UNIVERSITY OF PRETORIA

DOCTORAL THESIS

Fast temperature programmed gas chromatography coupled to supercritical fluid chromatography (SFC×GC)

by

Daniel MALAN

Submitted in partial fulfilment of the requirements for the degree

Doctor of Philosophy

In the Faculty of Natural & Agricultural Sciences
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Declaration of Authorship

I, Daniel MALAN, declare that the thesis, which I hereby submit for the degree PhD (Chemistry) at the University of Pretoria, is my own work and has not previously been submitted by for a degree at this or any other tertiary institution.

SIGNATURE: ....

DATE: ....

Ethics statement

The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval.

The author declares that he/she has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

"Learning is a peculiar compound of memory, imagination, scientific habit, accurate observation, all concentrated, through a prolonged period, on the analysis of the remains of literature. The result of this sustained mental endeavour is not a book, but a man."

Mark Pattison

UNIVERSITY OF PRETORIA

Abstract

Natural and Agricultural Sciences
Department of Chemistry

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The Thesis Abstract is written here (and usually kept to just this page). The page is kept centered vertically so can expand into the blank space above the title too…

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"Works well when under constant supervision and cornered like a rat in a trap."

Sasol Fuel, who saw the need for expertise, and started acting as a major funding source. This freed up so many resources.

The financial assistance of the South African National Energy Research Institute towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to SANERI.

Preface

Having studied at a South African university, I have seen first-hand the thirst for knowledge among South African students, and having grown up in a neoliberal economic society I see how the University is pressured to perform. The student, needing to be full of knowledge and skill after a fixed number of years, emerges from an honours course highly trained in chemistry, but deficient in context.

Inspired by my supervisor, prof Egmont Rohwer, and by the example of my predecessor students, the giants on whose shoulders I stood, this thesis is therefore written with the following audience in mind:

A young South African, who has passed through the South African school system, and has just completed an Honours course at the University of Pretoria, and is now embarking on a postgraduate degree. This student is highly trained in chemistry, in a fragmentary fashion, and has not had exposure to a variety of scientists from other institutions or other disciplines.

This thesis therefore starts from a very general base, and attempts to put the technical work done strongly in context of technology, history and society.

I beg my examiners to forgive me this indulgence.

Contents

List of Figures

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List of Abbreviations

EROEI	**E**nergy **r**eturned **o**n **e**nergy **i**nvested.
LCA	**L**ife **C**ycle **A**nalysis
LAH	**L**ist **A**bbreviations **H**ere
WSF	**W**hat (it) **S**tands **F**or
UNFCCC	**U**nited **N**ations **F**ramework **C**onvention on **C**limate **C**hange
IPCC	**I**ntergovernmental **P**anel on **C**limate **C**hange
CCGT	**C**ombined **c**ycle **g**as **t**urbine
HPLC	**H**igh **p**erformance **l**iquid **c**chromatography
GC	**G**as **c**chromatography
SFC	**S**upercirtical **f**luid **c**hromatography
CCGT	**C**ombined **c**ycle **g**as **t**urbine
CCGT	**C**ombined **c**ycle **g**as **t**urbine

Physical Constants

Constant Name $\quad Symbol = ConstantValue$ with units

List of Symbols

a	distance	m
P	power	W (J s^{-1})
ω	angular frequency	rad

Dedicated to the memory of Dr. Barbara Lotze

$C_nH_{2n+2} + O_2 \rightarrow CO_2 + H_2O + 45\,\mathrm{kJ\,g^{-1}}$
$CO_2 + H_2O + h\nu \rightarrow C_6H_{22}O_2$

Hydrocarbons give heat and sunshine makes sugar

1 Introduction: Climate, fuel, and biodiesel

⟨Chapter1⟩

1.1 Energy, fuel and the atmosphere

Industrialized societies depend on reliable sources of energy. For the purpose of this discussion, the main types of energy can be counted as electricity and fuels, although they are interconvertible. Fuels for industrialized societies are predominantly found as underground mineral deposits, from where they are extracted by mining or drilling. They are found as solids, liquids and gases, which the energy industry refers to as *coal*, *crude oil*, and *natural gas*. Because these fuels are of biological origin, deposited during previous geological eras, and then metamorphosed and preserved by geological processes, these fuels are commonly referred to as 'fossil fuels'.

The large-scale exploitation of fossil fuels started in the middle of the 18th century, when the plentiful coal from the coalfields of Great Britain drove a development that is is known to history as the Industrial Revolution. This development is closely associated with steam engines (Rosen 2012). The use of crude oil started in the middle of the 19th century, and is associated with the development of the automobile (Watts 2005, p. 42). The use of natural gas started in the middle of the 20th century and is associated with the introduction of gas-fired central heating in for homes in cold climates (Hanmer and Abram 2017) Fossil fuels also serve as feedstock to the chemical industry, but that topic lies outside the scope of this discussion.

There is no doubt that the use of fossil fuels as an energy source greatly improved the human lot. The mechanization of agriculture and the easy distribution of food by motorized transport have eliminated famine as a natural disaster (Angelis et al. 2007). The distribution of medical supplies by motorized transport and the rapid deployment of medical personnel have limited the impact and spread of epidemics (Ministere de la santé 2018). Heating and cooling of buildings have increased the habitable zone on earth. Artificial light has increased the hours available for mental activity, in particular extending the reach of entertainment, art and education.

However, in the context of chemistry, the uncontrolled use of fuels has at least two major problems.

The first problem with fossil fuel is that it is finite. There is only a certain amount of fossil fuel on earth. If all of it is extracted it will no longer be a reliable source of energy, and the existence of industrialized societies and the complex civilizations that depend on them will be in jeopardy. It is tempting to think that civilizations would have the foresight to prepare for such an eventuality, but the historical record shows that societies can collapse when at the height of their powers (Diamond 2006).

The second problem with fuels is that they produce pollution wherever they are produced, processed, transported and used. Pollution is injurious to the health and well-being of individuals, societies and nature. If pollution is serious enough, it not only degrades society and the environment, but also negates the benefits brought by the application of energy: modern hospitals are energy-intensive, but if they are filled with victims of pollution there is no nett benefit.

The first approach to pollution from fuels has been to ignore it. Pictures from the early industrial revolution shows English towns coated with soot and choked with smoke (Flick 1980), and rivers became toxic sewers (Halliday 2001).

The development of public health, social responsibility (Szreter 2003) and an embryonic environmental movement (Williams 1965) lead to political pressure for the implementation of pollution controls, which governments gradually introduced and increased in strictness.

The first generation of pollution control offered essentially two options: concentrate or dilute.

I will illustrate these two options using a typical South African coal-fired power station. In such a power station coal is burned to produce heat, which converts liquid water into high-pressure steam. The steam is allowed to expand through a turbine, which converts the energy in the steam into rotary motion, which is used to turn an alternator that produces an electric current by rotating a set of electrical conductors in a magnetic field. The furnaces of such a power station produce a flow of waste. This waste is an *aerosol*: finely divided solids suspended in a mixture of gases.

The solid part is typically separated from the gas-phase part by filter bags and electrostatic precipitators: the collected material is known as *fly ash*. The gas-phase part might be sent through scrubbers to remove some of the gas-phase pollutants, capturing it in a solid form. The solid part of the power station's furnace waste has now been concentrated. It is transported to a storage site, where it is stored indefinitely. It goes without saying that concentrated pollutants should be encapsulated during storage in some way, otherwise they just become more sources of pollution.

The gas-phase part of the pollutant is handled by diluting it. The outlet for the gas-phase stream of waste is through a tall stack, which ends high above ground level[1]. At this altitude the wind is strong and steady, which rapidly carries the gases and remaining aerosols away and disperses them.

The dilution of pollutants might seem like an abdication of responsibility, but it is an reasonable response to pollution. At low enough concentrations pollutants that enter the biosphere are broken down by sunlight and microbes which render it harmless. This makes dilution a reasonable first attempt at controlling pollution.

The devil is, of course, in the detail. For example, mercury that find its way into the environment is eventually converted by microbes to methyl mercury, which concentrates in aquatic animals. All rivers in the continental USA are now polluted by airborne mercury that originate from coal-fired power stations (Wentz et al. 2014). Some persistent organic pollutants, which also concentrate in the food chain, originate in fuel combustion. So while dilution was a reasonable first attempt at controlling pollution, it it certainly not the final answer.

[1]The chimneys of those sooty Victorian towns were not there to disperse the smoke, but to create a 'draft', a flow of air created by the buoyancy of hot air. The better the draft, the more efficient the fire.

The majority of fuels provide their energy as heat, which can be converted into useful work. This heat is obtained by combining the chemical compounds found in the fuel with atmospheric oxygen to form compounds with lower internal energy. The maximum amount of work that can be extracted from a given fuel can be estimated by examining the Gibbs free energy equation:

$$\Delta G = \Delta H - T\Delta S \tag{1.1}$$?eq:Gibbs?

For a given compound fuel compound, ΔH is determined by the difference in enthalpy of formation of the product waste compounds and the enthalpy of formation of the reactant fuel compounds. Since the reactant fuel compounds are given, ΔH is maximized by having product compounds with very low enthalpies of formations.

To maximize ΔS, the products should be as disordered as possible. This implies that *gas-phase* products composed of *small molecules* will yield more work.

The temperature T should also be as high as possible.

Because the industrial machinery in economically competitive, capital-intensive industries are highly efficient, the maximum amount of energy is extracted from their fuels for the lowest cost. Following Gibbs, the major compounds left over after extracting the energy from a fuel should have very low enthalpies of formation and be in the gas phase at the temperature of the process.

Because all fuels contain carbon as a major component, the extraction of energy yields compounds containing carbon. Most fuels also contain hydrogen. Reacting these fuels with the oxygen in air to extract maximal work will therefore yield water (H_2O) and carbon dioxide (CO_2).

(Of course the argument is not that Victorian engineers designed steam engines using the Gibbs energy equation, but the variation-selection process (Vincenti 1990, Chapter 8) by which engineering improvements accrue would inevitably drive the development of heat engines fuelled by fossil fuels to emit large quantities of carbon dioxide gas.)

Water is of course not a pollutant at all, and carbon dioxide is only toxic at very high concentrations, and therefore, for most of the industrial era, carbon dioxide was easily dealt with by by diluting it in the atmosphere, where it is also naturally present at low levels. To the extent that carbon dioxide was considered a pollutant it was assumed that it would be absorbed by the biosphere.

Photosynthesis, the process by which green plants capture the energy from sunlight to live and grow, does indeed remove carbon dioxide from the atmosphere, and an elementary model of the carbon cycle that assumes stability would seem to indicate that excess carbon dioxide in the atmosphere would be captured by photosynthesis and sequestered, leaving the carbon dioxide concentration in the atmosphere stable. Of course this model contains testable assumptions, which scientists could, and did, test.

The most famous of these tests is probably the “Keeling Curve” (Harris 2010). This is a continuous record of measurements of concentration of atmospheric carbon dioxide in the pristine air of the Pacific Ocean. This record starts in 1958 and shows that the carbon dioxide concentration of the atmosphere is increasing.

Paleoclimatologists have studied the hypothesis that the carbon dioxide concentration is stable over time. Not only have they found that the carbon dioxide concentration is *not* stable, they have also determined that the pre-industrial concentration of atmospheric carbon dioxide was lower than it was in 1958 (Petit et al. 1999).

So it is clear that the carbon dioxide concentration in the atmosphere is rising because of the biosphere is not absorbing all the carbon dioxide produced by the combustion of fossil fuels.

The projected concentration of carbon dioxide in the atmosphere is, however, still not at or even near toxic levels, which might make the continued dilution of carbon dioxide in the atmosphere seem a viable disposal method.

Emitted gases, however, do not only have chemical properties and biological impacts, they also have physical properties. Of concern for the current discussion is carbon dioxide's absorbance of electromagnetic radiation, in particular the radiation arriving from the sun, and the radiation from the earth's surface out to space, both which must pass through the atmosphere. A molecule can absorb parts of this radiation by having its electrons excited, or by changing its vibration. Carbon dioxide is electronically very stable, and therefore absorbs only extreme ultraviolet radiation, just like the major gases in the atmosphere, molecular nitrogen and molecular oxygen. However it poorly absorbs near ultraviolet and visible light, in which is it also similar to the major gases. Because there is a dipole moment between the carbon atom and the oxygen atoms of carbon dioxide, it has vibrational modes which can absorb radiation in the infrared region of of the electromagnetic spectrum. Oxygen and nitrogen do not have dipole moments, and therefore do not absorb infrared radiation strongly. This means that carbon dioxide will absorb infrared radiation in the atmosphere much more strongly than the major gases. The energy from the absorbed infrared radiation is of course turned into vibration, a form of kinetic energy, and this kinetic energy is randomly further distributed among the gases of in the atmosphere, appearing as an increase in temperature as the energy distribution moves towards equilibrium.

The most cursory understanding of the absorption of infrared radiation in the atmosphere by carbon dioxide therefore seems to say that an increase in the concentration of carbon dioxide in the atmosphere would lead to a higher average temperature in the atmosphere. This increase in temperature was first estimated by Svante Arrhenius in 1896 (Arrhenius 1897).

(The other major product of extracting energy from fuel is water. Its molecule also has a dipole moment, and it also absorbs infrared radiation strongly. However, its intermolecular properties sets an upper limit to its concentration: at high enough concentrations it will either condense into water or crystallize into ice and precipitate from the atmosphere to end up as surface water.)

Further research has only confirmed that industrial processes extracting energy from fuel is increasing the amount of carbon dioxide in the atmosphere by gigatons every year, and that this increase in concentration is leading to higher temperatures in the atmosphere. It had also become increasingly certain that this increase in temperature is inevitable and significant: it will change earth's climate[2]. This projected increase in temperature and the accompanying change in global climate is bound to have impact on societies within the lifetime of people alive today, and a larger impact on future generations. Some of these projected changes are incompatible with the maintenance of the complex civilizations that are supported by industrialized societies (IPCC 2014).

Scientists do not inhabit ivory towers, and their research is funded by public money in the expectation that the resulting science will benefit their societies. As the

[2]The atmosphere is in contact with the hydrosphere, and the two exchange carbon dioxide. The carbon dioxide diluted in the atmosphere is therefore also diluted in the oceans. This leads to *ocean acidification*, a gigaton problem in it's own right, but one that falls outside the scope of this discussion.

understanding of carbon pollution grew, it became obvious to scientists that industrialized societies could not just continue diluting gigatons of carbon dioxide in the atmosphere, and they alerted policymakers. The iconic moment of this development was the testimony of Dr James E. Hansen, at the time director of NASA's Goddard Institute of Space Studies, before the Senate of the US Congress in 1988 (Shabecoff 1988). In the same year the scientists of the world came together and established the Intergovernmental Panel on Climate Change (IPCC) to create a coherent body of knowledge to inform decision-making. The IPCC has so far produced a series of five Assessment Reports, which assesses the science of climate change, its impacts, and ways to mitigate it (Allen et al. 2014).

(Carbon dioxide is not the only greenhouse gas and the IPCC reports consider each in detail, but because the other gases are not produced in significant quantities by fuels and the energy industry they fall outside the scope of this discussion.)

Industrialized societies currently depend on energy from fossil fuels. Huge investments have been made in infrastructure, large numbers of people find employment in this industry, and available alternative sources of energy are not nearly sufficient. The health and well-being of the greater part of the world's population depend on it. But the future health and well-being of those same societies also depend on ending that dependence. It would be in the best interest of industrialized societies to create plans to reduce the dependence on energy from fossil fuels and exploit alternative energy sources.

But industrialized societies are also in economical competition with each other. This means that any society that spends resources on risky, expensive alternative sources of energy risks falling into a competitive disadvantage, and there is a surfeit of short-sighted politicians who will take advantage of this risk to create fear and so prevent planning for and investment in changes in energy production and use[3].

Fortunately there are enough leaders who have vision, and based on the recommendations of the IPCC the governments of the world have come together to create the UNFCCC. The United Nations Framework Convention On Climate Change is an international agreement that structures the response of nations to limit their emissions of greenhouse gases. Two international treaties have been agreed to so far: the Kyoto Protocol in 1997 (*Kyoto Protocol to the United Nations Framework Convention on Climate Change.* 1997) and the Paris Agreement in 2015 (*Paris Agreement* 2015).

These agreements attempt to reduce the amount of carbon dioxide released into the atmosphere by dividing the cost of reducing emissions fairly. While the Kyoto Protocol invoked a complex carbon credit trading scheme, in the Paris Agreement nations pledge to reduce emissions of carbon dioxide into the atmosphere, and each is free to do so in a way that suits them best.

The Republic of South Africa is a signatory to the Paris Agreement, and therefore the country is legally bound to limit and reduce its emissions of carbon dioxide and other greenhouse gases. The country has so far promised to stop increasing emissions between 2020 and 2025 (*South Africa's Intended Nationally Determined Contribution (INDC)* 2016).

1.2 Carbon footprints and carbon neutrality

Ending the emission of carbon dioxide by industrialized societies is an exercise in balancing effort with consequences. Every reduction in carbon dioxide emissions

[3]The impact of political corruption is not negligible, but I will abstain from commenting on it.

must necessarily have an effect on the economy, and conversely, every change in economic activity will have an effect on carbon dioxide emissions.

A rational, discerning society in searching for ways to carbon dioxide emissions will therefore attempt to change their economy in such a way that the emission of carbon dioxide is limited or reduced. But in most societies there are many ways to reduce carbon dioxide emissions, and decisions have to be made on which ones to implement. Such decisions must be based on sound information, and one way to generate that information is a discipline called *life cycle analysis* (LCA).

Life cycle analysis can yield rigorously-calculated data and comparisons, but it is a very general method. For analyses that are very similar and differ only in context, it is possible to develop simplified, standardized life-cycle analysis tools.

One such tool is the *carbon footprint*. Every economic activity in an industrialized society generates emissions of greenhouse gases. These emissions might be far removed from the activity in space and time. A most obvious example of this is the use of electricity: At the moment I'm using a computer to compose this paragraph and electric light illuminates the desk I'm working on here in my office in Pretoria. The electricity that powers the computer and the lamps are generated hundreds of kilometres from here, most of it on the Mpumalanga Highveld, in coal-fired power stations which emits carbon dioxide in the process. So my activity is far removed from the associated emissions **in space**. But even before the coal was burned, the machines that mined it and transported it to the power station emitted carbon dioxide, so my activity is also removed from the emissions **in time**. The sum of all these emissions connected with my activity constitute the carbon footprint of my activity.

Although carbon footprinting is conceptually straightforward, it is analytically rigorous and computationally complex. But there are standard protocols (World Resource Institute 2004) that can be followed, so that carbon footprints from different activities and different organizations are transparent and comparable.

Once an organization's activities have been footprinted, the organization can examine its operations and look at ways to change activities that will reduce that footprint.

If my university, for example, decides to install photovoltaic panels on the roof of my building, and use the electricity to illuminate my office, the activity will now most likely have a reduced carbon footprint, because photovoltaic electricity usually has a lower carbon footprint than coal-derived electricity.

To prevent a climate disaster, at some point industrialized societies will have to change all their activities until they no longer emit any greenhouse gases. Such activities will have carbon footprints of zero, and will be known as 'carbon neutral activities'. There are also activities that are nett removers of carbon from the atmosphere, which would be 'carbon negative'.

In truth, in industrialized societies there are very few carbon neutral activities. Even if I'm just sitting quietly in a pristine nature reserve, the food I am digesting and metabolizing have a positive carbon footprint: carbon was emitted to fix nitrogen from the air, which was used as fertilizer to help grow the food I ate, and is now in the proteins of my body. The very calcium and phosphorus in my bones were sourced from mines that were powered by fossil fuels.

1.3 Internal combustion engines.

Carbon footprinting exercises quickly show that a major source for emissions is transport. Most transport in industrialized societies is powered by internal-combustion engines. They are called 'internal-combustion' engines because the chemical transformation that extracts the energy from the fuel is internal to the engine. This is in contrast to the power station steam turbines mentioned earlier, where the extraction of the chemical energy from the fuel happens outside of the engine. The engine is the device that produces the mechanical energy. These engines are usually powered by liquid fuels derived from crude oil, although some designs run on gas, and of course in South Africa Sasol and PetroSA supply us with liquid fuels derived from coal and natural gas.

This discussion will use the term *noxious pollution* to describe pollution that is directly harmful to humans and *carbon pollution* to describe pollution that leads to industrial climate disruption.

The simplest way to reduce an activity's carbon footprint is to use the fuel that drives that activity more efficiently. Fortunately market forces are aligned with this, because more efficient use of a fuel also reduces running costs. Discussing engine designs therefore almost always invokes *efficiency*. Efficiency of an engine is calculated by taking the ratio output power:input power. The greek letter η is used as a symbol for efficiency:

$$\eta = \frac{P_{out}}{P_{in}} \tag{1.2}$$?eqn:efficiency?

Conservation of energy dictates that the output power cannot be more than the input power, and therefore $\eta \leq 1$. The power is 'lost' to two factors: firstly entropy, as dictated by the Second Law of Thermodynamics, and secondly losses, such as friction.

1.3.1 Scaling and efficiency

⟨par:scaling⟩ The efficiency of engines scales disproportionately with its linear dimensions, by which is meant that the larger an engine is, the more efficient it is. There are three factors that improve efficiency in larger engines (Brown, Menon, and Hagen 2015):

1. Longer residence time: In larger engines, the reacting fuel spends more time in the engine. This gives reactions more time to reach equilibrium, and hence more energy can be extracted.

2. Smaller surface area to volume ratio: The volume of an engine increases with the cube of its linear dimension, but the surface area increases with the square of its linear dimensions. This means that larger engines have a smaller surface-to-volume ratio than smaller engines. Since losses take place at the surface, the loss per unit volume is therefore larger for smaller engines than it would be for larger engines of the same type. If the losses in larger engines are comparatively smaller, we can expect larger engines to be more efficient than smaller ones.

3. Larger Reynolds number: The Reynolds number is a number that predicts the onset of turbulent flow, and is calculated from the viscosity, velocity and a characteristic linear dimension. The higher the Reynolds number, the higher the likelihood of turbulent flow. Turbulent flow promotes mixing, which leads

to improved combustion and hence higher efficiency. The larger the engine, the larger the characteristic linear dimension, and therefore the higher the likelihood of turbulent flow.

From the viewpoint of reducing carbon pollution, one should therefore aim to use a few large engines rather than a multitude of small engines.

1.3.2 Engine design and thermodynamic cycles.

⟨par:efficiency⟩ The final design of a successful internal combustion engine is determined by the variation-selection process described by Vincenti (Vincenti 1990). Such a design sufficiently satisfies a wide range of requirements. These requirements might be explicitly expressed in documentation, or they may be implied, or they may be practical. These requirements would include, but are not limited to, capital cost, running cost, maintenance cost, noise, sound, ease of maintenance, power output, emissions, surface finish, weight, mounting method, size, supply chain capability, shape, colour, fuel availability, operating temperature, altitude tolerance and torque.

From designers' attempts to fulfil these requirements arise the myriad of different engine designs, delivering anything from milliwatts to megawatts of power to anything from model aircraft to oil tankers. It is the good fortune of researchers who study engine efficiency that there are only a few conceptual systems that explain how the internal combustion engines convert the chemical energy of the fuel into mechanical work.

The principles on which the different engines operate are named 'cycles', because the way they convert heat into work can be describe in terms of a series of events that are repeated endlessly. The concept of a cycle is further entrenched because it is customary to explain the thermodynamic processes involved using at pV diagram, showing the changes of state of the working fluid during engine operation.

Theoretical thermodynamic cycles are useful because they can predict the performance of heat engines, allowing for comparison. In particular, it allows for comparison with theoretical maxima. The maximum efficiency of a heat engine is delivered by an engine running on the Carnot cycle. This describes a hypothetical engine that uses only reversible processes to extract useful work from the temperature difference between two reservoirs of heat. It has been shown that the Carnot cycle offers the maximum possible heat extraction, and that the maximum efficiency (η_C) is determined solely by the temperature difference between the hot (T_h) and the cold (T_c) reservoirs.

$$\eta_C = 1 - \frac{T_c}{T_h} \tag{1.3}$$?eqn:Carnot?

The Carnot cycle, however, can only deliver an infinitesimal amount of work, because the heat transfer must be reversible, and therefore infinitesimal. A more realistic maximum efficiency is given by the Chambadal-Novikov efficiency (η_{CN}) (Hoffmann 2008):

$$\eta_{CN} = 1 - \sqrt{\frac{T_c}{T_h}} \tag{1.4}$$?eqn:Chambadal-Nov

This theoretical efficiency takes into account the irreversible processes that are inevitable in engines delivering finite amounts of power.

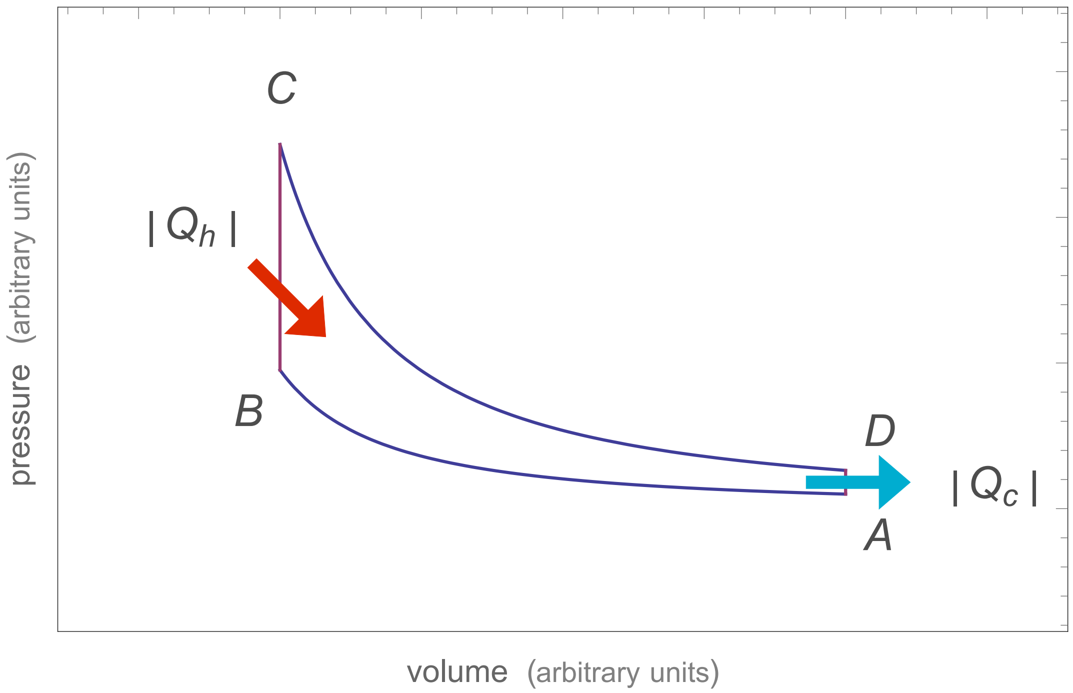


FIGURE 1.1: The pV diagram of the thermodynamic Otto cycle. The isochoric heat addition step (BC) corresponds to the burning of the homogeneous air-fuel mixture. Work is extracted from the engine during the adiabatic expansion CD (Wolfram | Alpha 2019a).

?⟨fig:otto-cycle⟩?

Barring revolutionary new discoveries and inventions, there will be only three thermodynamic cycles and their corresponding internal combustion engines on the market, as we enter the low-carbon era.

Otto engine and Otto cycle

The oldest of the internal combustion engines is the Otto engine. It is named after Nikolaus Otto, who developed the first working engine of this kind in 1876 (Cummins 1989, Chapter 9). In South Africa these engines are usually called 'petrol engines'.

In the Otto engine, a homogeneous mixture of atmospheric air and vaporized fuel is compressed. This mixture is then ignited by an electric spark. The energy released by the chemical reaction between the oxygen in the air and the fuel vapour causes the temperature of the compressed air to rise, and consequently the pressure. If this, now hot, gas is allowed to expand in an expandable vessel, the motion of the vessel can be captured to perform useful work. Once the useful work is extracted, the vessel can be collapsed again to expel the exhausted air, and re-filled with a compressed air/fuel vapour mixture. This completes the cycle. The expandable vessel is usually in the form of a cylinder and piston, with the piston connected to a crank that drives a shaft that transfers the work from the engine to the machine being powered.

(These legs of the cycle are only loosely related to the 'strokes' of a four-stroke engine, and should not be confused for them.)

The theoretical efficiency of the Otto Cycle is given by

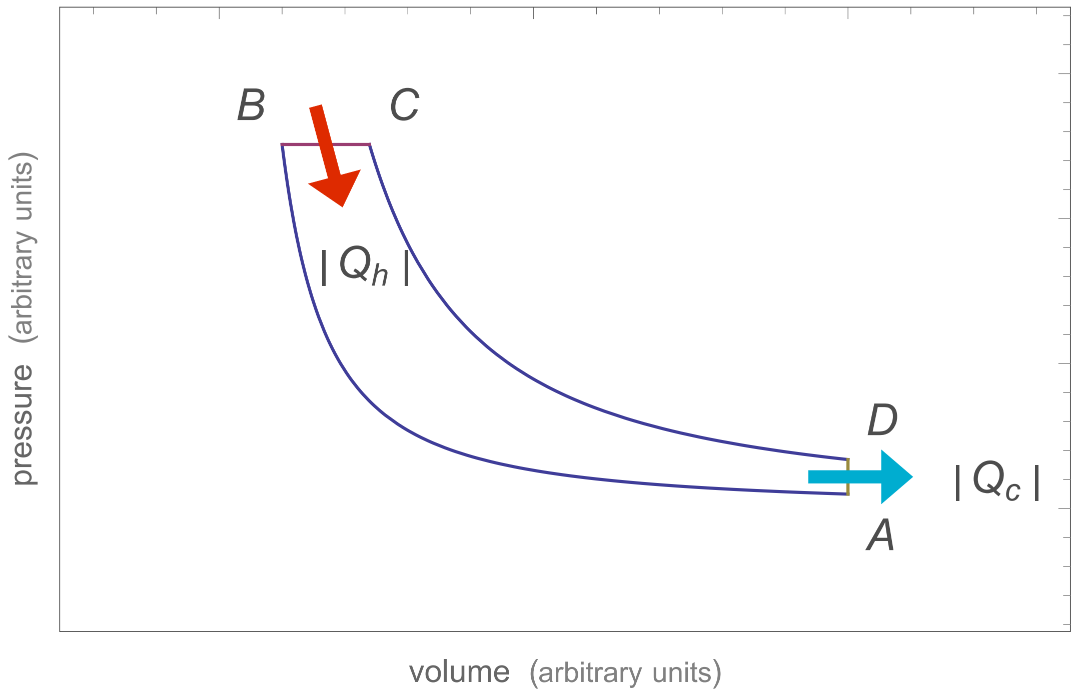


FIGURE 1.2: The pV diagram of the thermodynamic Diesel cycle. The isobaric process BC corresponds to the combustion of the finely divided fuel particles in air. Work is extracted from the engine both during this process and during the adiabatic process CD. (Wolfram | Alpha 2019b)

⟨fig:diesel-cycle⟩

$$\eta = 1 - \left(\frac{1}{r^{\gamma-1}}\right) \tag{1.5}$$

eqn:otto-efficien

where r is the *compression ratio*, $\frac{V_1}{V_2}$, and γ is the heat capacity ratio, which can considered a constant for the purposes of this discussion (Wolfram | Alpha 2019a).

This means that the efficiency of an Otto engine can be improved by increasing the degree of compression of the intake air before combustion.

Diesel Cycle

In the Diesel engine, named after Rudolf Diesel who demonstrated the first engine of this type in 1897 (Cummins 1989, Chapter 14), air is compressed, and then a finely divided solid or liquid fuel is injected into the system. The fuel then reacts with the oxygen in the air. The energy released in the reaction appears as a higher temperature in the gas, and — following Gay-Lussac's Law — the pressure of the gas rises. If the gases expands in the confines of a collapsible vessel, useful work can be extracted from expansion of the vessel. Once the work has been extracted, the vessel can be collapsed again to remove the now inert ('exhausted') gas and prepare for receiving the next charge of air, completing the cycle.

The theoretical cycle used to analyse the performance of the Diesel Engine is called the Diesel Cycle. (See figure 1.2)

The Diesel cycle differs from the Otto cycle in the heat addition step. In the Otto engine, the heat addition takes place when the homogeneous air/fuel mixture combusts. This combustion takes place in a short space of time during which the engine parts move only a negligible distance. Hence the pressure rises rapidly. In the Diesel engine, the fuel is injected into a volume of compressed air, where it combusts. (There is no separate ignition source: the temperature of the adiabatically compressed air is higher than the fuel's ignition temperature, so that the fuel ignites upon injection. While this is another difference between the two engines, it is of second-order importance when discussing thermodynamic cycles.) Because the combustion takes place at the surface of fuel particles, the combustion rate is lower than the combustion rate of the homogeneous mixture in the Otto cycle. Hence the rising temperature is balanced by the motion of the engine, and the heat addition is essentially isobaric.

The efficiency of the Diesel cycle is given by Equation 1.6

$$\eta = 1 - \frac{1}{r^{(\gamma-1)}}\left(\frac{\alpha^{\gamma}-1}{\gamma(\alpha-1)}\right) \tag{1.6}$$

eqn:diesel-efficiency

where α is the *cut-off ratio* and γ is the compression ratio.

The term $\frac{\alpha^{\gamma}-1}{\gamma(\alpha-1)}$ is always larger than 1, and therefore, when we compare equation 1.6 with equation 1.5 it is clear that for a given compression ratio the Diesel cycle is always less thermally efficient than the Otto cycle.

However, at high compression ratios Otto engines start suffering from 'knocking', when the homogenous air/fuel mixture detonates, instead of burning smoothly. The shock waves from this detonation will damage the engine and lead to faster wear. (The *octane number* of a fuel indicates the compression ratio it can accommodate.) Because Diesel engines do not suffer from this problem, they can, and usually are, designed to operate at higher compression ratios than Otto engines. Otto engines normally operate with a compression ration of up to 9:1, where as Diesel engines have compression ratios of up to 25:1. This makes Diesel engines significantly more efficient than Otto engines.

Gas turbines and the Brayton Cycle

The third internal-combustion engine important to industrialized society is the *gas turbine*. Gas turbines are used rarely in road transport, more often in marine and stationary applications, but thousands take to the sky every day, propelling aircraft (H. Morris 2017) carrying billions of airline passengers every year.

The first gas turbines were developed during wartime urgency to deliver pure jet thrust for military aircraft, but this proved to be an inefficient use of the available energy. Most modern turbine engines drive a shaft to extract rotational work. This shaft might drive a bypass fan (as used in airliner engines), a propeller (as used in smaller, low-speed aircraft), or a shaft which might drive a helicopter rotor or an electrical generator.

In operation, a gas turbine compresses air with one or more compressor stages. The compressed air passes through a combustion chamber, where fuel is added and combusted. As the now hot gases expand they pass through one or more turbine stages, which is connected to drive the compressor and the output shaft, which extracts work from the system. After the gas has passed through the turbine it returns to atmospheric pressure, optionally doing work on the engine in the form of thrust.

The theoretical thermodynamic cycle that is customarily used to analyse the gas turbine is called the Brayton cycle, named after George Brayton, who successfully manufactured a reciprocating engine based on this cycle. (See figure 1.3) Such engines are no longer manufactured (Cummins 1989, Chapter 10).

The efficiency of the Brayton cycle is given by equation 1.7

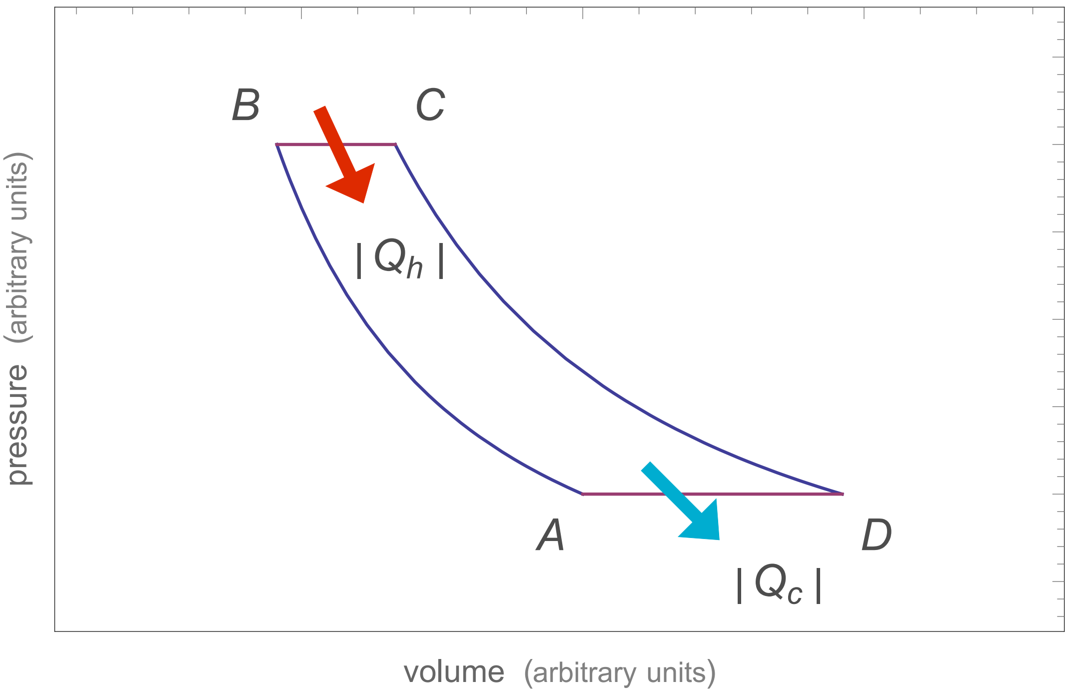


FIGURE 1.3: The pV diagram of the thermodynamic Brayton cycle. In the gas turbine the continuous combustion of the injected fuel and free expansion of the air through a turbine means that the heat addition (BC) is isobaric. Work is extracted during the adiabatic expansion process CD (Wolfram | Alpha 2019c).

⟨fig:brayton-cycle⟩

$$\eta = 1 - \frac{T_1}{T_2} = 1 - (\frac{P_1}{P_2})^{(\frac{\gamma-1}{\gamma})} \tag{1.7}$$

eqn:brayton-effic

where γ is the compression ratio (Wolfram | Alpha 2019c).

Because the fuel is continuously added to the compressed air and the combustion takes place in a heterogeneous mixture, gas turbines do not suffer from 'knocking' and therefore there is no upper limit to the compression ratio. The main figure determining the efficiency of the engine is the turbine inlet temperature, (*i.e.* the outlet temperature of the combustion chamber) which reaches 1600 °C in modern engines.

1.3.3 Noxious pollution from internal combustion engines.

Carbon pollution is not the only pollution emitted by internal combustion engines. There is also noxious pollution, which affects the societies in which they are used. There are ways to reduce or eliminate such pollution, but the implementation of such measures affect the efficiency of the engine.

Noxious pollution from internal-combustion engines come from different sources and have different effects.

Unburnt fuel

The combustion reactions in internal-combustion engines are very fast, but they are never at equilibrium. This means that at the end of a cycle, the exhaust gases expelled from the engine contains, besides the carbon dioxide, also chemical intermediates and unreacted fuel molecules.

Unreacted fuel is a noxious pollutant, not only in its own right, but also, during the decomposition process in the environment, chemical reactions induced by sunlight produces ozone, a reactive oxygen species that leads to respiratory problems in victims of pollution (Davidson 1998).

Some of the fuel-like pollutants from internal-combustion engines are not present in the fuel. These compounds are formed in a process that might be called *pyro-synthesis*. They are themselves more stable than the compounds from which they originate, but not as stable as the combustion end products, carbon dioxide and water. Because they are themselves quite stable, they require high temperatures to combust, which might not be achieved before they leave the engine as waste. Notable pollutants from this source are the policyclic aromatic hydrocarbons (PAH) and, if trace amounts of chlorinated substances are present in the fuel, dioxins

One of the products of pyro-synthesis is particles of soot. These particles are agglomerations of nano-sized particles of pure, amorphous carbon. The agglomerations might include adsorbed PAHs and acids. Soot particles are very stable and only react at very high temperatures. Diesel engines in particular have a reputation for producing soot (Mohankumar and Senthilkumar 2017).

The final pollutant that can be classed as originating from partially combusted fuel is carbon monoxide. It forms readily when fuels are burned in oxygen-poor environments. Modern, well-maintained engines is a relatively rare cause of acute carbon monoxide poisoning (Reumuth et al. 2018), but chronic exposure to low levels of environmental carbon monoxide has harmful effects (Wright 2002).

Contaminants and additives.

Crude oil and coal contains not only carbon and hydrogen, but also other elements, most notably sulfur and nitrogen. Depending on the refining process, these elements might find their way into fuels. Organic nitrogen and sulfur will easily oxidize and form stable oxides, yielding energy. Once outside the engine, however, the volatile oxides will dissolve in any water in the air, forming acids. (Duncan et al. 2016)

Fuel manufacturers add additives to fuels for various reasons, such boosting octane rating or preventing corrosion. The prime example of additives as a source of noxious pollution was tetraethyllead, which was added to petrol as an octane booster. The emitted lead compounds was shown to be a pollutant that lead to neurological impairment in its victims, and its use was phased out (Needleman 2000).

Side-reactions

Most internal combustion engines use atmospheric air as oxidant. This air is 21% oxygen, and 78% nitrogen. Although nitrogen is very stable and inert, at high temperatures it can react with oxygen. These reactions produce the oxides of nitrogen: NO, NO_2, NO_3 and N_2O, and N_2O_5. They are collectively called NO_x.

These oxides can participate in the cycle that causes the photochemical smog. They will also dissolve in atmospheric water and form acids: this might happen far from the point of emission, resulting in *acid rain*.

1.3.4 Mitigating pollution from internal-combustion engines.

Pollution from internal combustion engines is a social ill, and most governments have regulations in place to limit and reduce this pollution. Engine and fuel manufacturers are working hard to reduce this pollution.

The noxious pollution from internal combustion engines can be reduced by various improvements, but there are three complementary approaches: fuel formulation (Gertler et al. 1999), cleanup (Braun et al. 2018) and engine management (Reif 2015).

Fuel formulation

Adding oxygenates improves the octane rating of fuel and reduces NO_x formation.

Exhaust gas cleanup

⟨par:cleanup⟩ Exhaust gases from internal combustion engines can be "cleaned", and there are basically two approaches. The first is catalytic conversion, in which the exhaust gases that contain the pollutants are passed over a catalyst bed. The catalyst (a proprietary formulation of platinum, palladium and/or rhodium), adsorbs the uncombusted volatile organic carbons, and oxidizes them. It simultaneously catalytically decomposes NO_x to molecular nitrogen and oxygen.

Secondly, filter systems can be used to remove particulate matter.

Engine management

⟨par:engine-management⟩ As described above, the main source of noxious pollution from internal-combustion engines is uncompleted or undesired chemical reactions, and is not fundamental to the operation of the engine. By carefully managing the engine system, noxious pollution can be reduced.

The oldest engine management technology is the oxygen or 'lambda' sensor, which measures the oxygen in the exhaust gases. Such a sensor, coupled to an engine-management computer, allows the metering of the exact amount of fuel needed for optimum combustion (Frauhammer, Schweinsberg, and Winkler 2014).

A newer technology is known as exhaust gas recirculation. This mixes the intake air of the engine with exhaust gases, effectively diluting the oxygen. This reduces peak temperatures, and thereby NO_x formation.

In *stratified charge* engines the distribution of fuel in the volume of intake air is controlled by selective fuel injection. Carefully injecting the fuel at the right place at the right time can allow for higher compression ratios without inducing pinging. *Lean-burn* engines are Otto engines that use use extremely high air:fuel ratios.

Electronic engine management systems result in much more efficient and less-polluting engines than non-managed 'mechanical' engines, but because engines for automotive applications endure such a wide range of operating conditions, they can at best achieve a compromise between power, efficiency and emissions. It was this unsatisfactory compromise that lead to the Volkswagen emissions scandal: manufacturers chose to cheat on emissions test rather than admit to the relatively poor performance of a managed engine optimized for low emissions (Mansouri 2016).

Efficiency implications

Attempts to mitigate noxious pollution from internal-combustion engines mostly lead to losses in efficiency. For example:

- Exhaust gas flow through catalytic converters and filters consume energy.
- The engines cannot approach their theoretical maximum efficiencies, because reducing NO_x emissions is handled by limiting maximum combustion temperatures.

- NO_x reduction catalysts require the presence of hydrocarbons, *i.e.* incomplete reactions, which implies that not all energy is extracted from the fuel.
- Every treatment system added to the engine adds weight to the vehicle, which reduces payload and hence the total efficiency.

Before carbon dioxide pollution was a concern, it made sense to accept lower efficiencies as a necessary cost of reducing noxious pollution, but in a low-carbon future we cannot just keep on trading less noxious pollution for more carbon dioxide production.

1.3.5 Avoiding pollution from internal-combustion engines

ar:carbon-neutral⟩ The efficiency and cleanness of internal-combustion engines have dramatically increased over the last century, and more improvements are being implemented. But these improvements have not been fundamental to the engines in any way, and have been driven mostly by government regulation, at the cost of increased complexity and a higher purchase price.

It would seem obvious that it would be a good idea to introduce alternative technologies.

Electrification

In principle, there is nothing special about internal-combustion engines: they are not an end in themselves. They merely deliver a source of torque, which can be coupled to machinery to do useful work. Before the industrial era such torque was available from windmills and waterwheels, and today an alternative is the electric motor.

Electric motors are engines that use the interaction between electric current and magnetic fields to deliver useful torque to drive machines. Because they use electricity as a source of energy, they have no noxious emissions where they operate. Because they are not heat engines, their efficiencies are not subject to the Carnot limit, and efficiencies exceeding 95% are standard (Li and Curiac 2012).

Electricity, of course, is not necessarily carbon-neutral. Most electricity is generated in power plants that use fossil fuels as a primary source of energy. But because these plants are huge, and efficiency scales disproportionately with size, the energy output by the electric motor has a similar or lower carbon footprint than an equivalent internal-combustion engine (Doucette and McCulloch 2011).

Electricity grids are also increasingly being fed by solar and wind power, which are carbon-neutral at source. These renewable plants are also smaller and more flexible than their behemoth fossil-fuel counterparts, with lower capital costs and extremely low running costs.

Hence, in a low-carbon future, there is every reason to support or mandate the use of electrical motors for stationary applications wherever possible.

In automotive applications, *i.e.* in cases where the engine is used to move itself in addition to some form of payload, the application of electric motors is more demanding. In this case it is not easy to bring the electricity to the motor, although electric trains and buses fed by overhead conductors are splendid examples of the electrification of transport. So for electric vehicles to use the existing road network, they need to carry a source of electricity with them.

This source of electricity can be a either a chemical battery, or a fuel cell. In a chemical battery power from the grid is stored in the form of reversible electrochemical reaction, and in a fuel cell the chemical energy from a fuel is directly converted into electricity. There are fuel cells that can use hydrogen as a fuel, and fuel cells that can use methanol as a fuel. (It goes without saying that the hydrogen and the methanol need to be sourced from low-carbon sources for fuel cells to count as low-carbon energy sources.)

The storage and transport of hydrogen remain hurdles to the large-scale adoption of hydrogen-fuelled automobiles, although a market seems to be developing for hydrogen-fuelled electric trains (Agence France-Presse 2018). Hydrogen fuel cells emit no carbon or noxious pollution at point of use.

