EVALUATION OF EUCALYPTUS CITRIODORA DERIVED P-MENTHANE-3,8-DIOL-CITRONELLAL ACETAL AS A BIO-PLASTICIZER FOR COSMETIC APPLICATIONS.

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DECLARATION

I, Kirstin Burger 207097783, hereby declare that the dissertation for MSc (Chemistry) is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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Abstract

Plasticizers are generally added to cosmetic and personal care products to improve the film-forming abilities of the product and increase flexibility of the film formed on the skin or hair surface. For example, plasticizers are present in perfumes to prolong the release of the specific scent, which is the ultimate goal in a good quality perfume. Plasticizers in nail varnishes prevent chipping, improve the aesthetics by adhering to the keratin in the nail which means the coating stays on for much longer, which is the ultimate goal in nail products. Plasticizers improve the gloss, resist chipping and allow quick drying time. Therefore it can be seen that plasticizers play a vital role in personal care products like perfumes and nail varnishes.

Certain plasticizers e.g. phthalates, can cause problems associated with human health and can harm the environment. They are easily available and large volumes can be obtained at a low cost. These phthalates, for example, di-butyl phthalate (DBP) have been identified as carcinogenic. Nowadays the occurrence of cancer is rapidly increasing. The plasticizers present in a large number of consumer and personal care products, can possibly be linked to the ever increasing reports of cancer. Therefore a substitute to the traditional phthalate plasticizers must be investigated.

The aim of this research is to produce a plasticizer derived from naturally occurring Eucalyptus oil, which can be used to replace the existing plasticizers in cosmetic formulations. Para-menthane-3,8-diol (PMD), occurring naturally in the oil from the tree, Eucalyptus citriodora, forms an acetal with citronellal (PMD, acetal, citronellal all occur naturally in the oil). It has been previously shown that PMD-citronellal acetal will exhibit plasticizing properties similar to conventional plasticizers. The objective was to enhance the formation of the acetal in the Eucalyptus oil by reacting it with excess PMD. An effective synthesis method for the PMD-citronellal acetal enriched oil (~73.8%) was determined from optimization experiments.

The physical characterisation of the PMD-citronellal acetal enriched oil was done and compared with that of DBP. The acetal-enriched oil had a lower density, slightly higher solubility in water (at 25°C), lower refractive index (Brix %) and a higher boiling point (350°C) than DBP. The physical characteristics of the Eucalyptus oil source and the acetal-enriched Eucalyptus oil were very similar. This can be expected as the Eucalyptus oil consists of ~84.3% Citronellal, ~ 1.3% PMD and 2.7% PMD-citronellal acetal.
In this study the effectiveness of the acetal-enriched Eucalyptus oil (referred to from now on as the bio-plasticizer) was compared to a conventional plasticizer such as di-butyl phthalate (DBP), commonly used in cosmetic products. Two cosmetic formulations were produced: a nail varnish and a perfume formulation. Various tests were performed on these formulations to investigate the plasticizing properties of the bio-plasticizer. The objectives were to determine if the natural plasticizer is as effective as the potentially carcinogenic phthalate plasticizers and can be used as a substitute for the phthalates in personal care products.

The results indicate that the bio-plasticizer does behave similarly to di-butyl phthalate, however, the effectiveness of the bio-plasticizer is lower than that of di-butyl phthalate.

As the viscosity of the synthesized oil was high, this affected the overall consistency of the products. A more viscous nail varnish and perfume was produced in comparison to the DBP counterpart.

The stability of the bio-plasticizer in the cosmetic formulations of nail varnish and perfume was also investigated. The cosmetic products were incubated at 0°C, 25°C and 40°C over a period of two months. Any changes in colour, odour, pH, refractive index, separation and plasticizer peak change in the gas chromatogram trace were recorded. It was determined that the PMD-citronellal acetal-enriched oil was relatively unstable under elevated temperatures and light intensity. Storage under higher temperatures (40°C) tends to increase the acidity. Therefore the bio-plasticizer must be placed in a closed, covered bottle and stored in an environment away from light and elevated temperatures. According to the gas chromatogram peaks, it was clear that both the bio-plasticizer and the DBP were more unstable in the perfume formulation than in the nail polish and were especially sensitive to light when in the perfume. This could possibly be due to the interaction with the fragrance molecule, p-anisaldehyde.
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PLA</td>
<td>Polyactide</td>
</tr>
<tr>
<td>PMD</td>
<td>para-Menthane-3,8-diol</td>
</tr>
<tr>
<td>PMD-citronellal acetal</td>
<td>para-Menthane-3,8-diol-citronellal acetal</td>
</tr>
<tr>
<td>DBP</td>
<td>Di-butyl phthalate</td>
</tr>
<tr>
<td>DEP</td>
<td>Di-ethyl phthalate</td>
</tr>
<tr>
<td>BBP</td>
<td>Butyl-benzyl phthalate</td>
</tr>
<tr>
<td>DMP</td>
<td>Di-methyl phthalate</td>
</tr>
<tr>
<td>PVC</td>
<td>Poly-vinyl chloride</td>
</tr>
<tr>
<td>MEP</td>
<td>Monoethyl phthalate</td>
</tr>
<tr>
<td>MMP</td>
<td>Monomethyl phthalate</td>
</tr>
<tr>
<td>DEHP</td>
<td>Di-ethylhexyl phthalate</td>
</tr>
<tr>
<td>DINP</td>
<td>Diisononyl phthalate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>PAC</td>
<td>Corrected peak area</td>
</tr>
<tr>
<td>SABS</td>
<td>South African Bureau of Standards</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>GU</td>
<td>Gloss Units</td>
</tr>
<tr>
<td>k</td>
<td>Rate</td>
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Chapter 1

Introduction

A plasticizer is a material that when added to another yields a mixture which is easier to handle or has greater utility. The mixture’s physical properties such as flexibility, viscosity, fluidity and plasticity are modified. Plasticizers are used in cosmetic products as flexibility enhancers to resist chipping in nail varnish and to enhance film forming properties for perfumes. These chemicals are important as they prolong the use of the cosmetic product, since they allow for increased surface adhesion, resist smudging and allow for longer colour continuance.

The problem, however, is that some plasticizers, specifically phthalate-based plasticizers, can have devastating negative effects on a biological system such as the human body. Studies have indicated that plasticizers can be absorbed from personal care products, which may cause adverse health effects in humans, such as birth defects, and can disrupt hormones. Other studies have linked them to possible carcinogenic properties, such as breast cancer and tumours. Plasticizers may also be released into the air and can contaminate water, ultimately causing pollution.

In 2004, certain phthalate plasticizers were banned by the Cosmetic Directive and The EU Existing Substance Council. Examples of banned plasticizers include: di-ethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), butyl-benzyl phthalate (BBP) etc. In 2004, the world market for plasticizers reflected a total volume of approximately 5.5 million tons with phthalate plasticizers accounting for 363 thousand tons per year.

The industry has responded to the negative outcomes of these plasticizers by producing alternative, safer options. Recent examples include; Proviplast® 25422 and Proviplast® 25102, which are both 100% bio-based PLA (Polyactide) plasticizers, produced by the company Proviron. However, these options are limited compared to the abundant phthalate plasticizer options. Therefore there is an opportunity to research other molecules found or derived from nature which will not pose any health risks, will be safe for the environment, have low manufacturing costs and can exhibit similar, if not better, plasticizing properties than the phthalate-based plasticizers. Such molecules would be classified as bio-plasticizers.
since they are synthesized from natural sources, exhibit plasticizing properties and are biodegradable.\(^\text{10}\)

Eucalyptus oil isolated from the tree *Eucalyptus citriodora* contains many natural chemicals which have beneficial properties, for example, antimicrobial (aids to strengthen the body’s resistance to infection), antifungal, anti-inflammatory, antiseptic and bactericidal (destructive to bacteria) properties.\(^\text{11}\) The oil contains two chemicals of interest, namely: para-menthane-3,8-diol (PMD) and citronellal. With ageing of the leaves, an acetal product is naturally synthesized as shown in Figure 1.1 below.

![Figure 1.1: PMD and citronellal used to synthesize PMD-citronellal acetal, as a bio-plasticizer](image)

As can be seen in Figure 1.1, para-menthane-3,8-diol (PMD), occurring naturally in the oil from the tree, *Eucalyptus citriodora*, forms an acetal with citronellal. In a previous study conducted in this laboratory, it was shown that PMD-citronellal acetal exhibited plasticizing properties similar to conventional plasticizers when incorporated into selected cosmetic products.\(^\text{12}\)

The outcome of the study suggests that PMD-citronellal acetal can be considered a plasticizer as its performance characteristics are comparable to that of dibutyl phthalate.
1.1 Functions and uses of plasticizers

Plasticizers are very prevalent in the polymer industry. They are the cheapest and most common additives in lubricants, flame retardants, antioxidants and processing aids. Plasticizers are present in building materials (e.g. cable insulations, flooring), clothing (rainwear, shoes), dental materials (e.g. teething rings), personal care products (e.g. anti-wrinkle cream, nail varnish, conditioners), medical products and food packaging.\(^\text{13}\)

The function of plasticizers is to improve flexibility and processing occurring in polymers.

Plasticizers are compounds which are widely used for their film-forming properties as they produce transparent and intact films. The main fields of application for plasticizers are for sealants, cosmetics, coatings and adhesives.\(^\text{14}\)

Plasticizers are used in cosmetic formulations for the following reasons:\(^\text{15}\)

A plasticizer:
- Decreases glass transition temperature of the polymer
- Improves the mechanical properties
- Improves the resistance to impact
- Gives rise to more flexible films
- Increases the ability to resist rub-off
- Enhances film-forming properties
- Retains the properties of cosmetics
- Improves the optical clarity of the product
- Prevents film from chipping (desirable in nail varnishes)
- Improves adhesion to keratin (present in for example hair and nails)
- Exhibits the effect of permanence
- Exhibits a low vapour pressure

In some cases, when two plasticizers are combined they resist yellowing and produce gloss in cosmetics.\(^\text{16}\)

**Disadvantages of plasticizer use:**

- Phthalate plasticizers can cause allergies\(^\text{17}\)
- Some plasticizers form cloudy films which are undesirable in certain nail varnishes
- Some plasticizers can cause the product to age when exposed to heat
- Gloss deterioration
- Plasticizers may change the pH level of the product as they change the neutralization levels\textsuperscript{18}
- Plasticizers can be lost due to evaporation
- Excessive amounts can decrease the holding ability of the plasticizer\textsuperscript{19}

Internal and external plasticizers are available. Internal plasticizers, as the name suggests, are an integral part of the product. External plasticizers are easily lost to extraction, migration and evaporation.\textsuperscript{21}

\section*{1.2 Mechanism of action of plasticizers}

Plasticizers have relatively low molecular weights which allow them to alter the physical properties of a polymer to form a product which is more useful. Polymers are relatively brittle at room temperature. Plasticizers render the polymer more soft and pliable which will enhance plasticity and flexibility of the films. Plasticizers enhance the mobility of the polymer chain therefore modifying the polymer’s mechanical and physical properties. The strength of the interaction between the polymer and plasticizer will determine how efficient it is.

Plasticizers interpose between each strand present in the polymer which therefore breaks the polymer-polymer interactions. The polymer’s structure is modified to a less cohesive, flexible and more porous structure. There is swelling of the polymer due to latex spheres which causes the product to resist deformation. The end result is a plasticized polymer which has a lower tensile force compared to a non-plasticized polymer. This results in an enhanced plasticizer-polymer interaction. Polymers that have plasticizers incorporated within them will have a lower glass transition temperature ($T_g$) than those without the plasticizer.\textsuperscript{24}

The “proposed mechanisms” for plasticizers include a number of different theories. These include:\textsuperscript{25}

1. The free volume model
2. The lubrication theory
3. The gel theory
4. The solvation-desolvation equilibrium theory
The free volume model describes the effect of plasticizers by the amount of internal space which exists within a polymer. A greater volume means that there is more space available for polymer chain motion. The plasticizer increases the free volume by the small plasticizer molecules getting in between the polymer chains and forcing them apart.

The lubrication theory explains the effect of plasticizers as preventing rigid structures from forming. With plasticizer molecules present in between the polymer chains the resistance to sliding is lowered as a result of the weakening of the Van der Waals’ forces between each strand of the polymer.

In the gel theory, the addition of the plasticizer produces a polymer-plasticizer intermediate. The polymer strands are held together in the intermediate state by secondary bonding forces. As these forces are weak, they can easily be overcome therefore giving rise to the favourable characteristic of flexibility.

In the solvation-desolvation equilibrium theory, the plasticizer is temporarily bound to the polymer and equilibrium between solvation and desolvation occurs between the plasticizer and polymer. Depending on the family of plasticizer, there are different magnitudes of attraction with a specific polymer. A plasticizer molecule is attracted to an active group on the chain of the polymer and is dislodged, then replaced by another interacting plasticizer molecule. Therefore the plasticizer is capable of moving from one location to another in the polymer.26
1.3 General health issues of phthalate plasticizers

There are many health issues being linked to plasticizers and the products containing them. They are scrutinized for environmental issues by authorities and the scientific community. The following health issues have been reported:

- Carcinogenic effects\(^{27}\)
- Feminizing effects (reported in rats and fishes at low percentage exposure)\(^ {28}\)
- Toxic effects in the following organs: liver, reproductive tract, lungs, heart and kidneys. Respective examples: liver disease, vaginal cancer, asthma, ventricular dysfunction, renal disease.\(^ {29}\)

Studies have linked phthalates with infertility in males and their reproductive development as the sperm channel is affected.\(^ {30}\) They have been reported as endocrine disrupters as some of them mimic oestrogen and block normal pathways.\(^ {31}\) Phthalates alter the immune system causing allergies and increasing the risk of disease.\(^ {32}\) This study by Shigeno et al. showed that when dibutyl phthalate was rubbed onto rodents’ skin, the chemistry of the rodents’ immune system was altered making them prone to develop contact prone allergies.

1.3.1 Carcinogenicity

It has been reported that the increase in breast cancer is linked to phthalate plasticizer exposure.\(^ {33}\) The phthalates compromise cells’ normal functioning, as metabolites are accumulated and the result are the accumulation of abnormal cells, which are commonly seen in breast tissue. According to Lopez, a case study was performed on 233 women with breast cancer. The controls were woman of the same age and the levels of phthalates in their urine were measured. After other risk factors for breast cancer were taken into account, there were significant levels of monoethyl phthalate (MEP) detected in their urine. MEP is a metabolite of diethyl phthalate (DEP). The amounts were significantly elevated in young woman with breast cancer. Urine collections were made at home and in some cases from the hospital. The increase of phthalate exposure occurs via PVC medical tubing (refer to 1.4 Exposure). It was concluded that phthalates elevate the risk of breast cancer.\(^ {34}\)
1.3.2 Endocrine Disruption

Endocrine disrupters are chemicals which interfere with the “hormonal homeostasis” of the body. DBP is an endocrine disrupter as it binds to oestogens receptors. Phthalates alter gene expression therefore reducing testosterone synthesis. Phthalates are reported to disrupt the following processes.\(^{35}\)

1. Transport of cholesterol
2. Lipid and cholesterol homeostasis
3. Signalling insulin
4. Transcription
5. Oxidative stress

Many phthalates have structures similar to the 17-estradiol ligand. Therefore these phthalates are capable of binding to the oestrogen receptors thereby enhancing the oestrogen effect in the body. Males however, result in the delayed development of the reproductive tract and the formation of testes. This ultimately affects the onset of puberty and can result in infertility in males. Phthalate esters are capable of altering a receptor (pregnane X receptor), which is responsible to “generate” enzymes for steroid metabolism and detoxification. This study was performed on rodents.\(^{36}\)

1.3.2.1 Feminizing effects on males

Phthalate plasticizers are reported to enhance the following male reproduction problems from repeated exposure:

- Lowered sperm count
- Tumours in the testes
- Undescended testes
- Decreased testosterone levels
- Decreased prostate weight
- Diminished seminal vesicle
- Decreased sperm mobility
- Damage of the DNA in sperm

Infertility is ultimately the end result.
Obesity has been indicated as one of the outcomes from repeated phthalate exposure, due to their metabolites affecting the normal functioning in the body. The “feminizing effect” is enhanced by the accumulation of weight in the waist and breast areas.\textsuperscript{37}

1.3.2.2 Puberty

Phthalates have been said to be environmental agents, which are hormonally active. A study by Chou et al. (2009), investigated the link of phthalates on puberty. The Taiwanese girls which had premature pubertal timing had higher levels of phthalates (MMP = Monomethyl phthalate) in their systems.\textsuperscript{39}

1.3.3 Toxic effects on organs

Respiratory studies were performed on children who were exposed to plastics (toys, objects etc) and those that were not. It was established that there were increased cases of respiratory illnesses such as asthma, infections and bronchial infections. The most prevalent illness was asthma.\textsuperscript{40}

1.4 Exposure to phthalate plasticizers

Plasticizers have the tendency to leak to the surrounding environment (e.g. water and food). This is referred to as leaching and migrating. It is important to determine the “life-span” of the product, to prevent this from occurring. Therefore expiration dates must be adhered to.

Plasticizers tend to migrate as they tend to have low molecular weights and therefore have high mobility. Temperature, solubility, diffusion coefficients and plasticizer concentration influence their leakage.\textsuperscript{41}

Plasticizer mobility is important to investigate, especially as they are so widely used and easily capable of affecting one's health.

Phthalate plasticizers were reported to reach humans by the following ways according to DiGangi and Norin.\textsuperscript{42} Populations are exposed via:

a) Drinking contaminated water and contaminated food
b) Transfer via blood and saline buffer administered in the hospital via PVC tubing
c) Inhaling air ("off-gassing" of PVC products such as a car's dashboard and flooring)
d) Exposure from the skin (cosmetics and personal care products)

The use of these materials in food and packaging has been controlled by “the Food and Drug Administration, where DBP cannot be directly added to any food.” DBP is however allowed to be used when it can be incorporated into paper where it can come into contact with fatty foods and aqueous foods. The FDA (2002 a, b, c) has warned against plasticizers (such as DEHP) in medical apparatus such as tubing, catheters and storage items.

A concern has been for newborns who receive neonatal care via blood transfusions, respiratory therapy and membrane oxygenation. Phthalate plasticizers which are used in baby care products and toys make up to 1% of the plasticizer market. Toys put in their mouths, increases the child’s exposure by coming into direct contact with them.

According to Koo and Lee, plasticizers were suspected in the onset of premature puberty amongst young girls. High levels were found in baby toys. Ever since, the concentration of plasticizers has become regulated. Exposure to plasticizers for humans is a daily occurrence especially with the increased use in medicine and food packaging. Many studies have been focusing on the migration of plasticizers.

