# AN INVESTIGATION OF THE LONG TERM CHEMICAL STABILITY AND PHYSICAL PERFORMANCE OF PMD-CITRONELLAL ACETAL COMPARED WITH DIBUTYL PHTHALATE AND BIS(2-ETHYLHEXYL) TEREPHTHALATE AS PLASTICISERS IN SELECTED COSMETIC FORMULATIONS

**By**

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# DECLARATION

I, Amor Marx, hereby declare that the thesis 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.

…… (Signature)

# ABSTRACT

Plasticisers are used by cosmetic manufacturers to improve the film forming abilities of a product and increase flexibility of the film formed on the skin or hair surface, as is desired, for example, in nail lacquers and lip coats. In recent years authorities have banned several plasticisers in cosmetic products (mainly phthalates) since these substances may pose a wide range of health risks and can be harmful to the environment.

It is, therefore, necessary to find alternative, safe plasticisers, preferably of natural origin e.g. bio-plasticisers which can replace the toxic phthalates and still impart the same desirable properties to the cosmetic products in which they are used. In this study, the novel bio-plasticiser *para*-menthane-3,8-diol-citronellal acetal (PMD-citronellal acetal) was selected to compare its stability properties and plasticising behaviour with well-known non-phthalate bis(2-ethylhexyl) terephthalate (DEHT) and the problematic dibutyl phthalate (DBP).

The objectives were to determine if the novel bio-plasticiser PMD-citronellal acetal plasticising properties and chemical stability are similar or better than DEHT and DBP within two cosmetic formulations, viz. a nail lacquer and a lip coat formulation, after being incubated at elevated temperature (40 °C) over a three month period.

The results showed that flexibility for all plasticised formulations remained stable at room temperature (21 °C) and elevated temperature (40 °C). Adhesion performance of DEHT and PMD-citronellal acetal nail lacquer formulations outperformed DBP nail lacquer formulations. Elevated temperature and storage time had no influence on the organoleptic properties of any plasticised formulation.

PMD-citronellal acetal plasticised lip coat and nail lacquer formulations outperformed both DEHT and DBP nail formulations with regard to hardness.

Fourier Transform Infrared Spectrometry (FTIR) studies revealed that neat DPB, DEHT and Acetal were chemically stable at room temperature and elevated temperature over a three month incubation period. Furthermore, the three plasticised nail lacquer and lip coat formulations remained chemical stable over the three month incubation period at elevated temperature.

Chemical stability of the nail lacquer formulations was further evaluated by means of leaching tests using Solid Phase Extraction [1] and Ultra-Performance Liquid Chromatography (UPLC) at two temperatures (31 and 50 °C) and three time intervals (24, 48 and 72 hours). No leaching out of the nail lacquer formulation for Acetal and DEHT could be detected. It was observed that trace amounts of DBP leached from the nail lacquer formulation at 50 °C. DBP leaching decreased over time and was found to be statistically significant over the studied period.

It can be concluded that PMD-citronellal acetal can be selected as bio-plasticiser which exhibits similar properties to DEHT based on the performance stability and non-leaching criteria, and can be used as an alternative plasticiser to the toxic DBP in cosmetic formulations.

# ACKNOWLEDGEMENTS

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All the glory to Jesus Christ who put me into touch with individuals too many to name, you know who you are! Through all the troublesome times the Almighty Lord has shown me the light not to give up, but to persevere! He taught me that He is always on time with His wisdom, every time I felt it was too late.

Thanks to my family and friends, and of course, my dearest husband, Johannes, who believed in me and showed me so much support.

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# ABBREVIATIONS

| | | |
|---|---|---|
| AMA | - | American Medical Association |
| ASTM | - | American Standard Test Method |
| ATR | - | Attenuated Total Reflection |
| BBzP | - | Butyl benzyl phthalate |
| BzBP | - | Benzylbutyl phthalate |
| CDC | - | Center for Disease Control |
| CIR | - | Cosmetic Ingredient Review |
| CO | - | Coconut oil |
| DBP | - | Dibutyl phthalate |
| DEHP | - | Bis(2-ethylhexyl) phthalate |
| DEHT | - | Bis(2-ethylhexyl) terephthalate |
| DINCH | - | 1,2-cyclohexane dicarboxylic acid diisononyl ester |
| DINP | - | Di-isononyl phthalate |
| DMP | - | Dimethyl phtalate |
| DOP | - | Dioctyl phthalate |
| DOTP | - | Dioctyl terephthalate |
| EDC | - | Endocrine-Disrupting Chemicals |
| EWG | - | Environmental Working Group |
| FDA | - | Food and Drug Administration |
| FID | - | Flame Ionisation Detector |
| FTIR | - | Fourier Transform Infrared Spectrometry |
| GC | - | Gas Chromatography |
| GC-MS | - | Gas chromatography-mass spectrometry |
| HPC | - | Hydroxypropylcellulose |
| HPLC | - | High Performance Liquid Chromatography |

| | | |
|---|---|---|
| IR | - | Infrared |
| LOD | - | Limit of Detection |
| LOQ | - | Limit of Quantification |
| MF | - | Melamine Formaldehyde |
| MW | - | Molecular weight |
| MS | - | Mass Spectrometry |
| *mz* | - | Mass-to-charge-ratio |
| NOAEL | - | No observed adverse effect limit |
| NMU | - | Nelson Mandela University |
| NVC | - | Non-volatile content |
| *o-* | - | *Ortho* |
| *p-* | - | *Para* |
| PDA | - | Photo Diode Array Detector |
| PLA | - | Polylactic acid |
| PMD | - | *para*-menthane-3,8-diol |
| PMD-citronellal acetal (Acetal) | - | *para*-menthane-3,8-diol-citronellal acetal |
| PVC | - | Polyvinyl chloride |
| PVP | - | Polivinylpyrrolidone |
| RT | - | Room temperature |
| SPE | - | Solid Phase Extraction |
| UPLC | - | Ultra-Performance Liquid Chromatography |
| US | - | United States |
| UV | - | Ultraviolet |
| UV-VIS | - | Ultraviolet-visible |

# STATISTICAL ABBREVIATIONS

| | | |
|---|---|---|
| β | - | Standardized slope |
| *df* | - | Degrees of freedom |
| *F* | - | F value in a test |
| *M* | - | Mean or average |
| *MS* | - | Mean square |
| *n* | - | Population Size |
| *p* | - | Significance value |
| *R* | - | Correlation coefficient |
| $R^2$ | - | Correlation coefficient squared |
| *SD* | - | Standard Deviation |
| *STD* | - | Standard |
| *t* | - | T-test |
| *z* | - | Assessment value |

# METRIC STANDARDS

| | | |
|---|---|---|
| AU | - | Absorbance unit |
| cSt | - | Centistokes |
| H or hrs | - | Hours |
| kPa | - | Kilopascal |
| mm | - | Millimetres |
| $mm^2$ | - | Millimetres squared |
| MT | - | Metric Tons |
| N | - | Newton |

| | | |
|---|---|---|
| nm | - | Nanometre |
| pA | - | Picoampere |
| u | - | Atomic mass unit |
| µg | - | Microgram |
| µm | - | Micrometre |

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# CHAPTER 1
# INTRODUCTION TO PLASTICISERS

Since 1951 the International Union of Pure and Applied Chemistry (IUPAC) defines plasticisers as 'a substance or material incorporated into a material (usually a plastic or an elastomer) to increase its flexibility, workability or distensibility' [2].

In general, plasticisers are inert low molecular weight materials which exhibit high boiling points and low vapour pressures [3]. Plasticisers reduce the second order transition (glass transition temperature) of polymers and lower the melt viscosity, density, tensile strength and hardness of a polymer. They should also function as lubricants to improve ductility at the same time.

This chapter discusses the historical development of plasticisers, the different mechanisms of plasticisation, the plasticiser market as well as the most commonly used plasticisers in cosmetics. It also deals with the health and environmental concerns pertaining to phthalate plasticisers and the subsequent need for non-toxic alternatives.

## 1.1 Evolution of plasticisers

The concept of plasticisers was first introduced in the late 19$^{th}$ century. In the early days, natural camphor and castor oil were used for plasticisation purposes, but these were unsatisfactory for many end uses. Triphenyl phosphate, discovered in 1912, was later used as a substitute for camphor oil. This was the ultimate turning

point that made way for the era of ester plasticisers. The most important product that resulted from this discovery was tricresyl phosphate, still in use today. Tributyl phosphate was highly regarded for cellulose derivatives at one time, but less volatile products replaced this plasticiser at a later stage. Phthalic acid esters were used as plasticisers for the first time in 1920 and are still the largest class of plasticisers in the 21$^{st}$ century. Dibutyl phthalate (DBP) today is still the dominant plasticiser for polyvinyl acetate dispersions. The most commonly used phthalic acid ester is dioctyl phthalate (DOP), which constitutes 50% of plasticiser consumption worldwide [4].

Bis(2-ethylhexyl) phthalate (DEHP), as opposed to bis(2-ethylhexyl) terephthalate (DEHT), the latter molecule being the terephthalate thereof, is the most widely used plasticiser since it was introduced in the 1930's (see Section 1.11.2). The degree of plasticisation of polymers is dependent upon the chemical structure of the plasticiser, chemical composition, molecular weight as well as functional groups. In general, plasticisers with low molecular weight and a small number of polar groups provide higher flexibility and plasticisation [5].

Phthalates combine most of the desirable properties of a plasticiser, such as relatively low volatility at room temperature (RT), low cost and minimal interaction with resins at RT. In the case of phthalates, the polarizable benzene nucleus is highly effective with regard to compatibility with polyvinyl chloride (PVC), enabling great flexibility to the polymer chains. It is important to know that plasticiser-polymer compatibility decreases with the increasing length of the disubstituted alkyl esters. Shorter chain phthalates are easier to formulate with because of their faster diffusion. Unfortunately, the drawback is that they are more volatile. Plasticising effectiveness is reduced by branching. This effect is stronger the closer the branches are to the polar group, and the shorter the main chain becomes due to branching. This is paralleled by an increase in viscosity which explains the close relationship between viscosity and plasticiser effectiveness [8].

## 1.2 Classification of plasticisers

Plasticisers belong to different classes of organic compounds, such as hydrocarbons, fluorinated substances, esters, ketones, alcohols, amines, fats, oligomers and others. Plasticisers are commonly classified or divided based on their chemical structure or depending on the molecular weight (monomeric, polymeric) [6]. Plasticisers' chemical structures are very diverse. Reportedly there are 300 types of which less than 100 are commercially utilised [7]. Monomeric plasticisers include esters with a molecular weight of less than 500. These include: mono-, di-, and tri-esters of acids or the following anhydrides: terephthalic, phthalic, adipic, phosphoric, sebacic, citric, benzoic acid, trimellitic and monoalcohols [8]. In contrast, polymeric plasticisers have a molecular weight of more than 500. They form in a reaction between difunctional acids (usually adipic, sebacic acid) and ethyl glycol or propylene glycol. The most important feature of polymeric plasticisers is their very low migration from the softened plastic and their low volatility [5].

Although there are a wide variety of plasticisers used commercially, phthalates are the most widely used in most industries today [9, 10]. Some plasticiser structure differences are represented in Figure 1.1 [11].

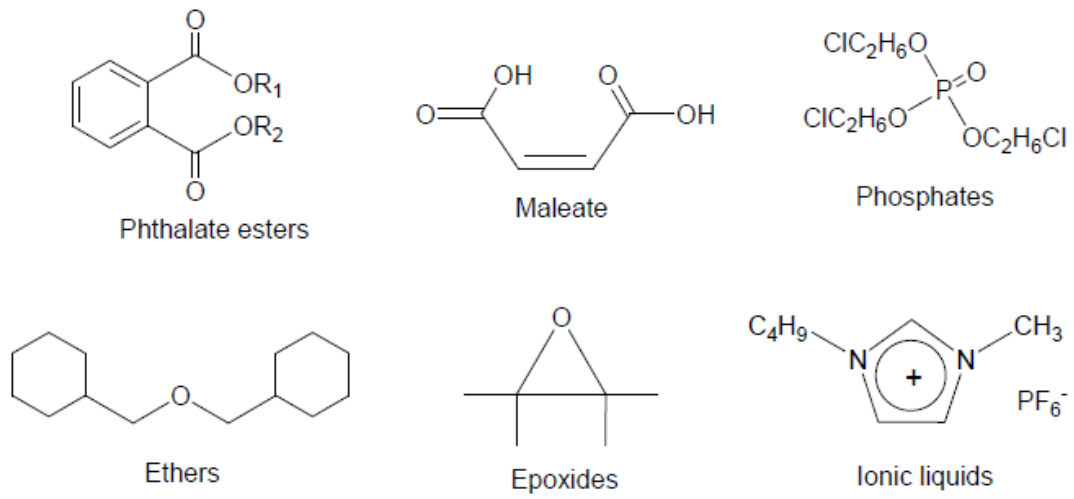



**Figure 1.1 Examples of plasticiser classes**

Plasticisers can be further classified on the basis of their solvation properties and their compatibility with PVC, namely, into primary and secondary plasticisers.

Primary plasticisers are compatible with PVC and those containing polar groups are characterised by high solvation capabilities. They are also defined as real solvents. These include sulphates and esters of alkylsulphite acids, alcohols and phenols. Diesters of *ortho*-phthalic acid produced from phthalic anhydride are the most common. They cause the polymer to gelate quickly, which facilitates the processing of PVC at typical temperatures. Similar properties are demonstrated by terephthalic diesters and alcohols containing 4 to 8 carbon atoms in the molecule, which thanks to the so-called safe, non-*ortho*-phthalic application profile, are becoming more and more popular among PVC manufacturers. The most important plasticisers belonging to the phthalic esters group include: dioctyl phthalate, di-isononyl phthalate and di-isodecyl phthalate. This group also includes: di-isoheptyl phthalate, di-isotridecyl phthalate, dibutyl phthalate, di-isobutyl phthalate, benzyl

butyl phthalate, di-iso undecyl phthalate and phthalic esters of linear alcohols with varying chain lengths.

Non-phthalate plasticisers also are classified as primary plasticisers. DEHT is the response to the ever-growing interest in non-phthalate plasticisers on the PVC processing market [12]. Primary class plasticisers are recognised as true plasticisers and are used solely because of their high compatibility and solvency properties with polymers.

Secondary plasticisers contain groups which are less polar. They demonstrate limited solubility and compatibility with PVC, which is why they are often used in mixtures with primary plasticisers. Such mixtures show a reduced tendency to migration, but increased strength at reduced temperatures and resistance to precipitation. This group includes aliphatic and aromatic chlorinated hydrocarbons (e.g. chlorinated paraffins) as well as epoxy esters of unsaturated fatty acids obtained from plants (e.g. epoxidised butyl and n-hexyl esters of unsaturated fatty acids). The secondary plasticisers require primary plasticisers to enter the polymer system and are diluents for primary classes [13]. Both primary and secondary plasticisers further enhance the final product as they have a synergistic relationship. Secondary plasticisers reduce the overall cost of the plasticisation process as they are cheaper than having higher quantities of primary plasticisers in the formulation. Secondary plasticisers are used to give the mixture special properties which include flexibility at low temperatures, reduced flammability and a lower price of the product.

## 1.3 Plasticisation process

Plasticisation occurs once a plasticiser is incorporated between polymer films in a formulation. Therefore, there would be a change in the mechanical and thermal properties of the polymer. Two processes occur during plasticisation, these include:

1. Internal plasticisation: structural features are introduced by the plasticiser which reduce intermolecular and intramolecular forces and decrease the order of the polymer. The plasticisers create less order within the polymer strands and are less in contact with one another. Flexibility of the polymer chains is achieved by allowing the polymer chains to slide past one another [14]. Internal plasticisers are inherently part of the plastic and remain part of the product.

2. External plasticisation: plasticisers are added to the polymers at increased temperature and this results in swelling of the polymer strands. External plasticisation creates a larger number of small structural units. The external plasticiser is less efficient in decreasing the stiffness of the polymer chains [15]. External plasticisers can be lost by means of evaporation, migration or extraction because they are not attached to polymer chains by primary bonds.

Both internal and external plasticisers exhibit a marked temperature dependence of material properties, which is more pronounced with internal plasticisers. At elevated temperatures, internal plasticisers have problems retaining dimensional stability. Internal plasticisation was the plasticisation mechanism in this study as the experimental process for plasticised lip coat and nail lacquer cosmetic formulations was formulated at RT [16].

## 1.4 Attributes of plasticisers

Plasticisers reduce polymer-polymer chain secondary bonding and in this way provide more mobility for the macromolecules, resulting in a more easily deformable mass. Plasticisers should be able to reduce the modulus, tensile strength, density and glass transition temperature of a polymer, while at the same time increase its flexibility, toughness and power factor [8].

Forces affecting polymer-plasticiser mixtures could be the result of hydrogen-bonding, dipole-dipole interactions as well as dispersion forces. The extent of polymer-plasticiser interactions can be measured by using methods such as:

- Gas chromatography
- Viscometry
- Melting point depression
- Fourier transform infrared (FTIR) spectroscopy

In general, plasticisers are compared to a reference plasticiser with well-known characteristics, such as DOP. A particular characteristic feature such as modulus or hardness is selected, and a value for this characteristic is determined. The effectiveness of the plasticiser is consequently determined as the ratio of plasticiser concentrations (test/DOP) for this specifically chosen characteristic [17].

## 1.5 Mechanisms of plasticiser action

Four significant theories have been postulated to explain how a plasticiser affects a polymer in both internal and external plasticisation. These are discussed in the subsequent sections.

### 1.5.1 The Lubricity Theory

This theory describes the effect of an external plasticiser on a polymer in terms of lubrication. A resin without any plasticiser (a 'dry' polymer) is rigid due to friction between its chains which bind together into a network. Upon heating the polymer, in order to be plasticised, the binding is weakened, and subsequently, the smaller plasticised molecules are able to slip in between the chains. When the polymer cools, the plasticiser molecules act as a lubricant between the chains, allowing them to 'slip'.

### 1.5.2 The Gel Theory

This is an extension of the one described above and implies that the plasticiser molecules break up the polymer-polymer interaction by forcing themselves in between the chains and 'obscuring' these interaction sites from the polymer molecules.

### 1.5.3 Free Volume Theory

This theory allows for some quantitative analysis of polymer-plasticiser interaction. The free volume of a polymer can be seen as the 'empty internal space' available for the polymer chains to move. It has been proved that the free volume of a polymer dramatically increases when it reaches the glass transition temperature. The glass transition temperature is the point at which significant molecular motion starts to occur. This motion is similar to an increase in the free volume of the polymer and could be the result of movement of the chain itself, or its ends, or due to side chains attached to it. The question now arises how this is facilitated by the addition of a plasticiser. Its lower molecular weight equates to an increase in the free volume per volume of material (not a lot of volume is added with these

small molecules, in fact, there is a lot more free space between them than between the polymer molecules). In short, the addition of a plasticiser allows a polymer to behave, at RT, in a way the pure resin would only behave at elevated temperatures.

### 1.5.4 Mechanistic Theory

The Mechanistic Theory considers that plasticiser molecules are not bound permanently to the polymer chains, but are free to associate and dissociate with the amorphous sites of polymer molecules. Since the interactions between plasticiser and polymer molecules are weak, there is a dynamic exchange process where one plasticiser molecule attached at a site is readily dislocated and replaced by another. Different plasticisers display different strength in plasticiser-polymer and plasticiser-plasticiser interactions. At low plasticiser levels, the plasticiser-polymer interactions are dominant, while at high plasticiser concentrations, plasticiser-plasticiser interactions predominate.

Figure 1.2 illustrates and compares the Lubricity, Gel and Free Volume Theories associated with plasticisation.

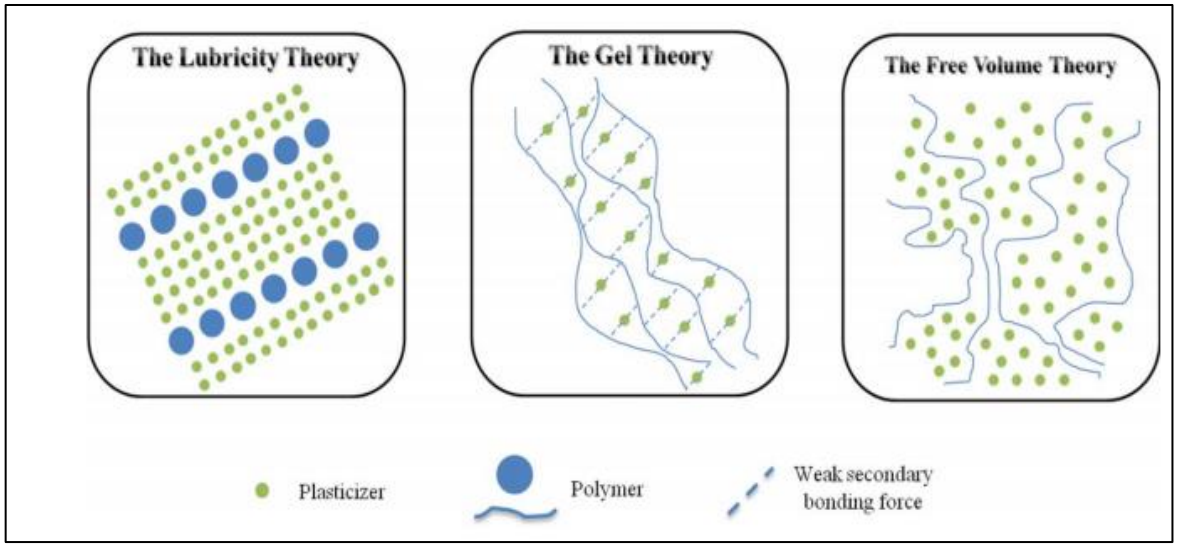



**Figure 1.2 Schematic illustration of plasticisation theories [18]**

## 1.6 Plasticiser market

Figure 1.3 shows that China has the single largest plasticiser market in the world, accounting for nearly 42% of world consumption in 2017 [19]. The Chinese market also has the highest estimated consumption growth between 2017 to 2022, spurred by increased plasticiser consumption in goods for both domestic and export markets. Overall, global plasticiser consumption will grow at a rate of about 3% per year in the next few years [2].

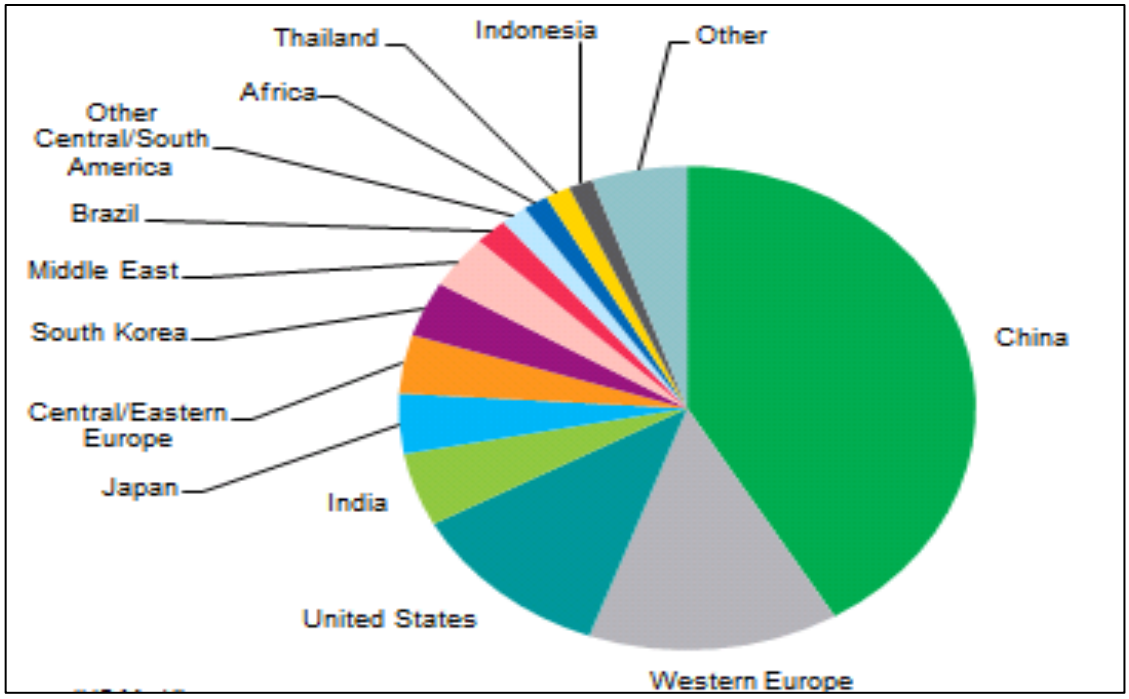



**Figure 1.3 World consumption of plasticisers (2017) [19]**

In 2014 the global plasticiser consumption was estimated at 8 million metric ton (MT). Figure 1.4 illustrates that 70% of global plasticiser consumption consisted of phthalates [20]. The global cosmetic products market was valued at 532,4 billion US Dollar in 2017. Worldwide the nail product market is 3,3 billion US Dollar of which 75% consists of nail lacquer [40].