Direct methanol fuel cells can react methanol with atmospheric oxygen in an electrochemical cell to yield electricity, with carbon dioxide as a waste product. Little is known about possible noxious pollution.

At this time it seems that the electrification of road transport will be by chemical batteries. Lithium-ion batteries can now store enough energy and deliver enough power to make electric motor vehicles practical and attractive (Hayes et al. 2011), and some governments are considering plans to no longer allow the production of passenger vehicles propelled by internal combustion engines (Burke-Kennedy 2018) (Reuters 2018) (Gabbatiss 2018).

Carbon-neutral fuels

Another way to avoid the carbon pollution associated with internal-combustion engines is to change the fuel. Not all fuels are fossil fuels, and it is possible to use fuels that are carbon neutral, and in some cases carbon negative.

Apart from using hydrogen in a fuel cell, as described above, *hydrogen* can also be used as a fuel in Otto engines, because it will combust in air to yield heat. The emissions are water and NO_x. Presently there are no hydrogen-fuelled Otto engines on the market.

Methanol is a common product of the fossil fuel industry, but work is underway to produce methanol by reducing carbon dioxide using solar energy. Such *solar methanol* might be used in direct conversion fuel cells, or in internal-combustion engines.

It is possible to harness the energy in contained in the reduced carbon in biological materials and use it as fuel. Such fuels are known as *biofuels*.

1.4 Biofuels

Biological processes are an integral part of the carbon cycle, because photosynthesis in plants reduces carbon dioxide in the atmosphere to sugars, which are converted by plant physiology into structural cellulose and other metabolites.

The prototypical biofuel is of course wood, used in all societies for cooking and heating. This familiarity makes biofuels seem an obvious and viable source of energy, but details matter, and switching from fossil fuels to biofuels to reduce carbon footprints of human activities is not a simple choice.

Firstly, the efficiency of photosynthesis is notoriously low: not above a few per cent, whereas the efficiency of a mass-produced silicon-based solar panel is in excess of 20%. In general it is much more efficient to capture solar energy in a PV panel and use an electric motor to provide the necessary mechanical power than to create a biofuel and use it to drive an internal-combustion engine.

Second, increased biofuel production has numerous impacts on the environment and society, which cannot be ignored.

Discussing all the factors that need to be studied to make such a decision is outside the scope of this work, but as an example a report prepared for stakeholders in the Netherlands (Smeets et al. 2006) uses the following criteria:

- GHG [greenhouse gas] emissions – the use of biofuels should cause reductions of GHG emissions. The comparison should be done regarding the average use of fossil fuels, considering the life cycle of fossil and biofuels (i.e., well-to-wheel basis) and in case of biofuels reduction should be at least 30%
- Impacts over food supply – the production of biomass for energy must not endanger the food supply and other local biomass applications. The analysis should be developed considering possible changes of land use in the region of biomass production.
- Biodiversity – Biomass production must not affect protected or vulnerable biodiversity.
- The basic criteria are that violation of national laws and regulations are unacceptable.
- Local environmental effects – Principles include (a) soil and soil quality, that must be retained or even improved, (b) ground and surface water supply, that must not be polluted.
- Local economic effects – The production of biomass must contribute towards local prosperity.
- Social well-being – The production of biomass must not decrease the well-being of local societies.

Nevertheless, there are cases where using biofuels is a good option.

1.4.1 Bio-gas

Anaerobic bacteria can convert biogenic carbon compounds to methane. This methane is identical to the methane obtained from natural gas, and can be used for the same applications.

An excellent application for the use of bio-gas is waste remediation. Waste material from the agri-food industry can be highly polluting if not treated properly, emitting noxious chemicals into water and the potent greenhouse gas methane into the atmosphere. But when used as feedstock for bio-gas production, it reduces carbon pollution by capturing methane and displacing natural gas, and also prevents water pollution (I. Venter 2014).

Bio-gas can be used to fuel Otto engines or gas turbines.

1.4.2 Bio-ethanol

⟨sec:BioEthanol⟩ Yeast and other microbes can convert sugar or starch from plants into ethanol. This technology is as old as civilization. Archaeologists may be in disagreement where beer was brewed first, but it is clear that between 3200 and 3000 BCE in ancient Sumeria the brewing of beer was an activity regulated by the government (Damerow 2012). By 2000 BCE in ancient Egypt the technology of fermentation was harnessed well enough that bakeries and breweries were co-located (*Model Bakery and Brewery from the Tomb of Meketre* 1920).

Beer and wine can best be described chemically as aqueous solutions of sugars, ethanol, flavourants and colourants, with or without suspended solids. It was not until the 8th century CE that Persian and Arab scientists mastered the art of distillation and purified ethanol (Modanlou 2008), and it was not until Pasteur that yeast was seen as a living organism, and ethanol a product of its metabolism (Barnett 2000).

It is unclear when ethanol was firsted as a fuel, but its flammability must have been noticed by the first distillers. By 1838 ethanol was common enough to be used in alcohol lamps as a source of heat in the chemical laboratory (Griffin 1838), and by the 1850s it was a major component of lamp oil used for illumination in the USA (Abebe 2008).

Ethanol as a fuel for internal combustion engines has a clear start date: in 1826 Samuel Morley was granted a patent for an engine designed to use ethanol as fuel (Cummins 1989, p. 79).

The industrial production of ethanol is a technologically advanced process. It is an active research field. A paper (Cardona and Sánchez 2007) reviewing the process technology of producing bio-ethanol written in 2007 had garnered 644 citations by January 2019.

The industrial production of ethanol consists of three steps.

1. Fermentation
2. Distillation
3. Dehydration

The fermentation step is the biological process by which the yeast organism *Saccharomyces cerevisiae* convert the sugar and starch in the biological material to ethanol and carbon dioxide. This needs to be done in a sterile environment to prevent contamination by other micro-organism.

The distillation step is the physical process of separating the produced alcohol from the aqueous mixture in which it is produced. This generally produces an azeotropic mixture that contains 95.6 % ethanol (Kumar, Singh, and Prasad 2010).

While the processes of fermentation and distillation of ethanol for fuel is in principle the same as that of producing alcoholic beverages, the emphasis of the processes are very different. In the beverage industry the emphasis is on the development of complex flavours and a consistent, recognizable drinking experience. In the fuel industry the emphasis is on efficiency and throughput.

To produce fuel-grade ethanol from the distilled azeotrope, dehydration is a necessary third step. This step can be implemented by various distillation processes that add a third compound, but in most modern plants the water is removed by adsorption onto molecular sieves (Kumar, Singh, and Prasad 2010).

Brazilian ethanol

Brazilian sugar-cane ethanol is an integral part of the country's energy network. Most Otto-engine vehicles have 'flex-fuel' engines, which can be fuelled with any blend of ethanol and petrol. The industry is regarded as sustainable ((Smeets et al. 2006).

US maize

US maize ethanol is primarily blended with petrol to meet legislative requirements of oxygenates in fuels. The production of maize is water-intensive and the fermentation process is carbon-intensive. The US ethanol from maize has a poorer energy balance and larger carbon footprint than Brazilian ethanol from sugar cane, but has a smaller water footprint (Mekonnen et al. 2018).

Ethanol can fuel Otto engines and gas turbines.

1.4.3 Fischer-Tropsch fuel from biomass

⟨sec:FT⟩ It is possible to heat woody biomass with steam in a low-oxygen environment to produce synthesis gas, which can be the converted to a mix of fuel products in the well-known Fischer-Tropsch catalytic process. Such a fuel could be called a biofuel, but it's production would be difficult to reconcile with the principles of *green chemistry*, described in Section 2.1.1.

1.4.4 Hydrotreated vegetable oil: "Green diesel"

⟨sec:GreenDiesel⟩ The petroleum industry has developed a collection of chemical processes, such as hydrogenation, oxygenation and cracking. This collection of processes can be applied to vegetable oils to produce a fuel for diesel engines and gas turbines. This path is being followed by the aviation industry (Chiaramonti et al. 2014).

1.4.5 Biodiesel

Oils and fats have been used as fuels since antiquity, most obviously as a fuel for lamps: olive oil has been identified as the fuel used in lamps dating from around 600 CE (Kimpe, P. A. Jacobs, and Waelkens 2001), and at the Paris World Fair in 1900 Rudolf Diesel demonstrated an engine that ran on peanut oil (Knothe 2010).

During the energy crisis of the 1970s, the South African government looked at alternative sources of fuel. Experiments were done with sunflower oil in tractors, but there was a problem with the formation of carbon deposits around the injector nozzles. Seeing that the clogging of the injectors might be caused by the high viscosity of the sunflower oil as a fuel, the transesterification of the oil was implemented, replacing the glycerol in the sunflower oil with methanol. This created an oily liquid with a lower viscosity, which proved to be a trouble-free replacement (Van Niekerk 1980).

Such a transformed oil is termed *biodiesel*. It consists primarily of a mixture of fatty acid methyl esters, often abbreviated into the acronym FAMEs.

Compared to ethanol, biodiesel production is relatively simple, the main method sharing much with the ancient technology of making soap. This simplicity makes the production of biodiesel attractive to small and decentralized manufacturing, and consequently governments consider biodiesel production an attractive proposition: it can create job opportunities in rural population and it can create a stable market for farmers who produce vegetable oil crops.

Biodiesel is not yet carbon neutral: the methanol used to create the methyl esters is a product of the petroleum industry, and therefore biodiesel emissions contribute to global warming. But replacing fossil diesel fuel with biodiesel results in a nett reduction of carbon emissions, with the future possibility of replacing fossil-derived methanol with carbon-neutral bio-methanol (Shamsul et al. 2014).

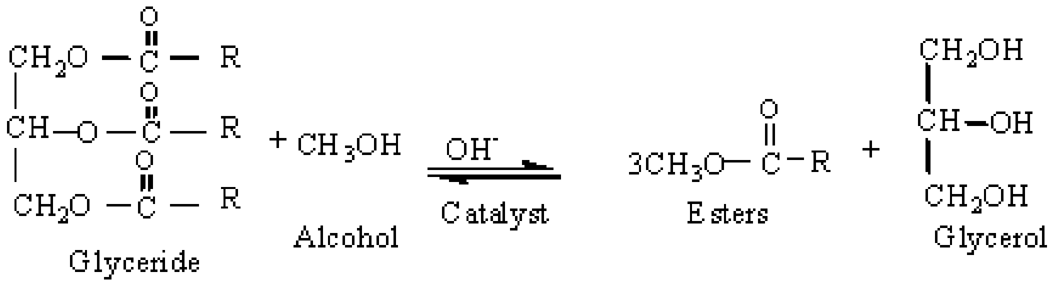


FIGURE 1.4: Transesterification

?⟨fig:Electron⟩?

1.5 The significance of biodiesel and its quality control.

In a complex technical, economic and social environment it is likely that there will always be a need that can be best met by internal combustion engines. The decision on the type of engine and the decision on the fuel for that engine are not independent, as Cummins reminds us (Cummins 1989):

> "Our generation faces a similar challenge in a real liquid fuel energy shortage that will come within the lifetime now living. As we plunge into the seeking of solutions to our dilemma, we must never forget that *an engine and the fuel it consumes are inseparable partners*; the one cannot progress without the full cooperation of the other. This precept is vital to the planning of future powerplants, since an engine's design determines its fuel and binds us to our future resource requirements."

While the variation-selection process by which real engineering progress is 'blind', *i.e.* the combination of factors that make a design successful is not known at the start of a development (Vincenti 1990), scientific knowledge provides guidance in the form of insight into processes and theoretically achievable targets for performance. Using the scientific knowledge we have today, we can risk a forecast: if society is determined that its carbon footprint should be reduced with minimal noxious pollution the application of internal combustion engines would tend towards the following:

1. Larger: Because larger engines are more efficient than smaller engines, larger engines will be preferred. (See section 1.3.1)
2. Low-carbon: The preferred engine would be fuelled by carbon-neutral fuels. (Section 1.3.5)
3. Constant speed: Engines are most efficient when they can work at constant load. (Section 1.3.4)
4. Efficient: Engines with high efficiency should be preferred, which implies engines with high compression or pressure ratios. (Section 1.3.2)
5. Cleanup: The exhaust should be amenable to cleanup, *i.e.* the exhaust gases of the fuel-engine system should not contain compounds that are incompatible with available converter or filter technologies. (Section 1.3.4)

From this it should be clear that the optimal engine of the future will not be an Otto engine, because it will always have a limited compression ratio and its catalytic exhaust cleanup will always require stoichiometric air:fuel ratios, which puts bounds to efficiency increases.

Because it is the most efficient engine, the gas turbine will play an important role. However, its exhaust temperature is very high, which begs for energy recovery to increase the efficiency of the system. Such systems are already seen in the *combined cycle gas turbine* (CCGT). But energy recovery systems are not light and small, so their best application outside aerospace (where the excess heat is utilized as thrust) appears to be stationary electricity generation.

For automotive applications the engine that will best contribute to reduce the carbon footprint of may be a large Diesel engine with exhaust cleanup to remove NO_x and soot, fuelled with a carbon-neutral fuel.

In choosing between refined vegetable oil and biodiesel as a fuel, it is most likely that biodiesel will have a lower carbon footprint. Refinery operations are energy intensive, which might add to the carbon footprint of the fuel, and refineries are not usually near the point of use, so that transport will also add to its carbon footprint. The carbon emissions of biodiesel production are comparatively low. (At this point it is important to note that these observations are not definitive: carbon footprints are not predicted, they must be calculated.)

Buying large, highly efficient diesel engines with sophisticated management systems and exhaust cleanup require high capital investment. In an economically competitive environment they therefore need to bring reliable returns, which implies high availability, as measured by frequency of breakdown and length of time between maintenance stops. Such high reliability can only be achieved if the manufacturer understands the fuel-engine system well and it behaves predictably.

In a low-carbon future, therefore, well-characterized biodiesel might be expected to play a central role in non-electric ground transport and industrial application where electrification is not possible.

1.6 Conclusion: the role of chromatography.

The development of the fuel-engine systems depends heavily on the chemical characterization of fuels and engine emissions, and chromatography has always played a central role: some of the earliest researchers developing gas chromatography were employees of a petrochemical company (Keulemans, Kwantes, and Zaal 1955).

This thesis explores the possibility of applying comprehensive two-dimensional (supercritical fluid × gas) chromatography to the chemical analysis of biodiesel for the purpose of characterization and quality control.

PV $\neq$ *nRT*

The ideal gas law does not apply to supercritical fluids.

2

Introduction: Carbon dioxide and chromatography

⟨Chapter2⟩

2.1 The chemical industry

Industrialized societies depend on chemicals. (In this discussion I define chemicals as pure substances that are produced by industry for industry.) Chemicals might be used in the processing of products, or blended with other chemicals in formulations that might be sold to users as products. In a familiar example, sugar is a chemical produced by the sugar industry from sugar cane or sugar beet. It is a pure substance (sucrose) that is mostly used in the industry as an ingredient for processed food. Other uses of sugar include coatings for medication, and feedstock for engineered micro-organisms that produce pharmaceuticals. (Sugar is a rare example of a chemical that is also sold to consumers.)

The chemical industry produces a huge variety of products, from compounds as as simple as hydrochloric acid to compound as sophisticated as cyanocobalamin (Figure 2.1). All of these chemicals help produce the products indispensable to industrialized societies. Nevertheless, the chemical industry is not held in high regard by people outside the industry. A part of this negative perception comes from the chemical industry's reputation for fatal accidents and pollution (Gumm 2015).

The list of incidents is long and examples easily spring to mind: In 1984 a leak at a chemical plant in Bhopal, India, caused the death of thousands of people and the injury of thousands more (R. Varma and D. R. Varma 2005). Stratospheric ozone is struggling to recover from depletion caused by the reckless emissions of chlorofluorocarbons (Ball et al. 2018). Plastics microparticles are now found everywhere in the oceans (Woodall et al. 2014), and the pesticide DDT is found in the breast milk of Inuit mothers (Gibson et al. 2016).

The chemical industry is also a prodigious producer of greenhouse gases. Apart from the carbon dioxide emitted by the production of the energy that power chemical processes, some chemical processes emit carbon dioxide as a waste product. Most notable of these is the reduction of atmospheric nitrogen as the first step in the production of nitrogen fertilizers. Of all the greenhouse gases monitored by the IPCC, only carbon dioxide, methane and nitrous oxide are found in nature: the others are exclusively products of the chemical industry (IPCC 2014).

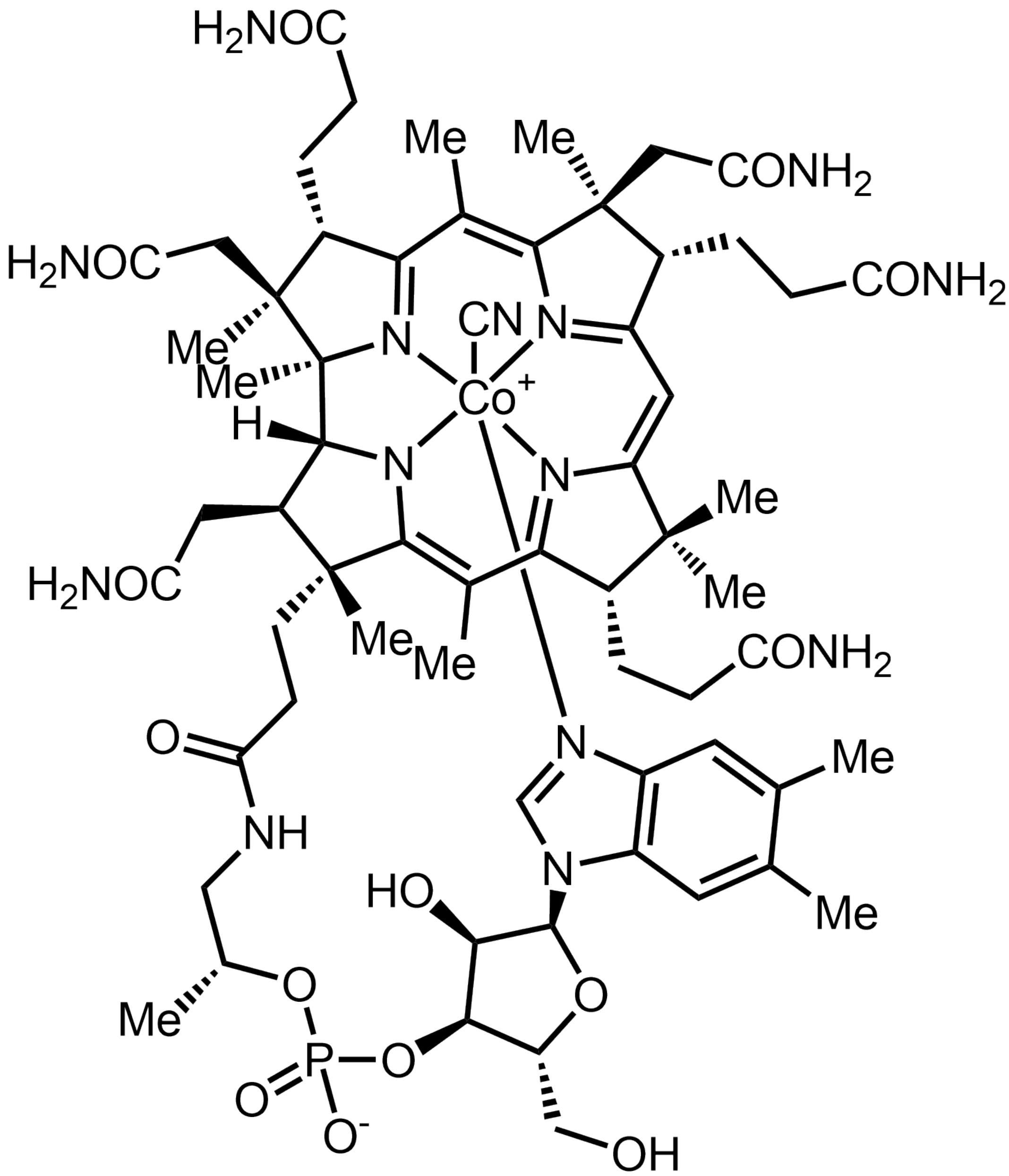


FIGURE 2.1: The chemical structure of cyanocobalamin, a form of vitamin B12. This compound is produced on the tonne scale by the chemical industry.

⟨fig:vitb12⟩

No chemical has ever jumped out of a lab, multiplied uncontrollably, and spread into the environment to poison or pollute: they have all been introduced into the environment by human ignorance, negligence, or recklessness. All chemicals behave well when used in properly controlled environments, but human actions can let them escape and damage and pollute. But while we try to solve the intractable problem of human behaviour, we as chemists cannot just lay blame: we must pay attention to the intrinsic safety of chemicals and chemical processes.

2.1.1 "Green chemistry"

The date of the birth environmental movement is conventionally set to 1962, when the biologist Rachel Carson published the book *Silent Spring*, which pointed out the destruction of nature by the unrestricted use of pesticides, and the dangers of overuse (Carson 2003). This was a direct imputation of the chemical industry, because the pesticide products contained many chemicals.

Chemists are human, and the realization uncontrolled that chemicals can have detrimental effects lead at least some chemists to reflect on their own work. This has given rise to the concept of *green chemistry*. Although the term has no rigorous definition or quantitative measure (Linthorst 2010), a set of 12 principles or guidelines are proposed:

1. It is better to prevent waste than to treat or clean up waste after it is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (*e.g.* solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
8. Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided whenever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for real-time, in process monitoring and control prior to the formation of hazardous substances.

?⟨itm:safe⟩?

12. Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions, and fires.

While these guidelines are clearly written with synthetic chemistry in mind, it does not mean that they do not apply to analytical chemistry. For example, Principle 7 suggests that, when possible, one should use hydrogen rather than helium as mobile phase in capillary gas chromatography: hydrogen is renewable, whereas helium is a finite resource and from time-to-time there are reports of shortages (Kornblut 2019).

One large area of the greening of chemistry is changing the use of solvents. Principle 5 recommends that solvents use be avoided, if possible — most solvents used in chemistry are ultimately derived from petroleum and are toxic to some degree. But solvents play a large role in many kinds of chemistry, and eliminating their use in the near future seems unlikely. Searching for and characterizing bio-derived, non-toxic, non-persistent solvents is an active field of research (Clarke et al. 2018).

But there are a few solvents in already current use that are inherently "green", such as water or ethanol.

One such naturally green solvent is carbon dioxide.

2.1.2 Carbon dioxide as a green chemical

Carbon dioxide as a chemical is used in industry in a few key areas.

- Carbon dioxide is often used in firefighting, in the form of portable fire extinguishers or room flooding systems. In this last use it is displacing the ozone-depleting halomethane (Halon).

- When liquid water is supersaturated with carbon dioxide, the gas desolvates slowly in the form of streams of tiny bubbles. This phenomenon makes beverages prepared from water supersaturated with carbon dioxide (or *carbonated water*) interesting to drink, and a large, international beverage industry is based on carbonated water.

- Carbon dioxide has a freezing point of -77 °C, and the solid can be conveniently obtained by evaporating liquid carbon dioxide at atmospheric pressure. The evaporating liquid rapidly cools the stream of carbon dioxide, lowering the temperature of the stream to below the freezing point, and the gas crystallizes into the solid. The resulting 'snow' can be compressed into blocks, which only slowly sublimates into gaseous carbon dioxide, keeping the temperature at the freezing point. Packing frozen food products together with this 'dry ice' allows for it to be transported cold.

- Pellets of dry ice can be entrained in a jet of air, and used to abrade surfaces for cleaning (Spur, Uhlmann, and Elbing 1999). This use of carbon dioxide can displace toxic solvents or abate the noxious dust produced by blasting operations.

- Carbon dioxide is a 'natural refrigerant' (Pearson 2005), and can be used to displace hydrofluorocarbon refrigerants, which are potent, long-lived greenhouse gases.

- Carbon dioxide can be used as a preservative and anti-oxidant in packaged food. If air in a packaged food is removed by purging the headspace with carbon dioxide, the growth of microbes can be discouraged, extending the shelf life of the product (Jacobsen and Bertelsen 2002).
- Carbon dioxide can be used to extract compounds from natural products.

Of these uses, extractions are economically the most important.

2.2 Extractions using carbon dioxide

2.2.1 Commercial extractions

There are several commercial processes that use carbon dioxide to extract valuable products from plant material.

Plant oils

Vegetable oils are obtained from various crops, and can be extracted from the plant material by pressing, heating or extraction. High-pressure carbon dioxide has been used to extract vegetable oils from various plants, although it seems that this process has only found niche applications so far (Eisenmenger et al. 2006; Grażyna and Anna 2018).

Hops

Hops is an essential component in the brewing of beer. It imparts a desired bitter flavour, stabilizes the beer during storage, and assists with foam formation (Schönberger and Kostelecky 2011). Hops is a seasonal crop with a limited growing range, but the demand for beer is not limited to certain areas or seasons. The creation of hops extract makes it possible for brewers to benefit from hops without owning a hops plantation or storing and transporting dried hops over long distances. All hops extracts produced today are extracted by carbon dioxide (Hunt et al. 2010).

Coffee

Coffee is an international industry, with coffee drunk in many cultures and in many forms. One of the attractions of coffee is the effects of the psychoactive substance found in the coffee bean, *caffeine*. Caffeine is a mild stimulant and promotes wakefulness. A small proportion of coffee drinkers enjoy drinking coffee, but prefer to avoid the stimulant effect, which might induce insomnia or irritability. For these coffee drinkers the market supplies *decaffeinated coffee*.

Given the large amount of coffee traded (an estimated 167.47 million bags of coffee in the 2018-2019 coffee year (*Coffee Market Report December 2018* 2018))[1], if only a small percentage of coffee needs to be decaffeinated, it will be a large amount of coffee to process, and industrial processes will be necessary to supply the demand.

Decaffeination of coffee is achieved by selectively extracting the caffeine from green (*i.e.* unroasted) coffee beans using carbon dioxide. This is the largest use of carbon dioxide for extraction (Ramalakshmi and Raghavan 1999). The extracted caffeine is sold for use in medication and 'energy' drinks.

[1]The factoid that "coffee is the second-most traded commodity after oil" has been proven to be untrue (Greenberg 2017).

2.2.2 Analytical Extractions

Extraction is not only an industrial process, but is part and parcel of analytical chemistry. The first extractions using carbon dioxide was not aimed at developing an industrial operation, but to develop methods for analytical chemistry. This method is usually called SFE, for **s**upercritical **f**luid **e**xtraction.

2.2.3 Why carbon dioxde?

But what makes carbon dioxide a suitable solvent for extraction?

There are two aspect to this question. The first is about the *greenness* of carbon dioxide. It is non-toxic, non-persistent, non-flammable, non-corrosive, inexpensive, commercially available, and a waste product. (It goes without saying that this carbon dioxide is sourced from a carbon-neutral source, perhaps the brewing industry.)

The second aspect of the desirability of carbon dioxide lies in its physical properties and the conditions under which we use it.

Chemists will intuitively understand that gaseous carbon dioxide has no solvating properties, and that liquid carbon dioxide should not behave much differently than any other solvent. Both these statements are true under 'normal' circumstances.

But consider the case of an isobaric cooling of a volume of gas. The gas-liquid transition takes place because energy is removed from the system. At some point the kinetic energy of some of the molecules becomes less than the energy of the intermolecular forces, and the molecules prefer to clump together, *i.e.* it condenses. The remaining gas molecules receive the excess energy, and therefore stay in the gas state, until more energy is removed, leading to more of the gas condensing. During this process the temperature remains constant, and this temperature is known as the boiling point.

Now consider a solute (solid or liquid) in the same volume of gas being cooled. In this case, as the gas cools, the gas-solute intermolecular forces can become stronger than than the gas-gas intermolecular forces at a temperature which is higher than the boiling point. In such a case the gas molecules will clump around solute molecules, while the kinetic energy of the gas molecules are still too high to allow condensation. Now the gas has obtained solvating properties and the solute will become truly dissolved in the gas.

The same effect can be imagined to happen during the isothermal compression of a gas.

If there are more than one solute in the volume of gas, some might dissolve in the gas, while others one might not. This means that the solvating gas can be *selective*. It can also be seen that the solvating power of the gas will depend on the temperature and the pressure of the gas. This means that the solvent becomes *tunable*.

While the dense gas has solvating properties, it still has the physical properties of a gas:

Diffusivity The solvating gas maintains its low diffusion coefficients, which means that it can easily diffuse into porous material, and that solutes will rapidly diffuse through it.

Surface tension The solvating gas has a low surface tension, which means that it will readily 'wet' surfaces and penetrate porous material.

Viscosity The solvating gas has a low viscosity, which means that it takes little energy to pump it.

Out[•]=

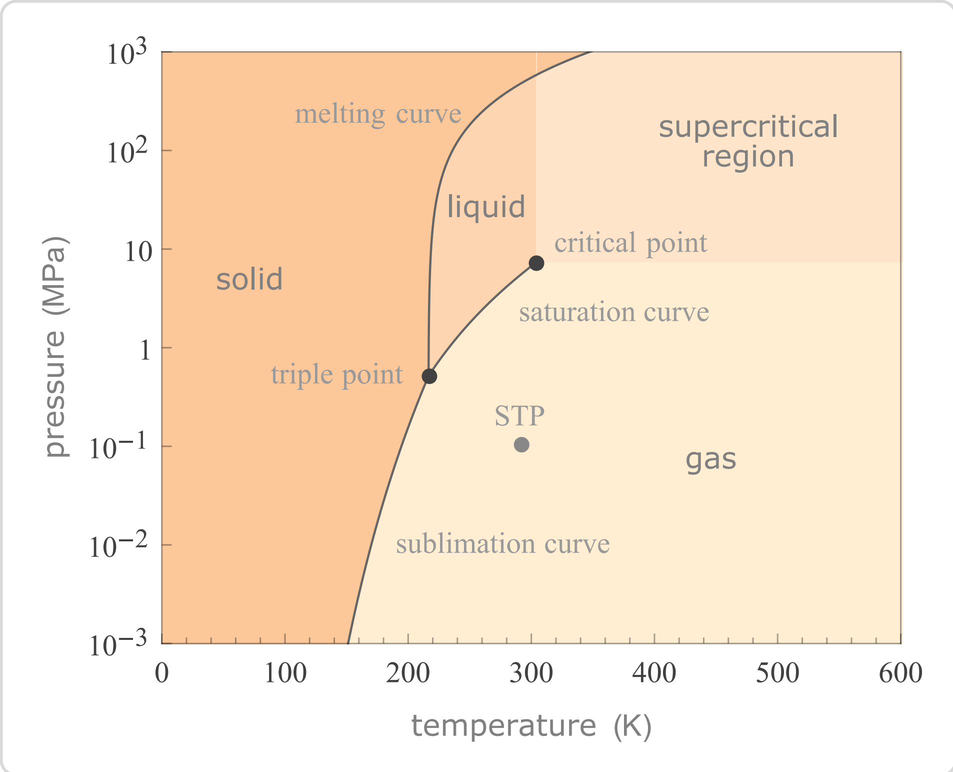


FIGURE 2.2: The phase diagram of carbon dioxide

⟨fig:co2phase⟩

For historical reasons such solvating gases are known as a 'supercritical fluids', because they were first obtained by heating a liquid at high pressure (Berche, Henkel, and Kenna 2009), so that the temperature and pressure of the substance is higher than it's *critical point*. The critical pressure of a substance is the pressure above which it is impossible to create a gas-liquid phase transition by isobaric cooling, and the critical temperature is the temperature above which it is impossible to create a gas-liquid phase transition by isothermal compression. When the gas is at its critical temperature and critical pressure, it is at its critical point. The critical point is very different from the *triple point*: there is no equilibrium involved (See figure 2.2). The terms 'supercritical fluid' and 'dense gas' are synonyms (Randall 1982) — the adjective 'dense' in 'dense gas' of course implies that the gas behaviour is far from that of an ideal gas.

There are no discontinuities in binary diffusivity when temperature is changed from above to below the critical temperature (Lauer, McManigill, and Board 1983). Hence extractions are often done under conditions which are not technically 'supercritical' but still yields its benefits.

The critical pressure of carbon dioxide is 304.12 K (31.10 °C) , and the critical pressure is 7.39 MPa (72.9 atm). This temperature and pressure are easy to achieve in the laboratory with standard chromatographic instrumentation, or in an industrial plant with standard process engineering technologies.

Carbon dioxide is gaseous at ambient conditions. This means that once it has been used in it's role as extractant, and it is exposed to the atmosphere, it will rapidly evaporate, without needing added heat, and leaving no residues.

Any pure compound will have a supercritical point. Compounds with technologically feasible supercritical points and useful chemical properties include ammonia, methanol, CFCs/Freon, hydrocarbons (propane, butane), water and sulfur hexafluoride. All of these lack green attributes: hyrocarbons pollute, the CFCs deplete ozone, sulfur hexafluoride is a potent greenhouse gas, and methanol and water are liquid at ambient conditions.

For these reasons the term supercritical fluid extraction is practically synonymous with extraction using high-pressure carbon dioxide.

It is also possible to use supercritical fluids as reactants. This topic falls outside the scope of this discussion.

Modifiers

⟨sec:modifiers⟩ The carbon dioxide molecule has zero dipole moment and the bulk fluid a low dielectric constant, so supercritical carbon dioxide is expected to be a non-polar solvent. (Although in practice the solvent behaviour is more complex, partly explained by the high quadrupole moment of the carbon dioxide molecule (Raveendran, Ikushima, and Wallen 2005).)

While the solvating power of a supercritical fluid is certainly 'tuneable' by adjusting its pressure and/or temperature, the range in solubility might be quite limited in practice. Just as with other solvents, it is possible to add a co-solvent or *modifier* to the supercritical carbon dioxide. This makes it possible to increase the solubility of polar compounds in the supercritical fluid. Methanol, ethanol, formic acid, and water are examples of suitable green modifiers for carbon dioxide (Herrero et al. 2010).

When modifiers are used the carbon-dioxide, modifier and solute forms a system with four degrees of freedom (modifier percentage, solute concentration, pressure, and temperature), which can become difficult to model. While this is a challenge for process engineers who need to design efficient industrial systems, analytical chemists can afford to be pragmatic and use heuristics to find suitable conditions (Wells, Zhou, and Parcher 2003).

Practical extractions

Figure 2.3 shows a schematic diagram of a system set up for supercritical fluid extractions.

Carbon dioxide (a) is readily available from suppliers of industrial gases, and high-purity grades are available. Chromatography-grade solvents are usually used as modifiers (d). High-pressure pumps (HPLC type) are used to compress the carbon dioxide (b) and the modifier (c), which are mixed together at the appropriate ratio. The mixture gets pumped into the (optionally heated (f)) extraction cell (e), which contains the material that needs to be extracted. Having extracted the extract from the material, the supercritical fluid passes through a pressure-control mechanism (g). This allows the pressure of the supercritical fluid to drop to ambient, turning it into a low-density non-solvating gas. The extract becomes desolvated, and precipitates in the collection vessel (h). The operation of the system might be either static or dynamic: in static operation the supercritical fluid is pumped into the system, the flow is stopped, and the matrix/fluid mixture is given time to approach equilibrium. Then the fluid is expelled and the extract collected. In dynamic operation the supercritical fluid is pumped through the extraction cell and the extract collected continuously.

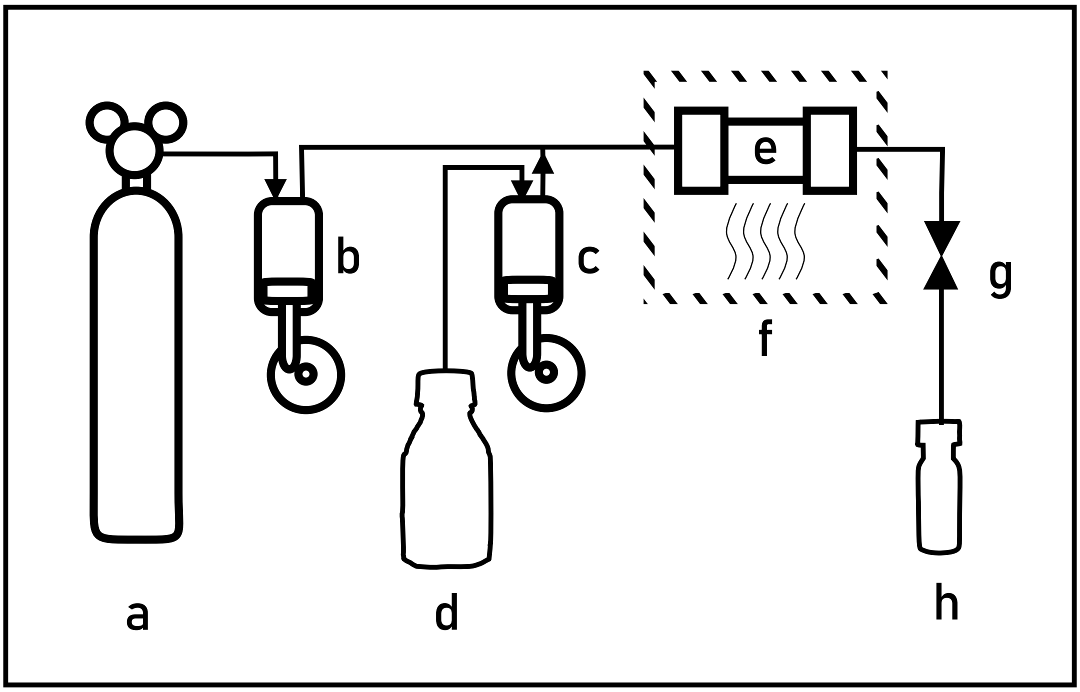


FIGURE 2.3: A diagram of an SFE system. (a) CO_2 supply (b) High-pressure SF pump (c) High-pressure modifier pump (d) Modifier reservoir (e) Extraction cell (f) Pressure control (g) Collection vessel

⟨fig:sfediagram⟩

2.3 Supercritical Fluid Chromatography

An analyte will extract out of a matrix into a given solvent with a certain efficiency and at a certain rate. While this is important while finding an optimum extraction method, otherwise its relevance is limited.

However, *different* analytes will extract out of a matrix with *different* efficiencies and at *different* rates. In a 1906 paper the Russian botanist Tsvet applied this observation to the dynamic extraction of a bed of calcium carbonate that had mixture of plant pigments applied at the inlet end, using petrochemical solvents. The different extraction efficiencies and rates of adsorption and desorption on the calcium carbonate surface lead to the *separation* of the compounds in the mixture. Tsvet called this method of separation *chromatography* (Ettre and Sakodynskii 1993a; Ettre and Sakodynskii 1993b). With time this method became generalized, and today chromatography is a major, established scientific field with many ramifications and a myriad of applications.

Because of the different technologies used in its applications, chromatography is conventionally classed by the state of it's mobile phase as either *gas chromatography* (GC) or *liquid chromatography* (LC). However, as we have seen, solvating gases can also extract analytes from solid stationary phases, and hence the term *supercritical fluid chromatography* (SFC) was created for these kinds of separations.

Supercritical fluid chromatography as practised today bears a great resemblance to *high performance liquid chromatography* (HPLC). The main reason for this is that there is a large overlap between the technology used for HPLC and the technology needed for SFC. In particular, both use high-pressure pumps and columns packed

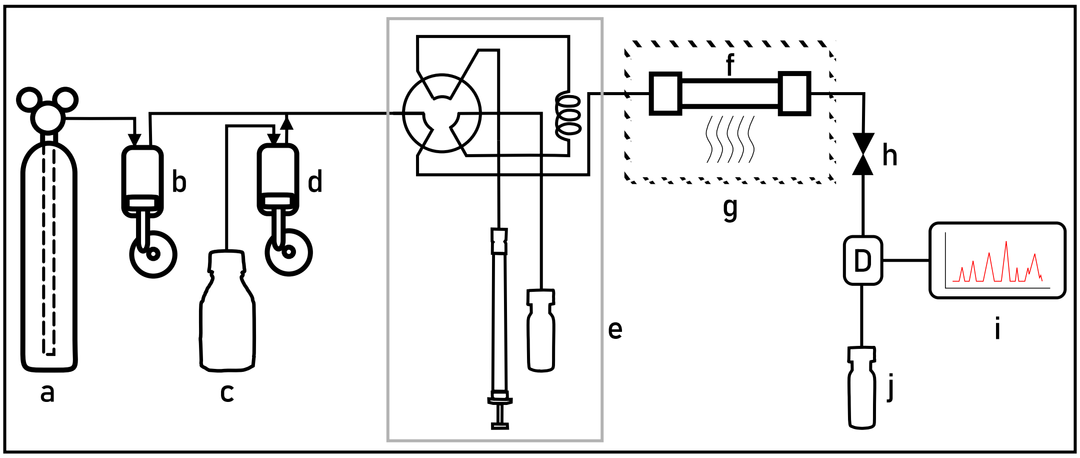


FIGURE 2.4: A diagram of an SFC system. (a) CO_2 supply (b) High-pressure SF pump (c) Modifier reservoir (d) High-pressure modifier pump (e) Injection system (f) Column (g) Optional heating (h) Back-pressure control (D) Detector (i) Data system (j) Optional fraction collection.

?⟨fig:sfcdiagram⟩?

with particles of very small diameter, and use optical detectors. The same instrument manufacturers who supply HPLC instrumentation also supply SFC instrumentation.

2.3.1 SFC and FID

But SFC was not always a technique based on HPLC technology. In the 1980s SFC was practised using open tubular columns and flame detectors, so the instrument designs looked more like GC instruments than HPLC instruments, and SFC was sold as a replacement for GC (Poole 2003).

During this era the detectors used for SFC was the flame-based detectors used for CG, in particular the flame ionization detector (FID).

The *flame ionization detector* was invented near-simultaneously in South Africa and New Zealand (Ettre 2008). The core of the system is a flame of hydrogen gas burning in pure air. The measured signal is the conductivity of the flame plasma, which is measured by applying a -100 V potential difference between electrodes at the tip and the base of the flame. There are very few free ions in the hydrogen flame, so the conductivity is normally low. But organic compounds introduced into the flame creates a number of free ions, which increases the conductivity of the flame gases. The change in conductivity is measured by measuring the current between the two electrodes, using an electrometer.

As a first approximation the signal produced by the FID is proportional to the number of carbon atoms in the analyte. This is rather surprising, until the mechanism of its working is elucidated. At high temperature in the hydrogen-rich core of the flame, all hydrocarbon atoms are reduced to methane (Holm and Madsen 1996). Once it gets into contact with the hot oxygen in the outer layers of the flame there is a chemi-ionization reaction. The electric field acting on the ions creates a flow of ions, which can be measured as an electric current.

The FID's shining strength as a detector is its tremendous linear dynamic range of 10^7. Combining the linear dynamic range with its carbon sensitivity makes it an excellent detector for quantifying organic compounds.

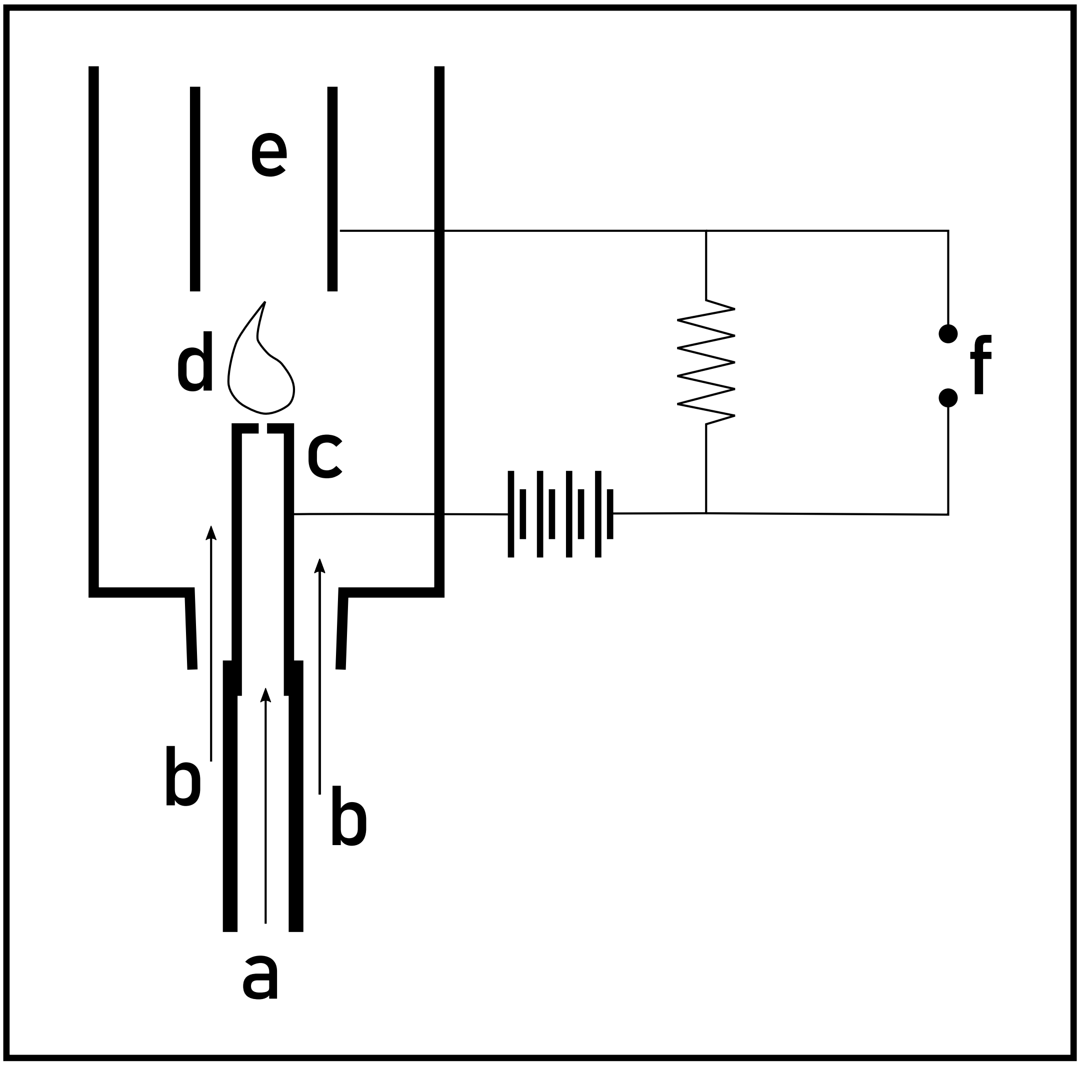


FIGURE 2.5: A schematic diagram of the flame ionization detector. (a) Mixture of column eluate and hydrogen gas (b) Clean air (c) Metallic flame tip electrode (d) Collector electrode (e) Conductivity signal

?⟨fig:fiddiagram⟩?

During period of history that SFC was seen as a variant of GC, the FID was the detector of choice. But when SFC started looking like HPLC, and the selectivity of the chromatography started being manipulated by adding modifiers (see Section 2.2.3), the FID lost its utility. The quantity of modifier added to the carbon dioxide mobile phase swamps the FID detector, making it useless as a detector. In contrast, if the chosen modifiers are transparent at the relevant wavelengths, optical detectors are more useful in SFC using modified carbon dioxide as a mobile phase.

2.3.2 SFC×GC

⟨sec:SFCxGC⟩ In chromatography, the analyte in the eluate can be detected to yield information about the sample, or fractions of eluate can be collected for further purification or characterization, or both. If fractions are collected, there is nothing that prevents the chromatographer from subjecting a collected fraction to another chromatographic separation.