1.5 The importance of plasticizers in cosmetic products

Cosmetics are formulations which are applied to the skin, nails and hair of an individual in order to beautify, enhance or protect these substrates. Most of the consumers of these products are females and are interested in the physical appearance of these products. The colour, odour, consistency and textures are of high importance. There are several thousand different cosmetic types which have been formulated for many aesthetic pleasing results. The importance of plasticizers in nail polish and perfumes will be discussed here.

There are a variety of nail products used to maintain and care for nails. Nail enamel is the most important of them. When nail polish was initially introduced, it was a colourless coating. Later coloured nail enamel was introduced. The most popular nail enamels are used to cover blemishes on the nails, add artificial colour and are smooth and shiny. Most nail enamels consist of:
- A film-forming base e.g. nitrocellulose
- A plasticizer e.g. di-butyl phthalate
- A solvent e.g. acetone
- A resin e.g. benzoin
- A dye or pigment e.g. aluminium
- A suitable fragrance

All these components are necessary in producing a favourable final product. If a base was used alone, the product would not adhere to the nail nor spread evenly and would crack. Resins aid film formation and adhesion to the nail. The solvents evaporate and enable the varnish to disperse evenly. The plasticizers generally are liquids with high boiling points. These plasticizers preserve the adhesion of the film onto the nail and preserve the film’s flexibility. Therefore the inclusion of a plasticizer is important in nail polish.47

Recent trends in products are the terms “eco-friendly” and “naturalness.” Consumers are becoming more aware and concerned of the ingredients in the products they use. Fragrances pose little health risk to the consumer as very few problems have been associated with their use except for allergic effects. However, plasticizers are used to prolong the scent of fragrances in perfumes.

1.6 Environmental impacts

Personal care products and cosmetics are marketed to be used on the human body to increase bodily aesthetics. These exclude pharmaceuticals and are therefore not ingested. Many chemicals, including plasticizers, are used in personal care products, each one with specific functions to yield the desired product. Some chemicals are used as preservatives or active ingredients in hair care products, soaps, dental care products, cosmetics, skin care products, insect repellents, fragrances, sunscreen agents and flame retardants. Many personal care products are overused by the consumer, leading to higher dosages of these chemicals than recommended.

These chemical compounds can be altered via biological transformation processes into other metabolites. Personal care products can be received into environments (soils, sludges, air, ground and surface water, sewage and landfills etc) by regular usage, such as bathing, showering, excretion, spraying or disposal of products. As they are released uncontrollably, they can bypass treatment systems. As these active ingredients and metabolites are
released into the environment in micro-concentrations, they bypass the treatment systems and especially are released into the aquatic systems as explained by Daughton and Ternes.\textsuperscript{48} These bioactive compounds disrupt the industrial ecology and are consumption-related environmental impacts. There have been reports of the accumulation of these bioactive compounds in aquatic organisms.\textsuperscript{49} Negative hormonal and effects which are toxic to these organisms have been detected in very low concentrations. In the late 1990’s, there was an interest in the possible effects of these bioactive compounds being released into the environment by these products.\textsuperscript{50} Plasticizers’ vapour pressures are low and they are non volatile, yet they are detected in air samples. This is especially due to building materials.

1.7 Regulations

In the 1990s, there was attention brought onto the phthalate plasticizers often used to soften PVC (poly vinyl chloride). As mentioned before, the phthalates do not “fix” themselves to the backbone of the polymer, therefore ensuring their release to the product’s surface. Children tend to put flexible “plastic toys” in their mouths. Therefore the effects of phthalates on the endocrine system of children were studied. A study by Sathyanarayana \textit{et al}, showed potential phthalate exposure from certain baby care products. According to the study, endocrine disruption was evident in exposed infants.\textsuperscript{51} In 2009, according to the “US Consumer Product Safety Commission” phthalate plasticizers were used in very low concentrations in products for children under twelve years. Information regarding the amount of potential endocrine disrupters which are currently used commercially is not yet established as most industrial chemicals have not been tested for endocrine disruption. Testing of chemicals for endocrine disruption was mandated under the Food Quality Protection Act of 1996 (1996).\textsuperscript{52}

As a result of studies like the above-mentioned regulations were passed to restrict using “common plasticizers and certain western countries have banned any plasticizer use.

In response to this ban and restriction, bio-plasticizers have been investigated as an alternative.\textsuperscript{54} When working with new compounds many questions are asked to ensure the safety of any emissions, whether the compounds are recyclable, and are not harmful to workers who have close encounters with them.

There are implications in place to limit the exposure to these chemicals especially the carcinogens. “The Occupational Safety and Health Administration (OSHA)” which is a
regulatory agency has tried to minimize the problem by setting exposure limits to plasticizers and finding alternatives. There has been a minimal risk level which has been instated.

Environmental, economical, industrial and safety aspects have thus influenced the development of plasticizers from renewable resources.

1.8 Importance of the oil extracted from *Eucalyptus citriodora*.

Eucalyptus oil is extracted from the leaves and branches of the tree, *Eucalyptus citriodora*. See Figure 1.2. The oil is known for its many remarkable properties for the treatment of health problems. It has antiviral, antifungal and antibacterial properties, making it an excellent remedy for many everyday health issues. Eucalyptus oil can be used orally to treat dental infections, sore throats, inflammation and dental cavities. Inhalation of the oil relieves mental stress and exhaustion. Regarding its antifungal and antibacterial properties it is used to alleviate many problems in the respiratory system, including colds, asthma, sinusitis and sore throats. Its anti-inflammatory properties make it ideal to be applied topically to treat minor wounds, burns, muscle pains, arthritis, skin infections and insect bites. Eucalyptus oil can be ingested in small quantities. Eucalyptus oil has been used in many applications including antiseptic, pharmaceutical, insect repellent, fragrance, flavouring and industrial uses.

As can be seen above, this oil is used in a variety of applications. It can be applied topically, inhaled and ingested. It is regarded as safe for external use, but ingestion of pure Eucalyptus oil of between 0.05-0.5ml/kg (of total body weight) is a lethal dose in adults. The possibilities of incorporating Eucalyptus oil derivatives in cosmetic applications would diminish the hype about dangerous ingredients already being used in these formulations. Such ingredients being replaced are di-butyl phthalate (DBP), a plasticizer used in many cosmetic and personal care formulations for its film-forming properties. The following question arises: Could Eucalyptus oil derivatives also display similar film-forming properties as conventional plasticizers? Perhaps the positive medicinal properties are still viable in the cosmetic formulations, producing a potent product not only satisfying aesthetic ideals.
The oil contains a large number of compounds, all occurring naturally. The compounds of interest include para-3,8-menthane-diol (PMD), para-3,8-menthane-diol-citronellal acetal (PMD-citronellal acetal) and Citronellal. The acetal is produced naturally as the Eucalyptus leaf matures. Synthetically PMD-citronellal acetal is the reaction product when PMD and pure citronellal react. Upon GC analysis of the oil, PMD-citronellal acetal is present but in small quantities. The major components of Eucalyptus citriodora oil are indicated in the gas chromatogram in Figure 1.3 below. The chromatogram was produced by a HP 5890 series gas chromatograph on a sample of Eucalyptus citriodora oil obtained from Merck. The percentage of each constituent depends individually on each tree.

Figure 1.3: Gas chromatogram of the Eucalyptus oil extracted from Eucalyptus citriodora.
Synthetically produced PMD-citronellal acetal has been previously tested as a plasticizer in cosmetic formulations and found to behave in a very similar manner to DBP which is commonly used in these formulations.\textsuperscript{62} The motivation for this research was to reduce the cost and effort of synthesising and isolating PMD-citronellal acetal by increasing the amount of naturally occurring PMD-citronellal acetal in Eucalyptus citriodora oil and using this modified oil without having to isolate the acetal. Therefore a synthetic reaction which optimises the yield of this acetal in Eucalyptus oil can be investigated to produce a bio-plasticizer, viz, para-menthane-3,8-diol-citronellal acetal enriched Eucalyptus oil. This bio-plasticizer could possibly have the following advantages:

- it can replace harmful phthalate plasticizers by performing the same functions (as seen with synthetically produced PMD-citronellal acetal),
- it is environmentally friendly and biodegradable,
- it is easily synthesized with a minimal amount of chemicals.

Therefore it is of interest to be able to synthesize the enriched oil containing PMD-citronellal acetal in high enough quantity and to determine if the PMD-citronellal acetal-enriched oil will show any measurable plasticizing properties. A synthesis reaction using \textit{Eucalyptus citriodora} oil as the source of citronellal and adding pure PMD could optimise the yield percentage of the acetal product in the oil.

For this dissertation the bio-plasticizer will be synthesised and tested as a replacement for a synthetic plasticizer in a nail varnish and a perfume formulation. These two cosmetic products will be formulated and the effectiveness of the bio-plasticizer will be compared to the conventional phthalate plasticizer (DBP).

1.9 Problem statement

Plasticizers cause many health problems therefore an alternative natural plasticizer needs to be investigated. PMD-citronellal acetal, which has been shown to exhibit plasticizing properties, is found in \textit{Eucalyptus citriodora} oil\textsuperscript{63} and can possibly be used as a replacement to conventional plasticizers in cosmetic and personal care products, thereby diminishing the health problems associated with conventional plasticizers such as dibutyl phthalate. The basis of this research is to enrich the Eucalyptus oil to contain a greater quantity of the PMD-citronellal acetal and then to incorporate this enriched oil into two formulations, the first being
a nail varnish and the second a perfume. The plasticizing properties of this acetal-enriched oil with those of dibutyl phthalate will be compared in these two formulation types.

1.10 Research hypothesis

Para-menthane-3,8-diol (PMD), occurring naturally in the oil from the tree, *Eucalyptus citriodora*, forms an acetal with citronellal. It is hypothesized that *Eucalyptus citriodora* derived PMD-citronellal acetal will exhibit plasticizing properties similar to the conventional plasticizer, dibutyl phthalate, and can be used as its replacement in cosmetic products.

1.11 Research questions

These are the following questions to be answered in this dissertation:

1. Can PMD-citronellal acetal be synthesized using *Eucalyptus citriodora* oil as the source of citronellal?

2. Can the yield of PMD-citronellal acetal in the oil be optimized by changing certain reaction variables (solvent, temperature, catalyst amounts, and reaction time)?

3. Does *Eucalyptus citriodora* derived PMD-citronellal acetal show similar plasticizing properties to DBP, a conventional plasticizer?

1.12 Research objectives

These are the following research objectives which will be needed to answer the research questions:

1. Determination of the physical and chemical properties of *Eucalyptus citriodora* oil.

2. Synthesis of PMD-citronellal acetal using *Eucalyptus citriodora* oil as the source of citronellal with high conversion of citronellal to acetal.
3. Comparison of the physical and chemical properties of the *Eucalyptus citriodora* derived PMD-citronellal acetal with those of DBP plasticizers which are usually used in cosmetic products.

4. Evaluation of the plasticizing effects of the *Eucalyptus citriodora* derived PMD-citronellal acetal with DBP in the following cosmetic products, namely:
   i) Nail polish
   ii) Perfume

5. Evaluation of the chemical stability of *Eucalyptus citriodora* derived PMD-citronellal acetal.

6. Determination of the physical stability of the nail polish and perfume formulations.
Chapter 2

Synthesis of *Eucalyptus citriodora* derived PMD-citronellal acetal

2.1 Materials

All reagents received for the synthesis of para-methyl-3,8-diol-citronellal acetal enriched Eucalyptus oil were used as is. Para-methyl-3,8-diol was previously synthesized. All the reagents sources and grades are summarized in Table 2.1.

Table 2.1: Reagents used for the synthesis of PMD-citronellal acetal and for analytical testing.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Source</th>
<th>Grade*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora oil</td>
<td>Not specified</td>
<td>Merck</td>
<td>84.3% Citronellal</td>
</tr>
<tr>
<td>p-Menthane-3,8-diol</td>
<td>C\textsubscript{10}H\textsubscript{20}O\textsubscript{2}</td>
<td>InnoVenton</td>
<td>Synthesized</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>Merck</td>
<td>AR</td>
</tr>
<tr>
<td>Conc. Sulphuric acid</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>Merck</td>
<td>95 – 98%</td>
</tr>
<tr>
<td>Anhydrous Sodium sulphate</td>
<td>Na\textsubscript{2}SO\textsubscript{4}</td>
<td>Merck</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Heptane (dry)</td>
<td>C\textsubscript{7}H\textsubscript{16}</td>
<td>Merck</td>
<td>AR</td>
</tr>
</tbody>
</table>

*AR = Analytical reagent

2.2 Synthesis

The various steps for the synthesis of PMD-citronellal acetal are discussed in this chapter. Optimization experiments were performed to determine the best synthetic method.

2.2.1 Summary of Synthetic method

p-Methyl-3,8-diol-citronellal acetal was synthesized by reacting Eucalyptus oil with p-Methyl-3,8-diol. A catalyst, concentrated sulphuric acid, was added drop-wise to accelerate the reaction to completion. The reaction mixture was stirred for 6 hours at room temperature. The reaction mixture was washed with brine solution and ice cold water. The separated organic layer was dried with anhydrous sodium sulphate. The solvent was removed by a vacuum. The experiment was repeated in triplicate. Optimization experiments
were performed by changing: the solvent, reaction time and catalyst amounts. Refer to section 2.6: Optimization experiments, on page 40.

2.2.2 Reaction step for Experiment 1.
Eucalyptus oil* (29 mg, 0.14 mol) and p-menthane-3,8-diol** (40 ml, 0.16 mol) were dissolved in the solvent, dichloromethane (50 ml). The reaction mixture was stirred at room temperature and conc. Sulphuric acid (0.368 g) was added drop wise over a period of 2 minutes. The reaction was stirred at room temperature for 6 hours. The reaction equation is shown in Figure 2.1.

Figure 2.1: Reaction of PMD with Citronellal.

The composition of the two reactants is given in Table 2.2 below:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Component</th>
<th>Peak area</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>Citronellal</td>
<td>815.092</td>
<td>84.3</td>
</tr>
<tr>
<td></td>
<td>PMD</td>
<td>11.4477</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Acetal</td>
<td>28.0494</td>
<td>2.7</td>
</tr>
<tr>
<td>PMD (crude liquid)</td>
<td>Citronellal</td>
<td>267.77</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>PMD</td>
<td>347.733</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>Acetal</td>
<td>229.438</td>
<td>25.6</td>
</tr>
</tbody>
</table>

*Eucalyptus oil contains 84.3% citronellal. With pure citronellal, 25 mg is added. Therefore 29 mg of Eucalyptus oil is required for the reaction.

**PMD liquid contains 41.8% pure PMD. With pure PMD, 25 ml is added. Therefore 40 ml of liquid PMD used is required for the reaction.
2.2.3 Product separation

The reaction mixture was placed in a separating funnel. The aqueous phase was discarded. The organic phase was washed twice with ice cold water (20 ml) and subsequently washed twice with a brine solution* (~ 13%). All aqueous washings were discarded. The dichloromethane was removed by vacuum distillation to afford crude p-Mentane-3,8-diol-citronellal acetal.

*Brine solution = 65.05 g NaCl dissolved in distilled water, filled up to the mark of a 500 ml volumetric flask = ~ 13 % brine.

2.3 Analytical Techniques

2.3.1 Gas-Liquid Chromatography – Mass Spectrometry (GC-MS)

GC-MS analysis was performed on a HP 5890 series gas chromatograph coupled to a HP 5972 series mass selective detector. The software used was HP 61034 and the detector was coupled with a Hewlett Packard computer. The GC housed a RTX 35 ms column (length 30 m x 9.25 mm ID x 0.25 mm thickness). The programme was set as follows:

- Initial column temperature : 70°C
- Initial column hold time : 5 minutes
- Final column temperature : 280°C
- Final column hold time : 5 minutes
- Heating rate : 10°C/min
- Injector temperature : 250°C
- Injector volume : 1μl
- Detector : HP 5972 series mass selective detector
- Split flow : 60 ml/min
- Carrier gas : Helium at constant flow (1 ml/min)
- Run time : 30 minutes
2.3.1.1 GC-MS conformation of p-menthane-3,8-diol-citronellal acetal

The GC-MS spectrum for the *Eucalyptus citriodora* oil derived PMD-citronellal acetal is shown in Figure 2.2.

Figure 2.2: GC-MS conformation for the presence of PMD-citronellal acetal in the synthesized oil.
The presence of the acetal in the reaction product was identified by determining the mass fragmentation pattern for PMD-citronellal acetal. In Table 2.3 below, the theoretical mass fragmentation was obtained from the software, ChemDraw Ultra 8.0. The actual mass fragmentation for PMD-citronellal acetal, was obtained from the GC-MS spectrum analysis.

Table 2.3: Mass fragmentation of PMD-citronellal acetal.

<table>
<thead>
<tr>
<th>M/Z theoretical pattern</th>
<th>M/Z from Fig 2.2</th>
<th>Mass fragmentation pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>154.14</td>
<td>154.4</td>
<td><img src="image1" alt="Mass fragmentation pattern" /></td>
</tr>
<tr>
<td>138.14</td>
<td>137.4</td>
<td><img src="image2" alt="Mass fragmentation pattern" /></td>
</tr>
<tr>
<td>183.14</td>
<td>183.2</td>
<td><img src="image3" alt="Mass fragmentation pattern" /></td>
</tr>
<tr>
<td>308</td>
<td>307.7</td>
<td><img src="image4" alt="Mass fragmentation pattern" /></td>
</tr>
</tbody>
</table>
It can be concluded that the acetal was present in the synthesized oil, as the various mass fragments that were determined theoretically are seen in the GC-Mass spectrum of the product.

### 2.3.2 Gas Chromatography

Analysis was performed on a HP 5890 series gas chromatograph. The data was processed by HP 61034 C software using a Hewlett Packard computer. The RTX 35 ms column was fitted in the GC (length: 30 m x 0.25 mm ID x 0.25 mm thickness). The various settings were programmed as follows:

- **Initial column temperature**: 70°C
- **Initial column hold time**: 5 minutes
- **Final column temperature**: 280°C
- **Final column hold time**: 5 minutes
- **Heating rate**: 10°C/min
- **Injector temperature**: 250°C
- **Injector volume**: 1µl
- **Detector**: FID detector
- **Split flow**: 60 ml/min
- **Carrier gas**: Helium at constant flow (1 ml/min)
- **Run time**: 30 minutes

### 2.3.3 Sample preparation

The reactants (Eucalyptus oil, PMD) and product liquids were diluted with the solvent heptane, for GC analysis (1µg/ml).