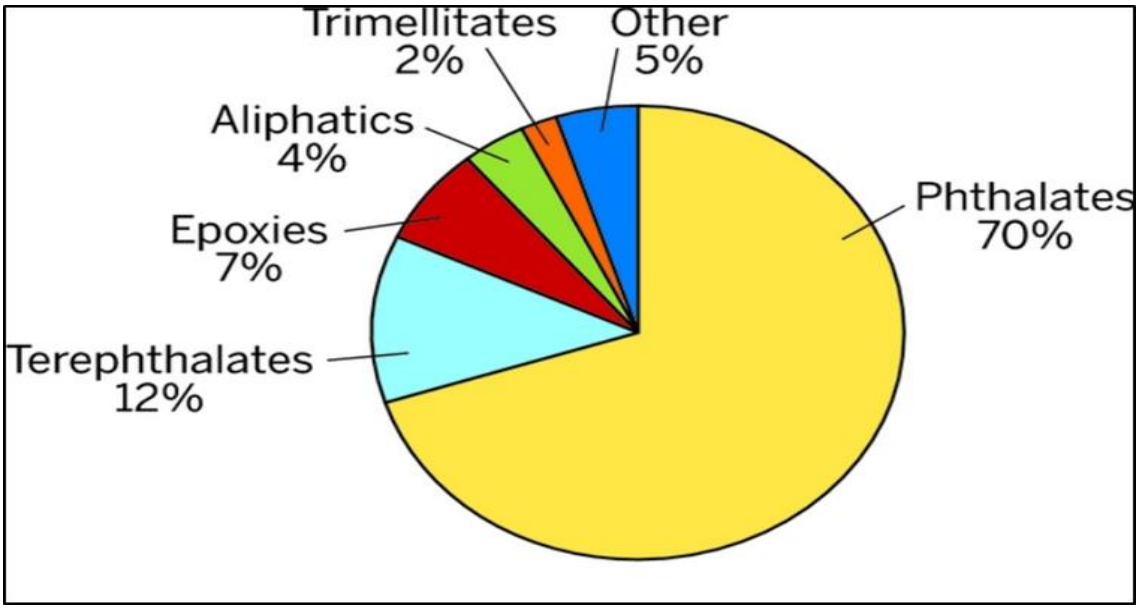



**Figure 1.4 Global plasticiser consumption (2014) [20]**

The world consumption of phthalate plasticisers is forecast to grow at an average annual rate of 1,3% during 2017 to 2022. World consumption of lower molecular weight phthalates, however, is predicted to decline in many regions as a result of replacement, mostly by non-phthalate plasticisers [21]. The demand for most downstream plasticiser markets is mostly influenced by economic conditions. For this reason, the demand for these plasticiser markets largely follows the patterns of the leading world economies.

## 1.7 The importance of plasticisers in cosmetics

Article 2 of the EU Cosmetics Regulation (Regulation (EC) No. 1223/2009) incorporates the following definition of a cosmetic product: 'Cosmetic products are defined as any substance or mixture intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively, or mainly, to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition'. Plasticisers are used by cosmetic manufacturers to improve the softening and deformation of a material, as is desired in nail polishes, glosses and shampoos [17]. However, the EU banned several plasticisers in cosmetic products (mainly phthalates) since these substances may cause a wide range of health problems [16].

The majority of consumers of cosmetics are females who are interested in the physical appearance of these products. The colour, odour, consistency and texture of these products are of high importance.

Recent trends in products are the terms 'eco-friendly', 'bio', 'green' and 'naturalness'. Consumers are becoming more aware and concerned about the ingredients in the products they use [19], thus influencing the manufacturers of raw materials and cosmetic products to produce safer, less toxic, natural ingredients and products, including plasticisers.

## 1.8 Phthalates in cosmetics

The most commonly used phthalate is DBP because of its ready availability and low price. A cosmetic product search was thus conducted to determine how many commercial products, and which types of product, use DBP as plasticiser [22].

### 1.8.1 DBP

The following findings were made:

- Alternatives to phthalates are indeed available to industry, as only a small percentage of any given type of cosmetic or beauty product contains phthalates
- The buyer of a cosmetic product has no practical way to choose phthalate-free products. The 'ingredients label' is printed so small as to be nearly unreadable, and a typical shopper will not know that 'DBP' is synonymous to 'butyl ester plasticiser'. Other expensive perfumes contain ingredient labels inside the packaging which cannot be read until the product has been purchased. Products were found on store shelves, mainly imported products, that lacked ingredient labels altogether. This is in direct violation of United States federal regulations
- It is not possible to develop a comprehensive list of cosmetic and beauty products that contain phthalates since this will require a product-by-product, label-by-label search of every single cosmetic and personal care container sold worldwide

As part of the Environmental Working Group (EWG) mission in search of DBP-containing cosmetics and beauty products, the United States patent office records were searched for products that contain DBP in the patent application. It was

found that DBP may be used in a broad range of beauty and personal care products, such as shampoos and conditioners, lotions, hair growth formulations, antiperspirants as well as sunscreen. It can also be used as an ingredient for gum, candy and orally-ingested pharmaceuticals.

The cosmetic industry adds phthalates to their products because of the useful properties DBP exhibits as an additive in many types of cosmetics. These properties include its ability to impart flexibility to thin films for mascara and nail polish, its oily texture that makes skin feel soft, and its ability to make lotions penetrate deeper into the skin. Electronic searches done in real-world drugstores showed that, for the consumer, the products most easily found are nail enamels containing DBP.

There are 38 companies and inventors holding 105 recent cosmetic-related patents proposing DBP as an additive. DBP in nail polishes is being added at more or less 5% by weight (for example, Maybelline nail enamel patent 5972095). A certain night cream invented by the Japanese contained a total of 20% DBP. Proctor and Gamble is the owner of more cosmetic patents containing phthalate-related products than any other company (37 of 105 patents analysed) [22].

Both the Elizabeth Arden Company (New York) and Chesebrough Ponds (Greenwich), have patents in which DBP is proposed as a penetration enhancer in cosmetic formulations. Elizabeth Arden claims DBP to be used as an additive to skin care products, whereas, instead, DBP is used to deepen product penetration into the skin and in this way 'improve' the product's delivery mechanism through the stratum corneum to its site of action in the epidermis. In the same fashion Chesebrough Ponds claims that DBP could be added to a hair growth formulation for men to assist its ability to penetrate deeper into the scalp, directly to the site of action at the hair follicles.

Further research from the chemical giant Zeneca provides more evidence that DBP acts as a penetration enhancer. This work reveals that when DBP is added to products for the skin, the enhanced allergic reaction evolves from DBP's ability to deliver the chemicals deeper into the skin [22].

The use of DBP as a penetration enhancer stands in direct contrast to the Centre for the Evaluation of Risks to Human Reproduction's assertion that 'Dermal contact with products containing DBP is possible, but absorption through the skin is most likely minimal' [23]. The Centre for Disease Control and Prevention (CDC) cites a study of DBP migration through rat skin. CDC, on the other hand, upon discovering high levels of DBP in women of child bearing age, postulates that dermal absorption is playing a role: 'Dermal absorption also occurs at a significant rate for phthalates with short side chains such as DBP,' citing the same rat study as evidence. Regardless of how various government agencies are interpreting the dermal absorption study in rats, the industry continues to use DBP specifically for its 'penetration enhancing' effect [22].

Products for which DBP is proposed as a possible additive are listed below [22]:

- The Procter & Gamble hairspray, mousse, gel, lotion, cream, pomade, hair spray, conditioner, spritz, hair tonic, facial moisturizers, foundations, lipsticks, mascaras, nail polishes, oral pharmaceuticals, hair loss treatments
- L'Oréal hair and nail products
- Lever Brothers Company deodorant, skin and hair cleansers
- LVMH Recherche nail varnish
- Shiseido Co., Ltd. skin cream for pharmaceuticals or night cream
- Kirker Enterprises, Inc. nail enamel
- Mansouri, Zari skin lotions
- Maybelline Cosmetics Corporation nail enamel
- Woodward Laboratories, Inc. (Los Alamitos, CA) nail products

- Almell, Ltd. (Dallas, TX) nail products
- Astra Aktiebolag (Sodertalje, SE) lotions and skin creams
- Yissum Research Development Company of the Hebrew University of Jerusalem product to treat tooth and gum disease
- Akzo Nobel NV (Arnhem, NL) fabric softeners and personal care compositions
- Anheuser-Busch, Incorporated (St. Louis, MO) gelled antiperspirant
- Chesebrough-Pond's USA Co., Division of Conopco, Inc. (Greenwich, CT) product to treat or prevent baldness
- Colgate Palmolive Company (New York, NY) antiperspirant and deodorant gels
- Digestive Care Inc. (Lebanon, NJ) coating ingredient for oral pharmaceutical
- Eastman Chemical Company (Kingsport, TN) nail product
- Elizabeth Arden Co., Division of Conopco, Inc. (New York, NY) skin products
- Goldiner, Arthur (1565 Strand Way, Oceano, CA 93445) Camplese, Linda custom fit teeth
- Henkel Kommanditgesellschaft auf Aktien (Duesseldorf, DE) skin care and hair care formulations
- Kao Corporation (Tokyo, JP) emulsion for general cosmetics
- Kao Corporation (Tokyo, JP), Taiyo Kagaku Co.,Ltd. (Yokkaichi, JP) hair care product
- Kraft General Foods, Inc. (Northfield, IL) sunscreen
- Laboratoires Virbac (Carros, FR) added to stabilize drugs
- Minnesota Mining and Manufacturing Company (St. Paul, MN) general cosmetics and personal care products
- Mitsui Toatsu Chemicals, Inc. (Tokyo, JP) hair care products
- Resler, Renee (3046 E. Marlette, Phoenix, AZ 85016) nail products
- Revlon Consumer Products (NY, NY) nail enamel
- Rhodia Chimie (Courbevoie, FR) hair and skin care products (sprays, tonic lotions, gels, mousses)

- Rhone-Poulenc Chimie (Courbevoie Cedex, FR) nail varnishes
- Unilever Patent Holding B.V. (Vlaardingen, NL) skin and hair care products, antiperspirants
- Wacker-Chemie GmbH (DE) nail varnish
- Warner-Lambert Company (Morris Plains, NJ) chewing gum and candy
- Witco Corporation (Greenwich, CT) conditioning products for skin and hair

DBP in product: Company and Product by weight [22]

- Procter & Gamble (Cincinnati, OH) long wear nail polish 7%
- Shiseido Company, Ltd. (Tokyo, JP) oil essence 10%
- Woodward Laboratories, Inc (Los Alamitos, CA) nail coating 3.4%
- L'Oréal treatment base for nails 3.8%
- Procter & Gamble (Cincinnati, OH) pump hair spray 0.2%
- Kirker Enterprises, Inc (Paterson, NJ) nail enamel 7%
- Maybelline Cosmetics Corporation (Wilmington, DE) nail enamel 5%
- Shiseido Company, Ltd. (Tokyo, JP) night cream 5%
- LVMH Recherche nail enamel 6-8%
- Shiseido Company, Ltd. (Tokyo, JP) skin cream 20%
- Wacker-Chemie GmbH (Denmark) nail varnish 2%
- L'Oréal nail varnish 5%
- Digestive Care Inc. (Lebanon, NJ) coating for oral drugs 2%
- Kao Corporation (Tokyo, JP) cosmetic emulsion 7%
- Procter & Gamble (Cincinnati, OH) oral drugs 5 mg per dosage unit
- Anheuser-Busch, Incorporated (St. Louis, MO) antiperspirant gel 10%

1.8.2 Molecular structure of DBP

Figure 1.5 illustrates the DBP molecule with the carboxylate chain attached to carbons 1 and 2 of the benzene ring.

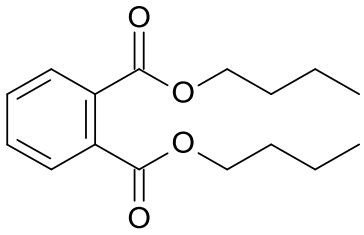



**Figure 1.5 Molecular structure of DBP**

## 1.9 Health concerns of phthalates

1.9.1 Exposure to phthalates

Soft vinyl products could contain more than 40% phthalates by weight [21]. People are widely exposed to phthalates due to the fact that vinyl is a ubiquitous plastic used to make anything from furnishings (for example, flooring and wallpaper), medical devices (for example, catheters, and blood bags), children's items (such as infant feeding bottles and squeeze toys) to packaging (disposable bottles and food wrapping). Often, children chew on toys, like teethers, which are made from highly phthalate-softened vinyl and in this way they take in more than the average amounts of phthalates.

Beyond vinyl, humans are even further exposed to phthalates in cosmetics and scented products, such as perfumes, soaps, shampoos, etc. Furthermore,

phthalates are added to insecticides, adhesives, sealants and car-care products [24].

Another way in which human phthalate intake can occur is through blood transfusion. Phthalates migrate from vinyl or polyvinyl chloride (PVC) medical devices into solutions that are then fed into the patient. Sick people, especially children with their immune system in the process of developing, may be susceptible to this type of exposure. In September 2001 the United States Food and Drug Administration (FDA) warned that medical devices made of vinyl might cause patients to be exposed to unsafe amounts of DEHP [22]. The American Medical Association (AMA) had concerns regarding DEHP-containing medical devices. Furthermore, a Health Canada Advisory Panel suggested that health care providers should not use DEHP containing medical devices in certain patient groups, including infants and males before puberty. The National Toxicology Program raised concerns that the developing male genital tract in humans may be adversely affected by high levels of DEHP. DEHP is used in scented products, such as soaps, lotions and perfumes. It is also found in plastic products, such as toothbrushes, toys and food packaging [25]. Another route of phthalate intake from personal care products, such as soap, is via skin contact. As shown by the CDC study of phthalates, the breakdown product of diethyl phthalate [26] was detected in the highest level in the tested population.

Worrisome for children in particular, is inhaling air and dust containing phthalates which have escaped from vinyl flooring, since they spend a lot of time indoors breathing close to the floor. An initial study conducted in Norway showed a higher incidence of bronchial obstruction in children living in houses with vinyl, as opposed to wooden-floors [27].

### 1.9.2 Leaching of phthalates

In a polymer-plasticiser system, there is continuous association and segregation between polymer and plasticiser molecules. It is therefore a possibility that the plasticiser could have a tendency of leaching from the system. Two primary factors could be possible for this leaching tendency, namely the diffusion rate of plasticiser molecules through the polymer matrix and the rate at which plasticiser molecules leach from the outer surface [28].

Researchers in Germany showed that DBP could leach out of the nail polish or lacquer as a result of hand washing [22]. This conclusion was made after they had measured leaching of DBP from nail polish. The polish becomes brittle as the plasticiser leaches out of the film. DBP could, therefore, be absorbed through a nail, as well as directly through the skin (*in vitro* dermal absorption). DBP exhibits both plasticising and film formation properties when used in a nail polish or nail lacquer. Upon applying nail polish, volatile chemicals as part of the formulation start to volatilise, leaving behind a film coating over the nail. DBP, being part of the non-volatile content of the film being left behind, reduces cracking and brittleness in the film. Subsequently, there is a possibility for DBP to be released into the body via dermal absorption [24].

Anaphylactic shock was reported in a 1999 case study as a severe allergic response to DBP. This patient was exposed to DBP in the coating of an oral pharmaceutical product [22]. According to the French cosmetic company L'Oréal, in patent 5,676,935, it states that alternative plasticisers are being used in order to substitute phthalates as plasticisers in nail varnishes. In this way, any allergic reactions could be prevented. An allergic reaction caused by DBP may induce a state of hypersensitivity in the immune system [25].

The loss of plasticiser from a plasticised polymeric system is a common way of leaching of plasticiser into physiological fluids after its application. This is a significant problem being encountered, since the loss of plasticiser results in modification of the properties and functions of the initially plasticised polymer system. Hence, the use of plasticisers is being questioned due to their possible toxicity as a result of leaching. Interest in the use of natural-based plasticisers, characterised by their low toxicity, is on the increase.

The dialkyl or alkylaryl esters of 1,2-benzene dicarboxylic acid, commonly referred to as phthalates, belong to an important class of endocrine-disrupting chemicals (EDC) which are characterised by their developmental toxicity, carcinogenicity, mutagenicity, neurotoxicity and immunotoxicity [29]. Of 289 volunteers tested in 2009 for phthalates, all of them tested positive for phthalates. Sexual abnormalities and deformities (low sperm counts) were also detected as a result of phthalates [30].

A study conducted by the National Health and Nutrition Examination Survey between 1999 and 2014 found phthalates in trace amounts in the urine of women [31]. Figure 1.6 presents median concentrations of DEHP, DBP, and butyl benzyl phthalate (BBzP) metabolites in urine over time for women of ages 16 to 49 years, using data from 1999 to 2014.

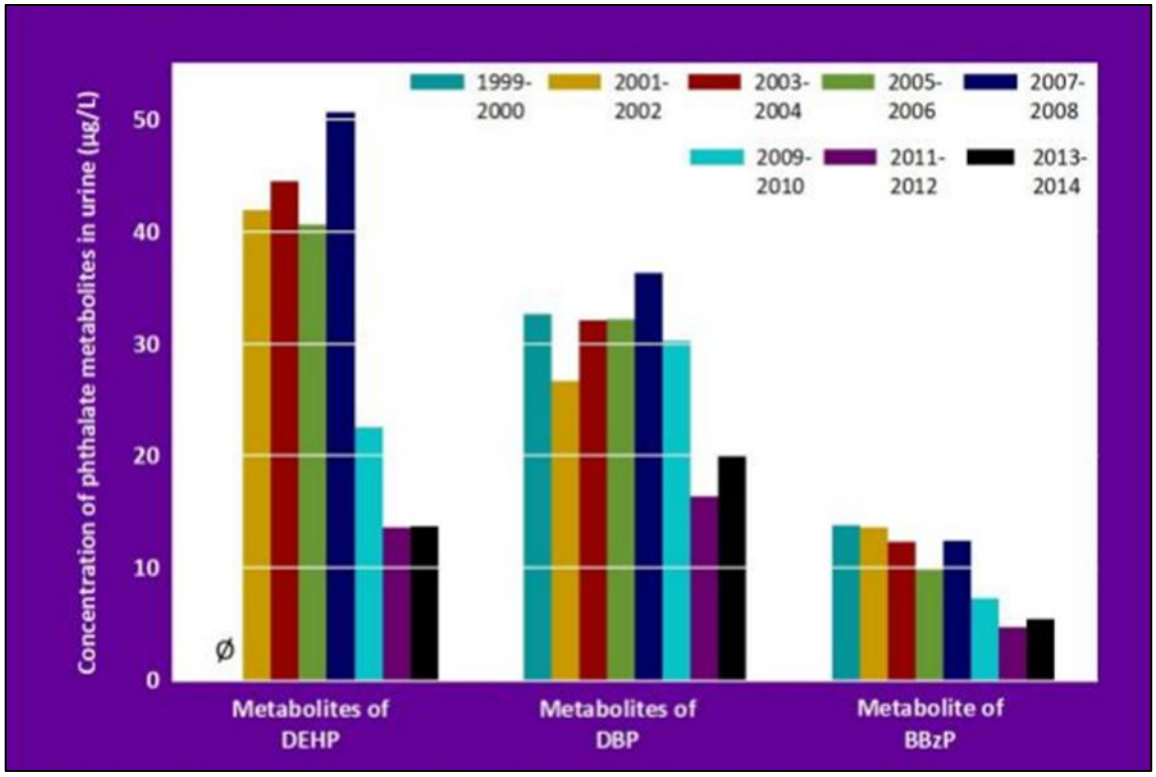



**Figure 1.6 Phthalate metabolites in women of ages 16 to 49 years [31]**

From 1999 to 2014, the median level of DEHP metabolites in urine of women ages 16 to 49 years varied between 41 and 51 µg/L, and was 51 µg/L in 2007 to 2008. Over the same period the median level of DBP metabolites in urine of test subjects varied between 27 and 36 µg/L (Figure 1.6) [31].

Huge concern has been raised in neonatal care applications since newborns receive high doses of DEHP via blood transfusions, respiratory therapy, etc. Adults are also subjected to DEHP exposure from medical plastics [32].

### 1.9.3 Toxicity of phthalates

DBP is regarded as a powerful reproductive and developmental toxicant in male laboratory animals. Currently, DBP is known to cause a broad range of birth defects and lifelong reproductive impairment in these laboratory animals exposed in utero and shortly after birth. Potential negative health effects of DBP is an ongoing threat to newborn animals exposed to DBP by breathing phthalate-contaminated air, by touching phthalate-containing objects, ingesting their mother's milk, which could possibly contain phthalates as a result of her exposures. In animal tests, DBP could prevent implantation of the fertilised egg, or even cause the loss of pregnancy [33]. In laboratory animals, it is also the culprit for the mother's body essentially 'dissolving' the foetus without miscarriage. Skeletal and external birth defects for male and female offspring are also caused as a result of animals being exposed to DBP during pregnancy. Other birth defects include deformity of vertebra and ribs, cleft palate, and fused breastbone [33]. A study by the European Commission found a 'safe' dose, called NOAEL (no observed adverse effect level) of 50 mg/kg/day [34]. Phthalates should be considered as being the main culprit attributing to the following human health effects:

- Average sperm count is on the decline in industrialized countries according to studies performed at the University of Missouri [35]
- Hypospadias refers to a physical deformity of the penis in which the opening of the urethra appears on the bottom of the penis instead of the tip. Studies done by the CDC show that hypospadias in the United States of America is on the increase since the last few decades [36]
- Undescended testicles are a birth defect, where testicles fail to descend into the scrotum during pregnancy, this defect is on the increase in Western countries. The risk of developing testicular cancer and breast cancer is greatly increased if men are born with this condition [36]

- Testicular cancer is the most common cancer found in young males in many countries [37, 38]

### 1.9.4 Regulation of phthalates

Haircare, skincare, personal care, oral care, perfumery, sunscreens and makeup products are all used in our daily routines to keep us clean, maintain healthy skin and teeth, to look good and smell nice. Several international laws ensure the safety of these types of products; all referred to under the umbrella term 'cosmetics'. Europe is a world leader in the cosmetics industry and dominant cosmetics exporter. The sector is highly innovative and provides significant employment in Europe. The European Commission (EC) involvement mainly concerns the regulatory framework for market access, international trade relations, and regulatory convergence. These all aim to ensure the highest level of consumer safety while promoting the innovation and the competitiveness of this sector. Regulation (EC) N1223/2009 on cosmetic products is the main regulatory framework for finished cosmetic products when placed on the EU market. It strengthens the safety of cosmetic products and streamlines the framework for all operators in the sector. The Regulation simplifies procedures to the extent that the internal market of cosmetic products is now a reality [39].

Phthalates are regulated as toxic pollutants in air and water. On the other hand, phthalates are not regulated in food and cosmetics.