For example, a synthetic chemist might use column chromatography to separate their target compound from side-reaction compounds, collecting fractions of the eluant. If the compounds are colourless, the chemist might use *thin-layer chromatography* (TLC) to examine the fractions for presence and purity of the relevant compounds.

If the product and the side-product were co-eluting it would not help to use the same chromatographic system (*i.e.* combination of stationary phase and mobile phase) for the TLC examination, because the co-elution would not become apparent. If, however, the chemist changed the mobile phase, or switched to a different stationary phase for the TLC examination, then it is quite likely that the two compounds would be separated.

For example, if a synthesis involving sugars were being attempted, two sugar enantiomers would likely co-elute on a silica clean-up column. Investigating the sugar fraction using a TLC plate with a chiral stationary phase would reveal the presence of stereoisomers.

The second, chiral separation (on TLC) is said to be *orthogonal* to the first (packed column) separation.

Such a separation is an example of a two-dimensional (2D) separation. In analytical chromatography, 2D separations are powerful tools.

The simplest 2D chromatography is called *heart-cutting*. In such a case a fraction of interest is collected from the first dimension (^{1}D) separation, and subjected to a second dimension (^{2}D) separation. For example, a fraction collected from an HPLC separation could be injected into a gas chromatograph. The separation on the GC dimension would obviously be very different from the separation on the HPLC dimension, and therefore we can say that the orthogonality is high.

Heart-cutting is a useful way to get detailed information from a sample, but it is technically demanding and labour-intensive. It is usually employed for challenging separations in well-understood samples, because peaks of interest in the first dimension must be captured for injection on the second dimension.

If, however, one collects the eluate in equal fractions and do identical separations on each of the fractions, then one enters the domain of *comprehensive multi-dimensional chromatography*. The comprehensive coupling of orthogonal chromatographies does not demand a previous understanding of the sample, and doing exactly the same separation for each fraction allows the process to be automated.

A 2D separation can be called comprehensive if the following three criteria are met (Giddings 1987):

1. Every part of the sample is separated by two distinct chromatograpic processes.
2. Equal percentages of all sample fractions are separated by the second process.
3. Compounds resolved by the first dimension separation remains resolved.

To effectively distinguish between heart-cut and comprehensive 2D couplings, a terse nomenclature was developed. Simple coupling between systems is designated by a hyphen ("-"), for example HPLC-GC. This corresponds to the usual notation of a coupled detector coupled to a chromatograph, for example GC-FID or GC-MS. Comprehensive coupling is denoted by a multiplex sign ("×"), for example GC×GC. If it is not clear from the context, the detector can be specified using a hyphen, as in GC×GC-MS.

Of the comprehensive coupled chromatography techniques, GC×GC is the most mature, with a selection of powerful instruments on the market.

This thesis further explores the implementation of SFC×GC, as first developed by Venter and Rohwer (A. Venter and Rohwer 2004; A. Venter, Makgwane, and Rohwer 2006).

In this implementation of SFC×GC, fractions of the eluate of the SFC is transferred to the GC. The mobile phase is changed from carbon dioxide to hydrogen in the modulating interface, and the GC dimension is a conventional open-tubular capillary separation with FID detection.

Any volatile modifiers or additives added to the SFC mobile phase will be separated from the analytes by the GC dimension. This makes in possible to use the FID as a detector in an SFC×GC chromatograph where the SFC is based on HPCL technology.

The ^{2}D separations are *fast* chromatographic separations, *i.e.* separations optimized for speed, sacrificing resolution. This is achieved using fast temperature programming of the GC separation.

The high orhtogonality of the SFC and the GC separations enables novel comprehensive 2D chromatography, which we applied to the chemical analysis of biodiesel.

The nice thing about standards is that you have so many to choose from.

Andrew S. Tanenbaum

3 Biodiesel, technical standards and chemical analysis

⟨Chapter3⟩

3.1 Introduction to standards

⟨Sec:Intro⟩

The fact that vegetable oil can be used to fuel Diesel engines has been known since the earliest days. Rudolf Diesel himself had exhibited an engine at the Paris Exhibition in 1998 (Knothe 2010) that ran on peanut oil. But the development of the petroleum industry late in the 19th century ensured an ample supply of fossil fuel for these engines.

The development of the diesel engine happened in parallel with the developing diesel fuel, for, as Cummins said "...we must never forget that *an engine and the fuel it consumes are inseparable partners*; the one cannot progress without the full cooperation of the other" (Cummins 1989). The first invention of an engine presupposes a supply of fuel, but the variation-selection process that searches for lower costs and higher efficiency then opens up the quest for more fuels. If a possible fuel is found, meticulous engine builders would then have to test each new fuel in their engines and approve of it. But fuel suppliers would like to see their fuels used in as many engines as possible. This convergence of interests gives rise to the establishment of *technical standards*. Technical standards allows engine builders to develop engines that will run on any fuel with certain agreed-upon standard qualities, and fuel producers can produce fuels knowing that they will work on any engine designed for that fuel.

A technical standard or just *standard* is a "document, established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context" (Hatto 2010). Standards are used in many aspects of industrialized societies, from implantable medical devices (ISO 2019) to coffins and caskets (SABS 1993). Standards are often associated with products, but also apply to procedures (ISO 2015) and systems (ISO 2017).

Standards are not mandatory, nor do they provide the 'best' way of doing something. The great strength of standards are their reliability. "Standards exist principally to provide a reliable basis on which common expectations can be shared regarding specific characteristics of a product, service or process" (BSI 2016).

TABLE 3.1: A few well-known standards organizations

Abbreviation	**Name**	**Country of origin**
SABS	South African Bureau of Standards	South Africa
ISO	International Organization for Standardization	International
CEN	European Committee for Standardization	Europe
ASTM	ASTM International	USA
BSI	British Standards Institution	UK
IEC	International Electrotechnical Commission	International
DIN	German Institute for Standardization	Germany
ANSI	American National Standards Institute	USA
UL	Underwriter's Laboratory	USA
ITU	International Telecommunication Union	International

Standards are published by *standards organizations*, which might be national or international in character, or might be established to serve a certain industry. Standards organizations are often known by their abbreviations, and a few of them are listed in Table 3.1. The authors of these standards documents are usually **technical committees**, comprised of individuals from a wide variety of stakeholder organizations, who work towards consensus.

Standards have a unique publication system. They are not published by publishing houses, but by the standards organizations themselves, who sell the documents directly to end users. Each standard is usually also better known by its number than by its title. For example, if I were to mention the document entitled "Quality management systems — Requirements" few people would know that I'm talking about the well-known quality system standard usually known as ISO 9001.

The South African national standards body is the South African Bureau of Standards (SABS), which was established by an act of parliament, Standards Act, 1945 (Act No. 24 of 1945)), as amended by the Standards Act, Act No. 8 of 2008 (South African Government 2008). The SABS issues South African National Standards.

Standards organizations not only write standards, they might also **adopt** them. Adoption happens when a suitable standard has already been issued by another standards organization. Standards very often refer to other standards, and standards are often based on published research. While standards are not mandatory, some legislation might refer to

The desire for engine designers for access to a reliable fuel and for fuel suppliers to have the largest possible market lead them to cooperate in the development of standards for fuels. In South Africa the relevant standard for petroleum-based diesel fuel is SANS 342, and in the USA the equivalent is ASTM D975.

Biodiesel is chemically very different from **petrodiesel** (diesel derived from fossil sources), and therefore the technical standards of biodiesel need to be different from the standards for petrodiesel.

3.2 SANS 1935: An overview

The current South African standard applicable to biodiesel is "South African National Standard 1935 Automotive biodiesel — Fatty Acid Methyl Esters (FAME) for diesel engines — Requirements and test methods." (SABS 2008) This is an adoption of the European Committee for Standardization's (CEN) standard EN 14214 (CEN 2008). In the USA is the equivalent standard is ASTM D 7651, which is largely similar but of different heritage.

SANS 1935 consists of a 18 pages. The first two pages are unnumbered: for the purposes of this discussion they will be numbered in small Roman numerals.

p(i) The first page is a title page, following the usual format for SABS standards. The top line of the page contains the ISBN (978-0-626-26349-2), and in large type the standard number (SANS 1935:2011). Then follows in capital letters "South African National Standards", and below that the title "Automotive biodiesel — Fatty Acid Methyl Esters (FAME) for diesel engines — Requirements and test methods". At the bottom edge of the page we find the SABS logo and some contact information.

p(ii) The second page is an informational page. It starts with a table of changes, which was still empty at the time of writing. Then follows a foreword, in which the technical committee who approved the standard is acknowledged (National Committee SABS SC 1018A). It also gives the date of publication (December 2011) and states that it supersedes SANS 1935:2004. Then there's very significant line, which states that the standard is referenced in the Petroleum Products Act (South African Government 1977).

p1 Contains the Table of Contents

p2 Is left blank

p3 Paragraph 1: This paragraph describes the scope of the standard, which is that it applies to biodiesel as an automotive fuel.

p3 Paragraph 2: This paragraph lists all the normative standards required to comply with SANS 1935. Thirty-five standards are listed.

p4 Continues the list of normative references

p5 Paragraph 3: This paragraph contains a list of definitions. The most important one is this: "biodiesel [is a] fuel comprised of methyl esters of long chain fatty acids derived from vegetable oils." This is a very specific description of biodiesel. It eliminates animal fats as a source of fatty acids, and it excludes methyl esters. But a note reads "Consideration for the inclusion of ethyl esters, animal fats and C8 – C12 carbon chains should be taken later." The significance of ethyl esters is that methyl esters are not usually carbon neutral. The methanol used in the transesterification reaction is usually obtained from the petrochemical industry, whereas ethanol from fermentation (See Section 1.4.2) could be carbon neutral. The definition also rules out hydrotreated vegetable oil (see Section 1.4.4) or biomass-derived Fischer-Tropsch diesel (see Section 1.4.3.

p5 Paragraph 4: This paragraph lists requirements

p5 Paragraph 4.1 discusses general requirements. According to these requirements biodiesel is a homogeneous liquid, free of adulterants or contaminants, to which additives might be added. It provides details regarding testing for oxidative stability and cold-flow properties

p6 Paragraph 4.2: This paragraph is about physical and chemical properties and states that biodiesel shall comply to the requirements of Table 1

p7 Paragraph 4.3: This paragraph concerns methods of test. It states that test methods shall be one of the test methods listed in Table 1.

p7 Paragraph 4.4 concerns disputes. It comes into effect when there is a dispute about product quality between, say, a biodiesel manufacturer and a biodiesel distributor. The contents of this paragraph prescribes which reference method shall be used.

p7-p8 These pages contain Table 1.

p9 Paragraph 5 describes a few simple rules for packing and marking biodiesel.

p10-p11 Annex A describes a method for the calculation of iodine value from chromatographic data. This calculation might be used instead of a measurement.

p12 Annex B prescribes how samples for testing must be taken.

p13 Annex C gives a list of values for calculating precision.

p14 Annex D provides an approved method for correcting density measurements. The prescribed tests requires density to be measured at 15 °C, which be might inconvenient. Instead, a different test may be made at a more convenient temperature, and a correction applied.

p15 Annex E is informative and recommends the implementation of quality management systems. It is followed by a bibliography.

p16 The final page contains information about the SABS and its services.

3.3 SANS 1935: Properties, requirements and methods.

On studying SANS 1935, it quickly becomes clear that the core of the document is Table 1. This table has three columns. The first column lists a **property**, the second column specifies a numerical value the property has to conform to, the **requirement**, and the third column prescribes the **test method** that must be used to obtain the value.

In the following discussion each of the requirements will be discussed, in order of increasing relevance to this thesis, grouped by method of determination (See Figure 3.1).

3.4 SANS 1935: Physical properties

3.4.1 Density

A diesel engine can only deliver work proportional to the heat of combustion of the fuel, and for a given chemical composition the energy will be proportional to the mass of the fuel. But diesel engines inject measures volumes of fuel, not measured masses. Therefore, an engine designer must specify the density of the fuel to specify the power output of the engine.

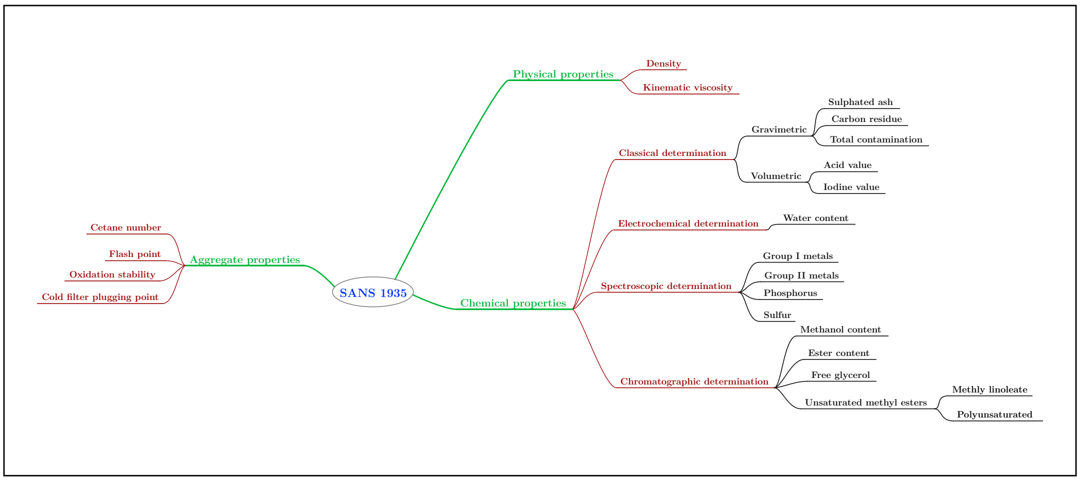


FIGURE 3.1: A mind map showing how the properties of biodiesel assessed by SANS 1935 is grouped for the purpose of discussion in this chapter.

⟨fig:MindMap⟩

According to SANS 1935 the density of biodiesel is required to be 860 kg m^{-3} to 900 kg m^{-3} at at temperature of 15 °C. The methods that can be used is described in ISO 3675 and ISO 12185.

ISO 12185 prescribes the measurement of density by using an electronic instrument known as the oscillating tube density meter. This measures the density of a liquid by measuring the mechanical vibrational frequency of a freely-oscillating tube filled with the liquid under test. The frequency depends on the weight of the filled tube, and therefore on the density of the liquid. These devices are easy to use and very accurate. The temperature of the liquid is controlled electronically.

ISO 3675 prescribes the use of a hydrometer, which is an instrument that measures density by measuring the buoyant force on a floating indicator. According to the law of Archimedes, the buoyant force on a body immersed in a liquid is equal to the weight of the displaced liquid. If the liquid is denser, the force is greater. Therefore, in a denser liquid a floating indicator will float with more of the indicator above the surface of the liquid. Hydrometer technology is mature, and the devices are simple and robust. If ISO 3675 is used at a temperature other than the specified one, a temperature correction is applied, as described in Annex D.

3.4.2 Kinematic viscosity

The viscosity of a fluid is a measure of its resistance to flow when a force is applied to it. Kinematic viscosity is the resistance to flow of a liquid when the force of gravity is applied to it. This flow of course depends on the density of the liquid, so that kinematic viscosity is determined by measuring the liquid's viscosity and dividing it by its density. It is important that the fuel for a diesel engine have the right viscosity, because the fuel must be finely divided for rapid combustion. The size of the droplets of fuel in the spray produced by the engine's injectors is strongly influenced by the fuel's viscosity.

SANS 1935 requires that kinematic viscosity be in the 3.5 to 5.0 The measurement is specified by ISO 3104, which uses a capillary viscometer: the time taken for a fixed volume of liquid to flow through a capillary. This time is them multiplied by an instrument-specific factor to yield the kinematic viscosity.

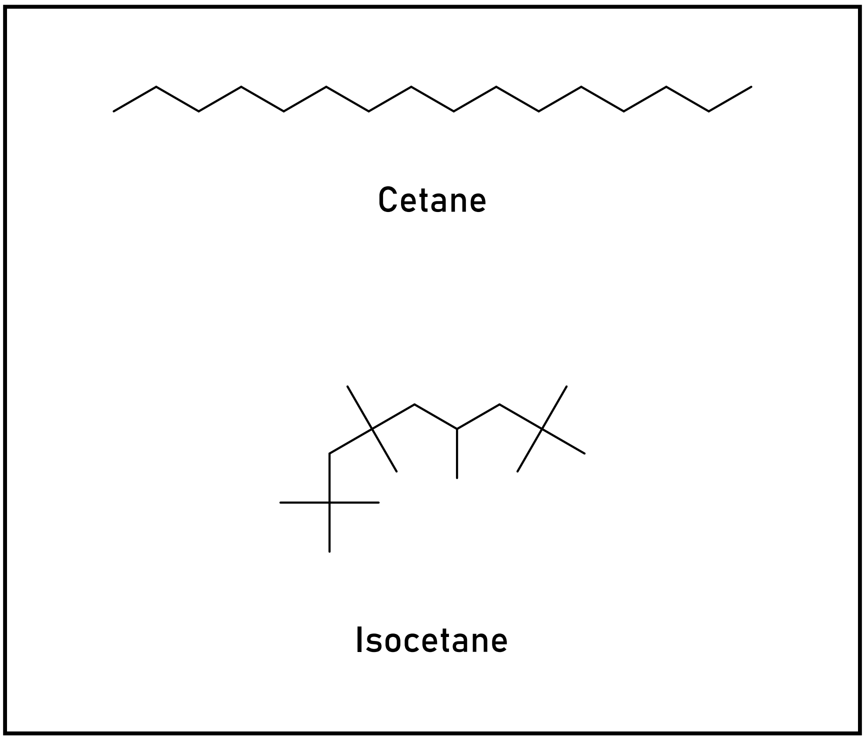


FIGURE 3.2: The chemical structures of the C16 hydrocarbon isomers cetane and isocetane.

⟨fig:Cetane⟩

3.5 SANS 1935: Aggregate properties: Specialized instrumentation

Some of the requirements specified in SANS 1935 are not values that have direct correspondence to physical or chemical quantities usually used in science, but are measures that have proved useful in practice.

3.5.1 Cetane number

For a diesel engine to operate according to design the fuel must combust in a reliable manner. The cetane number is a number that indicates the ease of ignition of a fuel in a diesel engine. Cetane is a synonym for hexadecane, and is a liquid compound that ignites easily when injected into a diesel engine. In contrast, its highly branched isomer 2,2,4,4,6,8,8-heptamethylnonane (also called isocetane, see Figure 3.2) ignites less easily. The intuitively-understood "ease of combustion" can be quantified as the **ignition delay**, the time between the moment the fuel is injected in to the engine and the moment the ignition starts. A mixture of the two compounds will have an ignition delay somewhere between that of the two compounds. A higher number indicates easier ignition.

SANS 1935 prescribes the test method of ISO 5165. The cetane number is obtained by measuring the ignition delay in a highly specialized test engine. The engine is equipped with the necessary instrumentation to measure ignition delay. It is a single-piston, four-stroke engine that has a combustion chamber of variable volume, which gives it a variable compression ratio. While running on the fuel under test, the compression ratio is changed until a prescribed ignition delay is achieved. Then the cetane/isocetane mixture that will give the same ignition delay at that compression ratio is found. If pure cetane will give the prescribed ignition delay, then the cetane number 100 is assigned. If pure isocetane gives the prescribed ignition delay, then the cetane number 0 is assigned. If it is found that a 50:50 cetane:isocetane mixture produces the prescribed ignition delay, then the cetane number of 50 is assigned.

Cetane number gives limited insight into the chemical properties of the fuel, or indeed, engine performance. But it is a trusted measure of the suitability of a fuel for use in a diesel engine and is therefore included in the standard. Biodiesel from common feedstocks usually easily meets the cetane specifications.

3.5.2 Flash point

Fuels are, naturally, flammable, and different fuels are flammable to different degrees. A fuel's **flash point** can be used to quantify its degree of flammability. The flash point is the temperature at which a fuel's vapour will ignite when it comes in contact with a flame.

The measurement of flash point is not primarily concerned with the performance of the fuel inside the engine, but is important to know because it determines how the fuel can be safely be handled during transport and storage (Worldwide Fuel Charter Committee 2009).

SANS 1935 specifies that either ISO 2719 (Procedure A) or ISO 3679 be used for determining the flash point. It seems that this aspect of SANS 1935 is out of date, because ISO 2719:2002 has been withdrawn and revised by ISO 2719:2016, which adds a Procedure C, specifically for FAME. ISO 2719 specifies the use of a Pensky-Martin closed-cup test, and automated device in which a temperature ramp heats an enclosed amount of liquid, while an ignition source is periodically introduced. The temperature at which the vapour ignites is the flash point. ISO 3679's test procedure works on a similar principle, but the device uses a smaller volume of liquid, and the liquid and its vapour are considered to be in thermal equilibrium.

3.5.3 Oxidation stability

⟨sec:Rancimat⟩ Fossil fuels for diesel engines are largely composed of alkanes, which are chemically very inert. This means that they can be stored for long periods without significant degradation. Biodiesel, in contrast, is by definition (SABS 2008, Paragraph 4.1.1) mostly composed of fatty acids, which are less inert than alkanes. This instability has been known since antiquity as fatty food going **rancid**. In particular, double bonds in the fatty acids are liable to reactions with atmospheric oxygen (Velasco, Dobarganes, and Márquez-Ruiz 2010). These are free radical reactions, illustrated in Figure 3.3. The hydroperoxides that form are highly reactive and can react with other fuel compounds to form polymers, soaps and acids.

The oxidative stability is measured by the Rancimat, an automated instrument. It works by bubbling a stream of hot air through a sample of biodiesel. This air is then passed through a conductivity measuring cell filled with deionized water. The conductivity of the water increases when volatile acids dissolves in it. At the beginning of the test period there are very few acids swept into the water, so the conductivity remains low. As the sample oxidizes and produce acids, the conductivity of the water slowly increases. But the oxidation reaction is **autocatalytic**, which means that the products of the oxidation reaction accelerates the oxidation. The result of the autocatalysis is that on the conductivity curve there is a sudden increase in conductivity. The time taken until this point is reached is called the **induction period**.

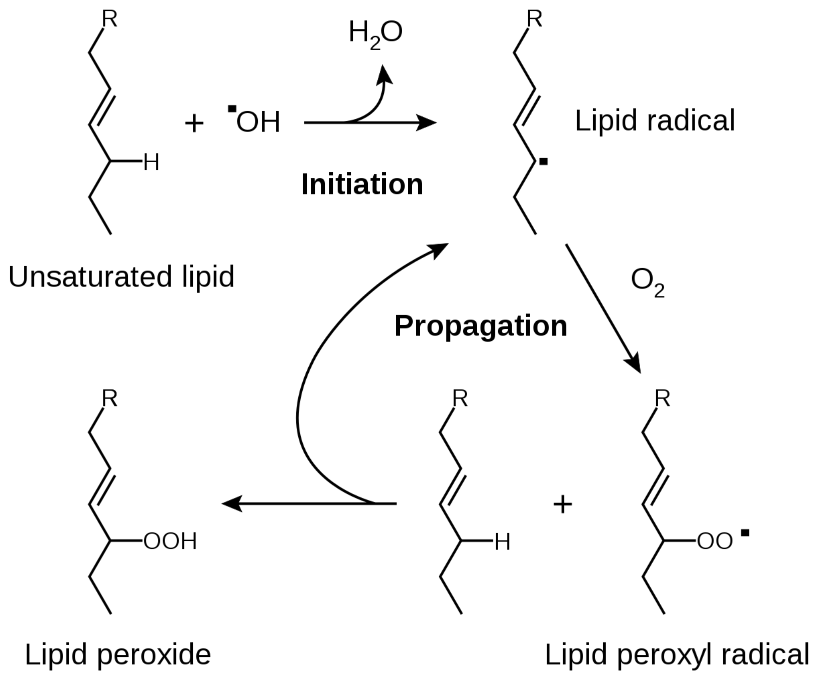


FIGURE 3.3: The peroxidation of lipids. By Tim Vickers, after (Young and McEneny 2001). Public Domain, https://commons.wikimedia.org/w/index.php?curid=1728531

⟨fig:RancidRadical⟩

SANS 1935 requires a minimum induction period of 6 minutes at 110 °C. Paragraph 4.1.2 permits the addition of antioxidants, compounds that prevent oxidation.

3.5.4 Cold filter plugging point

The flow of a liquid depends on its temperature. Most obviously, its viscosity depend on the temperature. But a liquid can freeze, which will also influence its flow. The operating temperature of a diesel engine is so high that the temperature of the fuel in the engine will be high enough to guarantee adequate flow for injection, but the biodiesel needs to be pumped from the fuel tank, through a fuel filter, to the engine. If crystals of fuel form at low temperatures, these crystals might plug the pores in the filter, which could lead to fuel starvation in the engine. It is therefore important that the biodiesel must be able to be pumped through the engine's fuel filter at all expected temperatures.

For this purpose SANS 1935 specifies a **cold filter plugging point**, measured according to SANS 50116. It is the lowest temperature at which a given volume of biodiesel still passes through a standardized filtration device in a specified time when cooled under certain conditions.

3.5.5 Copper strip corrosion

Many fuel storage and transfer systems parts and engines parts are made of metals, and under adverse conditions those parts are susceptible to corrosion. Chemical conditions that make corrosion more likely include acidity, water, and oxygen, but corrosion is a complex process, so it is very difficult to predict which combination of factors will result in unacceptable corrosion.

SANS 1935 prescribes ISO 2160 as the test for corrosiveness. In this test a strip of pure copper metal is polished and then immersed in a sample of the biodiesel at 50 °C for 3 h. The degree of corrosion is judged by visually comparing it to a standard card and then assigning it to a corrosion class.

3.6 SANS 1935: Chemical properties: Classical determination

3.6.1 Sulfated ash

Ash is the solids remaining after the complete combustion of a fuel. This is measure of the solids that will remain after use. Ash might originate from suspended solids, soluble metallic soaps, and residual catalyst that form refractory oxides during combustion. In addition to wear and deposits in the fuel system associated with ash, it also impairs modern diesel particulate filters.

SANS 1935 specifies ISO 3987 for the determination of sulfated ash. For the determination of ash it would not be practical to actually combust the sample and weigh the residue, because the ash might be carried away, either as volatile species or as a finely divided aerosol. Therefore the sample is oxidized using sulphuric acid, and then heated to drive off the sulphuric acid. What remains is metal oxides, which can be weighed.

3.6.2 Carbon residue

When a mixture of organic compounds is heated under conditions of low oxygen, it can form coke. Coke is a material that is practically pure carbon. Pure, solid carbon will combust, but the reaction is kinetically limited, so it will only combust slowly under conditions of high temperature and a large excess of oxygen. If a fuel tends to form coke inside an engine, it can cause problems in operation. Vegetable oil as diesel fuel, for example, tend to form coke on the injectors (van der Walt and Hugo 1982). This coking can cause problems with injection, which would affect engine performance. Carbon residue is not a scientific measure: from long experience it has been found to correlate with coking tendencies of oils in the petroleum industry, and so found its way into the biodiesel standard.

The test method prescribed for testing for carbon residue is ISO 10370. This involves heating a sample of biodiesel to high temperature in a crucible in air, using standardized apparatus. Most of the sample burns off, leaving a residue. The mass of the residue is determined and reported.

3.6.3 Total contamination

Ideally, biodiesel should be a homogeneous liquid. Some undissolved material can be tolerated, but too much can plug filters.

SANS 1935 specifies the test SANS 52662, which is synonymous with EN 12662. In this test total contamination is determined by filtering a sample of the biodiesel being tested through a glass-fibre filter, and determining the mass of the retained material.

3.6.4 Acid value

The end products of oxidation of biodiesel includes free organic acids, so the acidity of biodiesel is a good indicator of its quality. Measuring the acidity gives an indication of how much the biodiesel has already oxidized, whereas the oxidation stability (measured by Rancimat, see Section3.5.3) indicates how well the biodiesel will withstand oxidation on storage.

SANS 1935 specifies the test described in SANS 54104, which is equivalent to EN 14104. This test is a titration of a sample of biodiesel dissolved in a mixture solvents with alcoholic KOH. A glass pH electrode is used to follow the titration.

3.6.5 Iodine value

As discussed above (see Section 3.5.3) the oxidation of biodiesel is a major quality concern. The tendency of biodiesel to oxidize correlates with the number of double bonds in the fatty acids, or their **degree of unsaturation**.

The degree of unsaturation of oils and fats have long been measured by the **iodine value**, dating from 1884 (Knothe 2007). Halogens will rapidly add to double bonds, so that when a mixture of fatty acids is treated with a known excess of iodating reagent, the remaining iodine can be titrated to determine the amount of iodine absorbed by the fatty acids. The iodine value is the mass of iodine absorbed by 100 mass units of fat or oil.

The relevance of including iodine value in biodiesel standards has been questioned (Knothe 2002), because all the information regarding unsaturation of the fatty acids in biodiesel is contained in chromatographic data which need to be obtained in other requirements. SANS 1935 seems to acknowledge this, because Annex A allows that the iodine value can be calculated from chromatographic results (see Section 3.9.5).

Because determining iodine value is a mature technology and therefore relatively simple, it is tempting to think of it as a test that might be useful to small biodiesel producers. Unfortunately the iodine value is determined by the feedstock, so that for a feedstock from a certain vegetable oil crop there is unlikely to be any significant variation in iodine value, no matter the production process. Iodine value might be useful as a simple indicator of feedstock quality when the biodiesel is produced from waste vegetable oil, which might contain a variety of oils from different origins.

The prescribed method for the requirement is SANS 54111 (or, equivalently, EN 14111). In this method a known excess of Wijs's reagent (iodine chloride in acetic acid) is added to a weighed sample. The reaction mixture is then treated with potassium iodide, which converts the excess ICl to I_2, which is then titrated with potassium thiosulfate. The titration is followed potentiometrically and the equivalence point determined from the titration curve.

3.7 SANS 1935: Chemical properties: Electrochemical determination

3.7.1 Water content.

The compounds that comprise biodiesel are much more polar than those of petrodiesel. Therefore, much more water can dissolve in biodiesel than can dissolve in petrodiesel. This water has several deleterious effects on the quality of biodiesel. It encourages the growth of micro-organisms, allows hydrolysis, and accelerates corrosion.

The level of water specified in the requirements of SANS 1935 is lower than the solubility of water, and therefore refers to dissolved water. Free, visible water is excluded by paragraph 4.1.4.

The method specified by SANS 1935 for the determination of water in biodiesel is ISO 12937. This standard prescribes the well-known Karl Fischer titration used for the determination of water in solvents. This is a **coulometric** titration, which means that electricity is used as titrant. The titration curve is a plot of oxidation potential of a platinum electrode against the amount of charge (current integrated over time).

3.8 SANS 1935: Chemical properties: Spectroscopic determination

3.8.1 Group I metals

The most common catalysts in biodiesel production are sodium or potassium hydroxides (NaOH and KOH) or alkoxides (CH_3ONa and CH_3OK). These catalysts are polar and will dissolve in the glycerol byproduct of biodiesel production. But some may remain in the biodiesel itself, and needs to be removed. This cleanup can be done by washing with water, adsorbent columns, or selective membranes (Atadashi et al. 2011).

SANS 54108 is the method specified for the determination of sodium, and SANS 54109 the method specified for potassium. Both are flame atomic absorption spectroscopy methods: a sample of the biodiesel is aspirated into a gas flame, where the sodium and potassium are atomized. The atoms will absorb light at certain wavelengths, and measuring the amount of light absorbed will give a measure of the amount. Alternatively, EN 14538 may be used to determine sodium and potassium simultaneously with calcium and magnesium (see Section 3.8.2).

3.8.2 Group II metals

Fatty acids neutralized by alkali and alkaline earth metal hydroxides form **soaps**. If these metals are present in the feedstock, as catalyst, or in washing water they can form soaps with the fatty acids in the biodiesel. These soaps can form deposits in engines that can affect operations. For example, deposits of calcium soaps have been reported to cause injectors to stick (Pischinger, Schlag, and Mittelbach 2000).

SANS 1935 prescribes EN 14538 as the method for determining Group II metals. This is an optical emission spectroscopy method: a sample of the biodiesel is diluted in kerosene, and injected into a inductively coupled argon plasma. The emission intensities at certain wavelengths are compared to the emission intensities of solvents containing known concentrations of the metals.

3.8.3 Phosphorus

Phosphorus expected in biodiesel should not affect a diesel engine's performance, but it can have a detrimental effect on the exhaust treatment system by forming ash that can clog filters and reactive species that can reduce catalyst effectiveness. Trace levels of phosphorus will be expected in biodiesel, in the form of natural phospholipids. Normal biodiesel feedstock and production methods should yield acceptable phosphorus concentration, but inorganic phosphorus might be present in biodiesel produced from used cooking oil.

SANS 54107 is the prescribed method for determining phosphorus in biodiesel. A sample of biodiesel is dissolved in xylene, and the solution introduced in aerosol form into an inductively coupled argon plasma. The high temperature of the plasma causes phosphorus atoms and/or ions to emit radiation. This emission is measured at a certain wavelength, and compared to emissions from solutions with known concentrations.

3.8.4 Sulfur

The amount of sulfur in biodiesel is limited not because it affects the fuel's performance, but because the fuel must be compatible with emission control systems and not be more polluting in terms of sulfur emissions than petrodiesel. Most biodiesel feedstocks are naturally low in sulfur and are therefore unlikely to exceed the limits.

There are two prescribed tests for sulfur. ISO 20846 is a UV fluorescence method, while ISO 20884 is an X-ray fluorescence methods. The quantum-mechanical mechanism is the same for both methods: a chemical species absorbs energy from a photon which puts it in an activated state. The species then returns to a state of lower energy, emitting a photon of different energy. In the case of ISO 20846 the species is gaseous SO_2 (obtained by combusting the sample), and the activating photons are from the ultraviolet part of the electromagnetic spectrum. In the case of ISO 20884 the chemical species are the bound form of the sulfur as found in the biodiesel, and the activating photons are from the X-ray region of the electromagnetic spectrum.

3.9 SANS 1935: Chemical properties: Chromatographic determination

⟨sec:ChromDet⟩

3.9.1 Methanol content

Methanol in biodiesel increases its flash point, and it is an indicator of poor production process control.

SANS 1935 requires a maximum mass fraction fo 0.2 %. The prescribed test method is contained in SANS 54110, and involves heating a sealed vial partly filled with biodiesel to 80 °C. A portion of the headspace vapour is taken and injected into a gas chromatograph. The amount of methanol is quantified by comparing the methanol peak to either an internal or external standard.

3.9.2 Ester content and Linolenic acid methyl ester content

⟨sec:EsterContent⟩ As prescribed in Paragraph 3 of SANS 1935, biodiesel must consist of fatty acid methyl esters. The first line of Table 1 quantifies this requirement as a minimum of 96.5 % mass fraction.

The specified method is SANS 54103. This document refers to ISO 5508:1990, which has been withdrawn and superseded by ISO 12966-4:2015. ISO 5508 and ISO 12966 describe gas chromatographic methods for the determination of FAMEs. ISO 5508 is obsolete: it gives conditions for packed GC columns and thermal conductivity detectors, two technologies which are now rarely found in the chromatography laboratory. Both methods, however, require polar stationary phases. The quantity of esters is determined by integrating all the peaks in a certain retention time window and comparing it to the peak area of an internal standard.

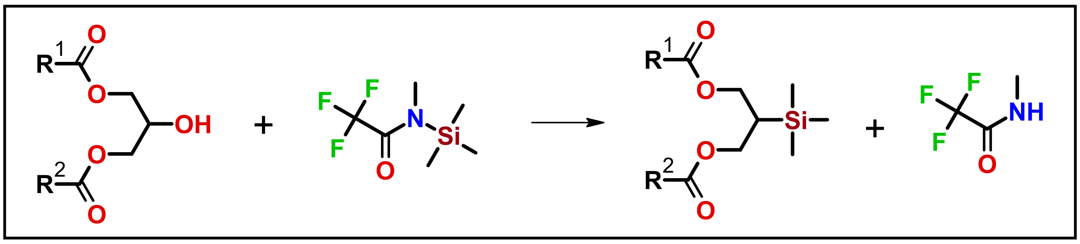


FIGURE 3.4: The derivitization of a diglyceride with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA). During GC the inert trimethylsilyl group does not interact with the stationary phase or its support, and the volatile trifluoroacetamide elutes with the solvent front.

?⟨fig:MSTFA⟩?

3.9.3 Glyceride content

⟨sec:Glycerides⟩ Glycerol (propane-1,2,3-triol) is the "backbone" of the oil molecules that constitute the feedstock for biodiesel production. Each hydroxyl group can form an ester bond with a fatty acid, and when all three have a fatty acid bound to it is an 'oil molecule'. IUPAC recommends that such a molecule is called a tri-O-acylglycerol (Nič et al. 2009), but by long-established custom they are called triglycerides. The conversion of a triglyceride to FAMEs is a stepwise process, with one ester bond at a time being methanolized. This means that during the reaction process, there will also be di- and monoglycerides (di- and mono-O-acylglycerols) in the reaction mixture. If the reaction is not well controlled then these glycerides will be found in the final product.

The presence of glycerides is of course an indicator of an incomplete transesterification reaction, but it has further negative effects. In particular, during cold weather, or in petroleum-blended biodiesel, some dissolved impurities might precipitate, in particular the monoglycerides (Dunn 2009; Plata, Gauthier-Maradei, and Kafarov 2015). This precipitate might block filters or otherwise interfere with engine performance.

SANS 1935 specifies that the mono-, di- and triglyceride content of biodiesel must determined with a procedure compliant with SANS 54105 (or, equivalently, EN 14105). In this method, the biodiesel sample is treated with MSTFA (2,2,2-Trifluoro-N-methyl-N-(trimethylsilyl)acetamide) before injecting it into a GC column.

MSTFA is a **derivatization reagent**: It reacts with the hydroxyl hydrogen atoms in the glycerides to trimethylsilyl derivatives. The molecule is then much more inert and will not interact with the column and the stationary phase, yielding peaks with better shapes.

The trifluoroacetamide group is a good leaving group, and upon nucleophilic attack by the hydroxyl oxygen its bond with the trimethylsilyl (TMS) group breaks, leaving the TMS bound to the oxygen. The labile hydrogen atom is now replaced by the TMS group, which is inert and will not interact with polar entities in the stationary phase or column, which will lead to improved peak shapes.

3.9.4 Free Glycerol

Free glycerol is one of the products of the transesterification of plant oils to produce biodiesel. It is a polar compound, which naturally separates from non-polar biodiesel, and any excess remaining dissolved in the biodiesel is removed during the washing step. Free glycerol contributes to injector coking.

Inappropriate processing may leave excess free glycerol in the biodiesel, and therefore determining free glycerol is an important quality-control step.

Free glycerol can be determined by the same chromatographic procedure prescribed for the determination of the other glycerides in SANS 54105 (see Section 3.9.3, but SANS 1935 also offers the option of SANS 54016. This standard uses a liquid-liquid extraction of biodiesel with a mixture of ethanol, water, and hexane. The free glycerol transfers quantitatively to the bottom layer, which is then analysed with a gas chromatographic method. The benefit of this method is that chromatogram is very simple: the glycerol is the only analyte peak in the chromatogram, which greatly simplifies quantification.

The column specified in SANS 54106 method is a PLOT column, *i.e.* a Porous Layer Open Tubular column, which is a capillary lined on the inside with a layer of particles coated with the stationary phase, in this case a polar polyethylene glycol.

3.9.5 Polyunsaturated methyl esters

⟨sec:ChromDetUnsat⟩ The degree of unsaturation of the constituent fatty acids are the main determinant of the oxidative stability of biodiesel. SANS 1935 limits the amount of highly unsaturated FAMEs by two lines in Table 1. In particular, the amount of methyl linoleate is limited to less than 12 % mass fraction, and the amount of polyunsaturated fatty acid methyl esters with four or more double bonds are limited to less than 1 % mass fraction.

Linolenic acid methyl ester

The prescribed method for the determination of methyl linoleate (C18:3) is SANS 54103, the same method as prescribed for total esters (see Section 3.9.2).

Highly unsaturated fatty acid methyl esters

Polyunsatured fatty acids (PUFAs) with more than four double bonds are not commonly found in plant oils, but are found in algae. Fish that feed on algae accumulate these oils, so that they are also found in fish oils, but fish oils are not a sustainable resource and should not be used for fuel(Kitessa et al. 2014). These compounds are highly oxidatively unstable, and are therefore undesirable in biodiesel.

The method prescribed for the determination of PUFA FAMEs is EN 15779. This method uses a capillary column with a wax stationary phase.

3.9.6 Conclusion: room for innovation.

The diversity of chromatographic biodiesel quality control methods causes the compliance with SANS 1935 to be expensive. To comply, a biodiesel producer will have to submit samples of biodiesel to at least four different kinds of chromatographic analysis:

- EN 14105 for free and total glycerol
- EN 14103 for FAME and methyl linoleate content
- EN 14110 for residual methanol

- EN 15779 for highly unsaturated FAMEs
- (Optionally) EN 14106 for free glycerol

This might prove costly, and there has been at least one innovation to reduce the number of instruments required (McCurry and Norman 2009).

As was emphasized in the introduction to this chapter (see Section 3.1), standards are not 'the best' way of determining a certain desirable property of biodiesel, it is merely a trusted method. As improved methods are developed and become trustworthy, they can be adopted as alternatives. As far as biodiesel is concerned, it seems that there is ample room for chromatographers to innovate and develop improved methods and instrumentation.

⟨todopage:1⟩ **To do** (1) **To do** (2)
⟨todopage:2⟩

$pV \neq nRT$
The universal gas law does not apply to supercritical fluids

4 Instrumentation: Supercritical Fluid Chromatography

⟨Chapter4⟩ This thesis discusses the development of a comprehensively coupled (supercritical fluid × gas) chromatograph and its application to the analysis of biodiesel. The discussion on the experimental work divides naturally into two parts: this chapter discusses the supercritical fluid chromatography (SFC) and the next chapter discusses the gas chromatography (GC).

4.1 SFC

As discussed in Chapter 2, an SFC chromatograph consists of a supply of mobile phase, a pump, a pressure control system, a modifier control system, a column, a pressure relief system and a detector

4.2 Mobile phase

As mobile phase we used carbon dioxide. The benefits of carbon dioxide as an solvent and mobile phase is discussed in Chapter 2.

We used 99.995 % pure carbon dioxide supplied by Air Produts. Our colleauges in industry also use food grade or technical grade carbon dioxide and they have not reported any significant impurities.

As modifier we used LiChrosolv®methanol from Merck. This is an 'HPLC-grade' solvent, of which the purification is optimized for the removal of UV-absorbing impurities. Using this grade of solvents is important when using optical aborbance or fluorescence detectors, because lowering levels of impurities improve limits of detection. It is quite likely that a less expensive grade of modifier would also be suitable for our SFC, because we do not use an optical detector.

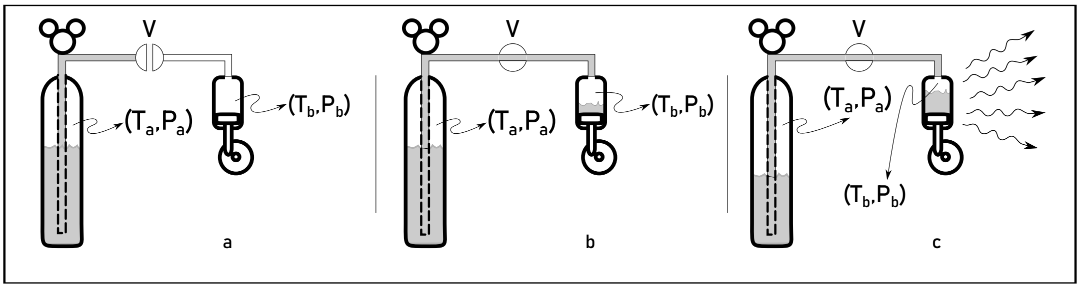


FIGURE 4.1: Diagram to help explain the filling of the carbon dioxide pump. (a) The pressure in the reservoir P_a is much higher than the pressure in the receptacle P_b. The valve is closed, so there is no flow. (b) The valve is open and some carbon dioxide has flowed from the reservoir to the receptacle. But $P_b = P_a$, so there is no flow. (c) When the temperature in the receptacle $T_b < T_a$, then $P_b < P_a$, following Gay-Lussac's law. Flow will continue until all the vapour in the receptacle has condensed.

⟨fig:co2fill⟩

4.3 Pump

⟨sec:CO2Pump⟩ As pump we used the robust, reliable Varian 8500 HPLC pump because it was available. This pump had its control electronics removed, and it was controlled from a personal computer by software written for the purpose.

The Varian 8500 pump is driven by a stepper motor. This kind of motor turns in discrete steps, instead of at a constant rate. It is driven by pulses of electric current, rather than a continuous current, and therefore the speed of the motor can be controlled by varying the rate of the pulses. This makes it relatively simple to control the speed of the motor from a computer.

The Varian 8500 pump has a built-in pressure transducer that provided an electronic signal proportional to the pressure at the pump outlet.

The Varian 8500 pump is a single-piston pump with a 250 ml capacity that needs to be refilled between strokes. Refilling means that one has to stop chromatography, which makes it important to fill the pump to full capacity, so that chromatographic runs are not interrupted. This means that the pump needs to be filled with carbon dioxide in the liquid phase rather than the vapour phase. Compressing either would create the appropriate phase for doing chromatography, but compressing a vapour would leave one with a much smaller volume of high-pressure carbon dioxide than compressing a liquid.

Filling a pump with liquid carbon dioxide is more difficult than one might imagine.

Imagine a reservoir of liquid carbon dioxide, equipped with a dip tube, connected to an empty receptacle via a valve (V). (See Figure 4.1) One can assume that the receptacle is empty and contains only carbon dioxide vapour at atmospheric pressure, say 1 atm. The vapour pressure of the vapour above the liquid in the reservoir is about 55.3 atm (5.6 MPa). When the valve (V) is opened the will high-pressure vapour in the reservoir will expel the liquid carbon dioxide through the dip tube and through the valve, into the receptacle. In the low-pressure environment of the receptacle the carbon dioxide will boil. Soon there will be some liquid carbon dioxide in the receptacle, with the rest of the receptacle volume filled with gaseous carbon dioxide. When the system comes to equilibrium the pressure in the receptacle (P_b)

will equal the pressure in the reservoir (P_a), and there will be no flow of liquid carbon dioxide. One can attempt to now increase the flow by increasing the volume of the receptacle (for example by withdrawing a pump piston), and hence decreasing the vapour pressure there. But any flow from the reservoir will lead to expansion of the headspace of the reservoir. This will lead to cooling of the vapour, and therefore lower pressure and therefore lower flow. The final result of this process is that the receptacle is never filled to capacity with liquid.

The only way to restore the flow from the reservoir to the recepacle is to create a pressure difference $P_a - P_b$. While it is possible to create an overpressure in the reservoir by adding a headspace gas, it is technically challenging and expensive. It is simpler to use the Guy-Lussac gas law. According to this law the ratio of the pressure to the temperature of a gas is constant so that $\frac{P_b}{T_b} = k$. This means that a decrease in temperature will lead to a decrease in pressure. The way to fill the reservoir to capacity is to ensure that the temperature of headspace vapour T_a is higher than the temperature of the headspace vapour T_b. Because safety regulations prohibit the heating of a cylinder of pressurized gas, the way to ensure $T_a < T_b$ is to cool the receptacle. This cools the headspace vapour, and following the Gay-Lussac law the pressure P_b decreases, the difference $P_a - P_b$ increases and the liquid flows until the receptacle is filled to capacity.