### 2.3.4 Thin Layer Chromatography (TLC)

The synthetic reaction was monitored by TLC plates. A TLC plate is coated with a thin layer of alumina or silica (the stationary phase). The mixture is spotted near the bottom of the plate. A solvent mixture of hexane: Ethyl acetate, 9.8:0.2 is prepared. The TLC plate is placed in a shallow amount of solvent (the mobile phase). The TLC plate absorbs the solvent and as it reaches the top of the plate, it is dried and analysed under UV light. The organic compound fluoresces and the components of the mixture can be identified. This method was
used to determine when PMD-citronellal acetal was synthesized and had reached completion.

2.4 Gas Chromatogram for p-Mentane-3,8-diol-citronellal acetal enriched Eucalyptus oil.

The gas chromatogram of the experiment which yielded the highest acetal product percentage is shown in Figure 2.3.

![Gas Chromatogram of p-Mentane-3,8-diol-citronellal acetal-enriched Eucalyptus oil](image)

**Figure 2.3: Gas Chromatogram of p-Mentane-3,8-diol-citronellal acetal-enriched Eucalyptus oil**

Table 2.4 and Figure 2.4 show the corrected GC peak areas and percentage components of the reaction product. The corrected peak areas, percentage composition and percent product yield calculations are described in sections 2.4.1 and 2.4.2. The peaks are split which is an indication of the isomers present in the Eucalyptus oil for each component. The peak areas used were the integrated peak areas for each compound (each isomer was taken into account).
Table 2.4: Percentage components of the product for Experiment 1 (Data from an average of 3 runs).

<table>
<thead>
<tr>
<th>Component</th>
<th>Peak area</th>
<th>Area %</th>
<th>Corrected Peak area*</th>
<th>Corrected area %*</th>
<th>Std dev</th>
<th>Product % Yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citronellal</td>
<td>222.795</td>
<td>9.6</td>
<td>242.178</td>
<td>10.17</td>
<td>0.51</td>
<td>-</td>
</tr>
<tr>
<td>PMD</td>
<td>382.506</td>
<td>12.2</td>
<td>438.735</td>
<td>12.12</td>
<td>0.27</td>
<td>-</td>
</tr>
<tr>
<td>Acetal</td>
<td>1788.427</td>
<td>73.8</td>
<td>2026.288</td>
<td>74.42</td>
<td>1.05</td>
<td>74.84</td>
</tr>
</tbody>
</table>

*Corrected peak areas and Product % Yield calculations are shown in sections 2.4.1 and 2.4.2 respectively.

Figure 2.4: Means graph of the various components present in the product liquid.

The product contained 10.17% citronellal ± 0.51% and 12.12% PMD ±0.27%. The acetal was the highest percentage component in the product with a percentage of 74.42% PMD-citronellal acetal ±1.05%. The synthesis results are reproducible (relative standard deviation < 2%) using the specific batch reactants mentioned in Table 2.1.
2.4.1 Calculation of GC corrected peak areas and response factors

The GC corrected peak areas and response factors were calculated for each individual component in the product mixture, according to a published procedure. Analysis of the products and reactants (Eucalyptus oil, PMD, PMD-citronellal acetal) were performed using a Gas Chromatograph. Refer to 2.3.1 for the relevant settings.

A maximum response is expected for carbon and hydrogen bonds in organic compounds. However when heteroatoms (e.g. oxygen) are present, the detector’s response is reduced. Therefore the response from the GC is lowered. The peak area is an indication of the intensity of the carbon atom ionisation and the amount of carbon eluted within a peak. Therefore the FID (flame ionization detector) signal needs to be corrected for any carbon atoms bonded to heteroatoms.

The average response factor \(f_i\) considers the signal when a carbon atom is bonded to another atom, other than hydrogen. To calculate the response factors, the molecular structures are used to determine the amount of carbon atoms in the structure \(C_n\). The ECN (effective carbon number) is calculated by subtracting the average reduction in signal from the number of carbon atoms \(C_n\). These average reduction values were obtained from a previously published procedure.

The average response factor \(f_i\), for each compound is obtained by dividing \(C_n\) by the ECN value.

\[
f_i = \frac{C_n}{ECN} \quad \text{eq 2.1}
\]

Example: The response factor \(f_i\) for p-menthane-3,8-diol can be calculated as follows:

\[
\text{Diagram of p-menthane-3,8-diol}
\]
Number of Carbon atoms: $C_n = 10$
Number of functionalities = $2 \times -OH$
Average reduction for alcohols = 0.64

Therefore:

$$fi = \frac{C_n}{ECN}$$

$$fi = \frac{10}{(10 - 2(0.64))} = 1.147$$

Table 2.5: Corrected GC peak areas and response factors.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Molecular structure*</th>
<th>Carbon number $(C_n)$</th>
<th>Reduction</th>
<th>ECN</th>
<th>Response factor $(f_i)$</th>
<th>Corrected peak area = (Peak area x $f_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citronellal</td>
<td><img src="Citronellal" alt="Molecular Structure" /></td>
<td>10</td>
<td>Ketone: 1 x 0.8</td>
<td>9.20</td>
<td>1.087</td>
<td>222.795 x 1.087 = 242.178</td>
</tr>
<tr>
<td>p-Menthan-3,8-diol</td>
<td><img src="p-Menthan-3,8-diol" alt="Molecular Structure" /></td>
<td>10</td>
<td>Phenol and Tertiary alcohol: 2 x 0.64</td>
<td>8.72</td>
<td>1.147</td>
<td>382.506 x 1.147 = 438.735</td>
</tr>
<tr>
<td>p-Menthan-3,8-diol-citronellal</td>
<td><img src="p-Menthan-3,8-diol-citronellal" alt="Molecular Structure" /></td>
<td>20</td>
<td>Ether and Furans: 3 x 0.78</td>
<td>17.66</td>
<td>1.133</td>
<td>1788.427 x 1.133 = 2026.288</td>
</tr>
</tbody>
</table>

*Note: Molecular structures obtained from ChemDraw Ultra 8.0.

The corrected peak areas (PAC$_i$) were calculated by multiplying the integrated peak areas for each component, obtained from the GC with the response factor from Table 2.5.

$$PAC_{\text{PMD-citronellal acetal}} = PA_{\text{PMD-citronellal acetal}} \times f_{\text{PMD-citronellal acetal}} \quad \text{eq 2.2}$$
2.4.2 Percentage yields

For this calculation, only major peaks were considered in the calculation. Unknown peaks and solvent peaks were excluded. The principle of the reaction is that all of the reactants were converted into products, intermediates, by-products and some starting material detected by the FID. The peak areas used for the calculation were the values obtained from integration (then corrected using eq 2.2). Therefore each peak shown in Figure 2.4 was considered.

The percentage yield was calculated by dividing the corrected peak areas for the product by the sum of all the corrected major peaks (x100), from Figure 2.4.

\[
% \text{ Yield} = \frac{PAC_{\text{PMD-citronellal acetal}}}{\sum PAC_{\text{PMD-citronellal acetal} + \text{Citronellal} + \text{PMD}}} \times 100 \quad \text{eq 2.3}
\]

2.5 Optimization experiments

The GC chromatogram in Figure 2.5 shows the expected retention times for the 3 main peaks corresponding to citronellal, PMD and acetal. The objective of the optimization experiments was to minimize the citronellal and PMD peaks and to maximize the acetal peak.

Reactant peaks must be minimized

Product peak must be maximized

Citronellal ~ 11.422 min
PMD ~ 14.888 min
PMD-citronellal acetal ~ 22.417 min

Figure 2.5: GC chromatogram average retention times for each component in the product oil.
The following parameters were used in the dissertation of Marthie-Postma Botha for the synthesis of PMD-citronellal acetal from a pure Citronellal source.

- Dichloromethane as the solvent;
- 25°C;
- Catalyst amount: 0.368 g;
- 6 hour reaction.

The objective of the current research is to synthesise PMD-citronellal acetal using *Eucalyptus citriodora* oil as a source of citronellal. Factors which could possibly increase the product percentage such as the effect of solvent, temperature, catalyst and time on this reaction were investigated. The synthesis of the acetal was performed in 5 different optimization experiments to increase the product yield (PMD-citronellal acetal) and decrease the amount of unreacted citronellal and PMD. The initial experiment used the parameters listed above. The subsequent experiments were similar to the initial one but differed in either solvent choice, temperature or time. All other reaction conditions were kept constant. These experiments are summarised in Table 2.6 and described below.

Table 2.6: Parameter settings for optimization experiments.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Catalyst amount (g)</th>
<th>Reaction time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dichloromethane</td>
<td>25</td>
<td>0.368</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>25</td>
<td>0.368</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Dichloromethane</td>
<td>50</td>
<td>0.368</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Dichloromethane</td>
<td>25</td>
<td>0.184, 0.368, 0.552, 0.736</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Dichloromethane</td>
<td>25</td>
<td>0.368</td>
<td>8</td>
</tr>
</tbody>
</table>
Experiment 1
The reaction conditions were as described in section 2.2.2.

Experiment 2: Investigation on the effect of changing solvents from dichloromethane to water.
The reactants, catalyst amount (0.368 g) and temperature (room temperature) remained constant. However, the solvent used was water.

Experiment 3: Investigation of increasing temperature.
The reactants, catalyst amount (0.368 g) and dichloromethane as the solvent were constant parameters. However, the effect of increasing the temperature from 25°C to 50°C was observed.

Experiment 4: Investigation on the effect of catalyst amount on acetal %.
The same amount of PMD and Eucalyptus oil were used as in the original experiment. Dichloromethane as the solvent was used in this reaction at room temperature. However, the effect of increasing the catalyst ($\text{H}_2\text{SO}_4$) was investigated. The original Experiment 1 produced a very high percentage (~74%) using the catalyst mass of 0.368g. Therefore the experimental design incorporated the masses: 0.184g, 0.368g, 0.552g and 0.736 g.

Experiment 5: Investigation of increasing reaction time on acetal %.
The reactants, catalyst amount (0.368 g) and dichloromethane as the solvent at room temperature were constant parameters. However, the effect of increasing reaction time to 8 hours was observed.

2.5.1 Data and results of the optimization experiments.

2.5.1.1 Solvent effect
Water was used to replace dichloromethane as the solvent. Upon GC analysis and peak correction, it was shown that by using an aqueous system the final acetal product did not reach the percentage range which could be obtained when dichloromethane was used. Figure 2.6 shows the comparison of Experiment 1 with Experiment 2 in terms of percentage composition of each product. Statistical analysis of the results was done in order to determine whether the differences were significant or not.
A series of formal t-tests, assuming unequal variances was performed in which each component's average percentage values were compared for Experiment 1 and Experiment 2. The following null hypothesis was tested: \( H_0: \mu_1 = \mu_2 \) (where 1, 2 represent the experiment number). Refer to the Tables 7.1 and 7.2 on page 105, in the Appendix for the statistical data used in the analysis.

A formal t-test comparing the citronellal values for Experiment 1 and 2 showed that the statistic does not fall within the critical t-values, thus we the hypothesis can be rejected (\( p = 0.0001 \)). Therefore there is evidence to claim that the average citronellal values of Experiment 1 and 2 differ significantly.

A formal t-test comparing the PMD values for Experiments 1 and 2 showed that the statistic does not fall with the critical t-values, therefore the hypothesis can be rejected (\( p = 0.00003133 \)). The observed differences between the PMD values for each experiment differ significantly.

A formal t-test which compared the PMD-citronellal acetal values for Experiments 1 and 2 showed that there was evidence that supports the rejection of the hypothesis (\( p = 0.0000003733 \)). The observed differences between the PMD-citronellal acetal values for each experiment differ significantly. Therefore the differences between water and dichloromethane cannot be explained by experimental variation and the solvent type affects the percentage composition. It can be concluded that dichloromethane was a better choice of solvent than water, in this particular experiment. A large product peak percentage was obtained in Experiment 1 (74.09% ± 1.05%) compared with Experiment 2 (23.97% ± 1.91%).
PMD-citronellal acetal is less soluble in water than in dichloromethane perhaps leading to the decreased product peak percentage.

2.5.1.2 Temperature effect

The effect of temperature increase was investigated in these experiments. Figure 2.7 shows the comparison of Experiment 1 with Experiment 3 in terms of percentage composition of each product. Statistical analysis of the results was done in order to determine whether the differences were significant or not. Refer to Experiment 3 on page 42.

![Figure 2.7: Means bar graph of the percentage components present in the product oil for Experiment 1 and Experiment 3.](image)

A series of formal t-tests, assuming unequal variances was performed in which the average percentage of each component was compared for Experiment 1 and 3. The following null hypothesis was tested: $H_0: \mu_1 = \mu_3$ (where 1, 3 represent the experiment number). Refer to the Tables 7.1 and 7.3 on pages 105 and 106 in the Appendix for statistical data.

A formal t-test comparing the citronellal values for Experiment 1 and 3 showed that the statistic falls within the critical t-values, Therefore there is little evidence to claim that the average citronellal values of Experiment 1 and 3 differ significantly, thus the null hypothesis is accepted ($p = 0.221$) and the differences can be explained by experimental variation.
A formal t-test comparing the PMD values for Experiments 1 and 3 showed that the statistic does not fall with the critical t-values, therefore the null hypothesis can be rejected \((p=0.000994)\) and the observed differences between the PMD values for each experiment are significant.

The formal t-test comparing Experiments 1 and 3 for the PMD citronellal acetal showed evidence that supports the rejection of the null hypothesis \((p = 0.000000481)\). The observed differences between the PMD-citronellal acetal values for each experiment are significant. A larger product peak percentage was obtained in Experiment 1 (74.09\% ± 1.05\% with 95\% confidence level) compared to Experiment 3 (26.95\% ± 1.97\% with 95\% confidence level). Therefore it can be concluded that a temperature of 25°C was better than 50°C for the synthesis reaction. The increased temperature possibly degrades the acetal therefore leading to a decreased yield.

### 2.5.1.3 Catalyst effect

A catalyst increases the rate of reaction by decreasing the activation energy. It combines with the reacting molecules and puts a strain on their chemical bonds thereby destabilizing it and allowing the reaction to occur at a lower energy. Catalysts are added to reactions which otherwise would take longer to come to completion. Sulphuric acid is a homogeneous catalyst as it is in the same phase as the reactants. The effect of various catalyst amounts was investigated in these experiments. Refer to Experiment 4 on page 42 for details. Figure 2.8 shows the effect that the catalyst amount has on the final product percentage. Statistical analysis of the results was done in order to determine whether the differences were significant or not.
A series of formal t-tests, assuming unequal variances was performed in which the PMD-citronellal acetal percentage produced at 0.368 g catalyst mass was compared to the PMD-citronellal acetal percentages produced at 0.184 g, 0.552 g and 0.736 g catalyst masses. Refer to Table 7.4 on page 106 in the Appendix for details.

The hypothesis: \( \mu_{0.368g} = \mu_{0.184g} \); was rejected as there was evidence that proved that the % acetal produced when 0.368 g of catalyst was used differed significantly from when 0.184 g of catalyst was used (\( p = 0.0000008 \)).

The hypothesis: \( \mu_{0.368g} = \mu_{0.552g} \); was rejected as there was evidence that proved that the values for % acetal differed significantly when 0.368 g and 0.552 g of catalyst were used (\( p = 0.000062 \)).

The hypothesis: \( \mu_{0.368g} = \mu_{0.736g} \); was rejected as there was evidence that proved that the values for the % acetal differed significantly when 0.368 g and 0.736 g of catalyst were used (\( p = 0.00001361 \)).

There is evidence to claim that the average values of each mass differ significantly from one another. The observed difference between these averages cannot be explained by experimental error.
As the catalyst amount increases from ~0.18 g to ~0.36 g, the acetal % increases by approximately 31.03 % ± 3.25. As can be seen by Figure 2.9, there is a decrease in acetal % as the catalyst amount increases from ~0.36 g to ~0.74 g. Therefore a maximum acetal percentage was achieved at the catalyst amount of 0.368g.

Generally it does not matter how much catalyst is added as it is constantly being regenerated. The catalyst is not the limiting factor in the reaction and by increasing the catalyst amount; the reaction rate should still be the same. Only very small amounts of catalyst are needed to achieve the desired reaction. The catalyst is regenerated during the reaction until the reactants are used up. A small amount of acid leads to catalysis whereby larger amounts can lead to unwanted by-products; this could have reduced the acetal product yield when more than 0.4 g of H₂SO₄ was used.

2.5.1.4 Time

The effect of reaction time on the Citronellal, PMD and PMD-citronellal acetal peaks was investigated. Refer to Experiment 5 on page 42 for details. The time required for the reaction to reach completion to produce high product and low reactant peak percentages was determined in this experiment. The data is plotted in Figure 2.9. From the curves in Figure 2.9 it appears as if the maximum percentage of PMD-citronellal acetal is reached at around 6 hours with only very small changes in peak percentages after this time. In order to determine whether the slight differences in peak percentages between 6 hours and 8 hours reaction time are significant, a series of formal t-tests, assuming unequal variances was performed. Refer to Table 7.5 on page 106 in the Appendix for details.
The null hypothesis for citronellal: $\mu_{6\ hr} = \mu_{8\ hr}$; was investigated. The hypothesis was rejected as there was evidence that proved the percentage citronellal at 6 hours differ significantly from the value at 8 hours ($p = 0.01631$).

The null hypothesis for PMD: $\mu_{6\ hr} = \mu_{8\ hr}$; was investigated. The hypothesis was accepted as there was evidence that proved that the value for the percentage PMD at 6 hours does not differ significantly from the value at 8 hours ($p = 0.19427$).

The hypothesis for PMD-citronellal acetal: $\mu_{6\ hr} = \mu_{8\ hr}$; was investigated. The hypothesis was accepted as there was evidence that proved that the value for the acetal at 6 hour sdoes not differ significantly from the acetal value at 8 hours ($p = 0.46703$). Therefore no significant decrease in product percentage yield was observed from 6 hours to 8 hours. Therefore it was concluded that the optimum time for the reaction to reach completion was 6 hours.

### 2.5.2 Conclusions

As can be seen from the above experiments, no changes in the original parameters are necessary to improve the yield of PMD-citronellal acetal.

Based on the results above, the optimum reaction conditions remain:

- Dichloromethane as the solvent;
- 25°C;
- Catalyst amount: 0.368 g;
- 6 hour reaction.
2.6 Physical properties of *Eucalyptus citriodora* oil, DBP and PMD-citronellal acetal enriched Eucalyptus oil

Density was recorded on a FLPH Viscometer. The refractive indices were determined by use of an ATAGO pocket refractometer. Distilled water was used as the blank. The viscosity was measured at 20°C in mPa.s with a HAAKE Viscotester. The boiling points were determined by distillation. The solubility of the compounds were determined by adding a known mass of sample to the solvent at room temperature (25°C).