The main phthalates used in cosmetics are DBP, dimethyl phthalate (DMP) and diethyl phthalate [26]. These are used at concentrations less than 10% as plasticisers in cosmetics, such as nail polishes (reducing cracking by making them less brittle), hair sprays (stiffness of the hair is prevented by means of a flexible film being formed on the hair), as well as solvents and perfume fixatives in many

other cosmetic products [40]. However, manufacturers in Europe have put restrictions on the use of DBP in nail polish and certain cosmetics in order to eliminate its use in certain nail products [40, 41].

Industries are obliged to keep track of transportation of DBP as well as spillages thereof. They are also limited as to the amounts of DBP released into the environment each year. The FDA, however, does not have a limitation on DBP used in cosmetics and beauty products. The FDA act does not require cosmetic manufacturers to test their products for safety. The labelled cosmetic product does not state the amount of phthalates in the product. Industry 'hides' phthalates in consumer products as being components of fragrances, or chemical mixtures that are seen as 'trade secrets' – these are all exempted from labelling requirements.

The buyer is thus unaware of the health effects the use thereof has on her health or the health of her foetus. Even women working in nail bars who are expected to get the highest exposures, are not aware of any labelling regulations.

## 1.10 Environmental impacts of cosmetics

Cosmetic and personal care products are used in vast quantities throughout the world, as a result of their regular use, they are continuously released into the environment in huge amounts. Many of these products are biologically active and are characterised by persistence and bioaccumulation potential, posing a threat to the ecosystem and human health. According to a widely accepted classification, cosmetics can be divided into leave-on and rinse-off products. A leave-on cosmetic is a product that for its functions is intended to stay on the skin for a somewhat extended period; examples are perfumes, decorative cosmetics, body and face creams, and antiperspirants. On the contrary, a rinse-off cosmetic is a product

designed to be rinsed off after a short stay on the skin or mucous membranes, such as shampoos, soaps, shower gels, and toothpaste. In the last few years, cosmetics, as well as pharmaceuticals and many other products for personal care that do not fall within cosmetic regulation (disinfectants, insect repellents, dietary supplements), have raised significant concerns as one of the most critical classes of emerging pollutants because they are continually released into the aquatic environment, their ecological and environmental impact is associated with the massive amounts used and with the fact that sometimes they are environmentally persistent, bioactive, and potentially able to bioaccumulate.

All these personal care and cosmetic products contain many chemicals, including plasticisers. Many personal care products are overused by the consumer, leading to higher dosages of these chemicals than recommended.

Cosmetics pose the most pressing ecological problems compared to pharmaceuticals because they are used in much larger quantities and throughout the course of life and, being intended for external application, are not subjected to metabolic transformation, therefore they are introduced unaltered into the environment in large amounts during washing, showering, or bathing [42]. Since relatively little is known about the fate and the toxicity of personal care products released into the environment, increasing attention is being placed on their occurrence, persistence, and their potential threat to ecosystems and human health. Ultimately, all these chemicals derived from personal care products can be received into environments (soils, sludges, air, ground and surface water, sewage, landfills, etc.) by regular usages, such as bathing, showering, excretion, spraying or disposal of products. As they are released uncontrollably, they can bypass treatment systems and these active ingredients and metabolites are then released into the environment and aquatic systems, in particular, in micro-concentrations [43]. The bioactive compounds disrupt the industrial ecology and there have been

reports of the accumulation of these bioactive compounds in aquatic organisms [44].

## 1.11 Alternative plasticisers (bio-plasticisers)

Traditionally, polymers are synthesised from petrochemical by-products. Researchers within the last decade are seeking alternatives to reduce the use of petroleum-based by-products. Petroleum based by-products have negative consequences both economically and environmentally including greenhouse gas emissions, and as they are non-degradable, create issues when disposed in landfills. As a result, the demand for the development of plasticisers from renewable resources has increased. Suppliers have responded to this higher demand, resulting in a search for new solutions to current and future requirements. Bio-plasticisers can be classified as compounds that have been derived or synthesised from one or more natural resources [45].

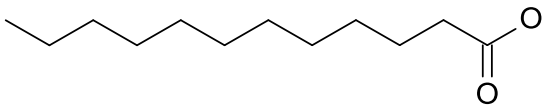
### 1.11.1 Overview of commercially used bio-plasticisers

Approximately 50 000 tons of bio-polymer based plastic packaging are currently produced in Europe. The packing industry currently contributes in the order of 5 to 10% to green packing material [46].

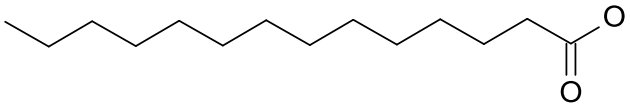
Bio-plasticiser synthesis from vegetable oils like soybean, linseed, sunflower [47], castor and canola oil [48] has increased in recent years. Generally, the synthesis of bio-plasticisers uses vegetable oils as the primary source. Vegetable oils are sustainable, renewable, abundantly available and bio-degradable with low toxicity [49]. Described below are some examples of alternative bio-plasticisers.

1.11.1.1 Coconut oil

A bio-based polymer such as polylactic acid (PLA) could be a sustainable bio-plastic alternative. However, PLA is found to be too brittle to be used, for example, in flexible food packaging. Bhasney *et al.* [50] investigated the use of coconut oil (CO) as a plasticiser for PLA and found that flexibility of PLA films improved and that CO could give suitable properties for food packing applications. CO is the oil extracted from the kernel or meat of the coconut. CO contains fatty acids which can serve as a good plasticiser even when used in small concentrations [50]. CO contains mostly medium chain (6 to 18 carbons) saturated fatty acids (caproic to stearic) in the form of triglycerides [51]. Lauric acid (48.4%) and myristic acid (18.43%) make up the bulk of CO (Figure 1.7) [52].




Lauric acid




Myristic acid

**Figure 1.7 Lauric and myristic acid chemical structures [53]**

1.11.1.2 Soybean oil

Soybean oil is an example of a vegetable oil which is extensively produced as a bio-based product particularly for polymeric use. The wide use of soybean oil is due to its large production, low cost and easy conversion of the double bonds to three-membered epoxide rings (Figure 1.8). It is important to achieve a high number of epoxide rings (or high oxirane content) that will result in increased cross-linking and consequently increased bio-plasticiser properties [54]. US patent 5578297A refers to epoxidised soyabean oil being a bio-plasticiser in a variety of cosmetic compositions including nail varnish and mascara [55].

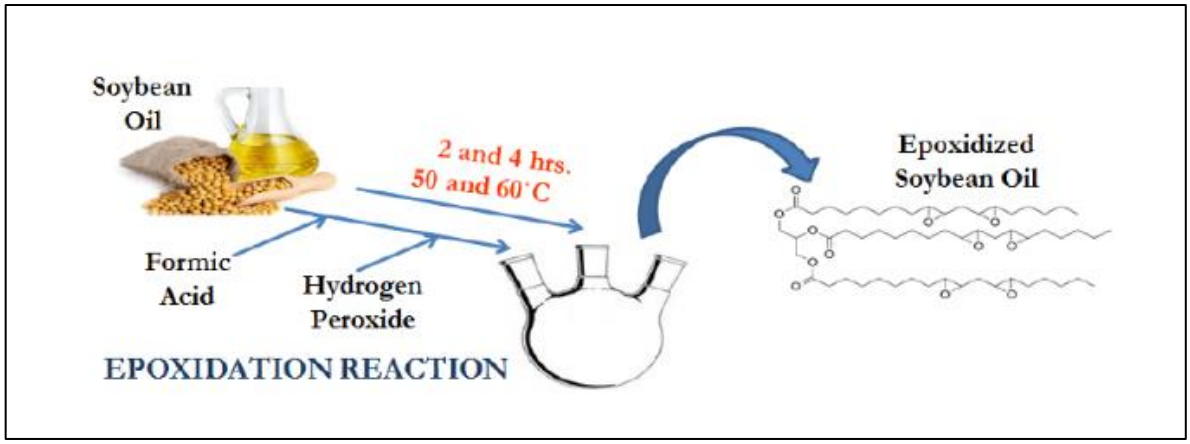
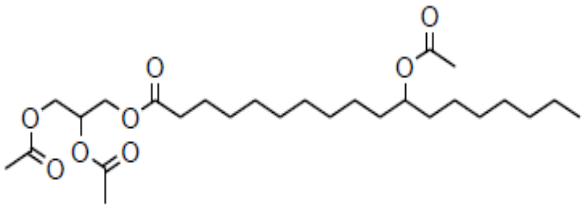


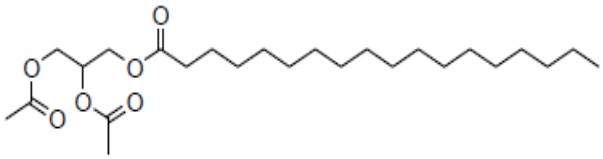

**Figure 1.8 Process for epoxidation of soybean oil [56]**

1.11.1.3 SOFT-N-SAFE™

Another bio-plasticiser that has been synthesised and tested is SOFT-N-SAFE™ derived from castor oil. Hydrogenated castor oil is esterified with excess glycerine and the resulting mixture distilled to create a product that typically contains 95 to 96% monoglyceride (Figure 1.9). The free hydroxyl groups on the monoglyceride are then esterified with acetic acid [57].



Monoester



Monoesterate

**Figure 1.9 Soft-N-Safe™ composed of monoester (left) and monoesterate (right)**

1.11.1.4 Hexamoll®

BASF manufactures 1,2-cyclohexane dicarboxylic acid di-isononyl ester (DINCH), a non-phthalate plasticiser known as Hexamoll® DINCH. Chemically, it belongs to the family of aliphatic esters. DINCH is a complex mixture of 9 carbon branched-chain isomers [58]. This chemical is produced by hydrogenation of di-isononyl phthalate (DINP) in the presence of a catalyst, or the Diels-Alder reaction of a maleic acid ester with 1,3-butadiene followed by hydrogenation [59, 60].

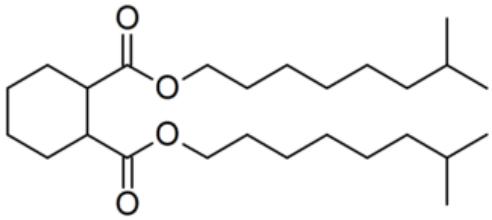


**Figure 1.10 Structural formula of DINCH**

DINCH has similar performance characteristics to DINP. DINCH provides low temperature performance compared to the phthalates. Figure 1.10 shows the

structure of DINCH while Figure 1.11 shows the hydrogenation of DINCH from DINP.

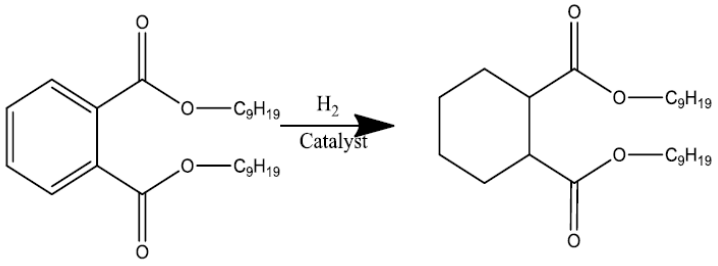
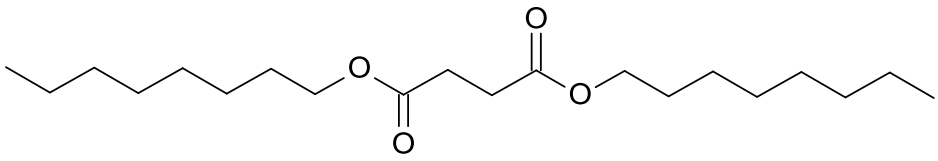


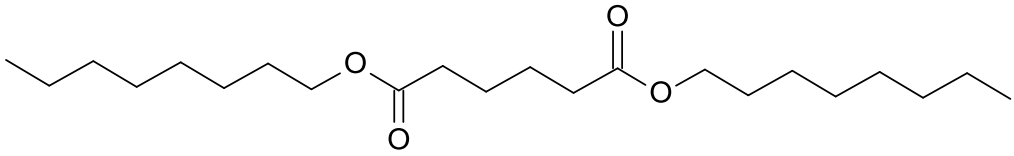

**Figure 1.11 Production of DINCH from hydrogenation of DINP**

1.11.1.5 OXEA Products

OXEA is another manufacturer that offers a wide range of plasticiser solutions to support the increasing market shift to phthalate-free and bio-based plasticisers. Examples of their commercially manufactured phthalate-free plasticisers include OXSOFT® DOA (dioctyl adipate) and bio-based OXBLUE® DOSX (dioctyl succinate). The structure of these plasticisers are illustrated in Figure 1.12 [61]. OXSOFT® DOA (MW: 370.6 g/mol) is a highly efficient plasticiser with excellent low temperature properties. Suggested applications are food cling wraps, garden hoses, gaskets, flooring and blended with other plasticisers to improve efficiency and/or low temperature properties. OXBLUE® DOSX (MW: 342.51 g/mol) is a bio-based plasticiser with outstanding performances for low temperature use and applications in an oily environment. OXBLUE® DOSX is a sustainable solution which enables producers to economically manufacture products with no compromise on performance. Suggested applications include flooring, food cling wraps and adhesives and sealants.



Dioctyl succinate



Dioctyl adipate

**Figure 1.12 Structures of Dioctyl succinate and Dioctyl adipate**

1.11.2 Overview of non-phthalate plasticisers used in this thesis

1.11.2.1 DEHT

The most common terephthalate is DEHT, see Figure 1.13, some also refer to this as dioctyl terephthalate (DOTP). Terephthalates actually are the non-toxic replacement, as well as an isomeric structure, for toxic phthalates. These are esters of terephthalic acid which are being produced by means of a reaction between terephthalic acid and monohydride alcohol in a similar way to phthalate synthesis.

Despite the word phthalate in the name, DEHT is not considered a phthalate ester. Phthalate esters are based on *o*-phthalic acid, whereas DEHT is based on *p*-phthalic acid. DEHT (Eastman™ 168 non-phthalate plasticiser) is thus a non-phthalate alternative which closely matches the performance requirement of the plasticiser in current coating formulations.

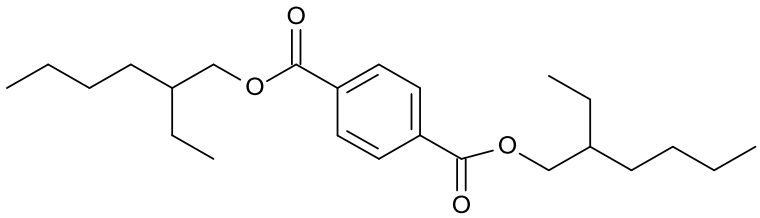


**Figure 1.13 Molecular structure of DEHT [62]**

Terephthalate esters do not possess the same toxicological issues as *o*-phthalate esters [63]. Figure 1.14 compares non-toxic *p*-terephthalate DEHT with the diester side chains emanating from the *p*-positions of the benzene ring versus toxic *o*-DEHP with its diester side chains in the *o*-position [64].

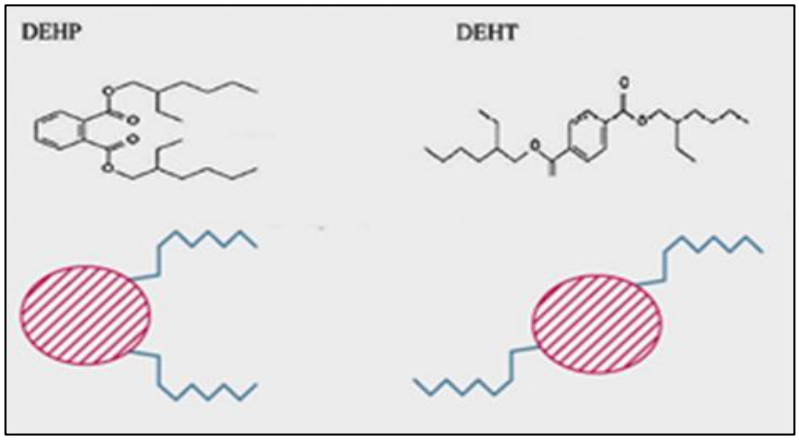



**Figure 1.14 DEHP [65] versus DEHT (non-toxic)**

DEHT offers one of the best cost-effective and performance efficient commercially available non-phthalate alternatives. It has been used to substitute phthalate esters into formulations with minimal production changes.

Eastman™ 168 (DEHT) is a very well-studied material with a robust set of data. These terephthalate esters have little or no potential to form an active monoester.

Terephthalate esters demonstrate a rapid and almost complete metabolic hydrolysis back to terephthalic acid and the corresponding alcohol.

#### 1.11.2.2 PMD-citronellal acetal

##### 1.11.2.2.1 Introduction

*p*-Menthane-3,8-diol-citronellal acetal (PMD-citronellal acetal) is a naturally occurring compound found in *Eucalyptus citriodora* oil. The leaves and branches of the *Eucalyptus citriodora* tree are utilised to extract Eucalyptus oil (Figure 1.15) [66]. These trees are readily grown in South Africa and are suitable for the climate. This oil is used in many applications, for example as an antiseptic, pharmaceutical, fragrance and insect repellent. It can be applied topically and be ingested, as long as ingestion of the pure Eucalyptus oil does not exceed 0,5 ml/kg of total body weight, since this is a lethal dose in adults. The PMD-citronellal acetal is produced in the leaf as the Eucalyptus leaves mature by reaction of PMD and citronellal, both present in the leaf. Synthetic PMD-citronellal acetal is formed when PMD is reacted with pure citronellal [67].

**Figure 1.15 *Eucalyptus citriodora* branch**

1.11.2.2.2 Background research on PMD-citronellal oil

At Nelson Mandela University (NMU), several researchers have conducted studies on *p*-menthane-3,8-diol derivatives as potential plasticisers. The work described in the thesis of Guyo forms one of the research focus areas within InnoVenton (the Institute of Chemical Technology). Guyo [68] concluded that plasticiser properties were evident when these derivatives were subsequently formulated in Methocel® (methylcellulose) polymer to form free films. Physical and thermal testing confirmed plasticiser potential.

The work described in the thesis by Mafu [69] shows a small production platform for citronellal processing and the development of a single continuous flow reactor system for the synthesis of novel derivatives of citronellal and isopulegol. *p*-Menthane-3,8-diol (PMD) was also used as an alternative to isopulegol.

A major research focus area at the Nelson Mandela University Flow Chemistry research group include the optimisation of pharmaceutical synthesis in a continuous flow reactor by performing economic and environmental analyses.

Previous work done by Ncanywa [70], incorporated the bio-plasticiser, PMD-citronellal acetal into a PVC formulation and found it to be a viable replacement for DBP in PVC plasticised films. Postma-Botha [71] and Burger [67] concluded in their MTech and MSc theses, respectively, that the novel bio-plasticiser, PMD-citronellal acetal, and eucalyptus-oil derived PMD-citronellal acetal exhibits plasticising properties and could be a suitable substitute for the toxic DBP in a nail polish and a perfume formulation.

1.11.2.2.3 Molecular structure of PMD-citronellal acetal

Attached to the cyclohexane ring of the PMD-citronellal acetal molecule is a 1,3-dioxane ring, which in turn is attached to an alkyl methylene carbon chain, adding to the hydrophobicity of the molecule (Figure 1.16). The polar and non-polar regions of the PMD-citronellal acetal are shown in Figure 1.17.

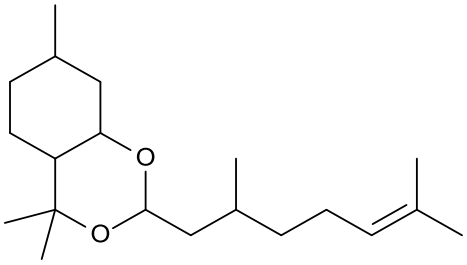



**Figure 1.16 Molecular structure of PMD-citronellal acetal [72]**

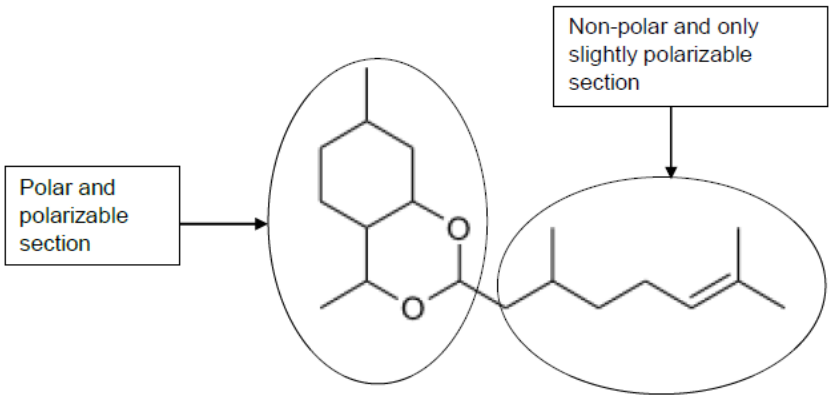



**Figure 1.17 Polarisable regions of PMD-citronellal acetal molecule [73]**

1.11.2.3 Comparison of the molecular structures of DBP, PMD-citronellal acetal and DEHT

Both plasticisers DBP and DEHT exhibit a 'flat' benzene ring (unsaturated) since the $\pi$-electrons are evenly delocalised in their aromatic rings. DEHT exhibits branching in both alkyl chains attached to the diester groups, whereas DBP has no branching in its alkyl chains, as can be seen from Figure 1.13 and Figure 1.5 respectively. Carbon atoms in the benzene ring have $sp^2$ hybridisation, whereas the cyclohexane ring in PMD-citronellal acetal being part of the chair conformation acetal molecular structure, is saturated and has carbon atoms with $sp^3$ hybridisation (see Figure 1.16) [62].

## 1.12 Problem Statement and motivation for study

The use of certain cosmetic products which contain phthalate plasticisers poses a health risk to humans, thus it is necessary to find alternative, safe plasticisers, preferably of natural origin, e.g. bio-plasticisers, which can replace the toxic

phthalates and still impart the same desirable properties to the cosmetic products in which they are used. In this study, the novel bio-plasticiser PMD-citronellal acetal was selected to compare its stability properties and plasticising behaviour with the well-known non-phthalate DEHT and the problematic DBP.

An external plasticiser may leach from a cosmetic formulation, such as a nail lacquer, upon washing of hands. The toxic phthalate plasticiser could, therefore, be absorbed by the area around the nail, via the cuticle, making its way into the bloodstream. The motivation for this research is to establish if PMD-citronellal acetal is chemically stable at elevated temperature and may possibly leach from the studied cosmetic formulations, in relation to the commercially available toxic DBP and non-toxic DEHT. A lip coat may leach from the lips into mouth saliva, and by orally ingesting it, subsequently travels into the bloodstream, causing adverse effects of phthalate 'build up' inside the human body. These possible dangers caused by the use of phthalate plasticisers in certain cosmetic products could be eliminated by substituting a conventional plasticiser (DBP) with a 'green' bio-plasticiser, PMD-citronellal acetal molecule, or a non-toxic ester plasticiser, namely DEHT.

Several phthalate replacement assessments conducted by competent authorities and private organisations have included DEHT as an alternative and suitable substitute for DEHP phthalate in products on the market today [74]. For this reason, it was decided to use DEHT as a non-phthalate plasticiser in conjunction with the novel bio-plasticiser, PMD-citronellal acetal. These two plasticisers were compared to the conventional toxic phthalate plasticiser, DBP.

## 1.13 Novelty of this research study

This research is considered to be novel since no study has been found pertaining to leaching of the novel plasticiser, PMD-citronellal acetal, from a cosmetic formulation, using analytical techniques, viz. Ultra-Performance Liquid Chromatography (UPLC) in conjunction with Solid Phase Extraction. Furthermore Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectroscopy has never been used as an analytical tool to establish the chemical stability of the neat plasticisers, namely PMD-citronellal acetal, DBP and DEHT. Neither was the FTIR-ATR technique found to be used as an analytical tool in previous research regarding the chemical stability of the novel bio-plasticiser, PMD-citronellal acetal, within cosmetic formulations.