In the case of the Varian 8500 pump the cooling of the pump was achieved by wrapping a coil of copper tubing around the cylinder, and pumping a chilled heat-exchange fluid through it. A chiller with a mechanically cooled tank with a 20 l capacity was filled with a solution of 5.0 l of diethyl glycerol in about 10 l of water. This mixture has a freezing point of -15 °C, which can be cooled by the chiller without freezing. (If the coolant freezes a layer of ice forms on the cooling plate of the chiller, which isolates the remaining liquid from the cooling plate and limits the minimum temperature of the coolant.) An inexpensive submersible water pump (designed for decorative water fountains) was used to pump the coolant through the circuit. This pump can deliver 800 l of water per hour at a head of 1.2 m.

For some experiments we also used a SFT-10 pump from Supercritical Fluid Technologies (Newark, Delaware). This is a purpose-built two-piston pump with a sapphire valve seats and a Peltier-cooled head. This is a much better technology than the HPLC pumps. It takes up less space and does not need refilling, since it feeds directly from the cylinder. This pump had its own microprocessor controllers on board, and flow and pressure could simply be commanded from the PC through a USB cable.

4.3.1 Pressure control system

The aim of chromatography is to separate chromatograms by a certain distance. But the days of separating coloured compounds in glass packed columns are long past and the direct measurement of distances are now relegated to thin-layer chromatography. Instead, we have to make do with proxies for distance such as *retention volumes* or *retention times*.

Retention times are particularly convenient today, because it can easily be measured by computer systems.

Retention times, however, depend on a known flow rate of the mobile phase through the column. The flow rate need not be constant, although for the sake of simplicity a constant flow rate is preferred. Ideally, the flow rate should also be adjustable.

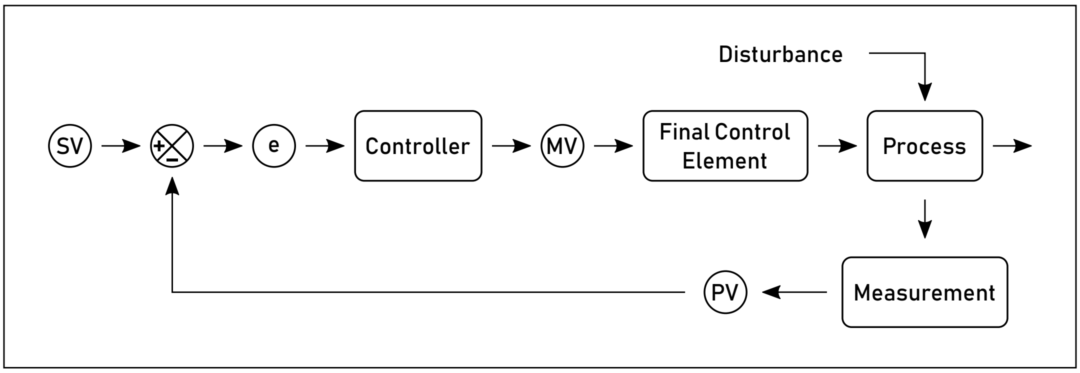


FIGURE 4.2: Schematic diagram of a closed-loop control system. (SV) Set Value, (PV) Process Variable, (e) Error, (MV) Manipulated Variable

⟨fig:processcontrol⟩

The need for an adjustable, constant flow rate can be met by using a control system. Control systems are well understood by engineers, among whom it is a major field of study (Koenig 2009).

Figure 4.2 shows a diagram of a simple process control system. Some aspect of the process under control is measured, which yields the *process variable* (PV). The PV is compared to the *set value* (SV), and the *error* (e) is obtained by finding the difference. The error is provided to the controller, which calculates the *manipulated variable* (MV). The MV is used to drive the final control element, which adjusts the process with the aim of producing a smaller error.

Chromatographic systems conventionally work under pressure control or flow control regimes. Either is suitable: constant pressure systems usually yield constant flow if other parameters are held constant. Because flow measurement is more complex than pressure measurement, pressure control is the older, simpler and less expensive control method. Also, in SFC the solvent strength of the mobile phase is pressure/density dependent. We therefore selected pressure control as our control regime.

In our system the pressure measured by the on-pump sensor was the process variable (PV) . This was compared to the set value (SV) set on the computer console. The controller was a software algorithm, implemented as a virtual instrument in the programming environment LabVIEW. The controller computed an output value for the manipulated variable (MV), which was the rate at which pulses were sent to the pump, in hertz. The digital input/output (DIO) interface of a National Instruments PCI-6014 multifunction data acquisition board created those pulses and sent them to the pump's electronic interface.

The software used a PID (Proportional-Integral-Derivative) controller module, with the Integral and Derivative contributions disabled. The simpler proportional control was found totally adequate for our purpose, and it can be improved in future by finding an appropriate tuning method and applying it with the integral and/or derivative activated.

4.3.2 Modifier Control

The modifier needs to be present in the mobile phase at a known and controlled concentration.

There are various SFC-modifier mobile phase supply units on the market. They tend to be a expensive and complex, because they require the use of two controlled,

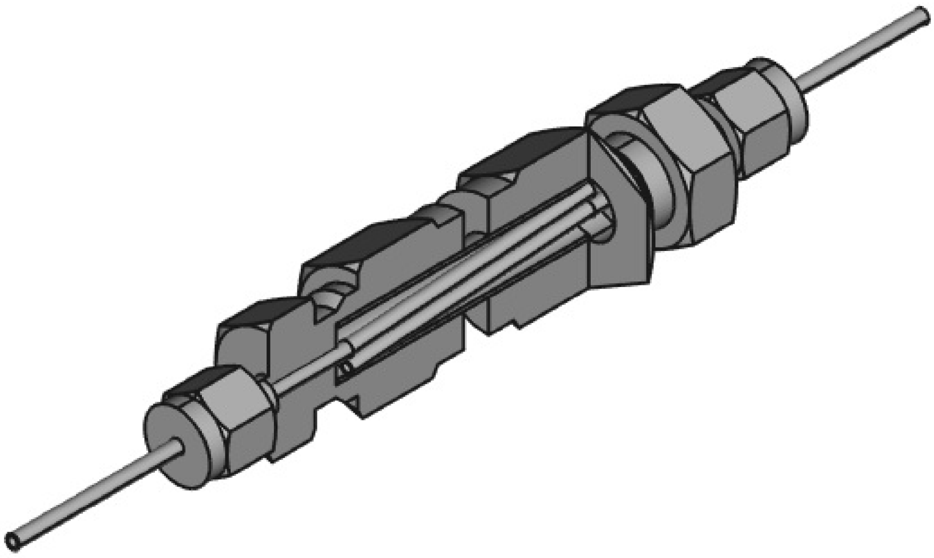


FIGURE 4.3: A cutaway diagram of the mixing chamber design. The chamber is designed for the mixing of the modifier and the supercritical carbon dioxide.

fig:mixingchamber⟩

high-pressure pumps. Given that our needs were rather modest, we elected to use a simpler system.

Instead of accurately pumping the supercritical fluid and the modifier, we only pump and control the supercritical carbon dioxide and add measured volumes of modifier.

The modifier control unit consisted of a six-port valve, a fixed-volume measuring loop, and a mixing chamber.

The unit operates by filling the measuring loop with modifier, and then switching the measuring loop into the flowing mobile phase. The modifier is washed into the mixing chamber, where it is intimately mixed with and dissolved in the supercritical carbon dioxide. The concentration of modifier in the mobile phase is determined by the switching rate of the sampling valve. Given that about 3 volumes of supercritical carbon dioxide is needed to wash the modifier out of the loop, the highest concentration of modifier that can be added in this manner is about 25 %.

Figure 4.3 shows the chosen design of the mixing chamber. The inlet and outlet pipes of the mixing chamber extend far into the chamber, to break up plug flow and encourage rapid mixing.

The modifier injection valve position was commanded from the controlling PC by electronic voltage pulses.

4.4 Sample injection

⟨sec:SFCInjection⟩ The sample inlet was a two-position rotary valve with an internal sampling volume (Figure 4.4). In one position the sampling volume was filled with a syringe, and

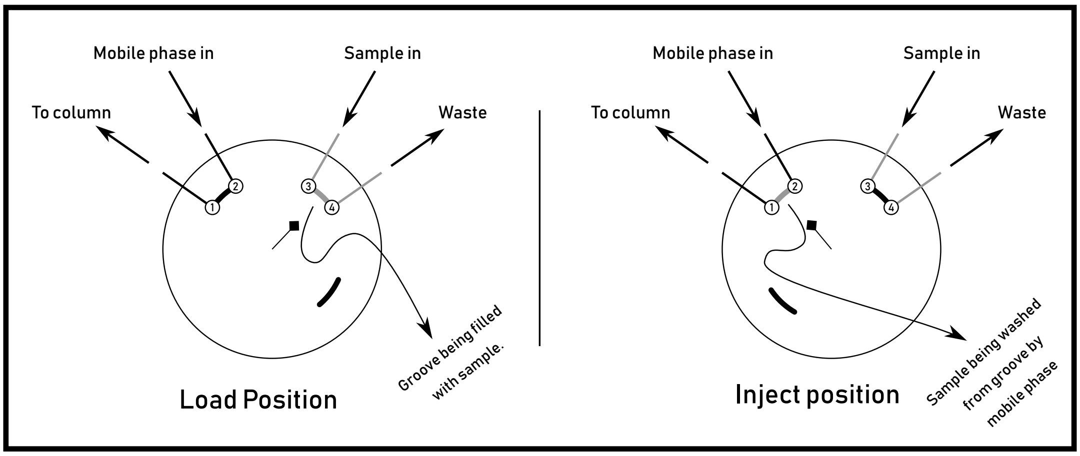


FIGURE 4.4: A schematic diagram of the sampling valve.

⟨fig:samplingvalve⟩

when the valve was commanded to inject the sampling volume was switched into the carrier mobile stream.

The reader might be more familiar with the six-port valve as an injection system. The benefit of the internal groove injection valve is that it allows a much smaller volume to be injected. Using this small volume makes it possible to inject samples without dilution, which simplifies sample preparation. A drawback of this design is that it is not possible to vary the volume of sample injected.

The sample volume was 5 μl

The injection valve position was commanded from the controlling PC by electronic voltage pulses.

4.4.1 Column

?⟨sec:SFCColumn⟩? The separation power of a chromatographic system can be modelled by the equation

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\bar{k}}{1+\bar{k}}\right) \tag{4.1}$$ {?}

were, R_s is the *peak resolution*, N is the *number of plates*, α is the *selectivity*, and $\bar{k}$ is the *retention factor*.

The number of plates N can be increased simply by making the column longer, at the cost of increasing the time of the run.

The maximum length of the column is determined by the pressure drop available that will still yield adequate flow. In capillary gas chromatography the openness of the column and the low viscosity of the gas-phase mobile phase routinely allows columns 100 metres long at inlet pressures of a few atmospheres. In HPLC the high viscosity of the mobile phase and the narrow, tortuous pathways between the small particles of the packing material means that the columns are typically 100 - 200 mm long, requiring hundreds of atmospheres of inlet pressure for adequate flow.

The low viscosity of an SFC mobile phase allows operation of much longer HPLC-type packed columns. This provides a much higher number of plates in the chromatographic system than HPLC.

The SFC column we used in the SFC×GC system was a set of five HPLC columns (150 mm × 4.6 mm, 3 μm particles) (Restek, Pinnacle DB Silica) connected in series.

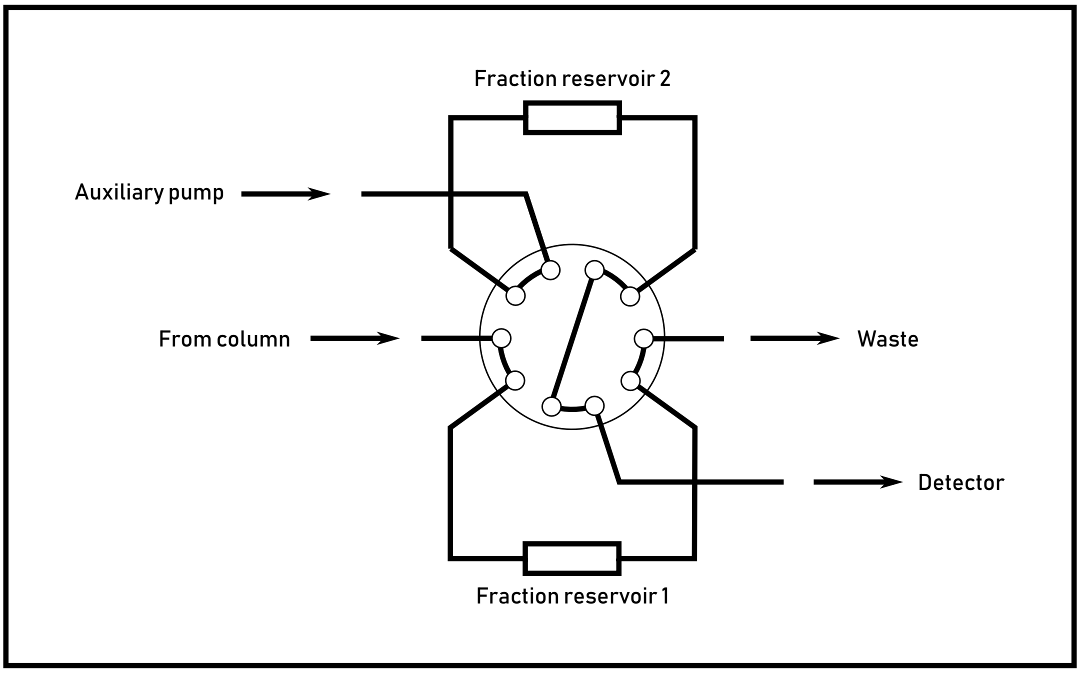


FIGURE 4.5: A schematic diagram of a valve configured to allow continuous-flow fraction collection.

ig:continuousflow⟩

The stationary phase in this column is ‘bare silica’, which is usually considered ’polar’. That means that the packing of the column consists of porous silica particles with no organic phase covering its surface, and hence one that will interact strongly with polar molecules. In contrast, the ubiquitous ‘C18’ stationary phase used in reverse-phase HPLC is ’non-polar’. The particles of such a stationary phase are coated with octadecyl chains bonded to its surface, and hence will tend to interact strongly with non-polar molecules. The base material of the particles is usually silica, but that is only because manufacturing uniform particles of a given size from silica is a mature technology and not because silica is important to the separation mechanism. (In fact, some care has to be taken to deactivate the silica so that it does not contribute to the retention. Such a mixed retention mechanism can lead to peak tailing.)

4.4.2 Stopped-flow

⟨sec:stopflow⟩ Among analytical chemists it is the convention that chromatographic runs are done without interruptions. In our SFC×GC chromatograph we collect fractions of SFC eluate and separate them by GC. It is possible to collect, store and inject fractions of SFC eluate while maintaining continuous flow, for example using a dual storage loop system like the one depicted in Figure 4.5. But such systems require careful selection of volumes, fraction collection reservoirs with matching volumes, and an auxiliary pump.

Instead of such a system, we opted for a more versatile and simpler stopped-flow system. In this system a fraction of the SFC eluate is collected, and then the flow through the column is stopped. While the flow is stopped the collected fraction is subjected to separation by GC.

In packed columns, "eddy diffusion is always the main cause for peak broadening at any temperature and at high velocities" (Gritti and Guiochon 2006). During the period that the flow is stopped only longitudinal diffusion contributes to peak broadening, which is small compared to eddy diffusion in dense mobile phases. Using the simple stopped-flow technique of sample collection will therefore not have a major effect on the resolution of the ^{1}D chromatography.

The flow in the SFC was stopped by a six-port rotary valve using three of the ports, directing flow either to a blocked-off port or to the depressurizer, in effect making it an on-off controller.

4.5 Pressure Relief

No matter the kind of SFC system one uses, at some point the eluate needs to be depressurized. This can be before or after the detector. If depressurization happens after the detector, then the details of the mechanism doesn't matter much, because the information has been obtained and the eluate can be discarded. If, as in our case, the depressurized eluate needs to still pass the detector, it is important to not lose the resolution achieved by the column. This means that the design of the depressurizer requires some care.

The main concern in depressurizer design is premature desolvation of analytes. This causes *discrimination*, which is the differential treatment of substances where identical treatments are required. In particular, compounds of higher molecular weight tend to desolve first from the supercritical fluid, and might precipitate in the wrong place if the depressurizer is not designed to prevent it.

Depressurization can be done either statically or dynamically. In a dynamic system there is an active element that controls the flow of the eluate in such a way as to maintain the pressure upstream of the active element of the controller. Such a device is often called a *back pressure regulator*, and is usually an electro-mechanical device with digital control. They tend to be complex and expensive.

In static depressurization there is no active pressure control. A simple *restrictor* is used to limit the flow between the high pressure of the SFC and the low-pressure outlet.

Textbooks often discuss different restrictor designs. (The book by Luque de Castro, Valcárcel, and Tena 1994, provides an example.). Our preferred depressurizer was the 'integral' or Guthrie design (Guthrie and Schwartz 1986). Figure 4.6 shows the steps in manufacturing the Guthrie restrictor.

We found this restrictor robust and fairly simple to manufacture. It was possible to adjust the flow to a given flow rate. This design of restrictor should eliminate discrimination, because the decompression takes place in a very short space.

However, the Guthrie design proved prone to blockage. These blockages could not be eliminated by incorporating a 0.5 µm filter before the restrictor, which prompted an investigation into the cause of blockages.

To rule out the possibility that it was particles that caused the Guthrie restrictor to become blocked, we first had to determine the diameter of the orifice. This proved to be harder than expected: optical microscopy was not able to give a simple, unambiguous measure of the orifice diameter.

Scanning electron microscopy (SEM) showed that the orifice diameter of a Guthrie restrictor is about 10 µm, as shown in Figure 4.7 This makes it very unlikely that particles with an origin in the SFC system blocked the restrictor: even the 3 µm particles from the column packing material should not block this restrictor.

Experience had taught us that the restrictor did not block if the flow was continuous. It was only when the pressure in the restrictor cycled that blockages occurred.

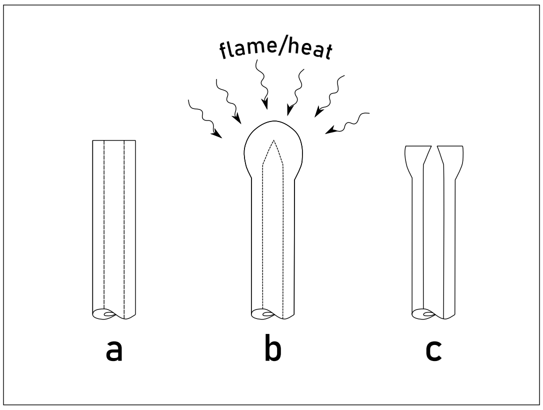


FIGURE 4.6: The steps of making a Guthrie restrictor. (a) Cut length of quartz capillary (b) Heat with flame to soften glass and create internal cone (c) Grind down end to expose orifice of the appropriate size.

⟨fig:restrictor⟩

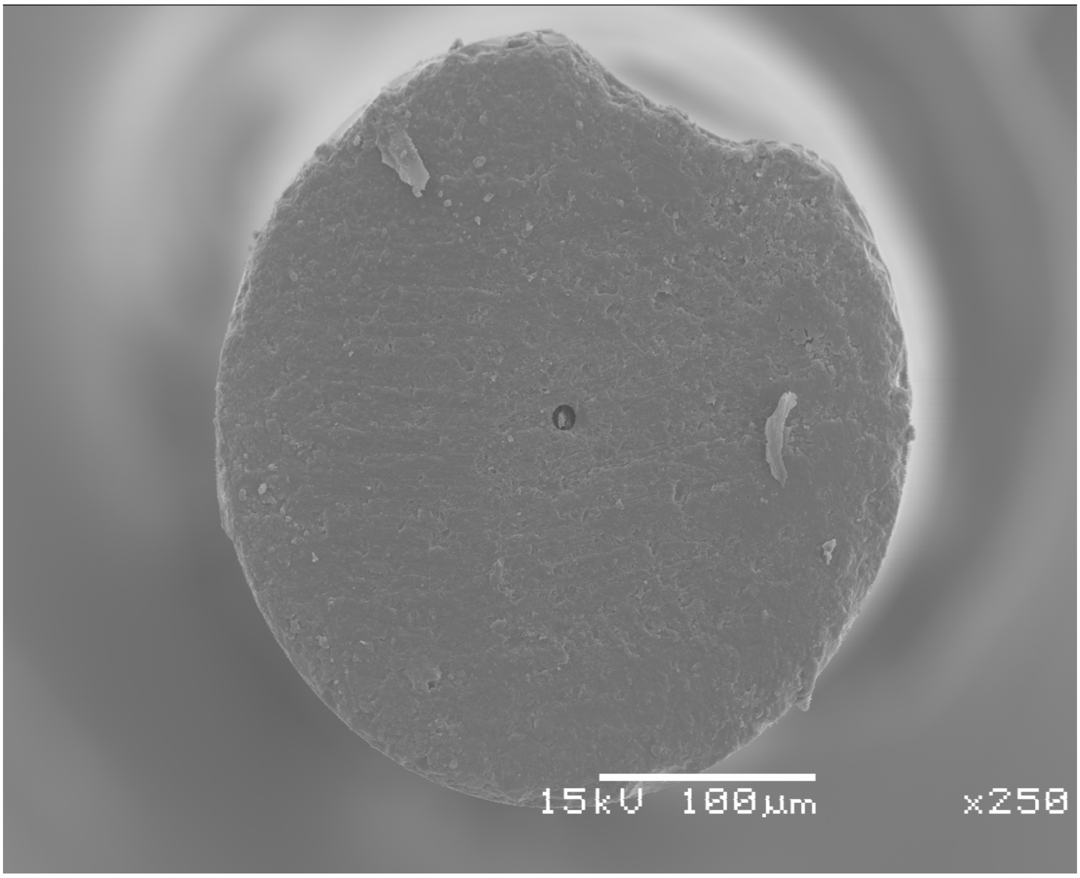


FIGURE 4.7: An electron microscope photograph of a restrictor tip, showing the size of the orifice.

restrictororifice⟩

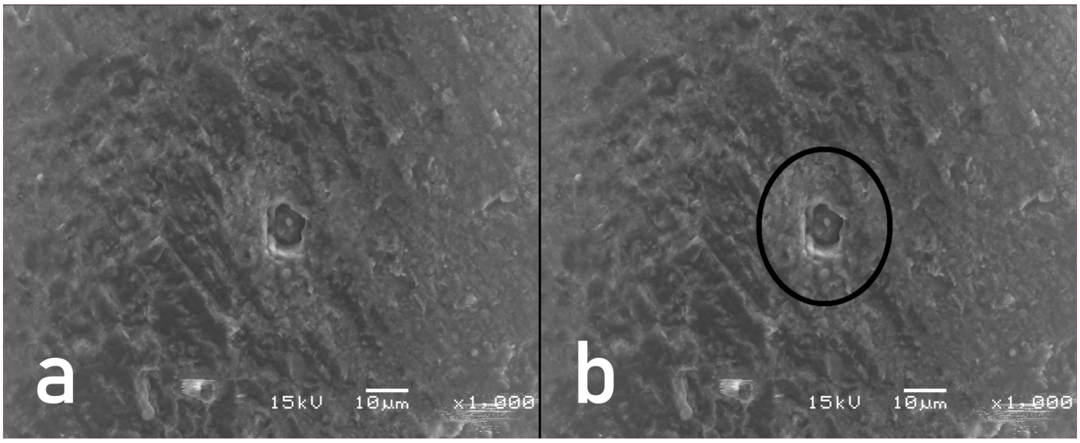


FIGURE 4.8: An electron microscope photograph of a blocked restrictor orifice. (a) Original image (b) Image with black circle showing estimated original orifice location

fig:restrictorblockage⟩

To eliminate the possibility that it was material from the stop valve (see Section 4.4.2) that caused the blockages, we cycled the pressure by switching the pump on and off, allowing the pressure to bleed off through the restrictor. This way of cycling the pressure did not prevent blockages.

Examination of blocked restrictors with electron microscopy revealed that the restrictors became blocked by a soft material. Backscatter SEM mode allows the energy of X-rays to be measured by energy-dispersive spectrometry, which yields information about elemental composition. While much care must be taken before this information can be used for quantitation, it revealed that the deposited material contains significant quantities of carbon and oxygen, and possibly some chlorine. This points to the probability that the material blocking the column is organic in nature, and possibly polymeric. See Figure 4.8.

We could not determine the origin of the material, but it seems likely to be the column, because cycling the pressure without a column did not cause a blockage. We eliminated the possibility of blockage by sample material by using a brand new column. We suspect that the blocking material might be remnants of surfactants used in the synthesis of the silica gel stationary phase. These surfactants are leached out of the column packing by the highly diffusive supercritical fluid mobile phase, and they then precipitate in the restrictor when the pressure drops and the compounds desolvate. Repetitive pressure cycles causes a build-up of material which gradually blocks the orifice.

The smallness of the orifice contributes to the plugging problem. With a diameter of 50 µm, a solid sphere that fits in this capillary will have a volume of .065 nl, or 65 pl. This means that nanogram quantities of material can easily block the restrictor.

Being satisfied that an unfortunate combination of restrictor design and column packing was the likely cause of the blockages, we decided to choose a different restrictor design. The choice was a simple linear restrictor and we trusted that heating the end of the restrictor would prevent discrimination.

The linear restrictor was 800 mm long and had an internal diameter of 0.050 mm.

4.6 Detector

When SFC was first developed, a capillary column was the usual column, and an FID was the usual detector. In current use, however, packed columns used with mobile phase modifiers predominate and flame detectors have lost their place: the high concentration of modifiers in the mobile phase would swamp the signal from analytes or saturate the detector. Therefore modern SFC uses predominantly UV/Vis optical detectors.

In the supercritical fluid chromatograph described in this chapter there was no dedicated detector: the role of the detector was taken by a gas chromatograph. This gas chromatograph collected fractions from the SFC, and separated them in fast chromatographic runs with an FID detector, yielding comprehensive 2D chromatograms. This chromatograph-as-a-detector is described in Chapter 5.

There are no significant technical limitations to column temperature programming in the order of a few hundred degrees per minute and equally rapid cool-down rates.

Wolfgang Bertsch, 1997

5

Instrumentation: Fast temperature programmed gas chromatography

This thesis discusses the development of a comprehensively coupled (supercritical fluid × gas) chromatograph and its application to the analysis of biodiesel. The discussion on the experimental work divides naturally into two parts: the previous chapter discusses the supercritical fluid chromatography (SFC) and this chapter discusses the gas chromatography (GC).

5.1 Speed of analysis

In principle, comprehensively coupled chromatography (as discussed in Section 2.3.2) could be performed by manually or mechanically collecting equal-sized fractions from a ^{1}D chromatograph, and then injecting a portion of each fraction into a different (^{2}D) chromatograph. This would meet all the criteria for comprehensively coupled chromatography. In practice, such an approach would be slow, labour-intensive, expensive, and error prone.

But reliable devices were invented that can repeatedly collect and re-inject fractions of eluate *while the ^{1}D chromatograph is still running*. These devices became known as *modulators* and made comprehensively coupled chromatography practical. Today GC×GC is an established technique: the annual GC×GC Symposium is in its 15th year, and major reviews are published regularly (J. V. Seeley and S. K. Seeley 2013; Prebihalo et al. 2018).

In GC×GC the entire 2D chromatogram is finished within the duration of the ^{1}D run. This is made possible by using short, narrow bore columns with thin-film stationary phases in the ^{2}D separation, which allows *fast chromatography*.

In LC×GC and SFC×GC with packed ^{1}D columns using stopped flow modulation (as described in Section 4.4.2) the total run time can be longer, but not indefinitely longer. Firstly, in dense mobile phases diffusion is not zero, so the stopped-flow time should be minimized to prevent unnecessary peak broadening. Secondly, total run times must be practical: while it's not inconceivable to have experiments that run for days, complex instrumentation can reliably run for such long periods is expensive.

The time it takes for a stopped-flow SFC×GC run can be calculated from

$$t_T = t_{SFC} + \frac{t_{SFC}}{t_m} \times t_{GC}$$

where t_T is the total time, t_{SFC} is the time the unmodulated SFC run would take, t_m is the interval between sampling events (better known as the *modulation period*), and t_{GC} is the time for each GC run.

Examining the expression shows that we can decrease t_T by increasing t_m, but there is limit to this: if the modulation time becomes too long, the separation obtained in the ^{1}D separation might be lost, which would mean it could no longer be considered comprehensively coupled chromatography. So, the only way to decrease the total run times is to reduce t_{GC}, the GC run time.

For example, if a typical SFC run takes 20 minutes, and fractions are collected for every 5 seconds of SFC run, it means there will be $20 \times 60/5 = 240$ fractions collected. Each of these fractions must be injected into a GC chromatograph. If each GC run took 1 minute, the SFC×GC run would last 240 minutes = 4 hours. Run times this long are not unheard of in analytical chromatography, but deciding on them depends heavily on context. Finding the proverbial biomarker for Alzheimer's disease would justify such a long run time: routine quality control of a commodity biofuel would not.

This discussion should make it clear that for successful SFC×GC the GC must be *fast*.

5.2 Fast gas chromatography theory

The theory of fast gas chromatography has been well developed (Leonid M. Blumberg 1997), and every chromatographer who has looked into faster chromatography has met the chromatographer's trilemma (See Figure 5.1): Any chromatographic method that involves decisions speed, resolution, and capacity can maximize only one at a time[1]. The fastest chromatogram will have low capacity and low resolution, the highest resolution chromatogram will be slow and have low capacity, and the chromatogram of a sample with a high concentration of analyte will be slow and have low resolution (Klee and L. M. Blumberg 2002).

We avoided the trilemma by not bothering with optimizing column capacity. Sample capacity in capillary GC is a function of film thickness and column diameter. We decided to use the workhorse of GC, the 0.25 mm internal diameter column with a 0.25 µm film thickness. The stationary phase was a proprietary cross-linked polysiloxane polymer (Restek Rxi®-5Sil MS), designed to mimic the behaviour of a 5 % diphenyl/95 % dimethyl polysiloxane stationary phase. With the phase ratio/column capacity fixed, the remaining trade-off is between speed and resolution.

If two peaks are satisfactorily separated on given column, it is not possible to run a faster chromatogram with higher resolution. This can be seen by examining the resolution equation (Sandra 1989)

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$$

Where the N is the *number of plates*, α is the *selectivity*, and k_2 is the *retention factor*.

[1]This trilemma applies only to capillary chromatography. When using packed columns, the capacity can be readily increased by using a column with a larger diameter and a larger amount of packing.

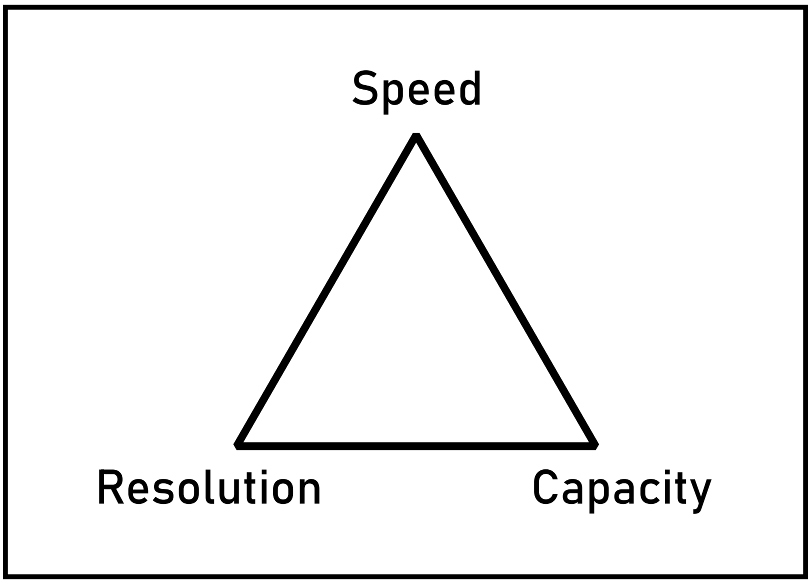


FIGURE 5.1: The chromatographer's trilemma.

⟨fig:trilemma⟩

N is can be calculated from $N = \frac{L}{H}$, where L is the *column length*, and H is the *plate height*.

Therefore $R_s \propto \sqrt{\frac{L}{H}}$, and obviously reducing the length of the column will reduce the resolution R_s.

H depends on gas flow, in a relationship described by the *Van Deemter Equation* $H = \frac{B}{\bar{u}} + C\bar{u}$ or, under conditions of high pressure drop, the equivalent equation by Blumberg (Leonid M. Blumberg 1997) $H = \frac{B}{\bar{u}^2} + C_1\bar{u}^2 + C_2\bar{u}$, where $\bar{u}$ is the average linear gas velocity in the column, and the coefficients B, C, C_1, and C_2 are constants relating to geometry and diffusion. These equations do have minima, but under most experimental conditions the flow will be at such a rate that increasing the flow will increase the plate height. Hence, increasing the flow while keeping the same column length will decrease the resolution R_s.

For this reason, the chromatographic separation of compounds with limited selectivity requires a long run time. For example, the official methods of separating the fatty acid methyl esters (FAMEs) found in biodiesel require columns 100 m long (AOCS 2017) and run times of hours. These systems have just enough theoretical plates to separate the chemically very similar FAMEs

For any given column and sample, the only way to make GC separations faster is to generate excess resolution. Fewer theoretical plates will then be necessary to obtain an optimal resolution. Excess resolution can be obtained by increasing the selectivity α, or by selective detection (*e.g.* an electron capture detector). In practical terms this means using a column with a different stationary phase or a different detector. A non-chromatographic way to generate excess resolution is often termed *sample clean-up*. While usually considered part of sample preparation, it is an additional, pre-chromatographic separation step that removes non-analyte compounds, so that only relevant compounds need to be separated from each other.

In SFC×GC excess resolution is generated in the ^{1}D SFC separation. Separating compounds by their group types first (A. Venter, Makgwane, and Rohwer 2006) is a kind of sample preparation step, so that the ^{2}D GC separation — in which separation is dominated by volatility — needs only resolve chemically similar peaks. The excess resolution generated by the ^{1}D SFC separation can be traded for faster chromatography using shorter columns, higher flow rates, temperature programming, or a combination of the above. Fortunately, the literature shows that deciding which approach to follow does not invoke a dilemma, and the recommendations are quite clear. It has been shown showed that superior resolution is obtained when shorter columns are used rather than higher flow rates (Klee and L. M. Blumberg 2002). In her thesis Gail Reed (Reed 1999) compared shorter columns against faster flow, and showed that shorter columns produce superior results over faster flow. Then she concluded:

> "Fast temperature programming should be used for fast GC rather than a smaller internal diameter. Fast temperature programming has been largely underutilized; however, new instrumentation will make it possible to more fully exploit fast temperature programming rates. Fast temperature programming rates allow for the use of short columns with normal internal diameters and film thicknesses which makes sample capacity less of a problem for this mode of fast GC compared to other means of fast GC."

In line with this recommendation, the fast GC described in this chapter used a short GC column (1 m long) that used a correspondingly fast temperature program.

5.3 Temperature programming

Temperature programming is, of course, not necessary only for fast chromatography, but has long been implemented to avoid the *general elution problem* (Skoog, Holler, and Crouch 2007, p. 779). This problem can be summarized as follows: in separations of complex mixtures, it becomes unlikely that one set acceptable operating conditions (temperature, flow and stationary phase) will give satisfactory (fast enough with acceptable resolution) separations for all the compounds of interest. As discussed above, there is only one optimum flow, and it is impractical to change the stationary phase during a run, so the only parameter to change is the temperature.

The technology for temperature programming in GC is mature, and every column manufacturer specifies temperature limits for isothermal and ramped temperature programs for every stationary phase.

5.4 Temperature ramp rates

⟨sec:RampRates⟩ Early experimenters realized the importance of temperature control in GC, and used vapour baths (Desty and Whyman 1957) or oil baths (Eggertsen, Knight, and Groennings 1956) to control temperature in their experiments, but they quickly realized that leaks could allow oil into the column. Oil that entered the column would then contaminate the stationary phase, rendering it useless. Therefore, in the modern, conventional gas chromatograph the column is heated in an air bath with very precise temperature control.

Blumberg and Klee (L. M. Blumberg and Klee 2000) recommend that a good initial temperature ramp rate is 10 C° per void time (t_m). For long columns the void times are long, and the ramp rates can be low. As illustration, Figure 5.2 shows the temperature ramp rates of a state-of-the-art chromatograph. By contrast, for short, narrow-bore columns, the ramp rate needs to be thousands of Celsius degrees per minute.

Table 1. Typical 7890B GC Oven Ramp Rates

Temperature range (°C)	120 V Oven* rates (°C/min)	Fast ramp rates** (°C/min) Dual-Channel	Single-Channel***
50 to 70	75	120	120
70 to 115	45	95	120
115 to 175	40	65	110
175 to 300	30	45	80
300 to 450	20	35	65

* Results obtained with line voltage maintained at 120V

** Fast ramp rates require power > 200 volts at > 15 Amps.

***Requires G2646-60500 oven insert accessory.

FIGURE 5.2: The temperature-rate table from the Agilent 7890B chromatograph data sheet (Agilent Technologies 2019).

fig:RampRate7890B⟩

The low ramp rate of conventional air baths is caused by three factors:

- The low heat capacity of air
- The poor thermal conductivity of air
- The mass of the oven that needs to be heated.

There is very little that can be done about these matters. It would be possible to switch oven gas to hydrogen for higher thermal conductivity, and construct a low-mass oven using, say, advanced resins for construction, but this would probably involve significant safety and cost issues. What is required is a complete redesign of the heating principle.

5.4.1 Resistive heating

Fortunately, the heating rate problem has a technologically simple solution, *resistive heating*[2]. When a constant electric field is applied to a metal, the free electrons in the metal will be accelerated by the electric field. But the electrons are within the crystal lattice of the metal, where their mean free path is very short. The electrons will therefore collide with atoms in the crystal lattice, scattering inelastically. The energy lost in the inelastic collisions will increase the vibrational frequency of atoms, and this energy will appear as heat.

The number of electrons and their average (drift) speed in combination is described by a measure called the *current* (I), and the electric field is can be described by the applied *voltage* (V). The current I is proportional to the applied voltage, and the ratio $\frac{V}{I}$ defines a proportionality constant R, called the resistance, which is a function of conductor material and dimensions. The total power dissipated to heat (P) is given by $P = IV$ or, equivalently, $P = I^2R$ or $P = \frac{R}{V^2}$.

Applying a voltage V to a metal element close to the column will heat the metal, and therefore the column in contact with it. The rate at which the piece of metal heats up depends on the power dissipated, the mass of the metal, and its heat capacity. If the volume of the metal is small enough, and the current high enough, the temperature of the metal element (and with it the temperature of the column) will increase at a rate high enough to be useful in chromatography. By suitable manipulation of V, then, the temperature of the column can then be changed at any desirable rate.

[2] Alternative methods of heating by electromagnetic fields are *inductive heating* and *dielectric heating*.

This technology has been reviewed (Wang, Tolley, and Lee 2012; M. R. Jacobs, Hilder, and Shellie 2013; Miranda 2010), and a few technologies for resistive heating of capillary columns have emerged:

- Direct heating of a metal column or a column coated with a metal layer
- Collinear heating
- Coaxial heating

The first SFC×GC work done (A. Venter and Rohwer 2004) used a directly heated metal column. While this approach proved the concept, experience showed two shortcomings. Firstly, metallic columns are usually designed with specific high-temperature applications in mind. This means that metal columns are not available with all the stationary phases available in fused-silica columns. Secondly, it was harder than expected to control the temperature accurately. The temperature was determined by a thermocouple glued to the column, which was sensitive to local variations.

An example of collinear resistive heating is Agilent™'s 'low thermal mass' column, which includes collinear heating wire and a collinear sensing element bundled with a short silica column. This approach requires that the collinear heating element be wrapped in close contact with the column.

For the work presented in this thesis, a coaxial heater was used. This heater is in the form of a thin-walled stainless-steel tube that carries the electric current. It was made of a 940 mm length of 304 stainless steel with d an outside diameter of 1.06 mm and an inside diameter of 0.80 mm. The column was threaded inside the stainless steel tube, which put it in close contact with the heater, giving reliable heat transfer. The coaxial heater can be coiled to fit inside a conventional GC oven.

This coaxial design has the advantage that the column can be changed without changing the resistive heater. This means that there is no need to re-calibrate the heater when columns are changed.

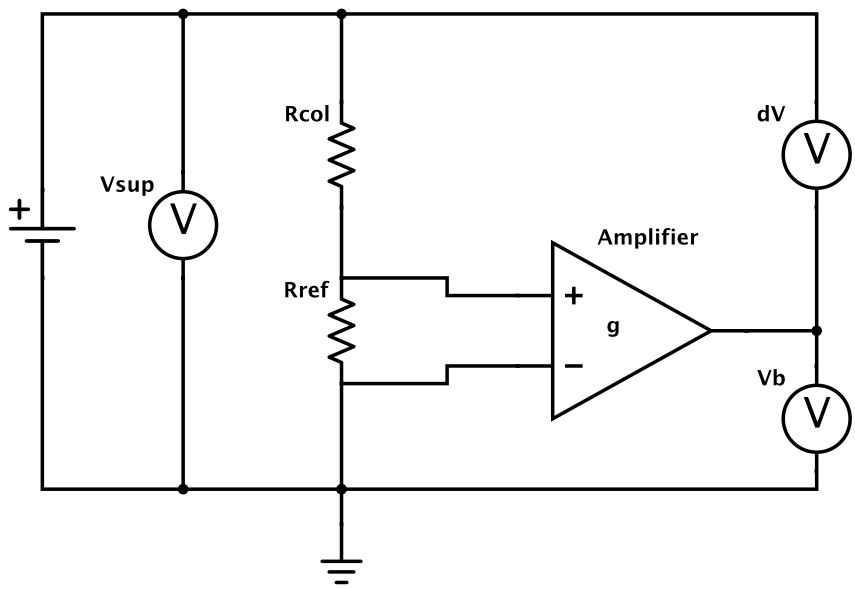


FIGURE 5.3: Electric circuit diagram of the coaxial heater.

⟨fig:HeaterDiagram⟩

The electrical resistance of a conductor is determined by its dimensions, the material it is made of, and the temperature of that material. In a conductor of given dimensions, therefore, a knowledge of the resistance implies a knowledge of the temperature. By following changes in the resistance, one can determine changes in the temperature, and by comparing resistance at certain temperatures with known temperatures, one can get a calibrated temperature from a given resistance.

High-powered resistive heating implies low resistance. Measuring the *absolute value* of low resistance is technologically challenging (Dyos 2012). But a simple electronic circuit can be used to *compare* the resistance of the coaxial heater with that of a reference resistor (Figure 5.3). The circuit is supplied by a voltage V_{sup}, in general an unknown value. V_{col} and V_{ref} represents the voltage drop over the respective resistors. Because the current I through the circuit is the same for both R_{col} and R_{ref}, it is true that $\frac{R_{col}}{V_{col}} = \frac{R_{ref}}{V_{ref}}$, and therefore

$$R_{col} = R_{ref} \frac{V_{col}}{V_{ref}} \tag{5.1}$$ {?}

The voltage drop across R_{ref} is too small to be directly digitized, therefore it is amplified by an amplifier with gain g, so that $V_b = gV_{ref}$. V_b is measured, as is dV, the potential difference between the supply and the amplifier output. We can show that the coaxial heater resistance R_{col} is a function of the voltage ratio $fracdVV_b$.

First,

$$V_{sup} = V_{col} + V_{ref}$$

but also

$$V_{sup} = dV + V_b$$

Therefore,

$$V_{col} + V_{ref} = dV + V_b$$

But $V_b = gV_{ref}$, so

$$V_{col} + V_{ref} = dV + gV_{ref}$$

$$V_{col} = dV + gV_{ref} - V_{ref}$$

$$V_{col} = dV + V_{ref}(g-1)$$

$$\frac{V_{col}}{gV_{ref}} = dV/gV_{ref} + V_{ref}(g-1)/gV_{ref}$$

$$\frac{V_{col}}{V_{ref}} = gdV/gV_{ref} + gV_{ref}(g-1)/gV_{ref}$$

$$\frac{V_{col}}{V_{ref}} = g\frac{dV}{V_b} + (g-1)$$

This proves that $\frac{V_{col}}{V_{ref}}$ is a linear function of dV/V_b. A quick check for correctness of the expression: for a unity-gain amplifier $g = 1$, and $\frac{V_{col}}{V_{ref}} = dV/V_b$.

Therefore

$$R_{col} = R_{ref}\left(\frac{gdV}{V_b} + (g-1)\right)$$

In practice the gain g might not be completely constant, but show a slight dependence on dV, so a general expression might be

$$R_{col} = f\left(\frac{dV}{V_b}\right)$$

5.5 Calibration

The assumption is that the temperature is a function of the resistance of the coaxial heater, or $T = g(R_{col})$. Because $R_{col} = f(\frac{dV}{V_b}$, we can see that $T = g(f(\frac{dV}{V_b}))$, or, because a function g of a function f is a function h ($f \circ g = h$), $T = h(\frac{dV}{V_b}))$. Through a calibration procedure $h-1$ can be approximated by a polynomial or lookup table.

5.5.1 Temperature uniformity

⟨sec:Uniformity⟩ It is highly desirable that the coaxial heater should give uniform heating, but there is no guarantee an electrically heated tube will heat uniformly, or will not. We did not analyse the problem theoretically, but the following factors will play a role:

Resistivity's dependence on temperature

The resistance of a metal increases with temperature. This means that if one section of the coaxial heater should get hotter than the rest, it's resistance will increase. If the current were to remain constant, more power would be dissipated in this section ($P = I^2R$). If more power is dissipated, the temperature will increase, leading to a higher resistivity, leading to higher power dissipation, leading to a higher temperature, in a runaway cycle. In the case of the supply voltage being held constant a higher resistance in one section would mean a lower current overall, but still a higher power dissipation in the section with higher temperature.

Thermal conduction

Each section of the coaxial heater is in thermal contact with its neighbours. If it were to get hotter, the heat will flow from the hotter section to the cooler neighbouring sections. This will tend to even out any temperature differences.

Radiation

An object radiates heat, which can be approximated by the Stefan-Boltzman law:

$$P = A\epsilon\sigma T^4 \tag{5.2}$$ eq:1

where A is the surface area of the object, ϵ is the *emissivity* of the surface, σ is the Stefan-Boltzman constant, and T is the absolute temperature of the object.

If a section of the coaxial heater were to become hotter than the other sections, it would therefore radiate more heat. The increased power dissipation by the higher resistance will therefore be partially offset by a higher radiation. This will tend to moderate temperature differences.

Convection

When a part of the coaxial heater is hot, it will heat the air around it, through radiation and conduction. This air might then move, through buoyancy or any other force, past another part of the coaxial heater. This second part could then be heated up by the transported air. For example, a vertically mounted coaxial heater can be expected to develop a temperature gradient from bottom to top, as natural convection lets hot air transfer heat from the lower end of the heater to the upper end. In this way temperature gradients could be established and maintained.

Examining thermal uniformity by imaging

The effect of the combination of resistivity's temperature dependence, thermal conduction, radiation, and convection on the temperature uniformity of the coaxial heater could be mathematically or numerically modelled, but such an endeavour would fall outside the scope of this project. Experience had not led us to expect any significant temperature non-uniformity, but we welcomed the opportunity to get empirical confirmation.