The physical properties of the *Eucalyptus citriodora* oil, DBP and Eucalyptus oil-derived PMD-citronellal acetal were determined and are compared in Table 2.7.

Table 2.7: Physical characterisation of the *Eucalyptus citriodora* oil, di-butyl phthalate and modified oil.

<table>
<thead>
<tr>
<th>Compound information</th>
<th>Eucalyptus oil</th>
<th>DBP</th>
<th><em>Eucalyptus citriodora</em>-derived PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td><em>Eucalyptus citriodora</em> oil</td>
<td>Di-butyl phthalate</td>
<td><em>Eucalyptus citriodora</em>-derived para-menthane-3,8-diol-citronellal acetal</td>
</tr>
<tr>
<td>Formula</td>
<td>Not specified</td>
<td>C(<em>{16})H(</em>{22})O(_4)</td>
<td>C(<em>{20})H(</em>{35})O(_2)</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>Not specified</td>
<td>278.34</td>
<td>307.5</td>
</tr>
<tr>
<td>*Molecular structure</td>
<td>Complex oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Pale yellow to greenish yellow liquid</td>
<td>Colourless liquid</td>
<td>Yellow-green oily liquid</td>
</tr>
<tr>
<td>Odour</td>
<td>Herbal odour type; high strength</td>
<td>Weak and aromatic</td>
<td>Slightly eucalyptus odour</td>
</tr>
<tr>
<td>Density (g/cm(^3) @ 20°C)</td>
<td>0.870</td>
<td>1.048</td>
<td>0.892</td>
</tr>
<tr>
<td>Refractive index (Pocket Refractometer ATAGO)</td>
<td>65.4 Brix %</td>
<td>80 Brix %</td>
<td>72.8 Brix %</td>
</tr>
</tbody>
</table>
The odour and colour of the Eucalyptus oil and the derived PMD-citronellal acetal are similar whereas DBP is completely different being a clear, weakly aromatic liquid. The densities of the Eucalyptus oil and the derived PMD-citronellal acetal are similar whereas DBP is more dense. The refractive index for DBP was determined as the highest in Brix %. The viscosities of the Eucalyptus oil and the derived PMD-citronellal acetal are similar where the Eucalyptus oil is slightly more viscous. DBP has a low viscosity in comparison. The boiling points for the DBP plasticizer and bio-plasticizer are similar and much higher than that of the *Eucalyptus citriodora* oil with the bio-plasticizer’s boiling point being 10°C higher than that of DBP. The compounds were regarded as relatively insoluble in water however; derived PMD-citronellal acetal is slightly more soluble than the Eucalyptus oil and DBP.

The Eucalyptus oil used comprises of approximately 84.3 % Citronellal, 1.3 % PMD and 2.7 % PMD-citronellal acetal. Therefore the similarities of Eucalyptus oil and Eucalyptus derived PMD-citronellal acetal in appearance, odour, density, refractive index and solubility can be accounted for.

Comparing the properties of the derived PMD-citronellal acetal with those of DBP, it can be seen that the PMD-citronellal acetal enriched oil can be expected to have similar plasticizing properties to that of DBP as the two compounds have similar molecular weights and boiling points, and a similar structure, however, the acetal lacks the aromatic ring of the DBP thus making it a less reactive and safer compound than DBP. They are both liquids with similar refractive indices, making them similar to process in cosmetic formulations. However, the lower density, higher viscosity and greater water solubility of the acetal must be taken into consideration when substituting it for DBP in a cosmetic formulation.

<table>
<thead>
<tr>
<th>Viscosity @ 20°C, mPas (HAAKE Viscotester 550)</th>
<th>1.88 - 17</th>
<th>0.017</th>
<th>1.62-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point</td>
<td>200°C</td>
<td>340°C</td>
<td>350°C</td>
</tr>
<tr>
<td>Solubility in H₂O (g/L) @ 25°C</td>
<td>0.036</td>
<td>0.013</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*Note: Molecular structure obtained from ChemDraw Ultra 8.0.*
Chapter 3

An investigation of *Eucalyptus citriodora* derived p-Menthane-3,8-diol-citronellal acetal as a plasticizer in a nail polish cosmetic formulation

3.1 Background

Nail polish is composed of coloured particles (pigments) and polymers which are suspended into a solvent. When the product is applied on the nails, the solvent must evaporate rapidly to leave a homogenous film. When a plasticizer is incorporated into the formulation the following properties are enhanced:

- Film forming properties;
- Chipping prevention;
- Rapid drying time;
- Increased gloss;
- Improved adhesion;
- Greater aesthetic properties.\(^7\)

A nail polish was formulated to investigate the effectiveness of the bio-plasticizer in a cosmetic product. Studies of three different nail polish formulations were undertaken. The first formulation was a blank, in which no plasticizer was present. The second formulation included the di-Butyl phthalate plasticizer and the third formulation included the bio-plasticizer viz p-Menthane-3,8-diol-citronellal acetal. Refer to Table 3.1 on page 52. The formulations were evaluated to determine if the bio-plasticizer was as effective as the conventional phthalate plasticizer formulation.

The following properties of each formulation were determined: hardness, drying time, flexibility, adhesion, gloss, non-volatile content and homogeneity.
3.2 Materials

The chemicals used for the nail polish formulations 1, 2 and 3 are listed in Table 3.1 below with their chemical formulas, sources and grades.

Table 3.1: Reagents used for the formulations and analysis.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Source</th>
<th>Grade*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearalkonium bentonite</td>
<td></td>
<td>Carst &amp; Walker</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>n-Butyl acetate</td>
<td>C_6H_{12}O_2</td>
<td>Merck</td>
<td>99% GC</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>C_3H_6O</td>
<td>Merck</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>C_6H_7(NO_2)_3O_5</td>
<td>Bergerac NC</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>Polyester resin</td>
<td></td>
<td>Arkema</td>
<td>Laboratory</td>
</tr>
<tr>
<td>di-Butyl phthalate</td>
<td>C_{16}H_{22}O_4</td>
<td>Aldrich</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>para-Menthane-3,8-citronellal acetal</td>
<td>C_{20}H_{35}O_2</td>
<td>Synthesized</td>
<td>74% GC</td>
</tr>
<tr>
<td>Aluminium powder</td>
<td>Al</td>
<td>Merck</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>CH_3COOCH_2CH_3</td>
<td>Merck</td>
<td>AR &amp; Laboratory</td>
</tr>
</tbody>
</table>

*AR = Analytical reagent
3.3 Experimental

3.3.1 Formulations

Table 3.2 summarises and compares the three nail polish formulations that were prepared.

Table 3.2: The comparison of nail polish formulations containing different plasticizers.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Function</th>
<th>Formula #1 % (w/w)</th>
<th>Formula #2 % (w/w)</th>
<th>Formula #3 % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stearalkonium bentonite</td>
<td>Thixotropic agent</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>n-Butyl acetate</td>
<td>Solvent: Evaporated off after application onto the nails</td>
<td>29.72</td>
<td>22.72</td>
<td>22.72</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Dilutes the formulation for easy application</td>
<td>29.05</td>
<td>29.05</td>
<td>29.05</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>Primary film former</td>
<td>26.22</td>
<td>26.22</td>
<td>26.22</td>
</tr>
<tr>
<td>Polyester resin</td>
<td>Secondary film former</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>DBP</td>
<td>Plasticizer</td>
<td>-</td>
<td>7.00</td>
<td>-</td>
</tr>
<tr>
<td>PMD-citronellal acetal</td>
<td>Bio-Plasticizer</td>
<td>-</td>
<td>-</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium powder</td>
<td>Pigment of choice in the nail polish</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Solvent</td>
<td>1.07</td>
<td>1.07</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Method of preparation:

Phase A

Stearalkonium bentonite was added to n-Butyl alcohol with intensive stirring (speed setting: 4 on heating apparatus: FMH Instruments, 2 cm magnetic stirrer, at room temperature). Isopropyl alcohol was added to this mixture. Nitrocellulose, Polyester resin and DBP or PMD-citronellal acetal were added to the mixture in a closed system over a period of 5 minutes. This mixture was stirred for a further 5 minutes.

Phase B

The aluminium powder was slowly added to Ethyl acetate.

Phase B was added to Phase A and slowly mixed for 5 minutes until the mixture was homogenous. For each formulation 100 g was produced.
3.3.2 Nail polish testing methods

The testing was performed in a constant temperature room of 22 ± 1°C and with a humidity of 50 ± 3%. One replicate for each formulation was performed, excluding the Gloss test where 5 replicates were performed and the percentage non-volatile components, where 3 replicates were performed to minimize standard deviations.

The following equipment was used for the various tests:

- 6 Aluminium Q-panels (150 mm x 100 mm x ≤ 0.8 mm, Apollo Scientific)
- Sand paper (3 M, p360)
- White spirits
- 0.5 mm metal applicator
- Stop watch
- Dry sand (size: 150 µm – 300 µm, Acid washed sand C.P 50150, MINEMA)
- Camel-hair brush (Bristles 25-40 mm)
- Cylindrical plunger
- Cylindrical Mandrel apparatus (mandrels with diameters ranging from 32 - 2 mm)
- Magnifying glass
- 2 mm metal cutter
- Adhesive tape
- Micro-TRI-gloss µ meter
- 8 Staedtler® tradition pencils: 3B, B, H, 2H, 3H,4H, 5H and 6H

3.3.2.1 Aluminium plate preparation

The 6 aluminium plates, two of which are shown in Figure 3.1 were cleaned as their surfaces were roughened by the application of sand paper in circular motions. The plates were wiped down with white spirits, an aliphatic solvent, and left to dry for 5 minutes. This renders a clean working surface for the various tests.

Figure 3.1: The Q-panel Aluminium plate used for the film tests to follow (Panel on left, unroughened, panel on right, roughened).
3.3.2.2 Surface drying test

A surface dry test was performed according to SABS (South African Bureau of Standards) Method 148,\textsuperscript{72} which is a method used to determine the time required for the product to dry. A prepared aluminium plate was smeared using a metal applicator to form a 0.5 mm thick nail polish layer. After each time interval of 1 minute, acid-washed sand was sprinkled onto a portion of this panel from a height of 150 mm. The adhesion of the sand onto the nail polish was evaluated. This procedure was repeated every minute until all the sand could be brushed off with a camel brush. The surface drying time is the time at which no sand particles adhere to the nail polish. Figure 3.2 illustrates the experimental method. One replicate for each formulation was performed.

![Illustration of plate smearing and brushing procedure to determine film drying time.](image)

3.3.2.3. Hard dry test

This test is a measure of the time which is required for a formulation to be dry on the surface as well as through the entire thickness of the film. It is the time required when the nail polish cannot be distorted or removed from the aluminium panel.

A cylindrical plunger which was fitted with a cloth covered rubber tip (approximately 25 mm) was used in the Hard-dry test.\textsuperscript{73} See Figure 3.3. The time from the Surface-drying test was

---

\textsuperscript{72} SABS (South African Bureau of Standards).

\textsuperscript{73} Cylindrical plunger with a cloth covered rubber tip.
continued for this test. The painted aluminium panel used to complete the Surface-drying test was placed on the base of the plunger and the handle was turned which caused the panel to rotate at 90°. This continued until the plunger did not distort any of the nail polish. The time for complete dryness was then recorded. One replicate for each formulation was performed.

![Cylindrical plunger used for the Hard-dry test.](image)

**3.3.2.4 Gloss test**

The Gloss test[^74] was used to determine the amount of light reflected from a surface. It is aesthetically pleasing for consumers to have a “glossy” final product. This device, shown in Figure 3.4 indicates the various glosses occurring at the angles: 20°, 60° and 85°. The angles of 20° and 60° are used for medium and high gloss formulations and not used for formulation comparison in this study as aluminium was used as the pigment of choice in the formulations and contributes to a lower gloss. For this study the gloss occurring at 85° was used for the comparison between formulations. The meter was calibrated and set with specific standards.

The painted aluminium panels were allowed to dry for 24 hours. The Micro-TRI gloss meter was placed over the panels and measured. The process was repeated to obtain 5 readings of which the average was recorded. The results are displayed on the screen and a fail or pass can be determined. The units for gloss are Gloss Units (GU), which vary between 1 and 100, a value above 50 being considered a pass. This procedure was repeated 5 times to ensure accuracy of the gloss readings.
3.3.2.5 Adhesion: Cross adhesion test

The principle of the Cross adhesion test\textsuperscript{75} is to determine the strength of adhesion. The American classification system of ASTM (American Society for Testing and Materials) was used, which is a scale of 1-5, where 5 is the strongest and 1 is the weakest.

A lattice pattern is cut with a 2mm metal cutter, to produce a square with particular dimensions. The cutter is used to mark the panel with horizontal and vertical lines at right angles to one another to produce a lattice with points. Adhesive tape is placed on the lattice and pressed down for 30 seconds. After this time interval, the tape is rapidly removed at 180°. See Figure 3.5. One replicate of this procedure was performed for each formulation.

The state of the lattice and thus strength of adhesion is evaluated by using the ASTM rating system illustrated in Table 3.3 below.
Table 3.3: The ASTM rating system used to evaluate the formulations for the Cross-hatch test for adhesion.

<table>
<thead>
<tr>
<th>ASTM rating</th>
<th>Surface</th>
<th>Description</th>
<th>Adhesion strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td><img src="image1.png" alt="Image" /></td>
<td>The edges of the cuts are completely smooth; none of the squares of the lattice are detached.</td>
<td>Strongest</td>
</tr>
<tr>
<td>4</td>
<td><img src="image2.png" alt="Image" /></td>
<td>There are small flakes which are detached, mostly at the intersections. The total affected lattice area is 5%.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image" /></td>
<td>There are small flakes which are detached at the edges and the intersections. Between 5-15% of the total lattice is affected.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><img src="image4.png" alt="Image" /></td>
<td>There are flakes along the edges and squares are removed. Between 15-35% of the total lattice is affected.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><img src="image5.png" alt="Image" /></td>
<td>The coating has flaked along the edges of the cuts in large ribbons and whole squares detached. The area is 35% - 65% of the lattice.</td>
<td>Weakest</td>
</tr>
</tbody>
</table>
3.3.2.6 Flexibility

The Cylindrical Mandrel method was used to determine the flexibility of the formulations. Flexibility is the property which allows the bending of the film without breaking or cracking. The mandrels are illustrated in Figure 3.6.

Aluminium plates for each formulation were prepared and allowed to dry for 24 hours. The test involves bending the aluminium plates for each formulation onto cylindrical mandrels of defined diameters. The mandrels diameters range from 32 mm – 2 mm. When distortion or cracking occurs at the specific diameter mandrel, this indicates a fail. The mandrels starting from 5 mm up to 2 mm were used for bending, as 5mm gave a pass. A magnifying glass was used to inspect the plates. The aim is to find the lowest diameter mandrel at which no cracking or distortion occurs. Figure 3.7 shows a bent plate containing the blank formulation which failed the test at 2 mm. One replicate for each formulation was performed.

Figure 3.6: The Cylindrical Mandrel test apparatus used to determine flexibility.

Figure 3.7: The Blank formulation illustrating a fail at 2 mm as cracking occurred.
3.3.2.7 Hardness

The Pencil-Hardness test is a measure of the resistance of the nail polish to scratching. Pencils with the lead hardness of 3B to 6H are used, where the 3B is the softest and 6H is the hardest.

Aluminium panels are prepared with the nail polish formulations and allowed to dry for 24 hours. The pencils are held at a 45° angle in a pencil holder apparatus and firmly pushed on the smeared aluminium panels. Every pencil was used on the panels starting from the softest to the hardest. The hardest pencil which does not indent or scratch the nail polish is then used to establish the ‘hardness.’ One replicate of this test was performed.

Figure 3.8: The Staedler® pencils used for the pencil hardness test, ranging from 3B to 6H.

3.3.2.8 Non-volatile contents

This procedure is performed to determine the percentage non-volatile contents in each of the formulations. The principle of this test is to determine the amount of non-volatile components by means of heating the nail polish and determining the difference in mass. Pre-weighed foiled petri dishes were prepared with 3 g of a formulation. These dishes were placed in a pre-heated oven at 110±5°C for 1 hour. A period of 1 hour was used as a time period to enable sufficient evaporation of all volatile components. A temperature of 110°C was used so as not to cause any decomposition of non-volatile components and to ensure all volatile components have evaporated. After the time period the dishes were weighed immediately. The solid residue which remained is the non-volatile content assuming that all the volatile solvent evaporated off. Figure 3.9 and 3.10 illustrate the three nail polish formulations used in the test. This procedure was repeated in triplicate for each formulation.
The non-volatile content is determined using the following formula:

\[
\% \text{ Non-volatile contents} = \frac{F - E}{I - E} \times 100
\]

\text{eq 3.1}

Where:
- \( F \) = Final mass of foiled petri dish + product after 1 hour
- \( E \) = Mass of foiled empty petri dish
- \( I \) = Initial mass of the foiled petri dish with ± 3 g of product.

A higher percentage non-volatile content is favourable since it indicates a lower amount of solvent in the formulations.

Figure 3.9: The nail polish formulations on each pre-weighed petri dish at time 0. From left to right: Formulation 1; Formulation 2; Formulation 3.

Figure 3.10: The nail polish formulations on each petri dish after 1 hour heating at 110°C. From left to right: Formulation 1; Formulation 2; Formulation 3.
3.3.2.9 Homogeneity

This test refers to the homogenous distribution of the formulation. A glass rod was dipped into each of the formulations and removed to be classified into one of three different categories.

   a) No settlement onto the glass rod.
   b) Soft settlement; the glass rod is slightly covered.
   c) Thick settlement; the glass rod is covered with the nail polish.

The homogeneity for nail polishes is regarded as acceptable in the cases of a) and b).79
### 3.4 Results and discussion

The results of the various tests described above are summarized in Table 3.4 for comparison of the three formulations. Replicate values have been included in the Appendix. See Table 7.7 on page 108, for further statistical data.

