatograph.

filter (Gas-clean filter, Chrompack London) and the gas stream split between two flow controllers F1 and F2 (see Figure 6.1). F2 was set to give a flow rate of approximately 100 ml/min and was used to purge the perfusion flask and the condenser for 30 minutes at 97°C immediately before headspace sampling. A Tenax-GC trap which had been conditioned at 250°C in a stream of helium for 30 minutes and cooled to room temperature was then connected to the condenser with the use of a Swagelok coupling. The elution of headspace was controlled by F1 at a rate of 35 ml/min to the perfusion flask containing the blood sample (1 ml) at 97°C. The function of the water-cooled condenser (10 - 12°C) was to reduce the water uptake by the Tenax-GC trap. Volatiles eluted from the perfusion flask were collected on a Tenax-GC trap and the elution volume (0.6 litre) was measured with a gas burette.

After the elution process had been completed, the trap was transferred to the inlet system of the gas chromatograph. Volatiles were desorbed at 200°C for 10 minutes with a helium flow rate of 20 ml/min and collected in the stainless steel sample loop (25 cm x 0.75 mm i.d.) which was cooled in liquid nitrogen. The sample was introduced on to the GC column by ohmic heating (70 volts A.C.) of the sample loop and transfer line for 15 seconds.

6.2.D) Gas chromatography-mass spectrometry

GC-MS analysis of volatiles was performed on a VG MM16F mass spectrometer interfaced to a Perkin-Elmer Sigma

3B gas chromatograph equipped with a 100 m x 0.5 mm i.d. glass SCOT column coated with Carbowax 20M. The column temperature was programmed at 4°C/min from 60°C to 170°C with initial and final isothermal periods of 8 and 10 min respectively. The linear helium flow rate was 20 cm/sec and the interface temperature was 200°C.

EI spectra were recorded repetitively during the entire chromatographic run using a VG 2035 data system and the data was stored on magnetic disc for post-run processing. The mass spectrometric conditions used were: electron energy, 70 eV; source temperature, 200 - 220°C; source pressure 2 x 10^{-6} torr; mass range, 350 - 20 amu; scan rate, 1 sec/dec (exponential down scan) with 1 sec interscan delay.

6.2.E) Quantitation of carbonyl compounds

An aqueous solution containing [$^2H_6$]-acetone (1 µl/dl, 10 µl) was added as the internal standard to the blood sample (1 ml) immediately before headspace sampling. Selected ions were chosen on the basis that each ion was one of the major ions in the mass spectra of the carbonyls and that it had minimum interference from the neighbouring chromatographic components (Table 6.1). The chromatographic peak areas of software-reconstructed selected ion chromatograms were integrated and quantitative measurements were made from the response ratios determined using an external standard solution under identical conditions. The external standard solution was freshly prepared by dissolving 10 µl of each carbonyl compound (Table 6.1) in

Table 6.1 The external standard solution used for the quantitation of carbonyls in blood and the selected ions chosen for the measurement of chromatographic peak areas.

Name	Concentration (ng/ml)	Selected ion (m/z)
Acetaldehyde	77.6	44
Propionaldehyde	77.4	58
Isobutyraldehyde	77.4	72
Acetone	78.6	58
Acrolein	83.3	56
n-Butyraldehyde	78.8	72
2-Butanone	80.2	72
Butandione	93.5	86
2-Pentanone	79.0	71
4-Methyl-2-pentanone	79.2	58
Hexanal	80.0	56
Cyclopentanone	94.0	55
Cyclohexanone	93.7	98
Benzaldehyde	104.0	105
Acetophenone	101.6	105

Table 6.2 Distribution of acrolein in blood.

	Aqueous control	Erythrocytes	Plasma
Area ratio of (m/z56)/(m/z64)	2.77	1.08	1.57
Percentage distribution	-	39	56
Total recovery of acrolein in both fractions	= (39 + 56) % = 95 %		

reboiled distilled water (500 ml) which was then further diluted 1 in 200 to give the required concentrations (approximately 100 ng/ml). The equation used for the calculation of carbonyl concentrations in blood was as follows:

Equation 6.

$$\text{Conc. of carbonyl in blood} = \frac{\frac{\text{Peak area of sample}}{\text{,, ,, ,, I.S.}} - \frac{\text{Peak area of blank}}{\text{,, ,, ,, I.S.}}}{\frac{\text{Peak area of ext. std.}}{\text{,, ,, ,, I.S.}} - \frac{\text{Peak area of blank}}{\text{,, ,, ,, I.S.}}} \times \text{Conc. in ext. std}$$

where I.S. stands for internal standard. The peak areas in the blank were measured from the analysis of reboiled distilled water (1 ml) under the same conditions.

6.2.F) Subject groups and blood samples

The analyses were carried out on blood samples obtained from four subject groups:

(i) 21 fire fatalities who died as a result of dwelling house, industrial or caravan fires,

(ii) 6 post-mortem controls who died from natural causes or as a result of road accidents;

(iii) 2 smoker controls;

(iv) 2 non-smoker controls.

In (iii) and (iv), venous blood samples were collected from healthy volunteers who had not consumed alcohol in the 24 hours preceeding sample collection. Blood samples were collected and stored at 4°C until analysed. If analysis was delayed by more than 24 hours, the samples

were frozen at -28°C until required.

6.3) Results and Discussion

When Tenax-GC was used as the trapping material in dynamic headspace analysis, the optimum volume of purge gas to be used was determined by the stripping efficiency of the perfusion flask and the break-through volumes of volatile compounds being studied. The break-through volume of a compound is the elution volume at which the compound begins to escape from the Tenax trap. An experiment was carried out in which different volumes of purge gas (0.1 - 1.7 litre) were used to elute the headspace of an aqueous solution (1 ml) containing acrolein, crotonaldehyde, acetonitrile, propionitrile and acrylonitrile, each at 0.5 µl/l. The peak area measurements obtained were compared with those from an analysis in which the same quantity of each compound was injected directly onto the GC column (Figure 6.3). The results obtained provide information on the stripping efficiency, recovery and break-through volume of these compounds using this headspace enrichment technique. The stripping efficiency of the perfusion flask used in this study was found to be better than the Bellar and Lichtenberg stripping apparatus which was considered to provide a very high stripping efficiency[163]. Recoveries of these compounds reached a maximum after an elution volume of between 0.3 and 0.5 litre with values ranging from 65 % to 97 %. The recovery of acetonitrile dropped after an elution volume of just 0.3 litre indicating its

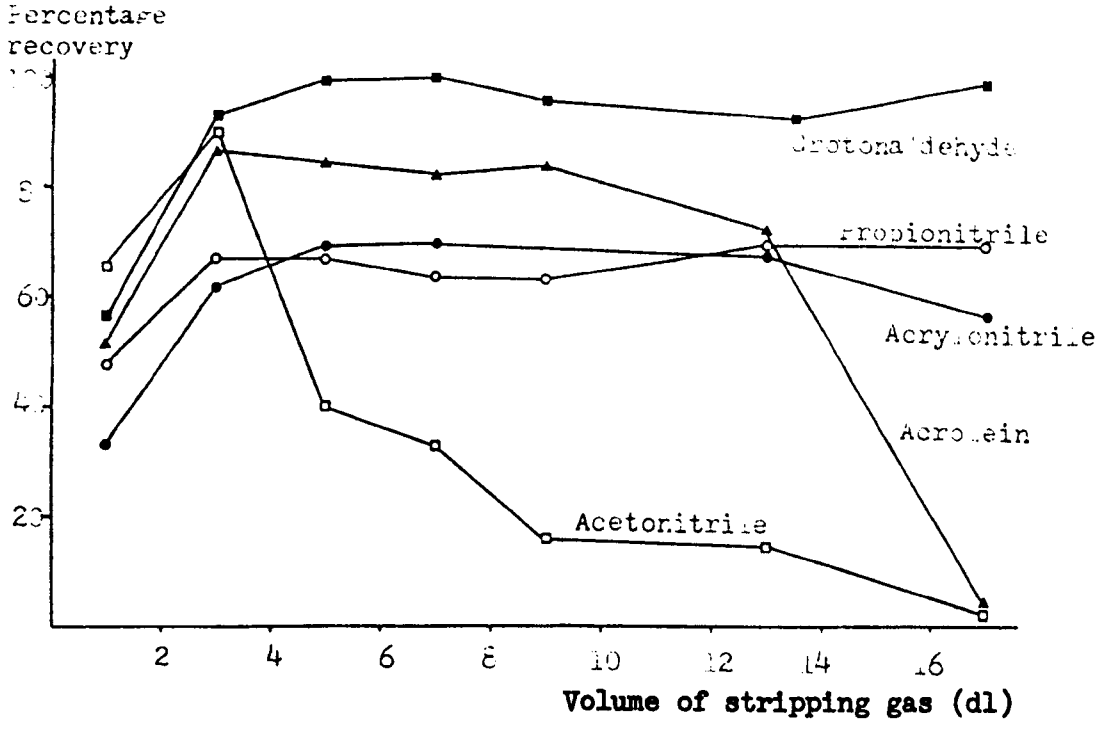


Figure 6.3 Recoveries of volatiles from water using different volumes of stripping gas.

low break-through volume on Tenax-GC. The optimum stripping volume for these compounds was found to be between 0.5 and 0.9 litre.

The experiment on the recovery of volatiles versus the purge gas volume was repeated only this time acetone, acrolein and crotonaldehyde were added to blood instead of water to a concentration of 5 µg/ml. The result of the analysis is shown in Figure 6.4. The patterns of the curves were very similar to those obtained from the aqueous samples except that acrolein was eluted from the blood at a slower rate. Considering the stripping efficiency of the apparatus used, recovery of volatiles on the Tenax-GC trap, and the break-through volumes of volatiles both obtained in this study and from published data [164], a suitable compromise between these factors was considered deemed to be 0.6 litre of purge gas at a flow rate of 35 ml/min and this was used in the study.

Acrolein was detected with the use of this headspace elution technique but not with the static headspace process (Chapter 5) strongly suggesting that acrolein and possibly other carbonyl compounds, were in fact bound reversibly to proteins in blood. The stable, but slowly-reversible binding of acetaldehyde to haemoglobin or other proteins has also been suggested by Stevens et al. in a recent paper[155]. Therefore, the rapid disappearance of acrolein in blood was an artifact in the static headspace analysis which was probably due to rapid protein binding rather than enzymetic metabolism, although the latter could also be occurring at the same time. The binding of acrolein to

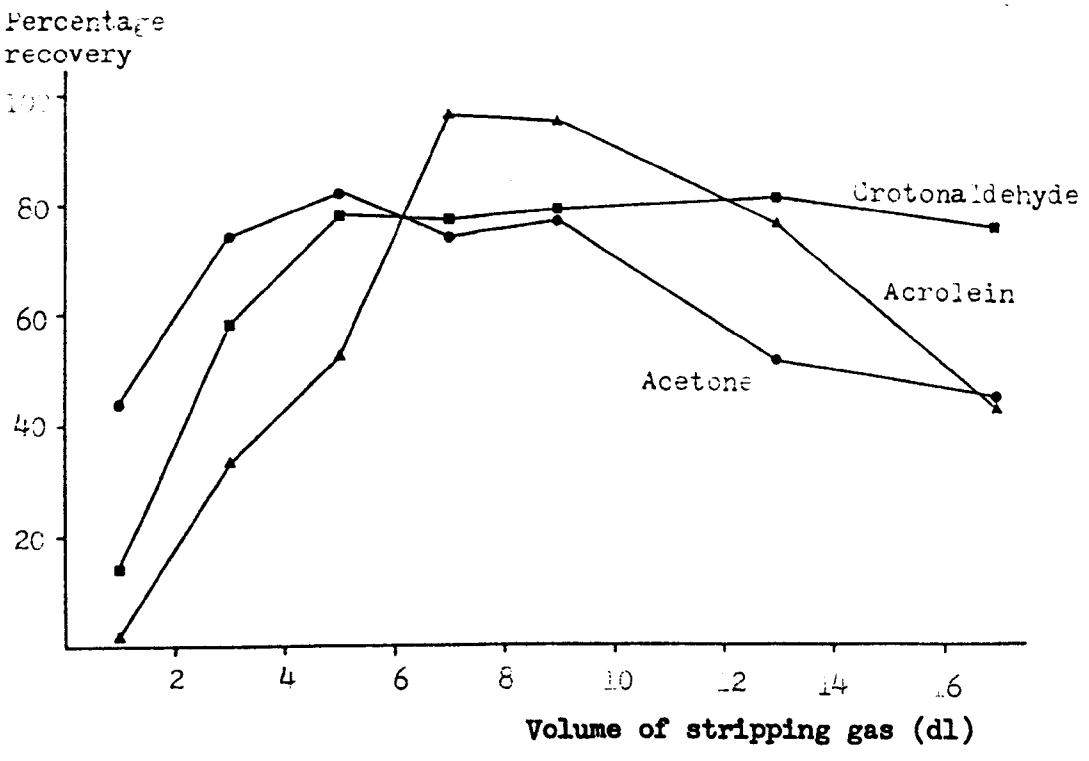


Figure 6.4 Recovery of carbonyl compounds from blood using different volumes of stripping gas.

proteins in blood may be represented by

$$[\text{Acrolein}]_{\text{free}} \underset{k2}{\overset{K1\ (k1)}{\rightleftharpoons}} [\text{Acrolein}]_{\text{bound}}$$

where K1 is the equilibrium constant and k1 and k2 are the forward and backward rate constants respectively. In a closed system, the vapour pressure of acrolein in the headspace is proportional to the free acrolein concentration in blood as shown in Figure 6.5. Thus if K1 was much greater than K2, only a very small percentage of the total acrolein would be present in the headspace and hence elude detection in static headspace analysis. However, in the process of headspace elution, acrolein vapour in the headspace is continously removed by the stripping gas, thus shifting the equilibrium from [acrolein]bound to [acrolein]vapour. Therefore, the headspace elution technique not only provides better sensitivity than the static headspace equilibration technique, but also solves the problem of the fast, but slowly reversible binding of acrolein in blood which otherwise would be difficult to analyse by other means.

In an earlier experiment (see previous chapter, Paragraph 5.3) in which changes in the absorbance of haemoglobin at 426 nm were monitored after the addition of acrolein to blood, the results (Figure 5.8) suggested that the binding rate of acrolein to haemoglobin was much faster than its binding to other proteins and that an equilibrium was reached in about 10 minutes. Using the headspace elution technique, a preliminary study of the distribution

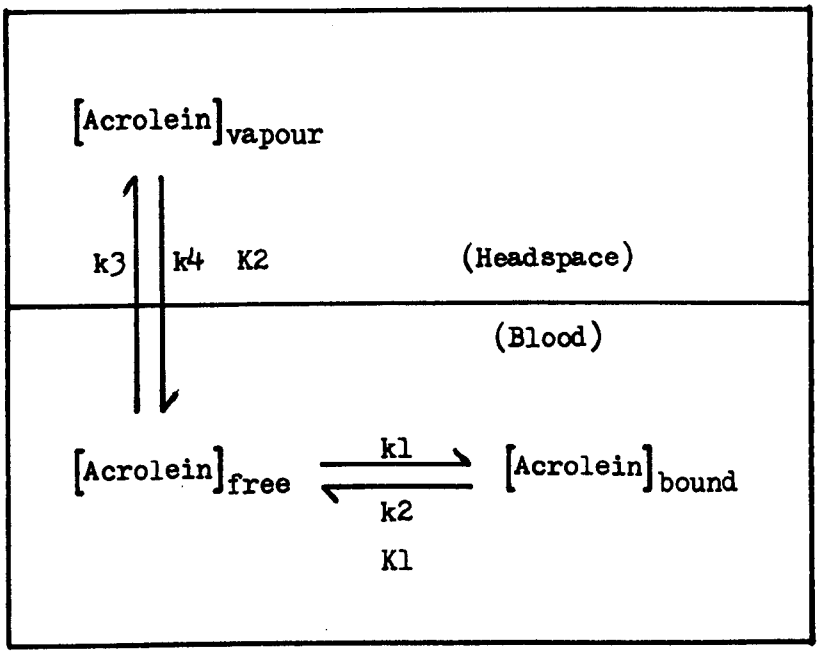


Figure 6.5 Partition of acrolein between headspace and blood.

of acrolein amongst erythrocytes and plasma 10 minutes after its addition to the blood sample showed that acrolein was present at more or less the same concentration in these two fractions (Table 6.2). The data on the total recovery of acrolein from the erythrocytes and plasma fractions also suggested that both types of binding were reversible.