Thermal imaging is the process by which infrared radiation from objects can be captured in a photographic process. At near-ambient temperatures objects emit copious amounts of infrared radiation, and higher the temperature, the more is emitted. (See Equation 5.2.) Specialized optics and sensors allow the capture of that radiation in a camera, which can produce an image of a scene that shows objects based on their surface temperature. The technological capability of thermal imaging has improved markedly over the past years.

Using a FLIR™ T660 thermal imaging camera we obtained a video of the coaxial heater executing a temperature ramp. This camera uses a 640×480 focal plane uncooled bolometer array as a detector, which is sensitive to radiation in the range 7.5 µm to 14 µm. Figure 5.4 shows the setup used to record the thermal video.

Figure 5.5 is a frame from the video, analysed to give estimates of the temperatures on spots on the coaxial heater. The maximum temperature difference between any two points is 17.5 °C, and there are no marked gradients.

This examination of the uniformity of the coaxial heater put to rest any fears that unexpected temperature gradients would interfere with the fast gas chromatography.

5.5.2 Temperature calibration

When doing temperature-programmed gas chromatography it is desirable to have an absolute measurement of the column temperatures. This makes it possible to translate and compare methods between instruments. It was therefore necessary to calibrate the temperature of the coaxial heater. Calibration is the comparison of measurement values from a device under test with those of a calibration standard of known accuracy. In the case of the temperature calibration of the coaxial heater, it means comparing the results of the temperature as measured by the coaxial heater with a standard temperature.

The problems of measuring a temperature inside a tube with a bore of 0.8 mm are not trivial.

The following temperature measurement technologies exist:

- Liquid-in-glass thermometers
- Sealed liquid or gas sensing instruments and bimetallic sensors.

FIGURE 5.4: This photograph shows the setup used to record the termal video.

⟨fig:ThermalImageSetup⟩

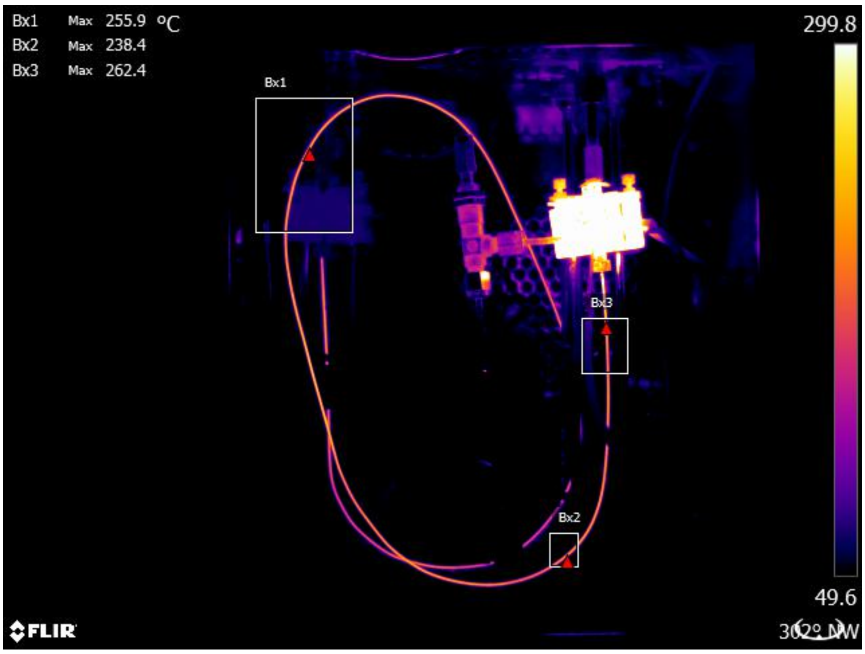


FIGURE 5.5: A thermograph of the coaxial heater at high temperature. It shows that there are no runaway hot spots.

⟨fig:ThermalImage⟩

- Electrical resistance temperature measurement using metallic sensors
- Thermistors and semiconductors
- Thermoelectric temperature measurement
- Disappearing filament optical pyrometer
- Photoelectric optical pyrometers
- Total radiation pyrometers.

Liquid-in-glass thermometry would not be applicable because of the size of the devices, and because they don't give a desirable electrical signal.

In recent years the technology for measuring temperature by radiant energy methods have improved markedly and has become affordable, in the form of thermal cameras. However, at lower temperatures the accuracy of the recorded temperature depends heavily on the emissivity of the measured material. Thermal imaging will also only measure the outside wall surface temperature of the heater, and not the temperature on the inside of the coaxial heater. So, while thermal imaging settled questions about heater uniformity (Section 5.5.1), it was not considered ready to serve as a calibration standard.

This leaves us with resistance temperature measurement with metallic sensors, thermistors and semiconductors, and thermoelectric temperature measurement.

Electrical resistance measurement using metallic sensors might have been feasible, if sensing elements of the appropriate dimensions were commercially available. A further difficulty with this method of temperature measurement is that long, thin conductors would be needed to connect the sensing element to the electronics. These conductors would add to the resistance measured by the sensing element, requiring complex correction or multi-wire measuring methods.

Thermistor and semiconductor devices could, in principle, be made small enough for the job, but commercially available devices come in 'packages' that are too large. Besides, the temperature range used in GC ($-50\,°C$ to $400\,°C$) does not fall inside the operating temperature range specified by manufacturers of semiconductor devices ($-55\,°C$ to $125\,°C$ for military applications).

Thermoelectric temperature measurement then remains, and was used to calibrate the coaxial heater. In particular, the *Seebeck effect* was exploited, which is the observation that a temperature gradient imposed on a metallic conductor will generate an electrical potential along the gradient. Therefore, a circuit of two dissimilar metallic conductors will generate a voltage when there is a temperature gradient along the conductors. The voltage generated is a function of the temperature difference between the hot junction of the two metals and their cold junctions at the voltmeter. Such a pair of dissimilar conductors used to generate a voltage is known as a *thermocouple*. Thermocouple technology is mature and widely used in industry for measuring temperatures. Thermocouples are usually constructed from well-characterized alloys that generate predictable voltages for given junction temperatures. Thermocouple alloys that have been standardized are known as 'types'. We selected the general-purpose Type K thermocouple. Thermocouple wire can be purchased in varying gauges, down to 25 µm in diameter, and the signal processing for thermocouple signals have been standardized.

According to McGee (McGee 1988) thermocouple junctions can be made by welding, crimping, soft soldering, hard soldering, bolting, or simply twisting the wires together.

Because of the temperature range expected to be measured (-50 °C to 400 °C) the option of soft soldering does not apply, because soft solders have melting points around 200 °C. The possibility of corrosion and mechanical vibration suggest that twisting the wires together will not form a reliable joint, and of course there are no sub-millimetre bolts on the market.

This leaves welding, crimping and hard soldering as methods for making thermocouple junctions. The option of crimping was not explored, chiefly because there are no devices that can crimp hair-fine wire. Our knowledge of crimping shows another problem: the final crimped connection has a diameter many times the diameter of the wire. This precludes the application of crimping in this context.

Hard soldering is usually done with high-temperature flames, and on contact the flames will rapidly burn the fine wires. The temperature required for hard soldering is still lower than the melting point of the wires, so that hard soldering is not excluded, but we did not have the knowledge or the technology to solve the associated problems.

Welding was found to be an accessible technology for forming small, reliable joints in fine thermocouple wire.

5.5.3 Thermocouple Welding

Welding is the process of joining two metal parts by melting a portion of each part, allowing the molten metals first to mix, and then to solidify. This creates a permanent joint between the two metals. Welding is widely practised as an industrial process in applications ranging from shipbuilding to microelectronics.

Various sources of heating can be used, and we decided to use electricity. A 24 V direct current, adjustable bench power supply was used to supply the current. The two wires of the thermocouple were twisted together, and the twisted pair was connected to one pole of the power supply. A carbon electrode was connected to the other pole. The carbon electrode was carefully brought closer to the thermocouple pair until a spark jumped across the air gap. When the spark turned into an arc the heat of the arc melted the end of the twisted pair. The molten metal would then contract into a spherical globule, which grew as the arc added more heat. As more of the metal of the wire melted, the globule would move away from the carbon electrode, until the gap became too large to sustain the arc. The current would then stop, leaving a spherical welded bead at the end of the wires. (See Figure 5.6.) The process could be repeated as often as necessary to obtain a bead of the desired size.

It is worth noting that it is necessary to form an arc: if the carbon electrode happened to touch the wire so that a current flowed directly from the wire to the carbon electrode the wire would rapidly heat up over its length, and melt. It is also interesting to note that it seemed necessary to have a roughly broken carbon electrode surface: a polished surface would not generate an arc, or even make electrical contact with the wires. This might be because the graphite used for the electrode was formulated for use in pencils.

The apparatus used to do the welding is depicted in Figure 5.7, and Figure 5.8 shows the image seen through the microscope when the welding is done.

5.5.4 Thermocouple probe construction

To measure the temperature inside the coaxial heater required the thermocouple had to be inserted into the coaxial heater. For this a fused silica capillary with an inside diameter of 0.25 mm and an outside diameter of 0.4 mm was used to construct a

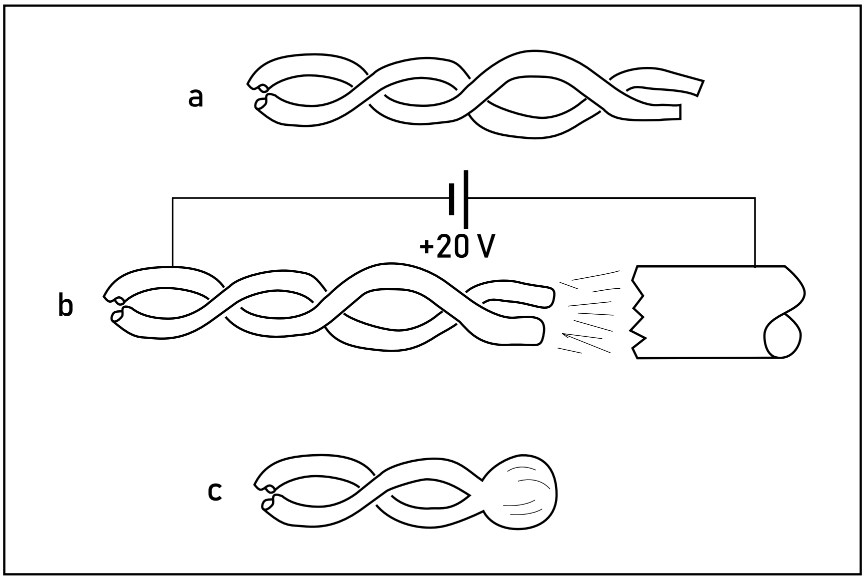


FIGURE 5.6: The process of welding fine wires to make thermocouples (a) Wires twisted together (b) Electric arc heating up the wires (c) Wires welded with a well-formed bead.

⟨fig:WeldingSteps⟩

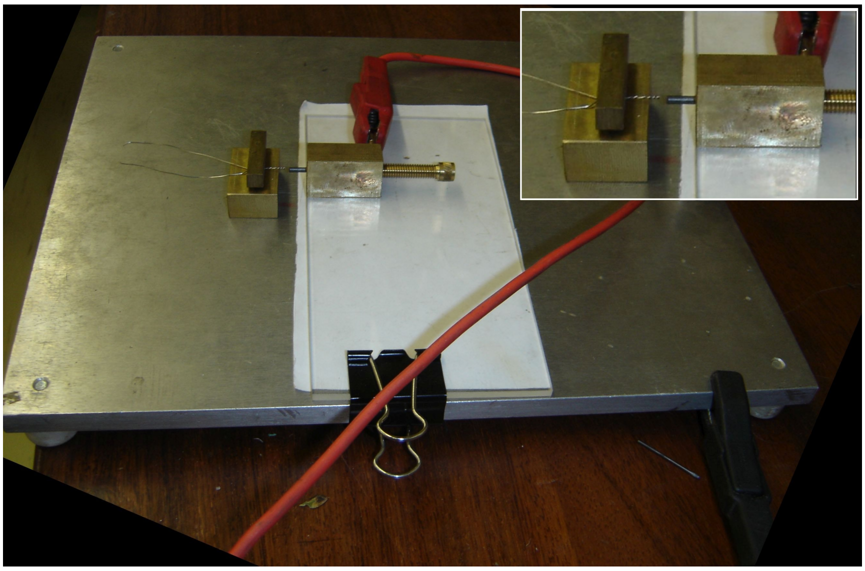


FIGURE 5.7: A view of the fine-wire thermocouple welder, as set up on a microscope base plate. The wire shown in the photograph is much thicker than that actually used. It is shown clamped between the clamping bar and the clamping weight. A thin sheet of acrylic serves to isolate the positive electrode from the negative base. The carbon electrode can be advanced towards the thermocouple twist using the screw. The black clamp at the bottom right-hand corner attached to the base plate and the red clamp attached to the screw housing provide a potential difference of approximately 20 V between the carbon electrode and the thermocouple.

ig:FineWireWelder⟩

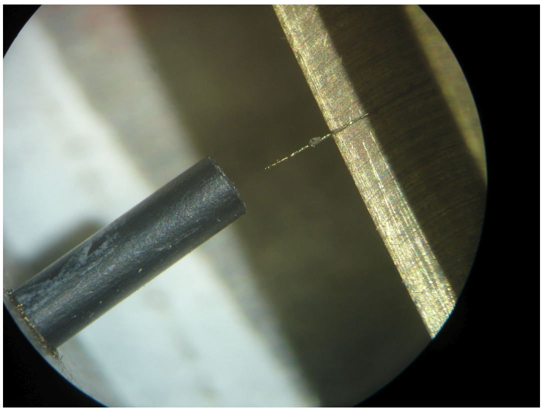


FIGURE 5.8: A microphoto of a twisted wire ready to be welded. The black carbon electrode is 2 mm in diameter.

⟨fig:TCWeldMicro⟩

probe. (These capillaries were easily obtained in the form of worn-out chromatographic columns.) A narrower capillary was used to draw the wires through the probe capillary, in a process described in Figure 5.9. The wires could then be welded to form a thermocouple, and then drawn into the probe capillary to the desired position.

5.5.5 Thermocouple interfacing

The Type K thermocouple has a sensitivity of approximately 41 µV °C^{-1}. This means the voltage generated at the temperature range of interest is too small to be conveniently digitized, and so needs to be amplified. Because the Type K thermocouple is so commonly used in industry, amplifiers have been developed specifically for thermocouple signal conditioning. We chose the AD595 integrated circuit amplifier. The component's data sheet explains its application concisely: "The AD595 is a complete instrumentation amplifier and thermocouple cold junction compensator on a monolithic chip. It combines an ice point reference with a pre-calibrated amplifier to produce a high level (10 mV/°C) output directly from a thermocouple signal." (*Monolithic Thermocouple Amplifiers with Cold Junction Compensation* 1999) The output of the AD595 can be directly digitized and fed to the computer.

5.5.6 Calibration procedure

The International Vocabulary of Metrology (*International vocabulary of metrology – Basic and general concepts and associated terms (VIM)* 2019) define *calibration* as "[an] operation that, under specified conditions, in a first step, establishes a relation between the **quantity values** with **measurement uncertainties** provided by **measurement standards** and corresponding **indications** with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a **measurement result** from an indication."

In the first step of calibration, the **quantity values** used in the calibration was the temperature value of the thermocouple probe as provided by the thermocouple voltage, the AD595 amplifier, the digitization and the subsequent calculations according to the AD595 data sheet. The **measurement standards** were the known responses of the thermocouple (Ripple 1995), the amplifier and the digitization system. The

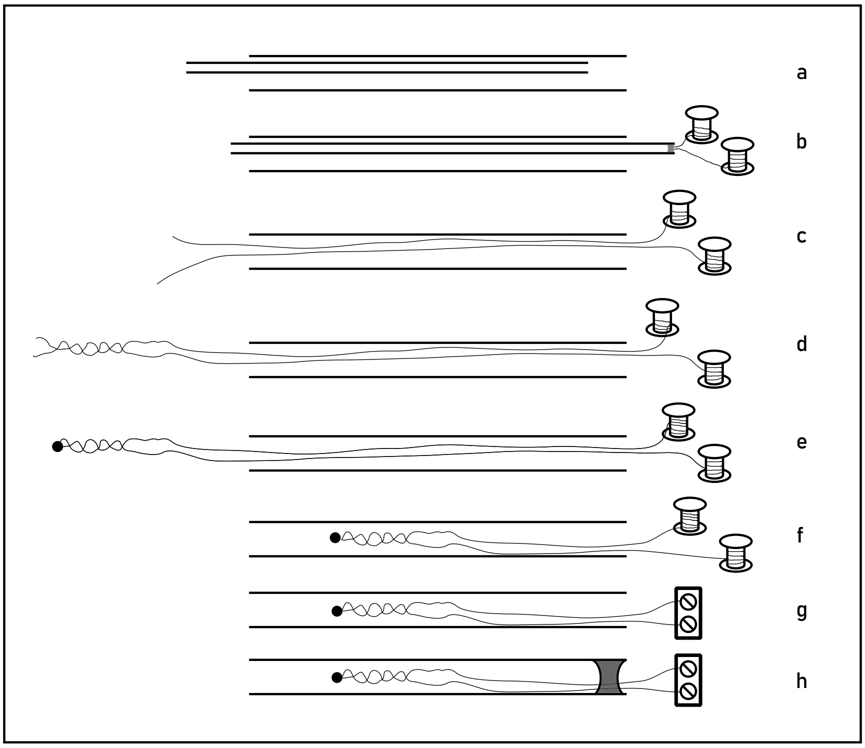


FIGURE 5.9: (a) A narrow capillary is threaded inside a wider one (b) The ends of a pair of thermocouple wires are fitted inside the end of the narrow capillary and anchored with cyanoacrylate adhesive. (c) The wires are pulled through the wider capillary using the narrow capillary. (d) The ends of the wires are twisted together, creating a mutual mechanical anchor. (e) The ends of the wires are welded together. (f) The wires are pulled back into the thick capillary, locating the junction at the desired position in the capillary. (g) The wires are trimmed and connected to a terminal block. (h) A drop of cyanoacrylate adhesive is used to anchor the wires permanently in the capillary.

eWireThermocouple⟩

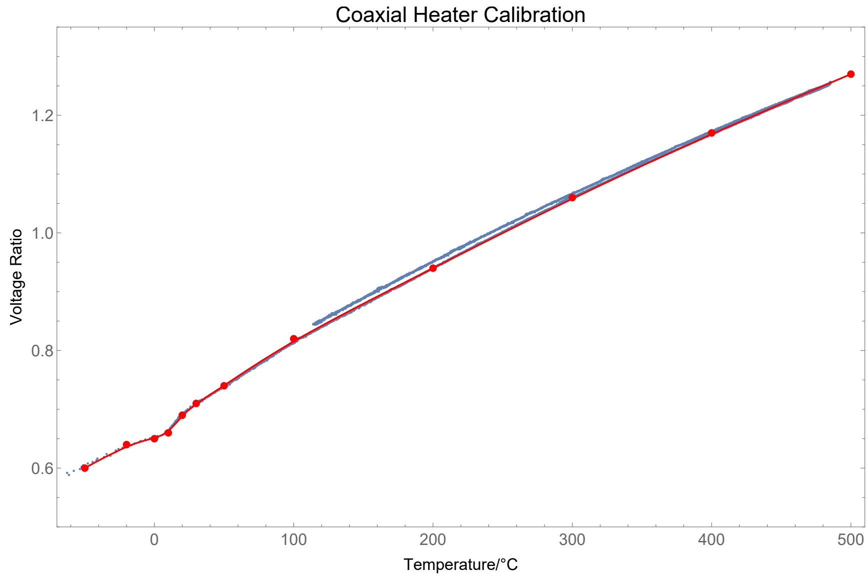


FIGURE 5.10: Calibration curve for the coaxial heater.

⟨fig:CalibrationCurve⟩

indication was the voltage ratio $\frac{dV}{Vb}$. As this was a first attempt a calibration, **measurement uncertainties** were omitted.

To calibrate the coaxial heater, the heater and its assembly was installed in the oven of the Varian 3300 GC, as it would be when in use. The detector was removed, and the thermocouple probe was threaded through the detector stem, through the heated T-piece block, and into the coaxial heater. The probe was inserted so far that the thermocouple junction was about half-way between the inlet end and the detector end of the coaxial heater.

When the system was set up as it would be in use, the coaxial heater was cooled down, and a power ramp applied. The temperature of the thermocouple was recorded, together with the voltages dV and Vb. A curve could then be plotted of thermocouple temperature T_{TC} against the voltage ratio $\frac{dV}{V_b}$ (Figure 5.10). This revealed the shape of the function $T(\frac{dV}{Vb})$, and completed the first step of the calibration.

The second step of a calibration is to establish a **measurement result** from an **indication**. It would be traditional to fit a mathematical function such as a polynomial to the curve, but a numerical method was chosen instead. We fitted a B-spline to the curve, and extracted coordinates on the curve from the B-spline (See Figure 5.11). An interpolation function was then used to obtain the measurement result T from the indication dV/V_b.

The calibration could be checked by setting the current through the coaxial heater to a minimum, at which not enough heat is generated to affect its temperature. Then the oven of the Varian 3300 GC could be set to a range of different temperatures. Once equilibrium was reached the reported temperature of the coaxial heater and the air bath temperature could be compared, and adjustments could be made to the calibration

5.5.7 Cold spots

⟨sec:ColdSpots⟩ For the fastest temperature programming with resistive heating the heating element should be as light as possible and carry the largest necessary current. The current doing the heating must be carried to the coaxial heater using a feed conductor. To

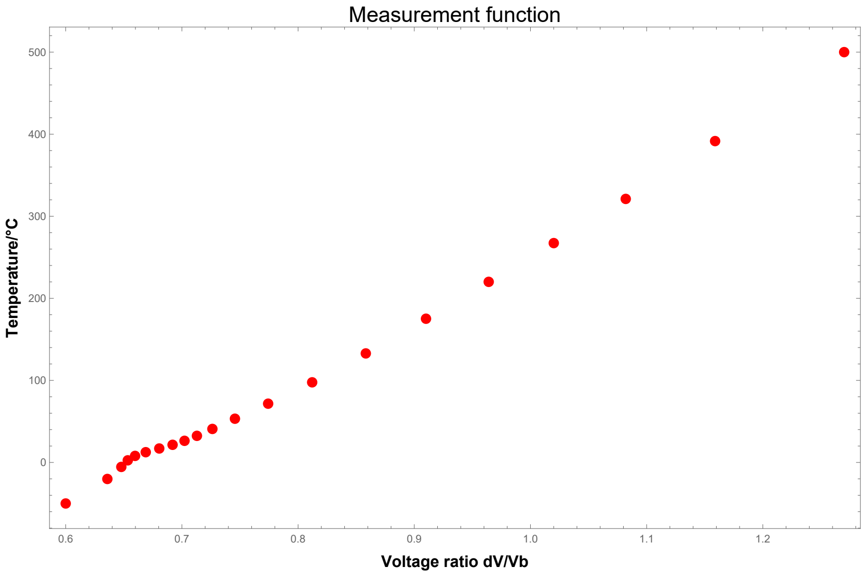


FIGURE 5.11: Measurement curve for the coaxial heater.

:MeasurementCurve⟩

prevent the feed conductor from heating up it must have a low resistance, and this low resistance is achieved by making the conductor as ’thick’ or as ’heavy’ as necessary, meaning that it is constructed of a material with a high mass per unit length.

Good electrical metallic conductors are invariably also good thermal conductors, and therefore, the area around the junction of the feed conductor to the coaxial heater will always have a lower temperature than the nominal temperature of the heater. In capillary GC this is undesirable: a cold spot in a column can wreak havoc with retention times and peak shapes.

Conversely, if an attempt is made to reduce the contact area between the feed conductor and the thin material of the coaxial heater, a hot spot might develop, which could burn a hole in the coaxial heater tube or damage the column.

The electrical connection between the feed conductor and the coaxial heater was therefore designed in the form of an externally heated block. This block was kept at a higher temperature than the highest expected temperature of the chromatographic temperature program. This prevented the formation of cold spots in the coaxial heater, which might lead to cold spots in the column, while also offering a large contact area so that hot spots do not develop. Each end of the coaxial heater therefore ended in one of two heated blocks, where it was brazed in place. Each block had a brass tail, to which an electric feed-wire was soft-soldered. Each block was heated by four 100 W Hotset™ electrical cartridge heaters, with dimensions of 6.5 mm × 40 mm. The cartridge heaters were switched on and off by solid state relays controlled from the computer. The temperature of the block was monitored through a thermocouple mounted in blind hole and the amount of power to the heaters was controlled by pulse width modulation (PWM) implemented in software.

5.5.8 Cold column

⟨sec:ColdColumn⟩

In a cold GC stationary phase, the retention factor k' is very high. This means that the analytes migrate slowly relative to the mobile phase. The lower the temperature, the higher k' becomes, so that for very low temperatures the migration of the analyte becomes negligible. In effect, the analytes are ’trapped’. This trapping, also called *cryo-trapping* or *cryo-focusing* is useful in various aspects of gas chromatography, such as two-stage thermal desorption or thermal modulation in GC×GC.

In the SFC×GC instrument described here, cryo-trapping was used as the second stage of a two-stage modulator. (The stop valve described in Section 4.4.2 represents the first stage.) The column was cooled down to very low temperatures, which trapped any analytes eluting from the first dimension in a narrow band on the GC column. Once the required amount of fraction had been collected, the eluate flow from the first dimension was be stopped by closing the stop valve. Then the temperature ramp of the fast GC would start. As the coaxial heater warmed up the column the values of k' would decrease, and the analytes would start migrating.

The first SFC×GC chromatograph cooled the column by using the cryo-cooling function of the Varian 3300 GC (A. Venter and Rohwer 2004; A. Venter 2003). The purpose of this function is to cool the GC column to sub-ambient temperatures, and is needed when analysing volatile compounds. In such cases the k' values at or near room temperature are too low to provide adequate retention, and the cryo-cooling function permits temperature programs to start at sub-ambient temperatures.

The Varian 3300 cryo-cooling function works by injecting liquid carbon dioxide into the column oven. The evaporating liquid carbon dioxide absorbs energy from the air, which lowers the temperature of the air in the oven. A control system controls the amount of carbon dioxide admitted and the amount of heat added through the oven heaters, thereby keeping the oven at the required temperature.

While the cryo-cooling function can cool down an oven to cryo-trap analytes, there are two reasons why it is not a suitable method for practical trapping in SFC×GC. The first reason is the quantity of coolant required: doing SFC×GC runs revealed that about 15 kg of carbon dioxide was consumed per run. A standard cylinder of carbon dioxide contains 33 kg, which implies that a new cylinder would be required every two runs. Such a rate of use is much too high for the intended application of the instrument. The second reason using the GC oven's cryo-cooling function was not suitable for SFC×GC is that it is much too slow. The time spent on cooling the oven and the column is time that cannot be spent doing separations, and cooling a conventional GC oven takes a lot of time: the Varian 3300's cryo-cooling function took 30 s to cool the column down to a low starting temperature. A commercial forced-convection system ("GC Chaser" supplied by Zip Scientfic) improves cool down time of an Agilent 6890 GC oven, taking 7 minutes instead of 16, cooling down the oven from 350 °C to 30 °C. Cooling the column in an air bath has the same drawbacks of low conductivity and low heat capacity as air-bath heating has (see Section 5.4).

⟨todopage:3⟩ **To do** (3)

A system was therefore developed by which liquid carbon dioxide is injected from one end into the space between the column and its coaxial heater. The other end is open to the atmosphere. When the valve opens the pressure of the carbon dioxide drops from 55 atm in the cylinder to 1 atm in the atmosphere and the liquid starts to boil, absorbing large quantities of heat from the surrounding column and coaxial heater in the process, so that their temperatures decrease rapidly. This system solves the speed and coolant quantity problems: because the coolant is in direct contact with the parts that need to be cooled, the cooling is rapid, and because the coolant is applied where it is needed, only a small quantity is required.

The carbon dioxide for cooling the coaxial heater was introduced through the same heated block that provided the electrical connection. (See Section 5.5.7.) A T-piece design allowed the liquid carbon dioxide to be admitted to the end of the coaxial heater, which was brazed to the block. A micro-union brazed to the block sealed the column's exit port, and the liquid carbon dioxide entered along the side of the T (Figure D.1 and Figure D.2). A metering valve allowed the flow rate of the coolant to be adjusted, and a solenoid valve could switch the flow on or off under computer control.

Cryogen supply

The carbon dioxide for cooling was supplied as by Afrox, in high-pressure cylinders each containing 33kg of technical grade carbon dioxide. Each cylinder was internally equipped with a *dip tube*, a tube that extends from the valve at the top of the cylinder to the bottom of the cylinder. This ensures that when the valve is opened, liquid carbon dioxide is delivered.

Experience taught that for repeatable cooling, the source of liquid carbon dioxide had to be near the solenoid valve. If this was not the case, when the valve was opened initially only carbon dioxide gas would be admitted, followed by a mixture of gas and liquid, and only finally liquid. (This is similar to the common experience of opening a water tap after a municipal water supply interruption: a lot of gurgling and spitting before a reliable stream of water flows from the tap.) Such unreliable coolant flow leads to unreliable cooling. To solve this problem we installed a reservoir for liquid carbon dioxide on top of the GC. The problem of filling a receptacle with liquid carbon dioxide was described in Section 4.3. The final design of the reservoir took the form of a coil of copper tube immersed in a circulating coolant (Figure D.3). Mounting the reservoir above the cut-off valve allows the liquid to collect at the bottom and allow gas to collect at the top, so that when the valve opens the flow into the coaxial heater contains only liquid.

5.5.9 Column mounting

The T-piece blocks described above (see Section 5.5.7, Section 5.5.8 and Figure D.2) acted as electrical connection for the coaxial heater and as injection point for coolant. The block is heavy compared to the coaxial heater and column, and also has to absorb the forces of the coolant inlet tube and the electrical connections. The column runs from the heated inlet/detector to the heated T-piece block, and in between it should not be exposed to any low-temperature cold spots, therefore the gap between the T-piece block and the inlet/detector should be quite small, but the gap cannot be zero, because electrical isolation needs to be maintained. Also fused-silica capillary columns are fragile, and misalignment causes them to break. Therefore, mechanically stiff and accurate mounting was needed for the T-piece blocks, to allow the precise but adjustable alignment of the T-piece blocks with the inlet/detector.

The final design for mounting the T-piece blocks was a pair of parallel rails. These rails were held in place in the Varian 3300 oven by friction, so that they could be adjusted and removed as necessary, yet was stiff enough to transfer the necessary forces without deflecting. The pointed ends of the rails pressed against a solid aluminium plate used as the floor of the oven, and at the top adjustable points pressed against pressure plates which pressed against the roof of the oven. Figure D.4 shows a technical drawing of the rails as designed.

The T-piece blocks were the electrical connections for the resistive coaxial heater, which meant they needed to be electrically isolated, but they were also heated, which meant that the insulation had to be resistant to heat. A commercially available material that met these requirements was found in the form of *silicon mica*, a composite material of mica and a silicone resin. This material has a continuous operating temperature of at least 500 °C, making it ideally suited to GC applications. The silicon mica is also machinable, and can easily be shaped to the required design.

The T-piece blocks were mounted on a pair of cars riding on the round-bar rails. Each car was designed as a sandwich of plates of stainless steel and silicon mica around a pair of brass bushes. Once assembled, the cars offered a set of studs on to which the user could fit and bolt down the T-piece blocks. The positions of the cars were determined by a locking collar on one of the rails. Figure D.5 shows technical drawings of the cars that explains the design.

5.5.10 Heating control

The amount of electrical power supplied to the coaxial heater was controlled by a bank of six PNP 2N2955 transistors, connected in parallel to distribute their heat dissipation. The final control signal was a voltage set either by a potentiometer from the front panel, or by the computer. An operational amplifier adjusted the current through the coaxial heater circuit so that a portion of the voltage applied to the coaxial heater was equal to the set-point voltage. By varying the set-point voltage the current through the coaxial heater can be controlled to provide any desired amount of heat ($P = I^2R$).

Temperature monitoring

Independent of the amount of power dissipated in the coaxial heater, the current through the reference resistor was compared to the current through the column. This ratio corresponds to the resistance of the coaxial heater. This resistance is a function of the temperature of the heater. Through the calibration procedure described earlier, the temperature of the coaxial heater can be calculated. The computer can do this fast enough so that a temperature measurement is available to continuously feed to a control system.

PID tuning

A proportional-integral-derivative (PID) controller was used to calculate the amount of power necessary to keep the temperature of the coaxial heater as close to the temperature set point as possible. The temperature set-point, in turn, was given by the desired chromatographic temperature ramp.

The process of determining the best calculation by the PID is called *tuning*, and usually consists of determining the optimum values of a few parameters. Tuning PID controllers is a complex sub-discipline of process engineering, and outside the scope of this project, but for practical purposes we used a privately published step-by-step tuning method (Peacock 2008) which is a version of the Cohen-Coon tuning method.

Figure 5.12 shows the effect of an improved loop tuning.

A properly tuned heater helps to improve the repeatability of the chromatography through reliable temperature programs and prevents damage to the column due overheating during set point overshoots.

Heating rates

As discussed in Section 5.4, for fast temperature-programmed gas chromatography the ramp rate needs to be in the order of thousands of degrees Celsius per minute.

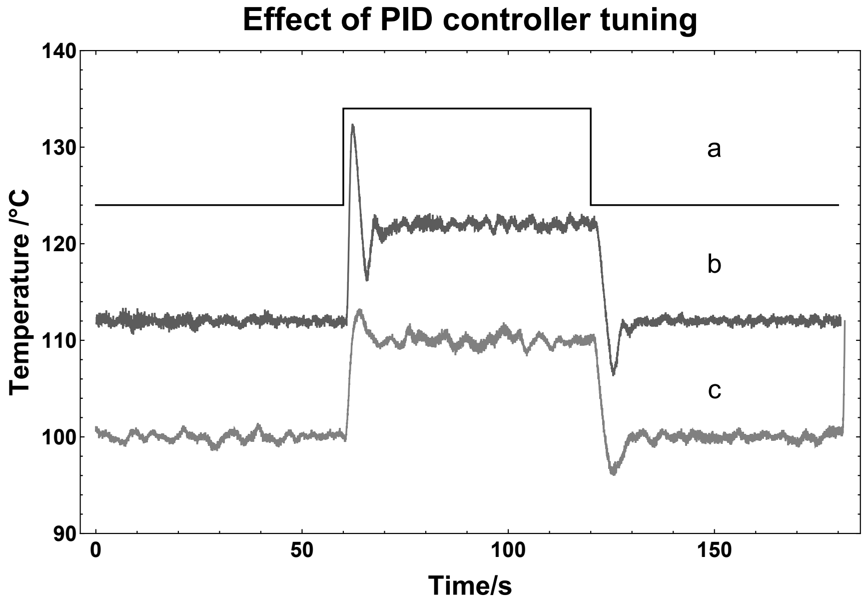


FIGURE 5.12: An illustration of effective PID controller tuning. The trace (a) represents the set point change over time and includes a step change. (b) Before tuning the temperature overshoots and then undershoots the step change in the set point. (c) After the controller has been tuned the overshoot in response to a step change in the set point is minimized.

⟨fig:LoopTuning⟩

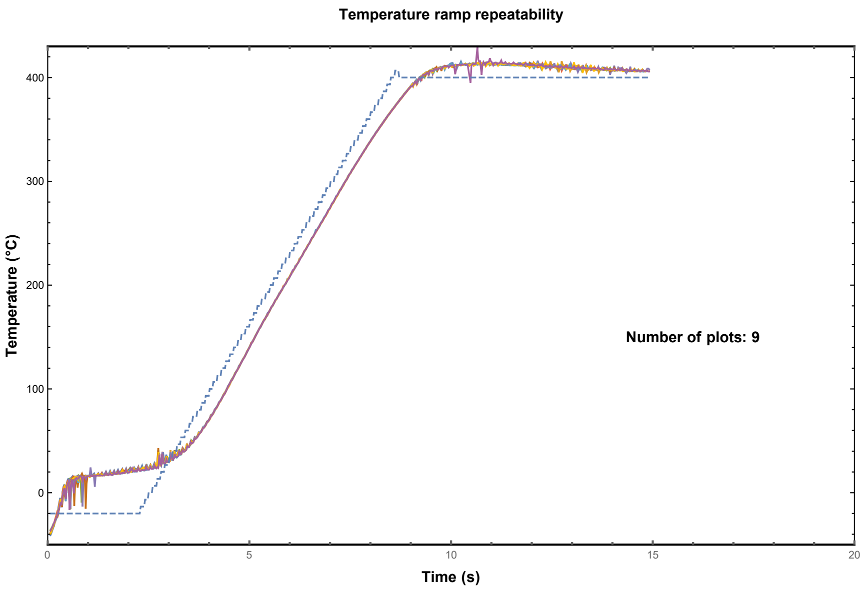


FIGURE 5.13: A graph of 9 identical, consecutive temperature ramps overlaid. The heating rate is 4000 C°/min. The temperature follows the set point faithfully up to 350 °C.

⟨fig:4000K/min⟩

Figure 5.13 illustrates heating ramps executed by the coaxial heater. The heating rate was 4000 C°/min, and there are no significant differences between the 9 consecutive ramps.

Cooling rates

The project did not demand a precise knowledge of, or control over, the cooling rate of the coaxial heater. The only requirement was that cooling should be as fast as possible. The cooling rate could be adjusted through the metering valve, and an optimum cooling rate of 5100 C°/ min was achieved with a carbon dioxide flow rate of around 30 g/ min. This allowed the column to be cooled down from 350 °C to -20 °C in about 2 seconds. At this rate the portion of the chromatographic cycle that is not used for separation is dominated by fraction collection, and further cooling rate increases will not significantly reduce run times.

5.6 Detector

The detector used in this fast GC was an unmodified Varian™ 3300 flame ionization detector (FID). The detector bias voltage was supplied by the original electronics, but a stand-alone high-speed electrometer (V.G. Micromass Ltd, Model M406-H) captured the signal, which was then conditioned by a bench-top amplifier (V.G. Micromass Ltd, Model M406) before it was sent to the computer. This electrometer and amplifier were fast enough to detect and amplify the signals generated by the fast GC.

5.7 Data acquisition and control software

The whole SFC×GC instrument was controlled from a single PC, running a single program. The program was written in LabVIEW 7.1™ (National Instruments). This software was designed to interact very easily with the National Instruments PCI-6014 multifunction data acquisition board.

LabVIEW is a *visual programming language*, so called because programs are created by manipulating icons and wires on a screen, instead of typing text. This visual aspect of it makes it very easy to develop user interfaces as *virtual instruments* and get quick results. Figure 5.14 shows the interface of the program used to control the SFC×GC instrument.

5.8 Data structure

In GC×GC, 2D data is recorded as a continuous FID output stream — as if it is a 1D GC chromatogram — and later converted into a 2D chromatogram using knowledge of the modulation period. For two reasons we could not use this approach. First, in our instrument the first (SFC) dimension runs in a stop-flow mode making continuous data recording inappropriate. Second, the duration of the cooling cycle varies, which would introduce unacceptable variation in ^{2}D retention times.

We therefore constructed 2D chromatograms by recording a GC run for each SFC fraction injected. The ^{1}D retention times were recorded as the start times of each individual GC run. The ^{2}D retention times were measured from the time the GC fast temperature program started.

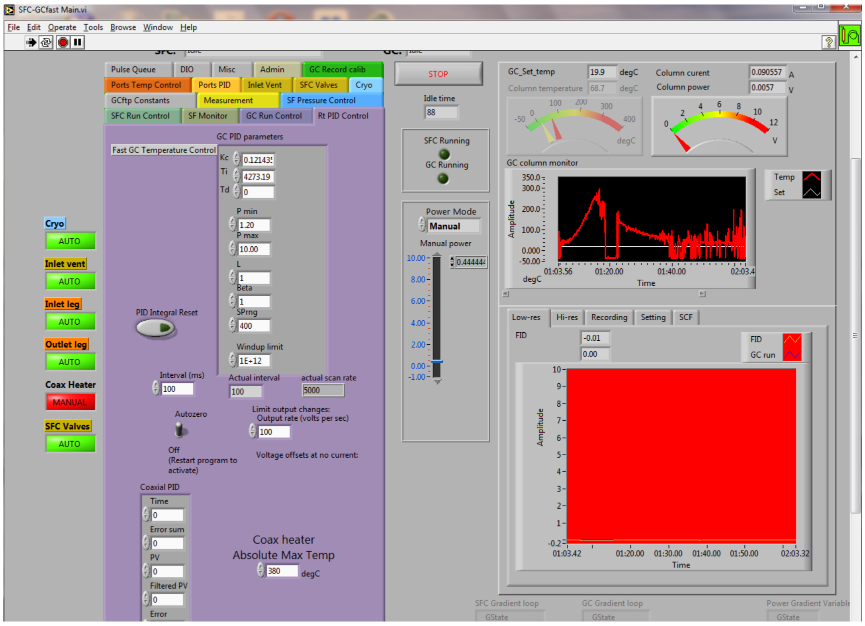


FIGURE 5.14: A screenshot of the LabVIEW virtual instrument used to control the SFC×GC instrument.

⟨fig:SFCGCFastVI⟩

5.9 Data visualization

For data visualization we used the technical computing system Wolfram Mathematica 11.3™. Mathematica is an extremely powerful and broad system, and we found it useful for its data manipulation and visualization tools.

The collected data could be handled in different ways. One way was to split it up into separate GC runs. Each individual GC run and its associated data could then be examined using the `Manipulate[]` function (Figure 5.15).

Then the data could be re-arranged into a list of three-element lists, with ^{1}D retention time, ^{2}D retention time, and detector signal as the elements of the inner lists. The Mathematica functions `List3DPlot[]` and `ContourPlot[]` could then be used to plot 3D chromatograms (Figure 5.16) or contour plots (Figure 5.17) respectively.

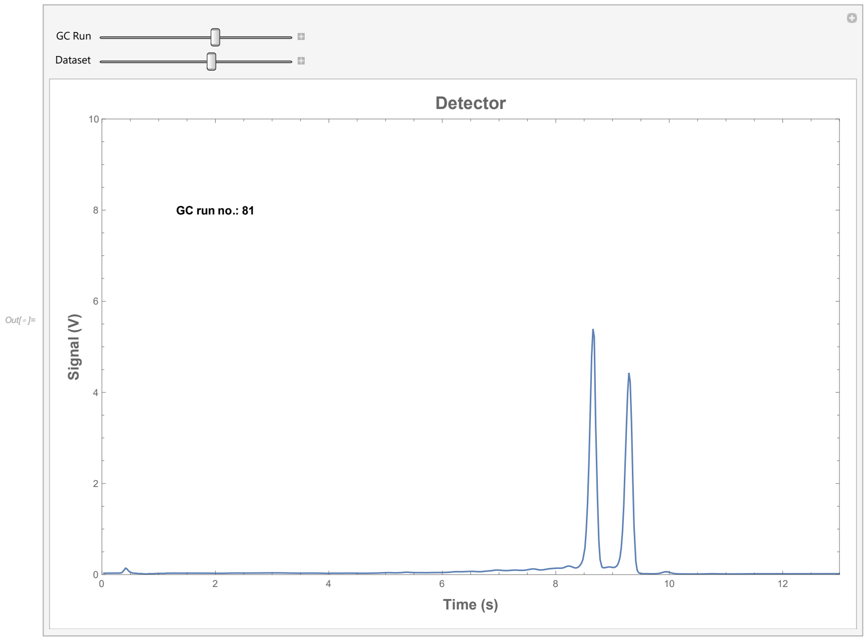


FIGURE 5.15: A single fast GC chromatogram, in a Mathematica `Manipulate[]` environment. The sliders can be used to select the which GC run to view, and which data of that run.

⟨fig:SingleGC⟩

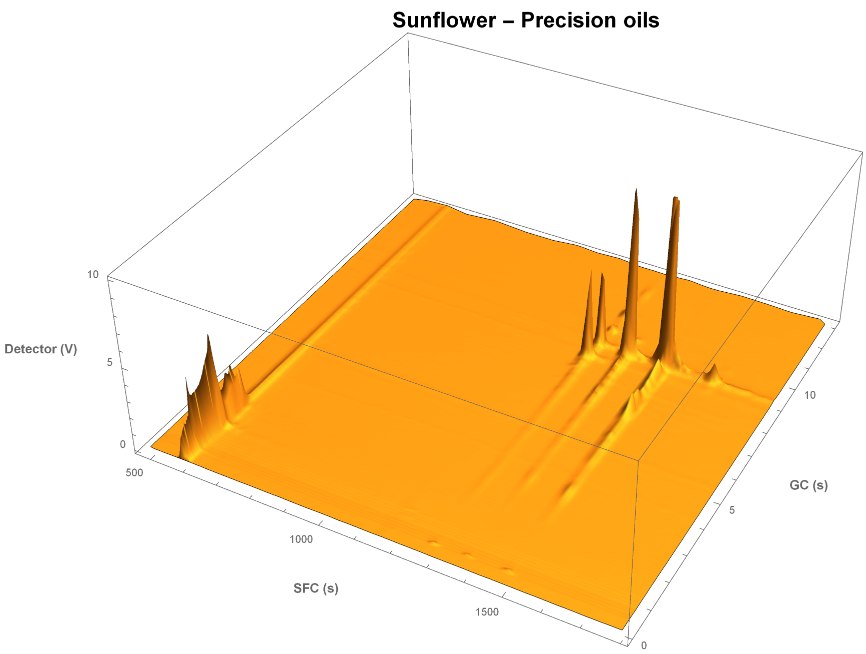


FIGURE 5.16: A 2D SFC×GC chromatogram.

⟨fig:2DChromatogram⟩

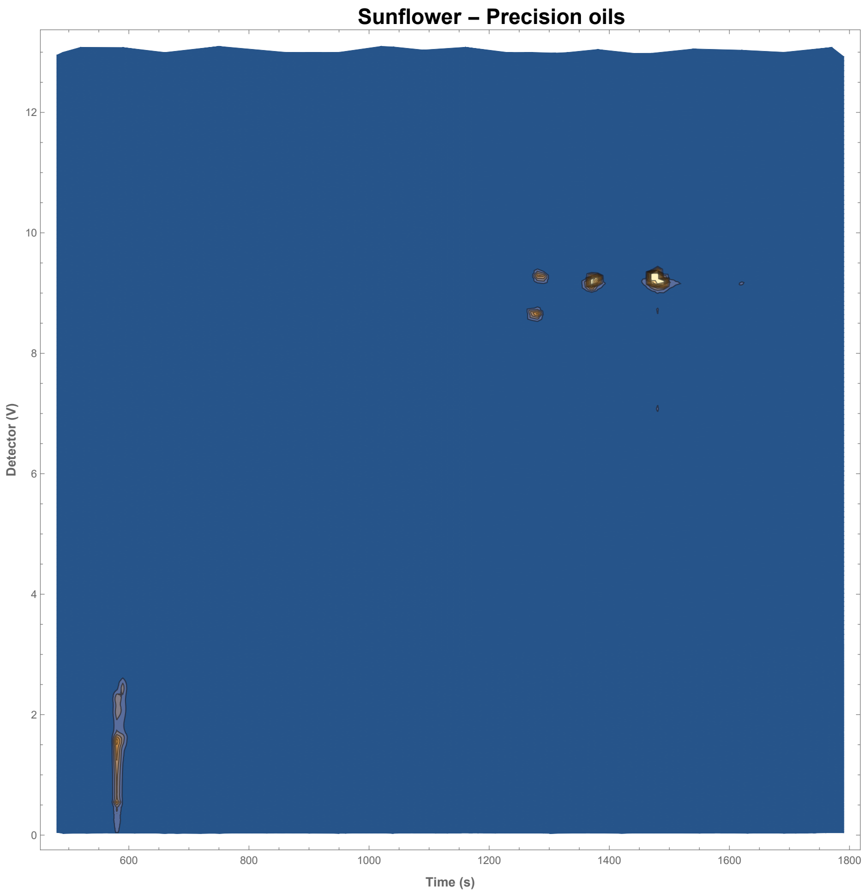


FIGURE 5.17: An SFC×GC contour plot.