Table 3.4: The results from the various nail polish tests for each formulation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Formulation # 1 (Blank)</th>
<th>Formulation # 2 (DBP)</th>
<th>Formulation #3 (PMD-citronellal acetal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Surface-drying test</td>
<td>11 minutes</td>
<td>4 minutes</td>
<td>5 minutes</td>
</tr>
<tr>
<td>2. Hard-dry test</td>
<td>13 minutes</td>
<td>5 minutes</td>
<td>6 minutes</td>
</tr>
<tr>
<td>3. Gloss at 85°</td>
<td>Fail: 47.4 GU</td>
<td>Pass: 53.3 GU</td>
<td>Pass: 50.23 GU</td>
</tr>
<tr>
<td></td>
<td>SD: 0.2236</td>
<td>SD: 0.1414</td>
<td>SD: 0.1504</td>
</tr>
<tr>
<td></td>
<td>Pass: 80.0 GU</td>
<td>Pass: 80.4 GU</td>
<td>Pass: 91.1 GU</td>
</tr>
<tr>
<td></td>
<td>SD: 0.4848</td>
<td>SD: 0.1885</td>
<td>SD: 0.3361</td>
</tr>
<tr>
<td></td>
<td>Fail: 30.9 GU</td>
<td>Fail: 32.5 GU</td>
<td>Fail: 35.2 GU</td>
</tr>
<tr>
<td></td>
<td>SD: 0.2449</td>
<td>SD: 0.0612</td>
<td>SD: 0.1904</td>
</tr>
<tr>
<td>4. Adhesion</td>
<td>Weak: 1</td>
<td>Strong: 5</td>
<td>Strong: 5</td>
</tr>
<tr>
<td>5. Flexibility</td>
<td>Pass: 4 mm</td>
<td>Pass: 3 mm</td>
<td>Pass: 3 mm</td>
</tr>
<tr>
<td></td>
<td>Fail: 3 mm</td>
<td>Fail: 2 mm</td>
<td>Fail: 2 mm</td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Acceptable</td>
</tr>
<tr>
<td>7. % non-volatile components</td>
<td>58.1%</td>
<td>65.7%</td>
<td>64.4%</td>
</tr>
<tr>
<td></td>
<td>SD: 1.213</td>
<td>SD: 0.8468</td>
<td>SD: 0.5568</td>
</tr>
</tbody>
</table>

Surface-drying time is considered a very important parameter in nail polish formulations. A very important aesthetic quality for customers is rapidly drying nail polish. Therefore, the lower the Surface-drying time, the better it is. Formulation 2 obtained the best drying time of 4 minutes. Formulation 3 followed at 5 minutes. However, the positive effect of a plasticizer on surface-drying time is noted, since drying time is significantly lower in presence of either plasticizer.
Hard-drying time indicates how long it takes for the formulation to be completely dry and resist any distortion. Formulation 2 was again better than formulation 3: the formulation incorporating DBP as a plasticizer completely dried 1 minute faster than the formulation with the bio-plasticizer.

Gloss is an indication of the luminous reflection of the product. Gloss is considered acceptable above 75% and usually occurs at 90%. However, Aluminium was used as the pigment in all three formulations and contributes to lower gloss readings regardless of the angles (20°, 60° and 85°) which the Micro-TRI gloss meter reads. For comparison purposes the readings at 85° were used to determine the effectiveness of the formulations in terms of gloss. Both formulations 2 (53.3%) and 3 (50.23%) are considered passes; however the formulation with DBP fairs slightly better as can be seen by the higher percentage value.

Adhesion and flexibility are predicted to increase as the amount of plasticizer in the formulation increases. The blank formulation was shown to have very weak adhesion to the plate. The coating had flaked along the edges of the cuts in large ribbons with whole squares of film detached. The area of 35% - 65% of the lattice was affected. Formulations 2 and 3 were considered to have very strong levels of adhesion as they both were evaluated having the highest ASTM score of 5. The edges of the cuts were completely smooth; none of the squares of the lattice was detached.

Flexibility is considered favourable when the lowest diameter mandrels are used and no cracking or distortion of the film occurs. The lowest diameter mandrel available with the Cylindrical Mandrel apparatus was 2 mm. Formulation 2 and 3 scored the same as they both passed using the 3 mm mandrel and failed at the 2 mm mandrel. Therefore both formulations are considered to have very good flexibility. The blank formulation cracked and peeled when the 3 mm mandrel was used.

Formulation 3 had an acceptable hardness. The hardness test criteria ranges from 6H to H, therefore it is classified as acceptable because 2H is less than 6H (very hard) and more than 3B (very soft). It can be seen from the results that the formulation which contains PMD-citronellal acetal (2H) has a lower hardness than the formulation which contains DBP as the plasticizer (3H) and both were lower than the formulation with no plasticizer. The hardness, however, is still considered an acceptable pass.
According to the results obtained, the bio-plasticizer formulation has a lower percentage of non-volatile components in the formulation. Therefore the formulation has more volatile ingredients than the DBP formulation.

Formulation 3 was shown to have hard settlement. This is due to the viscosity of the bio-plasticizer. The viscosity of PMD-citronellal acetal oil is much higher than that of DBP, therefore contributing to its “harder settlement” on the glass rod during the homogeneity evaluation. Formulation 3 was considered unacceptable, as hard settlement occurred on the glass rod.

In conclusion, PMD-citronellal acetal can be considered as a natural alternative to DBP. Their performance characteristics are very similar, with the exception of homogeneity. This could negatively affect consumers’ acceptance of a product containing the acetal. However, for health and environmentally conscious consumers the bio-plasticizer offers peace of mind, while only compromising slightly on the physical properties.
Chapter 4

An investigation of p-Menthane-3,8-diol-citronellal acetal as a bio-plasticizer in a perfume formulation

4.1 Background

For many centuries man has used natural oils and extracts as a means to mask body odour. Natural compounds included sweet smelling flowers or fruit, natural spices and herbs, pungent bark and plants. Examples of plants include lavender, rose, cinnamon and jasmine flowers etc. Scents which give fragrances their unique characteristics are often short lived as they dissipate over time. This is especially the case with aerosol sprays which are often used for perfumes, colognes and other hair or skin cosmetic applications.

A perfume formulation comprises of different notes. A note is described as a distinctive odour. These notes include:

- Top note; the first fragrance impression.
- Middle note; the main fragrance theme of the perfume, with immediate volatility.
- Base note; the longest lasting fragrance theme of the perfume, with low volatility.

To maintain the fragrance balance of a perfume formulation, slow release properties need to be incorporated into the formulation. Therefore a suitable plasticizer and polymer fixative mixture needs to be addressed. The topical application of the fragrance requires an appropriate carrier to the skin to allow for easy dispersion and evaporation of the solvent to ensure the successful delivery of the fragrance. This ensures the aesthetic satisfaction to the consumer.
Controlled released systems such as perfumes and fragrances should fulfil the following criteria:  

1. The formulation should contain stable polymers with prolonged storage periods.  
2. The polymer should maintain the constant release of the signature fragrance over a period of time.  
3. The release period should be between 6-12 hours.  
4. Polymers which are non-toxic and non-carcinogenic should be used.

In this study, the perfume formulation consists of a polymer fixative, plasticizer, solvent and the fragrance. The fixative comprises of various polymers. Polymers are used as they are very stable, allow for the slow release of fragrance molecules and decrease the evaporation rate of the fragrance. The plasticizer of interest (DBP or PMD-citronellal acetal) boosts the properties of the fixative by enhancing the slow release of the fragrance molecules, thereby further decreasing the evaporation rate. The plasticizer also plays an important role in film formation. This property is desired in a perfume as this enables the prolonged adhesion of the fragrance to the skin. This results in decreased evaporation of the active ingredient (fragrance) and minimises rub-off.
4.2 Materials

The materials used in this study are listed in Table 4.1.

Table 4.1 List of chemicals required for the perfume formulations.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Chemical Formula</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merquat 10 (Polyquaternium -10)</td>
<td>Nalco</td>
<td>-</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>SF 1288 emulsifier (PEG 1,2-dimethicone)</td>
<td>Durotec</td>
<td>C_{2n+2}H_{4n+6}O_{n+2} + (CH_3)_{2}O_nSi(CH_3)_3</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose</td>
<td>Uniresins</td>
<td>-</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>Propylene glycol (Propane-1,2-diol)</td>
<td>Aldrich</td>
<td>C_3H_8O_2</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Baycusan® C1003 (Polyurethane -32)</td>
<td>GE Bayer Silicone</td>
<td>-</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>Sharon Bolel</td>
<td>-</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>DBP (di-Butyl phthalate)</td>
<td>Aldrich</td>
<td>C_{16}H_{22}O_4</td>
<td>99% RG</td>
</tr>
<tr>
<td>PMD-citronellal acetal (para-Menthane-3,8-diol-citronellal acetal)</td>
<td>Synthesized</td>
<td>C_{20}H_{36}O_2</td>
<td>73.8% Synthesized</td>
</tr>
<tr>
<td>p-Anisaldehyde</td>
<td>Aldrich</td>
<td>CH_3OC_6H_4CHO</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Ethanol (Ethanol (95%) Industrial)</td>
<td>Associated Chemical Enterprises</td>
<td>C_3H_5OH</td>
<td>99% AR*</td>
</tr>
<tr>
<td>Methyl benzoate</td>
<td>Aldrich</td>
<td>C_8H_8O_2</td>
<td>99% GC</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>NMMU laboratory</td>
<td>H_2O</td>
<td>Laboratory</td>
</tr>
</tbody>
</table>

*AR = Analytical reagent
4.3 Experimental

4.3.1 Aim

The aim of the experiment was to determine if PMD-citronellal acetal had similar plasticizing properties to the conventional DBP used in certain perfume formulations. This was determined by measuring the degree of evaporation of a fragrance molecule in a perfume formulation containing either plasticizer over a certain time period in a heated chamber. The data was analysed with a GC-MS and the various fragrance peaks were tabulated.

4.3.2 Preparation of the fixative

A fixative was prepared prior to preparing actual perfume formulations. The fixative “holds” the scent together once applied topically to the skin. Phase A and B of the fixative were prepared separately then B was added to A to form the final fixative mixture. Table 4.2 shows the fixative formulation with weight percentage of the ingredients.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>% (w/w)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHASE A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyquaternium-10</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>78.15</td>
<td>98</td>
</tr>
<tr>
<td>PEG-1,2-dimethicone</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td><strong>PHASE B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxypropyl cellulose</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>15.9</td>
<td>20</td>
</tr>
<tr>
<td>Polyurethane - 32</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>0.32</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>125.4</td>
</tr>
</tbody>
</table>

**Phase A**

Phase A was prepared by adding 2 g of Polyquaternium-10 to 98g of distilled water and the mixture stirred (speed setting: 4 on apparatus: FMH Instruments, at room temperature, 2 cm magnetic stirrer). To the mixture, 1g of PEG-1,2-dimethicone was added and stirred. This completes Phase A.
Phase B
Phase B was prepared by adding 2g of Hydroxypropyl cellulose to 20g of Propylene glycol until the mixture was clear. To the clear mixture, 2g of Polyurethane-32 was added and stirred till clear. The 0.4g of Coconut oil was added to the mixture with constant stirring. Phase A and B were mixed together. This completes the fixative mixture.

4.3.3 Perfume formulations

Three perfumes were formulated, one containing no plasticizer, one containing DBP and one containing PMD-citronellal acetal. Each perfume contains the relevant plasticizer, a highly volatile solvent, the fixative and a fragrance. The blank formulation contained no plasticizer therefore the difference in weight was made up with the solvent, Ethanol. A total of 100g was prepared for each formulation. Table 4.3 compares the three formulations in terms of their composition.

Table 4.3: Reagents used for each perfume formulation, measured in grams.

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula 1 (Blank)</th>
<th>Formula 2 (DBP)</th>
<th>Formula 3 (PMD-citronellal acetal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fixative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>-</td>
<td>2.02</td>
<td>-</td>
</tr>
<tr>
<td>PMD-citronellal acetal</td>
<td>-</td>
<td>-</td>
<td>2.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>93.00</td>
<td>90.98</td>
<td>90.98</td>
</tr>
<tr>
<td>p-Anisaldehyde (Fragrance)</td>
<td>2.0225</td>
<td>2.0225</td>
<td>2.0225</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.03</strong></td>
<td><strong>100.03</strong></td>
<td><strong>100.03</strong></td>
</tr>
</tbody>
</table>

Method of preparation:

The fixative, p-anisaldehyde and plasticizer were weighed in separate beakers. A small volume from the total ethanol was used to “dissolve” the chemicals to ensure that all remainder residues were placed into each formulation’s separate 100 ml volumetric flask. Small volumes of ethanol were poured and stirred to ensure that no chemicals adhere to the
walls of the volumetric flask. The 100 ml volumetric flask was filled to the mark with ethanol and a homogenous solution was made.

4.3.4 Evaporation

The evaporation rate of p-anisaldehyde in each perfume formulation was investigated by GC analysis using methyl benzoate as the internal standard. The experiment was performed over 3 consecutive days. An 8 hour experiment was performed at 31°C, since this is close to the skin’s temperature. Each formulation was incubated at 31°C for the time intervals of: 0, 2, 4, 6 and 8 hours. The experiment was performed in triplicate at each time interval.

Procedure for each formulation:

- The oven was preheated at 31°C for 20 minutes.
- 12 petri dishes containing 2 ml of formulation (corresponding to 3 replicates at each time interval) were placed in the preheated oven.
- For the time zero experiment 2 ml of a perfume formulation was added to a 10 ml volumetric flask. 0.5 ml of Methyl Benzoate solution (0.2 M) was added to the flask and the volume made up with Ethanol. This sample was poured into a GC vial and used for GC analysis. Repeated in triplicate.
- After each incubation time interval; the petri dishes were taken out the oven and 2 ml of ethanol was used to dilute the perfume residue (performed in duplicate for each petri dish). The washings were placed into a 10 ml volumetric flask. A volume of 0.5 ml Methyl Benzoate solution (0.2 M) was added into each volumetric flask. The volume was made up with Ethanol. This sample was poured into a GC vial and used for GC analysis. Repeated in triplicate.
After incubation, the GC analysis procedure was performed as quickly as possible to deter any further evaporation. Figures 4.1 and 4.2 show the residues of the formulations after 8 hours of incubation.

![Image 1](image1.png)

**Figure 4.1:** The petri dish with 2 ml of the DBP formulation, after the 8 hour incubation period at 31°C.

![Image 2](image2.png)

**Figure 4.2:** The petri dish with 2 ml of the PMD-citronellal acetal formulation, after the 8 hour incubation period at 31°C.

### 4.4 Analytical Techniques

Gas Chromatography was used as an analytical technique to quantify the following peaks: Methyl benzoate, p-anisaldehyde, PMD-citronellal acetal and DBP.

#### 4.4.1 Gas Chromatography

Analysis was performed on a HP 5890 series gas chromatograph. The data was processed by HP 61034 C software using a Hewlett Packard computer. The RTX 35 ms column was fitted in the GC (length: 30 m x 0.25 mm ID x 0.25 mm thickness). The various settings were programmed as follows:
Initial column temperature : 70°C  
Initial column hold time : 5 minutes  
Final column temperature : 280°C  
Final column hold time : 5 minutes  
Heating rate : 10°C/min  
Injector temperature : 250°C  
Injector volume : 1µl  
Detector : FID detector  
Split flow : 60 ml/min  
Carrier gas : Helium at constant flow (1 ml/min)  
Run time : 30 minutes  

4.5 Calculations  

The initial concentration of the fragrance in each formulation was calculated.  
Let:  
\[ C = \text{concentration (mol/dm}^3\text{)} \]  
\[ m = \text{mass (g)} \]  
\[ M_m = \text{molecular mass (g/mol)} \]  
\[ v = \text{volume (L)} \]  

Concentration of the fragrance in each formulation  

The fragrance incorporated into the formulations was p-anisaldehyde with a molecular mass of 136.15 g/mol. A 100ml (0.1 L) volumetric flask was used to prepare the perfume formulations. The actual mass of p-anisaldehyde in each formulation was 2.0225g.  

Therefore the concentration of p-anisaldehyde is calculated as follows:  

\[ C_{\text{Fragrance}} = \frac{m}{M_m \times v} \]  
\[ C = \frac{2.0225}{(136.15 \times 0.1)} \]  
\[ C = 0.148 \text{ moles/dm}^3 \]
Concentration of the internal standard: 0.2 M Methyl benzoate

Methyl benzoate was used as the internal standard, for the GC analysis studies. Methyl benzoate solution (0.2M) was prepared in 100 ml (diluted with ethanol) as follows:

\[ C_{\text{internal standard}} = \frac{m}{M \cdot m \cdot \nu} \] \hspace{1cm} \text{eq 4.1}

\[ C_{\text{internal standard}} = \frac{2.72146}{(136.1 \times 0.1)} \]

\[ C_{\text{internal standard}} = 0.1998 \text{ moles/dm}^3 \]

Concentrations of solutions for analysis

For the GC analysis at time zero, 0.5 ml of 0.2M Methyl benzoate and 2 ml of perfume were added to a 10 ml volumetric flask and made up with Ethanol.

Therefore:

\[ \text{Dilution factor} = \frac{10}{0.5} = 20 \]

\[ C_{\text{Methyl Benzoate}} = \frac{0.1998}{20} \]

\[ C_{\text{Methyl Benzoate}} = 0.01 \text{ moles/dm}^3 \]

Concentration of p-anisaldehyde in the 10 ml volumetric flasks

\[ \text{Dilution factor} = \frac{10}{2} = 5 \]

\[ C_{\text{p-anisaldehyde}} = \frac{0.148}{5} \]

\[ C_{\text{p-anisaldehyde}} = 0.03 \text{ moles/dm}^3 \]

Response factor

The concentration of p-anisaldehyde was determined in fragrance samples using methyl benzoate as the internal standard. The relative response factor for each formulation was determined.

The fragrance (p-anisaldehyde) and internal standard (methyl benzoate) had retention times of ±7.9 and 5.4 minutes, respectively. This indicates that the peaks are clearly defined and distinguished from one another and that no overlapping of peak areas occurs.
Analytical methods validation is an important regulatory requirement for analysis. The response factor is calculated to determine the accuracy of the results.

\[
R = \frac{A_{Is} \times C}{A_x \times C_{Is}} \quad \text{eq 4.2}
\]

- \( R \) = Response factor for each formulation.
- \( A_{Is} \) = Peak area for the Internal standard, obtained from the GC data.
- \( C \) = Concentration of the fragrance (mol/dm\(^3\)).
- \( A_x \) = Peak area for the fragrance obtained from the GC data.
- \( C_{Is} \) = Concentration of the Internal standard.