It was mentioned earlier that the rapid "disappearance" of acrolein in blood was an artifact due to protein binding. However, metabolism of acrolein could also occur in blood at the same time since NAD-dependent aldehyde dehydrogenases exist in virtually every tissue and there are at least four enzymes that are capable of oxidizing various aldehydes of endogenous and exogenous origin[118]. Although, in general, the liver contains the greatest amount of total aldehyde oxidizing capacity, degradation of acrolein in blood cannot be excluded. Figure 6.6 shows the rate of degradation of acrolein in haemolysed blood at room temperature measured by dynamic headspace GC-MS. Haemolysed blood rather than whole blood was used in this experiment because aldehyde dehydrogenase in human blood is known to be present in the erythrocytes[165], therefore, the rate of acrolein metabolism would be affected by the rate of its entry to the erythrocytes. In order to avoid the latter being the limiting factor, whole blood haemolysed by an equal volume of distilled water was used in this study. As shown by the results, almost 30 % of the acrolein was lost in the first hour. The degradation curve appears to consist of two portions, a fast degradation of acrolein in the first hour after which, the rate of

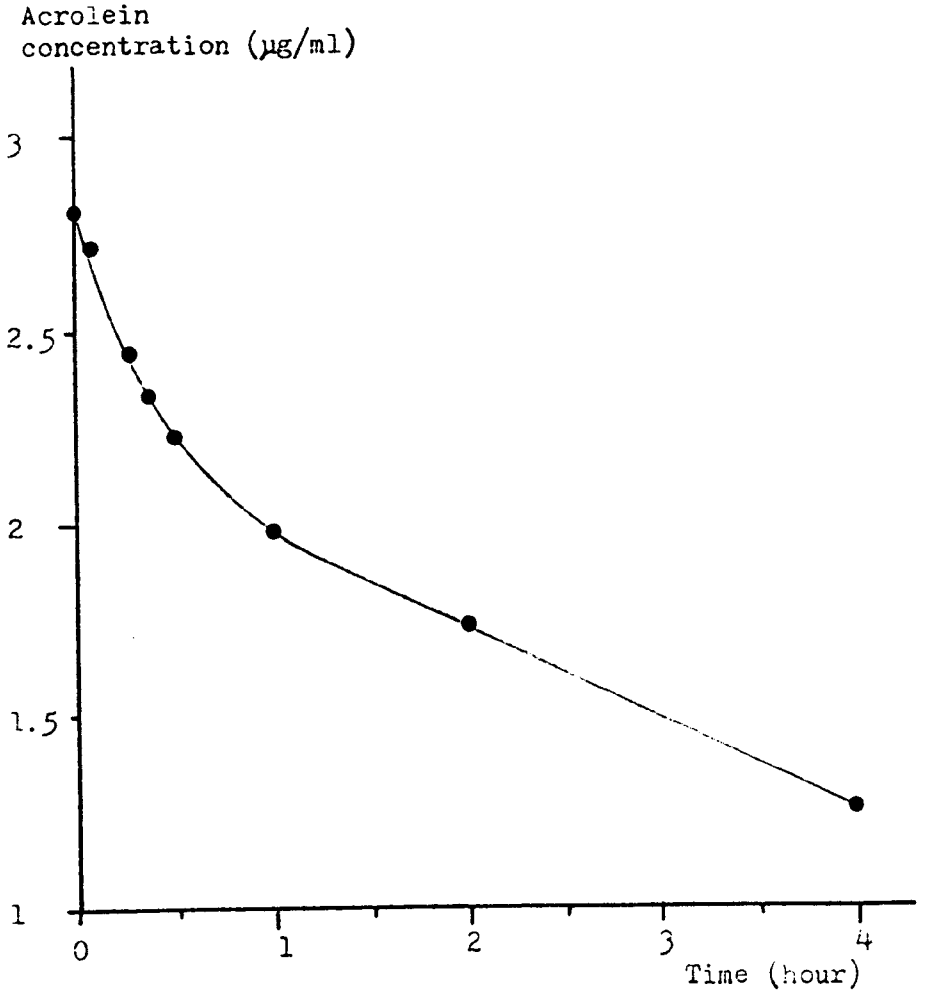


Figure 6.6 Degradation of acrolein in lysed blood.

degradation appears to be linear with time. Analysis of a sample after 23 hours showed no acrolein was present.

The main aim of this experiment was primarily to demonstrate that degradation of acrolein occurred in blood rather than to study the enzyme kinetics or to find out how, where and by what enzyme the oxidation of acrolein took place. It is not possible at this time to say exactly what happens to acrolein in blood, bearing in mind that the possibilities of protein binding, adduct formation, metabolism and polymerisation of acrolein may all occur simultaneously.

The use of dynamic headspace gas chromatography has proved to be a feasible way of analysing volatiles including carbonyls in blood. Some of the major problems normally associated with volatile profile studies are the separation of the volatiles and the identification of individual components in the profile. The separation of volatiles required the use of an efficient capillary column, but even then, multi-component GC peaks were quite common. The volatile constituents in blood were so complex that the GC peaks differed very little in retention time, therefore identification of the GC peaks by retention time alone was practically impossible and inaccurate. The use of gas chromatography-mass spectrometry provided valuable structural information on the identity of volatile components and the use of a dedicated data system allowed handling of the vast amount of data from each sample.

Repetitive scanning GC-MS was used in this study which

allowed both qualitative and quantitative analysis of blood volatiles to be carried out simultaneously. Although quantitation by the repetitive scanning method was not as sensitive nor as accurate as selected ion recording, it was more versatile and it did not have a restriction on the maximun number of ions which could be monitored at any one time. A fast scan rate of 1 sec/dec and a total cycle time of just 3.2 sec permitted some six to seven scans across the GC peaks even at the early part of the chromatogram which is generally accepted as the minimum for quantitative measurements. The reproducibility of the analytical technique was improved by the addition of three deuterated internal standards, i.e., [$^2H_6$]-acetone, 1,1-[$^2H_2$]-propionitrile and [$^2H_3$]-acetonitrile to the blood sample prior to headspace elution.

Analyses of standard solutions containing propionaldehyde, acrolein and crotonaldehyde at concentrations ranging from 10 ng/ml to 5 µg/ml using [$^2H_6$]-acetone as the internal standard have shown that linear relationships between the response ratio and the concentration were obtained with correlation coefficients (n=5) of 0.9991, 0.9988 and 0.9827 for propionaldehyde, acrolein and crotonaldehyde respectively. Repeated analysis (n=5) of a standard containing these compounds at 100 ng/ml gave coefficients of variation between 5 - 10 %, the sort of values normally accepted when using quantitative repetitive scanning GC-MS. Analysis of added acrolein in blood in the concentration range 10 ng - 5 µg/ml also gave a linear relationship in the calibration curve with a correlation

coefficient of 0.9918, only slightly lower than that obtained for aqueous solutions. The detection limits of volatiles in blood were in the nanogram per millilitre region.

Volatiles profiles for samples from different subjects varied widely in the number and relative intensities of the components. In general, blood samples from fire fatalities showed more complex profiles than those from other control groups. Over 140 compounds have been identified in blood samples obtained from fire fatalities. These include a series of carbonyls, organic nitriles, esters, alcohols, aliphatic hydrocarbons, halogenated hydrocarbons, aromatic compounds, heterocyclic compounds and sulphur-containing compounds. It has been suggested that the volatiles in these blood samples may have four possible origins: as normal metabolites, as experimental artefacts, as products of post-mortem decay or as materials originating from the fire atmosphere. Some compounds may have more than one origin. To distinguish the species which originated from the fire atmosphere, it was therefore necessary to examine post-mortem controls (putrefaction), fresh blood controls (normal metabolities) and blank samples (experimental artefacts). A typical blood volatiles profile of a fire fatality is shown in Figure 6.7. Only the analytical results on the carbonyl compounds are discussed in this chapter, the results on nitriles and profile comparision will be discussed in the next two chapters.

The carbonyl compounds which have been identified included acetaldehyde, propionaldehyde, isobutyraldehyde,

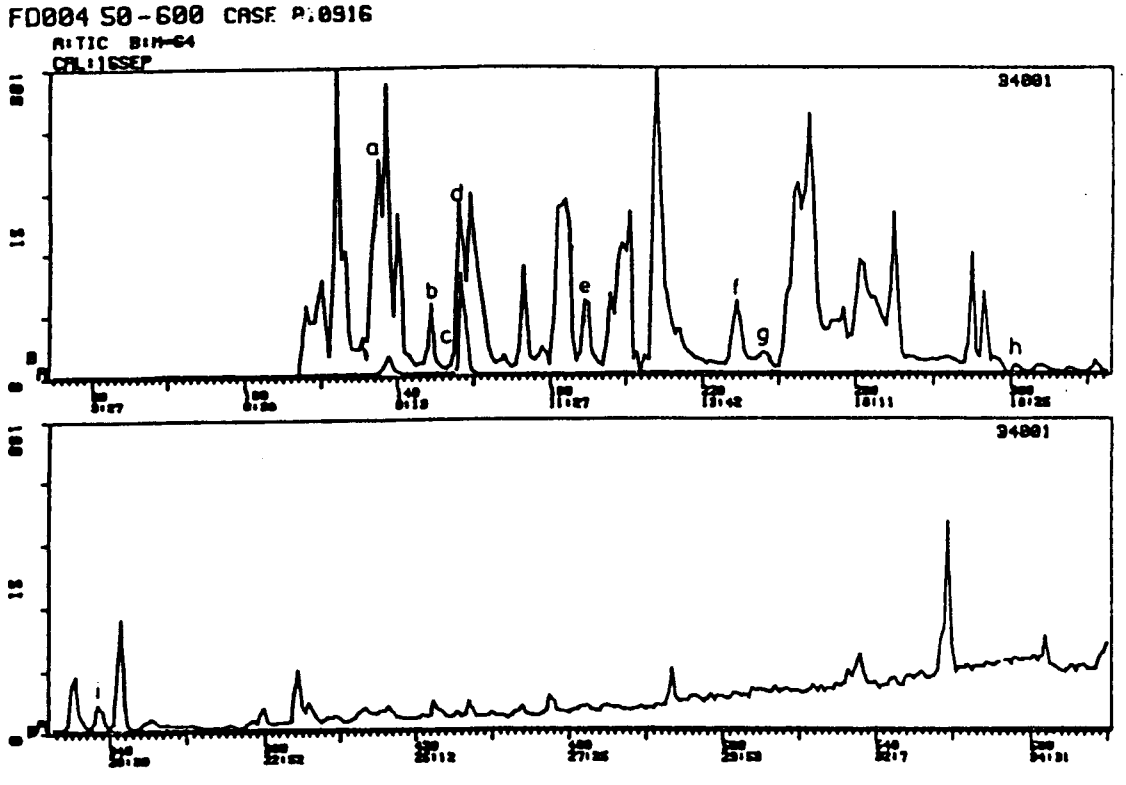


Figure 6.7 Computer reconstructed total ion current chromatogram and mass chromatogram of m/z 64 (for internal standard) of a volatiles mixture extracted from a fire death blood sample. Gas chromatography was carried out using a 100 m Carbowax 20M SCOT capillary column with helium carrier gas flow rate 2 ml/min. Column temp. 60°C (8 min) programmed at 4° per min to 160°C. Carbonyl components : a ethanal, b propanal, c 2-methyl-propanal, d $[^2H_6]$-acetone (internal standard) + acetone, e 2-butanone, f 2-pentanone, g 3-methyl-but-3-ene-2-one, h hexanal, i C_7-ketone.

Table 6.3 Blood carbonyl levels in fire fatalities, post-mortem and normal controls. All concentrations in ng/ml.

	Fire fatalities				Post-mortem controls				Normal controls			
Compound name	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Propionaldehyde	21	0-85	27.1	21.0	6	13-266	79.2	99.4	4	17-33	23.8	6.8
Isobutyraldehyde	21	5-99	41.0	26.8	6	49-103	65.8	20.1	4	84-137	112.3	28.2
Acrolein	21	0-46	4.8	11.6	6	0-0	0	0	4	0-0	0	0
n-Butyraldehyde	21	0-31	6.7	8.1	6	0-32	15.7	15.0	4	0-9	4.3	3.8
2-Butanone	21	0-249	48.6	51.6	6	7-33	17.8	9.2	4	11-25	18.3	5.9
Butandione	17	13-1720	257.5	416.9	6	55-133	86.5	28.1	4	10-58	31.5	20.2
2-Pentanone	17	2-353	73.1	116.7	6	3-9	5.5	2.3	4	6-13	8	3.4
4-Methyl-2-pentanone	18	0-9	2.1	2.8	6	0-1	0.2	0.4	4	0-1	0.8	0.5
Hexanal	19	0-486	141.4	135.2	6	9-87	31.2	28.9	4	26-56	45.3	13.9
Cyclopentanone	19	0-282	46.0	76.1	6	0-3	0.5	1.2	4	0-2	1	0.8
Cyclohexanone	21	0-56	6.8	16.2	6	0-18	4.2	7.2	4	0-1	0.3	0.5
Benzaldehyde	21	0-256	98.4	68.7	6	55-165	118.5	39.8	4	98-175	134.3	32.2

acetone, acrolein, n-butyraldehyde, 2-butanone, butandione, 2-pentanone, 4-methyl-2-pentanone, 2,3-pentanedione, hexanal, cyclopentanone, 3-octanone, cyclohexanone, furfural, 2-furylmethyl ketone, benzaldehyde and acetophenone. Other carbonyl compounds which have been tentatively identified included methyl-butanal, 3-methyl-but-3-en-2-one, 2-methyl-3-pentanone, 3-hydroxy-2-butanone and C6, C7 and C8 ketones. It was believed that formaldehyde might also be present but since it was not retained in the Tenax-GC trap, it has not been found in these profiles. The levels of blood carbonyls which have been quantified are shown in Appendix 1 and a summary of the results is shown in Table 6.3.

Several problems were experienced during the course of the analyses:

(i) The quantitation of volatiles was limited by the dynamic range of the 12-bit analogue to digital converter used in the mass spectrometer-computer interface. Therefore, compounds such as acetaldehyde and acetone which were present at high levels were overloaded in the chromatograms.

(ii) Amongst the fire fatalities in our study, a large proportion of adults were found to have blood alcohol levels above the U.K. legal maximum for drivers of motor vehicles (80 mg/dl). In these blood samples, compounds which had retention times close to ethanol were masked by the huge ethanol peak in the chromatogram. It was therefore decided to exclude those samples which showed a positive ethanol measurement from the volatiles analysis

during the latter part of this study. High levels of ethanol also gave rise to high levels of acetaldehyde due to its enzymic conversion by alcohol dehydrogenase[118, 119].

(iii) Most of the blood samples when received were already haemolysed, and in a few cases completely clotted. In the latter cases, the recovery of volatiles from clotted blood was uncertain.

Despite its limitations, the analytical method was still the most versatile and comprehensive way for the study of blood volatiles. The results indicated that there was very little difference between the two normal control groups, ie., the smokers and non-smokers controls. That was probably because the two subjects in our study only smoked about five cigerettes per day, not enough to cause any significant differences in their blood carbonyl compositions. For this reason, the smokers and non-smokers were treated as a single normal control group. Statistical comparisions of the differences in the blood carbonyl concentrations between the fire fatalities and the two control groups, using Wilcoxon's Sum of Ranks Test are shown in Table 6.4.

With the exception of isobutyraldehyde, the levels of 2-butanone, butandione, 2-pentanone and cyclopentanone were all significantly higher in samples from the fire fatalities than those from the control groups. The mean levels of acrolein, cyclohexanone and hexanal were also found to be higher in the fire fatalities (Table 6.3)

Table 6.4 Comparision of the blood carbonyl levels in fire fatalities (F.D.), post-mortem (P.M.) and normal controls (N.C.) using Wilcoxon's Sum of Ranks Test.