No data is currently available in order to evaluate and compare the chemical stability of the novel bio-plasticiser, PMD-citronellal acetal, versus that of the toxic, conventional DBP, and the commercially available bis(2-ethylhexyl) terephthalate (DEHT) which is considered to be the safe, non-toxic alternative for bis(2-ethylhexyl) phthalate (DEHP).

## 1.14 Research Hypothesis

The plasticising properties and chemical stability of PMD-citronellal acetal, hereafter also referred to as Acetal, are similar or better than bis(2-ethylhexyl)-terephthalate (DEHT) and dibutyl phthalate (DBP) within two cosmetic formulations, after being incubated at elevated temperature over a three month period.

## 1.15 Study objectives

The following objectives were identified and are listed below:

- quantitative determination of previously synthesised PMD-citronellal acetal by means of gas chromatography
- comparison of the physical performance of PMD-citronellal acetal, DBP and DEHT plasticised nail and lip formulations against unplasticised nail and lip formulations (referred to as the Nail Blank and Lip Blank) respectively - tests include flexibility, adhesion, homogeneity, organoleptic, hardness, non-volatile content, viscosity and pH
- characterisation of neat plasticisers, viz. PMD-citronellal acetal, DBP and DEHT using FTIR-ATR spectroscopy as analytical tool
- determination of the chemical stability of PMD-citronellal acetal, DBP and DEHT using FTIR-ATR spectroscopy
- determination of the chemical stability of plasticised cosmetic formulations by means of FTIR-ATR analysis
- verifying whether SPE is a suitable extraction method for the plasticisers under scrutiny by means of their respective recovery rates obtained by UPLC equipped with diode array detector
- evaluating and comparing of leaching rates of plasticisers from nail and lip formulations into an aqueous medium by means of SPE and UPLC analyses

It is important to take cognisance of the fact that the emphasis of this thesis is not based upon optimum formulation dynamics of the cosmetic products under review, but rather focuses on the chemical stability of each plasticiser and its performance as part of the plasticised formulations.

# CHAPTER 2

# SYNTHESIS OF PMD-CITRONELLAL ACETAL

This chapter provides details of the synthesis of PMD-citronellal acetal and its characterisation, as performed by Ncanywa at NMU [70].

## 2.1 Synthesis of PMD-citronellal acetal

### 2.1.1 Materials

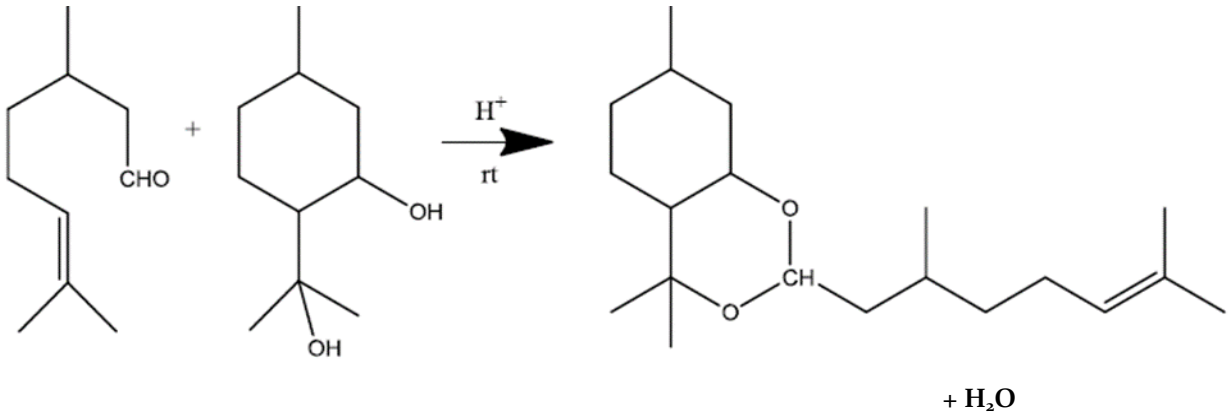
All the chemicals used for the synthesis are listed in Table 2.1 and were used as received.

**Table 2.1 Reagents used for the synthesis of PMD-citronellal acetal**

| Chemical name | Formula | Source | Grade |
|---|---|---|---|
| *para*-Menthane-3,8-diol | $C_{10}H_{20}O_2$ | NMU | 99% |
| Citronellal | $C_{10}H_{18}0$ | Merck | AR |
| Hexane | $C_6H_{14}$ | Merck | AR |
| Sulphuric Acid (conc.) | $H_2SO_4$ | Merck | 98% |
| Anhydrous sodium sulphate | $Na_2SO_4$ | Merck | AR |

2.1.2 Synthesis of *p*-menthane-citronellal acetal

A batch of *p*-menthane-3,8-diol-citronellal acetal was synthesised by the cyclisation of citronellal with *p*-menthane-3,8-diol in a double-walled 2000 ml glass reactor. The reaction was acid-catalysed with sulphuric acid ($H_2SO_4$) [75]. The reaction equation is shown in Figure 2.1.




**citronellal** **_p_-menthane-3,8-diol** **_p_-menthane-3,8-diol-citronellal acetal**

**Figure 2.1 PMD-citronellal acetal synthesis**

Citronellal (500 ml) was pre-heated to 60 °C and added rapidly to the double-walled glass reactor (2000 ml) containing *p*-menthane-3,8-diol (500 ml) in hexane (1000 ml) maintained at 60 °C by circulating warm water from a temperature controlled bath. In order to speed up the reaction, 4 ml of concentrated sulphuric acid ($H_2SO_4$) was added slowly and cautiously. Sampling was performed at hourly intervals for analysis by means of gas chromatography (GC). The glass batch reactor is shown in Figure 2.2.

**Figure 2.2 Double-walled glass reactor used to synthesize *p*-menthane-3,8-diol**

After completion of the reaction (4 hours) the reactor contents were transferred into a separating funnel and the mixture washed three times with brine and cold water. Any residual $H_2SO_4$ in the organic phase was neutralized with a 10% (w/v) solution of sodium bicarbonate ($NaHCO_3$).

Thereafter the organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. PMD-citronellal acetal was obtained as a crude oil of 67% yield. The crude material was subsequently vacuum distilled and purified (see Figure 2.3). The oil was then washed with hot de-ionised water and the mixture allowed to settle in a separating funnel. The bottom aqueous phase was drained off whilst carbon dioxide was allowed to escape from the bottom of the funnel. After drying the oil, over anhydrous sodium sulphate it was again analysed by means of GC.

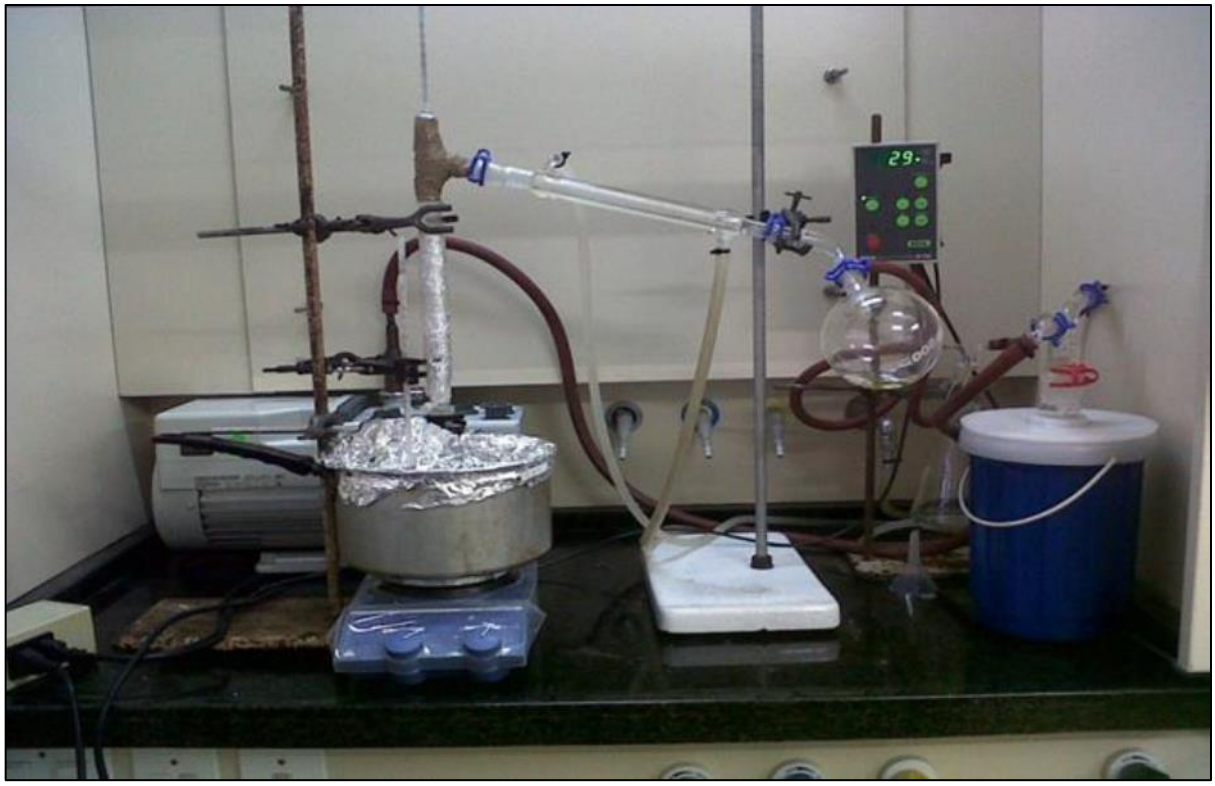


**Figure 2.3 Vacuum distillation set up for purification of PMD-citronellal acetal**

## 2.2 Analysis of PMD-citronellal acetal

### 2.2.1 Gas chromatography (FID)

An Agilent gas chromatograph, equipped with a flame ionization detector (FID), was used for analysis of the oil. An RTX MS column with dimensions (length 30 m x 0.25 mm ID) was used. Recording and integration of chromatograms were performed by means of Empower software version 3.0.

Instrumental conditions used were as follows:

| | |
|---|---|
| FID temperature: | 300 °C |
| Carrier gas: | Nitrogen |
| Carrier gas flow rate: | 0.5 ml/min |
| Injector temperature: | 270 °C |
| Initial column temperature: | 70 °C |
| Initial column hold time: | 5 minutes |
| Column ramp rate: | 10 °C /minute |
| Final column temperature: | 270 °C |
| Final column hold time: | 5 minutes |
| Total run time: | 30 minutes |

Figure 2.4 illustrates a gas chromatogram of PMD-citronellal acetal (~67%) prior to vacuum distillation in which reactants still present were citronellal (~23%) as well as PMD (~9%). After the product was purified by means of vacuum distillation, it can be seen from Figure 2.5 that PMD-citronellal was present in a much higher amount, i.e. 96.2% whereas the presence of the other reactants, citronellal and PMD, were present in very small amounts, i.e. 1.5% and 1.9%, respectively. These results were obtained by adding the areas of the integrated peaks and are in good agreement with the results obtained by Ncanywa [70].

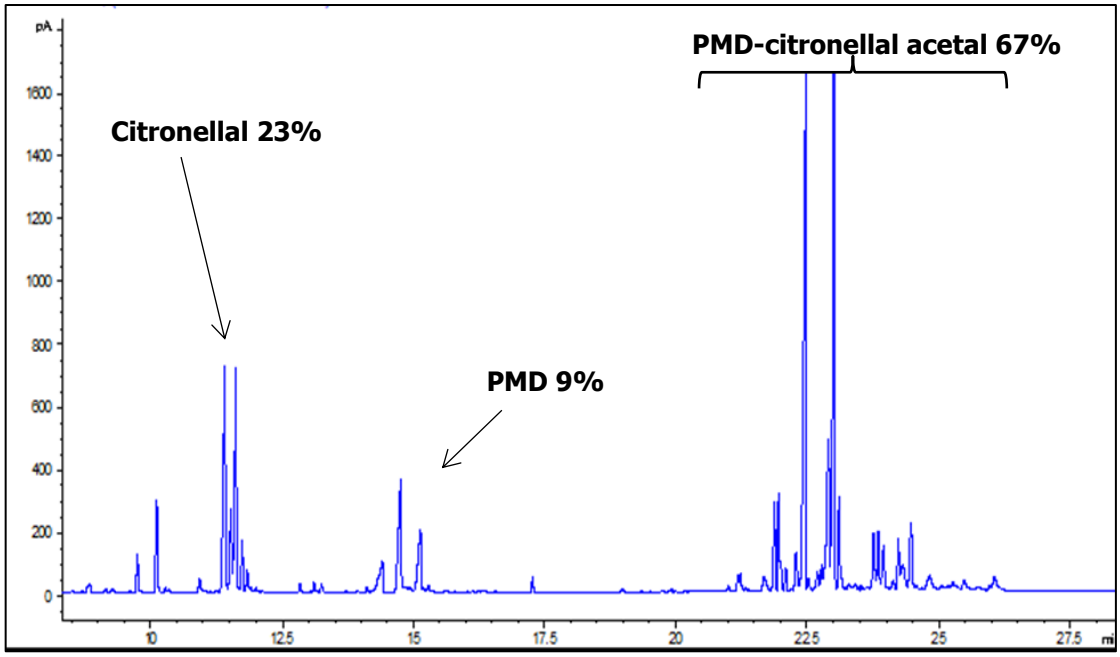



**Figure 2.4 Gas chromatogram of PMD-citronellal acetal (crude product)**

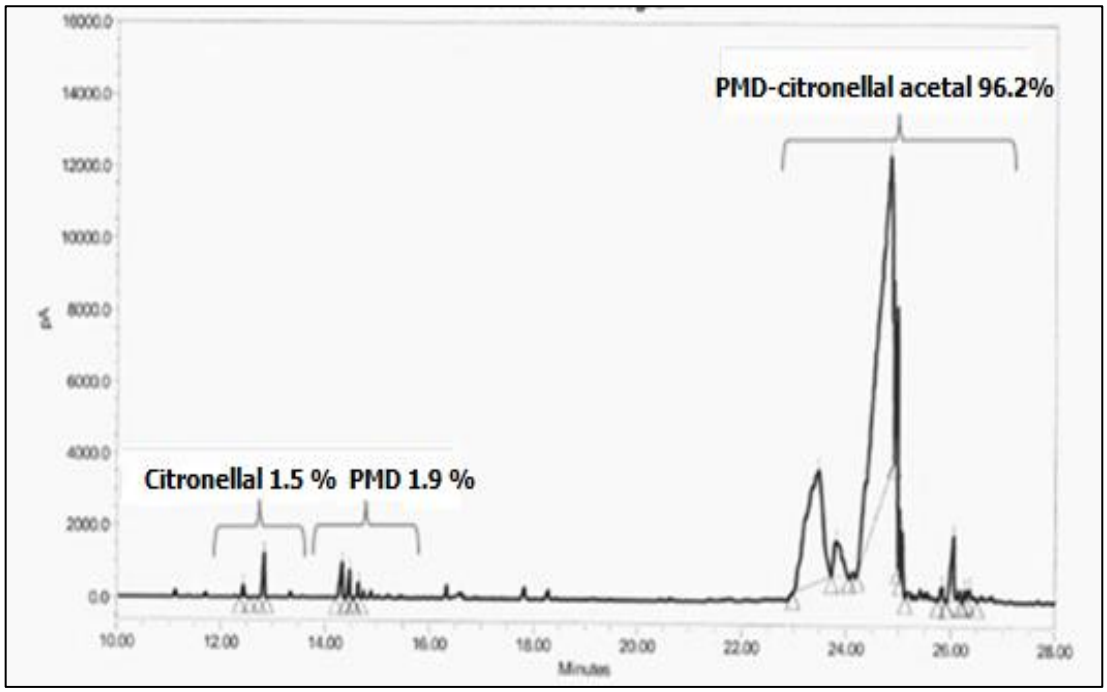



**Figure 2.5 Gas chromatogram of PMD-citronellal acetal (purified)**

The final product formed was oily and a yellowish colour. The GC-MS was used to identify the PMD-citronellal acetal produced upon reacting citronellal and *p*-menthane-3,8-diol. The presence of PMD-citronellal acetal was also confirmed by means of FTIR-ATR analysis results of which are shown in Chapter 5. The ether single bond vibration at 1107 $cm^{-1}$ was proof thereof. Table 2.2 summarises the physical properties of the Acetal obtained and compares its properties to those of the bio-plasticiser DEHT and the commonly used phthalate plasticiser DBP.

**Table 2.2 Chemical and physical properties of PMD-citronellal acetal compared with DEHT and DBP [67, 70, 76]**

| **Plasticiser name** | Dibutyl phthalate (DBP) | Bis(2-ethylhexyl) terephthalate (DEHT) | *p*-Menthane-3,8-diol-citronellal acetal (PMD-citronellal acetal) |
|---|---|---|---|
| **Synonyms** | Dibutyl 1,2 benzene-dicarboxylate and Phtalic acid dibutyl ester | Bis(2-ethylhexyl) 1,4-dioxane dicarboxylate | 2-(2,6-Dimethyl-5-hepten-1-yl)-4,4,7-trimethylhexahydro-4H-1,3-benzodioxin |
| **Chemical Formula** | $C_{16}H_{22}O_4$ | $C_{24}H_{38}O_4$ | $C_{20}H_{35}O_2$ |
| **CAS Number** | 84-72-2 | 6422-86-2 | To be established |
| **Molecular weight (g/mol)** | 278.35 | 390.57 | 307.50 |
| **Colour** | Colourless, oily liquid | Colourless, oily liquid | Yellowish, oily liquid |
| **Odour** | Weak, aromatic | None (odourless) | Slightly eucalyptus |
| **Density (g/cm) @ 20 °C** | 1.048 | 0.984 | 0.892 |
| **Boiling Point** | 340 °C | 375 °C | 350 °C |
| **Water solubility (g/l)** | 0.013 | <0.00001 | Insoluble |

Table 2.3 displays the mass fragmentation pattern for PMD-citronellal acetal. The theoretical mass fragmentation was obtained from ChemDraw Ultra 8.0 software, whereas the actual mass fragmentation was obtained from the GC-MS spectrum for PMD-citronellal acetal. The presence of Acetal in the synthesised oil was confirmed from the various mass fragments that were detected in the GC-MS spectrum obtained.

### 2.2.2 Gas chromatography-mass spectrometry (GC-MS)

A HP 5890 gas chromatograph coupled to a HP 5972 series mass selective detector were used to perform the analysis of the PMD-citronellal acetal oil. Data from the detector was acquired by means of a Hewlett Packard computer with HP 61034 software. The GC, fitted with a RTX 35 MS column (30 m x 0.25 mm ID), was programmed as follows:

| | |
|---|---|
| Initial column Temperature: | 70 °C |
| Initial column hold time: | 5 min |
| Heating rate: | 10 °C/min |
| Final column temperature: | 280 °C |
| Final column hold time: | 5 min |
| Injector temperature: | 250 °C |
| Split flow: | 60 ml/min |
| Carrier gas: | Helium at 1 ml/min (constant flow) |
| Run time: | 30 min |
| MS-mass range: | 30 – 550 u |

The GC-MS spectrum obtained for PMD-citronellal acetal is shown in Figure 2.6.

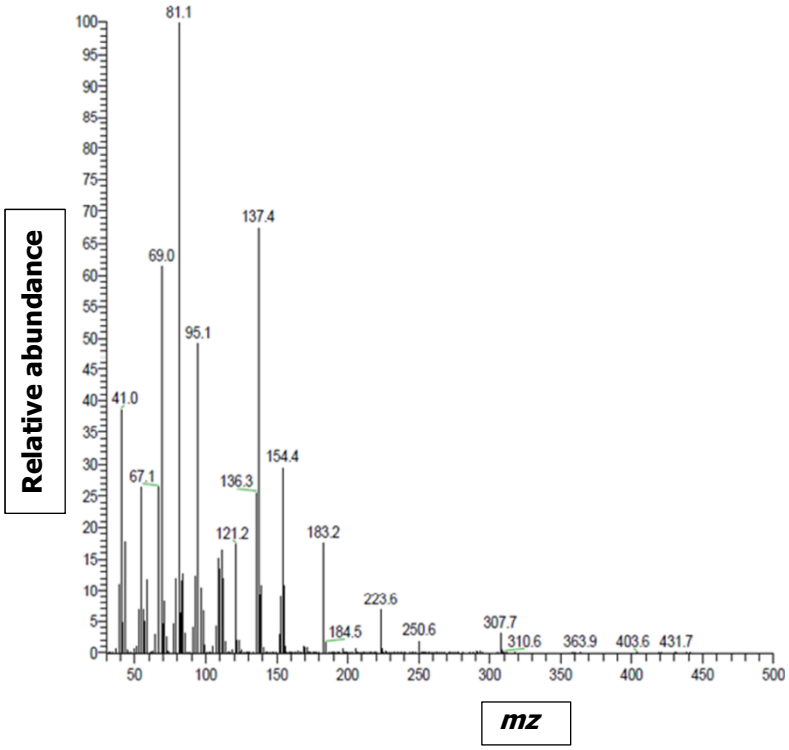
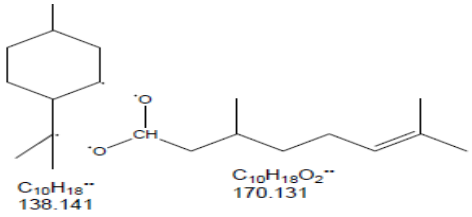
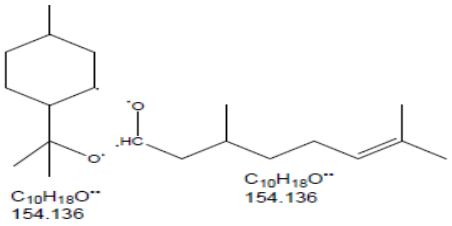
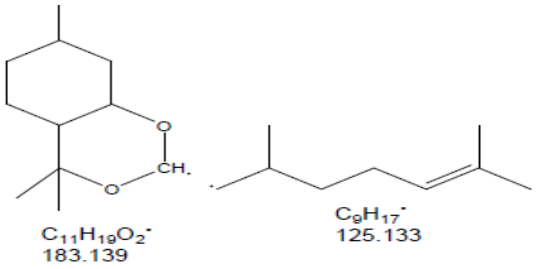
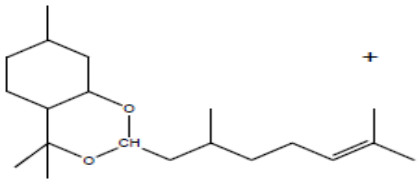



**Figure 2.6 GC-MS confirmation of the presence of PMD-citronellal acetal**

## Table 2.3 Mass fragmentation of PMD-citronellal acetal [67, 73]

| M/Z theoretical pattern | M/Z from Fig 2.6 | Mass fragmentation pattern |
|---|---|---|
| 82 | 81.1 |  |
| 138.14 | 137.4 |  |
| 154.14 | 154.4 |  |
| 183.14 | 183.2 |  |
| 308 | 307.7 |  |

# CHAPTER 3
# COSMETIC FORMULATIONS: INGREDIENTS AND PREPARATION

## 3.1 Nail lacquers

### 3.1.1 Introduction

Nail polish originated in China 3000 years Before Christ [77]. These early mixtures consisted of beeswax, gelatine, gum arabic as well as egg whites. The Chinese added colour by making use of flower petals. In Egypt, during this same time period, only the upper-class people made use of nail polish [78].

It then became a symbol of social rank and was subsequently used in various colour and form (cake, powder and paste) in its developing area. The creation of nail polish was also inspired by the development of automobile paint with the invention of the car in 1920. Nail lacquer soon followed [14]. It could, therefore, be deduced that nail lacquer is an interesting example of a cosmetic product that is more closely related to industrial coatings than traditional cosmetics [39].