⟨fig:Contourplot⟩

I'm really into coconut oil for everything. I cook it, eat it, put it in my hair, and use it as body lotion. I put it on my face, too - day cream, night cream, whatever. I love the smell. It reminds me of the beach. I'm not particular on what brand as long as it's organic.

Juliette Lewis

6 Investigating biodiesel feedstock using SFC×GC

⟨Chapter6⟩

6.1 Introduction

Biodiesel consists mostly of a mixture of methyl esters of fatty acids obtained from plant oils. To comply with the relevant technical standard, SANS 1935, (SABS 2008) (see Chapter 3), it must consist of a mass fraction of 96.5 % or more of fatty acid methyl esters (FAME), but not more than 12 % linolenic acid methyl ester, and not more than 1 % of FAMEs with more than 4 double bonds.

The prescribed methods for determining the quantities of the compounds are chromatographic, but the method for determining the *total amount* of FAMEs is a completely different method from that for determining the amount of *unsaturated* FAMEs. Both these methods generate complex chromatograms that need highly skilled and experienced chromatographers to interpret.

There is no doubt that the use of artificial intelligence and other technological innovation for interpreting chromatograms will grow, but the paradox of automation[1] predicts that as biodiesel production grows, feedstocks proliferate, and complexity increase, the analytical chemist will need better tools, methods, and instruments to understand the problems that arise when automation fails.

Comprehensively coupled chromatography offers a way to better exploit chemistry for improved analytical separations. It does this in three ways: the first is by increasing the peak capacity of the system, the second is by improved sensitivity, and the third is by generating patterns in the data.

6.2 SFC of FAMEs

The power of comprehensive chromatography is unlocked by orthogonality. Orthogonality is the difference in separation mechanism between the two dimensions (Marriott, Schoenmakers, and Wu 2012). When FAMES are chromatographically separated by SFC in a system using neat carbon dioxide as a mobile phase and unmodified silica as a stationary phase, then the separation is according to the number

[1] Automation helps you least when you need it most (Strauch 2018; Bainbridge 1983).

of double bonds, independent of chain length (Robertson et al. 1991; R. M. Smith and Cocks 1994; R. M. Smith, Hyytiänen, et al. 2001). This stands in strong contrast to the separation of FAMEs by GC, where the major separation is according to volatility, which can be adjusted, but not overridden, by changing the polarity (or other chemical aspect) of the stationary phase. (See Figure 6.1.)

Silver ions are often used in stationary phases to separate unsaturated compounds, and this includes stationary phases for SFC (Sandra et al. 2002; Potgieter et al. 2013). The retention mechanism is quite complex (Nikolova-Damyanova 2019), but it offers a powerful technique for the elucidation of lipid structures, as reviewed as early as 1966 (L. J. Morris 1966). Nevertheless, in this chapter the use of stationary phases modified with silver ions was neither necessary nor attempted.

The utterly different retention behaviours of FAMEs on silica with a carbon dioxide mobile phase and on GC offers high orthogonality, which promises to make comprehensive coupling worthwhile.

6.3 Performance of the coaxial heater.

Practical SFC×GC depends on reliably repeating fast temperature programs on a capillary gas chromatography column. The effect of these conditions on the column will be discussed in the following sections.

6.3.1 Column lifetime

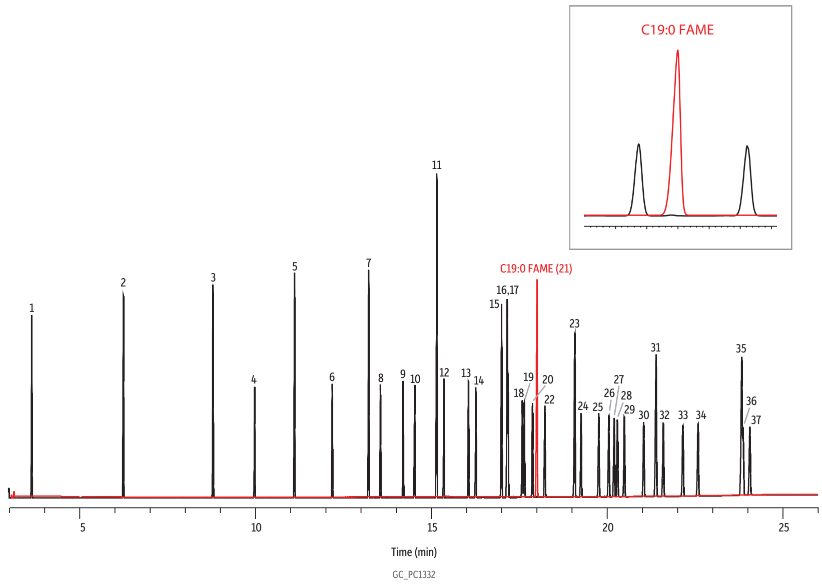
As discussed in Section 3.9, FAMEs can be separated by gas chromatography using a relatively non-polar columns with high-temperature programs. For this purpose column manufacturers supply special 'high temperature' columns, which address two aspects of column lifetime: mechanical degradation and stationary phase degradation. The SFC×GC instrument did not use such columns, and the following discussion explains why their use could be avoided.

Mechanical degradation

Fused silica has a high **tensile strength**, but also a low **fracture toughness**. This means that it is strong enough to resist the forces involved in its use as a column, but very liable to fracture if it gets damaged. Very small flaws, less than a micron in depth, will cause fused silica (or any other glass) to fracture under loads far under what would be expected from its tensile strength. Such flaws can be caused by mechanical scratches or reaction with atmospheric water. Therefore the fused silica capillary is coated on the outside with a layer of polyimide, which protects it from environmental damage. The strength of the a capillary column therefore depends on the integrity of the polyimide coating. The polyimide, like all resins and polymers, degrade faster at higher temperatures, so the longer the column spends at high temperature the sooner the polyimide will degrade to the point where it fails to protect the fused silica, and the shorter the overall mechanical lifetime of the column will be.

Traditional advice to chromatographers is to use temperature programs that minimize the time spent at high temperature, which will contribute to longer column lifetimes. The usual way to do it is to to use a temperature program with a maximum temperature no higher than necessary, but the same cumulative time at high temperature can be obtained by using higher temperatures but for shorter periods.

Food Industry FAME on FAMEWAX by EN14103 (2011)



	Peaks	t_R (min)	Conc. (mg/mL)	Structural Nomenclature
1.	Methyl capronate	3.629	1.2	C6:0
2.	Methyl caprylate	6.237	1.2	C8:0
3.	Methyl caprate	8.787	1.2	C10:0
4.	Methyl undecanoate	9.971	0.6	C11:0
5.	Methyl laurate	11.105	1.2	C12:0
6.	Methyl tridecanoate	12.179	0.6	C13:0
7.	Methyl myristate	13.215	1.2	C14:0
8.	Methyl myristoleate	13.549	0.6	C14:1 (*cis*-9)
9.	Methyl pentadecanoate	14.196	0.6	C15:0
10.	Methyl pentadecenoate	14.524	0.6	C15:1 (*cis*-10)
11.	Methyl palmitate	15.152	1.8	C16:0
12.	Methyl palmitoleate	15.355	0.6	C16:1 (*cis*-9)
13.	Methyl margarate	16.052	0.6	C17:0
14.	Methyl heptadecenoate	16.261	0.6	C17:1 (*cis*-10)
15.	Methyl stearate	16.995	1.2	C18:0
16.	Methyl oleate	17.156	1.2	C18:1 (*cis*-9)
17.	Methyl elaidate	17.168	1.2	C18:1 (*trans*-9)
18.	Methyl linoleate	17.583	0.6	C18:2 (all-*cis*-9,12)
19.	Methyl linolelaidate	17.641	0.6	C18:2 (all-*trans*-9,12)
20.	Methyl γ-linolenate	17.874	0.6	C18:3 (all-*cis*-6,9,12)
21.	Methyl nonadecanoate	18.052	2.0	C19:0
22.	Methyl α-linolenate	18.223	0.6	C18:3 (all-*cis*-9,12,15)
23.	Methyl arachidate	19.075	1.2	C20:0
24.	Methyl (Z)-11-eicosenoate	19.255	0.6	C20:1 (*cis*-11)
25.	Methyl 11,14-eicosadienoate	19.761	0.6	C20:2 (all-*cis*-11,14)
26.	Methyl eicosa-8,11,14-trienoate	20.046	0.6	C20:3 (all-*cis*-8,11,14)
27.	Methyl heneicosanoate	20.197	0.6	C21:0
28.	Methyl arachidonate	20.290	0.6	C20:4 (all-*cis*-5,8,11,14)
29.	Methyl 11,14,17-eicosatrienoate	20.488	0.6	C20:3 (all-*cis*-11,14,17)
30.	Methyl 5,8,11,14,17-eicosapentanoate	21.036	0.6	C20:5 (all-*cis*-5,8,11,14,17)
31.	Methyl behenate	21.39	1.2	C22:0
32.	Methyl erucate	21.595	0.6	C22:1 (*cis*-13)
33.	Methyl docosadienoate	22.150	0.6	C22:2 (all-*cis*-13,16)
34.	Methyl tricosanoate	22.584	0.6	C23:0
35.	Methyl lignocerate	23.826	1.2	C24:0
36.	Methyl docosahexaenoate	23.863	0.6	C22:6 (all-*cis*-4,7,10,13,16,19)
37.	Methyl nervonate	24.055	0.6	C24:1 (*cis*-15)

Column	FAMEWAX, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12497)
Sample	Food industry FAME mix (cat.# 35077) Methyl nonadecanoate (cat.# 35055)
Diluent:	Standard cat.# 35055 was dissolved in toluene.
Injection	
Inj. Vol.:	1 µL split (split ratio 100:1)
Liner:	Topaz 4.0 mm ID Precision inlet liner w/wool (cat.# 23305)
Inj. Temp.:	240 °C
Oven	
Oven Temp.:	60 °C (hold 2 min) to 200 °C at 10 °C/min to 240 °C at 5 °C/min (hold 7 min)
Carrier Gas	H_2, constant flow
Flow Rate:	1.7 mL/min
Detector	FID @ 250 °C
Instrument	Agilent 7890B GC
Notes	This chromatogram is an overlay of two injections: food industry FAME standard (black) and C19:0 methyl ester in toluene (red). An excellent separation of C19:0 (used in EN 14103 as an internal standard) and the most prevalent FAMEs found in biodiesel blends was achieved. Note that C4:0 from the food industry FAME standard elutes in the solvent front.

FIGURE 6.1: This figure shows that when separating FAMEs by GC, using specialized column, separation is primarily by fatty acid chain length, and secondarily by number of double bonds. Reproduced with permission of Restek Corporation.

fig:RestekFAMEsGC⟩

Fast temperature programs expose the columns for only very brief periods at high temperature.

(Column mechanical lifetime can also be improved by using metal capillaries. Metals are not afflicted by brittle fracture the way fused silica is, but they don't offer the chemical inertness of fused silica. To exploit the fracture toughness of metal technologies have been developed to deactivate metal surfaces (D. A. Smith 2002), which makes metal columns a modern possibility. Column manufacturers offer metal columns specifically designed for biodiesel analysis.)

Another durability benefit of the stainless steel coaxial heater is that the outer surface of the capillary column is protected from mechanical damage by encasement in the stainless steel tube of the coaxial heater. During development of the coaxial heater there were occasions where the column was accidentally overheated by poorly-controlled resistive heating. Even though the polyimide coating had been completely charred away and the capillary was fragile, the column was intact and still provided separations.

Using carbon dioxide as a between-program coolant also protects the polyimide coating from oxidative damage by purging the column environment of oxygen.

Stationary phase degradation

The stationary phases inside the GC columns face degradation similar to the that of the polyimide. Column manufacturers minimize stationary phase degradation by using improved technologies like cross-linked resins, or polymers with backbones resistant to certain degradation mechanisms (Day et al. 2003). Using fast temperature programs help extend stationary phase lifetime by minimizing the cumulative time the column spends at high temperature.

Column bleed

Short columns and fast temperature programs have another beneficial side effect: reduced **column bleed**. Any resin- or polymer-based stationary phase degrades over time, and this degradation is faster at higher temperatures (McNair, Miller, and Snow 2019, p. 66). It is observed as a rise in baseline during the high temperature part of a temperature program. Various stationary phase technologies can be employed to reduce column bleed, but because the amount of column bleed depends on the amount of stationary phase it is clear that a 1 m column will have 1/30th of the column bleed of a 30 m column with the same stationary phase. Despite using temperature programs that went up to the temperature limit recommended by the column manufacturer, column bleed was not a significant feature in the obtained chromatograms.

6.3.2 Thermal shock

An aspect of fast temperature programming with such a temperature range as used in this study (−30 °C to 400 °C) is **thermal shock**. Thermal shock occurs when an object is subjected to a rapid change in temperature. Under such non-equilibrium conditions different parts of the object will have different temperatures. The material that the object is made of has a certain **coefficient of thermal expansion**, which means that the relative sizes of the parts of the object at different temperatures will differ. This will cause stress between the parts, and if the stress exceeds the strength

⟨tab:RetentionTimeVariance⟩ TABLE 6.1: A summary of retention time repeataility of alkanes separated on the fast temperature programmed chromatograph.

Compound	n	t_r (s)	S.D. of t_r (s)	R.S.D. of t_r (%)
Dodecane	73	5.07	0.023	0.46
Hexadecane	73	6.58	0.052	0.78

of the material, the material will fracture. Fused silica has a low coefficient of expansion ($0.55 \times 10^{-6}\,K^{-1}$, compared to the $9.0 \times 10^{-6}\,K^{-1}$ of soda lime glass or the $4.0 \times 10^{-6}\,K^{-1}$ of borosilicate glass) and therefore experiences low thermal shock. The amount of material in a capillary is also relatively small, which keeps temperature gradients small and therefore stresses low. We didn't observe any column failures that could be ascribed to thermal shock.

6.3.3 Thermal fatigue

Another cause of material failure due to temperature is **thermal fatigue**. "Thermal fatigue (TF) is the gradual deterioration and eventual cracking of a material by alternate heating and cooling during which free thermal expansion is partially or completely constrained" (Rao and Raj 2001). The expansion of the portion of the fused silica column subjected to alternate heating and cooling is not constrained, and the part of the fused silica column that is constrained (in the sealing ferrules) is not subjected to alternate heating and cooling (it is held at constant temperature in the heated T-piece block) therefore we do not expect thermal fatigue. We did not explicitly test for the occurrence of thermal fatigue, but we did not encounter column failure that could be attributed to thermal fatigue.

6.3.4 Corrosion

One failure of the coaxial heater could be attributed to corrosion. The joint between the coaxial heater and the heated T-piece block is brazed, and there was a failure of the thin-walled stainless steel tube near that joint, in the portion heated during the brazing operation. Visual inspection seemed to indicate thinning caused by corrosion. An acid flux was used during the brazing, which might have contributed to the corrosion, especially in the presence of water condensed from the atmosphere during the cooling of the column.

6.3.5 GC retention time precision

During the development of the resistive heater it was shown that the measured temperature ramps are highly repeatable. But the retention times of of compounds being separated by GC are highly sensitive to temperature, so it was necessary to obtain a measure of the retention time repeatability.

To estimate the retention time variance, two alkanes (dodecane and hexadecane) were added to the SFC mobile phase at low concentration. These compounds are not retained on the silica at all, and are therefore present in all the GC runs at the same concentration and all the peaks should be identical. Variance in peak retention, -area, and -height therefore indicate the repeatability of the fast GC system. Variability of retention time is summarized in Table 6.1

The peak widths were about 500 ms, so the 20 ms standard deviation for hexadecane means that the variation in retention time is only about 10 % of the peak width.

TABLE 6.2: Oils used for FAME analysis

⟨tab:OilSamples⟩

Oil	**Brand**	**Species**
Canola	Spar	*Brassica napus*
Sunflower oil	Pick n Pay	*Helianthus annuus*
Coconut oil	Lemcke	*Cocos nucifera*
Flax seed oil	Lemcke	*Linum usitatissimum*

The relative standard deviations (RSD) of retention times were similar to those obtained in GC×GC (Shellie, Xie, and Marriott 2002).

6.4 Study of potential biodiesel feedstock by SFC×GC

Biodiesel can be produced from any combination of a variety of vegetable oils. Indeed, to ensure conformance to standards it might be necessary to produce biodiesel from blends or combinations of oils. But as an introduction it is instructive to start by analysing the FAMEs obtained from unblended oils.

6.4.1 Samples

Various samples of edible vegetable oil were obtained from supermarkets. (See Table 6.2)

6.4.2 Sample preparation

Fatty acids in oils might be either free or bound to glycerol. To quantitatively convert them to FAME therefore requires that the bound fatty acid be transesterified, and the free fatty acids esterified. Both transesterification and esterification can be achieved by acid catalyst, but this reaction is quite slow. Basic catalysts can rapidly transesterify acyl glycerols, but will not esterify free fatty acids.

The method used involved first treating the oil sample with sodium hydroxide dissolved in dry methanol. The methanol acts as a solvent but also provides an excess of methanol so that the transesterification reaction is driven to completion. On completion of the reaction an excess of acid is added, which neutralizes the base and esterifies any free fatty acids. Then an organic solvent and water are added, which provides two phases: the non-polar FAMEs dissolve in the organic layer, and the polar glycerol and salts dissolve in the water layer.

Method

This method is based on an official method (AOCS 2017), modified in two respects. First, the acidic catalyst boron trifluoride is replace by sulphuric acid, and second, hexane is used instead of heptane.

1. Transfer 4 drops of melted sample to a 20 ml glass stoppered test tube. Add a few boiling stones

2. Add 2 ml NaOH/Methanol solution (0.5N) and boil for 11 min under reflux.

3. Add 2 ml H_2SO_4/Methanol solution via condenser and boil for 2 min

4. Add 2 ml hexane via the condenser and boil for 1 min.
5. Remove the test tube from the heat source and leave to cool.
6. Add 4 ml saturated NaCl and mix gently.
7. Allow the phases to separate. Transfer the hexane layer to a vial and use for injection.

6.4.3 SFC

A 0.5 µl volume of the hexane layer was injected into the neat carbon dioxide mobile phase, as described in Section 4.4. The column was five HPLC columns (150 mm × 4.6 mm, 3 μm particles) (Restek, Pinnacle DB Silica) connected in series.

6.4.4 Modulation

The modulation period was 10 s during which the fractions of the SFC eluate were transferred to the GC inlet via a linear restrictor. In the hot (350 °C) inlet the fractions were evaporated and were swept onto the cold (−20 °C column they were trapped in the stationary phase. 3 s after the collection period ended the vent valve opened for 1 s to vent excess pressure from the GC inlet.

6.4.5 GC

The fast temperature program ramped the GC column temperature from −20 °C to 350 °C in 10 s (2200 $°C\,s^{-1}$), maintaining 350 °C for 2 s. Then the cooling system would activate and cool the column to −20 °C or below, ready to trap the next SFC fraction. The FID detector was kept at 350 °C.

In this way a series of GC chromatograms of SFC fractions were recorded, which were assembled into a 2D chromatogram. Figure 6.2 shows a 2D chromatogram of a sample of FAME prepared from canola oil. This 2D chromatogram consists of 132 fast GC chromatograms collected in approximately 90 minutes.

6.4.6 Results and discussion

The chromatogram shown in Figure 6.2 is very simple to interpret. In the SFC dimension, separation is by number of double bonds. This means that FAMEs without double bonds elute first in this dimension, followed by FAMEs with one, two and three double bonds respectively. There is good resolution between the peaks in the SFC dimension.

The volatility of a FAME depends primarily on the length of the carbon atom chain, therefore the separation in the GC dimension is by chain length. Because these FAMEs are of biological origin, we expect their chains to have an even number of carbon atoms. From the literature we know that oleic acid (C18:1) is the most common fatty acid in canola oil (Joint FAO/WHO Codex Alimentarius Commission 2019), and that therefore the major peak in the chromatogram is a C18 FAME. This allows us to easily identify the peaks for C16, C18, C20 and C22 in the GC dimension.

By inspecting this chromatogram we can confirm that canola oil is a viable biodiesel feedstock: The unsaturated compounds have mostly a single double bond, which should make it oxidatively stable, and the quantities of unsaturated FAMEs are low, which means that it will most likely have suitable cold-flow properties, because unsaturated FAMEs have higher freezing points than saturated FAMEs.

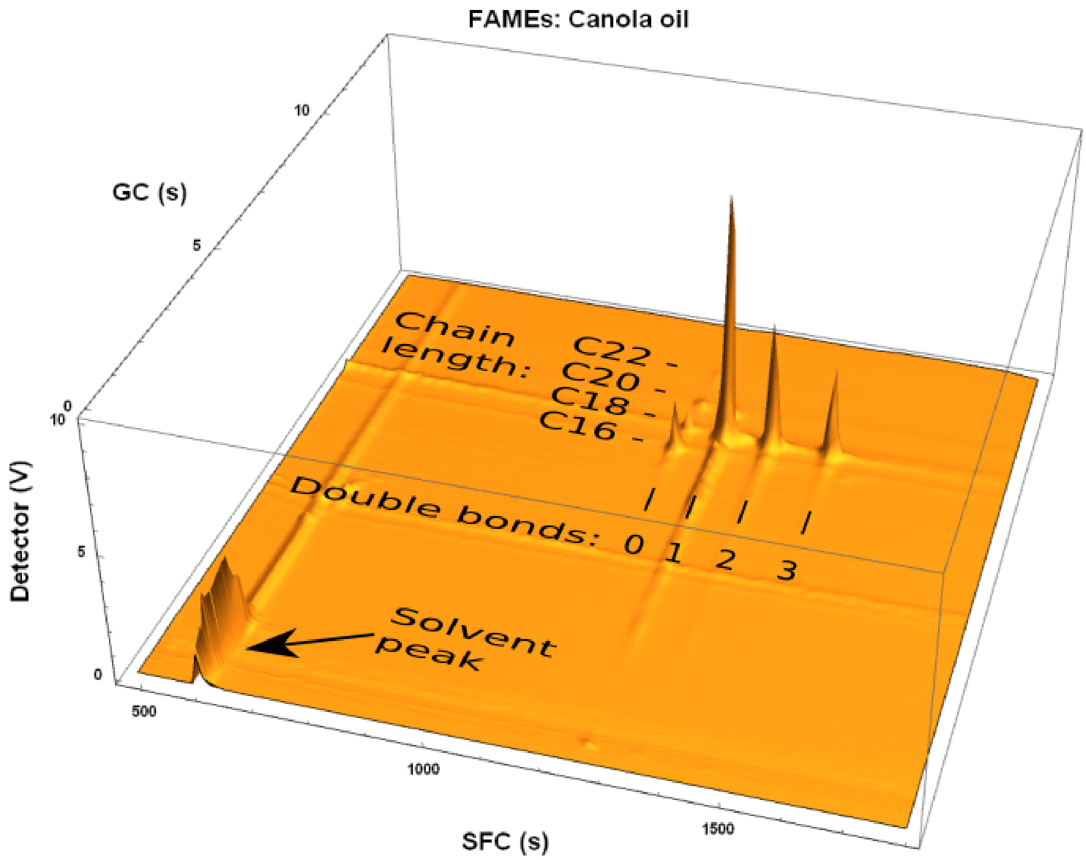


FIGURE 6.2: A 2D chromatogram of FAMEs derived from canola oil. It is clear that the oil consists mostly of unsaturated fatty acids.

⟨fig:2DCanola⟩

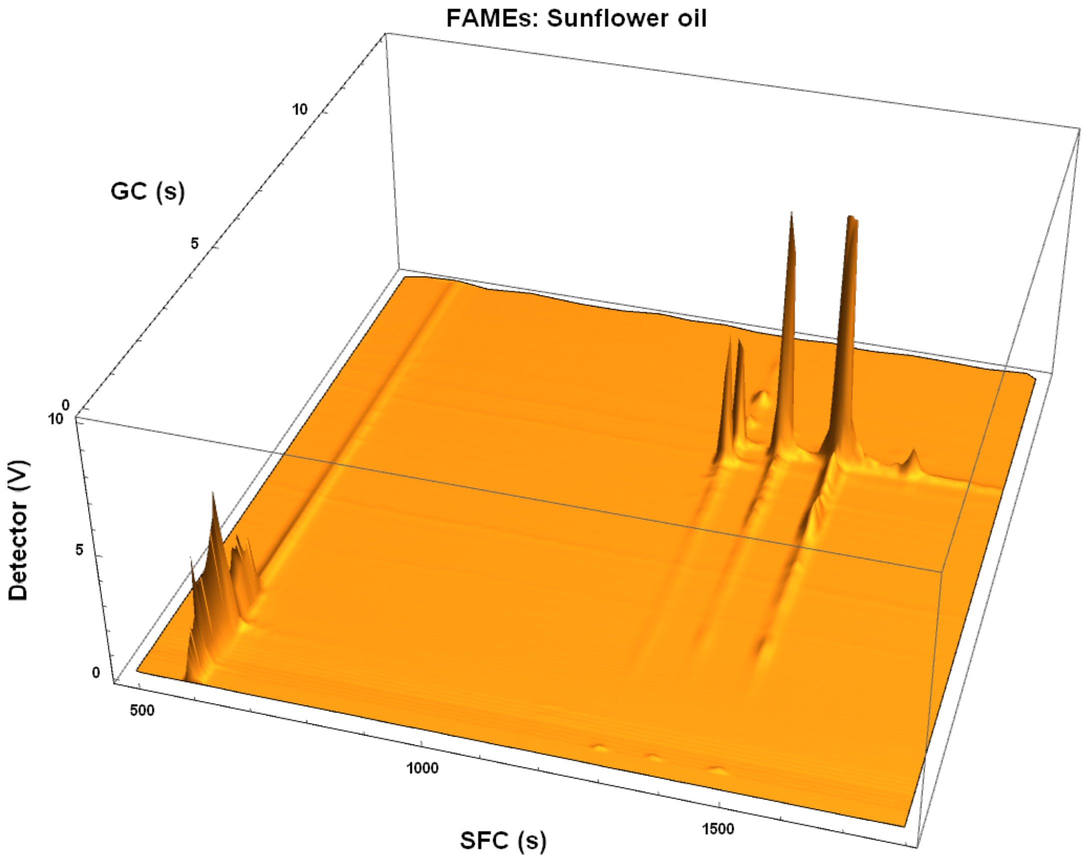


FIGURE 6.3: A 2D chromatogram of FAMEs derived from sunflower oil. It is clear that the oil consists mostly of unsaturated fatty acids, with a high proportion of linoleic acid (C18:2)

⟨fig:2DSunflower⟩

6.4.7 Sunflower oil

Figure 6.3 shows an SFC×GC chromatogram of FAMEs obtained from sunflower oil. The biggest peak in the chromatogram corresponds to linoleic acid methyl ester (C18:2). This agrees with the literature, which indicates that the main, traditional cultivar of sunflower produced in South Africa is of the high-linoleic type, with a fatty acid profile of 69 % linoleic acid, 20 % oleic acid, and 11 percent saturated fats (Joint FAO/WHO Codex Alimentarius Commission 2019).

This sunflower oil chromatogram was compared to a fatty acid profile obtained by a professional oil analysis laboratory[2]. (See Table E.1.) The fatty acid profile data was plotted on a 3D bar chart (See Figure 6.4). The axes of the bar chart were chosen to plot the fatty acid profile information in a similar space and scale as the chromatogram (Figure 6.3). It can be seen that the bar chart shows the same information as the chromatogram. The information in the bar chart has been obtained from a 1D chromatogram by integrating peaks, calculating amounts of FAMEs from peak areas, and then plotting those amounts in the bar chart, whereas the SFC×GC chromatogram is a plot of FID response and involves no quantification.

This chromatogram suggests that sunflower oil will make a suitable feedstock for biodiesel: the relatively high amount of unsaturated FAs would seem to imply suitable cold flow properties. The relatively high degree of unsaturation does suggest caution regarding oxidative stability.

6.4.8 Coconut oil

Figure 6.5 shows the SFC×GC chromatogram of FAMES from coconut oil (*Cocos nucifera L*). It is clear that the dominant FAMEs in coconut oil are saturated.

The high saturation of coconut oil makes it oxidatively stable, but the high melting point of the saturated FAMEs also means that it might not meet cold flow requirements.

The high oxidative stability of coconut oil makes it resistant to going rancid, which means it has been used since antiquity as a natural ingredient for cosmetics (Berdick 1972).

6.4.9 Flax seed oil

Figure 6.6 shows an SFC×GC chromatogram of FAMEs obtained from flax seed oil. This oil is obtained from the seeds of the annual plant *Linum usitatissimum*, and was sold as an edible oil.

The chromatogram shows that the major fatty acid component of this oil is linolenic acid (C18:3). This will immediately inform a producer that flax seed oil would not be a suitable feedstock for biodiesel: SANS 1935 limits the linolenic acid fraction to less than 12 % (See section 3.9.5).

Flax seed oil only recently became considered a food or a food supplement: the 1911 edition of the Encyclopaedia Britannica only mentions it as a food by saying "The oil is to some extent used as food in Russia and in parts of Poland and Hungary" (*Linseed* 1911), and remarks that the oil is edible when it is cold pressed. This is not surprising, because the oil goes rancid very quickly, leaving it unpalatable.

[2] Precision Oil Laboratories, info@precisionoils.co.za, +27 15 307 7208, SANAS Testing Laboratory T0802

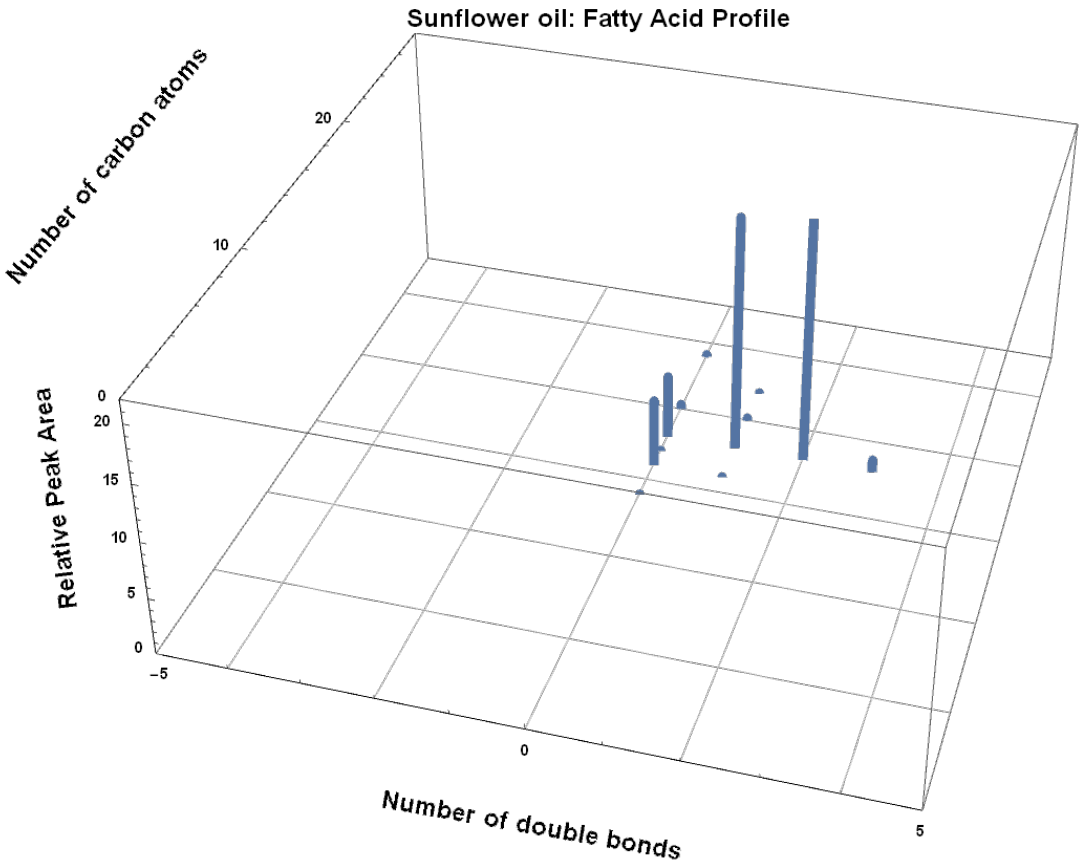


FIGURE 6.4: The fatty acid profile of a sunflower oil, when plotted on a suitably-scaled bar chart, shows how well the SFC×GC chromatogram of the same oil (Figure 6.3) represents the same data without any processing.

SunflowerBarChart⟩

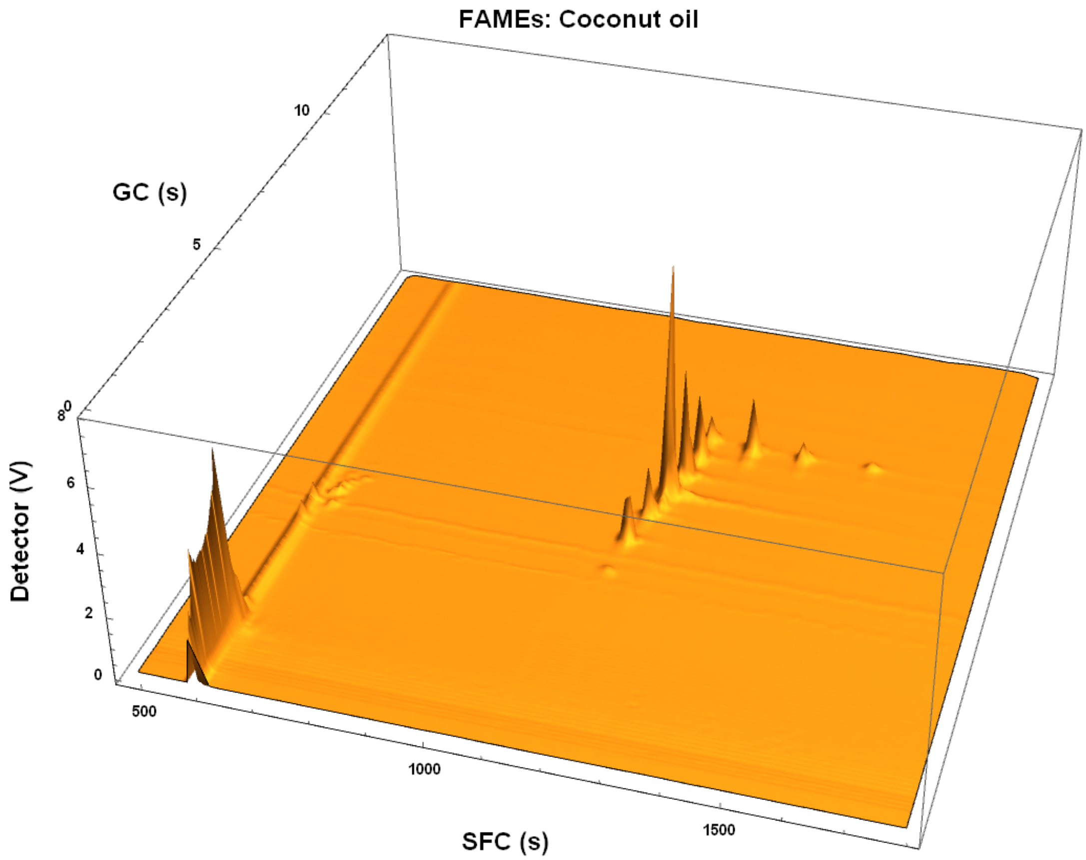


FIGURE 6.5: A 2D chromatogram of FAMEs derived from coconut oil. It is clear that the oil consists mostly of saturated fatty acids.

⟨fig:2DCoconut⟩

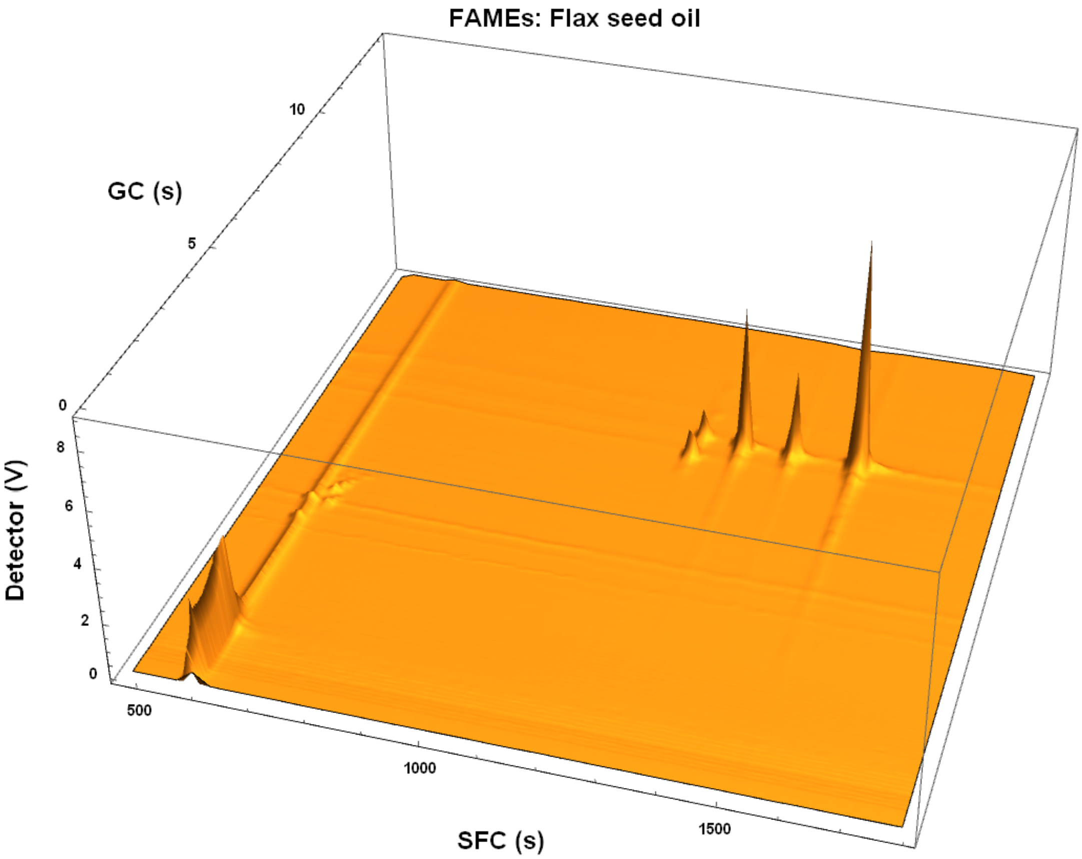


FIGURE 6.6: A 2D chromatogram of FAMEs derived from flax seed oil. It is clear that the oil consists mostly of highly unsaturated fatty acids.

⟨fig:2DFlax⟩

Historically the more common name for the oil has been **linseed oil** and it was mostly used for industrial purposes, particularly paint and varnish. As the encyclopedia puts it: “Commercial linseed oil has a peculiar, rather disagreeable sharp taste and smell.” The oil of used in artists’ oil paints is most commonly refined and prepared linseed oil. This oil is classed as a **drying oil**, because a layer of this oil exposed to air becomes solid, or “dry". This is the result of the high degree of unsaturation of the oil, which rapidly causes oxygen-mediated polymerization. Exposure to air over time makes the oil become more viscous and eventually solid, making it a suitable base for paint, particularly if the oil has been pre-treated. In biodiesel such polymerization could cause clogging or the formation of objectionable sludge.

6.5 Biodiesel blends

6.5.1 Introduction

Biodiesel can be blended in all proportions with diesel obtained from petroleum (petrodiesel). The volatility of biodiesel is slightly lower than that of petrodiesel, but in 1D gas chromatography the biodiesel compounds with higher volatility overlap significantly with the petrodiesel compounds of lower volatility. One example of an attempt to overcome this problem is the use of a highly polar ionic liquid column, which increases the retention of the FAMEs in the blend relative to the retention of the petrodiesel hydrocarbons (Ragonese et al. 2009).

On a polar stationary phase like bare silica with a non-polar stationary phase like carbon dioxide, the hydrocarbons from petrodiesel are not expected to be significantly retained. But, as shown in the in the previous section, under such conditions FAMEs are retained and separated. When we inject a blend of biodiesel and petrodiesel on SFC×GC, we therefore expect the biodiesel portion to be completely separated from the petrodiesel portion.

6.5.2 Sample

A B50 biodiesel blend was prepared by mixing equal volumes of biodiesel and petrodiesel in the laboratory. The biodiesel sample was donated by a commercial testing laboratory and had been produced from waste cooking oil by an anonymous producer. The petrodiesel sample (Shell Extra Diesel 500 ppm) was obtained from a consumer filling station.

The injection system described in Section 4.4 was used to inject a 0.5 µl volume of this undiluted B50 blend.

6.5.3 SFC

The SFC used neat carbon dioxide at 200 bar inlet pressure as mobile phase. The column consisted of five HPLC bare silica columns (150 mm $\times$ 4.6 mm, 3 μm particles) (Restek, Pinnacle DB Silica) connected in series.

6.5.4 Modulation

The SFC eluate fractions 2 s were collected on the GC column cooled to a temperature of -20 °C. Tto he inlet vent was held closed for 4 s, and then opened for 1 s to release excess pressure.

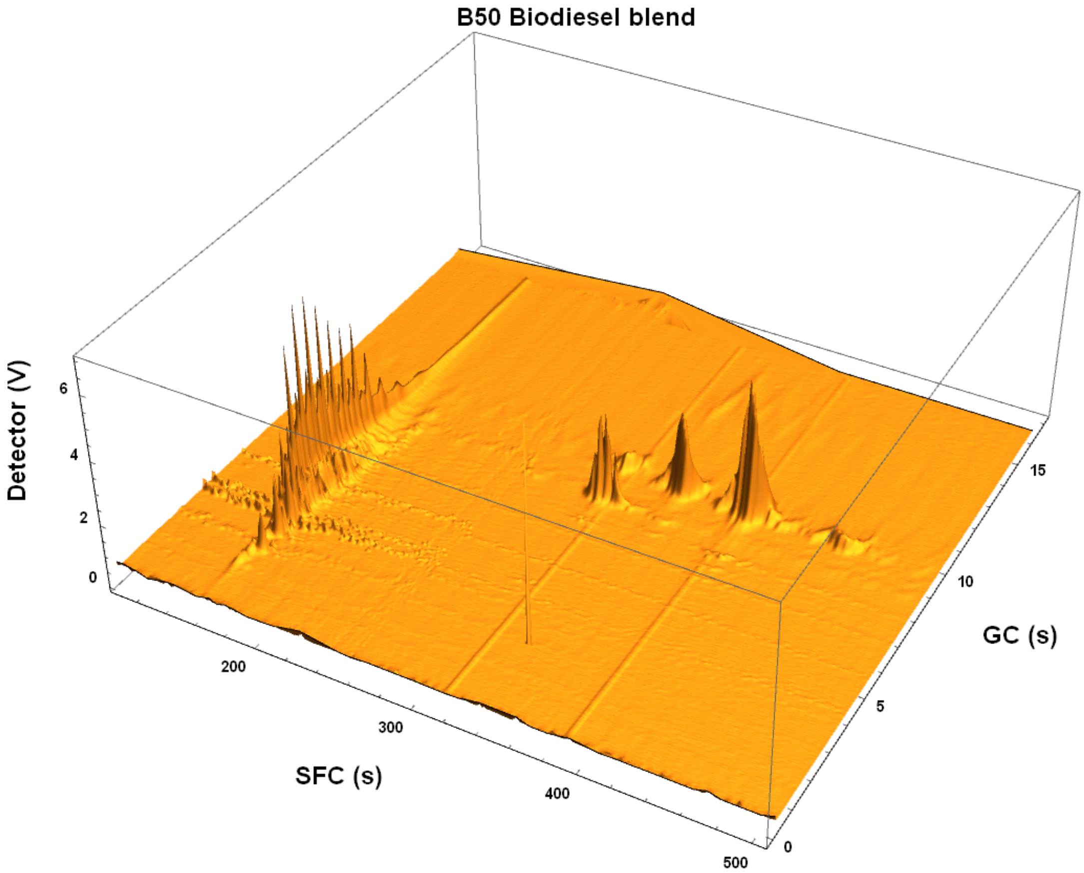


FIGURE 6.7: The separation of the biodiesel fraction and the petroleum fraction of a biodiesel blend. SFC: Neat CO_2 at 200 bar on Restek Pinnacle DB Silica column, 5 × 150 mm × 4.6 mm, 3 µm particles. GC: Hydrogen carrier on OV-5 1 m × 0.25 mm × 0.25 µm, temperature program −20 °C to 250 °C at 1800 °C min^{-1} hold for 6 s. GC runs: 371.

⟨fig:B50⟩

6.5.5 GC

The column used in the fast gas chromatograph was an OV-5 column, which has a stationary phase comprised of 5 % diphenyl, 95 % dimethylpolysiloxane. It was 1 m long, with an internal diameter of 0.25 mm. The thickness of the stationary phase was 0.25 µm.

The temperature was ramped from −20 °C at a rate of of 1800 °C min^{-1} to 250 °C, where it was maintained for 6 s. After the temperature program had ended the column was cooled to −20 °C again and the next fraction was collected.

The detector was an FID at 250 °C. The FID response was recorded, and so a total of 371 fast GC chromatograms were collected to compile the 2D chromatogram.

6.5.6 Results and discussion

⟨sec:B50Discuss⟩ The chromatogram of the biodiesel/petrodiesel blend (Figure 6.7) shows that the blend has been separated into two distinct groups of compounds. At a retention time in the ^{1}D (SFC) dimension of about 160 s the hydrocarbons from the petrodiesel part of the blend elutes. To those familiar with petrochemical chromatography the characteristic unresolved complex mixture (“hydrocarbon hump”) topped by alkane

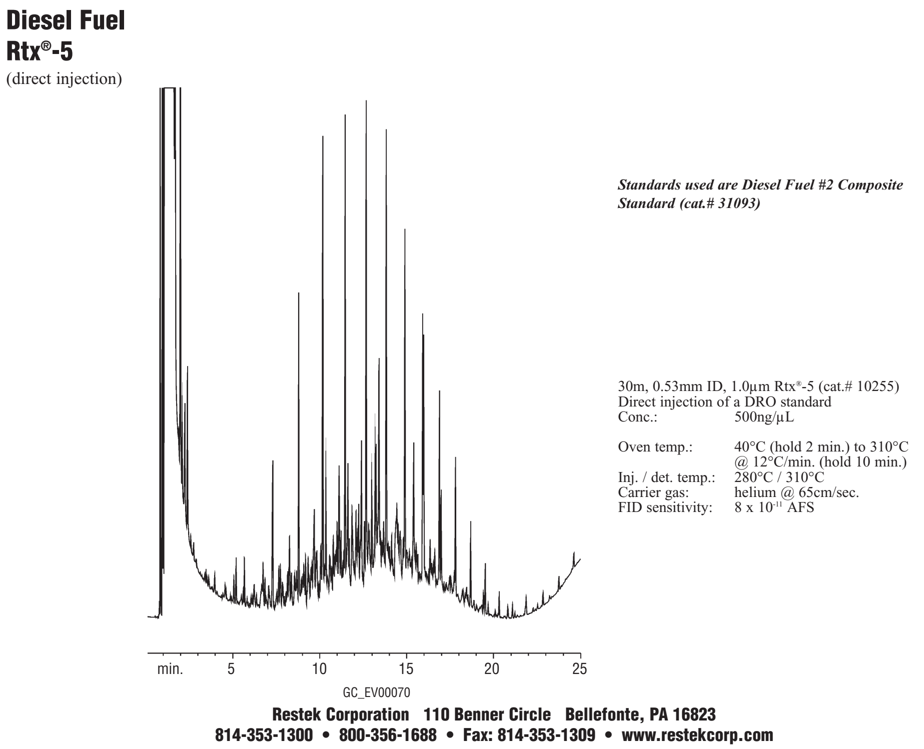


FIGURE 6.8: An example of a GC chromatogram obtained from a petroleum-based diesel fuel sample, showing the unresolved complex mixture topped by alkane peaks.