Compound name	F.D. versus P.M.	F.D. versus N.C.
Propionaldehyde	N.S. (1.02)	N.S. (0.44)
Isobutyraldehyde	P.S. (2.04)	S.S. (2.93)
Acrolein	N.S. (0.87)	N.S. (0.74)
n-Butyraldehyde	N.S. (1.20)	N.S. (0.26)
2-Butanone	P.S. (1.98)	N.S. (1.70)
Butandione	N.S. (0.60)	P.S. (2.02)
2-Pentanone	P.S. (2.00)	N.S. (1.39)
4-Methyl-2-pentanone	N.S. (1.67)	N.S. (0.43)
Hexanal	N.S. (1.94)	N.S. (1.22)
Cyclopentanone	V.S. (3.25)	S.S. (2.60)
Cyclohexanone	N.S. (0.20)	N.S. (0.85)
Benzaldehyde	N.S. (1.08)	N.S. (1.41)

Note1 : Figures in brackets are the calculated z values.
V.S. = very significant ($P < 0.002$).
S.S. = statistically significant ($P < 0.01$).
P.S. = probably significant ($P < 0.05$).
N.S. = not significant ($P > 0.05$).

Note 2 : These comparisons were made for interest only and are not considered to have any real significance, due to the small size of the groups.

although these did not achieve statistical significance.

In general, high levels of carbonyls in blood were associated with high levels of carboxyhaemoglobin which suggested that these compounds were associated with inhalation of fire gases. However, it is difficult to draw a direct relationship between elevated levels of carbonyls and carboxyhaemoglobin or even with cyanide levels for that matter, since, as discussed in Chapter 1, the physical and chemical parameters such as temperature, air flow, availability of oxygen and the nature of the combustible materials, which govern the production of these compounds in fires, are quite different.

Acrolein, the most strongly irritant and toxic compound in the carbonyl family, has been found in 24 % of the fire fatality samples. This compound was not found in any of the control subjects. Three of the samples which showed higher acrolein levels were also found to have carboxyhaemoglobin levels higher than 60 % which can be taken as an additional indication that acrolein was inhaled in the fire environment.

Published data on the toxicity of carbonyl compounds in relation to their concentrations in blood are not available, therefore interpretation of these results is not possible and their role in causing fatalities is still unknown. It is likely that there is an additive effect, or even a synergistic effect in the toxicity of aldehydes since most aldehyde dehydrogenases, the enzymes responsible for the removal of aldehydes, possess a broad substrate specificity[118,119]. The possibility of competition for

enzymes therefore exists and this fact leads to the possibility that these exogenous aldehydes can result in a build up of the steady state concentrations of both endogenous and exogenous compounds. However, with reference to the toxicity data from animal studies[60, 62,107-114] which have already been discussed in Chapter 3, the levels of blood carbonyls found in fire fatalities might not produce any lethal effect.

The major role of these compounds in fires was probably one of irritancy. Some carbonyl compounds, particularly the unsaturated aldehydes, are known to be strong sensory irritants. Exposure to these compounds may result in severe lachrymation which can hinder vision. Inhalation of the vapour can also cause intense irritation to the respiratory tract. All these, together with other factors considered, would impair the ability to escape from a fire.

Studies on simulated "real-fires" under laboratory controlled conditions have shown that the production of carbonyl compounds from thermal degradation of polymeric materials occurs at low temperatures, ie., < 700°C[46]. It is therefore obvious to propose that the carbonyl compounds play a more significant role at the early stages of a fire. The presence of these compounds together with other toxicants in the fire atmosphere may lead to incapacitation and ultimately death if escape is not accomplished.

Within the time available for the present study, thirty-one samples in total were analysed. Statistical

analysis of the measurements from a small number of samples could give an inaccurate interpretation especially when the measurements in the parent group showed large coefficients of variation. As the study progressed, more and more unknown components in the profiles were identified. It would therefore be of interest if those compounds were included in the quantitation. For all these reasons, it would certainly be worthwhile to carry out further investigation of this subject if time were available. Animal exposure studies might provide valuable information on inhalational toxicities and changes in blood carbonyl levels in relation to exposure concentrations.

6.4) Conclusions

The use of capillary gas chromatography-mass spectrometry with the aid of a data system has been shown to be useful for both qualitative and quantitative analysis of volatiles in blood samples. Those carbonyl compounds which eluded detection in static headspace analysis were probably involved in fast, but slowly reversible, binding of the compounds to blood proteins.

Many of the carbonyl compounds which have been found in the thermal degradation products of polymeric materials were found in blood samples from fire fatalities but not from the control groups. While the toxicological significance of these compounds is still unknown, they are thought to play an important role in causing sensory irritation and may contribute to incapacitation at the

early stages of a fire.

SECTION III: ANALYSIS OF NITRILES AND OTHER VOLATILES IN BLOOD

CHAPTER 7: ANALYSIS OF ORGANIC NITRILES IN BLOOD

7.1) Introduction

Organic nitriles of the general formula R-C≡N have been found in the combustion and thermal degradation products of many nitrogen containing polymeric materials, such as polyacrylonitrile[46], polyurethanes[11], wool, silk and nylon[38]. Many of these compounds are known to be highly toxic [49,166] and concern has been expressed that they may be produced in dangerously high concentrations in the course of fires in buildings. Hydrogen cyanide, the simplest member in the nitriles family, has been quantitatively measured in blood samples obtained from fire fatalities and the results showed a significant elevation in the mean blood cyanide concentration with respect to control groups[162].

In the present study, several organic nitriles have been identified and, in some cases, quantified in blood from fire fatalities. The results were compared with those obtained from various control groups in an attempt to evaluate their potential importance in causing fatalities in fires.

7.1.A) Toxicity of organic nitriles

Organic nitriles are in general highly toxic, although they are not as strongly irritant as certain aldehydes. Inhalation of these vapours may cause severe intoxication similar to hydrogen cyanide poisoning[49,50,166]. The predominant acute effects of nitriles appear to be exerted

on the nervous system and may produce symptoms such as laboured breathing, convulsions, paralysis, asphyxia, necrosis, gastrointestinal irritation and bronchoconstriction. Information on the human toxicity of nitriles is rare and for this reason, the threshold limit values (TLV) recommended by ACGIH[61,62] are used as a guide to their relative toxicities. Table 7.1.

Recently, it has been suggested[167,168] that the toxicities of organic nitriles are due to in vivo cyanide liberation as a result of microsomal hydroxylation at the α-methylene group to form an unstable cyanohydrin. The liberated cyanide inhibits mitochondrial cytochrome C oxidase and causes respiratory arrest.

Willhite reported that mice exposed to acetonitrile, propionitrile or n-butyronitrile vapours exhibited the same syndrome regardless of the nitrile to which they were exposed[167]. The animals commonly exhibited intense dyspnea, convulsions and corneal opacity. The toxic effects on animals exposed to different concentrations of nitriles is shown in Table 7.2.

Pozzani et al. conducted studies with human volunteers who inhaled acetonitrile for 4 hours at concentrations of 40, 80, or 160 ppm[170]. One subject complained of tightness in the chest several hours following exposure to 40 ppm acetonitrile. Inhalation of 160 ppm acetonitrile was associated with a transient facial flush 2 hours post-exposure in a second volunteer. Dequidt et al. reported the death of a 19-year old male who was exposed to an unknown concentration of acetonitrile[171]. In spite of

Table 7.1 Threshold limit values for nitriles recommended by ACGHI[61,62].

Nitrile	TLV (ppm)
Acetonitrile	20
Propionitrile	6
n-Butyronitrile	8
Isobutyronitrile	8
Acrylonitrile	2

Table 7.2 Toxic effects on animals exposed to different levels of nitriles[169].

Nitrile	Conc. in air (ppm)	Animal	Response
Acrylonitrile	636	Rat	Fatal after 4 hr.
	258	Rabbit	Fatal during and after exposure.
	152	Cat	Markedly toxic, sometimes fatal.
	110	Dog	Fatal to 75%.
	98	,,	Convulsions & coma, no death.
	55	,,	Transitory paralysis.
	29	,,	Very slight effects.
Propionitrile	500	Rat	Fatal to 33% rats (2/6).
	LLC*(rat) = 500 ppm for 4 hr.		
Acetonitrile	8000	Dog	No death.
	16000	,,	Death occurred.
	LLC*(rat) = 7500 ppm for 8 hr.		
	LLC*(rat) = 8000 ppm for 4 hr.		

*LLC = lowest published lethal concentration.

intensive treatment, he died 6 days after the onset of poisoning. On the 3rd day after exposure, the acetonitrile in the blood was 11.76 µg/ml. Analysis of the post-mortem samples showed elevated cyanide levels in blood, urine and other tissues. In an experiment with human volunteers, Dalhamn et al. reported that acetonitrile in cigarette smoke is absorbed by the oral and pulmonary tissue with a retention of 74% and 91% respectively[172,173].

No reports of effects on humans following exposure to propionitrile, n-butyronitrile, acrylonitrile or crotonitrile have been published.

7.1.B) Analysis of organic nitriles in blood

As mentioned earlier (Chapter 6), the volatile constituents in blood can be extracted non-selectively by gas-stripping techniques, thereby allowing the study of the volatiles profile as well as the simultaneous quantitation of individual compounds. This is particularly useful for compounds which have high vapour pressures and low solubilities in water. Reproducibility of the measurements can be improved by the addition of an internal standard labelled with stable isotopes for each family of compounds being quantified. In the present study, [1,1-$^2H_2$]-propionitrile was used for the quantitation of acrylonitrile, propionitrile, crotonitrile and benzonitrile, and [$^2H_3$]-acetonitrile was used specifically for quantitation of acetonitrile since the latter had a very low retention on Tenax-GC (see Figure 6.3).

7.2) Experimental

7.2.A) Synthesis of [1,1-$^2H_2$]-propionitrile

This was prepared using the method described by Leitch[174]. Deuterium oxide (99.8 atom%) was obtained from Aldrich Chem. Co. (Dorset, U.K.). Calcium oxide (Reagent grade), propionitrile (Reagent grade) and anhydrous potassium carbonate (AnalaR grade) were obtained from BDH (Dorset, U.K.). Calcium oxide was heated in a silica crucible over a strong Bunsen flame for 45 minutes before use.

Redistilled propionitrile (2.5 ml), deuterium oxide (2.5 ml) and heat-treated calcium oxide (0.25 g) were heated with magnetic stirring in a pressure reaction vessel at 120°C for 18 hours. The suspension was then distilled and the distillate filtered through anhydrous potassium carbonate (0.1 g). The resultant colourless liquid was recycled through the deuterium exchange reaction followed by the same purification procedures. The yields of [1,1-$^2H_2$]-propionitrile after the first and second deuterium exchange process were 64% and 16% respectively.

7.2.B) Identification and quantitation of organonitriles in blood

Details of the extraction, sorption and desorption procedures, and finally the separation of volatiles by capillary gas chromatography have already been described in Chapter 6. Nitriles in the volatiles profile were identified by comparison of their GC retention indices and

mass spectra with those of authentic standards. Two deuterium-labelled internal standards, [1,1-$^2H_2$]-propionitrile and [$^2H_3$]-acetonitrile, were used for quantitative measurements. The latter was obtained from Prochem. BOC Ltd. (London, U.K.). Quantitative measurements were made using calibration curves or response ratios determined using standard solutions under identical conditions. The selected ions chosen for monitoring the nitriles are shown in Table 7.3.

7.3) Results and Discussion

The method described by Leitch[174] was found to be a simple and economical method to synthesise deuterium-labelled nitrile. The process involves the exchange of the acidic hydrogen atoms at the α-position with deuterium atoms from deuterium oxide using calcium oxide as the catalyst. Determination of the percentage incorporation of deuterium in the synthesised sample by mass spectrometry was difficult because of the crossed ion contributions amongst [1-$^2H_1$]-propionitrile, [1,1-$^2H_2$]-propionitrile and the undeuterated material. The isotopic abundance was, however, more easily determined by nuclear magnetic resonance (NMR) spectroscopy. The NMR spectrum of the sample indicated that it contained 90 per cent [1,1-$^2H_2$]-propionitrile and 10 per cent of [1-$^2H_1$]-propionitrile with no undeuterated propionitrile detected.

Analysis of whole blood samples containing added propionitrile in the concentration range 1 to 100 ng/ml

Table 7.3 The selected ions chosen for monitoring nitriles in blood.

Nitrile	Selected ion (m/z)
[$^2H_3$]-Acetonitrile (I.S.)	44
[1,1-$^2H_2$]-Propionitrile (I.S.)	56
Acetonitrile	41
Acrylonitrile	53
Propionitrile	54
Cis-crotonitrile	67
Trans-crotonitrile	67
Benzonitrile	77

gave a linear calibration curve with a correlation coefficient of 0.9995 (Figure 7.1). This linear relationship was assumed to apply for the other nitriles. The detection limits of nitriles in blood were in the nanogram per millilitre region.

A series of organic nitriles were found in the blood samples from fire fatalities. Those nitriles which have been identified included acrylonitrile, acetonitrile, propionitrile, n-butyronitrile, crotonitrile (both cis- and trans- isomers), benzonitrile and tolunitrile. All of these have also been found in the combustion and thermal degradation products of nitrogen-containing polymeric materials[11,38,46]. A typical blood volatiles profile from a fire fatality showing the presence of nitriles is shown in Figure 7.2. Detailed comparision of the volatiles profiles of various control groups will be discussed later in Chapter 8. The levels of some of the nitriles in blood samples from fire fatalities, post-mortem controls and normal healthy controls are shown in Appendix 2. A summary of the results and the statistical comparision of the nitriles levels between the fire fatalities and the control groups are shown in Tables 7.4 and 7.5 respectively.

Acrylonitrile was present only at trace quantities (0 - 14 ng/ml) in the fire fatality samples, with only two samples (10%) showing significantly elevated levels compared to the control groups.

The levels of acetonitrile were found to be much higher in smoker controls than in non-smoker controls, possibly as a result of inhalation of acetonitrile in

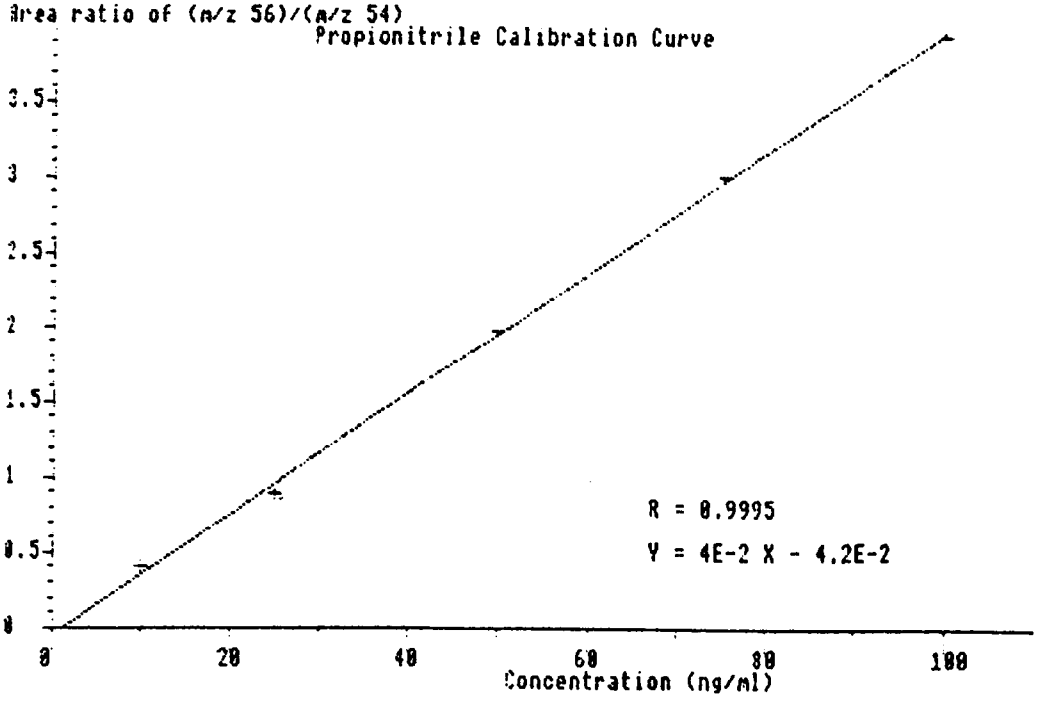


Figure 7.1 Blood propionitrile calibration using 1,1-[$^2H_2$]-propionitrile as internal standard.