**Formulation without plasticizer (Blank)**

\[
R = \frac{A_{Is} \times C}{A_x \times C_{Is}} \quad \text{eq 4.2}
\]

\[
R = \frac{1061427 \times 0.03}{3049651.33 \times 0.01}
\]
R = 1.04

**Formulation containing DBP**

\[
R = \frac{A_{is} \times C}{A_x \times C_{is}} \quad \text{eq 4.2}
\]

\[
R = \frac{1065378 \times 0.03}{4434446 \times 0.01}
\]

R = 0.72

**Formulation containing PMD-citronellal acetal**

\[
R = \frac{A_{is} \times C}{A_x \times C_{is}} \quad \text{eq 4.2}
\]

\[
R = \frac{1166256 \times 0.03}{4764720 \times 0.01}
\]

R = 0.73

**Results**

The concentration of the fragrance at each time interval is calculated using the following formula in each formulation.

\[
C = \frac{R \times C_{is} \times A_x}{A_{is}} \quad \text{eq 4.3}
\]

- **C** = Concentration mol/dm\(^3\) of fragrance.
- **R** = Response factor for each formulation.
- **C_{is}** = Concentration of the internal standard mol/dm\(^3\).
- **A_x** = Area under the peak for the fragrance.
- **A_{is}** = Area under the peak for the internal standard.

**Sample calculation for Blank formulation:**

\begin{align*}
R \text{ value} & = 1.04 \\
\text{Average area for internal standard, time 0} & = 1069190 \\
\text{Average area for fragrance, time 0} & = 3049651.33 \\
\text{Concentration of internal standard} & = 0.01 \text{ mol/dm}^3
\end{align*}
\[ C = \frac{R \times C_{\text{is}} \times A_{x}}{A_{\text{is}}} \]
\[ C = \frac{0.01 \times 1069190}{10.6256.33} \]
\[ C = 0.029664 \text{ mol/dm}^3 \]

**Sample calculation for DBP formulation:**

R value = 0.72

Average area for internal standard, time 0 = 1066256.333

Average area for fragrance, time 0 = 4434445.667

Concentration of Internal standard = 0.01 \text{ mol/dm}^3

\[ C = \frac{R \times C_{\text{is}} \times A_{x}}{A_{\text{is}}} \]
\[ C = \frac{0.72 \times 0.01 \times 4434445.667}{1066256.33} \]
\[ C = 0.029944 \text{ mol/dm}^3 \]

**Sample calculation for PMD-citronellal acetal formulation:**

R value = 0.73

Average area for internal standard, time 0 = 1065378.333

Average area for fragrance, time 0 = 4764720.1

Concentration of Internal standard = 0.01 \text{ mol/dm}^3

\[ C = \frac{R \times C_{\text{is}} \times A_{x}}{A_{\text{is}}} \]
\[ C = \frac{0.73 \times 0.01 \times 4764720.1}{1065378.33} \]
\[ C = 0.032648 \text{ mol/dm}^3 \]

**Calculation of error**

\[ E = \frac{t \times SD}{\sqrt{n}} \] ............................eq 4.4

\[ E = \frac{2.262 \times 3.21136 \times 10^{-5}}{\sqrt{3}} \]
\[ E = 4.1939 \times 10^{-5} \]
4.6 Results

The results obtained from the evaporation experiments are illustrated in Figure 4.4 with a trend line fitted to each data set. The fragrance concentrations were calculated from peak areas, R values and the internal standard concentration. Sample calculations were shown on page 76-77. Refer to Table 7.8 on page 109 in the Appendix for the actual data and statistical analysis.

In Figure 4.4, Control is the same as Blank (Formulation #1). The fragrance concentration is the concentration of p-anisaldehyde in the perfume formulation. Triplicate values have been plotted for each data point.

![Figure 4.4: Exponential fit for the three formulations.](image)

The general equation for the change of concentration of a substance with time is described as follows:

\[
\frac{dC}{dt} = -kC \text{ .......................... eq 4.5}
\]

where \( C \) = concentration

\( k \) is the rate constant

Integration of this equation gives the following exponential equation:

\[
C = C_0 e^{-kt} \text{ .......................... eq 4.6}
\]
where $C_0$ is the initial concentration at $t = 0$

It can be seen that the exponential trend lines for PMD-citronellal acetal and DBP perfume formulations are similar from approximately 5 – 8 hours, since they overlap. It can be seen that each formulation follows a similar trend. However, their similarity needs to be proved statistically.

### 4.6.1 Comparing the Blank formulation to DBP and PMD-citronellal acetal

A model with dummy variables was set up and tested in order to determine whether the differences in evaporation rates between the different formulations were significant or not. See Table 4.4. For comparing three sets of data, two dummy variables are needed e.g. $D_1$ and $D_2$.

**Table 4.4: Dummy variables for model comparing three sets of data**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>$D_1$</th>
<th>$D_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetal</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DBP</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

An exponential model does not provide a perfect fit, however, it was used to describe the model with dummy variables for each perfume formulation. The data for this model is found in Table 7.7 on page 107 in the Appendix. This model explains 97.8% in the observed fragrance concentrations ($R^2 = 0.978$). Refer to Table 4.5. The model validation is shown in Fig 7.1 and 7.2 in the Appendix on page 112. A statistician was consulted regarding the analysis, where Statistica version 11 was used.

The general exponential equation described in the previous section for the fragrance evaporation experiments incorporating dummy variables can be written as follows:

$$ Y = e^{\beta_0 + \beta_1 T + \beta_2 D_1 + \beta_3 D_2 + \beta_4 D_1 \text{Time} + \beta_5 D_2 \text{Time}} + \epsilon \quad \text{..................eq 4.7} $$

Where:

- $Y$ = Concentration
- $e^{\beta_0}$ = Initial concentration
- $\beta_1$ = Rate constant
- $\beta_2, \beta_3$ = True coefficients of the intercept
- $\beta_4, \beta_5$ = True coefficients of the rate constant
\[ T \] = Time
\[ \varepsilon \] = Residual
\[ D_1, D_2 \] = Dummy variables (= 1 or 0).

**Blank formulation equation**
Substituting the dummy variable values from Table 4 into equation 4.7 reduces the equation to:

\[ Y = e^{\beta_0 + \beta_1 T} + \varepsilon \] ................. eq 4.8

**PMD-citronellal acetal formulation equation**
Substituting the dummy variable values from Table 4 into equation 4.7 reduces the equation to:

\[ Y = e^{\beta_0 + \beta_1 T + \beta_2 + \beta_4 T} + \varepsilon \]

This simplifies to:

\[ Y = e^{\beta_0 + \beta_2 + (\beta_1 + \beta_4)T} + \varepsilon \] ................. eq 4.9

Note: The differences between equation 4.8 (Blank) and equation 4.9 (PMD-citronellal acetal) are the true coefficients: \( \beta_2 \) and \( \beta_4 \).

**DBP formulation equation**
Substituting the dummy variable values from Table 4 into equation 4.7 reduces the equation to:

\[ Y = e^{\beta_0 + \beta_1 T + \beta_3 + \beta_5 T} + \varepsilon \]

This simplifies to:

\[ Y = e^{\beta_0 + \beta_3 + (\beta_1 + \beta_5)T} + \varepsilon \] ................. eq 4.10

Note: The differences between equation 4.8 (Blank) and equation 4.10 (DBP) are the true coefficients: \( \beta_3 \) and \( \beta_5 \).

In order to determine which coefficients are significant, exponential regression analysis is done. The data used for the dummy model regression analysis is shown in Table 7.7 on page 108 in the Appendix. The results of the regression analysis are shown in Table 4.5 below.
Table 4.5: Exponential regression analysis comparing the blank to each formulation.

<table>
<thead>
<tr>
<th>True Coefficient</th>
<th>Estimate</th>
<th>Standard</th>
<th>t-value</th>
<th>p-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>0.076025</td>
<td>0.001568</td>
<td>48.4803</td>
<td>0.000000</td>
<td>0.072853</td>
<td>0.079197</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>-0.187798</td>
<td>0.007964</td>
<td>-23.5796</td>
<td>0.000000</td>
<td>-0.203908</td>
<td>-0.171689</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>-0.005767</td>
<td>0.028683</td>
<td>-0.2010</td>
<td>0.841706</td>
<td>-0.063783</td>
<td>0.052250</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.008579</td>
<td>0.028320</td>
<td>0.3029</td>
<td>0.763550</td>
<td>-0.048703</td>
<td>0.065861</td>
</tr>
<tr>
<td>$\beta_4$</td>
<td>0.061921</td>
<td>0.010036</td>
<td>6.1698</td>
<td>$p &lt; 1 \times 10^{-6}$</td>
<td>0.041621</td>
<td>0.082221</td>
</tr>
<tr>
<td>$\beta_5$</td>
<td>0.077766</td>
<td>0.009745</td>
<td>7.9804</td>
<td>$p &lt; 1 \times 10^{-6}$</td>
<td>0.058055</td>
<td>0.097476</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.978</td>
<td></td>
</tr>
</tbody>
</table>

To compare the blank formulation with the acetal formulation, two null hypotheses were determined:

$H_0 : \beta_2 = 0$ and $H_0 : \beta_4 = 0$

To test the hypotheses the p-values were evaluated from the exponential regression analysis. See Table 4.5. If the p value is greater than 0.05, the null hypothesis must be accepted and the coefficients are insignificant.

**Conclusion:**

As can be seen in Table 4.5, $\beta_2$ is found to be insignificant ($p = 0.841$). Thus the initial concentrations of the blank and the PMD-citronellal acetal fragrances are found to be equal. However, $\beta_4$ is significant ($p < 0.05$). Thus the PMD-citronellal acetal fragrance has a statistically significant different rate constant from that of the blank.

To compare the blank formulation with the DBP formulation, two null hypotheses were determined:

$H_0 : \beta_3 = 0$ and $H_0 : \beta_5 = 0$

To test the hypotheses the p-values were evaluated from the exponential regression analysis. See Table 4.5.

**Conclusion:**

As can be seen in Table 4.5, $\beta_3$ is found to be insignificant ($p = 0.763$). Thus the initial concentrations of the blank and the DBP fragrances are found to be equal. However, $\beta_5$ is significant ($p < 0.05$). Thus the DBP fragrance has a different rate constant from that of the blank.
4.6.2 Comparing the PMD-citronellal acetal formulation to the DBP formulation

When two sets of data are compared, one dummy variable, viz D, must be used. The variable has a value of either 0 or 1. Thus for DBP, the value is set at 0 and for the acetal the value was set at 1.

The exponential equation used was a simpler version of equation 4.5 below, as only one dummy variable was present in the equation.

\[ Y = e^{\beta_0 + \beta_1 T + \beta_2 D_1 + \beta_3 D_2 + \beta_4 D_4 Time + \beta_5 D_5 Time} + \epsilon \] ............................eq 4.7

\[ Y = e^{\beta_0 + \beta_1 T + \beta_2 D + \beta_3 DT} + \epsilon \] .............................................................eq 4.11

There are only four coefficients, viz \( \beta_0, \beta_1, \beta_2, \) and \( \beta_3 \).

**PMD-citronellal acetal formulation equation**

Let:

\( D = 1 \)

Substitute the Dummy variable values into equation 4.11:

\[ Y = e^{\beta_0 + \beta_2 + (\beta_1 + \beta_3)T} + \epsilon \] .............................................................eq 4.12

**DBP formulation equation**

Let:

\( D = 0 \)

Substitute the Dummy variable value into equation 4.9

\[ Y = e^{\beta_0 + \beta_1 T} + \epsilon \] .............................................................eq 4.13

Note: The differences between equation 4.12 (PMD-citronellal acetal) and equation 4.13 (DBP) are the true coefficients: \( \beta_2 \) and \( \beta_3 \). From the calculated p values for the formulations, two null hypotheses were determined:

\( H_0: \beta_2 = 0 \) and \( H_0: \beta_3 = 0 \)
- If both hypotheses are accepted, $\beta_2$ and $\beta_3$ are insignificant (i.e. equal to zero) and equation 4.12 will resemble the equation 4.13, therefore the two perfume formulations have the same evaporation curve.

- If both hypotheses are rejected, $\beta_2$ and $\beta_3$ are significant in equation 4.12; therefore the acetal and the DBP equations (4.12 and 4.13 respectively) are not the same. This would prove that the two perfumes have different evaporation curves.

From the data in Table 7.10 (page 111) in the Appendix, an exponential regression analysis was calculated to determine which coefficients describe each formulation. From the p-values the true coefficients are determined. See Table 4.7.

### Table 4.6: Exponential regression analysis comparing PMD-citronellal acetal to DBP

<table>
<thead>
<tr>
<th>True Coefficient</th>
<th>Variables</th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t-Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>Intercept</td>
<td>-3.563</td>
<td>0.02572</td>
<td>-138.5</td>
<td>7.91E-39</td>
<td>-3.616</td>
<td>-3.510</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>Time</td>
<td>-0.100821</td>
<td>0.005250</td>
<td>-19.20</td>
<td>7.04E-17</td>
<td>-0.1116</td>
<td>-0.09002</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>D</td>
<td>0.1061</td>
<td>0.03637</td>
<td>2.917</td>
<td>0.007179</td>
<td>0.03136</td>
<td>0.1809</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>D.Time</td>
<td>-0.01697</td>
<td>0.007425</td>
<td>-2.286</td>
<td>0.03064</td>
<td>-0.03223</td>
<td>-0.001712</td>
</tr>
</tbody>
</table>

The following deductions can be made to determine if the evaporation trends of DBP is different to PMD-citronellal acetal. Using the p-values in Table 4.6 the following simplifications can be made:

**DBP formulation vs PMD-citronellal acetal formulation**

$H_0$: $\beta_3 = 0$

The null hypothesis cannot be accepted ($p = 0.030642$) and this term is significant in the PMD-citronellal acetal equation 4.12.

$H_0$: $\beta_2 = 0$

The null hypothesis cannot be accepted ($p = 0.007179$) and this term is significant in the PMD-citronellal acetal equation 4.12.

Therefore the two perfumes have different true coefficients determining their rate constants and would have different evaporation curves.
Conclusions:
There is evidence to suggest that the rate constants of the fragrances PMD-citronellal acetal and DBP differ significantly.

4.6.3 Evaporation rate constant determination
Using the data from Table 4.5 in section 4.5.1 and the statistical conclusions reached, the true coefficients which are statistically significant can now be summarised below:

PMD-citronellal acetal
The hypothesis $H_0: \beta_2 = 0$ was accepted ($p = 0.841706$). Thus eq 4.9 reduces to

$$Y = e^{\beta_0 + (\beta_1 + \beta_4)T} + \varepsilon \quad \ldots \ldots \quad \text{eq 4.14}$$

Thus the rate constant $= \beta_1 + \beta_4$

DBP
The hypothesis $H_0: \beta_3 = 0$ was accepted ($p = 0.763550$). Thus eq 4.10 reduces to

$$Y = e^{\beta_0 + (\beta_1 + \beta_5)T} + \varepsilon \quad \ldots \ldots \quad \text{eq 4.15}$$

Thus the rate constant $= \beta_1 + \beta_5$

Blank
The equation for the Blank is eq 4.8

$$Y = e^{\beta_0 + \beta_1T} + \varepsilon \quad \ldots \ldots \quad \text{eq 4.8}$$

Thus the rate constant $= \beta_1$

The evaporation rate of each perfume formulation could now be calculated by substitution of the values for the relevant Dummy variables from Table 4.5. The calculated rate constants are shown in Table 4.7 below.

<table>
<thead>
<tr>
<th>Table 4.7: Calculated rate constants.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Blank</td>
</tr>
<tr>
<td>DBP</td>
</tr>
<tr>
<td>PMD citronellal acetal</td>
</tr>
</tbody>
</table>
4.6.4 Half-life

The rate constant can be used to determine the percentage of fragrance remaining in the formulation after a given time using the following equation:

\[
\text{Rate} = \frac{\ln \left( \frac{C}{C_0} \right)}{t}
\]

where \( C_0 \) is the initial concentration of fragrance, \( C \) is the concentration at time \( t \) measured in hours.

Using eq 4.16, the percentage of fragrance remaining after 8 hours was calculated and the results are shown in Table 4.8.

**Calculation of the half-life of the fragrance evaporation**

The half-life \( T_{1/2} \) is the time observed when the perfume in the formulation is reduced to half its initial concentration. Half-life is measured in hours.

Rearranging equation 4.16, the half-life can be determined as follows:

\[
T_{1/2} = \frac{\ln(0.5)}{k}
\]

Where \( k \) is the rate constant.

The half-life for each formulation was calculated and tabulated in Table 4.8 below.

Table 4.8: Half-life values and percentage fragrance remaining after 8 hours for each formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Rate constant</th>
<th>Half-life (hours)</th>
<th>% fragrance remaining after 8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-0.1877</td>
<td>3.7</td>
<td>22</td>
</tr>
<tr>
<td>Acetal</td>
<td>-0.1258</td>
<td>5.5</td>
<td>36.6</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.1100</td>
<td>6.3</td>
<td>41.5</td>
</tr>
</tbody>
</table>
4.7 Conclusion

The following conclusions can be drawn from the statistical analysis of the data.

The initial concentrations of the fragrance in the blank and the PMD-citronellal acetal formulations were found to be equal (p = 0.841, from regression analysis). The PMD-citronellal acetal fragrance evaporation has a different rate constant from that of the blank formulation (p < 1 x 10^{-6}, from regression analysis).

The initial concentrations of the fragrance in the blank and the DBP formulations were found to be equal (p = 0.763, from regression analysis). The DBP fragrance has a different rate constant from that of the blank (p < 1 x 10^{-6}, from regression analysis).

The perfume formulations of DBP and PMD-citronellal acetal were compared to determine if their evaporation trends were similar. Both hypotheses were not accepted, i.e β_2 and β_3 are significant (i.e not equal to zero) and equation 4.13 does not resemble equation 4.12, therefore the two perfume formulations do not have the same evaporation curve. This would prove that the two perfumes have different evaporation curves.

From the data in Table 4.8, it can be concluded that the evaporation rate was the slowest for the DBP perfume, as the amount of fragrance remaining after the 8 hour incubation experiment was 41.5 %. The PMD-citronellal acetal perfume closely followed with 36.6% remaining fragrance after 8 hours. Both formulations containing plasticizers were significantly more effective in retaining the fragrance than the blank formulation.

Therefore it was concluded that DBP was slightly more effective than PMD-citronellal acetal as a plasticizer and fragrance fixative in a perfume formulation. DBP had the “slowest” evaporation rate (Rate = -0.110033) and a half-life of 6.3 hours while the PMD-citronellal acetal perfume closely followed with an evaporation rate of -0.1258 and a half-life of 5.5 hours. These differences were statistically significant.
Chapter 5

Stability testing of the cosmetic formulations.

5.1 Stability testing

The nail polish formulations were tested to determine their stabilities. These tests are performed to ensure that the cosmetic products meet their intended physical and chemical quality standards. The product must maintain its functionality and aesthetics. Stability tests were done under accelerated conditions and in real time to determine how stable the product was.