⟨fig:HCHump⟩

peaks will be instantly recognizable as a representing a petroleum product. (See Figure 6.8 for an example.)

Between the ^{1}D retention times of 300 s and 480 s the peaks of the FAMEs are seen, clearly separated by number of double bonds in the SFC dimension, and by volatility in the GC dimension. When compared to Figure 6.3, one can surmise that the feedstock for the biodiesel was waste sunflower oil.

As an aside, it is worth pointing out that the hydrocarbons from the petrodiesel fraction of the blend were also separated on the SFC dimension. This is not surprising: the standard ASTM D 5186 provides a method for the determination of aromatic compounds in diesel by SFC (ASTM 2019). The method separates the unretained non-aromatics from the monoaromatics and from the polynuclear aromatics. The SFC×GC method provides additional information about the volatility of those aromatics which could help to quantify or identify them, but that falls outside the scope of this discussion.

This separation shows that SFC×GC can be used to investigate the biodiesel component of petrodiesel/biodiesel blends without sample pretreatment or specialized columns.

Being able to determine the biodiesel content of biodiesel blends may prove useful in at least two scenarios:

The first is in monitoring blending. Biodiesel, essentially a mixture of esters, is quite polar compared to petrodiesel, essentially a mixture of hydrocarbons. This means that blending might not be a matter of just pumping the relevant volumes of the respective fuels into a tank and relying on diffusion to complete the mixing.

Being able to determine the amount of biodiesel in different samples of a blend will provide assurance that the blend is homogenous and should perform according to expectations. While such a test is perhaps better performed using a spectroscopic method, SFC×GC can provide information for calibration, and will of course be invaluable during trouble shooting.

The second application of SFC×GC is in regulating biodiesel content. In some political environments fuels might be taxed according to their biologically-derived content, for example to promote agriculture or to meet carbon emissions targets. Sometimes biological content is not encouraged by taxation, but simply mandated. Such incentives and mandates are of course liable to corruption, and therefore the ability to monitor the blending of the fuels is needed. The most reliable way to differentiate between organic matter derived from biological sources and organic matter derived from fossil sources is a radiochemical method, where the content of radioactive ^{14}C is determined. (Organic material from fossil sources contains no ^{14}C.) The technical standard ASTM D6866 provides an approved method. But this requires specialized equipment and trained staff, whereas fuel laboratories more often have experience with chromatographic techniques and might find SFC×GC a useful technique to provide evidence that a diesel fuel blend contains the stated amount of biodiesel.

6.6 Conclusion

Comprehensively coupled chromatography offers better analytical separations in three ways: improved peak capacity, improved sensitivity, and more structured data. The examples presented here does not pretend to prove improved peak capacity: biodiesel is not normally considered a chromatographically-challenging complex sample. Neither is improved sensitivity relevant: determining biodiesel components is not a trace analysis problem for which high sensitivity is necessary. What these examples do show is that the high orthogonality between SFC and GC provides data that contains a clear and powerful pattern that greatly simplifies interpretation.

In the example of FAMEs derived from various vegetable oils the pattern shows how the degree of unsaturation provides separation on the first dimension, and the volatility provides separation on the second dimension. These separations, combined in one 2D chromatogram, provides intuitive understanding of the chemical composition of the oils which facilitates prediction about the contribution these oils will make to the final biodiesel product.

In the example of examining the FAME content of a biodiesel blend, the pattern shows that SFC chemically separates the hydrocarbons from petroleum from the FAMEs of biological origin. The biodiesel content can be determined by focusing data analysis efforts on the relevant parts of the chromatogram.

In terms of compliance, SFC×GC can provide a chromatographic method that combines the determination of total FAMEs and unsaturated FAMEs in a single method, and might provide a way to quantify the biodiesel component in blends with petrodiesel.

The provided examples also prove that it is possible to design a system that solves the problems of doing practical, rapidly-repeated fast temperature programmed GC, ramping temperatures from below ambient to the limits of the column in hundreds of consecutive, identical runs. This high performance is possible without resorting to advanced or expensive technology.

It is a bad plan that admits of no modification.

Publilius Syrus

7 Application: Modified SFC

⟨Chapter7⟩

7.1 Introduction

In the previous chapter the SFC×GC was run with neat carbon dioxide as mobile phase for the SFC separation. Carbon dioxide as a mobile phase is compatible with a flame ionization (FID) detector: under the conditions found in the hydrogen-rich portion of the flame the carbon dioxide cannot be reduced to methane, the first step in the process that produces the ions that gives the FID it's name. But most modern SFC development involves the use of modifiers and additives[1]. These modifiers are usually organic compounds, and in the FID flame they produce ions. Because the modifiers are present in much higher quantities than the analytes, in an FID they will produce many more ions than than the analyte, and therefore the analyte signal will be swamped by the modifier signal. Mixing organic additives and modifiers with the carbon dioxide mobile phase therefore renders SFC incompatible with flame ionization as a detection method. Therefore, most modern SFC chromatographs use optical detectors, most commonly UV spectrophotometry borrowed from HPLC.

While the use of optical detectors have not hampered the development of SFC, they do have a few limitations, which might be imposing artificial limits on exploiting the versatility of carbon dioxide as a mobile phase:

- While SFC is compatible with optical detection (carbon dioxide is does not absorb visible or ultraviolet radiation), there is a wide range of potential modifiers that are not compatible with spectrophotometric detection because they absorb strongly in the UV, but might have chemical properties useful for manipulating SFC separations.
- Optical detectors can only detect analytes with chromophores, and the intensity of the signal depends strongly on the species.
- Optical detectors have a limited dynamic range, which complicates quantification.

[1] Modifiers are added to the carbon dioxide to manipulate the solubility of the analytes in the mobile phase, in exactly the same way that mixtures of solvents are used in, say, gradient elution in HPLC. Additives are added in small amounts, and manipulate the interaction of the analyte with the stationary phase.

In comparison, the FID can detect practically any organic compound over a wide concentration range, giving a signal proportional to the mass of the compound.

If only there were a way to separate the the modifiers from the analytes before detection . . .

When GC is comprehensively coupled to SFC, the modulator will focus each fraction of SFC eluate, and then the GC will separate all the compounds in the fraction, analytes and modifiers included. If the modifiers are less volatile than the analytes then the modifiers will elute before the analyte, and the analyte can be detected or quantified using an FID, and the signal from the modifier can be ignored.

Of course it is quite likely that the peak from the modifiers will **overload** the GC. Both the detector and the column may be overloaded. If the *detector* is overloaded, then it just means that the detector no longer responds linearly to the amount of material on the column — this might include responding with its maximum output, leaving flat-topped peaks. If the *column* is overloaded, then the peaks will **front**: they will be asymmetrical, with the side of the peak towards earlier elution times having a lower slope than the side of the peak towards later elution times. The side of the peak towards later elution times will have a larger slope.

The requirement that the modifiers added to the SFC mobile phase be volatile (so that they can elute before the analyte) is not too onerous a restriction. Higher volatility correlates with lower diffusivity, so that the preferred high-diffusivity modifiers for SFC would also tend to be be volatile enough to elute early on GC.

A practical SFC×GC chromatograph opens up the field for the use of carbon dioxide with modifier as a mobile phase for analysing complex mixtures found in biodiesel production, biodiesel quality control, and compliance monitoring.

7.2 SFC×GC using modified carbon dioxide.

In this section a SFC×GC chromatogram is presented that shows that methanol added as a modifier to the SFC mobile phase does not interfere with the fast GC any more than a sample's solvent interferes in the usual 1D GC. The 2D separation space of SFC×GC therefore gains in control over elution in the SFC dimension at the cost of losing the capability to detect compounds more volatile than methanol on the GC dimension.

7.2.1 Sample

The sample was a 1:1 blend of petrodiesel and biodiesel, prepared in the laboratory. The biodiesel sample was donated by a commercial testing laboratory, and had been produced from palm oil by an anonymous producer. The petrodiesel (Shell Extra Diesel 500 ppm) was obtained from a commercial filling station.

7.2.2 SFC

The SFC used carbon dioxide at 200 bar inlet pressure and room temperature as mobile phase. The flow rate is of carbon dioxide at atmospheric pressure was reportedly 175 ml min^{-1}. The carbon dioxide mobile phase was modified with 5 % mass fraction of HPLC-grade methanol (Merck LiChrosolv). The column consisted of five HPLC bare silica columns (150 mm $\times$ 4.6 mm, 3 μm particles) (Restek, Pinnacle DB Silica) connected in series.

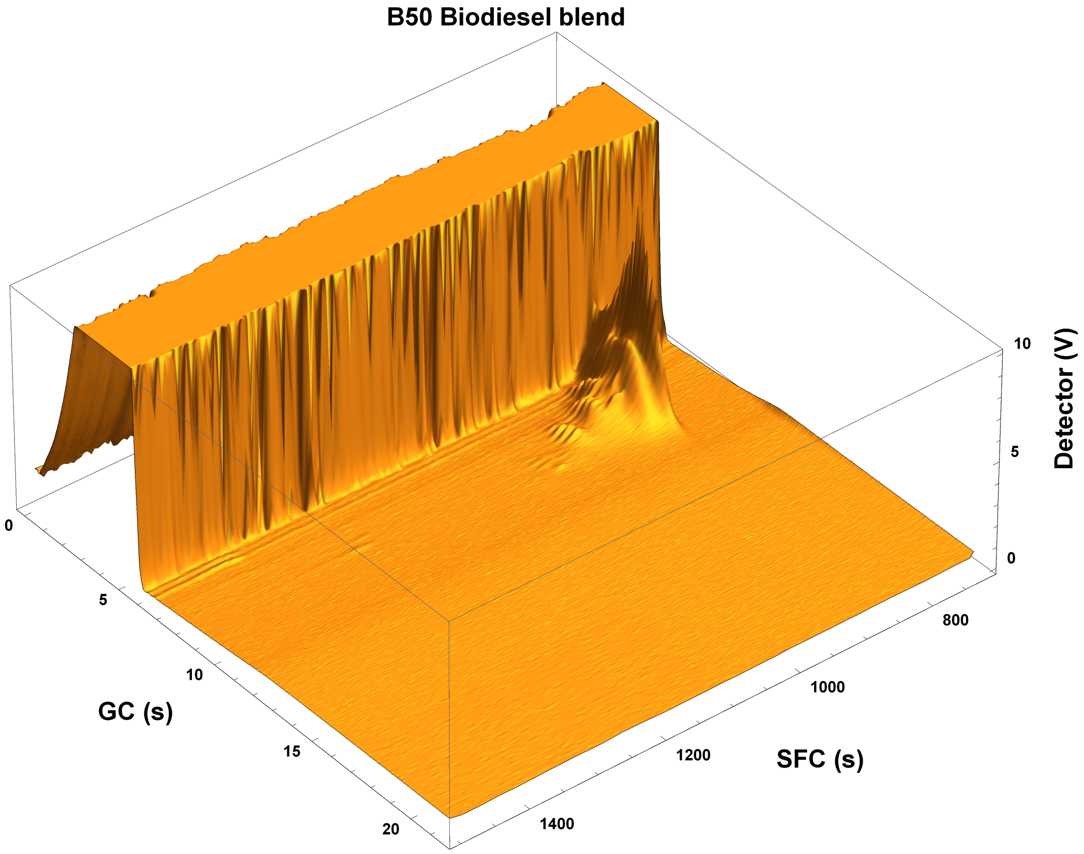


FIGURE 7.1: The modifiers used in the SFC dimension elutes as a solvent front on the GC dimension, and does not otherwise affect the separation.

⟨fig:Modifier⟩

3595 7.2.3 Modulation

Each fraction collected from the SFC was 5 s of flow time. After the flow from the SFC had been stopped, 3 s was allowed for the last vapour in the inlet to be swept into the cold (−20 °C) column to be trapped, and then the inlet vent valve was opened for 1 s to relieve excess inlet pressure.

3600 7.2.4 GC

The GC column was a Restek Rxi-5Sil MS 0.250 mm x 0.25 µm × 1 m. The hydrogen carrier gas flowed at a rate of 11.6 ml min^{-1}.

For each fast GC program the temperature was ramped from −20 °C to 320 °C in 10.3 s (33 °C s^{-1}) and kept there for 10 s before cooling the column down to −20 °C
3605 again, in preparation of trapping the next fraction.

A total of 233 fast chromatograms were recorded and combined into a 2D chromatogram.

7.2.5 Results and discussion

The chromatogram obtained for this run is shown in Figure 7.1. Note the unusual
3610 orientation of the chromatogram, chosen to best show the data: In the SFC dimension the later elution times are closer to the reader.

The intention of this chromatographic run was to explore the effect of the methanol modifier on the retention behaviour of the biodiesel fraction of the B50 blend. However, the run time was not long enough for the FAMEs to elute and the only peaks on the chromatogram are the group of hydrocarbons from the petrodiesel. Nevertheless, the chromatogram shows the possibilities that arise when a modifier is added to the SFC mobile phase.

The 'wall' that appears on the chromatogram is the tailing edge of the peak of the modifier added to the SFC. These peaks are present in all the fractions at equal concentration. The flat top of the peak is caused by detector overload.

It should be noted that the detector overload is not caused by saturation of the FID response, but by the limits imposed by the chosen signal amplifier. The FID has a dynamic range of 10^6, and could comfortably accommodate the modifier peak, but the amplifier gain was chosen to best match the analyte signal with the input range of the 16-bit analog-to-digital converter.

The 'corrugations' on the wall can be ascribed to two sources. First is variations in retention time of the modifier peak, which can be caused by variations in GC gas flow and variations in the fast temperature programs. Second, variations in the amount of modifier collected by the modulator will also cause slight changes in the apparent position of the peak. This second reason is probably the major contributor for this run, caused by variations in the timed collection period introduced by the imprecision of the electromechanical valve actuator.

The chromatogram shows the hydrocarbon "hump" in the GC dimension and the separation of the aromatics in the SFC dimension, as discussed in Section 6.5.6. It can be seen that the 'solvent peak' of the modifier interferes with the more volatile of the hydrocarbons, but the rest of the separation space is available for separation.

7.2.6 Conclusion

Adding modifiers to SFC expands the versatility of carbon dioxide as a mobile phase, but in 1D SFC the use of modifiers precludes the use of the universal flame ionization detector (FID) because the modifier signal will swamp the analyte signal.

Adding GC-FID as a ^{2}D separation allows the use of any volatile modifier to manipulate retention in the SFC dimension, including modifiers that might not be UV-transparent. Modifiers present in the fraction collected by the modulator elute like the customary solvent peak in the GC dimension. This means that the signal from the modifier does not interfere with the signal of the analyte, having been separated by the GC column.

The example shown above shows that SFC×GC will reliably separate any modifiers used in the SFC dimension from the analytes, allowing the FID to be used as a detector for gradient-elution SFC separations.

"Begin at the beginning," the King said, very gravely, "and go on till you come to the end: then stop".

Lewis Carroll

8 Conclusion

⟨todopage:4⟩ To do (4) To do (5)
?⟨Chapter 8⟩?

8.1 Introduction

It has been shown that an SFC×GC instrument can be built using mainly 20th century technology.

8.2 Synopsis

8.2.1 Chapter 1: Introduction: Climate, fuel, and biodiesel

The molecular basis of sustainability (Anastas and Zimmerman 2016) demands that chemists discuss sustainability in terms of compounds. Chapter 1 opens with the idea that energy is an essential component of industrialized societies, but that choosing of fossil fuels as our source of energy causes pollution, which threatens to nullify the benefits they bring. It describes the process which causes carbon dioxide to be a major emittant, and how its interaction with planetary radiation makes it a pollutant. The concept of "carbon footprint" is introduced, which allows the comparison of activities in terms of their carbon pollution, which allows decision makers to select the least polluting option. The discussion then focuses on internal-combustion engines, which is a major source of carbon and noxious pollution, and it is shown that higher-efficiency engines have lower carbon footprints. A discussion of ways to reduce the carbon footprint of internal combustion engines shows that, in the cases where they cannot be replaced by electric motors, a reduction of carbon footprint can be obtained by preferring large, high-performance Diesel engines fuelled by biodiesel. The discussion concludes with the idea that the success of such engines will demand high quality biodiesel, and that chromatography will plary a central role in ensuring that quality.

8.2.2 Chapter 2: Introduction: Carbon dioxide and chromatography

Chapter 2 starts with a discussion of the chemical industry and the need to move towards "green" chemistry. It introduces carbon dioxide as a renewable resource, discusses its various uses in industry, and then focuses on its application in extraction and chromatography. It describes how carbon dioxide becomes a solvent at high pressures and densities, and then introduces supercritical fluid chromatography (SFC). Fractions of eluate from SFC can be analysed by gas chromatography, and if a suitable set of criteria is met, then the combination is called SFC×GC.

8.2.3 Chapter 3: Biodiesel standards

As discussed in Chapter 1, a reliable high-performance engine requires a reliable fuel. Chapter 3 discusses the concepts of technical standards, which establishes requirements with which fuels must comply. The discussion then focuses on the technical standard SANS 1935, which lists the requirements that biodiesel offered for sale in South Africa must comply with, concluding with the

8.2.4 Chapter 4: Instrumentation: Supercritical Fluid Chromatography

Chapter 4 explains the experimental equipment used for chromatography using high-pressure carbon dioxide as a mobile phase. It starts with describing the mobile phase, how it is stored and pumped, how modifier is added, and how the sample is injected. It describes problems with designing the restrictor that maintains the pressure, and concludes with remarks about using a gas chromatograph as a detector.

8.2.5 Chapter 5: Instrumentation: Fast GC

Chapter 5 opens with a discussion on the time aspect of comprehensive SFC×GC chromatography, and shows that for practical analysis the GC dimension must be *fast*. The theory of fast GC is discussed, which leads to the the need for fast temperature programming. The design of a resistively heated coaxial heater is described, including its calibration and control. The discussion then covers the need for a cold column, and the design of coaxial cooling using boiling liquid carbon dioxide is described. Next, the discussion covers the design of hardware to mount the coaxial heater in a conventional GC oven. The chapter concludes with a description of the detector and the data flow from signal to final chromatogram.

8.2.6 Chapter 6: Investigating biodiesel feedstock using SFC×GC

Chapter 6 discusses the application of the developed SFC×GC instrument to study the fatty acid profile of various potential biodiesel feedstocks.

8.2.7 Chapter 7: Application: Modified SFC

Chapter 7 demonstrates that the use of SFC with modifiers does not preclude the use of the flame ionization detector when GC is used as a second dimension.

8.3 Strengths of the SFC×GC

8.3.1 Length of SFC column

We could separate paraffins, olefins and aromatics, using the long column.

8.3.2 Variable modulation period

It is possible to vary the modulation period during an SFC×GC run. This would allow one to speed up analysis, by collecting fewer fractions where less information would be expected.

8.4 Limitations of this SFC×GC design

8.4.1

8.5 Suggested design improvements

8.5.1 Four-wire resistance measurement

The resistive heater had a design that depended on measuring the resistance of the thin-walled stainless steel tube. The resistance was measured by comparing the potential difference between the ends of the heater with the potential difference over a reference resistor carrying an identical current. The circuit that carried the current also measured the potential difference. Because the current was high, the circuit had to be constructed in such a way that it no other significant resistance in it, which meant that care had to be taken to used heavy-guage cable and only use soldered joints.

These troubles could have been avoided if the current circuit and the measuring circuit were separated, using ’four wire’ resistance measurement. In such a design the potential difference is measure using a circuit that connects directly to the voltmeter. The voltmeter has a high input impedance, which means that the current that the circuit carries is low, and stray resistances will play much less of a role. Also, this would have made it possible to use bolted joints on the current-carrying circuit, which could have simplifed operations.

8.5.2 Legs heating and cooling integrated in detector and inlet

8.5.3 Siltek-treated coaxial heater

⟨todopage:6⟩ **To do** (6)

To do…

⟨todolbl:1⟩ ☐ 1 (p. 51): Not mentioned in SANS 1935: Lubricity

⟨todolbl:2⟩ ☐ 2 (p. 51): Not mentioned in SANS 1935: Blends

⟨todolbl:3⟩ ☐ 3 (p. 82): Cite Calidus ultrafast GC

⟨todolbl:4⟩ ☐ 4 (p. 113): Move zlabelchap:pdfstartpage to appropriate chapter. Remove before publication

⟨todolbl:5⟩ ☐ 5 (p. 113): Remove usepackagestampinclude before publication.

⟨todolbl:6⟩ ☐ 6 (p. 115): Discuss material that passes through column during trapping.

A
Frequently Asked Questions

?⟨AppendixA⟩?

A.1 How do I change the colors of links?

The color of links can be changed to your liking using:
`\hypersetup{urlcolor=red}`, or
`\hypersetup{citecolor=green}`, or
`\hypersetup{allcolor=blue}`.
If you want to completely hide the links, you can use:
`\hypersetup{allcolors=.}`, or even better:
`\hypersetup{hidelinks}`.
If you want to have obvious links in the PDF but not the printed text, use:
`\hypersetup{colorlinks=false}`.

B
Glossary

?⟨AppendixB⟩? A list of technical terms that might be unfamiliar to the reader.

Azeotropic mixture A mixture of liquids in which the vapour phase and the liquid phase have the same composition. The mixture cannot be further purified by simple distillation.

Payload The portion of the weight of a vehicle's load that is in excess of what it needs to complete a journey. This

C Thermocouple Production

?⟨AppendixC⟩? How the thermocouple probe was made:

1.
2. A piece of 0.25 mm polyimide-coated fused-silica capillary of about 500 mm length was cut and mounted with sticky tape on a wooden metre stick.
3. A longer length of 0.1 mm fused-silica capillary was threaded through the 0.25 mm capillary.
4. The end of one of the thermocouple wires was inserted into the end of the 0.1 mm capillary. A drop of cyanoacrylate adhesive was touched to the end of the capillary. Capillary action drew the liquid adhesive into the capillary and fixed the wire in place.
5. The wire was drawn carefully into the capillary by pulling on the 0.1 mm capillary.
6. Once the end of the wire protruded through the end of the 0.25 mm capillary the end of the 0.1 mm capillary was cut off.
7. The wire was anchored at one end with adhesive tape, pulled tight, and anchored at the other end.
8. The procedure was repeated for the other wire.
9. The two thermocouple wires (Goodfellow) was clamped in a twisting bar. The twister bar has a square profile, 8 mm on a side.
10. The wires were flamed with a cigar lighter until they were red a dull red hot. (At any higher temperature the wires would melt.) This chars the polyimide coating.
11. The flamed portion of the wires were lightly sanded with 1200 grit water paper. A pair of small pliers had its beak lined with the abrasive, and lightly stroked up and down the wire to remove the char.

12. A 6 mm tube was inserted between the wires to serve as spacer. and moved until about 10mm away from the twister bar.

13. The wires were twisted by turning the twister bar until the twister portion was about 5mm long.

14. The spools were rewound to retract the wire, until the start of the twist rested on the clamping bar.

15. The clamping weight was lowered onto the clamping bar, keeping the pair or wires in place.

16. A small pair of scissors was used to snip off the end of the

17. The welding electrode was brought into position. This was a carbon rod in the form of a pencil lead (Schwann Stabilo), 2 mm in diameter, mounted on a screwing connector. The welding circuit consisted of a bench power supply set to approximately 20V. connected. The negative terminal was clamped to the aluminium base plate of the microscope, on which rested the brass clamp bar. The positive terminal was clamped to the carbon electrode. A voltage of approximately 23 V was applied.

18. It was discovered that the carbon electrode should not have a polished end, but a roughly broken end.

19. The electrode was moved closer to the clamped twisted wire.

20. At the right point a spark would jump from the carbon to the wire, melting the end of the wire. The molten wire would draw into a globule on the end of the wire, withdrawing from the electrode and so breaking the spark, ending the heating.

21. If the wire would actually touch the electrode the wire would heat up red hot and melt off, usually destroying the twist and requiring making a new twist.

22. If all went well, there would be a hemispherical weld at the end of the twist where the two wires would be joined.

23. The thermocouple wires was withdrawn into the capillary until the end just protruded, kept in place with a pair of rubber-tipped self-closing tweezers.

24. If two sets of thermocouples were needed, the procedure would be repeated for another pair of wires.

25. The wires would be pulled back, one pair at a time.

26. The other end of the capillary was taped to the connector pad.

27. The wires was flamed and scraped to remove the polyimide isolation, and screwed down on a screw connector block.

28. The resistance between the protruding end of the thermocouple and the connector block was measured to ensure electrical connection. The resistance for the Chromel is 1440 Ωm^{-1}, and for the Alumel 600 Ωm^{-1}

29. A drop of cyanoacrylate adhesive was put on the end of the capillary to anchor the wires and to prevent high-pressure gas from blowing the wires out of the capillary.

D
Engineering Drawings

?⟨AppendixD⟩?

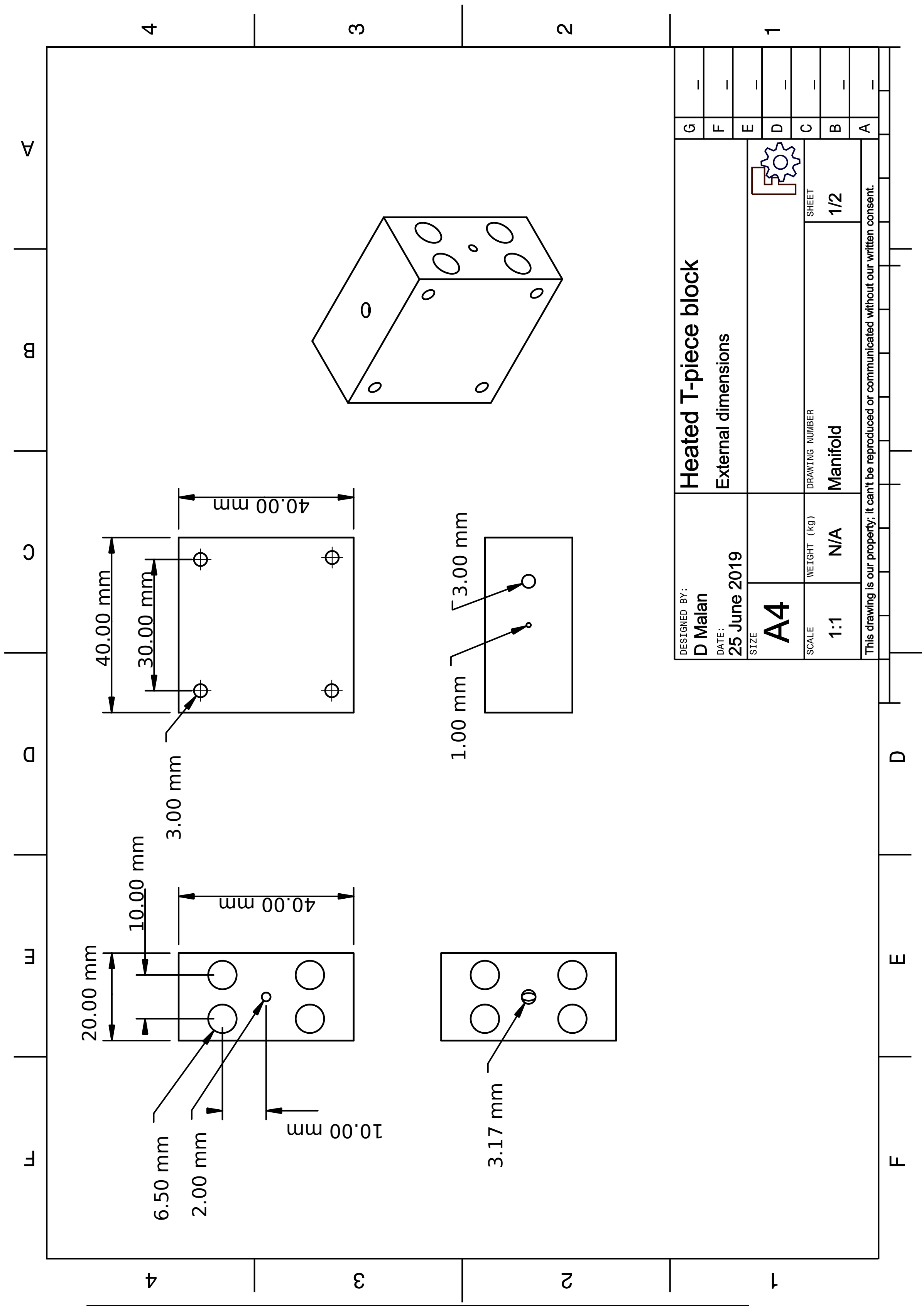


FIGURE D.1: A technical drawing showing the dimensions of the heated T-piece blocks.

⟨fig:ManifoldDims⟩

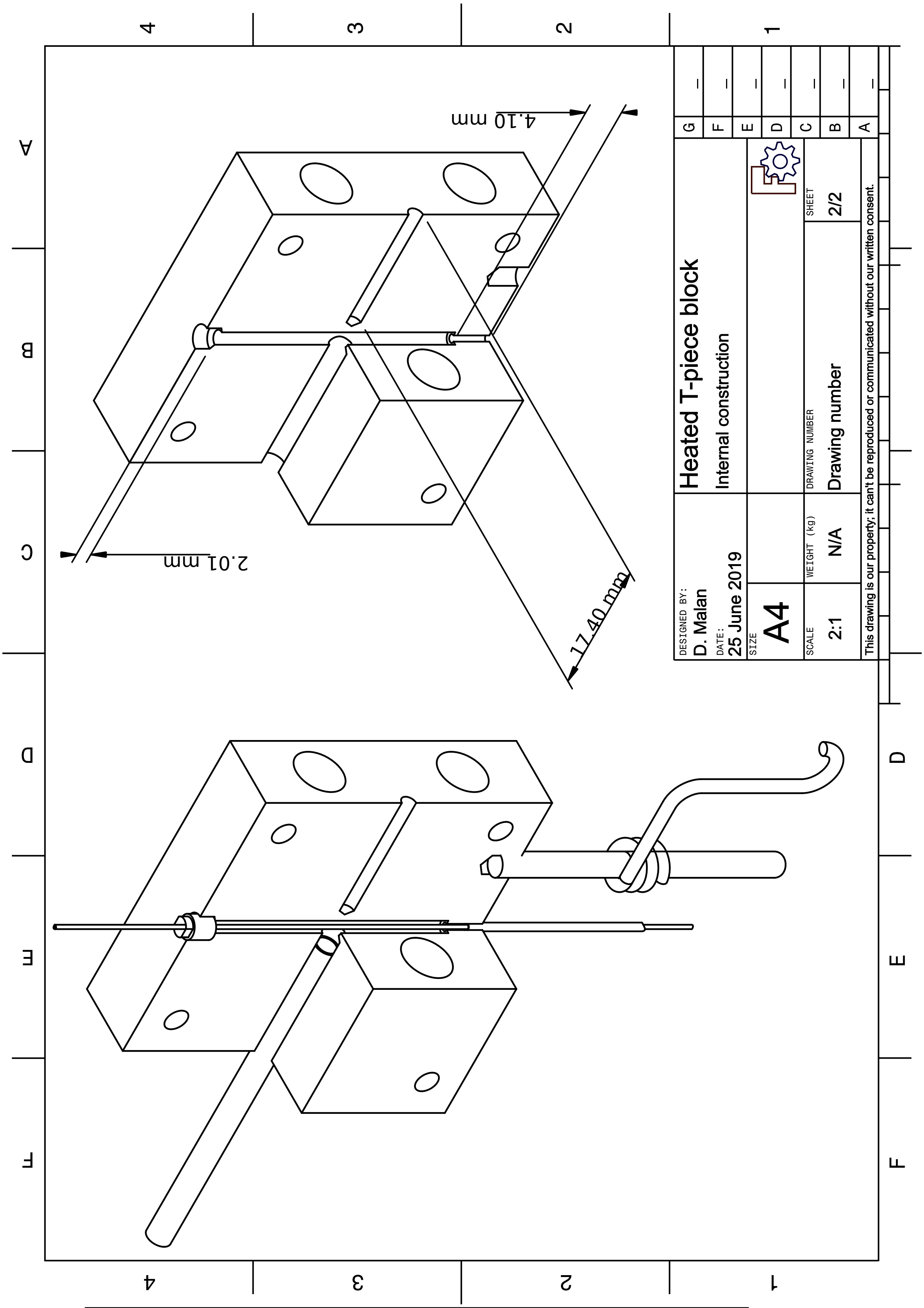


FIGURE D.2: A technical drawings of the internal construction and assembly of the heated T-piece blocks.

⟨fig:ManifoldAssy⟩

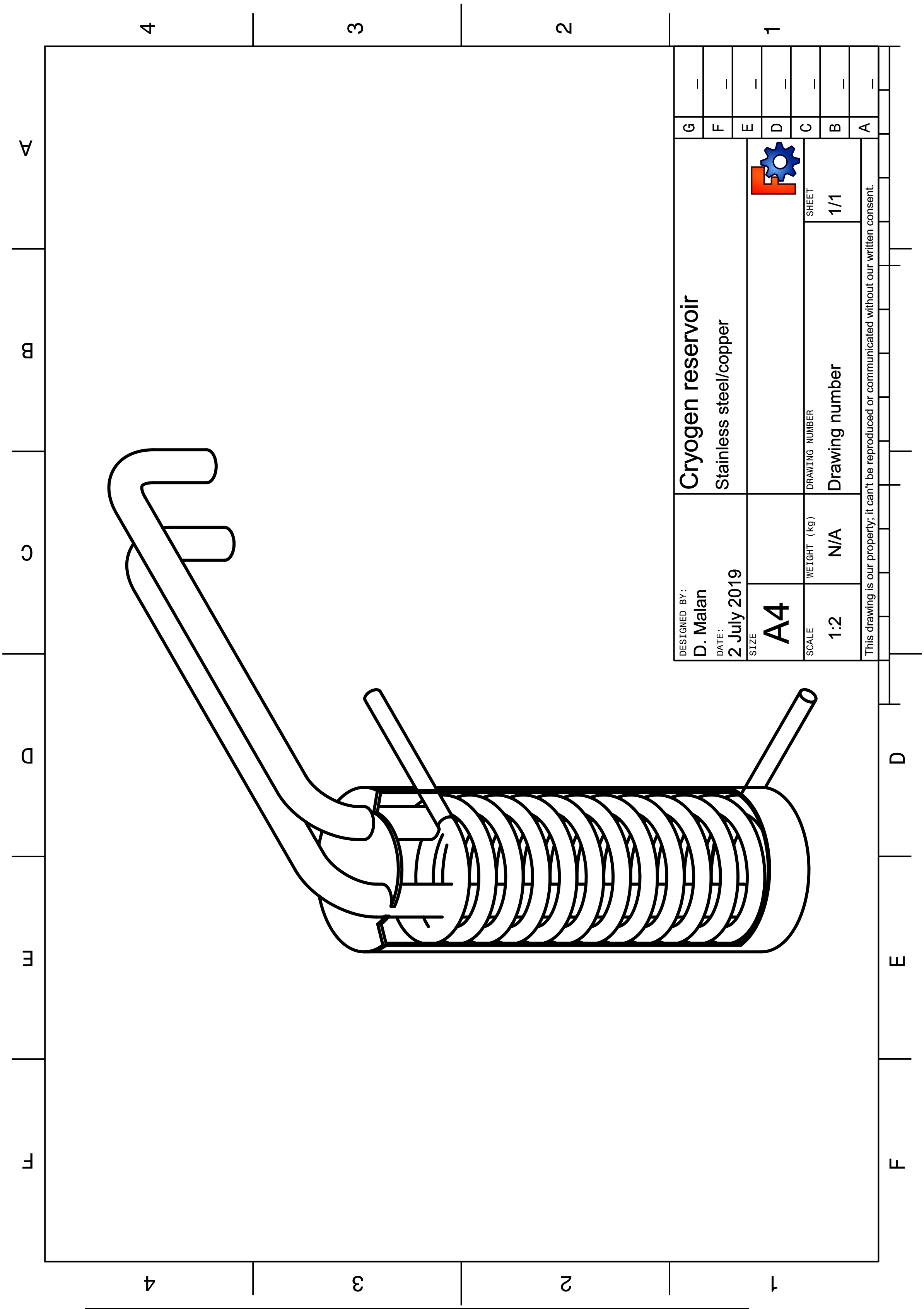
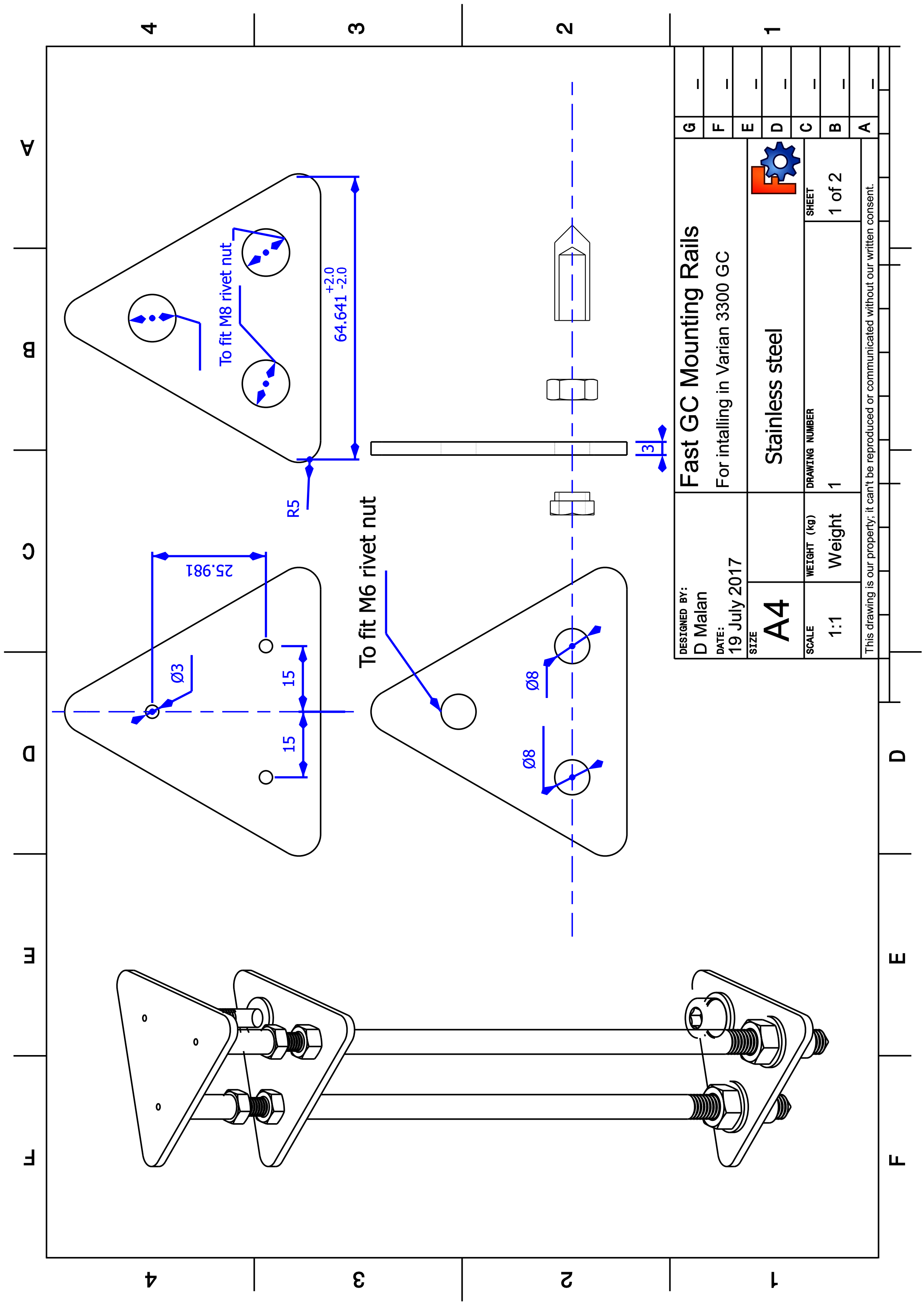


FIGURE D.3: Cut-away technical drawing of coolant reservoir.

⟨fig:CryogenReservoir⟩



⟨fig:RailsDrawing⟩ FIGURE D.4: A technical drawing of the rails carrying the T-piece mounting block.

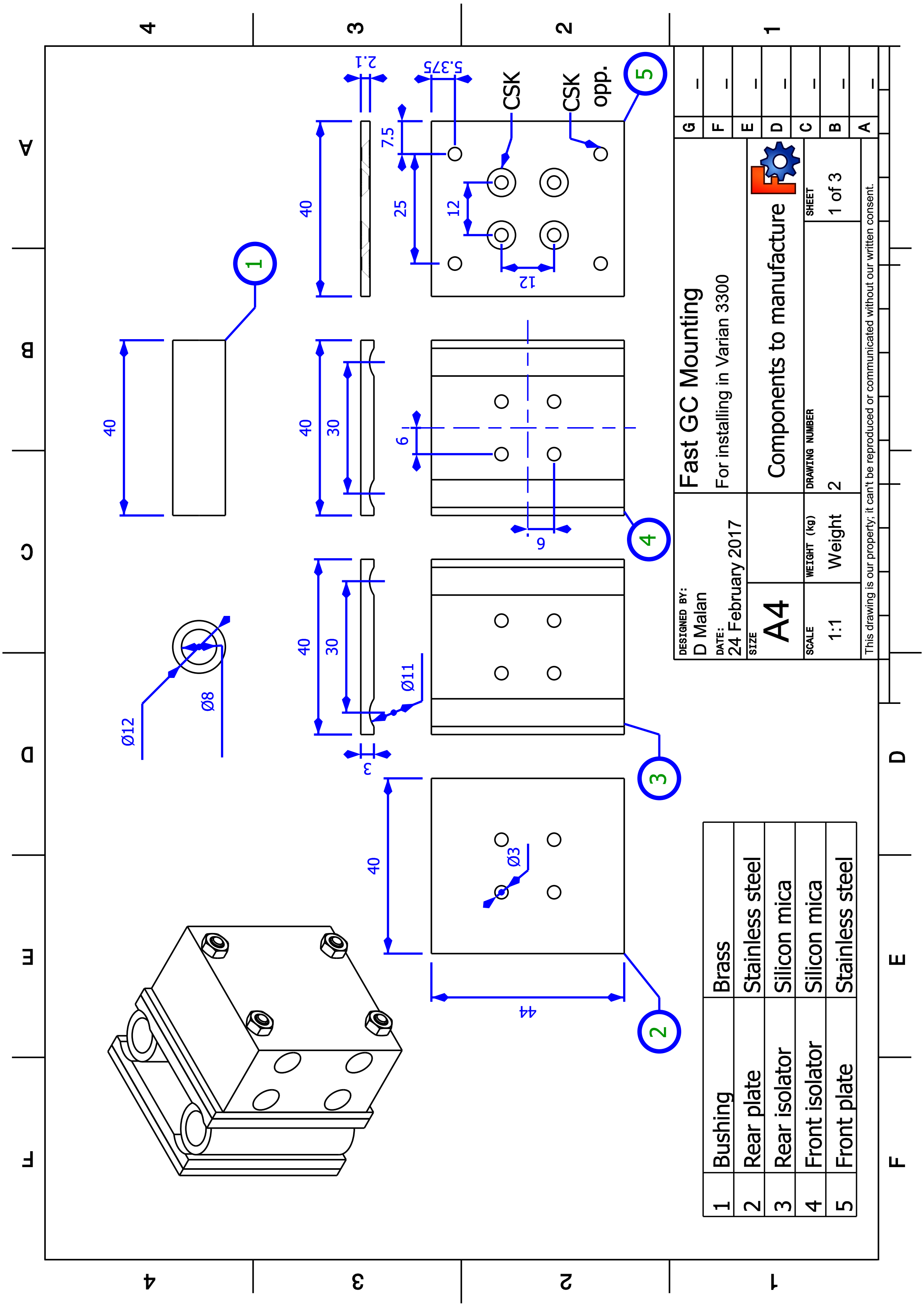


FIGURE D.5: A technical drawing of the T-piece block mounting, showing parts and dimensions.

⟨fig:CarsDrawing1⟩

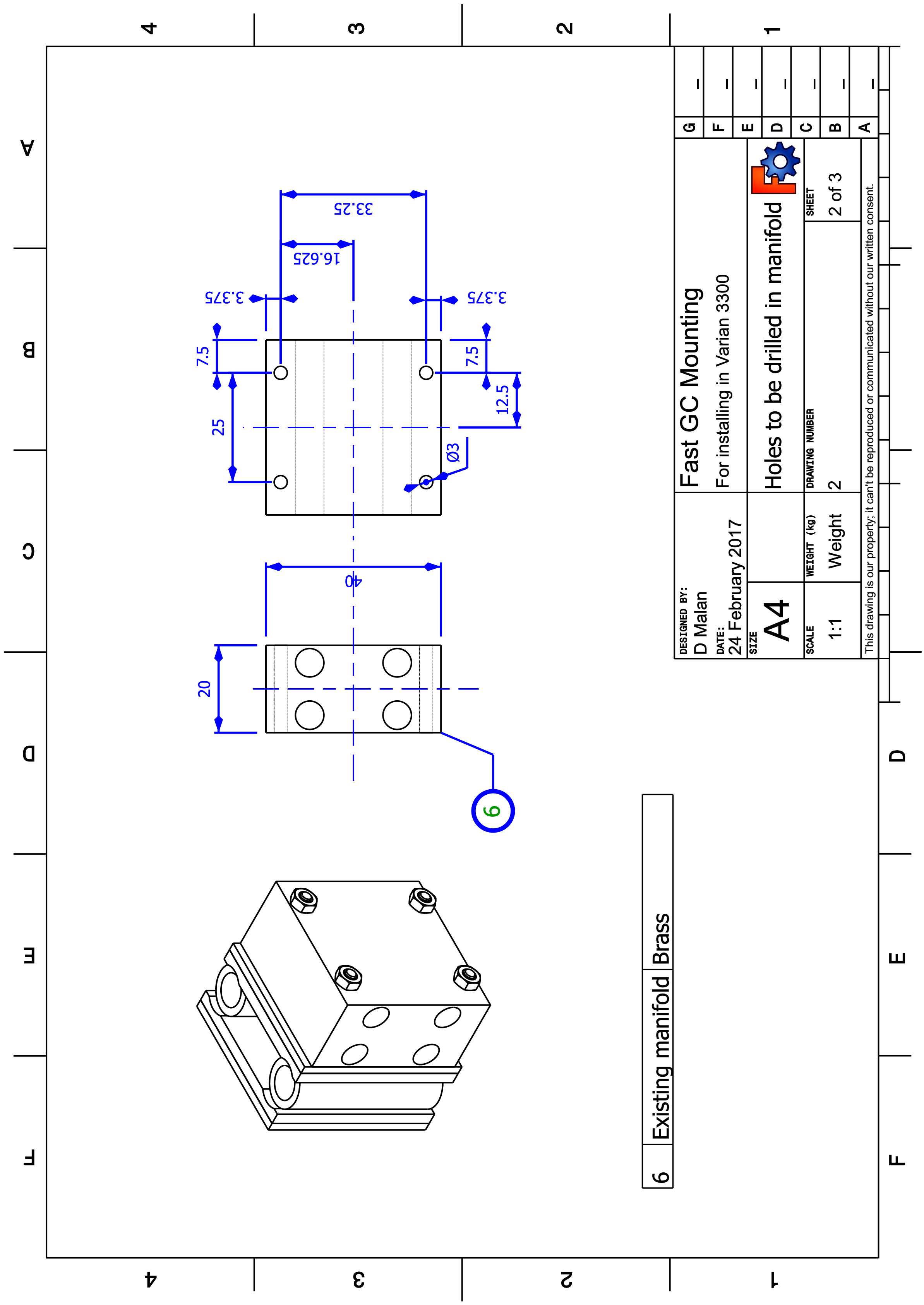


FIGURE D.5: (continued) A technical drawing of the T-piece block mounting, showing final assembly and dimensions.