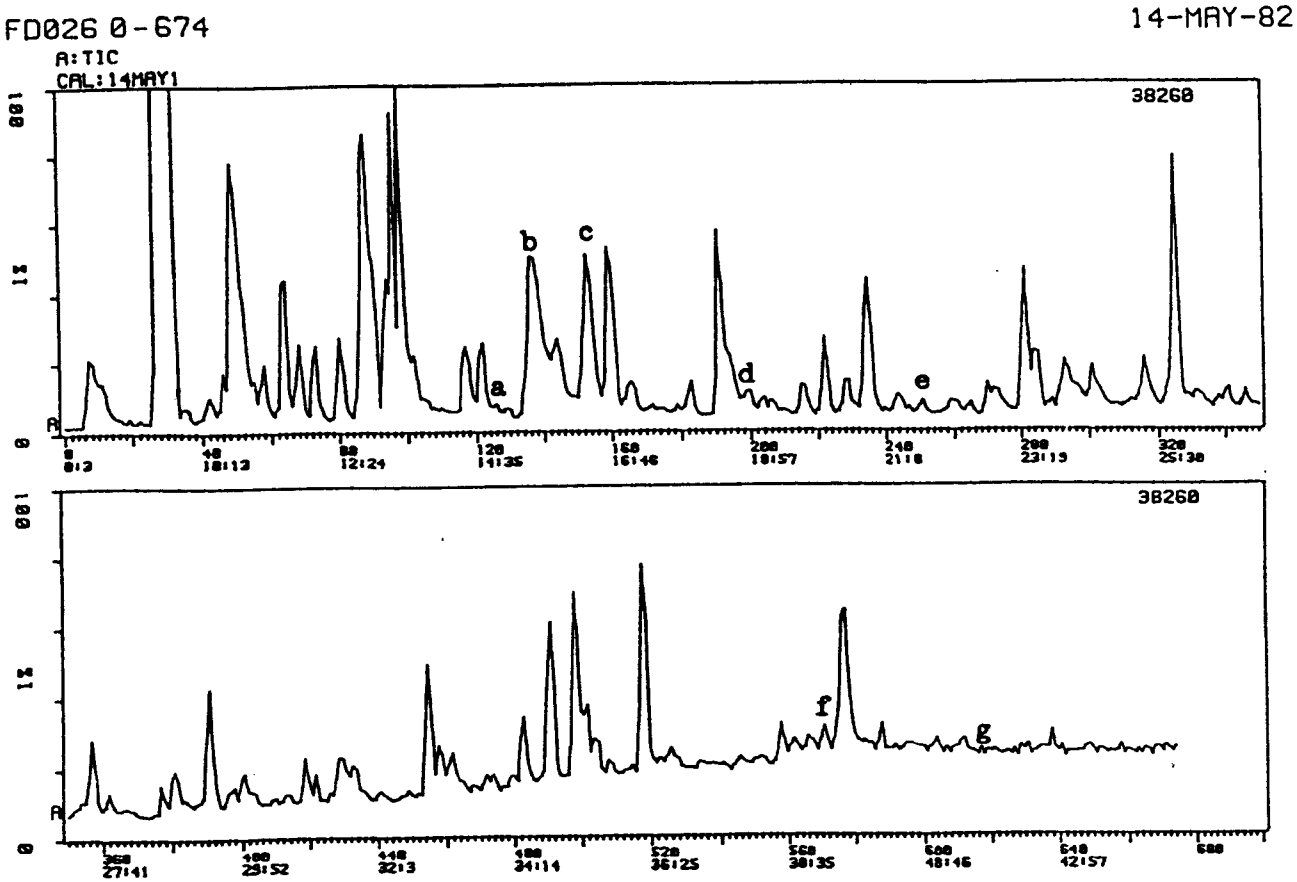


Figure 7.2 Total ion chromatogram of volatiles extracted from the blood of a fire fatality showing the presence of organic nitriles. The identities of the peaks are: a, acrylonitrile; b, acetonitrile + $[^2H_3]$-acetonitrile; c, propionitrile + $[1,1\text{-}^2H_2]$-propionitrile; d, cis-crotonitrile; e, trans-crotonitrile; f, benzonitrile; g, tolunitrile.

Table 7.4 Levels of nitriles in blood from fire fatalities, post-mortem and normal controls. All concentrations in ng/ml.

	Fire fatalities				Post-mortem controls			
Nitrile	n	Range	Mean	SD	n	Range	Mean	SD
Acrylonitrile	17	0-14	2.4	3.6	6	0-<1	-	-
Acetonitrile	17	0->215	-	-	6	2-24	8.2	8.5
Propionitrile	20	1-114	16.1	24.8	6	0-<1	-	-
Crotonitrile	21	0-67	17.1	22.7	6	0-<1	-	-
Benzonitrile	21	0-78	17.7	22.4	6	0-0	-	-

Normal controls

Nitrile	n	Range	Mean	SD
Acrylonitrile	4	<1-3	-	-
Acetonitrile	4	1-67	23	30.7
Propionitrile	4	0-0	-	-
Crotonitrile	4	<1-2	-	-
Benzonitrile	4	0-0	-	-

Table 7.5 Statistical comparision of nitrile levels in fire fatalities (FD), post-mortem (PM) and normal controls (NC) using Wilcoxon's Sum of Ranks Test.

Nitrile	FD versus PM	FD versus NC
Acrylonitrile	P.S. (2.21)	N.S. (0.36)
Propionitrile	V.S. (3.59)	V.S. (3.10)
Crotonitrile	V.S. (3.50)	S.S. (2.63)
Benzonitrile	S.S. (2.97)	P.S. (2.52)

Note 1 : Figures in brackets are the calculated z values.
V.S. = very significant ($P < 0.002$).
S.S. = statistically significant ($P < 0.01$).
P.S. = probably significant ($P < 0.05$).
N.S. = not significant ($P > 0.05$).

Note 2 : These comparisons were made for interest only and are not considered to have any real significance, due to the small size of the groups.

cigarette smoke. McKee et al. reported that acetonitrile was present in urine samples from smokers at concentrations ranging from 22 to 200 ng/ml[175]. Over half the samples from fire fatalities showed overloaded levels of acetonitrile and up to 94% of the samples showed higher levels than the mean of the smoker controls. The only sample (subject no. 19) which showed a level of acetonitrile similar to the smoker controls was also found to have a normal level of carboxyhaemoglobin and blood cyanide. This strongly suggested that high levels of acetonitrile were the result of inhalation of toxic gases in fires. The blood concentration of acetonitrile may therefore be used as an additional indication of the degree of exposure to smoke and toxic gases.

Propionitrile, the most toxic compound in this series of organic nitriles was found in fire fatality samples at concentrations ranging from less than 1 to 114 ng/ml. This compound was not found in any of the control samples. Crotonitrile, probably the strongest irritant in this series of nitriles was also found in samples from fire fatalities but not from any of the control groups. However, no significant correlation (Spearman's Test) between these two nitriles was observed.

Although benzonitrile was found in the profile of all the samples including the blank, any contribution from experimental artefacts was subtracted in the calculation to give the actual levels in blood (see Equation 6.1). However, since the level of benzonitrile in the blank was quite high (estimated several ng/ml), low levels of

benzonitrile in blood may therefore not have any significance. Taking this into consideration, about half of the samples from fire fatalities still showed levels of benzonitrile which were significantly higher than controls.

In general, these organic nitriles are highly toxic, and inhalation of their vapours causes dizziness, dyspnoea, convulsion and in severe cases, unconsciousness and coma. Although the significance of in vivo cyanide liberation in acute aliphatic nitrile intoxication has been controversial in the past[176], recent evidence supports the view that the toxicity of these compounds is associated with the in vivo metabolism of the parent compounds to liberate cyanide, hence giving toxic effects very similar to those caused by cyanide poisoning[50,167]. Therefore, it is possible that nitriles and hydrogen cyanide may have additive toxic effects. In this study, high levels of nitriles were in general associated with high levels of carboxyhaemoglobin or elevated levels of blood cyanide but no statistically significant correlation (Spearman's Test) was demonstrated.

Woolley et al. studied the nitrogen-containing pyrolysis products of polyacrylonitrile and polyurethane foam at various temperatures and found that a series of nitriles were present in addition to hydrogen cyanide[46]. At 700°C, the total yield of acetonitrile, acrylonitrile and benzonitrile from polyacrylonitrile was 14.1% of the available nitrogen. The presence of these highly toxic organic nitriles in the blood samples of fire fatalities lends support to the growing belief that inhalation of

smoke and toxic gases is the major cause of death in fires. Many of these samples showed very high levels of acetonitrile, estimated in the microgram per millilitre range which would undoubtly play an important role in fire toxicology. The combined toxic effects from these compounds might be as significant as carbon monoxide or hydrogen cyanide which were considered in the past to be the only major toxicants in fires. However, until further data becomes available concerning the toxicity of organic nitriles in relation to their concentration in blood, the interpretation of these results will be difficult.

7.4) Conclusions

A series of highly toxic organic nitriles was found in blood samples from fire fatalities but not in the samples from other control groups except for acetonitrile which was also found in smoker control samples. The results provide important additional evidence of the significance of inhalation of smoke and toxic gases in fires. The toxicological significance of these results is still unknown but it is possible that their role in fires will lie in an additive contribution to the effects of other toxicants in causing incapacitation or intoxication.

CHAPTER 8: BLOOD VOLATILES PROFILE ANALYSIS

8.1) Introduction

Recently, there has been a growing interest in the study of volatiles as components of body fluids or as environmental pollutants. Such studies have been helped by the availabilty of commercial capillary columns and better methods of volatiles sampling. The techniques most frequently used for the collection of volatiles have been cryogenic condensation, solvent extraction and adsorption on solid adsorbents. Cryogenic methods tend to collect large quantities of water, which presents a major problem in the subsequent chromatographic analysis. Solvent extraction techniques normally require subsequent concentration procedures, hence are unsuitable for low molecular weight compounds which have high vapour pressures.

The advantages of using sorption techniques in conjunction with gas stripping or headspace extraction, are high sensitivities (parts per billion range) and the absence of a solvent peak in the chromatographic analysis. This technique has been widely used in areas such as analysis of volatile metabolites in biological fluids[157-160,177], environmental pollutants[161], water analysis[163], odour analysis[178] and volatiles composition in cigarette smoke[179]. The study of abnormalities in volatiles profiles of body fluids has been used recently in clinical chemistry as a diagnostic aid for certain metabolic and viral diseases[157-160]. With few

exceptions, most of these studies have focussed on a pattern recognition method or identification of several major components as the "profile markers" for comparision with various control groups. The most suitable instrumental technique for this type of study is undoubtedly capillary gas chromatography-mass spectrometry with a dedicated data system. Capillary gas chromatography is used for its efficiency in separation, mass spectrometry for providing structural information on individual components and the data system for handling the vast quantity of data obtained from each profile.

While combustion toxicology has become a subject of increasing importance in recent years for assessing the hazards associated with combustion and thermal degradation products, little attention has been given to the analysis of these toxic compounds in fire fatalities apart from the measurement of carboxyhaemoglobin or, in a few studies, blood cyanide. In the present study, profiles of volatile constituents in blood samples obtained from fire fatalities have been examined in an attempt to recognise materials from the fire environment which were inhaled, ingested or absorbed through the skin by the fire victims. Over 140 compounds have been positively or tentatively identified in blood samples from fire fatalities. The profiles were compared with those from control groups in an attempt to evaluate their significance in fire toxicolgy.

8.2) Experimental

A detailed description of the procedure for adsorption, thermal desorption and analysis of volatiles has been reported in the previous two chapters. In general, "organic volatiles" in the profiles refers to the compounds which could be extracted from one millilitre of blood by 0.6 litre of stripping gas (helium) and which could subsequently be chromatographed without prior derivatisation.

8.2.A) Identification of volatiles

The software-reconstructed total ion chromatogram (35 - 350 amu) was used as the chromatographic trace of the volatiles profile. On average, approximately 680 mass spectral scans were acquired over the entire chromatographic run of 45.5 minutes, excluding those before the void time. Volatile components were identified by comparision of retention indices and mass spectra with those of authentic standards. Where the latter were not available, tentative identifications were made on the basis of their mass spectral data only.

The profiles were examined in great detail, on an individual scan basis, to permit the detection of as many of the components present as possible. This involved the appropriate use of background subtraction facilities available in the software package and also the discrimination, in many cases, of individual components in overlapping peaks using either manual or software-based detection of maxima in mass chromatograms. Reconstructed

ion chromatograms were also used for "screening" for the presence of minor components which had low intensities in the total ion chromatogram. A library of mass spectra of identified compounds and of authentic standards was created in the data system in order that computer-based library searches could be performed to speed up the identification process during the latter course of the study.

A computer program was written for the calculation of the retention index of each component in the chromatogram. This was based on the retention time of the component relative to those of n-alkanes chromatographed under the same GC conditions and during the latter course of the study, compounds in the chromatogram which had known retention indices were used as references.

Comparision of the profiles was done manually after compensating for changes in the absolute sensitivity of the instrument using the intensities of the internal standards. In the case of multi-component peaks or peaks that had low intensities in the total ion chromatogram, peak area measurements of selected ion chromatograms were used for comparision.

8.3) Results and Discussion

Tenax-GC functioned as an efficient adsorbent for the volatiles encountered in this work, although methanol, formaldehyde and hydrocarbons below pentane were not retained. An additional advantage was its extremely low affinity for water which saturated the vapour during gas

stripping. The use of a water-cooled condenser further reduced the uptake of water in the Tenax-GC trap and yet did not have any significant effect on the recovery of volatiles. It was also found that the Tenax-GC traps could be re-used without any memory effect after conditioning at 250 - 300°C in a stream of helium.

A report by Michael et al. described the problem of foaming when blood or urine was purged at elevated temperatures, and the problem could not be easily solved by the use of anti-foaming agents[161]. The problem of foaming was also experienced in this study. However, it was found that this problem could simply be overcome by careful control of the purge gas in the first 30 seconds or so of the extraction, that is, by shutting off the purge gas when the foam almost reached the top of the perfusion flask and then turning the purge gas back on when the foam had hardened (after about 10 to 15 seconds). Recovery of the volatiles from the foamed blood clot was found to be comparable to that from aqueous solution.

Although retention indices have long been used as criteria for identification of chromatographic peaks, the chromatogram of a typical blood volatiles profile, however, was so complex that the use of retention data alone would have been difficult and inaccurate. Normally, the degree of confidence can be increased by utilizing columns of higher efficiency. In quantitative repetitive scanning GC-MS, the price to pay is poorer precision resulting from fewer scan across a GC peak. As a suitable compromise, the chromatographic conditions employed in this work were found

to give adequate separation of the volatile components in the profile and yet gave at least eight scans per peak. Although a few multi-component peaks were still present, this problem was readily overcome when the mass spectrometer and the data system were used in conjunction.

Volatiles profiles of samples from different subjects varied widely in the number and relative intensities of the components. In general, blood samples from fire fatalities showed more complex profiles than those from healthy or non-fire death post-mortem controls. A typical software-generated total ion chromatogram (TIC) showing the blood volatiles profile of a fire fatality is shown in Figure 8.1. Over 140 compounds were identified in blood samples from fire fatalities. The chromatographic and mass spectral data are summarised in Table 8.1 at the end of this chapter.