Lacquer is a coating that dries by means of evaporation only, and not by oxidation nor by polymerisation. No chemical change is necessary for film formation since the solvents evaporate quickly [1]. It is therefore scientifically incorrect to state that nail enamel and nail polish are the same as nail lacquer.

Nail lacquers perform two functions, i.e. protecting the nail and aesthetically enhancing the image thereof. The film-forming polymer should exhibit attributes of good adhesion to nails, form a hard but flexible film, be toxicologically safe and have regulatory compliance with good chemical, mechanical, thermal resistance and ease of removal [79].

Nitrocellulose, also called gun cotton, cellulose nitrate, pyroxylin or collodion, is the most common film former in nail lacquers and has been used since the 1920's. It is a naturally derived polymer, produced by nitration of cellulose derived from cotton fibres or wood pulp.

The film-forming polymers of nail lacquer formulated in this thesis consist of nitrocellulose (the primary film former) as well as a secondary film former, melamine formaldehyde (MF). This is a thermosetting resin that changes irreversibly, via the formation of a covalently cross-linked, thermally stable network [80].

Thermosetting resins, such as nitrocellulose and melamine formaldehyde, form tiny crystals after mixing with solvent. After solvent evaporation, these crystals do not re-dissolve upon the addition of the same solvent. As a result this is referred to as an irreversible reaction.

### 3.1.2 Nail lacquer ingredients

#### 3.1.2.1 Nitrocellulose

##### 3.1.2.1.1 Product description and safety

Nitrocellulose (also known as cellulose nitrate, flash paper, flash cotton, guncotton, and flash string) is a highly flammable compound and is therefore sold as a mixture, e.g. with isopropanol, in order to reduce its flammability [42].

Nitrocellulose is produced by causing cellulose to react with nitrating acid (a mixture of nitric acid and sulfuric acid). Following complex washing and stabilising stages, damping agents (alcohols or water) or plasticisers are added to the nitrocellulose.

As described above, nitrocellulose, being a derivative of natural cellulose, has an outstanding range of properties. It dissolves readily in organic solvents and, with its relatively rigid molecule chain, it forms a hard but flexible film, ideal for a good surface finish [43].

#### 3.1.2.2 Melamine Formaldehyde

##### 3.1.2.2.1 Introduction and formation

Liebig prepared melamine (2,4,6-triamino-1,3,5-triazine) in 1834 for the first time and it reached the market in 1939. Melamine is a white crystalline, heterocyclic aromatic compound. The primary amino groups react with formaldehyde resulting in derivatives containing one to six methylol groups. These methylol hydroxyl

groups may further react with unsubstituted melamine amino groups which in turn form methylene-linked derivatives (Figure 3.1) [80].

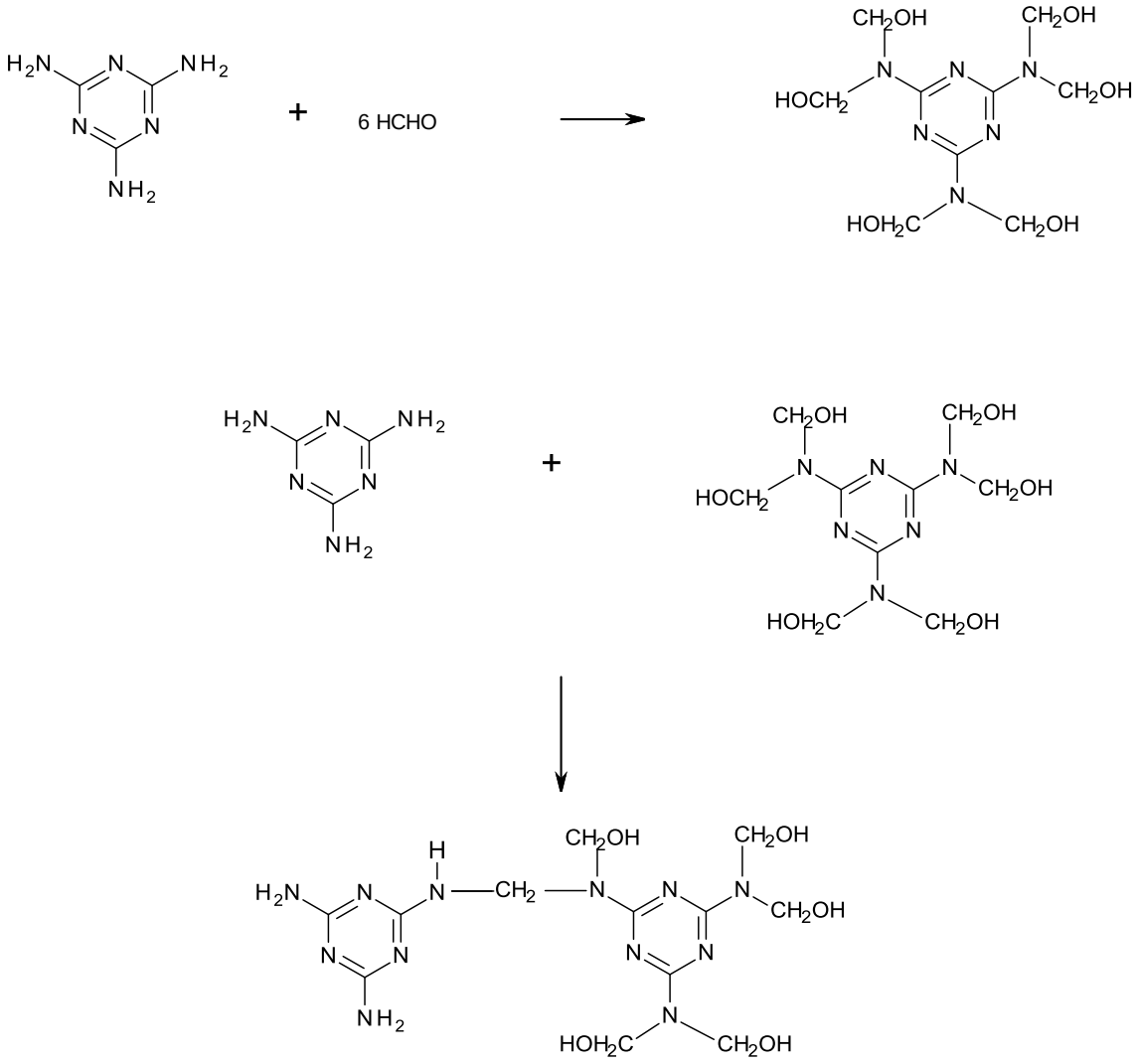



**Figure 3.1 Melamine formaldehyde formation [80]**

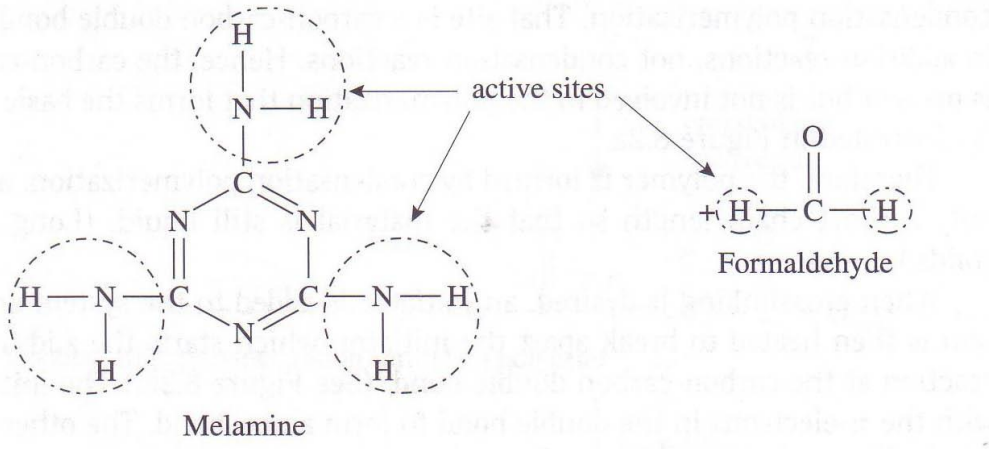



**Figure 3.2 Melamine formaldehyde**

Figure 3.2 shows the reaction taking place between melamine and formaldehyde. Note the active sites on both the molecules. MF is referred to as 2,4,6-triamino-1,3,5 triazine [80].

3.1.2.2.2 Product description and safety

The function of MF as a resin in a formula is to provide films with good hardness and resistance properties. MF is one of the hardest and stiffest thermosetting polymers. MF is an amino resin and has various material advantages, such as transparency, improved hardness, thermal stability, excellent boil resistance, scratch resistance, abrasion resistance, flame retardant, moisture resistance and surface smoothness. These material advantages lead MF to be used in large quantities for industrial applications [81]. As described above, MF is a n-butylated melamine-formaldehyde resin with a medium degree of alkylation, medium methylol content, and a relatively low molecular weight and viscosity. Melamine has a good compatibility with a range of backbone polymer resins, including alkyd

resins, polyester resins and acrylic resins. Melamine resins in formulations are stabilised with primary alcohols, amines or combinations of these. Melamine resin is very stable when stored under normal ambient (RT) conditions.

Adverse effects of formaldehyde used as a film former were summarised from the Cosmetic Ingredient Review. These included respiratory damage, skin irritation and sensitisation, and carcinogenesis. Data was insufficient to support the safety of MF [82].

## 3.2 Lip coat

### 3.2.1 Introduction

A lip coat creates a transparent barrier when applied over a lipstick, consequently prolonging the wear thereof. It also prevents the lipstick from smudging or being transferred onto other objects [83-85]. Consumer expectations of lip products include aspects such as safety, aesthetics and performance. Since lips are classified as mucous membranes, the lip product is expected to be dermatologically safe [85].

Lip cosmetic sales in the United States (US) in 2016 have generated huge sales, of which the lip coat segment was responsible for approximately 155 million US dollars [86].

Previous research done by NMU has shown that PMD-citronellal acetal exhibits plasticising properties [67] very similar to that of DBP. This could be due to the fact that these molecules have more or less the same boiling point and molecular

weight. The PMD-citronellal acetal molecule consists of a safer, less reactive cyclohexane ring, whereas DBP consists of a more reactive benzene ring.

In the lip coat described below, mixing of the thermoplastic resin, polyvinylpyrrolidone (PVP), with hydroxypropylcellulose (HPC) as per formulation, the solvent added evaporates to dryness. Upon adding solvent, the crystalline mass dissolves completely and is, therefore, referred to as a reversible reaction [41].

### 3.2.2 Lip coat ingredients

#### 3.2.2.1 Polyvinylpyrrolidone (PVP)

##### 3.2.2.1.1 Safety

PVP is the linear polymer of 1-vinyl-2-pyrrolidone monomers. Based on the data available (short-term PVP inhalation, animal studies, tests conducted to test for sensitisation of skin, oral tests, etc.), PVP is safe to be used in cosmetic applications. Pyrrolidones exhibit a planar structure and as a result, the oxygen with its high electronegativity can easily cause an electron to delocalise (Figure 3.3). As a result, a strong dipole moment is formed. Chemically binding a flexible non-polar alkyl chain with a compact hydrophilic head is responsible for alkyl pyrrolidones to be soluble in both polar and non-polar solvents [87].

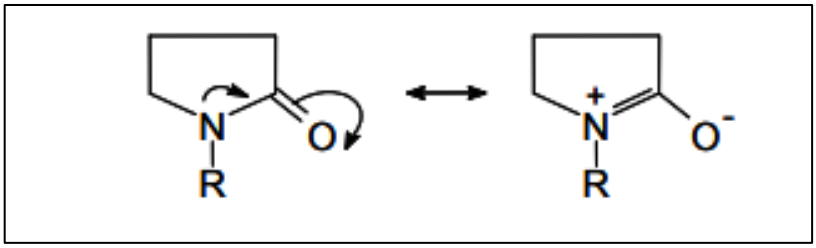



**Figure 3.3 Electron delocalisation in pyrrolidones**

3.2.2.1.2 Applications

PVP has a wide array of functions and applications in many industries including pharmaceuticals, cosmetic products and paints. It is frequently used as a binder, emulsion stabiliser, film former, hair fixative, and suspending agent (non-surfactant). It is also widely used due to its excellent hygroscopic properties, complexing ability and physiological compatibility. In the field of cosmetics, in particular, PVP is used as a filming-agent, viscosity-enhancement agent, lubricant and adhesive, forming key components in hair sprays, mousse gels and lotions, shampoos, lipsticks, sunscreens, and lotions in skin care products, eye make-up and deodorants.

This polymer is also often used in conjunction with other polymers in order to provide improved adhesion to the substrate [88, 89].

3.2.2.2 Hydroxypropylcellulose (HPC)

3.2.2.2.1 Introduction

Cellulose is the most abundant organic renewable resource in the plant kingdom, and HPC forms part of these cellulose derivatives. HPC (Figure 3.4) has excellent film-making properties. HPC is a derivative of cellulose (ether) and is soluble in

both water and organic solvents. It is a free-flowing, granular powder which can be dispersed in water at 60 °C. It is a highly flexible polymer which has the ability to form a film characterised by high elongation and moderate tensile strength. It also displays thermoplastic behaviour, and when manufactured with high hydroxypropyl substitution it requires little or no plasticiser. According to the Cosmetic Ingredient Review (CIR) Expert Panel, HPC is safe to use [90].

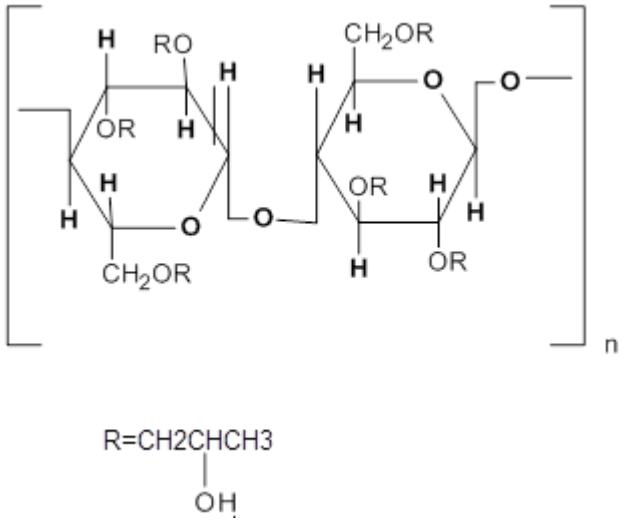



**Figure 3.4 Molecular structure of HPC**

3.2.2.2.2 Applications

HPC functions as a thickener, foam stabiliser, rheology modifier and film former [91].

## 3.3 Experimental procedure

### 3.3.1 Preparation of nail lacquer

The nail lacquer formulation is shown in Table 3.1. Three different nail lacquer formulations were prepared, each one containing a different plasticiser and one formulation was prepared without plasticiser as a control.

**Table 3.1 Nail lacquer formulation - adapted from Baran *et al.* [92]**

| Composition | % w/w | Function | Supplier |
|---|---|---|---|
| n-Butyl acetate | 40 | Solvent | Merck |
| Isopropyl alcohol | 11.3 | Diluent | Merck |
| Ethyl acetate | 18 | Solvent | Merck |
| Nitrocellulose | 15 | Film former (primary) | Bergarac NC |
| Melamine resin (Butoxymethyl methylol melamine) | 9 | Film former (secondary) | Cytek |
| Plasticiser | 6.7 | Plasticiser | See Note* |

*Note: Three plasticisers, namely DBP (supplier: Protea Chemicals), DEHT (supplier: Eastman Chemical Co.) and PMD-citronellal acetal (supplied by NMU) were added respectively as 6.7% w/w to each formulation as set out in Table 3.1.

The nail lacquer bulk formulation (10 litre) was made by mixing n-butyl acetate, ethyl acetate and isopropyl alcohol under slow stirring with an Arrow air stirrer (adjustable height lab stand, 120 W at 350 kPa fitted with a stainless steel triple blade propeller). The stirrer speed was varied by adjusting a valve (0 to 1200 rpm speed range). The nitrocellulose and melamine resin were added to the solvent

mixture in a 15 litre stainless steel vessel, mixed under slow stirring conditions within this closed system (to avoid solvent evaporation) for 60 minutes.

Each bulk nail lacquer mixture was prepared by individually adding 6.7% plasticiser to 2 litre of the bulk mixture, while slowly stirring within the closed system for 20 minutes.

3.3.2 Preparation of lip coat

The lip coat formulation is shown in Table 3.2. Four different lip coat formulations were prepared, each containing a different plasticiser and one was prepared without plasticiser as a blank.

**Table 3.2 Lip coat formulation [93]**

| **Composition** | **% w/w** | **Function** | **Supplier** |
|---|---|---|---|
| Ethanol absolute | 84 | Solvent | Sigma-Aldrich |
| PVP K-30 | 10 | Adhesive | BASF |
| HPC | 1 | Film former, thickener | Ashland |
| Plasticiser | 5 | Plasticiser | See Note below * |

*Note: Three plasticisers, DBP (supplier: Protea Chemicals), DEHT (supplier: Eastman Chemical Co.) and PMD-citronellal acetal (supplied by NMU) were added as 5.0% w/w to each formulation, respectively, as set out in Table 3.2.

The bulk lip coat mixture (10 litre) was prepared by adding PVP K-30 at a slow rate to absolute ethanol under slow stirring with an Arrow air stirrer until all the powder was wetted. The HPC was added while increasing the stirring speed until a vortex

formed in the mixing liquid. Stirring continued in a closed system for 60 minutes until a homogenous mixture was obtained.

Each plasticised lip coat solution was formulated by using 2 litre of the bulk mixture and adding 5% of each respective plasticiser to the bulk solution, stirring under slow speed. The process was continued for a further 5 minutes until a homogenous mix was achieved.

Nail lacquer and lip coat formulations were decanted into clearly labelled air tight 500 ml Consol® glass jars, acting as inert storage bulk containers.

A different glass jar was filled with each formulation and firmly closed. Thereafter, each jar represented its respective testing time interval. Sealed jars were placed in a desiccator containing silica gel, ensuring absorption of any possible moisture (moisture absorption could have possible adverse effects on the intensity of FTIR-ATR spectral bands with regard to the hydroxyl functional group, as explained later in this thesis).

# CHAPTER 4
# ACCELERATED AGEING AND PHYSICAL PERFORMANCE TESTS OF COSMETIC FORMULATIONS

## 4.1 Introduction

Physical performance tests are done to determine whether the cosmetic formulations will last on store shelves. These are also important quality tests that need to be performed to ensure that customer expectations are met if using the product for its purpose [94]. A lip coat formulation should, for example, form a homogenous, flexible film over the lipstick and as a result have good adhesion properties in order to prolong the wear of lipstick without smudging. If the lip coat forms a film that is not flexible enough, it could cause brittleness and forms cracks over the lipstick. For the same reasons physical performance tests are to be performed on a nail lacquer, i.e. to ensure enhanced film forming properties with respect to adhesion of a homogenous film to the nail keratin bed and prevention of chipping of the nail lacquer film. It can therefore be seen that plasticisers play an important role in fulfilling overall greater aesthetic properties in cosmetic formulations [95]. The nail lacquer and lip coat formulations were tested at accelerated temperature conditions. This was done to ensure that the cosmetic products meet their intended physical and chemical quality standards. The products should maintain their functionality and aesthetics.

Theory and practice of accelerated ageing relate to the chemical changes which polymers undergo. These changes are useful in order to compare the physical and chemical stability of one material relative to that of another. An example of how closely physical performance is related to chemical changes, is the loss of molecular weight in polymers due to oxidation.

Physical ageing, on the contrary, refers to changes in the physical structure of the polymer, with no changes to the molecular chains. On the other hand, chemical degradation results in modification of the chemical structure of the molecular chains. Elevated temperature accelerates physical ageing processes, also referred to as accelerated stress. A change in the physical structure is accompanied by mechanical stresses in the material due to the inhibition of elongation or shrinkage. The result of such stresses causes fractures or cracks [96, 97].

Due to the wide variety of cosmetic products and their inherent complexity, standard stability tests cannot be prescribed [98]. General guidelines for cosmetic storage conditions and thermal stability are shown in Table 4.1. It is up to the manufacturer to decide on appropriate humidity conditions based on the packaging type and cosmetic formulation indicated by asterix in Table 4.1 [99].

**Table 4.1 General thermal stability storage conditions for cosmetic products**

| Study | Storage conditions | Min. suggested test period (months) |
|---|---|---|
| Long term | 25 ± 2 °C RH* | 12 |
| Intermediate | 30 ± 2 °C RH* | 3 |
| Accelerated | 40 ± 2 °C RH* | 1 |

## 4.2 Methodology

When cosmetic products are exposed to factors such as temperature, light, and humidity their physical and chemical attributes as well as the functionality and appearance may be influenced. In this study only one aspect, namely temperature over a time period was considered.

The cosmetic formulations described in Chapter 3, viz. nail lacquer and lip coat, containing the different plasticisers as well as the Blank were tested to compare their physical performance properties before and after accelerated ageing. Studies of the four nail lacquer formulations and the four lip coat formulations were undertaken over a three month period at elevated temperature (40 ± 1 °C).

The formulations were stored and tested at RT and 40 °C. Samples stored at 40 °C were tested at monthly intervals, i.e. Stage T1, Stage T2 and Stage T3, respectively. The control sample (T0), stored at RT (23 ± 1 °C), was only tested after a three month period [Stage T0 (end)] - adapted from Tatariants *et al.* [100]. Blank sample formulations (unplasticised samples) were subjected to and tested at the same time intervals.

In order to minimise standard deviations, five samples of one batch per formulation were prepared and incubated at the relevant temperatures. The following physical tests were performed on both nail lacquer and lip coat sample formulations at each time interval during the testing period, namely:

- Film hardness
- Viscosity
- Non-volatile content (NVC)
- Film flexibility
- Film adhesion

- Homogeneity
- Organoleptic properties

## 4.3 Physical Performance Tests

### 4.3.1 Preparation of films of nail lacquer and lip coat

The casting film method was applied by using a standard prescribed steel rod used in the paint industry. The rod ensured a uniform casted coating of the formulations onto the steel plates. 'Draw downs' were made of each nail lacquer and lip coat formulation onto separate clean, mild steel plates (200 mm x 100 mm). The plates were left to hard dry since most physical tests can only be done on hard dried film coatings. Tests were performed 48 hours after the 'draw downs' were done. These are briefly described below.

### 4.3.2 Flexibility

The flexibility conical mandrel method was adapted from the American Standard Test Method (ASTM) D522 standard [101]. Flexibility was measured by placing a coated mild steel plate (100 mm x 180 mm) with a maximum thickness of 0,8 mm, in a conical mandrel apparatus (Elcometer 1510), bending this coated panel over a conical-shaped mandrel (Figure 4.1). By doing so, the resistance of the coating to cracking, elongation and/or detachment from the test panel was being assessed. The conical shaped bending area allows the deformation of the steel panel as well as examination of the elasticity range over any diameter between 3,1 mm and 38 mm in a single test. The test steel plate was visually observed for the appearance of cracks. If any cracks occurred, the diameter of the beginning and end of the crack was noted. A 'Pass' result refers to excellent flexibility, i.e. no signs of

cracking, elongation or detachment from the test steel plate. A 'Fail' result, on the other hand, refers to the opposite signs showing on the mild steel plate.