Table 5.1: List of the equipment used for stability testing.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specifications</th>
<th>Equipment Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>@ Room temperature</td>
<td>pH-meter</td>
</tr>
<tr>
<td>Temperature</td>
<td>0°C, 40°C</td>
<td>Deepfreeze, InterCo Sanas Oven</td>
</tr>
<tr>
<td>Refractive index</td>
<td>@ Room temperature</td>
<td>Pocket Refractometer ATAGO</td>
</tr>
<tr>
<td>Peak area percentage</td>
<td>@ Room temperature</td>
<td>Gas Chromatogram</td>
</tr>
</tbody>
</table>

The following tests were performed.

5.2 Analytical Techniques

For the stability testing the formulations were analysed by GC to determine their various percentage compositions at time 0 and again after 2 months incubation. Refer to section 5.4, for the stability results. Each of the formulations was diluted with the solvent n-Butyl acetate (1µg/ml) for GC analysis.

5.2.1 Gas Chromatography

Analysis was performed on a HP 5890 series gas chromatograph. The data was processed by HP 61034 C software using a Hewlett Packard computer. The RTX 35 ms column was
fitted in the GC (length: 30 m x 0.25 mm ID x 0.25 mm thickness). The various settings were programmed as follows:

Detector Temperature : 300°C  
Injector temperature : 280°C  
Initial column temperature : 70°C  
Initial column hold time : 5 minutes  
Final column temperature : 260°C  
Final column hold time : 5 minutes  
Heating rate : 10°C/min  
Injector volume : 1µl  
Detector : FID detector  
Split flow : 60 ml/min  
Carrier gas : Helium at constant flow (1 ml/min)  
Run time : 20 minutes  
Sensitivity : 1

5.3 Physical and chemical tests

The synthesized PMD-citronellal acetal, all three nail polish formulations and all three perfume formulations were incubated at three different temperatures. The samples were incubated at 0°C, 25°C and 40°C over a period of two months. The following characteristics were recorded initially and after incubation:

- Colour  
- Odour or fragrance  
- pH  
- Refractive index (Brix %)  
- Emulsion stability (any signs of separation)  
- Gas Chromatogram concentrations (1 µg/ml concentrations were prepared using ethanol, butyl acetate and heptane as the solvents). Ethanol was used for the perfume samples, butyl acetate for the nail polish samples and heptane for the PMD-citronellal acetal samples. Refer to section 5.2.1.
5.3.1 Light testing
This test is used to determine how stable the formulations are when exposed to UV light. Discolouration of the product can be due to fragrances or sensitive ingredients. If the product is sensitive to UV light, a UV absorber Benzophenone (0.1%) should be added to the formulations. Glass vials were filled with the products and placed at a window sill for a period of two months. Initial and final evaluations of physical and chemical characteristics listed in Section 5.3 were determined.

5.3.2 Freeze thaw testing
Freeze thaw testing provides information that regular testing does not. It will indicate whether a formula will remain stable under varied conditions. These extreme temperature conditions could be experienced during shipping or storage. Trucks which lack temperature control facilities could potentially be a problem for products. Therefore it is crucial that the formulas can withstand extreme temperature changes over a short period of time. Freeze thaw testing demonstrates this.

Freeze thaw cycle:
Samples were prepared in glass vials sealed with aluminium foil. The initial readings of physical characteristics were recorded. The samples were incubated in the freezer (0°C) for 24 hours until all samples were frozen. The samples were removed and allowed to thaw at room temperature. The samples were then incubated in an oven at 50°C for 24 hours. The samples were removed and allowed to equilibrate at room temperature. The final evaluations of physical characteristics were recorded as per section 5.3.
5.4 Stability test results

An important parameter was to determine the effects that the stability tests had on the various GC peaks. Examples of the perfume formulations subjected to light intensity tests are represented in Figures 5.1-5.4 below, showing the retention time for the respective peaks in two of the formulations.

**Figure 5.1:** Gas chromatogram example of the peaks present in the DBP perfume formulation at time 0.

**Figure 5.2:** Gas chromatogram example of the peaks present in the DBP perfume formulation after the 2 month incubation period for Light intensity testing.
Figure 5.3: Chromatogram example of the peaks present in the PMD-citronellal acetal perfume formulation at time 0.

Figure 5.4: Chromatogram example of the peaks present in the PMD-citronellal acetal perfume formulation after the 2 month incubation period for Light intensity testing.
In Figures 5.2 and 5.4, there appears to be degradation in the peak height of the components. The results obtained for the stability tests of the synthesized oil, PMD-citronellal acetal after the 2 month incubation period, are shown in Table 5.2. All stability tests were performed in triplicate.

Table 5.2: Stability test results for the synthesized oil, PMD-citronellal acetal.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time</th>
<th>pH</th>
<th>Refractive index (Brix %)</th>
<th>Colour</th>
<th>Odour</th>
<th>*GC peak decrease of PMD-citronellal acetal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0</td>
<td>0.37</td>
<td>72.5</td>
<td>Slightly green-yellow</td>
<td>Eucalyptus odour</td>
<td>-</td>
</tr>
<tr>
<td>Room Temperature 0°C</td>
<td>2 months</td>
<td>0.10</td>
<td>73.0</td>
<td>Yellow</td>
<td>No change</td>
<td>3.62</td>
</tr>
<tr>
<td>Room Temperature 40°C</td>
<td>2 months</td>
<td>0.19</td>
<td>72.4</td>
<td>Yellow</td>
<td>No change</td>
<td>5.52</td>
</tr>
</tbody>
</table>

*Let:

% Acetal = GC peak decrease of PMD-citronellal acetal

\[ a = \% \text{PMD-citronellal acetal at time = 2 months} \]

\[ b = \% \text{PMD-citronellal acetal at time = 0} \]

% Acetal = b – a ................................................................. eq 5.1

The stability test results of the perfume formulations and the nail polish formulations are shown in Table 5.3 and Table 5.4 respectively.
Table 5.3: Stability testing results for the perfume formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature</th>
<th>Time</th>
<th>pH</th>
<th>Refractive index (Brix %)</th>
<th>Colour</th>
<th>Odour</th>
<th>Emulsion stability</th>
<th>Plasticizer GC peak decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Perfume</td>
<td>Initial (RT)</td>
<td>0</td>
<td>5.63</td>
<td>22.2</td>
<td>Clear</td>
<td>Sweet odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>5.91</td>
<td>24.3</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>5.65</td>
<td>24.7</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>4.1</td>
<td>22.0</td>
<td>Clear</td>
<td>No change</td>
<td>Solidifies with a liquid layer</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>2.75</td>
<td>29.3</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>4.95</td>
<td>25.6</td>
<td>Clear</td>
<td>No change</td>
<td>Unstable, solid</td>
<td>-</td>
</tr>
<tr>
<td>DBP Perfume</td>
<td>Initial (RT)</td>
<td>0</td>
<td>5.50</td>
<td>23.9</td>
<td>Clear</td>
<td>Sweet odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>5.54</td>
<td>26.8</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>0.02%</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>5.69</td>
<td>25.4</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>2.14%</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>2.42</td>
<td>12.3</td>
<td>Clear</td>
<td>No change</td>
<td>Solidifies with a liquid layer</td>
<td>8.08 %</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>2.96</td>
<td>30.00</td>
<td>Clear</td>
<td>No change</td>
<td>Solid white crystals, unstable</td>
<td>21.92%</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>6.17</td>
<td>25.7</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>1.02%</td>
</tr>
<tr>
<td>PMD-citronellial acetal Perfume</td>
<td>Initial (RT)</td>
<td>0</td>
<td>3.93</td>
<td>23.6</td>
<td>Clear</td>
<td>Sweet minty odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>4.97</td>
<td>25.3</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>9.6%</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>4.45</td>
<td>24.2</td>
<td>Clear</td>
<td>No change</td>
<td>No change</td>
<td>6.2%</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>1.96</td>
<td>8.4</td>
<td>Yellow</td>
<td>Eucalyptus odour</td>
<td>No change</td>
<td>10.74%</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>3.36</td>
<td>26.4</td>
<td>Slightly yellow liquid</td>
<td>Pungent eucalyptus odour</td>
<td>No change</td>
<td>23.26%</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>5.65</td>
<td>24.6</td>
<td>Slightly yellow liquid</td>
<td>Pungent eucalyptus odour</td>
<td>No change</td>
<td>6.2%</td>
</tr>
</tbody>
</table>
Table 5.4: Stability testing results for the nail polish formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature</th>
<th>Time</th>
<th>pH</th>
<th>Refractive index (Brix %)</th>
<th>Colour</th>
<th>Odour</th>
<th>Emulsion stability</th>
<th>Plasticizer GC peak decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Nail polish</td>
<td>Initial (RT)</td>
<td>0</td>
<td>3.75</td>
<td>44.1</td>
<td>Metallic silver</td>
<td>Acetate odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>3.69</td>
<td>43.4</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Unstable, solidifies</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>2.53</td>
<td>29.5</td>
<td>Separation, metallic sedimentation on the bottom</td>
<td>No change</td>
<td>Unstable, separation</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>4.21</td>
<td>41.8</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Solidifies</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>1.56</td>
<td>46.3</td>
<td>Separation, metallic sedimentation</td>
<td>No change</td>
<td>Unstable, separation</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>3.55</td>
<td>45.0</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Unstable, solid formation</td>
<td>-</td>
</tr>
<tr>
<td>DBP Nail polish</td>
<td>Initial (RT)</td>
<td>0</td>
<td>3.52</td>
<td>54.5</td>
<td>Metallic silver</td>
<td>Acetate odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>3.31</td>
<td>55.5</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Solidifies</td>
<td>1.30%</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>2.94</td>
<td>54.1</td>
<td>Slight separation, best metallic dispersion</td>
<td>No change</td>
<td>Sedimentation</td>
<td>2.28%</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>5.33</td>
<td>43.6</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Hard solid</td>
<td>1.31%</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>2.20</td>
<td>54.5</td>
<td>Slight separation, best metallic dispersion</td>
<td>No change</td>
<td>Sedimentation</td>
<td>1.38%</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>3.58</td>
<td>54.8</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Hard solid</td>
<td>1.34%</td>
</tr>
<tr>
<td>PMD-citronellal acetal Nail polish</td>
<td>Initial (RT)</td>
<td>0</td>
<td>0.37</td>
<td>72.5</td>
<td>Metallic silver</td>
<td>Acetate odour</td>
<td>Homogenous liquid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>2 months</td>
<td>3.74</td>
<td>52.8</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Sedimentation</td>
<td>1.34%</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>2 months</td>
<td>2.63</td>
<td>28.4</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Sedimentation</td>
<td>1.81%</td>
</tr>
<tr>
<td></td>
<td>40°C</td>
<td>2 months</td>
<td>3.61</td>
<td>41.2</td>
<td>Metallic silver</td>
<td>No change</td>
<td>Solidifies</td>
<td>1.24%</td>
</tr>
<tr>
<td></td>
<td>Light intensity</td>
<td>2 months</td>
<td>1.77</td>
<td>53.0</td>
<td>Yellow- metallic silver</td>
<td>No change</td>
<td>Sedimentation</td>
<td>1.81%</td>
</tr>
<tr>
<td></td>
<td>Freeze thaw</td>
<td>2 day cycle</td>
<td>0.40</td>
<td>72.9</td>
<td>Yellow-metallic silver</td>
<td>Pungent eucalyptus odour</td>
<td>Unstable, solid formation</td>
<td>1.03%</td>
</tr>
</tbody>
</table>
5.4.1 Graphical representation of stability results

5.4.1.1 Synthesized PMD-citronellal acetal

Figure 5.5: The effects of temperature on refractive index for PMD-citronellal acetal.

Figure 5.6: The effects of temperature on pH for PMD-citronellal acetal.
5.4.1.2 Perfume formulations

Figure 5.7: The effects of temperature on refractive index on each perfume formulation. Key: RT = Room temperature; LT = Light intensity; FT = Freeze thaw.

Figure 5.8: The effects of temperature on pH on each formulation. Key: RT = Room temperature; LT = Light intensity; FT = Freeze thaw.
5.4.1.3 Nail polish formulations

![Graph showing refractive index and pH changes with temperature for different nail polish formulations.]

Figure 5.9: The effects of temperature on refractive index for each nail polish formulation. Key: RT = Room temperature; LT = Light intensity test; FT = Freeze Thaw.

![Graph showing pH changes with temperature for different nail polish formulations.]

Figure 5.10: The effects of temperature on pH for each nail polish formulation. Key: RT = Room temperature; LT = Light intensity test; FT = Freeze thaw.
5.5 Discussion

5.5.1 PMD-citronellal acetal

pH is the measure of hydrogen ion activity in the sample which determines the intensity of acidity or alkalinity. An electrometric method was used to determine the pH in the samples. This involved the use of an electromotive force to generate a response to the present hydrogen ions, whilst a glass electrode was used as the indicator electrode. The response generated was pH.\(^88\)

The high acid level (0.37) of the initial oil is an indication of the acidic naturally occurring chemicals which remained in the product oil after synthesis. The constituents of *Eucalyptus citriodora* oil include:\(^89\) citronellal, α-citronellol, isopulegol isomers, citronellyl acetate, p-cymene, citronellal dimmers, α-pinene, β-caryophyllene. Several esters of isobutyric acid (4), monoterpene, sesquiterpene, hydrocarbons, oxides (6), monoterpenic alcohols (10), esters (8) and aldehydes (4), (Z)-jasmone, citronellyl citronellate, citronelic acid, eugenol.

Upon analysis of the gas chromatogram peaks, several naturally occurring components remained in the product oil (PMD-citronella acetal). The natural acid components in the oil are: isobutyric acid, citronelic acid. This could possibly influence the low pH of the synthesized oil. Isobutyric acid is a strong acid. A variety of carboxylic acids are abundant in nature. Carboxylic acids can be synthesized when aldehydes are oxidized.\(^90\) The low pH can be attributed to perhaps unreacted catalyst (H\(_2\)SO\(_4\)).

Due to the effects of temperature, accurate measurements of pH have been problematic. When a solutions’ temperature increases, the viscosity will decrease causing an increase in the mobility of the ions in the solution. Increasing temperature may lead to an increase in the number of ions in solution due to dissociation. Therefore pH is a measure of hydrogen ion concentration and a change in temperature should have an effect on pH.\(^91\)

The effect of heating at a high temperature namely, 40°C on the acidity of the synthesized oil, PMD-citronellal acetal was investigated. The pH was very low at 0.19 and regarded as highly acidic. Temperature has a number of significant effects on pH measurement. Increasing the temperature may have an effect by decreasing the pH. This can be seen when PMD-citronellal acetal was heated to 40°C (A decrease from 0.37 to 0.19).
The effect of incubating PMD-citronellal acetal at low temperatures such as 0°C was investigated. Colder temperatures tend to cause a decrease in ion mobility and dissociation. Therefore possibly resulting in an increase of pH. According to the data obtained, the pH value decreased thereby illustrating an exception to this explanation. A decrease in pH was observed (from 0.37 to 0.10). Increased light intensity during room temperature incubation affected the pH of PMD-citronellal acetal. An increase in pH (from 0.37 to 0.53) was observed.

The acetal was regarded as relatively stable under the different temperature conditions. However, a colour change was noted under elevated temperatures such as 40°C and increase in light intensity. When incubated at elevated temperatures the pH decreases. Therefore the oil becomes more acidic, which could possibly become problematic in cosmetic formulations. Increased light intensity results in the increase in acidity of the oil. When incubated at 40°C the PMD-citronellal acetal becomes very acidic (0.19). Acetals can be hydrolysed to aldehydes in acidic solutions.\(^9\)

The odour of the oil remains constant after incubation. The refractive index readings remained fairly unchanged, however, a colour change occurs. The change in colour is most probably a result of some aldehyde formation. The PMD-citronellal acetal peak occurring at retention time of approximately 22.4 minutes was analyzed. Incubating the oil at 0°C and 25°C appears to retard the degradation of the acetal peak. The acetal peak decreased the most after the 40°C incubation period. It can be seen that the acetal oil is sensitive to heat and light. It is recommended to store PMD-citronellal acetal in a closed, covered bottle away from light and elevated temperatures.
5.5.2 Perfume

The blank perfume was regarded as stable. There were no changes in odour or colour. However at 40°C and after freeze thaw, the perfume solidified as evaporation occurred. When the temperature increased the product became more acidic, especially after the light intensity test. Refractive index increased by 7 units after the light intensity test. The formulation was mostly affected by light.

After the incubation time period, no colour or odour changes occurred in the DBP perfume. However at elevated temperatures, 40°C and increased light intensity the emulsion was considered unstable. The perfume solidified. The refractive index decreased by approximately half the value when the sample was incubated at 40°C. Increased light intensity caused the refractive index to increase by approximately 7 units. The pH was slightly affected when frozen or left at room temperature. The pH is in the region of ~5.5. When the sample was heated the formulation became more acidic. Freeze thaw cycles saw an increase in pH of ~0.67 units. The DBP peak remains fairly constant after the 0°C incubation period. However, increased heating and increased light intensity caused the DBP peak to decrease by 8.08% and 21.92% respectively. Freeze thaw caused a slight decrease in the plasticizer peak. Therefore it can be concluded that light and heat decrease the DBP present in the product to a large extent. Benzophenone (1%) can be incorporated into the formulation to resist instability caused by light.

Physically the PMD-citronellal acetal perfume became slightly yellow after the 40°C incubation period, increased light intensity and the freeze thaw test. However, the perfume remained a homogenous liquid after all the stability testing. Changes in odour were noted when heating was introduced. The characteristic odour was particularly a pungent eucalyptus scent. This compound could possibly overpower the scents incorporated into a perfume. Further work to mask this scent should be considered. The refractive index values for the tests remained fairly close to the original value of 23.6 Brix %. The values increased slightly. However, after the 40°C incubation period, the refractive index was approximately 3 times smaller than the original value. At the following incubation periods: 0°C, room temperature and freeze thaw, the pH of these samples showed a decrease in acidity. When heat was introduced, as in the 40°C and light intensity tests, the pH of these samples showed an increase in acidity. Increased acidity can cause irritation to the outer epidermis upon application of the perfume formulation. Possible buffers could be incorporated to control the pH changes upon heating. The PMD-citronellal peaks experienced substantial percentage degradation especially after the light intensity test. The compound was
considered light sensitive and formulations should be packaged into dark stained perfume bottles and stored in cool environments. High temperatures of 40°C caused substantial degradation (~10%) of the acetal peak. This further supports that the temperature of storage is of utmost importance.