Several series of compounds were observed amongst the identified components and these included carbonyls, organic nitriles, esters, alcohols, hydrocarbons, halogenated hydrocarbons, simple aromatic hydrocarbons, heterocyclic compounds and sulphur-containing compounds. Many of these species were present as normal metabolites and a few were present as artefacts from post-mortem putrefaction and apparatus background (Figures 8.2 to 8.4). It was thought that many of the "abnormal" components in fire fatality samples originated from the fire atmosphere as the result of inhalation of smoke and fire gases. A closer examination of the profiles indicated that many of the "normal" components in the profiles of fire fatalities were

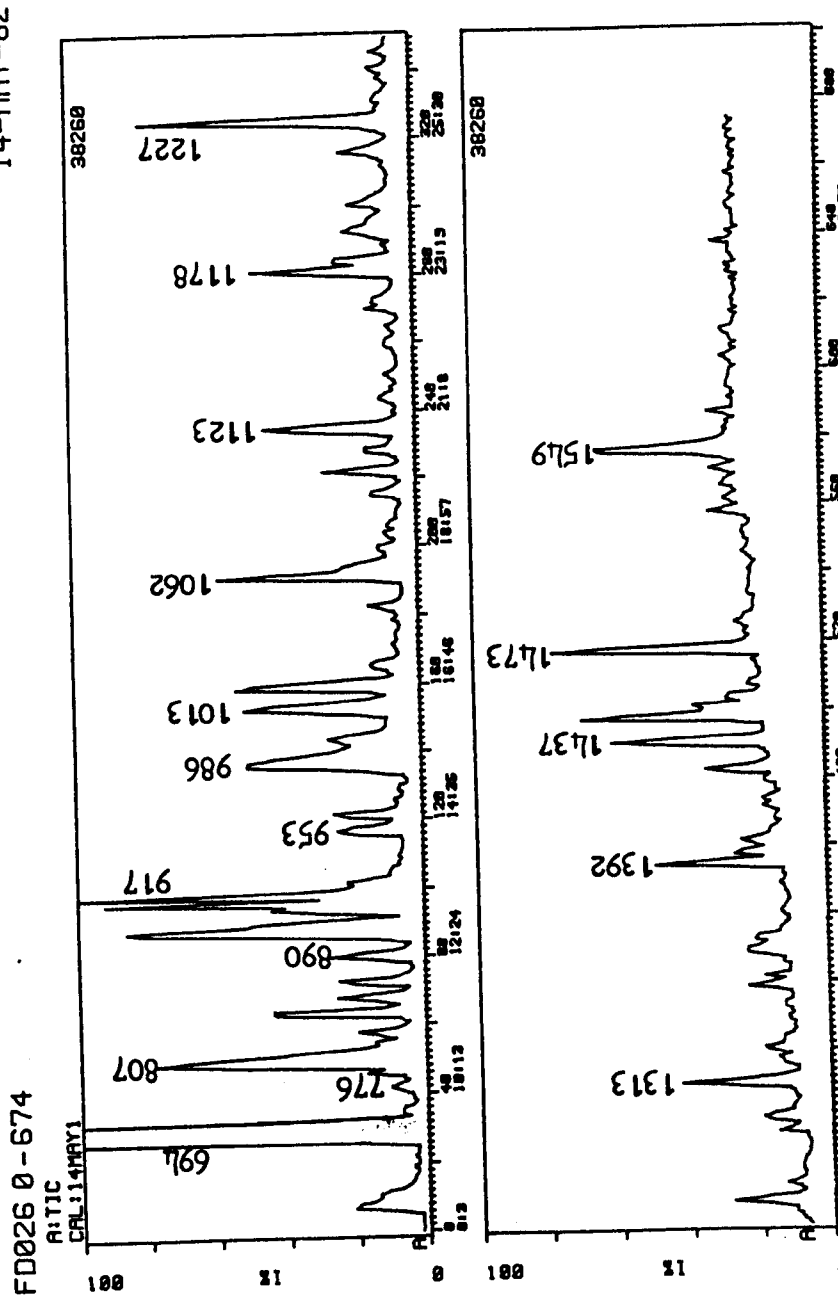


Figure 8.1 Total ion chromatogram of volatiles extracted from the blood of a fire fatality. Retention indices are given for major peaks which may be identified by refering to Table 8.1.

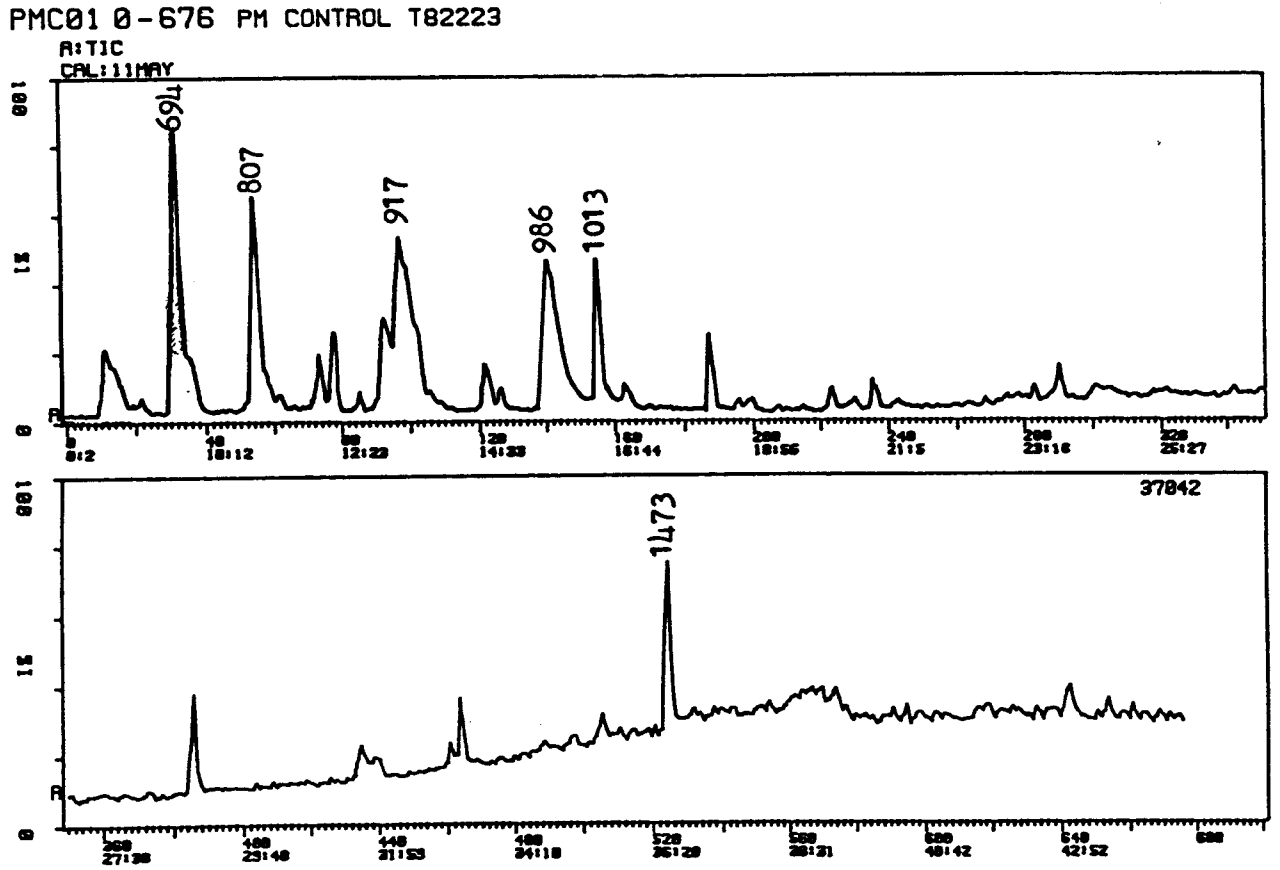


Figure 8.2 Total ion chromatogram of volatiles extracted from the blood of a post-mortem control. Retention indices are given for major peaks which may be identified by referring to Table 8.1.

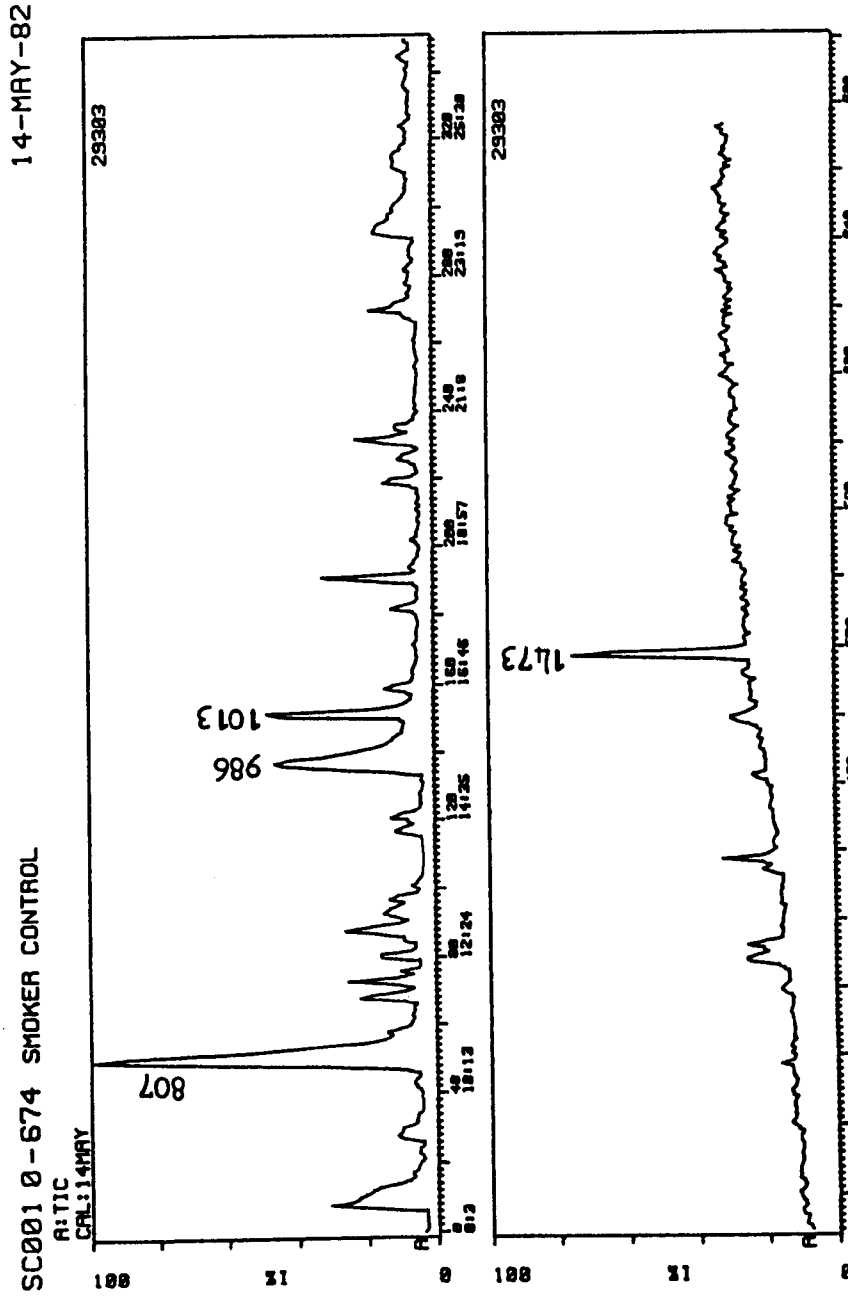


Figure 8.3 Total ion chromatogram of volatiles extracted from the blood of a healthy subject (smoker). Retention indices are given for major peaks which may be identified by referring to Table 8.1.

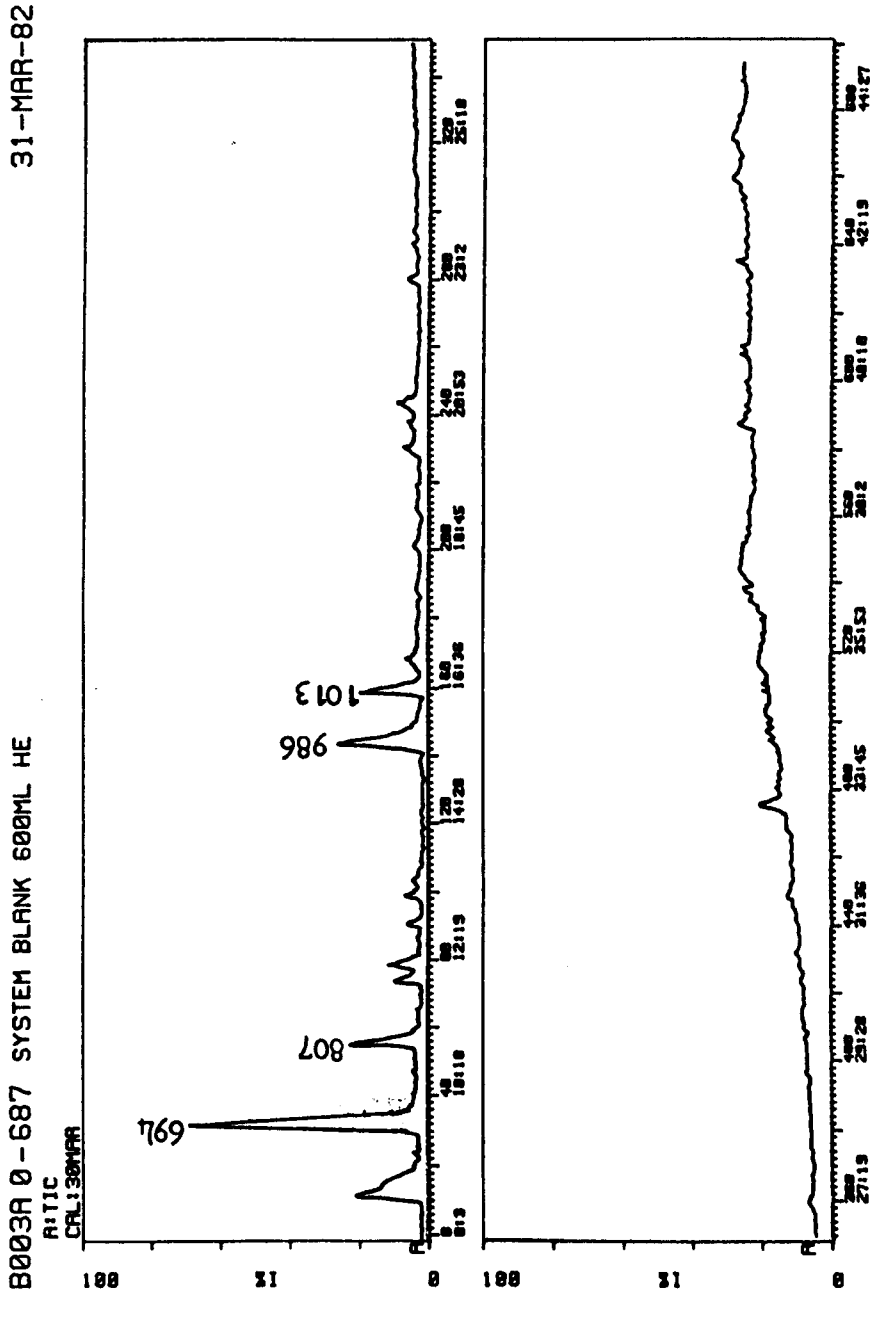


Figure 8.4 Total ion chromatogram of volatiles present in the procedure blank. Retention indices are given for major peaks which may be identified by referring Table 8.1.

in fact at much higher levels than in the controls. The comparision of volatiles profiles from fire fatalities with the control groups are also summarised in Table 8.1 at the end of this chapter.

In general, more complex profiles were associated with elevated levels of carboxyhaemoglobin, an additional indicator that these compounds were inhaled from the fire environment. Also of significance was the fact that most of the series of compounds listed above have already been identified in the combustion and thermal degradation products of both natural and synthetic polymeric materials[4, 12, 37, 59, 99, 100, 102, 180]. The species observed in blood from fire deaths were therefore consistent with the proposal that they originated in the course of a fire.

With very few exceptions, no published data are available on the toxicological effects of these compounds in relation to their concentrations in blood. Even if these data were available, it would still be difficult to interpret the present results which relate to not just one, but a large number of toxicants, each of which may have additive or synergic effects on the others. A more simplistic approach to the problem is therefore obligatory in which each toxicant is considered either in isolation or as one member of a mixture of components whose toxicities may be summated in a purely additive procedure. Several toxicity index summation procedures have been examined for use in toxicity model studies[181,182].