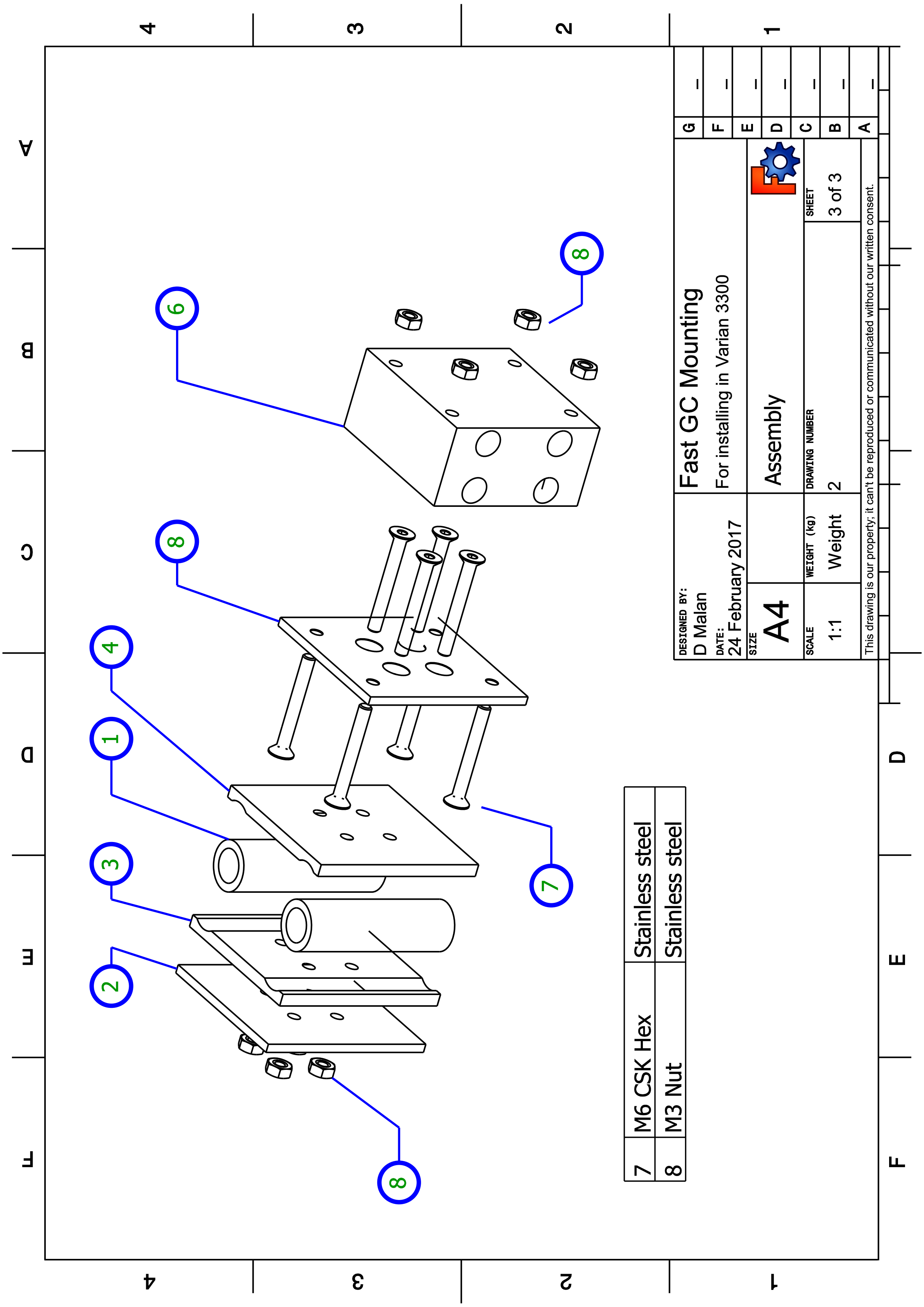


FIGURE D.5: (continued) A technical drawing of the T-piece block mounting, showing assembly method.

E
Fatty acid profiles

?<AppendixE>?

TABLE E.1: The fatty acid profile of sunflower oil sample

SunflowerPrecisionOils⟩

Fatty acid	Fraction
C14:0	0.05
C16:0	6.14
C16:1	0.04
C17:0	0.05
C17:1	0
C18:0	5.77
C18:1 t	0
C18:1 c	21.21
C18:2 t	0
C18:2 c	63.91
C18:3n6	0.11
C18:3n3	1.04
C20:0	0.47
C20:1	0.24
C20:2	0
C21:0	0
C22:0	0.77
C22:1	0
C24:0	0.19
C24:1	0

TABLE E.2: Fatty acid profile of coconut oil according to the FAO (Joint FAO/WHO Codex Alimentarius Commission 2019)

?⟨tab:CoconutFAO⟩?

Fatty acid	Lower	(Upper)
C6:0	ND	0.7
C8:0	4.6	10
C10:0	5	8
C12:0	45.1	53.2
C14:0	16.8	21
C16:0	7.5	10.2
C16:1	ND	
C17:0	ND	
C17:1	ND	
C18:0	2	4
C18:1	5	10
C18:2	1	2.5
C18:3	ND	0.2
C20:0	ND	0.2
C20:1	ND	0.2
C20:2	ND	
C22:0	ND	
C22:1	ND	
C22:2	ND	
C24:0	ND	
C24:1	ND	

Bibliography

Abebe, Mimi (Oct. 2008). "History of Ethanol". In: *Journalism & Mass Communications: Student Media*.

Agence France-Presse (Sept. 17, 2018). "Germany launches world's first hydrogen-powered train". In: *The Guardian*. URL: https://www.theguardian.com/environment/2018/sep/17/germany-launches-worlds-first-hydrogen-powered-train.

Agilent Technologies (2019). *Agilent 7890B gas chromatography data sheet*. URL: https://www.agilent.com/cs/library/datasheets/public/5991-1436EN.pdf.

Allen, Myles et al. (Nov. 2014). *IPCC fifth assessment synthesis report - Climate Change 2014 synthesis report*. Intergovernmental Panel on Climate Change (IPCC), pp. –116. URL: http://www.ipcc.ch/pdf/assessment-report/ar5/syr/SYR_AR5_LONGERREPORT.pdf.

Anastas, Paul T. and Julie B. Zimmerman (July 2016). "The Molecular Basis of Sustainability". In: *Chem* 1.1, pp. 10–12. DOI: 10.1016/j.chempr.2016.06.016.

Angelis, Vanda De et al. (June 2007). "Multiperiod integrated routing and scheduling of World Food Programme cargo planes in Angola". In: *Computers & Operations Research* 34.6, pp. 1601–1615. DOI: 10.1016/j.cor.2005.07.012.

AOCS, ed. (2017). *AOCS Official Method Ce 2-66*.

Arrhenius, S. (1897). "On the Influence of Carbonic Acid in the Air upon the Temperature of the Earth". In: *Publications of the Astronomical Society of the Pacific* 9.54, p. 14. URL: http://stacks.iop.org/1538-3873/9/i=54/a=14.

ASTM (2019). *Standard Test Method for Determination of the Aromatic Content and Polynuclear Aromatic Content of Diesel Fuels By Supercritical Fluid Chromatography1*. Tech. rep. ASTM International.

Atadashi, I. M. et al. (Dec. 2011). "Refining technologies for the purification of crude biodiesel". In: *Applied Energy* 88.12, pp. 4239–4251. DOI: 10.1016/j.apenergy.2011.05.029.

Bainbridge, Lisanne (Nov. 1983). "Ironies of automation". In: *Automatica* 19.6, pp. 775–779. DOI: 10.1016/0005-1098(83)90046-8.

Ball, William T. et al. (Feb. 2018). "Evidence for a continuous decline in lower stratospheric ozone offsetting ozone layer recovery". In: *Atmospheric Chemistry and Physics* 18.2, pp. 1379–1394. DOI: 10.5194/acp-18-1379-2018.

Barnett, James A. (2000). "A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850-1880". In: *Yeast* 16.8, pp. 755–771. DOI: 10.1002/1097-0061(20000615)16:8<755::aid-yea587>3.0.co;2-4.

Berche, Bertrand, Malte Henkel, and Ralph Kenna (June 2009). "Fenômenos críticos: 150 anos desde Cagniard de la Tour". In: *Revista Brasileira de Ensino de Física* 31.2, pp. 26021–26024. DOI: 10.1590/s1806-11172009000200015.

Berdick, Murray (July 1972). "The role of fats and oils in cosmetics". In: *Journal of the American Oil Chemists' Society* 49.7, pp. 406–408. DOI: 10.1007/bf02582522.

Blumberg, L. M. and M. S. Klee (2000). "Optimal Heating Rate in Gas Chromatography". In: *Journal of Microcolumn Separations* 12.9, pp. 508–514.

Blumberg, Leonid M. (Nov. 1997). “Theory of fast capillary gas chromatography. Part 1: Column efficiency”. In: *Journal of High Resolution Chromatography* 20.11, pp. 597–604. DOI: 10.1002/jhrc.1240201106.

Braun, Peter et al. (May 2018). “Potential Technical Approaches for Improving Low-Temperature NOx Conversion of Exhaust Aftertreatment Systems”. In: *Chemie Ingenieur Technik* 90.6, pp. 762–773. DOI: 10.1002/cite.201700122.

Investigation of scaling laws for combustion engine performance. (Oct. 5, 2015). Fall Technical Meeting of the Western States Section of the Combustion Institute (WSS/CI 2015 Fall Meeting) (Provo, Utah, USA). URL: http://sites01.lsu.edu/faculty/smenon/wp-content/uploads/sites/133/2017/02/WSSCI_Provo_v5.pdf (visited on 01/18/2019).

BSI (2016). *A standard for standards – Principles of standardization*. BSI Standards Limited. ISBN: 978-0-580-94429-1. URL: https://www.bsigroup.com/Documents/standards/guide-to-standards/BSI-BS-0-2016.pdf.

Burke-Kennedy, Eoin (Feb. 16, 2018). “Ireland 2040: €22bn to turn State into low-carbon economy”. In: *The Irish Times*.

Cardona, C.A. and O.J. Sánchez (2007). “Fuel ethanol production: Process design trends and integration opportunities”. English. In: *Bioresource technology* 98.12. Cited By :644, pp. 2415–2457. URL: www.scopus.com.

Carson, Rachel (Oct. 23, 2003). *Silent Spring*. Houghton Mifflin. 400 pp. ISBN: 9780618249060. URL: https://www.ebook.de/de/product/1865596/rachel_carson_silent_spring.html.

CEN (2008). *Automotive fuels – Fatty acid methyl esters (FAME) for diesel engines – Requirements and test methods*. Tech. rep. European Committee for Standardization.

Chiaramonti, David et al. (Dec. 2014). “Sustainable bio kerosene: Process routes and industrial demonstration activities in aviation biofuels”. In: *Applied Energy* 136, pp. 767–774. DOI: 10.1016/j.apenergy.2014.08.065.

Clarke, Coby J. et al. (Jan. 2018). “Green and Sustainable Solvents in Chemical Processes”. In: *Chemical Reviews* 118.2, pp. 747–800. DOI: 10.1021/acs.chemrev.7b00571.

Coffee Market Report December 2018 (2018). Tech. rep. International Coffee Organzation.

Cummins Jr., C. Lyle (1989). *Internal Fire*. Society of Automotive Engineers. ISBN: 978-0898837650. URL: https://www.amazon.com/Internal-Fire-Lyle-Jr-Cummins/dp/0898837650?SubscriptionId=AKIAIOBINVZYXZQZ2U3A&tag=chimbori05-20&linkCode=xm2&camp=2025&creative=165953&creativeASIN=0898837650.

Damerow, Peter (2012). “Sumerian Beer: The Origins of Brewing Technology in Ancient Mesopotamia”. In: *Cuneiform Digital Library Journal* 2012:002. ISSN: 1540-8760. URL: http://www.cdli.ucla.edu/pubs/cdlj/2012/cdlj2012_002.html.

Davidson, Arthur (1998). “Photochemical oxidant air pollution: A historical perspective”. In: *Air pollution in the 21st century - Priority issues and policy*. Elsevier, pp. 393–405. DOI: 10.1016/s0166-1116(98)80024-1.

Day, G. M. et al. (Mar. 2003). “Thermally Stable Siloxane Polymers for Gas Chromatography”. In: *ACS Symposium Series*. American Chemical Society, pp. 127–136. DOI: 10.1021/bk-2003-0838.ch012.

Desty, D. H. and B. H. F. Whyman (Mar. 1957). “Application of Gas-Liquid Chromatography to Analysis of Liquid Petroleum Fractions”. In: *Analytical Chemistry* 29.3, pp. 320–329. DOI: 10.1021/ac60123a001.

Diamond, Jared M. (2006). *Collapse : how societies choose to fail or succeed*. English. New York: Penguin.

Doucette, Reed T. and Malcolm D. McCulloch (Feb. 2011). "Modeling the CO2 emissions from battery electric vehicles given the power generation mixes of different countries". In: *Energy Policy* 39.2, pp. 803–811. DOI: 10.1016/j.enpol.2010.10.054.

Duncan, Bryan N. et al. (Jan. 2016). "A space-based, high-resolution view of notable changes in urban NOx pollution around the world (2005-2014)". In: *Journal of Geophysical Research: Atmospheres* 121.2, pp. 976–996. DOI: 10.1002/2015jd024121.

Dunn, Robert O. (Dec. 2009). "Effects of minor constituents on cold flow properties and performance of biodiesel". In: *Progress in Energy and Combustion Science* 35.6, pp. 481–489. DOI: 10.1016/j.pecs.2009.07.002.

Dyos, G. (2012). "Handbook of Electrical Resistivity - New Materials and Pressure Effects - 2.1 Introduction". In: *Handbook of Electrical Resistivity - New Materials and Pressure Effects*. Institution of Engineering and Technology, p. 11. ISBN: 978-1-84919-149-4. URL: https://app.knovel.com/hotlink/khtml/id:kt00B0HS35/handbook-electrical-resistivity/measuremen-introduction.

Eggertsen, F. T., H. S. Knight, and Sigurd. Groennings (Mar. 1956). "Gas Chromatography Use of Liquid-Modified Solid Adsorbent to Resolve C5and C6Saturates". In: *Analytical Chemistry* 28.3, pp. 303–306. DOI: 10.1021/ac60111a004.

Eisenmenger, Michael et al. (Oct. 2006). "Pilot-scale supercritical carbon dioxide extraction and fractionation of wheat germ oil". In: *Journal of the American Oil Chemists' Society* 83.10, pp. 863–868. ISSN: 1558-9331. DOI: 10.1007/s11746-006-5038-6. URL: https://doi.org/10.1007/s11746-006-5038-6.

Ettre, L. S. (June 2008). "The Invention, Development, and Triumph of the Flame-Ionization Detector". In: *Chapters in the Evolution of Chromatography*. PUBLISHED BY IMPERIAL COLLEGE PRESS AND DISTRIBUTED BY WORLD SCIENTIFIC PUBLISHING CO., pp. 303–320. DOI: 10.1142/9781860949449_0023.

Ettre, L. S. and K. I. Sakodynskii (Feb. 1993a). "M. S. Tswett and the discovery of chromatography I: Early work (1899–1903)". In: *Chromatographia* 35.3-4, pp. 223–231. DOI: 10.1007/bf02269707.

— (Mar. 1993b). "M. S. Tswett and the discovery of chromatography II: Completion of the development of chromatography (1903–1910)". In: *Chromatographia* 35.5-6, pp. 329–338. DOI: 10.1007/bf02277520.

Flick, Carlos (1980). "The Movement for Smoke Abatement in 19th-Century Britain". In: *Technology and Culture* 21.1, pp. 29–50. ISSN: 0040-165X. DOI: 10.2307/3103986. URL: http://www.jstor.org/stable/3103986 (visited on 01/15/2019).

Frauhammer, Jörg, Alexander Schenck zu Schweinsberg, and Klaus Winkler (July 2014). "Catalytic emission control". In: *Gasoline Engine Management*. Springer Fachmedien Wiesbaden, pp. 268–283. DOI: 10.1007/978-3-658-03964-6_18.

Gabbatiss, Josh (Oct. 19, 2018). "Ban new petrol and diesel cars by 2032 instead of current 'unambitious' target, MPs say". In: *The Independent*.

Gertler, Alan W. et al. (Nov. 1999). "The Impact of California Phase 2 Reformulated Gasoline on Real-World Vehicle Emissions". In: *Journal of the Air & Waste Management Association* 49.11, pp. 1339–1346. DOI: 10.1080/10473289.1999.10463965.

Gibson, Jennifer et al. (Jan. 2016). "Levels and trends of contaminants in humans of the Arctic". In: *International Journal of Circumpolar Health* 75.1, p. 33804. DOI: 10.3402/ijch.v75.33804.

Giddings, J. C. (May 1987). "Concepts and comparisons in multidimensional separation". In: *Journal of High Resolution Chromatography* 10.5, pp. 319–323. DOI: 10.1002/jhrc.1240100517.

Grażyna, Wejnerowska and Ciaciuch Anna (Feb. 2018). "Optimisation of oil extraction from quinoa seeds with supercritical carbon dioxide with co-solvents". In: *Czech Journal of Food Sciences* 36.No. 1, pp. 81–87. DOI: 10.17221/122/2017-cjfs.

Greenberg, Jon (May 17, 2017). *No, coffee is not the second-most traded commodity after oil*. URL: https://www.politifact.com/global-news/statements/2017/may/08/starbucks/no-coffee-not-second-most-traded-commodity-after-o/ (visited on 01/31/2019).

Griffin, John Joseph (Jan. 1, 1838). *Chemical Recreations: A Compendium of Experimental Chemistry, Part 1*. R. Griffin and Company.

Gritti, Fabrice and Georges Guiochon (Aug. 2006). "General HETP Equation for the Study of Mass-Transfer Mechanisms in RPLC". In: *Analytical Chemistry* 78.15, pp. 5329–5347. DOI: 10.1021/ac060203r.

Gumm, Brian (Oct. 22, 2015). *Blowing Smoke: Chemical Companies Say 'Trust Us,' But Environmental and Workplace Safety Violations Belie Their Rhetoric*. Tech. rep. Center for Effective Government.

Guthrie, E. J. and H. E. Schwartz (1986). "Integral Pressure Restrictor for Capillary SFC". In: *Journal of Chromatographic Science* 24.6, pp. 236–241. ISSN: 0021-9665. URL: https://UnivofPretoria.on.worldcat.org/oclc/5127807429.

Halliday, Stephen (2001). "Death and miasma in Victorian London: an obstinate belief". In: *BMJ: British Medical Journal* 323.7327, pp. 1469–1471. ISSN: 0959-8138. DOI: 10.1136/bmj.323.7327.1469. eprint: https://www.bmj.com/content/323/7327/1469.full.pdf. URL: https://www.bmj.com/content/323/7327/1469.

Hanmer, Clare and Simone Abram (Dec. 2017). "Actors, networks, and translation hubs: Gas central heating as a rapid socio-technical transition in the United Kingdom". In: *Energy Research & Social Science* 34, pp. 176–183. DOI: 10.1016/j.erss.2017.03.017.

Harris, Daniel C. (Oct. 2010). "Charles David Keeling and the Story of Atmospheric CO2 Measurements". In: *Analytical Chemistry* 82.19, pp. 7865–7870. ISSN: 0003-2700. DOI: 10.1021/ac1001492. URL: https://doi.org/10.1021/ac1001492.

Hatto, Peter (2010). *Standards and Standardization Handbook*. European Commission Directorate-General for Research Industrial Technologies.

Hayes, John G. et al. (Sept. 2011). "Simplified electric vehicle power train models and range estimation". In: *2011 IEEE Vehicle Power and Propulsion Conference*. IEEE. DOI: 10.1109/vppc.2011.6043163.

Herrero, Miguel et al. (Apr. 2010). "Supercritical fluid extraction: Recent advances and applications". In: *Journal of Chromatography A* 1217.16, pp. 2495–2511. DOI: 10.1016/j.chroma.2009.12.019.

Hoffmann, Karl Heinz (Feb. 2008). "An introduction to endoreversible thermodynamics". In: *Atti della Accademia Peloritana dei Pericolanti - Classe di Scienze Fisiche, Matematiche e Naturali* 1, pp. 1–18. ISSN: 1825-1242. DOI: 10.1478/C1S0801011.

Holm, Torkil and Jørgen Øgaard Madsen (Jan. 1996). "Methane Formation by Flame-Generated Hydrogen Atoms in the Flame Ionization Detector". In: *Analytical Chemistry* 68.20, pp. 3607–3611. DOI: 10.1021/ac960556y.

Hunt, Andrew J. et al. (Jan. 2010). "Generation, Capture, and Utilization of Industrial Carbon Dioxide". In: *ChemSusChem* 3.3, pp. 306–322. DOI: 10.1002/cssc.200900169.

International vocabulary of metrology – Basic and general concepts and associated terms (VIM) (2019). 3rd ed. Joint Committee for Guides in Metrology, International Bureau of Weights and Measures. URL: https://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2012.pdf (visited on 07/06/2019).

IPCC (2014). "2014: Summary for policymakers". In: *Climate Change 2014: Impacts,Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Ed. by C. B. Field et al. IPCC. URL: https://www.ipcc.ch/site/assets/uploads/2018/02/ar5_wgII_spm_en.pdf.

ISO (2015). *BS EN ISO 18134-3:2015 Solid biofuels. Determination of moisture content. Oven dry method. Moisture in general analysis sample*.

— (2017). *ISO/IEC 17025:2017: GENERAL REQUIREMENTS FOR THE COMPETENCE OF TESTING AND CALIBRATION LABORATORIES*.

— (2019). *ISO 14708-2: IMPLANTS FOR SURGERY – ACTIVE IMPLANTABLE MEDICAL DEVICES – PART 2: CARDIAC PACEMAKERS*.

Jacobs, Matthew R., Emily F. Hilder, and Robert A. Shellie (Nov. 2013). "Applications of resistive heating in gas chromatography: A review". In: *Analytica Chimica Acta* 803, pp. 2–14. DOI: 10.1016/j.aca.2013.04.063.

Jacobsen, M. and G. Bertelsen (June 2002). "The use of CO2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: a review". In: *Journal of Muscle Foods* 13.2, pp. 143–168. DOI: 10.1111/j.1745-4573.2002.tb00326.x.

Joint FAO/WHO Codex Alimentarius Commission (2019). *Codex Alimentarius: STANDARD FOR NAMED VEGETABLE OILS CXS 210-1999*. World Health Organization : Food and Agriculture Organization of the United Nations. ISBN: 92-5-104682-4. URL: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B210-1999%252FCXS_210e.pdf (visited on 09/24/2019).

Keulemans, A. I. M., A. Kwantes, and P. Zaal (1955). "The selectivity of the stationary liquid in vapour phase chromatography". In: *Analytica Chimica Acta* 13, pp. 357–372. DOI: 10.1016/s0003-2670(00)87957-x.

Kimpe, K., P. A. Jacobs, and M. Waelkens (Dec. 2001). "Analysis of oil used in late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat". In: *Journal of Chromatography A* 937.1-2, pp. 87–95. DOI: 10.1016/s0021-9673(01)01304-8.

Kitessa, Soressa et al. (May 2014). "DHA-Containing Oilseed: A Timely Solution for the Sustainability Issues Surrounding Fish Oil Sources of the Health-Benefitting Long-Chain Omega-3 Oils". In: *Nutrients* 6.5, pp. 2035–2058. DOI: 10.3390/nu6052035.

Klee, M. S. and L. M. Blumberg (May 2002). "Theoretical and Practical Aspects of Fast Gas Chromatography and Method Translation". In: *Journal of Chromatographic Science* 40.5, pp. 234–247. DOI: 10.1093/chromsci/40.5.234.

Knothe, Gerhard (Sept. 2002). "Structure indices in FA chemistry. How relevant is the iodine value?" In: *Journal of the American Oil Chemists' Society* 79.9, pp. 847–854. DOI: 10.1007/s11746-002-0569-4.

— (Feb. 2007). "Arthur von Hübl and the iodine value". English. In: *INFORM* 18.2. Copyright - Copyright AOCS Press Feb 2007; Document feature - Photographs; Equations; Last updated - 2010-07-17, pp. 136–138. URL: https://search-proquest-com.uplib.idm.oclc.org/docview/223601381?accountid=14717.

— (2010). "History of Vegetable Oil-Based Diesel Fuels". In: *The Biodiesel Handbook*. Elsevier, pp. 5–19. DOI: 10.1016/b978-1-893997-62-2.50007-3.

Koenig, David M. (Jan. 11, 2009). *Practical Control Engineering: A Guide for Engineers, Managers, and Practitioners*. MCGRAW HILL BOOK CO. 474 pp. ISBN: 0071606130. URL: https://www.ebook.de/de/product/7882280/david_m_koenig_practical_ control_engineering_a_guide_for_engineers_managers_and_practitioners.html.

Kornblut, Phil (Mar. 1, 2019). “Helium Shortage 3.0”. In: *Gasworld*.

Kumar, Santosh, Neetu Singh, and Ram Prasad (Sept. 2010). “Anhydrous ethanol: A renewable source of energy”. In: *Renewable and Sustainable Energy Reviews* 14.7, pp. 1830–1844. DOI: 10.1016/j.rser.2010.03.015.

Kyoto Protocol to the United Nations Framework Convention on Climate Change. (Dec. 11, 1997). URL: https://unfccc.int/resource/docs/convkp/kpeng.pdf (visited on 01/25/2019).

Lauer, Henk H., Douglass. McManigill, and Robert D. Board (July 1983). “Mobile-phase transport properties of liquefied gases in near critical and supercritical fluid chromatography”. In: *Analytical Chemistry* 55.8, pp. 1370–1375. DOI: 10.1021/ac00259a041.

Li, Harry and Radu Curiac (Jan. 2012). “Energy Conservation: Motor Efficiency, Efficiency Tolerances, and the Factors That Influence Them”. In: *IEEE Industry Applications Magazine* 18.1, pp. 62–68. DOI: 10.1109/mias.2011.943105.

Linseed (1911). In: *Encyclopædia Britannica*. 11th ed. Cambridge University Press, pp. 734–735.

Linthorst, J. A. (Apr. 2010). “An overview: origins and development of green chemistry”. In: *Foundations of Chemistry* 12.1, pp. 55–68. ISSN: 1572-8463. DOI: 10.1007/s10698-009-9079-4. URL: https://doi.org/10.1007/s10698-009-9079-4.

Luque de Castro, Maria D., Miguel Valcárcel, and Maria T. Tena (1994). *Analytical Supercritical Fluid Extraction*. Springer. ISBN: 3-540-57495-6. URL: https://www.amazon.com/Analytical-Supercritical-Fluid-Extraction-Lecture/dp/3540574956?SubscriptionId=AKIAIOBINVZYXZQZ2U3A&tag=chimbori05-20&linkCode=xm2&camp=2025&creative=165953&creativeASIN=3540574956.

Mansouri, Nazanin (2016). “A Case Study of Volkswagen Unethical Practice in Diesel Emission Test”. In: *International Journal of Science and Engineering Applications* 5.4, pp. 211–216. URL: https://UnivofPretoria.on.worldcat.org/oclc/6158205082.

Marriott, Philip J., Peter Schoenmakers, and Ze-ying Wu (2012). “Nomenclature and conventions in comprehensive multidimensional chromatograpy — an update”. In: *LC-GC Europe*.

McCurry, James D. and Wesley Norman (May 2009). “5-in-1 biodiesel: an approach to combining five biodiesel gas chromatographic methods on a single instrument”. In: *Biofuels, Bioproducts and Biorefining* 3.3, pp. 296–298. DOI: 10.1002/bbb.155.

McGee, Thomas D. (1988). *Principles and methods of temperature measurement.* Wiley-Interscience.

McNair, Harold M., James M. Miller, and Nicholas H. Snow (Sept. 11, 2019). *Basic Gas Chromatography*. WILEY. 284 pp. ISBN: 1119450756. URL: https://www.ebook.de/de/product/36215945/harold_m_mcnair_james_m_miller_nicholas_h_snow_basic_gas_chromatography.html.

Mekonnen, Mesfin M. et al. (2018). “Water, Energy, and Carbon Footprints of Bioethanol from the U.S. and Brazil”. In: *Environmental Science & Technology* 52.24, pp. 14508–14518. DOI: 10.1021/acs.est.8b03359.

Ministere de la santé (2018). *Strategic response plan for the ebola virus disease outbreak. Democratic Republic of Congo*. World Health Organization.

Miranda, Jesse Alberto Contreras (2010). “Axial Temperature Gradients in Gas Chromatography”. PhD thesis. Brigham Young University.

Modanlou, H. D. (2008). "A tribute to Zakariya Razi (865 - 925 AD), an Iranian pioneer scholar." In: *Archives of Iranian medicine* 11.6, pp. 673–7. ISSN: 1029-2977. URL: https://UnivofPretoria.on.worldcat.org/oclc/269410950.

Model Bakery and Brewery from the Tomb of Meketre (1920). Metropolitan Museum of Art The Met Fifth Avenue 1000 Fifth Avenue New York, NY 10028: The Metropolitan Museum of Art. URL: https://www.metmuseum.org/art/collection/search/544258 (visited on 01/14/2019).

Mohankumar, S. and P. Senthilkumar (2017). "Particulate matter formation and its control methodologies for diesel engine: A comprehensive review". In: *Renewable and Sustainable Energy Reviews* 80, pp. 1227–1238. DOI: 10.1016/j.rser.2017.05.133.

Monolithic Thermocouple Amplifiers with Cold Junction Compensation (1999). AD594/AD595. Rev. C. Analog Devices.

Morris, Hugh (Aug. 16, 2017). "How many planes are there in the world right now?" In: *The Telegraph*. URL: https://www.telegraph.co.uk/travel/travel-truths/how-many-planes-are-there-in-the-world/# (visited on 12/18/2018).

Morris, L. J. (1966). "Separations of lipids by silver ion chromatography". In: *Journal of Lipid Research* 7.6, pp. 717–732. eprint: http://www.jlr.org/content/7/6/717.full.pdf+html. URL: http://www.jlr.org/content/7/6/717.abstract.

Needleman, Herbert L. (Sept. 2000). "The Removal of Lead from Gasoline: Historical and Personal Reflections". In: *Environmental Research* 84.1, pp. 20–35. DOI: 10.1006/enrs.2000.4069.

Nič, Miloslav et al., eds. (June 2009). *IUPAC Compendium of Chemical Terminology*. IUPAC. DOI: 10.1351/goldbook.

Nikolova-Damyanova, Boryana (Sept. 10, 2019). *Multiple Interactions in Silver Ion Chromatography of Lipids*. URL: https://lipidlibrary.aocs.org/lipid-analysis/silver-ion-chromatography-of-lipids/multiple-interactions-in-silver-ion-chromatography-of-lipids.

Paris Agreement (Dec. 12, 2015). UNTC XXVII 7.d. URL: https://treaties.un.org/doc/Publication/MTDSG/Volume%20II/Chapter%20XXVII/XXVII-7-d.en.pdf (visited on 01/25/2019).

Peacock, Finn (2008). *PID Tuning Blueprint*. URL: http://www.pidtuning.net.

Pearson, Andy (Dec. 2005). "Carbon dioxide—new uses for an old refrigerant". In: *International Journal of Refrigeration* 28.8, pp. 1140–1148. DOI: 10.1016/j.ijrefrig.2005.09.005.

Petit, J. R. et al. (June 1999). "Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica". English. In: *Nature* 399.6735. Copyright - Copyright Macmillan Journals Ltd. Jun 3, 1999; Last updated - 2017-10-31; CODEN - NATUAS; SubjectsTermNotLitGenreText - Antarctica, pp. 429–436. URL: https://search-proquest-com.uplib.idm.oclc.org/docview/204481126?accountid=14717.

Pischinger, Rudolf, Stefan Schlag, and Martin Mittelbach (2000). *Chemische und motortechnische Untersuchungen der Ursachen der Einspritzpumpenverklebung bei Biodiesel. Endbericht zum Forschungsauftrag*. Research rep. URL: http://www.abfallwirtschaft.steiermark.at/cms/beitrag/10211037/135047763/ (visited on 08/06/2019).

Plata, Vladimir, Paola Gauthier-Maradei, and Viatcheslav Kafarov (Mar. 2015). "Influence of minor components on precipitate formation and filterability of palm oil biodiesel". In: *Fuel* 144, pp. 130–136. DOI: 10.1016/j.fuel.2014.12.043.

Poole, Colin F. (2003). "Supercritical Fluid Chromatography". In: *The Essence of Chromatography*. Elsevier, pp. 569–617. DOI: 10.1016/b978-044450198-1/50020-3.

Potgieter, H. et al. (June 2013). "Hyphenation of supercritical fluid chromatography and two-dimensional gas chromatography–mass spectrometry for group type separations". In: *Journal of Chromatography A* 1294, pp. 137–144. DOI: 10.1016/j.chroma.2013.04.020.

Prebihalo, Sarah E. et al. (Jan. 2018). "Multidimensional Gas Chromatography: Advances in Instrumentation, Chemometrics, and Applications". In: *Analytical Chemistry* 90.1, pp. 505–532. ISSN: 0003-2700. DOI: 10.1021/acs.analchem.7b04226. URL: https://doi.org/10.1021/acs.analchem.7b04226.

Ragonese, Carla et al. (Dec. 2009). "Conventional and fast gas chromatography analysis of biodiesel blends using an ionic liquid stationary phase". In: *Journal of Chromatography A* 1216.51, pp. 8992–8997. DOI: 10.1016/j.chroma.2009.10.066.

Ramalakshmi, K. and B. Raghavan (July 1999). "Caffeine in Coffee: Its Removal. Why and How?" In: *Critical Reviews in Food Science and Nutrition* 39.5, pp. 441–456. DOI: 10.1080/10408699991279231.

Randall, L. G. (1982). "The Present Status of Dense (Supercritical) Gas Extraction and Dense Gas Chromatography: Impetus for DGC/MS Development". In: *Separation Science and Technology* 17.1, pp. 1–118. ISSN: 0149-6395. URL: https://UnivofPretoria.on.worldcat.org/oclc/4661041191.

Rao, K. Bhanu Sankara and B. Raj (2001). "Fatigue Testing: Thermal and Thermomechanical". In: *Encyclopedia of Materials: Science and Technology*. Elsevier, pp. 2999–3001. DOI: 10.1016/b0-08-043152-6/00534-9.

Raveendran, Poovathinthodiyil, Yutaka Ikushima, and Scott L. Wallen (June 2005). "Polar Attributes of Supercritical Carbon Dioxide". In: *Accounts of Chemical Research* 38.6, pp. 478–485. DOI: 10.1021/ar040082m.

Reed, Gail L. (Sept. 15, 1999). "Fast GC: applications and theoretical studies". PhD thesis. Virginia Polytechnic.

Reif, Konrad, ed. (2015). *Gasoline Engine Management*. Springer Fachmedien Wiesbaden. DOI: 10.1007/978-3-658-03964-6.

Reumuth, Georg et al. (Aug. 2018). "Carbon monoxide intoxication: What we know". In: *Burns*. DOI: 10.1016/j.burns.2018.07.006.

Reuters (Nov. 13, 2018). "Spain to propose ban on sale of petrol and diesel cars from 2040". In: *Sunday Times*.

Ripple, D. C. (1995). *ITS-90 Thermocouple Database, NIST Standard Reference Database 60*. eng. DOI: 10.18434/t4s888.

Robertson, Alan M. et al. (1991). "Research and development topics in Analytical Chemistry". In: *Analytical Proceedings* 28.1, p. 8. DOI: 10.1039/ap9912800008.

Rosen, William (Mar. 11, 2012). *The Most Powerful Idea in the World: A Story of Steam, Industry, and Invention*. UNIV OF CHICAGO PR. 370 pp. ISBN: 0226726347. URL: https://www.ebook.de/de/product/16779670/william_rosen_the_most_powerful_idea_in_the_world_a_story_of_steam_industry_and_invention.html.

SABS (1993). *CKS 440: Coffins and caskets.*

— (2008). *Automotive biodiesel — Fatty Acid Methyl Esters (FAME) for diesel engines — Requirements and test methods.* Standard. South African Bureau of Standards.

Sandra, P. (Feb. 1989). "Resolution-definition and nomenclature". In: *Journal of High Resolution Chromatography* 12.2, pp. 82–86. DOI: 10.1002/jhrc.1240120204.

Sandra, P. et al. (Oct. 2002). "Characterization of triglycerides in vegetable oils by silver-ion packed-column supercritical fluid chromatography coupled to mass spectroscopy with atmospheric pressure chemical ionization and coordination ion spray". In: *Journal of Chromatography A* 974.1-2, pp. 231–241. DOI: 10.1016/s0021-9673(02)01311-0.

Schönberger, C. and T. Kostelecky (2011). "125th Anniversary Review: The Role of Hops in Brewing". In: *Journal of the Institute of Brewing* 117.3, pp. 259–267. DOI: 10.1002/j.2050-0416.2011.tb00471.x.

Seeley, John V. and Stacy K. Seeley (Jan. 2013). "Multidimensional Gas Chromatography: Fundamental Advances and New Applications". In: *Anal. Chem.* 85.2, pp. 557–578. ISSN: 0003-2700. DOI: 10.1021/ac303195u. URL: https://doi.org/10.1021/ac303195u.

Shabecoff, Philip (June 24, 1988). "Global Warming Has Begun, Expert Tells Senate". In: *New York Times*, p. 1. URL: https://nyti.ms/2GvL7QI.

Shamsul, N. S. et al. (May 2014). "An overview on the production of bio-methanol as potential renewable energy". In: *Renewable and Sustainable Energy Reviews* 33, pp. 578–588. DOI: 10.1016/j.rser.2014.02.024.

Shellie, Robert A., Li-Ling Xie, and Philip J. Marriott (Aug. 2002). "Retention time reproducibility in comprehensive two-dimensional gas chromatography using cryogenic modulation". In: *Journal of Chromatography A* 968.1-2, pp. 161–170. DOI: 10.1016/s0021-9673(02)00961-5.

Skoog, Douglas A., F. James Holler, and Stanley R. Crouch (2007). *Principles of instrumental analysis*. Sixth edition. Belmont, CA: Thomson Brooks/Cole. URL: https://UnivofPretoria.on.worldcat.org/oclc/77224390.

Smeets, Edward et al. (Aug. 1, 2006). *Sustainability of Brazilian bio-ethanol*. Research rep. Universiteit Utrecht, Copernicus Institute, Department of Science, Technology and Society.

Smith, David Abbott (Sept. 3, 2002). "Surface modification of solid supports through the thermal decomposition and functionalization of silanes." US 6,444,326 B1. URL: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=6,444,326.PN.&OS=PN/6,444,326&RS=PN/6,444,326.

Smith, Roger M. and Simon Cocks (1994). "Separation of saturated and unsaturated fatty acid methyl esters by supercritical fluid chromatography on a silica column". In: *The Analyst* 119.5, p. 921. DOI: 10.1039/an9941900921.

Smith, Roger M., Minna Hyytiänen, et al. (2001). "High resolution packed column supercritical fluid chromatography of fatty acid methyl esters". In: *Journal of Separation Science* 24, pp. 208–212. DOI: https://doi.org/10.1002/1615-9314(20010301)24:3%3C208::AID-JSSC208%3E3.0.CO;2-P. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/1615-9314%2820010301%2924%3A3%3C208%3A%3AAID-JSSC208%3E3.0.CO%3B2-P (visited on 08/17/2018).

South Africa's Intended Nationally Determined Contribution (INDC) (Nov. 1, 2016). English. UNFCCC. URL: https://www4.unfccc.int/sites/ndcstaging/PublishedDocuments/South%20Africa%20First/South%20Africa.pdf (visited on 12/08/2018).

South African Government (1977). *Petroleum Products Act, 1977 (Act No. 120 of 1977)*. URL: https://www.gov.za/sites/default/files/gcis_document/201409/a8-2008.pdf.

— (2008). *Standards Act 2008*. URL: https://www.gov.za/sites/default/files/gcis_document/201409/a8-2008.pdf.

Spur, G., E. Uhlmann, and F. Elbing (Dec. 1999). "Dry-ice blasting for cleaning: process, optimization and application". In: *Wear* 233-235, pp. 402–411. DOI: 10.1016/s0043-1648(99)00204-5.

Strauch, Barry (Oct. 2018). "Ironies of Automation: Still Unresolved After All These Years". In: *IEEE Transactions on Human-Machine Systems* 48.5, pp. 419–433. DOI: 10.1109/thms.2017.2732506.

Szreter, Simon (Mar. 2003). "The population health approach in historical perspective". In: *American journal of public health* 93.12604486, pp. 421–431. ISSN: 1541-0048. URL: https://www.ncbi.nlm.nih.gov/pmc/PMC1449802/.

van der Walt, A.N. and F.J.C. Hugo (Jan. 1982). "Attempts to prevent injector coking with sunflower oil by engine modifications and fuel additives". In: *ASAE Publ.; (United States)*.

Van Niekerk, P. S. (1980). *Doenlikheidstudie ; die gebruik van sonneblomolie as plaasvervanger vir diesel in die R S A.* Die outeur. URL: https://books.google.co.za/books?id=CcbuoQEACAAJ.

Varma, Roli and Daya R. Varma (Feb. 2005). "The Bhopal Disaster of 1984". In: *Bulletin of Science, Technology & Society* 25.1, pp. 37–45. DOI: 10.1177/0270467604273822.

Velasco, J., C. Dobarganes, and G. Márquez-Ruiz (2010). "Oxidative rancidity in foods and food quality". In: *Chemical deterioration and physical instability of food and beverages*. Elsevier.

Venter, Andre (2003). "Comprehensive two-dimensional supercritical fluid and gas chromatography (SFC×GC)". PhD thesis. University of Pretoria.

Venter, Andre, Peter R. Makgwane, and Egmont R. Rohwer (Mar. 2006). "Group-type analysis of oxygenated compounds with a silica gel porous layer open tubular column and comprehensive two-dimensional supercritical fluid and gas chromatography." In: *Analytical chemistry* 78.6, pp. 2051–4. ISSN: 0003-2700. DOI: 10.1021/ac051693a. URL: http://www.ncbi.nlm.nih.gov/pubmed/16536445.

Venter, Andre and Egmont R. Rohwer (July 2004). "Comprehensive two-dimensional supercritical fluid and gas chromatography with independent fast programmed heating of the gas chromatographic column." In: *Analytical chemistry* 76.13, pp. 3699–706. ISSN: 0003-2700. DOI: 10.1021/ac035538c. URL: http://www.ncbi.nlm.nih.gov/pubmed/15228344.

Venter, Irma (Oct. 29, 2014). "Renewable energy project set to power BMW's Rosslyn plant". In: *Engineering News*.

Vincenti, Walter G. (1990). *What engineers know and how they know it : analytical studies from aeronautical history*. English. Baltimore: Johns Hopkins University Press. URL: http://catdir.loc.gov/catdir/description/jhu051/89049003.html.

Wang, Anzi, H. Dennis Tolley, and Milton L. Lee (2012). "Gas chromatography using resistive heating technology". In: *Journal of Chromatography A* 1261, pp. 46–57.

Watts, Steven (2005). *The People's Tycoon: Henry Ford and the American Century*. Knopf. ISBN: 978-0-375-40735-2. URL: https://www.worldcat.org/title/peoples-tycoon-henry-ford-and-the-american-century/oclc/829332863.

Wells, P. S., S. Zhou, and J. F. Parcher (Jan. 2003). "Unified Chromatography with CO2-Based Binary Mobile Phases". In: *Analytical Chemistry* 75.1, 18 A–24 A. DOI: 10.1021/ac031212s.

Wentz, Dennis A. et al. (2014). *Mercury in the nation's streams - Levels, trends, and implications. U.S. Geological Survey Circular 1395*. Tech. rep. DOI: 10.3133/cir1395. URL: http://dx.doi.org/10.3133/cir1395.

Williams, William Henry. (1965). *The Commons, Open Spaces & Footpaths Preservation Society, 1865-1965 : a short history of the society and its work.* English. [London]: [The Society]. ISBN: 978-0-375-40735-2.

Wolfram | Alpha (2019a). URL: https://www.wolframalpha.com/input/?i=otto+cycle (visited on 07/09/2019).

— (2019b). URL: https://www.wolframalpha.com/input/?i=diesel+cycle (visited on 07/09/2019).

— (2019c). URL: https://www.wolframalpha.com/input/?i=brayton+cycle (visited on 07/09/2019).

Woodall, L. C. et al. (Dec. 2014). "The deep sea is a major sink for microplastic debris". In: *Royal Society Open Science* 1.4, pp. 140317–140317. DOI: 10.1098/rsos.140317.

World Resource Institute (2004). *The Greenhouse Gas Protocol: A Corporate Accounting and Reporting Standard*. World Resources Inst. ISBN: 1-56973-568-9. URL: https://www.amazon.com/Greenhouse-Gas-Protocol-Corporate-Accounting/dp/1569735689?SubscriptionId=AKIAIOBINVZYXZQZ2U3A&tag=chimbori05-20&linkCode=xm2&camp=2025&creative=165953&creativeASIN=1569735689.

Worldwide Fuel Charter Committee (2009). *Biodiesel guidelines*. Worldwide Fuel Charter Committee. URL: http://oica.net/wp-content/uploads/biodiesel-guideline-final-26mar09.pdf.

Wright, J. (Sept. 2002). "Chronic and occult carbon monoxide poisoning: we don't know what we're missing". In: *Emergency Medicine Journal* 19.5, pp. 386–390. DOI: 10.1136/emj.19.5.386.

Young, I. S. and J. McEneny (May 2001). "Lipoprotein oxidation and atherosclerosis". In: *Biochemical Society Transactions* 29.2, pp. 358–362. DOI: 10.1042/bst0290358.