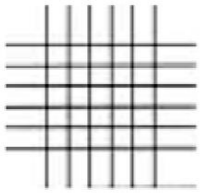
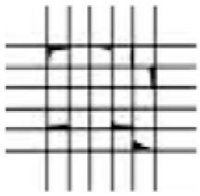
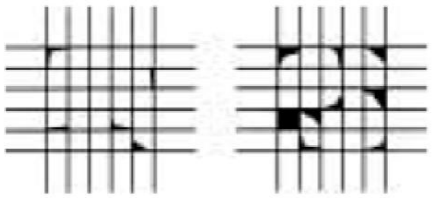
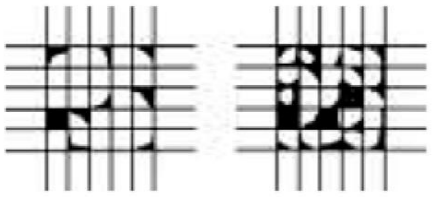
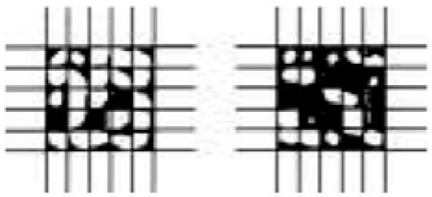
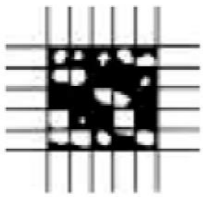
**Figure 4.1 Conical mandrel bend tester (Elcometer 1510) [102]**

4.3.3 Adhesion

A cross cutter was used to make two sets of six cross cuts in a lattice pattern on dried film coatings covering a steel test panel. These sets of cross cuts were made at a right angle to each other. Specially defined adhesion tape was placed over the area of the grid and then firmly rubbed with a finger. This tape was removed in a rapid fashion, pulling it off as close to a right angle as possible, i.e. at 180 °. The grid area was visually inspected for removal of any coating from the substrate. Adhesion strength is indicated according to ASTM 3359 Class (1B to 5B) which defines strength of adhesion as follows [103]: ASTM class 5B indicates no film pull-off, representative of the highest level of adhesion obtained. ASTM class 1B illustrates that 35 to 65% of the film is removed, representing the poorest adhesion. Class 2B to 4B represent varying and sometimes slightly subjective

degrees of adhesion properties that fall between class 1B and 5B (Refer to Table 4.2.)

**Table 4.2 The ISO/ASTM rating system used to evaluate the formulations for the Cross-hatch test for adhesion**

| | |
|---|---|
| **ISO Class: 0/ASTM Class: 5B**<br>The edges of the cuts are completely smooth; none of the squares of the lattice is detached. |  |
| **ISO Class: 1/ASTM Class: 4B**<br>Detachment of small flakes of the coating at the intersections of the cuts. A cross-cut area not significantly greater than 5% is affected. |  |
| **ISO Class: 2/ASTM Class: 3B**<br>The coating has flaked along the edges and/or at the intersections of the cuts. A cross-cut area significantly greater than 5%, but not significantly greater than 15%, is affected. |  |
| **ISO Class: 3/ASTM Class: 2B**<br>The coating has flaked along the edges of the cuts partly or wholly in large ribbons, and/or it has flaked partly or wholly on different parts of the squares. A cross-cut area significantly greater than 15%, but not significantly greater than 35%, is affected. |  |
| **ISO Class:4/ASTM Class: 1B**<br>The coating has flaked along the edges of the cuts in large ribbons, and/or some squares have detached partly or wholly. A cross-cut area significantly greater than 35%, but not significantly greater than 65%, is affected. |  |
| **ISO Class: 5/ASTM Class: 0B**<br>Any degree of flaking that cannot even be classified by classification 4. |  |

### 4.3.4 Homogeneity

A thin spatula was inserted into each container of nail lacquer and lip coat formulation. The spatula was rapidly removed, and the retained sample was inspected and evaluated according to the three different classes of settlement:

- No settlement
- Soft settlement if a little collation of particles is observed
- Settlement where a hardened colloidal matter is formed

### 4.3.5 Organoleptic properties

The organoleptic properties of the nail lacquer and lip coat formulations (qualities relating to colour and odour) were evaluated using sight and smell.

### 4.3.6 Hardness

A BYK Gardner hardness tester fitted with a König pendulum was used to perform this test (Figure 4.2). The pendulum is free to swing on two balls which rest on the coated test steel panel. The number of oscillations of the swinging pendulum will decrease more quickly on a soft coated surface. The amplitude was determined by means of accurately positioned photo sensors. An electronic counter displayed the number of oscillations made by the pendulum. Any possible draughts in the vicinity were excluded by means of a transparent acrylic case surrounding the tester.

**Figure 4.2 König pendulum hardness tester [104]**

4.3.7 Non-Volatile Content (NVC)

The test was performed on a Mettler Toledo halogen HR 73 instrument (Figure 4.3), set at 105 °C. About 1 g of sample (mass accurately recorded by instrument) was decanted into the instrument's tarred weighing pan fitted with filter paper. The percentage NVC is automatically displayed on the instrument panel upon completion of solvent evaporation. The lower this value, the more solvents have evaporated, resulting in a higher percentage of NVC [105], also referred to as solid matter.

**Figure 4.3 Mettler Toledo halogen HR 73 analyser [106]**

4.3.8 Viscosity

A Thermo Haake K20, equipped with a Gardco/DIN 4 mm viscosity cup (Figure 4.4), was used at the same sample temperature as the sample tested, i.e. at 25 ± 0.5 °C. Gravity is the driving force causing a liquid in a viscosity cup to flow through its orifice. A stopwatch was used to record the time (rounded off to the nearest 0.5 seconds) at the first break in the stream of material flowing from the orifice of the cup.

**Figure 4.4 Gardco viscosity cup and stand**

Viscosity is determined as the elapsed time in seconds. In order to convert this efflux time in seconds to centistokes, the following formula is used [107]:

$$V = 4.57\ x\ T - (\frac{452}{T}) \qquad \text{.....equation 4.1}$$

where

$T = efflux\ time\ in\ seconds$

$V = viscosity\ in\ centistokes$

Figure 4.5 illustrates the relationship between centistokes (cSt) and seconds using the above equation.

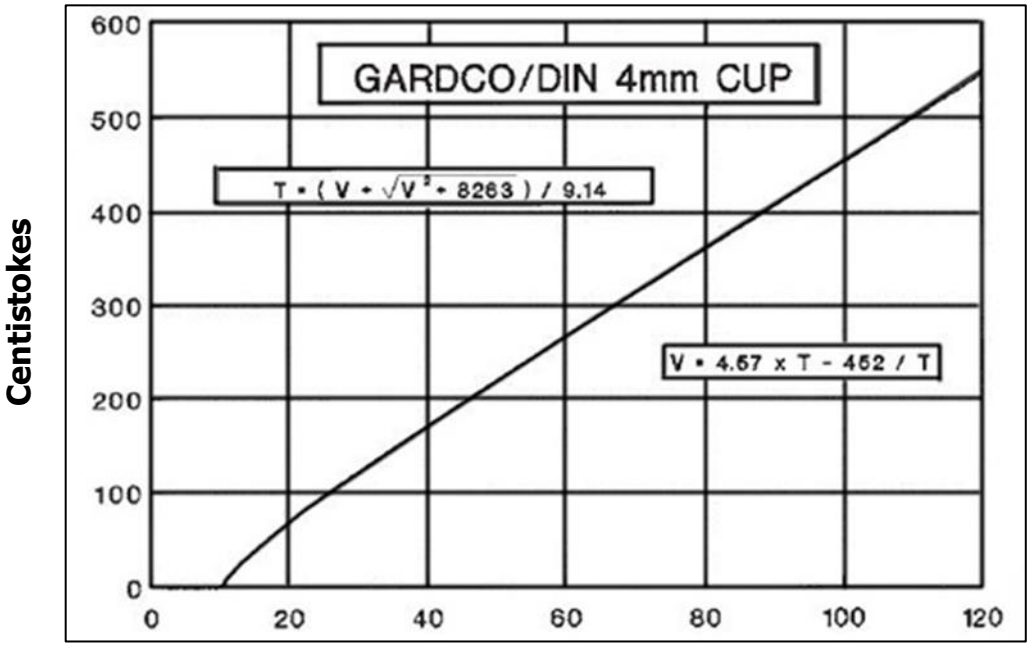



**Figure 4.5 Viscosity in centistokes (converted from seconds)**

4.3.9 pH

The pH of both lip coat and nail lacquer formulations was determined by means of a double junction pH meter with glass electrode (Hanna HI1043P) [108].

## 4.4 Results and Discussion

The results of the various tests described are summarised in Tables 4.2 to 4.8 for comparison of the nail and lip formulations with their respective Blank formulations.

4.4.1 Flexibility

For simplicity only 1 of 6 replicate results are tabulated per stage for each nail and lip formulation, since each result is similar. The full set of results can be seen in the Appendix (see Appendix A: Table 1).

**Table 4.3 Summarised flexibility results for lip coat and nail lacquer formulations during accelerated ageing tests**

| | FLEXIBILITY | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| **Stage** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **T0** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **T1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **T2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **T3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **T0 (end)** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

Table 4.3 shows the summarised results of the flexibility test carried out on the conical mandrel apparatus described in Section 4.3.2. As can be observed from Table 4.3 the Blank nail lacquer formulations and Blank lip coat formulations failed at all 5 stages compared to the plasticised formulations.

Flexibility is considered favourable ('pass') when no cracking of the film occurs. As expected, the Blank formulations cracked and peeled because of the absence of plasticiser in these formulations. It can be concluded that flexibility for all plasticised formulations remained stable at RT and elevated temperature, over the three month period as no cracking of any films was observed using the conical mandrel test.

The DEHT molecule has the longest hydrocarbon chains emanating from each side of the ester in the *p*-positions of the benzene ring and therefore imparts additional free volume in formulation 3. Theoretically, DEHT (formulation 3) should therefore result in the most flexible film, in comparison to the films obtained from DBP (formulation 2) and Acetal (formulation 4), respectively. Therefore, the longer the alkyl chain of the plasticiser (as in the case of DEHT) the more free volume it will occupy inside the polymer matrix, and the more efficient the plasticised polymer should behave. However, in the case of cross-linking in the nail lacquers, where the plasticiser is being 'trapped' by the polymer chains, the opposite effect takes place. Therefore it is hypothesised that cross-linking decreases the free volume in which the plasticiser resides causing less mobility thereof, resulting in a 'stiffer' (less flexible) film. However, an increase in temperature causes free volume to increase, which compensates for the reduced mobility of the plasticiser caused by cross-linking [109]. It is therefore postulated that elevated temperature compensates for the effect of cross-linking. As a result, all plasticised nail lacquer films remained flexible over the three month elevated incubation period.

4.4.2 Adhesion

**Table 4.4 Adhesion results of lip coat and nail lacquer formulations during accelerated ageing tests**

| | ADHESION RATING | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| **Stage** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **T0** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **T1** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **T2** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **T3** | Fail - 1 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **T0 (end)** | Fail - 1B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

From Table 4.4 it can be observed that the Blank lip coat formulation fails adhesion (2B) at Stages T0, T1, T2 and T3. At Stage T3 the Blank nail lacquer failed adhesion (1B). All the formulations with plasticisers passed the adhesion tests (refer to Figure 4.1). The full set of adhesion results can be seen in Appendix A: Table 2.

The Blank formulation fails adhesion, i.e. the edges of the film detached. The area of 35% to 65% of the lattice was affected. DBP, DEHT and Acetal formulations were considered to have very strong levels of adhesion over the first 2 months elevated incubation period and scored the highest ASTM value of 5B. The edges of the cuts were completely smooth; none of the squares of the lattice was detached. However, at Stage T2 and Stage T3 incubation at elevated temperature, the adhesion performance of Nail DBP (formulation 2) deteriorated to an ASTM value of 4B, i.e. not significantly more than 5% flaking occurred. It is postulated that this performance characteristic is related to the leaching of DBP plasticiser from the nail lacquer at elevated temperature. DEHT and Acetal formulations remained

stable as did the control sample which was again evaluated after the three month RT storage condition.

4.4.3 Homogeneity

The homogeneity test performed at all storage time intervals did not show any settlement on the spatula for all formulations.

4.4.4 Organoleptic properties

The evaluated organoleptic properties for the nail lacquer and lip coat formulations are summarised in Table 4.5.

**Table 4.5 Organoleptic properties of the various plasticised nail lacquer and lip coat formulations**

| Organoleptic Property | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| Odour | Acetate | Ethanolic | Acetate | Ethanolic | Acetate | Ethanolic | Acetate | Ethanolic |
| Colour | Clear and colourless | Clear and colourless | Clear and colourless | Clear and colourless | Clear and colourless | Clear and colourless | Straw colour | Slightly yellowish |

Table 4.5 displays the colour and odour obtained for the four different nail and lip coat formulations. For all formulations, Stages T0 to T3 were not tabulated individually, since no odour nor was colour change detected over the three month incubation period.

All formulations remained unchanged with regard to colour and odour over the entire incubation period. Elevated temperature and storage time therefore had no influence on the organoleptic properties of any formulation.

4.4.5 Hardness

Table 4.6 summarises the hardness results obtained with the pendulum hardness tester. Figure 4.6 and Figure 4.7 graphically illustrate the hardness results obtained over the test period. The full set of results can be seen in Appendix A: Table 3.

**Table 4.6 The hardness results of the various nail lacquer and lip coat formulations during accelerated ageing tests**

| | NO. PENDULUM SWINGS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| **Stage T0** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| Mean | 53 | 41 | 31 | 24 | 26 | 22 | 35 | 20 |
| STD | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |
| **Stage T1** | | | | | | | | |
| Mean | 63 | 43 | 31 | 23 | 26 | 20 | 34 | 18 |
| STD | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Stage T2** | | | | | | | | |
| Mean | 67 | 43 | 35 | 23 | 28 | 21 | 36 | 18 |
| STD | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| **Stage T3** | | | | | | | | |
| Mean | 72 | 43 | 35 | 24 | 31 | 21 | 38 | 18 |
| STD | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| **Stage T0 (end)** | | | | | | | | |
| Mean | 53 | 41 | 32 | 23 | 26 | 22 | 36 | 20 |
| STD | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |

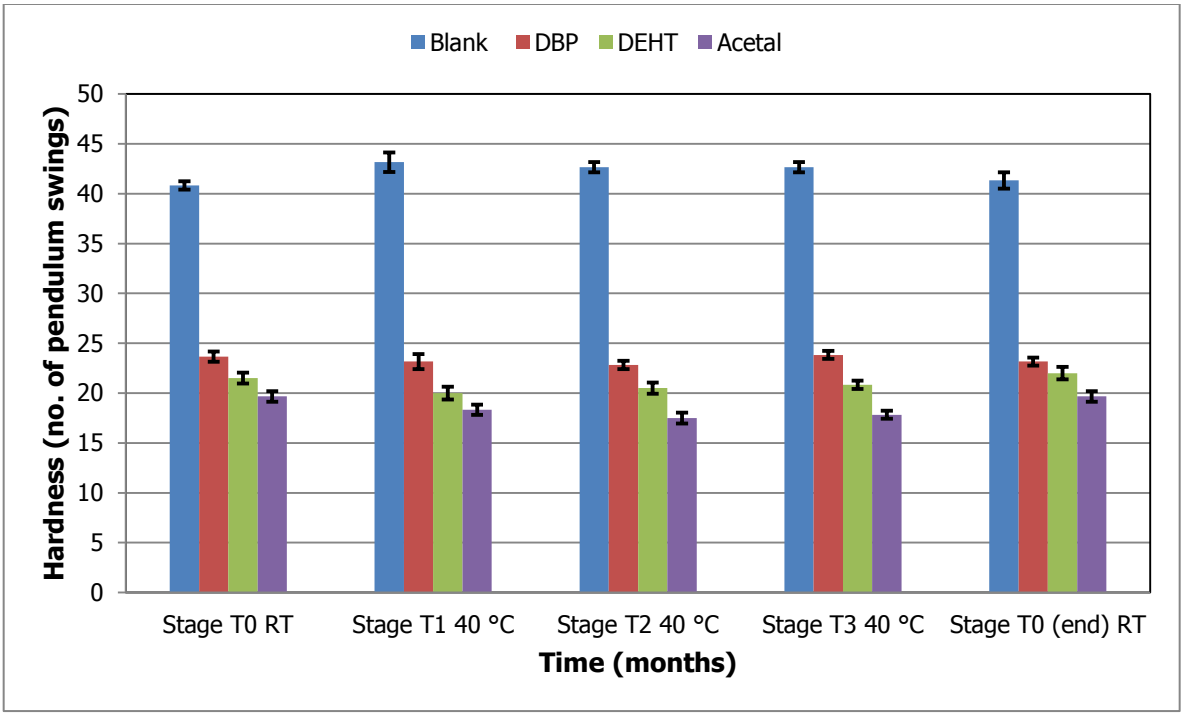



**Figure 4.6 Hardness results for lip formulations during accelerated ageing**

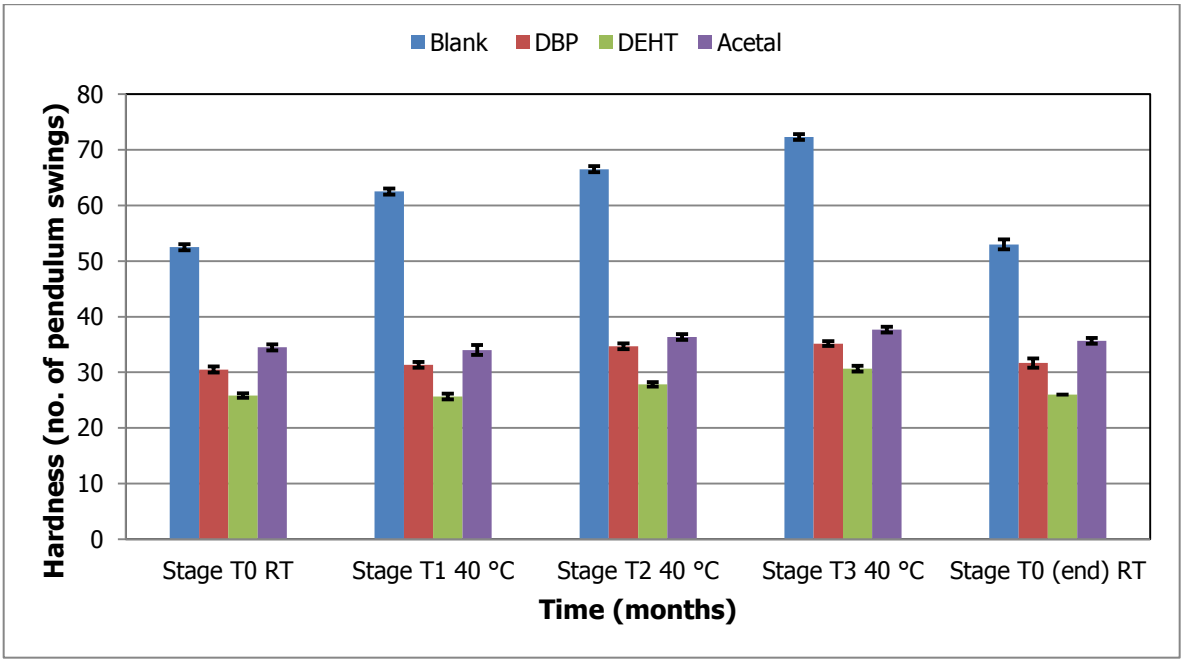



**Figure 4.7 Hardness results for nail lacquer formulations during accelerated ageing**

Both unplasticised nail lacquer and lip coat formulations showed [110] an increased amount of pendulum swings indicative of harder films (Figure 4.6 and 4.7). This is attributable to the absence of plasticiser in both Blank formulations as expected since the presence of plasticiser induces softer films. Film hardness remained stable over time and at elevated temperature for these Blank formulations.

Acetal plasticised nail lacquer formulations outperformed both DEHT and DBP formulations with regard to hardness throughout the three month elevated and RT incubation period (more pendulum swings were obtained in the same amount of time, showing less resistance on the harder films). This could be due to cross-linking of the film formers, nitrocellulose and melamine formaldehyde, which prevented segmental mobility of the plasticiser in the polymer matrix, leading to harder films. In a previous study by Ru [111] it was observed that molecular mobility of the polymer chain led to restriction of the freedom of portions of the polymer molecule, side chains as well as segments.

The plasticisation efficiency of the Acetal molecule was therefore being hindered by cross-linking, which is more pronounced at a higher temperature. On the contrary, elevated temperature causes the free volume to increase, which results in greater plasticisation efficiency and 'softer' films. Plasticisation efficiency was therefore being outperformed by cross-linking.

Lip coat formulations lack cross-linking between PVP and HPC, instead, hydrogen-bonding takes place between these film formers. The mobility of plasticiser within the lip formulations is, therefore, less restricted than in the case of cross-linked nail lacquers. The absence of cross-linking in all lip coat formulations resulted in excellent adhesion and flexibility throughout the three month incubation period at RT and elevated temperature. Films were all 'softer' (fewer pendulum swings obtained) than those of plasticised nail lacquers, with Acetal lip coat formulation

showing the softest films obtained (fewest pendulum swings). This is in good agreement with the molecular structure of Acetal, i.e. the ether (O-C-O) moiety of the 1,3-dioxane ring attached to the cyclohexane ring of PMD-citronellal acetal, exhibits electronegativity due to the lone pairs of electrons surrounding the oxygen atoms, leading to a 'softer' film, in comparison to the DBP and DEHT lacking the 1,3-dioxane ring as part of their molecular structures. The ether oxygen of Acetal forms bonds with two carbon atoms, and as a result, the lack of hydrogen atoms indicates that ether linkages are surrounded by a lot of free volume [112].

### 4.4.6 Non-Volatile Content (NVC)

The NVC test results obtained are summarised in Table 4.7 and Figures 4.8 and 4.9 for lip coat and nail lacquer, respectively. The full set of results can be seen in Appendix A: Table 4.