The importance of carbonyls and nitriles in fire

fatalities has already been discussed in Chapters 6 and 7 and therefore is not repeated in this discussion.

Simple alkenes, in general, are of low toxicity, but inhalation of the vapour at high concentrations may cause irritation to the respiratory system. Most of these compounds are known to have a narcotic effect and cause depression of the central nervous system when inhaled[39]. The most important alkene is cyclopentadiene which has a TLV of only 75 ppm[62]. Exposure to the vapour at high concentrations may also cause liver damage and leukaemia[66].

The simple aromatic hydrocarbons encountered included benzene, toluene and substituted benzenes from C8 to C10 including most of the possible isomers. Over twenty identified compounds belonged to this category. The TLVs and inhalation toxicities of some of these compounds are shown in Table 8.2. General symptoms of intoxication are dizziness, mental confusion and, in severe cases, unconsciousness. Some of them are also strong sensory irritants. Although many of these compounds were also found in blood samples from the control groups, probably originating from evironmental sources, they were normally present at lower levels than in the fire fatalities.

The heterocyclic compounds encountered included furan, pyridine, pyrrole, thiophene and series of substituted compounds derived from these. Pyridine and its related compounds were found in blood samples from fire fatalities at similar levels to those from the control groups.

Table 8.2 Threshold limit value and inhalational toxicity of aromatic hydrocarbons[39,62].

Name	TLV (ppm)	Inhalational toxicity
Benzene	10	Causes dizziness and headache; high concentrations may cause unconsciousness. It is a chemical carcinogen which may cause leukaemia.
Toluene	100	Causes dizziness, headache, nausea and mental confusion.
Xylene	100	)
Styrene	100	)As above. The vapour also
Ethyl benzene	100	)irritates eyes and mucous
Isopropyl benzene	50	)membranes.
Trimethyl benzene	25	)

Information on the toxicity of these compounds is rare, but since they all give unpleasant odour, inhalation of these vapours causes irritation to eyes and respiratory tract.

The sulphur-containing compounds included carbon disulphide and dimethyl sulphide. Carbon disulphide is very toxic by inhalation with a TLV of only 10 ppm. High concentrations when inhaled produces narcotic effects and may result in unconsciousness. The vapour also gives an unpleasant odour and irritates the eyes. Although carbon disulphide and dimethyl sulphide have been found in the thermal decomposition products of sulphur-containing polymeric materials[37,64], it was found in our study that these compounds are also formed by putrefaction, probably from proteins. Therefore, it is difficult to evaluate their significance in putrefied blood.

Although a series of lower aliphatic alcohols from ethanol to hexanol, including most of the possible isomers, was found in some of the profiles, their presence was usually associated with high levels of ethanol. Therefore, it is thought that they originated mainly from the congeners present in alcoholic beverages. Their significancies in relation to fire toxicology is unknown.

In one case, high levels of trichlorofluromethane and 1,1,2-trichlorotrifluroethane were found in the blood of a fire fatality. These two compounds are used in fire extinguishers. It is thought that these vapours might have been inhaled during attempts to put out the fire. Unfortunately, the fire brigade report for this case was unavailable to confirm this.

So far, only the inhalational toxicity of these combustion products has been dealt with. The physiological and behavioural effects of these toxicants have not been taken into account because of the limited information available. Most of the toxicants encountered were either strong sensory irritants or depressants of the central nervous system, or a combination of both. It is most likely that they play a vital role in causing incapacitation of casualties in fires, which may account for the increasing proportion of fire fatalities who have died as a result of smoke and toxic gas inhalation.

In this study, a large amount of time has been spent on the identification of individual components in each profile and profile comparisions could only be carried out manually. Therefore, only a small number of samples could be studied within the time available. With the chromatographic and mass spectral data for these volatiles which are now available, it is hoped to develop a computer-aided profile comparision procedure which may drastically cut down the analytical time. During the past few years, a library of mass spectra of volatile compounds has been created in our data system. Therefore, computer-aided library search routines may be used more extensively in the future. Apparatus for headspace extraction is now becoming available commerically, in some cases in disposable form, which may save time in cleaning the apparatus, particularly the perfusion flasks used in this study. This would mean that blood samples from fire fatalities could be analysed routinely for their volatiles

composition, in order to provide a better view and more information, such as the trend or pattern of the blood volatiles profile with respect to the type of fire. The study of volatiles may also be extended to include those thermal decomposition products of higher molecular weight than those considered here, such as phosphate esters and polyaromatic hydrocarbons. Many of these compounds are known to be potentially dangerous to life.

8.4) Conclusions

The volatiles profile for different subjects varied widely in the number and relative intensities of the components. In general, blood samples from fire fatalaties showed more complex profiles than those from healthy or non-fire death controls. Over 140 compounds have been identified and these included series of carbonyls, nitriles, esters, alcohols, hydrocarbons, halogenated hydrocarbons, simple aromatic hydrocarbons, heterocyclic compounds and sulphur-containing compounds. Many of these compounds were only found in fire deaths or were found at elevated levels in fire fatalaties than in control groups. The exact toxicological effects caused by these toxicants are still unknown but it is likely that they play a significant role in causing incapacitation in fires.

Table 8.1 Volatiles observed in blood samples from fire deaths (FD), post mortem controls (PM), smoker controls (SC) and non-smoker controls (NSC).

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
500	Pentane	72	43	43	+				
541	1,3 Butadiene	54	39	54	+				
600	Hexane	86	57	57	+	+	+	+	
614	2,4-Dimethylpentane	100	43	41	+				
616	Trichlorofluoromethane	136	101	101	+				
621	1,1,2-Trichlorotrifluoroethane	186	101	151	+				
625	Diethyl ether	74	31	59	+	+	+	+	+
661	2-Methyl-1,3-butadiene	68	67	67	+				
678	Methylcyclopentane	84	56	56	+				
681	C_4H_4	52	52	52	+				
694	Acetaldehyde	44	44	44	+	+	+	+	+
700	Heptane	100	43	57	+				
717	Carbon disulphide	76	76	76	+	+	+	+	
724	Cyclopentadiene	66	66	66	+				
735	Dimethylsulphide	62	47	62	+	+	+	+	
760	?C_7H_{14}	98	-	-	+				
773	Furan	68	39	68	+				
776	Propionaldehyde	58	29	58	+	+	+	+	
800	Octane	114	43	57	+				
805	Isobutyraldehyde	72	43	72	+	+	+	+	
806	[$^2H_6$]Acetone (I.S.)	64	46	64	+	+	+	+	+
807	Acetone	58	43	58	+	+	+	+	+
820	Cyclohexadiene	80	79	79	+				

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
827	Unknown	112	55	-	+	+	+	+	
829	Acrolein	56	27	56	+				
840	Unknown	112	41	41	+				
848	2-Methylfuran	82	82	82	+				
852	Tetrahydrofuran	72	42	72	+				
857	Carbon tetrachloride	152	119	152	+				
858	n-Butyraldehyde	72	43	72	+				
862	1,1,1-Trichloroethane	132	97	97	+				
867	Hexamethylcyclotrisiloxane	222	207	207	+	+	+	+	+
870	Ethyl acetate	88	43	61	+	+	+	+	+
872	?1-Octene	112	70	-	+				
879	Methanol	32	31	31	+	+	+	+	
890	2-Butanone	72	43	72	+	+	+	+	
897	?Methylbutanal	86	41	58	+				
901	Dichloromethane	84	49	49	+	+	+	+	+
908	2-Propanol	60	45	59	+	+	+	+	
917	Ethanol	46	31	45	+	+	+	+	+
922	Benzene	78	78	78	+	+	+	+	
929	?Propyl isothiocyanate	101	101	101	+				
932	2,5-Dimethylfuran	96	96	96	+				
940	2-Methyl-1,3-dioxolane	88	73	73	+				
953	Butandione	86	43	86	+	+	+	+	
961	2-Pentanone	86	43	71	+	+	+	+	
964	Trichloroethylene	130	95	95	+				
967	Unknown	67	41	67	+				

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
970	Acrylonitrile	53	53	53	+		+		
975	3-Methyl-3-buten-2-one	84	43	84	+				
976	?2-Methylpentan-3-one	100	57	57	+				
985	[$^2H_3$]-Acetonitrile (I.S.)	44	44	44	+	+	+	+	+
986	Acetonitrile	41	41	41	+		+		
986	?C_4-nitrile	69	42	68	+				
991	Chloroform	118	83	83	+	+	+	+	+
1000	Decane	142	57	57	+				
1004	2-Butanol	74	45	45	+		+		
1004	4-Methyl-pentan-2-one	100	43	43	+				
1005	Thiophene	84	84	84	+				
1006	Tetrachloroethylene	164	166	166	+				
1012	Ethyl isobutyl ether	102	59	59	+				
1013	[$^2H_2$[-Propionitrile (I.S.)	58	56	56	+	+	+	+	+
1014	Propionitrile	55	54	54	+				
1017	Unknown	-	93	-	+				
1018	1-Propanol	60	31	59	+	+			
1018	Toluene	91	91	91	+	+	+	+	+
1032	$C_6H_{12}O$ Ketone	100	43	58	+	+			
1037	2,3-Pentanedione	100	43	57	+	+			
1047	Octamethylcyclotetrasiloxane	296	281	281	+	+	+	+	+
1050	Dimethyl disulphide	94	94	94	+	+	+	+	
1062	Hexanal	100	44	56	+	+	+	+	
1068	Unknown	-	45	-	+			+	
1072	2-Methyl-propan-1-ol	79	43	43	+				
1082	cis-Crotonitrile	67	41	67	+				

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
1090	n-Butyronitrile	69	41	41	+				
1100	C_{11}-Alkane	156	57	57	+				
1103	2-Pentanol	88	45	45	+				
1104	$C_7H_{14}O$-Ketone	114	43	58	+				
1106	Ethylbenzene	106	91	91	+	+	+	+	+
1109	p-Xylene	106	91	91	+	+	+	+	+
1116	m-Xylene	106	91	91	+	+	+	+	+
1121	$C_{10}H_{16}$?Gamma-terpinene	136	93	93	+				
1123	1-Butanol	74	31	56	+	+	+	+	
1129	Hydrocarbon	-	43	-	+				
1131	Nitromethane	61	61	61	+				
1135	Unknown	-	57	-	+	+	+	+	
1142	2-Hexanol	94	45	69	+				
1144	trans-Crotonitrile	67	41	67	+				
1149	Isopropylbenzene	120	105	105	+	+	+	+	
1157	$C_{10}H_{16}$-Terpene	136	121	121	+	+			
1158	Unknown	-	73	-	+	+	+	+	
1162	o-Xylene	106	91	91	+	+	+	+	+
1164	Unknown	154	43	111	+				
1175	Limonene	136	68	68	+	+	+	+	
1178	Cyclopentanone	84	55	55	+				
1184	Amyl alcohol	88	42	55	+	+			
1186	n-Propylbenzene	120	91	91	+	+			
1190	C_8-Ketone	128	43	58	+				
1194	Pyridine	79	79	79	+	+	+	+	

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
1201	m-Ethylmethylbenzene	120	105	105	+	+	+	+	
1211	1-Pentanol	88	42	42	+				
1217	1,3,5-Trimethylbenzene	120	105	105	+				
1218	Unknown	-	73	-	+				
1223	p-Ethylmethylbenzene	120	105	105	+	+	+		
1225	2-Methylpyridine	93	93	93	+	+	+	+	
1227	Styrene	104	104	104	+	+	+	+	
1232	3-Octanone	128	43	57	+				
1237	o-Ethylmethylbenzene	120	105	105	+	+	+	+	
1245	$C_{10}H_{14}$ *	134	119	119	+	+	+		
1253	$C_{10}H_{16}$-Terpene	136	93	93	+		+		
1254	3-Hydroxybutan-2-one	88	45	45	+				
1255	Methylpyrazine	94	94	94	+				
1256	1,2,4-Trimethylbenzene	120	105	105	+	+			
1272	Unknown	-	119	-	+				
1274	$C_{10}H_{14}$ **	134	105	105	+	+			
1282	Cyclohexanone	98	55	98	+	+			
1294	Tetramethylbenzene	134	119	119	+	+			
1300	Methylstyrene	118	118	118	+	+	+	+	
1309	1,2,3-Trimethylbenzene	120	105	105	+	+			
1313	1-Hexanol	102	56	56	+	+			
1315	3-Methylpyridine	93	93	93	+	+	+		
1321	$C_{10}H_{14}$ *	134	119	119	+				
1323	Phenylacetylene	102	102	102	+				
1325	Methylstyrene	118	118	118	+	+			

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
1329	$C_{10}H_{14}$ *	134	119	119	+				
1335	4-Methylpyridine	93	93	93	+		+		
1336	$C_{10}H_{14}$ *	134	119	119	+				
1341	o-Methylstyrene	118	117	117	+	+	+		
1349	Unknown	96	96	96	+				
1350	Unknown	-	126	-	+	+	+	+	
1363	Cyclohexanol	100	57	57	+		+		
1378	Unknown	-	75	-	+				
1392	Hydrocarbon	-	57	57	+	+	+	+	
1397	Dichlorobenzene	146	146	146	+	+	+	+	+
1416	Furfural	96	96	96	+	+			
1437	Indene	116	116	116	+	+			
1443	4-Vinylpyridine	105	105	105	+				
1447	Pyrrole	67	67	67	+	+	+	+	
1452	Benzofuran	118	118	118	+				
1455	? Di-tert-butylmethylphenol	220	205	205	+		+	+	
1456	2-Furyl methyl ketone	110	95	95	+				
1473	Benzaldehyde	106	77	77	+	+	+	+	
1480	Camphor	152	95	95	+				
1508	$C_{10}H_{16}$-Terpene	136	-	-	+	+			
1514	? Dichlorocyclohexane	152	81	81	+				
1523	Benzothiazole	135	135	135	+				
1524	Methylindene	130	130	130	+				
1533	Methylbenzofuran	132	131	131	+				
1549	Benzonitrile	103	103	103	+				

Table 8.1 (Continued)

Retn. Index	Compound Name	Mol. Wt.	Base Peak	S.I.	Occurrence FD	PM	SC	NSC	Blk
1563	Diphenylamine	169	169	169	+				
1575	Trichlorobenzene	180	180	180	+				
1580	Phenylbenzene	154	154	154	+				
1590	Phenol	94	94	94	+				
1595	Acetophenone	120	105	105	+	+			
1598	Tolunitrile	117	117	117	+	+			
1624	Alpha-terpineol	154	59	59	+				+
1898e	2-Methylnaphthalene	142	142	142	+				
1915e	1-Methylnaphthalene	142	142	142	+				

$C_{10}H_{14}$ * = Dimethylethyl- or methylisopropylbenzene
$C_{10}H_{14}$ ** = sec-Butyl- or methyl-n-propylbenzene
e = Retention time outside the temperature programme range, retention index was estimated from naphthalene.

CHAPTER 9: ROUTINE FIRE TOXICOLOGY AND PATHOLOGY

9.1) Introduction

This chapter describes the pathological examination and toxicological analyses routinely carried out on each fire fatality at the Department of Forensic Medicine and Science, University of Glasgow, during the period from July 1976 to February 1982. The study was originally confined to the Strathclyde region of Scotland and was later extended to cover other areas in the United Kingdom with the assistance of pathologists in the British Association in Forensic Medicine. During this period, 227 fire fatalities which occurred in Strathclyde and 71 fire fatalities which occurred in other parts of the United Kingdom were included in this study. These were cases who had died in fires in buildings either at the fire scene or during the journey to the hospital, but excluded those deaths which had resulted from arson or which involved fires in vehicles following road traffic accidents[6].

9.2) Experimental

9.2.A) Pathology

1) Strathclyde study

A detailed examination of each fire death was carried out by forensic pathologists of the Department of Forensic Medicine and Science, Glasgow University. The body was examined for physical injuries, such as burns or broken bones and for microscopic features, such as bacterial

infection or damage to the respiratory epithelium. Other pre-existing diseases such as heart disease or chronic bronchitis were also taken into account to establish the cause of death, since the condition of these vital organs may have affected the ability of the individual to survive in a fire situation.