5.5.3 Nail polish

Physically the blank nail polish formulation appeared to be unstable as solidification occurred after heat was introduced or sedimentation of the aluminium pigment occurred after the following stability tests: room temperature, light intensity test and incubation at 0°C. The odour remained unchanged. Based on physical appearance, the formulation was regarded as unstable. The refractive index for each test remained fairly constant approximately near the initial samples value (~44.1 Brix %). However, the refractive index of the sample at room temperature dropped to approximately half the initial value. This can be attributed to the physical change of this formulation. There was an increase in acidity for the following tests: increased light intensity, 0°C, freeze thaw and room temperature incubations. However, upon heating at 40°C, the formulation decreased in acidity. The samples were analyzed with GC but were not included in Table 5.4, as no plasticizer was present.

After the incubation time period, no odour changes occurred in the DBP nail polish. However at elevated temperatures (40°C) and increased light intensity the emulsion was considered to have slight separation of the pigments. The nail polish solidified as expected, after freeze thaw, 0°C and 40°C. The refractive index decreased by approximately 10 units after the 40°C incubation. The remainder of the tests remained fairly close to the original refractive index value. The formulation increased in acidity by ~ 1 pH unit, for the room temperature and light intensity tests. When the sample was heated the formulation became less acidic, with a pH of 5.33. The DBP peak remains fairly constant after each incubation period with an average decrease of not more than ~2.30%.

After freeze thaw, the odour of the acetal nail polish changed to a pungent eucalyptus scent. The product changed colour to a yellow-metallic silver after freeze thaw and light intensity test. After each stability test, the emulsion was regarded as unstable as it either solidifies or sedimentation of the pigment occurred. The refractive index values decreased greatly for the majority of the tests. The freeze thaw data remained fairly similar to the initial samples data. For all the tests, excluding the freeze thaw sample, saw an increase in pH values. For the room temperature incubation, the refractive index decreased by 44.1 Brix % units. The PMD-
citronellal acetal peaks remained fairly constant after each incubation period with an average decrease of not more than ~1.81%. Based on the GC data the acetal content remains at acceptable levels.

5.6 Conclusions

Judging from the GC peaks plasticizer degradation, it is clear that both plasticizers are more unstable in the perfume formulation than in the nail polish and are especially sensitive to light when in the perfume (Refer to Tables 5.3 and 5.4 on pages 93 and 94 respectively). This could possibly be due to an interaction with the fragrance molecule, p-anisaldehyde. If one aldehyde (RCHO) molecule reacts with one alcohol (R'OH) molecule a hemiacetal (RCH(OH)OR') is formed. When a second alcohol molecule reacts with the hemiacetal, a full acetal (RCH(R'O)R')₂ is formed. This reaction however is reversible in acidic solutions, the acetal can be hydrolyzed back to an aldehyde. ⁹⁴

The perfume formulation is composed of an aldehyde, acetal and alcohol, namely: p-anisaldehyde, PMD-citronellal acetal and ethanol. The GC peaks for PMD-citronellal acetal were considerably low. This degradation can be accounted for. The acetal peak decreased in the presence of the acidic medium and the aldehyde peak increased due to the increase of aldehyde formation in this perfume formulation. This can be seen over the 2 month incubation period, especially under increased temperatures in the light intensity test and the 40°C incubation period. The PMD-citronellal acetal oil itself becomes highly acidic (~0.19) at the 40°C incubation period. Therefore, this accounts for the acidic medium.
Chapter 6

Summary and conclusions

The research questions can be answered. PMD-citronellal acetal can be synthesized using Eucalyptus oil as the source of citronellal. This synthetic reaction yields a product which contained 10.17% citronellal ± 0.51% (95% confidence), 12.12% PMD ±0.27% (95% confidence level) and 74.42% PMD-citronellal acetal ±1.05% (95% confidence). Therefore the synthesis of PMD-citronellal acetal using Eucalyptus oil as the source of citronellal, results in high conversion of citronellal to acetal. Refer to Figure 2.3 on page 36.

The yield of PMD-citronellal was observed to be high when the following reaction conditions are defined: dichloromethane as the solvent, constant H₂SO₄ catalyst amount of 0.368g, 6 hour reaction and performed at room temperature (25°C). Refer to Section 2.6.1 on page 42. No further optimization could be achieved and the initial parameter settings were the most optimal ones investigated in this work.

The physical characterisation of the modified *Eucalyptus citriodora* oil and di-butyl phthalate was done. Refer to Table 2.7 on page 49. The *Eucalyptus citriodora* derived PMD-citronellal acetal oil has a lower density, higher viscosity, is slightly more soluble in water (at 25°C), has a slightly lower refractive index (Brix %) and a slightly higher boiling point (350°C) than DBP. The physical characteristics of the Eucalyptus oil source and the derived acetal were very similar, except for the boiling point which was much higher for the derived acetal and more similar to that of DBP. This can be accounted for as the oil consists of ~84.3% Citronellal, ~ 1.3% PMD and 2.7% PMD-citronellal acetal.

The plasticizing effects of the *Eucalyptus citriodora* derived PMD-citronellal acetal compared with DBP were determined.

The bio-plasticizer based nail polish had acceptable hardness, gloss and very strong adhesion levels. The product had good flexibility however longer surface and hard drying times than DBP. It had a harder settlement due to its increased viscosity. Lower percentage non-volatile components were present. Refer to Table 3.4 on page 63.
The bio-plasticizer based perfume exhibits a slightly higher evaporation rate and shorter half-life compared to the DBP based perfume. Therefore the PMD-citronellal acetal perfume does not last as long as DBP and evaporates slightly faster. Refer to Table 4.7 on page 84.

*Eucalyptus citriodora* derived PMD-citronellal acetal thus shows similar plasticizing properties to the conventional plasticizer, DBP. With the experimentation of the cosmetic formulations, DBP fared slightly better than the acetal product. However the bio-plasticizer behaves in a similar manner to that of DBP.

The acetal was regarded as relatively unstable under the different temperature conditions. The acetal becomes very acidic when heated to 40°C (pH = 0.19) and storing it at 0°C also increases the acidity. It is recommended to store PMD-citronellal acetal in a closed, covered bottle away from light and elevated temperatures. Refer to Section 5.3.1 in Chapter 5.

The cosmetic products were incubated at 0°C, 25°C and 40°C over a period of two months. Any changes in colour, odour, pH, refractive index, separation and plasticizer peak change were recorded. It was determined that PMD-citronellal acetal was relatively unstable under elevated temperatures. Storage under colder temperatures (0°C) and elevated temperatures tends to increase the acidity. Therefore the bio-plasticizer must be placed in a closed, covered bottle and stored in an ambient environment away from light. According to the gas chromatogram peaks, it was clear that both plasticizers were more unstable in the perfume formulation than in the nail polish and were especially sensitive to light when in the perfume. This could possibly be due to the interaction with the fragrance molecule, p-anisaldehyde. In the PMD-citronellal acetal perfume formulation a large decrease in acetal peak was noted at the 40°C incubation test. It was seen that the acetal oil becomes very acidic after the 40°C incubation period (0.19). Therefore, in the presence of an acidic medium the acetal can be hydrolyzed to an aldehyde. This can explain the decrease of the acetal peak and the increase of an aldehyde peak.

Future work should include improvement to the stability of the oil and cosmetic products. If the acetal is used in fragrance formulations the odour must be masked to prevent over empowerment of the fragrance incorporated into the perfume.
7. Appendix

7.1. Statistical data from Chapter 2

In the Tables below (Table: 7.1. – 7.6) are the summarized statistical data for the experiments in Chapter 2. The corrected peak area percentages were recorded.

Table 7.1: Data used for Experiment 1 (% of each component in product).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citronellal</th>
<th>PMD</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.17</td>
<td>12.19</td>
<td>74.12</td>
</tr>
<tr>
<td>2</td>
<td>10.28</td>
<td>12.11</td>
<td>74.3</td>
</tr>
<tr>
<td>3</td>
<td>10.399</td>
<td>12.07</td>
<td>73.84</td>
</tr>
<tr>
<td>Average</td>
<td>10.283</td>
<td>12.12333</td>
<td>74.08667</td>
</tr>
<tr>
<td>SD</td>
<td>0.114529</td>
<td>0.061101</td>
<td>0.231805</td>
</tr>
<tr>
<td>Error</td>
<td>0.519732</td>
<td>0.277275</td>
<td>1.051923</td>
</tr>
<tr>
<td>Upper limit</td>
<td>10.80273</td>
<td>12.40061</td>
<td>75.13859</td>
</tr>
<tr>
<td>Lower limit</td>
<td>10.16847</td>
<td>12.06223</td>
<td>73.85486</td>
</tr>
</tbody>
</table>

Table 7.2: Data used for Experiment 2 (% of each component in product).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citronellal</th>
<th>PMD</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.218</td>
<td>46.639</td>
<td>23.505</td>
</tr>
<tr>
<td>2</td>
<td>5.721</td>
<td>46.258</td>
<td>24.111</td>
</tr>
<tr>
<td>3</td>
<td>5.258</td>
<td>45.991</td>
<td>24.313</td>
</tr>
<tr>
<td>Average</td>
<td>5.399</td>
<td>46.296</td>
<td>23.97633</td>
</tr>
<tr>
<td>SD</td>
<td>0.279576465</td>
<td>0.325667008</td>
<td>0.420497</td>
</tr>
<tr>
<td>Error</td>
<td>1.268710484</td>
<td>1.477868126</td>
<td>1.908202</td>
</tr>
<tr>
<td>Upper limit</td>
<td>6.667710484</td>
<td>47.77386813</td>
<td>25.88454</td>
</tr>
<tr>
<td>Lower limit</td>
<td>4.130289516</td>
<td>44.81813187</td>
<td>22.06813</td>
</tr>
<tr>
<td>p-values (comparing Exp. 1 and 2)</td>
<td>0.0001</td>
<td>0.00003133</td>
<td>0.0000003733</td>
</tr>
</tbody>
</table>
### Table 7.3: Data used for Experiment 3 (% of each component in product).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citronellal</th>
<th>PMD</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.108</td>
<td>20.92</td>
<td>27.369</td>
</tr>
<tr>
<td>2</td>
<td>10.314</td>
<td>20.823</td>
<td>26.967</td>
</tr>
<tr>
<td>3</td>
<td>11.179</td>
<td>20.08</td>
<td>26.503</td>
</tr>
<tr>
<td>Average</td>
<td>11.20033</td>
<td>20.60767</td>
<td>26.94633</td>
</tr>
<tr>
<td>SD</td>
<td>0.89719</td>
<td>0.459539</td>
<td>0.43337</td>
</tr>
<tr>
<td>Error</td>
<td>4.086965</td>
<td>2.093336</td>
<td>1.974126</td>
</tr>
<tr>
<td>Upper limit</td>
<td>15.2873</td>
<td>22.701</td>
<td>28.92046</td>
</tr>
<tr>
<td>Lower limit</td>
<td>7.113368</td>
<td>18.51433</td>
<td>24.97221</td>
</tr>
<tr>
<td>p-values (comparing Exp. 1 and 3)</td>
<td>0.221053</td>
<td>0.000994</td>
<td>0.000000481</td>
</tr>
</tbody>
</table>

### Table 7.4: Data used for Experiment 4 (% values for PMD-citronellal acetal in product oil).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.184 g</th>
<th>0.368 g</th>
<th>0.552 g</th>
<th>0.736 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42.2</td>
<td>72.9</td>
<td>51.8</td>
<td>35.1</td>
</tr>
<tr>
<td>2</td>
<td>41.8</td>
<td>74.1</td>
<td>53.5</td>
<td>32.9</td>
</tr>
<tr>
<td>3</td>
<td>43.4</td>
<td>73.5</td>
<td>53.2</td>
<td>34.1</td>
</tr>
<tr>
<td>Average</td>
<td>42.4667</td>
<td>73.5</td>
<td>52.8333</td>
<td>34.03333</td>
</tr>
<tr>
<td>SD</td>
<td>0.83267</td>
<td>0.6</td>
<td>0.90738</td>
<td>1.1015141</td>
</tr>
<tr>
<td>Error</td>
<td>3.77862</td>
<td>2.72278</td>
<td>4.11765</td>
<td>4.9986414</td>
</tr>
<tr>
<td>Upper limit</td>
<td>46.2453</td>
<td>76.2228</td>
<td>56.951</td>
<td>39.031975</td>
</tr>
<tr>
<td>Lower limit</td>
<td>38.688</td>
<td>70.7772</td>
<td>48.7157</td>
<td>29.034692</td>
</tr>
<tr>
<td>p-values (comparing 0.368 g to 0.184 g; 0.552 g and 0.736 g)</td>
<td>0.0000008</td>
<td>-</td>
<td>0.000062</td>
<td>0.00001361</td>
</tr>
</tbody>
</table>

### Table 7.5: Data used for Experiment 5 (% of each component in product).

<table>
<thead>
<tr>
<th>Average values in hours (triplicate)</th>
<th>Citronellal</th>
<th>PMD</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.223</td>
<td>31.120</td>
<td>36.630</td>
</tr>
<tr>
<td>3</td>
<td>24.117</td>
<td>29.288</td>
<td>42.605</td>
</tr>
<tr>
<td>4</td>
<td>19.870</td>
<td>25.629</td>
<td>49.501</td>
</tr>
<tr>
<td>5</td>
<td>15.381</td>
<td>19.381</td>
<td>62.811</td>
</tr>
<tr>
<td>6</td>
<td>9.205</td>
<td>12.421</td>
<td>73.405</td>
</tr>
<tr>
<td>7</td>
<td>9.063</td>
<td>12.183</td>
<td>72.730</td>
</tr>
<tr>
<td>8</td>
<td>7.112</td>
<td>11.532</td>
<td>73.118</td>
</tr>
</tbody>
</table>
Table 7.6: Data used for Experiment 5, for the statistical analysis assuming unequal variances at time 6 hours and 8 hours (% of each component in product).

<table>
<thead>
<tr>
<th>Hours</th>
<th>Runs</th>
<th>Citronellal</th>
<th>PMD</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>8.62</td>
<td>12.1</td>
<td>73.72</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>9.32</td>
<td>12.78</td>
<td>74.42</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>10.1</td>
<td>13.1</td>
<td>74.1</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>9.205</td>
<td>12.42167</td>
<td>73.405</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.74036</td>
<td>0.510686</td>
<td>0.350428</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>3.359735</td>
<td>2.317478</td>
<td>1.590234</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>7.087</td>
<td>11.231</td>
<td>72.781</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7.432</td>
<td>11.108</td>
<td>73.093</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6.817</td>
<td>12.257</td>
<td>73.48</td>
</tr>
<tr>
<td>Av</td>
<td></td>
<td>7.112</td>
<td>11.532</td>
<td>73.118</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.30826</td>
<td>0.63087</td>
<td>0.35017</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>1.39888</td>
<td>2.86289</td>
<td>1.58906</td>
</tr>
<tr>
<td>p values (comparing 6 hours to 8 hours)</td>
<td>0.01631</td>
<td>0.19427</td>
<td>0.46703</td>
<td></td>
</tr>
</tbody>
</table>
### 7.2 Statistical data for Chapter 3

In Table 7.7 below, are the summarized statistical data for nail polish testing for Chapter 3.

Table 7.7 Replicate values for nail polish testing.

<table>
<thead>
<tr>
<th>Test</th>
<th>Replicates</th>
<th>Formulation #1 (Blank)</th>
<th>Formulation #2 (DBP)</th>
<th>Formulation #3 (PMD-citronellal acetal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloss at 85° (Unit: GU)</td>
<td>1</td>
<td>47.4</td>
<td>53.5</td>
<td>50.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.5</td>
<td>53.2</td>
<td>50.22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>47.3</td>
<td>53.4</td>
<td>50.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.1</td>
<td>53.1</td>
<td>50.51</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>47.7</td>
<td>53.3</td>
<td>50.1</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>47.4</td>
<td>53.3</td>
<td>50.226</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.2236</td>
<td>0.1414</td>
<td>0.1504</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1.131</td>
<td>0.7153</td>
<td>0.7608</td>
</tr>
<tr>
<td>Gloss at 60° (Unit: GU)</td>
<td>1</td>
<td>80.8</td>
<td>80.5</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80</td>
<td>80.2</td>
<td>91.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>79.8</td>
<td>80.6</td>
<td>90.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>79.9</td>
<td>80.51</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>79.5</td>
<td>80.2</td>
<td>91.1</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>80</td>
<td>80.402</td>
<td>91.06</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.4848</td>
<td>0.1885</td>
<td>0.3362</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>2.452</td>
<td>0.9533</td>
<td>1.700</td>
</tr>
<tr>
<td>Gloss at 20° (Unit: GU)</td>
<td>1</td>
<td>31</td>
<td>32.45</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.7</td>
<td>32.5</td>
<td>35.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31.2</td>
<td>32.45</td>
<td>35</td>
</tr>
<tr>
<td></td>
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<td>32.5</td>
<td>35.4</td>
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<td>5</td>
<td>30.6</td>
<td>32.6</td>
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</tr>
<tr>
<td></td>
<td>Average</td>
<td>30.9</td>
<td>32.5</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.2449</td>
<td>0.0612</td>
<td>0.1903</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1.239</td>
<td>0.3097</td>
<td>0.9630</td>
</tr>
<tr>
<td>% non-volatile components</td>
<td>1</td>
<td>59.2</td>
<td>65.64</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58.31</td>
<td>64.89</td>
<td>63.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>56.8</td>
<td>66.58</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>58.1</td>
<td>65.7</td>
<td>64.4</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.213</td>
<td>0.8468</td>
<td>0.5568</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>4.753</td>
<td>3.318</td>
<td>2.181</td>
</tr>
</tbody>
</table>
7.3 Statistical data from Chapter 4

The actual data and statistical analysis of the evaporation experiments for all three perfume formulations as follows:

Table 7.8: The concentrations (mol/dm³) of p-anisaldehyde for each formulation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blank</th>
<th>DBP</th>
<th>PMD-citronellal acetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
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7.3.1 Model validation

The observed perfume concentrations are similar to the predicted perfume concentrations as the observed points are closely on or scattered around the predicted linear trend line.

The residuals are the difference of observed concentration from the predicted concentration. The residuals are closely scattered around zero.
References


2 Postma-Botha, M., 2011. Evaluation of p-menthane-3,8-diol-citronellal acetal for plasticizing properties, Port Elizabeth: NMMU.


11 Postma-Botha, M., 2011. Evaluation of p-menthane-3,8-diol-citronellal acetal for plasticizing properties, Port Elizabeth: NMMU.


Alistair, M.F., 2006. Shape Selective Methylation of meta-Cresol, s.: University of Cape Town, MSc (Engineering), Department of Chemical Engineering.


SABS., Drying time of paint film. s.l.:SABS Method 148, First Revision.


90 www.chemicalland21.com


92 Acetals. [Online]

94 Acetals. [Online]