**Table 4.7 The NVC results from the various nail lacquer and lip coat formulations during accelerated ageing tests**

| | PERCENTAGE (%) NVC | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| **Stage T0** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| Mean | 10.80 | 11.01 | 17.77 | 16.04 | 18.26 | 16.08 | 17.56 | 16.03 |
| STD | 0.02 | 0.04 | 0.06 | 0.01 | 0.05 | 0.03 | 0.06 | 0.02 |
| **Stage T1** | | | | | | | | |
| Mean | 10.80 | 11.01 | 17.82 | 16.04 | 18.25 | 16.06 | 17.57 | 15.99 |
| STD | 0.03 | 0.02 | 0.04 | 0.02 | 0.03 | 0.04 | 0.05 | 0.03 |
| **Stage T2** | | | | | | | | |
| Mean | 10.82 | 11.02 | 17.82 | 16.01 | 18.20 | 16.01 | 17.58 | 16.02 |
| STD | 0.02 | 0.03 | 0.05 | 0.04 | 0.02 | 0.04 | 0.06 | 0.03 |
| **Stage T3** | | | | | | | | |
| Mean | 10.82 | 11.03 | 17.79 | 16.00 | 18.21 | 16.07 | 17.62 | 15.97 |
| STD | 0.07 | 0.03 | 0.05 | 0.04 | 0.04 | 0.03 | 0.04 | 0.03 |
| **Stage T0 (end)** | | | | | | | | |
| Mean | 10.77 | 11.04 | 17.79 | 16.04 | 18.27 | 16.12 | 17.56 | 15.99 |
| STD | 0.05 | 0.03 | 0.06 | 0.02 | 0.03 | 0.02 | 0.06 | 0.03 |

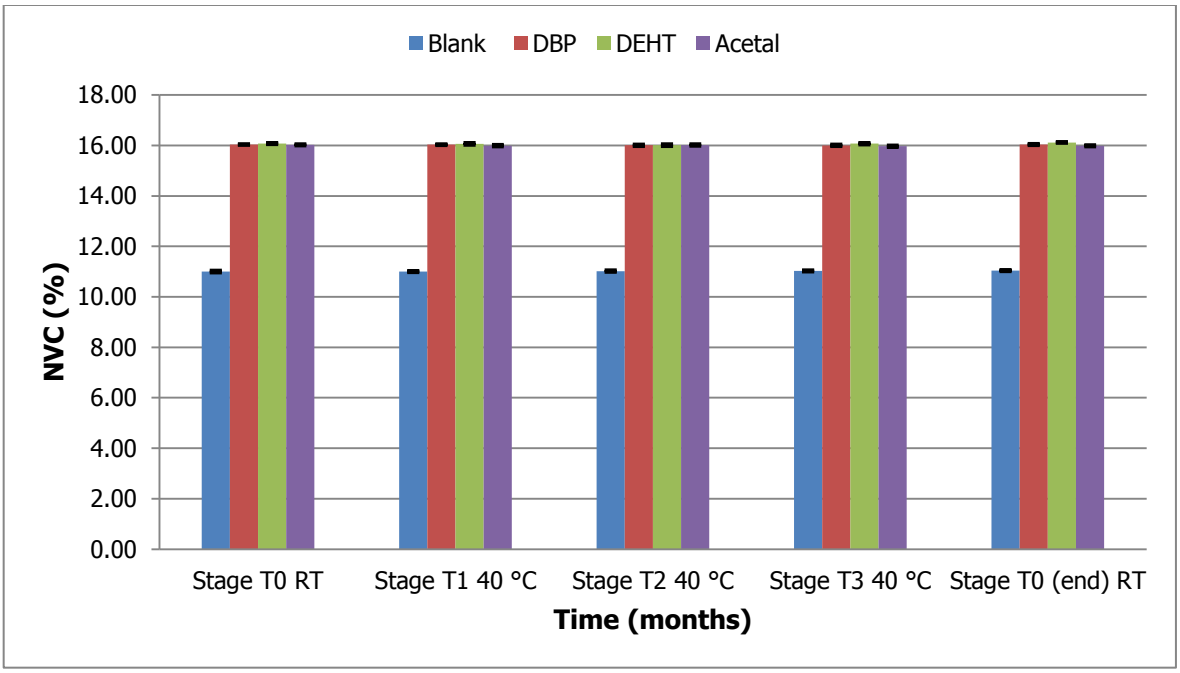



**Figure 4.8 NVC results for lip coat formulations during accelerated ageing**

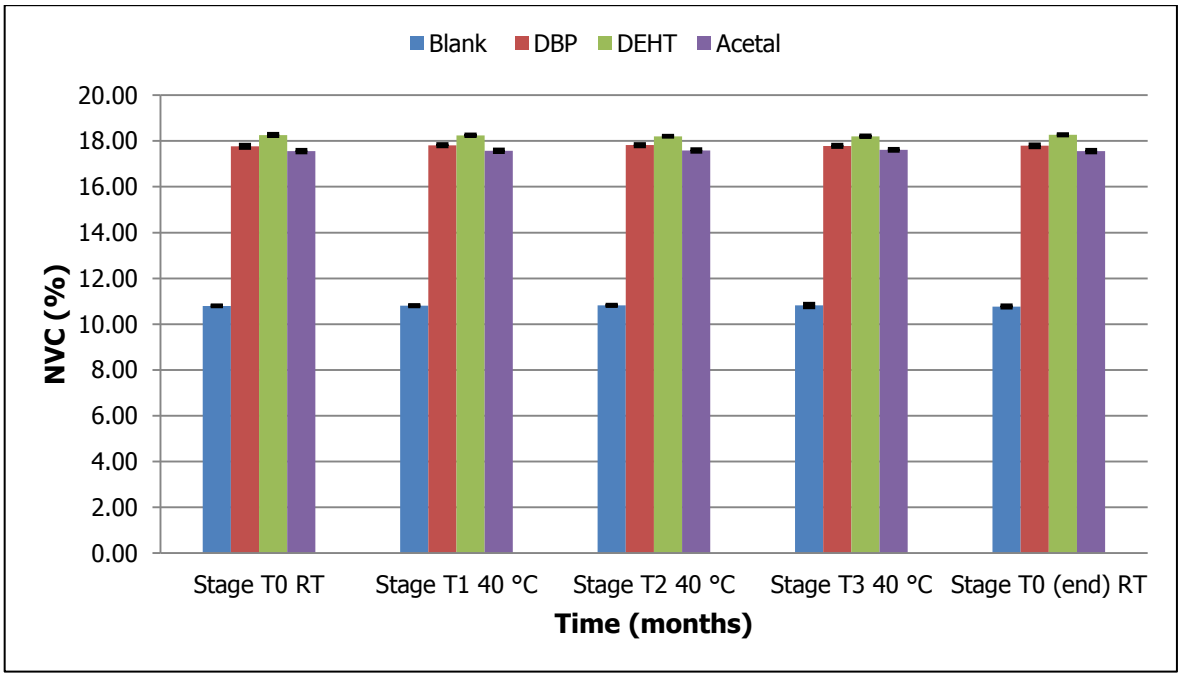



**Figure 4.9 NVC results for nail lacquer formulations during accelerated ageing**

The NVC of all the three plasticised nail and lip formulations are similar and higher than Blank formulations due to the absence of plasticiser in the formulations.

4.4.7 Viscosity

The viscosity results of both nail and lip formulations are presented in Figure 4.10 and Figure 4.11 and Table 4.8. The full set of results can be seen in Appendix A: Table 5.

**Table 4.8 Viscosity (cSt) results from the various nail lacquer and lip coat formulations during accelerated ageing tests**

| | VISCOCITY (cSt) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| **Stage T0** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| Mean | 67.85 | 50.59 | 50.59 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| STD | 1.48 | 1.26 | 1.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Stage T1** | | | | | | | | |
| Mean | 68.32 | 51.61 | 51.10 | 43.80 | 45.92 | 37.87 | 57.65 | 25.24 |
| STD | 1.17 | 1.25 | 0.00 | 1.65 | 1.63 | 1.36 | 1.21 | 1.46 |
| **Stage T2** | | | | | | | | |
| Mean | 69.67 | 51.61 | 57.65 | 44.34 | 51.10 | 38.42 | 63.52 | 25.83 |
| STD | 1.35 | 1.25 | 1.21 | 1.31 | 0.00 | 0.00 | 1.18 | 1.84 |
| **Stage T3** | | | | | | | | |
| Mean | 79.53 | 57.65 | 59.63 | 44.87 | 56.15 | 38.42 | 69.67 | 26.43 |
| STD | 1.13 | 1.21 | 1.21 | 0.00 | 1.55 | 0.00 | 1.35 | 1.96 |
| **Stage T0 (end)** | | | | | | | | |
| Mean | 75.37 | 51.10 | 57.65 | 44.34 | 51.61 | 38.96 | 63.52 | 24.64 |
| STD | 2.26 | 0.00 | 1.21 | 1.31 | 1.25 | 1.33 | 1.18 | 0.00 |

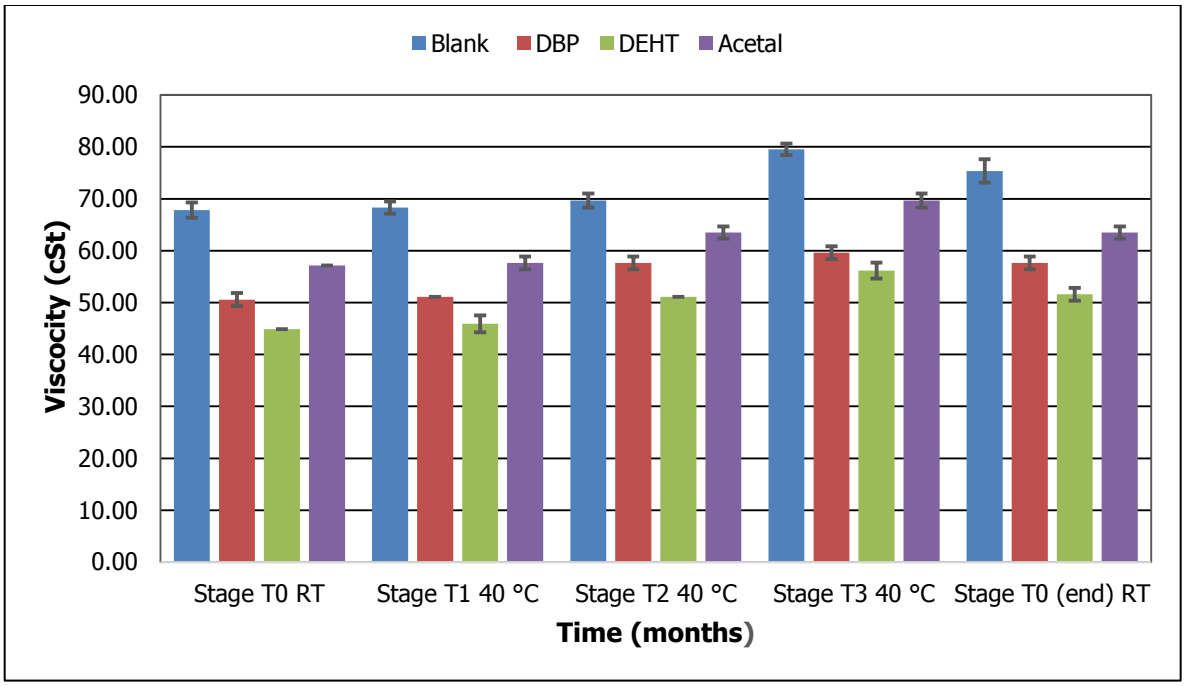



**Figure 4.10 Viscosity of nail lacquer formulations during accelerated ageing**

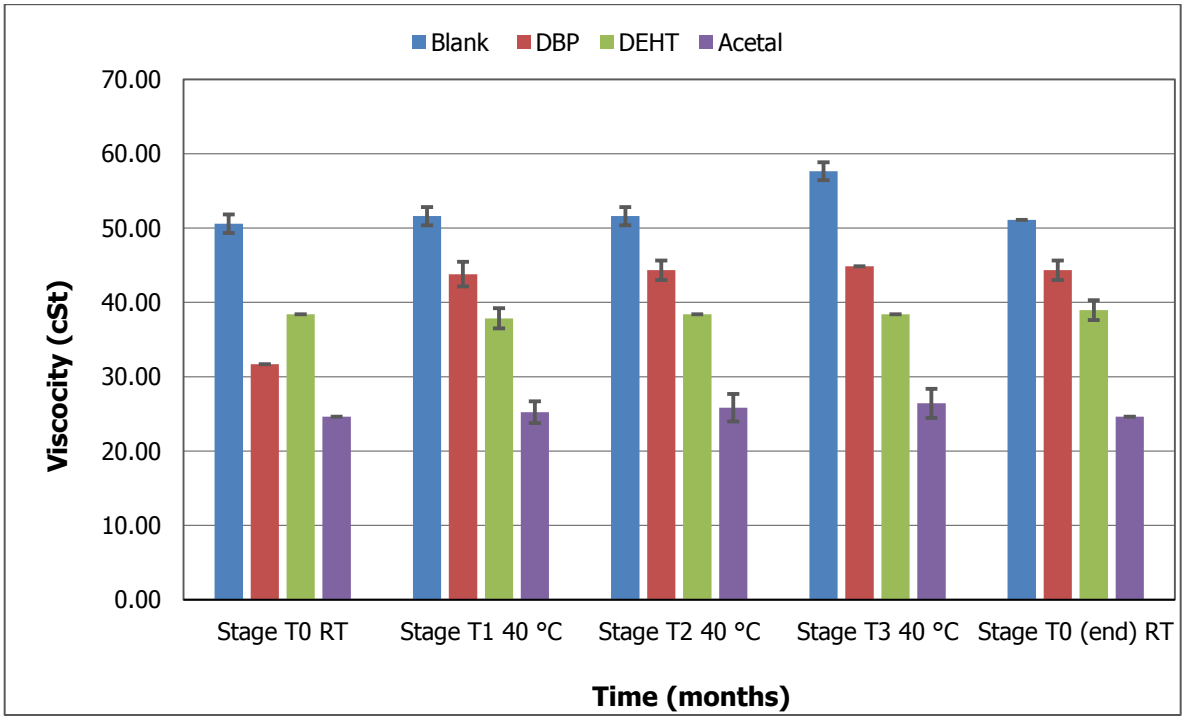



**Figure 4.11 Viscosity of lip coat formulations during accelerated ageing**

From the observed results the viscosity of both Blank lip coat and Blank nail lacquer formulations were higher compared to the corresponding plasticised formulations. This correlates well with theory that plasticisers impart lower viscosity of polymer formulations.

All unplasticised nail lacquer formulations showed an increase in viscosity at elevated temperature and over time due to cross-linking of film formers nitrocellulose and MF which increases at higher temperature (Figure 4.10). Viscosity of unplasticised lip coat formulation showed an increased trend at elevated temperature due to hydrogen-bonding between PVP and HPC (Figure 4.11).

DBP, DEHT and Acetal nail lacquer formulation viscosity increased over time at elevated temperature. It is postulated that cross-linking of the film formers caused the viscosity to increase preventing the plasticisers to impart lower viscosity in the formulations. Furthermore, Acetal nail lacquer viscosity was higher than DBP and DEHT. The same trend was observed for hardness results of these plasticised formulations. It is therefore postulated that there is a relationship between hardness and viscosity of these formulations due to cross-linking of the film formers, nitrocellulose and MF, restricting segmental mobility of plasticiser causing viscosity to increase instead of imparting a lower viscosity. It is postulated that cross-linking outperforms plasticisation efficiency of the Acetal molecule in this plasticised formulation.

DEHT plasticised nail lacquer formulations took slightly longer to move through the orifice of the Gardco Din cup, indicative of this formulation being slightly more viscous than DBP formulation. It is hypothesised that the plasticisation efficiency of DEHT outperformed cross-linking as in the case of Acetal nail lacquer and DPB due to the DEHT molecule exhibiting longer hydrocarbon alkyl chains.

Acetal plasticised lip coat formulation exhibited the lowest viscosity of all the lip coat formulations. This lower viscosity compared well with the lowest hardness results obtained for this formulation. The molecular structure of the Acetal molecule exhibits an ether moiety (O-C-O) as part of its 1,3-dioxane ring. This contributes to more electronegativity due to the lone pairs of electrons on the oxygen atoms, resulting in a higher fluidity. The Acetal molecule therefore imparts a lower viscosity for the lip coat.

The Acetal lip coat formulation viscosity remained constant over the three month accelerated storage period, as did DEHT lip coat and DBP lip coat, but DBP lip coat viscosity increased after one month storage. For DPB lip coat it is postulated that the short alkyl chains attached to the ester carbonyl groups of the DBP molecule do not impart more plasticisation efficiency (more free volume) in order to lower the viscosity as compared to Acetal and DEHT with their longer hydrocarbon chains. The increased viscosity result for the DBP lip coat after one month storage could not be attributable to elevated storage condition, since the same increased result was obtained for the control sample stored at RT after three months. Plasticisation efficiency with regard to viscosity is therefore less efficient for the DBP lip coat than for DEHT and Acetal plasticised lip coat formulations. This could possibly be due to the molecular structure of the DBP molecule, which exhibits short alkyl chains attached to its ester carbonyl groups, therefore not imparting enough free volume to execute the plasticisation efficiency of lowering the viscosity as efficiently as the DEHT and Acetal plasticiser molecules within their respective lip coat formulations.

4.4.8 pH

pH results are presented in Table 4.9 and Figure 4.12 and Figure 4.13 for the nail lacquer and lip coat formulations, respectively. The full set of results can be seen in Appendix A: Table 6.

**Table 4.9 pH results for the various nail lacquer and lip coat formulations during accelerated ageing tests**

| | pH | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
| | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| Stage T0 | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| Stage T1 | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| Stage T2 | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| Stage T3 | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| Stage T0 (end) | 5.55 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |

It can be observed from Table 4.9 that the pH values for the individual formulations remained constant over the test period. The Blank nail lacquer formulation remained at an average pH of 5.60 over the tested period compared to the pH of 6.2 for the Blank lip coat formulation over the same period. For the DBP formulation, the pH values observed were pH 3.9 (nail formulation) and pH 5.80 (lip formulation). DEHT formulations had pH values of 4.0 (nail formulation) and pH of 5.90 (lip formulation) over the studied period. PMD-citronellal acetal formulation was observed to have a pH of 3.80 (nail formulation) and pH 5.70 (lip formulation) over the studied period.

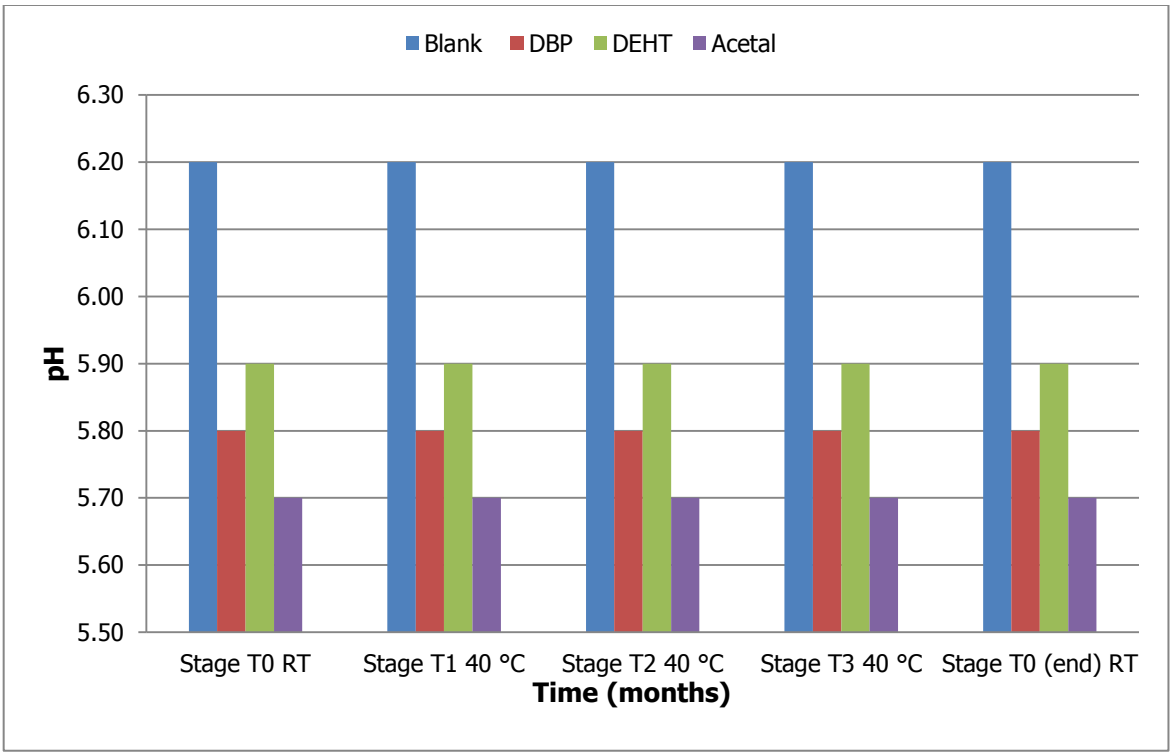



**Figure 4.12 pH of lip coat formulation during accelerated ageing**

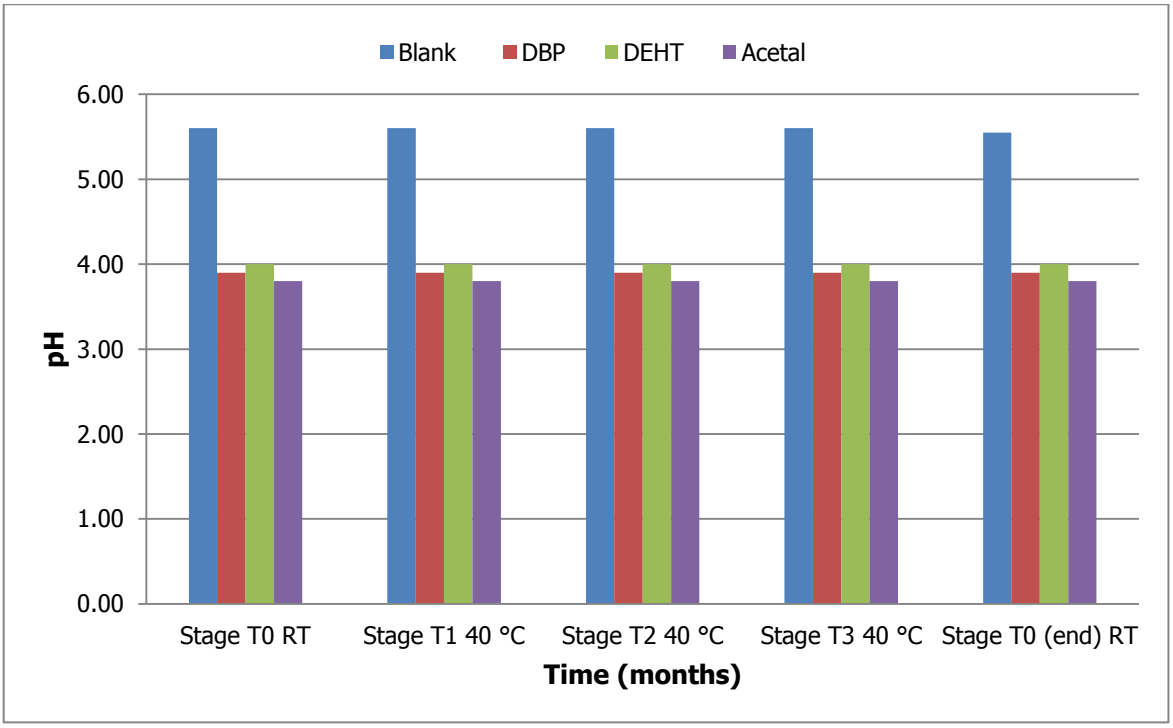



**Figure 4.13 pH of nail lacquer formulations during accelerated ageing**

Neither RT nor elevated temperature had an effect on the pH of any cosmetic formulation tested at the different time interval periods. Addition of all plasticisers caused a lowering of the pH of the formulation. Plasticised nail formulations exhibited lower pH values than plasticised lip formulations. It is postulated that the lower pH values of the nail lacquer formulations could be due to the deprotonation of MF in the cross-linking film forming process with nitrocellulose rendering a more acidic environment.

From Figure 4.13 stable pH trends were observed for the plasticised and unplasticised nail lacquer formulations over time and elevated temperature storage conditions. Acetal nail lacquer exhibited the lowest pH value whereas the pH values of DBP and DEHT nail lacquers formulations were very similar.

The plasticised lip coat and Blank formulations remained stable over time and at elevated temperature (Figure 4.12). The plasticised lip coat formulations were more acidic compared to the Blank lip coat formulation. The Acetal lip coat formulation had the lowest pH value compared to DBP and DEHT lip coat formulations, possibly due to the deprotonation of MF in the cross-linking film forming process with nitrocellulose, leading to a more acidic environment. The pH values of DBP and DEHT nail lacquers formulations were very similar.

## 4.5 Conclusions

Flexibility for all plasticised formulations remained stable at RT and elevated temperature at all testing intervals, as no cracking of any films was observed except for Blank formulations which cracked and peeled at all testing intervals due to the absence of plasticiser.

DBP, DEHT and Acetal nail and lip formulations were considered to have very strong levels of adhesion over the first two months elevated incubation period and scored the highest ASTM value of 5B. The edges of the cuts were completely smooth, and none of the squares of the lattice was detached. However, at Stage T2 and Stage T3 incubation at elevated temperature, the adhesion performance of Nail DBP (formulation 2) deteriorated to an ASTM value of 4B, implying that not more than 5% flaking or detachment was observed. This adhesion performance of DBP at elevated temperature could possibly be related to the leaching of DBP plasticiser from the nail lacquer at elevated temperature (Chapter 6). The Blank nail lacquer formulation failed adhesion with an ASTM rating of 1B after the three month elevated incubation period, since cross-linking increases with elevated temperature and as a result, the entire film cracked due to brittleness.

Acetal plasticised nail lacquer formulations outperformed DEHT and DBP nail lacquer formulations with regard to hardness throughout the three month elevated and RT incubation period (more pendulum swings were obtained in the same amount of time showing less resistance on the harder films). This could be due to cross-linking of the film formers, nitrocellulose and melamine formaldehyde, which prevented segmental mobility of the plasticiser in the polymer matrix, leading to harder films. The plasticisation efficiency of the Acetal molecule was therefore being outperformed by cross-linking, causing the formation of the hardest films. Conversely, DEHT nail lacquer formulation resulted in the softest plasticised nail lacquer, which could be attributed to this molecule exhibiting the longest hydrocarbon chains emanating from the ester carbonyl group occupying the *p*-positions on the benzene ring, hence imparting a higher degree of plasticising efficiency to the formulation. It is therefore postulated that within the DEHT nail lacquer formulation, plasticisation efficiency of the DEHT molecule outperformed cross-linking between the nail lacquer film formers resulting in the 'softest' nail lacquer formulation.