Burn injuries were measured in terms of the percentage of the body surface area affected using the 'Rule of Nine' [183], by which the head and each arm are allotted 9 per cent of the body surface, the front and the back of the trunk or each leg 18 per cent and the neck or external genitalia 1 percent. Taking into the account the burn injuries observed only, the 'probability of mortality' was assessed using clinical tables[184].

The respiratory tract was examined for injuries such as pulmonary oedema, congestion, haemorrhage, broncho-constriction, sloughing of the epithelial lining, collapse of the lung and production of mucopurulent material.

2) U.K. study

Pathologists who participated in this study were sent a questionaire to provide information on the circumstances in each case and were requested where possible, to provided a copy of the autopsy report.

Specially washed vials were provided for the collection of blood samples, which were returned by first-class post in containers complying with Post Office requirements. The average delay between the fire and receipt of the sample was 5 days (range 0.5 - 18 days).

Toxicological analyses were normally carried out immediately on receipt or, if this was not possible, samples were stored at 4°C overnight.

9.2.B) Toxicology

Samples of blood, urine, trachea and major organs were removed at the autopsy for histology and toxicology. The toxicological aspect of the project included the analysis of carboxyhaemoglobin and cyanide in blood, alcohol in blood or urine, inorganic elements in trachea and the presence of drugs in liver or blood.

Carboxyhaemoglobin levels were measured using the CO-Oximeter (Model 282, Instrumentation Laboratory)[185] with confirmation by gas chromatography where necessary, for example in cases with high methaemoglobin levels[156]. The level of carboxyhaemoglobin was expressed as the percentage of the total haemoglobin present. Cyanide measurements were carried out by gas chromatography-mass spectrometry or gas chromatography with electron capture detection[186]. Blood cyanide concentrations were expressed in micromoles of cyanide per litre of blood (µmole/l). These can be converted to µg hydrogen cyanide per 100 ml blood by multiplying with a factor of 2.7. Alcohol in blood or urine was measured by gas chromatography[187] and the result was expressed in milligrams of alcohol per 100 millilitre of blood or urine (mg/dl). Samples of blood or liver were analysed for the presence of drugs using a spectroscopic procedure for barbiturates, or by high-pressure liquid chromatography for

benzodiazepines[188]. Concentrations of inorganic elements in tracheal samples were determined using neutron activation analysis and atomic absorption spectroscopy[67,189].

9.3) Results and Discussion

The types of fatal fire included in this study are shown in Table 9.1. As in the national pattern of fire statistics, the majority of fatal fires in occupied buildings occurred in dwellings. Amongst these fatalities, older people, particularly those over 60, formed a much higher proportion of the population of fire fatalities than the normal population distribution in U.K. (Table 9.2). The fatal fires included in this study occurred mostly in the winter months and during the weekend period, and in this respect followed the pattern observed in the national fire statistics[190]. Recent work in the Fire Research Station has shown a remarkably linear inverse relationship between the incidence of fires and temperature[191].

A summary of the pathological features observed in fire fatalities is shown in Table 9.3. Burn injuries had been sustained in 229 cases (82 %) but it was often not possible to establish whether burns were the main cause of death as charring of the body continued to take place after death.

It is generally assumed that thermal injury to the respiratory tract is limited to the upper part[192,193] and that injury to the deeper parts is likely to be caused by

Table 9.1 Types of fatal fire included in the study.

Type of fire	No. of fires	Fatalities No.	Fatalities %
Dwellings	233	263	88
Caravans	4	4	1
Hotels, hostels, homes	6	14	5
Industrial premises	2	3	1
Other	4	14	5
Total	249	298	100

Table 9.2 Age distribution of fire fatalities.

Age group	Normal population distribution (%) [a]	Fatalities in this study No.	Fatalities in this study %	National fire statistics [b] No.	National fire statistics [b] %
< 10	15.8	37	12	90	10
10 - 19	17.8	23	8	59	6
20 - 29	13.8	17	6	69	7
30 - 39	11.6	11	4	64	7
40 - 49	11.5	27	9	63	7
50 - 59	11.5	40	13	98	10
60 - 69	10.2	48	16	113	12
70 - 79	6.0	33	11	164	17
> 79	1.9	45	15	155	16
Unknown	–	17	6	71	8
Total	100.1	298	100	946	100

[a] Figures taken from the Annual Report of the Registrar-General (1976).
[b] Figures taken from Reference 190.

Table 9.3 Pathology of fire fatalities.

Pathological feature	No. of cases	% of cases
Burn injuries present	229	82
Burn injuries absent	50	18
Burn injuries not known *	19	-
Respiratory system injury	209	83
Soot deposition in airways	232	92
Cardiovascular disease implicated in cause of death	4	2

* These are excluded from the calculation of the percentage of cases showing burns.

Table 9.4 Carboxyhaemoglobin levels in fire fatalities.

HbCO (%)	No. of cases	% of cases	Toxicological significance	% of total
< 1	5	2	) Normal range[a]	15
1 - 9	38	13	)	
10 - 19	20	7	) Below fatal level[b]	47
20 - 29	24	8	)	
30 - 39	27	9	)	
40 - 49	24	8	)	
50 - 59	31	10	) Above fatal level[b]	52
60 - 69	57	19	)	
70 - 79	53	18	)	
> 79	15	5	)	
Unknown	4	1		
Total	298	100		

[a] In our control study, the mean HbCO level in non-smokers (n=252) was 3.3 %, and that in smokers (n=270) was 5.0 %.

[b] The threshold for fatal carbon monoxide poisoning is assumed to be 50 % HbCO (see text).

chemical agents rather than by heat, although it may be difficult to distinguish between these in some cases.

Soot deposition on the walls of the air passages provided evidence of smoke inhalation. The soot was found as a mucous-bound layer and was readily visible to the naked eye as far down as the small bronchi of the lung. Soot was very rarely observed beyond the terminal bronchi. In cases where soot particles were observed in the bronchioles and alveolar spaces, it was often difficult to distinguish between the "new" soot from the fire and "old" soot resulting from cigarette smoking and air pollution. One exception to this problem was a young infant who had been brought up in a smoke-free environment. In this case, alveolar soot particles were considered to have originated in the fire alone.

Most of the fatalities (92 %) showed the presence of soot in the airways and 83 per cent of the fatalities showed evidence of injury to the respiratory system. Four fatalities in the present study were found to have sustained recent heart attacks. In two cases, the carboxyhaemoglobin level was low (<1 % and 5%) and no soot was found in the upper air passages, suggesting that death occurred either prior to or very soon after the start of the fire. This is important from a medico-legal viewpoint in determining the cause of death.

Normally, the accepted fatal level for carbon monoxide poisoning is 60 per cent saturation of the total haemoglobin[194]. However, fire victims are subjected to additional stress by heat, depleted oxygen supply and

elevated carbon dioxide. Therefore, in the present study, the fatal threshold has been assumed to be 50% HbCO, in accordance with other workers in the field of fire toxicology[195,196]. Based on this assumption, carbon monoxide alone could account for the deaths of about 50 per cent of the cases (Table 9.4). Many of the fatalities in this group had little or no burn injuries. In fact, of those who showed no burn injury at all, over 90 % had fatal levels of carboxyhaemoglobin. Carbon monoxide intoxication was considered to be a contributing factor in the cause of death of a further 32 per cent of the cases in which the carboxyhaemoglobin level was above the normal control range but below the fatal threshold level. Some evidence was observed that advanced cardiovascular disease increased susceptibility to carbon monoxide poisoning with a consequent lowering of the threshold for fatal poisoning (Chi square test, $1\% < P < 5\%$). No other significant additive effects were observed between carbon monoxide and other factors operating in the fire.

Over half of the cases were found to have a blood cyanide level above the maximum found in the controls (Table 9.5). Considerable uncertainty still exists in interpreting blood cyanide levels and in establishing the fatal threshold for cyanide poisoning[48]. With reference to published data[197,198], significant but non-fatal toxic effects from cyanide poisoning appear to begin in the region of 50 μmol/l and serious life risk occurs above 100 μmol/l. In this study, 20 per cent of the fatalities may have suffered significant incapacitation as a result of

Table 9.5 Blood cyanide concentrations in fire fatalities.

Cyanide level (μmol/l)	No. of cases	% of cases	Estimated toxicological significance
0 - 9	67	26	Normal range
10 - 19	53	21	Normal range in cigarette smokers
20 - 49	66	26	Possible toxic effects
50 - 99	51	20	Significant but non-fatal effects
> 99*	17	7	Potentially fatal
Unknown	44	-	
Total	298	100	

* Highest level observed was 238 umol/l.

Table 9.6 Blood alcohol concentrations in fire fatalities.

Concentration (mg/100ml)	No. of cases	% of cases
Negative	151	51
< 50	25	8
50 - 100	15	5
100 - 150	11	4
150 - 200	19	6
200 - 300	47	16
> 300*	30	10
Total	298	100

* Highest concentration observed was 585 mg/100ml.

cyanide poisoning and a further 7 per cent were likely to have had severe life risk from cyanide intoxication.

It must be noted that these figures do not take into account the effects of other factors such as heat, oxygen depletion and the presence of carbon dioxide, which cannot be determined after the fire. However, the data were examined for possible interactions between cyanide and carbon monoxide which may have influenced the susceptibility of the victim to the fire, but no significant correlation between the two was observed. In the early stages of the study, plasma thiocyanate levels were measured as a potential index of metabolised cyanide[199]. The results obtained were examined using Student's t-test. No direct correlation between blood cyanide and plasma thiocyanate levels in fire fatalities was observed (n=112, r=0.31). The plasma thiocyanate level is therefore of little value as an indicator of transformed cyanide.

In our study, one of the most prominent contributory factors in fire deaths was the consumption of alcohol before the fire. About half of the fatalities were found to have alcohol in their blood (Table 9.6), and in 32 per cent of the cases the level was above 150 mg/100ml (the current legal maximum limit for drivers of motor vehicles in U.K. is 80 mg/100ml). At these levels, the effects of alcohol would have been sufficient to cause marked symptoms of intoxication and would severely impair the ability to cope with a fire situation either in fighting the fire or escaping from it. Perhaps in many of these cases, the fire

was started accidentally under the influence of alcohol. A high percentage of the fatalities who had blood alcohol concentrations above 200 mg/100ml were expected to be severely intoxicated with alcohol at the time of death. Three other studies of fire fatalities in America, Norway and Denmark were also found to have a high incidence of alcohol abuse[34,196,200].

In the Glasgow study, only 18 cases (8%) showed the presence of drugs and these were mostly tranquillizers and sedatives, all at therapeutic levels.

Details of the results of the analysis of inorganic elements in the tracheas of fire fatalities has already been published[67]. These can be briefly summarised as follows: the tracheal concentrations of inorganic elements including antimony, bromine, zinc, chromium, cobalt, iron, lead, cadmium and gold were increased in fire fatalities, compared with controls. The raised elemental levels suggest that there may be a potential risk from such inorganic compounds during exposure to smoke and fire gases. An elevated level of antimony was found in 50 per cent of cases studied although the chemical form has not yet been established. The toxicological significance of these elements with respect to fire deaths remains unknown.

9.4) Conclusions

Most of the fire fatalities (82%) in this study had severe burn injuries. It was not possible in many cases to establish the role played by burns in the cause of death

as charring of the body had continued after death. Another prominent pathological feature of the fatalities was that 92 per cent of the cases showed evidence of smoke inhalation and 83 per cent showed subsequent injuries to the respiratory system. Carbon monoxide is still considered to be the most important toxicant in fires. In this study, 52 per cent of the deaths could be attributed to carbon monoxide poisoning alone. Hydrogen cyanide has also been shown to play a significant role in causing deaths in fires. Seven per cent of the cases studied were likely to have suffered severe cyanide poisoning. No additive or synergistic effects between cyanide and carbon monoxide were observed. Alcohol abuse was found to be a prominent contributory factor in fire deaths. About a quarter of the fatalities were likely to have been severely intoxicated with alcohol at the time of death. Although elevated levels of inorganic elements were found in tracheal samples from fire fatalities, their significance in fire toxicology is still unknown.

SECTION IV: GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 10: DISCUSSION AND CONCLUSIONS

10.1) Discussion

10.1.A) Fire problem

The awareness of increased hazards associated with fires has led to extensive studies of combustion toxicology. A great deal of progress has been made in understanding the basic physics and chemistry of fires and combustion products. However, post-mortem studies on fire fatalities have been extremely few in number and chemical analysis on post-mortem samples has in the past been focussed only on carboxyhaemoglobin, cyanide and alcohol. The role played by other toxicants in causing fire fatalities has not yet been established. It would seem to present an imbalance when considerable effort is being devoted to the identification and measurement of toxicants produced in fires whereas little work is being done on the measurement of these materials in fire casualties. The aim of the project within the context of which the present study was carried out was to provide an overall picture as to the nature of inhalational toxicity resulting from fire exposure.

It has been well recognised that one of the major hazards associated with fire is obscuration of vision caused by smoke. However, the inability to find an exit due to impairment of vision caused by irritants may be just as vital for the individual caught in a fire. In an experiment carried out by Lopez to study smoke emission

from burning aircraft cabin materials[201], it was concluded that the dominating factor on human visibility was the irritating effects of combustion products as opposed to light obscuration.

It must be understood that many people died in fires only because they were unable to escape. It is therefore important to relate the escape times and the rates of generation of hazardous fire products. It has been suggested that at the early stages of a fire, risk to life is from smoke and other degradation products rather than from the fire temperature. It is a common belief that when modern synthetic polymers are burnt, they produce deadly poisonous fumes, but recent studies have shown that the combustion and degradation products from synthetic polymers are no more toxic than those from conventional materials[182,202]. The increased fire hazard with synthetic polymers is in fact associated with the ignitability and flame spread rates. In an attempt to reduce the fire hazard, fire retardant additives have been used. However, the incorporation of fire retardants often leads to the formation of highly toxic products and of copious amounts of smoke. For example, organophosphorous compounds have been used in a rigid polyurethane foam which, when it is burnt, gives off a highly neurotoxic bicyclophosphate ester[203,204]. Also, when halogenated polyolefins are burnt, they generate greater amounts of smoke than the corresponding non-halogenated polymers[205].

So what can be done to reduce the fire hazards? In the past, the world effort has been confined to laboratory

measurements to assess the toxicological hazards associated with fire, in an attempt to impose controls by legislation on the use of materials in buildings. All these studies fall into two basic categories: i) measurement of smoke and toxic products, and ii) toxicological assessment involving the use of animal models.

Measurement of smoke production and detailed chemical analysis of gaseous products has been extensively studied in both small scale and large scale laboratory tests and still comprises the largest proportion of studies in this field. Test methods which are currently employed in these studies are too numerous to be mentioned here (see Reference 206). Some of the difficulties in adapting these test methods in legislation are that they involve a complex multivariant system and it is often difficult to relate laboratory tests to real fire situations.

Inhalation toxicity of combustion products is also a complex problem involving a reliable animal model, a controlled combustion process and a sensitive analytical method. Some of the problems associated with determining the toxicology of combustion products have been discussed by MacFarland[207]. He pointed out that in these studies, the agents and dosage usually remain unknown due to the difficulty in measuring the complex products formed and due to the fact that the composition and concentration of products change with time and experimental conditions. Measurements and correlation of toxicants in the environment and in the biosystem have not been done to any great extent in the biological assessments. Biochemical

analysis, predominately blood chemistry, has so far been limited to CO and perhaps HCN.

10.1.B) Blood volatiles analysis

The analysis of blood volatiles reported in this thesis began in August 1981. Within the time available, thirty-one profiles (including 10 controls) were studied. Of the 21 fire fatalities for which volatiles profiles were examined, 17 cases (81%) showed severe burn injuries. Elevated levels of carboxyhaemoglobin were found in 19 cases (90%) and 13 cases (62%) had fatal levels of carboxyhaemoglobin. Elevated levels of blood cyanide were also found in 12 cases (63%) with evidence of severe cyanide intoxication in 1 case (5%). These statistics resemble closely those of the whole set of fire fatalities which were studied in our Department, indicating a true random sample of the whole set. The results from the blood volatiles study may therefore be used as an indication of the whole population.

The analysis of organic volatiles in blood has indicated that more complex profiles and higher levels of carbonyls and nitriles were found in fire fatalities than in controls. Of particular concern were the very high levels of acetonitrile found in most of the fire deaths. This toxic compound might play a very significant role in causing intoxication of the victims in fires and could possibly be one of the most important fire gases next to carbon monoxide and hydrogen cyanide. Close examination of the volatiles profiles also indicated that those compounds

which might be of significance fell into two main catagories: those which were strong sensory and respiratory irritants, such as the aldehydes, styrenes and xylenes; secondly, those which were depressants of the central nervous system, such as the nitriles, benzene and toluene. The concept of incapacitation following exposure to fire gases at sublethal levels has long been recognised. Although the degree of intoxication due to inhalation of toxic gases has yet to be established, the results obtained in this study have clearly demonstrated that most people dying in fires have been exposed to a wide range of highly toxic fire gases.

While it is unlikely that any one of these toxicants alone (apart from CO and CN^-) would account for the deaths, the possibility of additive or synergistic effects cannot be neglected. The possibility of synergistic action between two or more toxicants in the fire environment has frequently been suggested in the literature[208-210]. To establish whether any synergism exists between two toxicants can sometimes be very difficult, let alone the complex mixture of toxicants that are present in the fire environment. However, recent studies on animals exposed to thermal degradation products from various polymeric materials have shown that despite the complexity in chemical composition of the test atmospheres, the basic toxic effects on the animals were relatively simple and were always dominated by carbon monoxide, hydrogen cyanide or irritants[211].

10.1.C) Future work

Fire fatality studies during the last decade or so have provided valuable information on the importance of carbon monoxide and hydrogen cyanide in fires. However, very little is known about the role played by other fire toxicants due to the lack of reliable and sensitive assays for their measurement. Without a doubt, this area is the one which exhibits both the greatest neglect in the past and also the greatest promise of rapid development in the future given even a modest investment of effort and resources.

Studies on animals exposed to combustion and thermal degradation products from various polymeric materials have so far been limited to the correlation of observed toxicological effects with the carboxyhaemoglobin levels in blood. From the viewpoint of combustion toxicology, it is still an incomplete and unsatisfactory assessment of fire hazards. A more logical approach would be to include the correlation of toxicants in the test environment and in the biosystem. The analytical method developed in this study has shown some success in measuring these gaseous toxicants in blood. This, in conjunction wih animal exposure studies, would undoubtedly lead to a better understanding of how people died in fires.

With respect to the technical aspects of this work, one of the greatest needs is in reducing the time required to carry out each sample, thereby making it a routine procedure. Automation would be of great value in this connection. Further development of the analytical method

may be required for the quantitation of major volatile components in blood which were overloaded in the mass chromatograms. These volatiles included acetaldehyde, acetone and acetonitrile. This was mainly due to the limited dynamic range of the analytical instrument, particularly the analogue to digital converter. The study of volatiles may also be extended to include thermal decomposition products of higher molecular weight than those considered in this project. The new generation of chemically bonded fused silica chromatographic columns shows promise in being suitable for both low and middle range molecular weight materials with an operating temperature range from sub-ambient to 300°C. Many of these compounds such as phosphate esters and polyaromatic hydrocarbons are known to be potentially dangerous to life. Also, analysis of metabolites of reactive species, eg. acrolein.

10.2) Conclusions

1) The extraction of organic volatiles from biological fluids using the sorption technique has shown some success in measuring the gaseous toxicants which are produced during the course of a fire.

2) The assay was found to be more sensitive and versatile than the DNP-derivatisation technique (specific for carbonyl compounds) or the static headspace gas chromatography used in this study. The experimental conditions used permitted detection of volatiles down to the ng/ml level and the use of stable-isotopically labelled

internal standards improved the precision of the assay to around 5 to 10 per cent.

3) Blood volatiles profiles from different subjects varied widely both in the number and the concentration of components. In general, blood samples from fire fatalities showed more complex profiles than those from controls.

4) Over 140 chemical species in these profiles have been identified by mass spectrometry. These include series of carbonyls, nitriles, alcohols, esters, aliphatic hydrocarbons, halogenated hydrocarbons, aromatic hydrocarbons, heterocyclic compounds and sulphur-containing compounds.

5) Quantitative measurements of carbonyls and nitriles in blood have shown that many of these compounds were either absent in the control samples or present at a lower concentration than in the fire fatalities.

6) Of particular concern were the very high levels of acetonitrile and the presence of acrolein in the blood of some fire fatalities. Since degradation of acrolein has been shown to occur in blood, many other samples might have had high levels of acrolein at the time of death.

7) Although the toxicological significance of these gaseous toxicants in causing fire fatalities has not yet been established, it is likely that they played a contributory role in causing incapacitation or even intoxication in fires.

8) Results from the analysis of blood volatiles, carboxyhaemoglobin and cyanide have provided strong evidence that smoke and toxic gases account for more deaths

than those resulting directly from burn injuries.

APPENDICES AND REFERENCES

Appendix 1 Concentrations of carbonyl compounds in blood samples from fire fatalities, post-mortem controls and normal human controls. All concentrations are expressed in ng/ml. Levels of blood ethanol, carboxyhaemoglobin and blood cyanide are included for comparision purposes (see Chapter 9 for experimental details).

(A) Fire fatalities

Compound name	Subject number 1	2	3	4	5	6	7	8	9	10	11
Acetaldehyde	>271	>480	>231	>117	>407	>363	>500	>482	234	>417	96
Propionaldehyde	85	26	23	14	11	16	12	27	14	21	17
Isobutyraldehyde	29	56	15	6	43	33	31	25	43	84	27
Acetone	>282	>148	>271	>73	>123	>246	>525	>326	>126	>239	>141
Acrolein	14	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	28	N.D.
n-Butyraldehyde	N.D.	8	14	7	1	3	9	31	N.D.	20	7
2-Butanone	249	N.D.	3	60	19	29	68	57	32	53	36
Butandione	13	B	B	131	73	B	29	B	81	359	58
2-Pentanone	27	B	B	19	7	B	14	B	6	N.D.	9
4-Methyl-2-pentanone	2	B	B	6	2	B	N.D.	9	<1	N.D.	3
Cyclopentanone	15	5	1	24	11	N.D.	4	13	23	282	28
Cyclohexanone	N.D.	N.D.	N.D.	<1	4	A	N.D.	2	13	N.D.	2
Hexanal	29	7	16	93	96	141	11	126	17	165	138
Benzaldehyde	20	75	16	62	132	87	67	39	165	120	60
Acetophenone	A	C	A	C	A	A	A	A	A	N.D.	1
Blood ethanol (mg/dl)	-ve	193	113	-ve	-ve	67	-ve	49	-ve	72	-ve
HbCO (%)	63	16	69	59	61	31	58	71	45	81	71
Blood cyanide (uM)	1	15	101	7	18	64	5	14	18	3	C

Notes : N.D. = not detected
A = found, but peak area equal to or smaller than that in the blank sample.
B = peak area could not be measured because of a large overloaded ethanol peak in the chromatogram.
C = was not measured.

Appendix 1 continued.

Compound name	Subject number 12	13	14	15	16	17	18	19	20	21
Acetaldehyde	123	49	>187	>298	>263	>111	>610	53	47	>87
Propionaldehyde	23	41	53	10	8	64	A	48	16	41
Isobutyraldehyde	23	74	21	52	54	99	8	5	53	81
Acetone	>108	>235	>141	>155	>196	>287	>176	>222	>122	>156
Acrolein	N.D.	N.D.	4	N.D.	N.D.	N.D.	N.D.	8	46	N.D.
n-Butyraldehyde	A	6	1	8	A	11	14	A	A	A
2-Butanone	24	57	4	39	77	52	59	3	34	66
Butandione	20	185	463	669	1720	205	13	166	60	132
2-Pentanone	12	25	2	124	226	310	353	2	17	17
4-Methyl-2-pentanone	N.D.	2	N.D.	6	6	N.D.	N.D.	N.D.	<1	N.D.
Cyclopentanone	18	>105	3	C	C	34	201	2	7	>98
Cyclohexanone	53	3	A	9	N.D.	N.D.	56	A	A	A
Hexanal	100	349	381	174	C	486	C	A	184	173
Benzaldehyde	77	256	65	193	160	217	36	A	95	125
Acetophenone	A	3	A	3	A	A	19	A	A	2
Blood ethanol (mg/dl)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	12	-ve	-ve
HbCO (%)	56	32	3.1	71	38	15	83	1	71	77
Blood cyanide (uM)	32	20	8.6	5	6	19	29	1	C	37

Appendix 1 continued.

	(B) Post-mortem controls						(C) Smoker controls		(D) Non-smoker controls	
	Subject number									
Compound name	1	2	3	4	5	6	1	2	1	2
Acetaldehyde	>163	111	76	107	>180	>84	40	53	189	81
Propionaldehyde	6	114	13	48	266	28	24	21	17	33
Isobutyraldehyde	54	53	49	63	103	73	84	92	137	136
Acetone	>168	>231	>263	>150	>178	>565	>236	>214	>217	>301
Acrolein	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
n-Butyraldehyde	A	24	6	32	31	1	3	5	A	9
2-Butanone	7	33	12	23	14	18	17	20	11	25
Butandione	55	96	133	96	66	73	34	24	10	58
2-Pentanone	6	9	3	7	5	3	6	13	7	6
4-Methyl-2-pentanone	N.D.	N.D.	N.D.	1	N.D.	N.D.	<1	A	<1	<1
Cyclopentanone	A	N.D.	A	3	N.D.	N.D.	1	A	<1	2
Cyclohexanone	N.D.	6	1	A	18	A	A	1	A	A
Hexanal	9	23	11	22	87	35	56	26	55	44
Benzaldehyde	88	138	55	130	135	165	124	140	98	175
Acetophenone	A	A	A	A	A	3	A	A	A	A

Appendix 2 Concentrations of nitriles in blood samples from fire fatalities, post-mortem controls and normal human controls. All concentrations are expressed in ng/ml. Levels of blood ethanol, carboxyhaemoglobin and blood cyanide are included for comparision purposes (see Chapter 9 for experimental details).

(A) Fire fatalities

	Subject number										
Nitrile	1	2	3	4	5	6	7	8	9	10	11
Acrylonitrile	N.D.	B	B	<1	<1	B	<1	B	2	N.D.	3
Acetonitrile	53	B	B	121	69	B	56	B	54	>215	>85
Propionitrile	19	27	4	6	5	1	11	2	5	22	9
Crotonitrile	N.D.	8	6	6	2	2	<1	<1	2	14	7
Benzonitrile	1	23	12	A	A	17	3	2	15	A	12
Blood ethanol (mg/dl)	-ve	193	113	-ve	-ve	67	-ve	49	-ve	72	-ve
HbCO (%)	63	16	69	59	61	31	58	71	45	81	71
Blood cyanide (uM)	1	15	101	7	18	64	5	14	18	3	?

Notes : N.D. = not detected
A = found, but peak area equal to or smaller than that in the blank sample.
B = peak area could not be measured because of a large overloaded ethanol peak in the chromatogram.

Appendix 2 continued.

Nitrile	Subject number 12	13	14	15	16	17	18	19	20	21
Acrylonitrile	<1	8	3	2	2	A	A	A	3	14
Acetonitrile	66	>135	>85	>106	>61	>187	>47	45	129	208
Propionitrile	6	>35	10	11	11	37	6	<1	15	114
Crotonitrile	3	12	12	26	56	67	61	2	11	60
Benzonitrile	16	44	33	10	4	73	9	A	19	78
Blood ethanol (mg/dl)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	12	-ve	-ve
HbCO (%)	56	32	3.1	71	38	15	83	1	71	77
Blood cyanide (uM)	32	20	8.6	5	6	19	29	1	?	37

	(B) Post-mortem controls						(C) Smoker controls		(D) Non-smoker controls	
Nitrile	Subject number 1	2	3	4	5	6	1	2	1	2
Acrylonitrile	N.D.	N.D.	<1	N.D.	N.D.	N.D.	2	<1	<1	3
Acetonitrile	2	4	3	4	12	24	67	21	1	3
Propionitrile	N.D.	N.D.	N.D.	N.D.	<1	N.D.	N.D.	N.D.	N.D.	N.D.
Crotonitrile	N.D.	N.D.	<1	<1	<1	N.D.	<1	<1	N.D.	2
Benzonitrile	A	A	A	N.D.	A	A	A	A	N.D.	A

Appendix 3 Structural formula and mass spectral data of carbonyls and nitriles.

Name	Structural formula	Principal ions in mass spectrum m⁺(I%)
Acetaldehyde	CH_3CHO	29(100), 44(77), 43(42), 42(12), 26(5), 41(5), 27(3), 25(3)
Acetone	CH_3COCH_3	43(100), 58(35), 28(7), 42(7), 27(5), 59(2)
Acetophenone	$C_6H_5COCH_3$	105(100), 77(75), 120(28), 51(25), 43(15), 50(9), 78(8), 106(8)
Acrolein	$CH_2=CHCHO$	27(100), 56(64), 55(49), 26(49), 28(38), 29(37)
Benzaldehyde	C_6H_5CHO	77(100), 106(91), 105(89), 51(33), 50(16), 40(16), 29(10), 78(9)
Butandione	$CH_3COCOCH_3$	43(100), 86(16), 28(8), 42(6), 44(2), 32(2), 41(1), 87(1)
2-Butanone	$CH_3COC_2H_5$	43(100), 29(43), 72(25), 27(23), 42(11), 57(9), 44(9), 26(6)
Isobutyraldehyde	$(CH_3)_2CHCHO$	43(100), 41(66), 72(43), 27(40), 29(13), 39(12), 40(9)
n-Butyraldehyde	C_3H_7CHO	43(100), 72(90), 44(89), 27(80), 41(78), 57(70), 29(63), 55(54)
Cyclohexanone	$C_5H_{10}CO$	55(100), 28(82), 42(55), 98(37), 41(28), 69(27), 70(20), 32(19)

Appendix 3 continued.

Name	Structural formula	Principal ions in mass spectrum m⁺(I%)
Cyclopentanone	C_4H_8CO	55(100), 28(55), 84(47), 41(42), 56(31), 27(22), 42(16), 39(15)
4-Heptanone	$(C_3H_7)_2CO$	71(100), 43(75), 114(21), 41(14), 27(8), 58(6), 72(5), 39(5)
Hexanal	$C_5H_{11}CHO$	44(100), 56(78), 41(72), 43(51), 57(44), 29(35), 72(17), 82(12)
4-methyl-2-pentanone	$(CH_3)_2CHCH_2COCH_3$	43(100), 58(39), 57(18), 41(15), 18(13), 85(12), 29(10)
2-Pentanone	$CH_3COC_3H_7$	43(100), 86(30), 28(23), 27(21), 41(21), 58(19), 71(16)
3-Pentanone	$(C_2H_5)_2CO$	57(100), 29(89), 86(18), 27(17), 28(9), 56(6), 32(5)
Propionaldehyde	C_3H_7CHO	29(100), 58(69), 27(43), 57(19), 26(11), 40(7), 30(5)

Appendix 3 continued

Name	Structural formula	Principal ions in mass spectrum m^+(I%)
Acetonitrile	$CH_3C{\equiv}N$	41(100), 40(27), 39(11), 38(6), 31(2)
Acrylonitrile	$CH_2{=}CHC{\equiv}N$	53(100), 26(82), 52(75), 51(27), 27(9), 50(6), 54(4)
Benzonitrile	$C_6H_5C{\equiv}N$	103(100), 76(33), 50(11), 104(8), 75(6), 51(5), 77(5)
Crotonitrile	$CH_2{=}CHCH_2C{\equiv}N$	41(100), 65(65), 39(42), 40(23), 66(21), 38(16), 27(16)
Propionitrile	$C_2H_5C{\equiv}N$	54(100), 26(24), 27(21), 52(16), 55(15), 51(12), 53(11)

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