The absence of cross-linking in all lip coat formulations resulted in excellent adhesion and flexibility throughout the three month incubation period at RT and elevated temperature. Films were all 'softer' (fewer pendulum swings obtained) than those of plasticised nail lacquers with Lip Acetal formulation showing the softest films obtained (the least pendulum swings). This is in good agreement with the molecular structure of Acetal, i.e. the ether (O-C-O) moiety of the 1,3-dioxane ring attached to the cyclohexane ring of PMD-citronellal acetal, exhibits electronegativity due to the lone pairs of electrons surrounding the oxygen atoms, leading to a 'softer' film. The ether oxygen of this 1,3-dioxane ring forms bonds with two carbon atoms, therefore, the lack of hydrogen atoms indicates that ether linkages are surrounded by a lot of free volume [112] further contributing to a 'softer' lip formulation.

All nail lacquer and lip coat formulations exhibited stable pH values throughout their incubation period. The addition of plasticiser to the Blank nail lacquer formulation and Blank lip coat formulation, respectively, lowered the pH values of all cosmetic formulations. Plasticised nail formulations exhibited lower pH values than the lip formulations and could, therefore, be considered to be more acidic. The pH values of the lip formulations were closer to neutral.

The nail lacquer and lip coat plasticised and unplasticised formulations remained unchanged with regard to colour and odour over the three month incubation period. Elevated temperature and storage time therefore had no effect on the organoleptic properties of any formulation. The homogeneity performed at all stages did not show any settlement on the spatula for any of the plasticised lip coat nor nail lacquer formulations. The unplasticised formulations also showed no settlement at any stage.

The viscosity results of the Blank lip coat and Blank nail lacquer formulations were higher compared to the corresponding plasticised formulations due to the absence of plasticiser.

All unplasticised nail lacquer formulations showed an increase in viscosity at elevated temperature and over time due to cross-linking of film formers nitrocellulose and MF. DBP, DEHT and Acetal nail lacquer formulation viscosity increased over time at elevated temperature. It is postulated that cross-linking of the film formers caused the viscosity to increase, preventing the plasticisers to impart lower viscosity in the formulations.

Unplasticised lip coat formulations showed an increase in viscosity due to hydrogen-bonding between PVP and HPC.

Acetal plasticised lip coat formulation exhibited the lowest viscosity of all the lip coat formulations. The Acetal, DEHT and DBP lip coat formulations viscosity results remained constant over the three month accelerated storage period.

# CHAPTER 5
# FTIR-ATR ANALYSIS TO DETERMINE CHEMICAL STABILITY OF NEAT PLASTICISERS

## 5.1 Introduction

Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectrophotometry is a valuable analytical technique to study materials in the gas, liquid or solid phase. FTIR is a powerful method used to identify chemical functional groups of polymers for screening and profiling of samples. Furthermore, most substances exhibit a characteristic spectrum and can be identified by this, similar to the human fingerprint.

The infrared spectra vary according to the chemical composition of a material tested and can indicate the complex and possible interaction between its constituents.

Infrared (IR)-measurements are mainly performed in ATR (Attenuated Total Reflection) mode these days since the ATR mode technique is more user-friendly. Most types of samples (e.g. solids, liquids, powders, pastes, pellets, slurries, fibers, etc.) are placed undiluted on the ATR crystal, thereafter the measurement is performed within seconds.

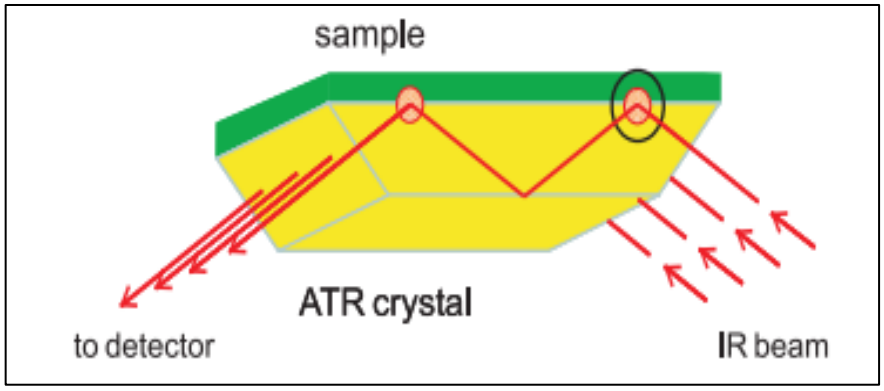



**Figure 5.1 Principle of ATR beams**

The basic principle of how ATR works is shown in Figure 5.1 [113]. The ATR crystal consists of an IR transparent material with a high refractive index and has polished surfaces. The infrared beam enters the ATR crystal at an angle of 45 degrees (relative to the crystal surface) and is totally reflected at the crystal to sample interface. Due to its wave-like properties, the light is not reflected directly on the boundary surface but by a virtual layer within the optically less dense sample. The fraction of the light wave that reaches into the sample is called the evanescent wave. The depth at which the light penetrates the sample depends on the wavelength, the refractive indices of the ATR crystal and sample as well as the angle of the entering light beam. The penetrating light into the sample is typically in the region of a few microns. In those spectral regions where the sample absorbs energy, the evanescent wave will be attenuated. After one or several internal reflections, the IR beam exits the ATR crystal and is directed to the IR-detector. In ATR measurements, the thickness of the sample does not affect the intensity of the absorbance bands [113, 114].

Polymer interactions can be studied in different ways, e.g. a shift in peaks, changes in peak intensities, the appearance of other peaks, development of a

shoulder band attached to the peak, etc. Peaks shifted to higher wavenumbers are indicative of an increase in bond strength [115].

There are a number of ways in which chemical instability could be detected using FTIR as an analytical tool. Degradation of polymers could, for example, take place via chain scission at ester linkages due to hydrolysis leading to the formation of terminal alcohol and carboxylic acid groups. Progression of hydrolysis would be evident from the FTIR spectrum due to an increase in the hydroxyl (OH) band intensity. Reduction of the carbonyl (C=O) peak intensity could also be the result of hydrolysis [116].

Another advantage of using the FTIR-ATR methodology is that the position of substitution on a benzene ring (*ortho-, meta-, para-*) can be determined by focussing on specific bands in the fingerprint region [117].

In an infrared spectrum, the intensity of absorption is directly related to the change in dipole that takes place during the vibration. Hence, vibrations which produce a large change in dipole, e.g. a carbonyl (C=O) group, cause a more intense absorption than those that result in a smaller change in dipole, e.g. an alkene (C=C) [118].

The carbonyl (C=O) stretch is one of the most distinctive bands in the IR spectrum. It can be found at 1640 $cm^{-1}$ to 1820 $cm^{-1}$ and is generally a very strong peak. Carboxylic acids show a very broad hydroxyl (OH) band that frequently obscures the CH stretch around 3000 $cm^{-1}$ [119].

## 5.2 Methodology

An Alpha Bruker FTIR-ATR spectrophotometer, equipped with OPUS software version 7.2, was used to determine the chemical stability of the neat plasticisers in their liquid form over a three month period.

FTIR scans of each of the three neat plasticisers were done as an analytical tool in order to detect any shift in spectral bands (representing functional groups) at elevated temperature (40 ± 1 °C) at each monthly interval over the accelerated storage period. The control samples stored at RT (23 ± 1 °C) were used as a control at the start (Stage T0) and end of the three month period [Stage T0 (end)].

Transmittance infrared spectral bands (also referred to as 'peaks') were measured in the range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. Any changes in spectral intensities would be an indication of the formation, destruction or interaction of functional groups within the polymer [116, 120].

## 5.3 Results and Discussion

Tables 5.1 to 5.3 summarise the functional groups of the neat plasticisers. Figures 5.2, 5.3, and 5.4 illustrate the FTIR spectra of the neat plasticisers. The three time intervals spectra per plasticiser are shown in Figures 5.5, 5.6 and 5.7, namely, Stage T0, Stage T3 and Stage T0 (end). Stage T0 represents the control stored at RT for each plasticiser incubated at elevated temperature. The individual spectra at all the time intervals can be found in Appendix B (Figures 1 to 55).

The FTIR spectra obtained for DBP, DEHT and Acetal resulted in identical spectra for all time intervals at RT and elevated temperature for each plasticiser.

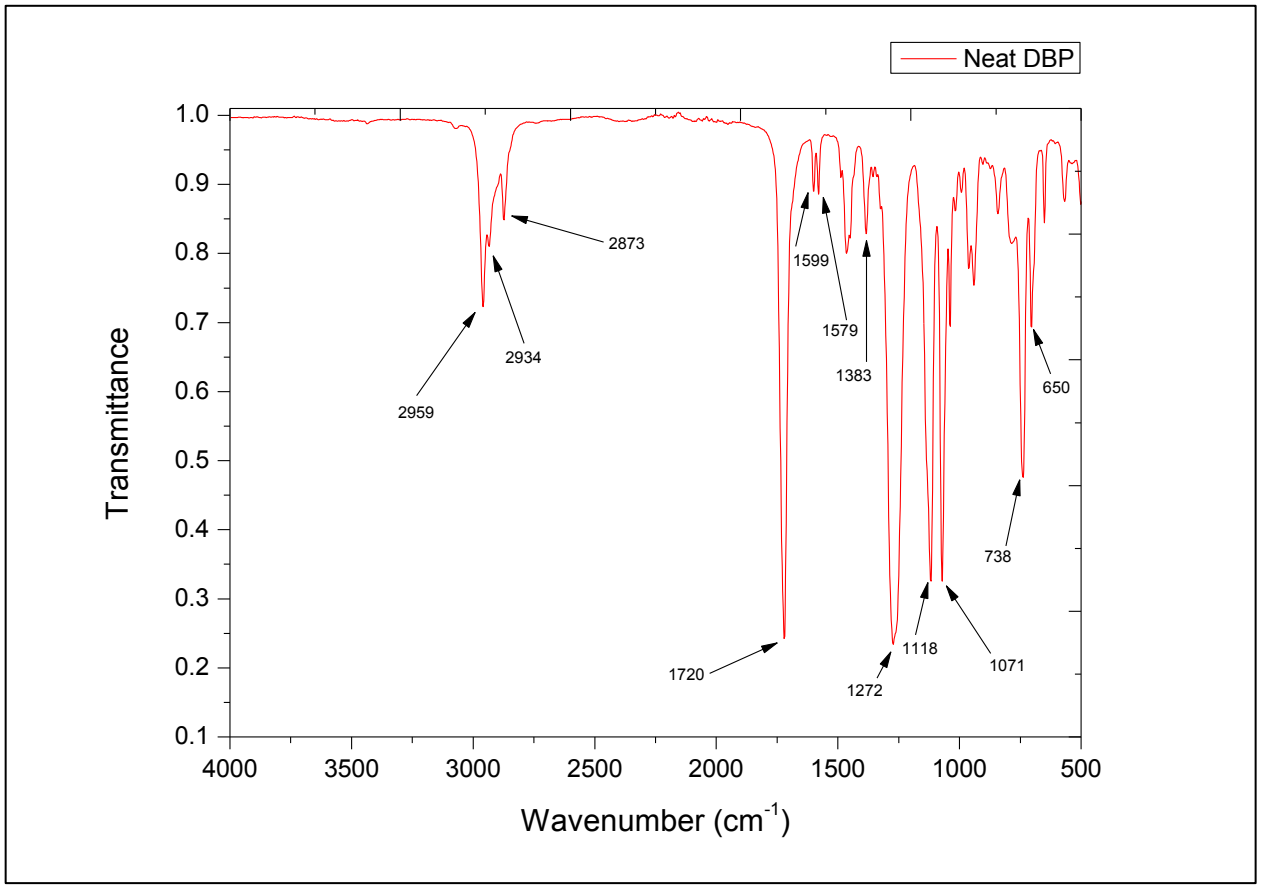



**Figure 5.2 FTIR-ATR spectrum of neat DBP**

Figure 5.2 displays the neat IR spectrum of DPB. It shows C-H stretches at ~2959 $cm^{-1}$, ~2934 $cm^{-1}$, ~2873 $cm^{-1}$, C=O carbonyl ester at ~1720 $cm^{-1}$, semi-quadrant aromatic doublet ring modes at ~1599 $cm^{-1}$ and ~1579 $cm^{-1}$, $CH_3$ umbrella deformation bend at ~1383 $cm^{-1}$, O-C-C aromatic ester stretch at ~1272 $cm^{-1}$ and ~1118 $cm^{-1}$, CO ether stretch at ~1071 $cm^{-1}$ and C-H aromatic out-of-plane doublet bend at ~738 $cm^{-1}$ and ~650 $cm^{-1}$. Table 5.1 summarises the main spectral bands of DPB [121-123].

**Table 5.1 Summary of functional groups of neat plasticiser, DBP [121-123]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 2959, 2934, 2873 | C-H stretches |
| 1720 | C=O carbonyl ester |
| 1599, 1579 | C=C semi-quadrant aromatic ring modes (doublet) |
| 1383 | $CH_3$ umbrella deformation bend |
| 1272 | O-C-C stretch (aromatic ester) |
| 1118 | O-C-C stretch (aromatic ester) |
| 1071 | C-O stretch (ether) |
| 738, 650 | C-H bend, doublet (aromatic out-of-plane bend) |

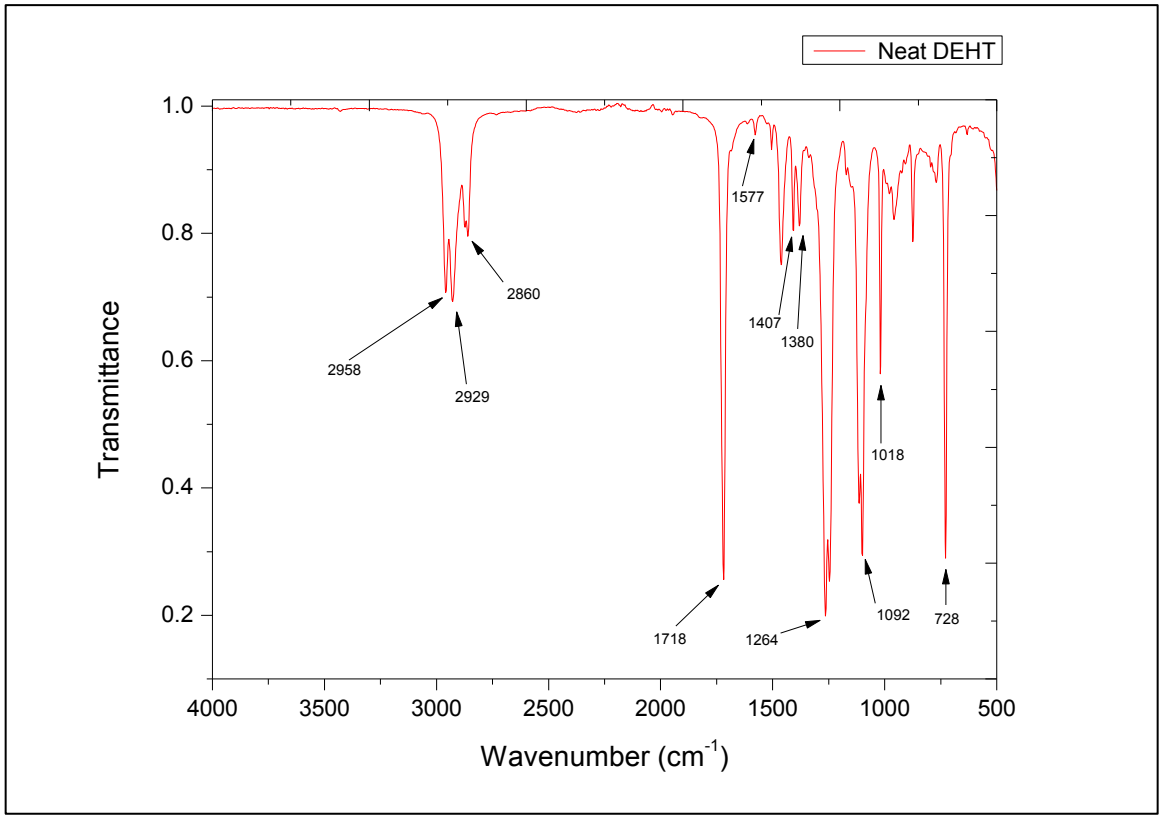



**Figure 5.3 FTIR-ATR spectrum of neat DEHT**

Figure 5.3 illustrates the neat IR spectrum of DEHT. It shows C-H stretches at ~2958 $cm^{-1}$, ~2929 $cm^{-1}$ and ~2860 $cm^{-1}$, carbonyl ester (C=O) at ~1718 $cm^{-1}$, alkene (C=C) of the benzene ring at ~1577 $cm^{-1}$, $CH_3$ umbrella deformation bend at ~1407 $cm^{-1}$ and ~1380 $cm^{-1}$, C-O ether stretch at ~1264 $cm^{-1}$, ~1092 $cm^{-1}$ and ~1018 $cm^{-1}$ and C-H bend at ~728 $cm^{-1}$. Table 5.2 summarises the main spectral bands of DEHT [121-123].

**Table 5.2 Summary of functional groups of neat plasticiser, DEHT [121-123]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 2958, 2928, 2860 | methyl C-H stretches |
| 1718 | C=O carbonyl ester |
| 1577 | C=C alkene of benzene ring |
| 1407, 1380 | $CH_3$ umbrella deformation bend (doublet) |
| 1264 - 1018 | C-O ether stretch (two or more bands) |
| 728 | C-H bend (aromatic out-of-plane bend) |

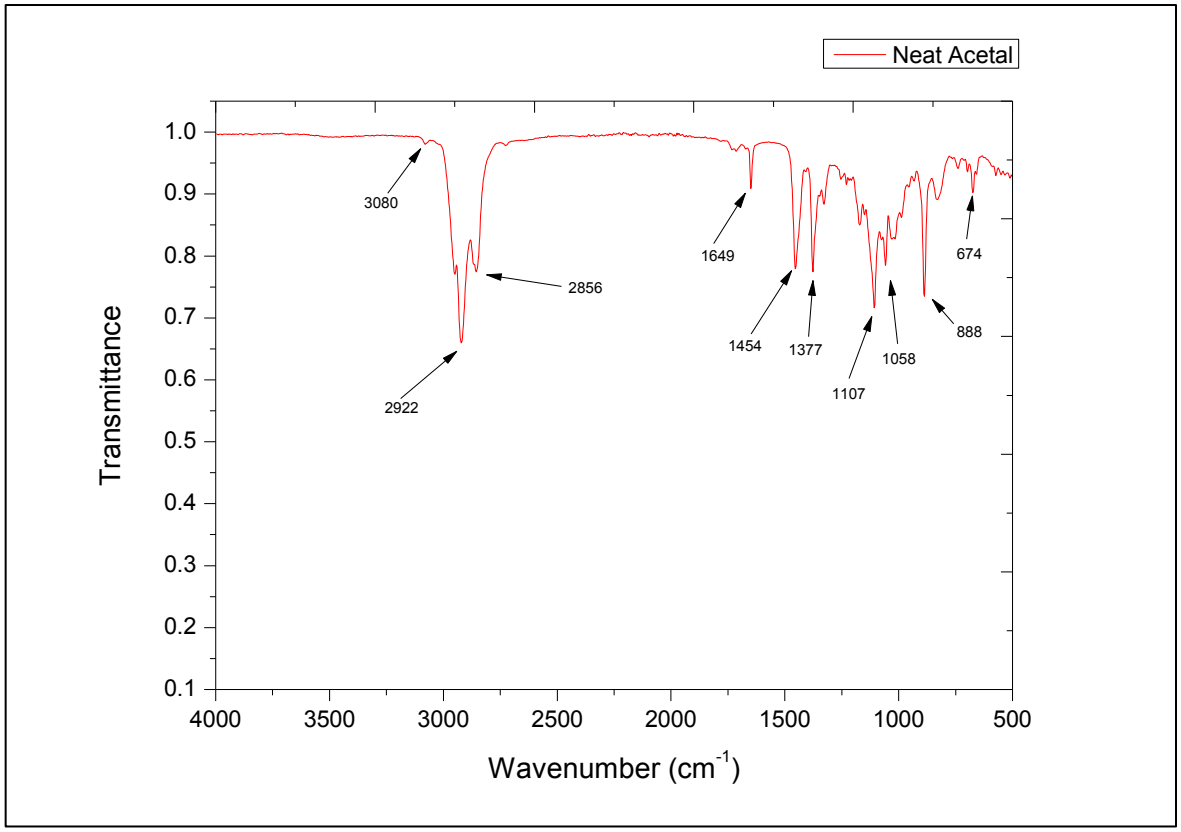



**Figure 5.4 FTIR-ATR spectrum of neat PMD-citronellal acetal**

Figure 5.4 depicts the neat IR spectrum of PMD-citronellal acetal. It displays C-H stretches at ~3080 $cm^{-1}$, alkyl C-H multi-peaks at ~2922 $cm^{-1}$ and ~2856 $cm^{-1}$, C=C non-aromatic peak at ~1649 $cm^{-1}$, $CH_3$ umbrella doublet deformation bend from the isopropyl groups at ~1454 $cm^{-1}$ and ~1377 $cm^{-1}$, C-O ether single bond vibration at 1107 $cm^{-1}$, C-O ether stretch at ~1058 $cm^{-1}$, C-C deformation and stretching at ~888 $cm^{-1}$ and a C-H bend at ~674 $cm^{-1}$. Table 5.3 summarises the main spectral bands of PMD-citronellal acetal [70, 121, 123].

**Table 5.3 Summary of functional groups of neat plasticiser, PMD-citronellal acetal [70, 121, 123]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3080 | CH stretch |
| 2922, 2856 | Alkyl C-H multi-peaks |
| 1649 | C=C alkene (non-aromatic) |
| 1454, 1377 | $CH_3$ umbrella deformation bend (doublet for isopropyl groups) |
| 1107 | C-O ether single bond vibration |
| 1058 | C-O ether stretch |
| 888 | C-C deformation and stretching |
| 674 | CH bending |

From FTIR spectra the ester carbonyl (C=O) spectral band of neat plasticiser, DBP, is observed at ~1720 $cm^{-1}$ (Figure 5.2), whereas this carbonyl band appears at a slightly lower wavenumber for DEHT, i.e. at ~1718 $cm^{-1}$ (Figure 5.3). This is in agreement with literature that states that the same functional group is likely to appear at a lower wavenumber if the molecular structure has a longer carbon chain in its backbone (more hydrophobic), as in the case of DEHT (compare Figure 5.2 and Figure 5.3) [119, 122, 123]. DBP (Figure 5.2) shows a slightly broader carbonyl (C=O) band at ~1720 $cm^{-1}$ than DEHT at ~1718 $cm^{-1}$ (Figure 5.3), being indicative of a more polar plasticiser [121]. The fewer carbon atoms in the backbone of DBP in comparison to the backbone of DEHT and Acetal, respectively, contributes to the higher polarity of DBP [124, 125].

The alkene (C=C) part of the aromatic ring of DBP is observed as a doublet at ~1600 and ~1580 $cm^{-1}$, representing itself as an aromatic quadrant stretch due to the *ortho*-position of the carbon-ester chain attached to the benzene ring (Figure 5.2). On the contrary, in the case of DEHT both carbon-ester chains are in the *p*-position of the molecule, hence leading to the symmetry of chains on each side of

the benzene ring. DEHT exhibits a small, single spectral band at ~1580 $cm^{-1}$ due to its carbon-ester chains being in the *p*-position (Figure 5.3), as opposed to the carbon-ester chain of DBP being in the *o*-position. Similar observations were found in literature which differentiates between semi-circular stretching of the benzene ring of DEHT as opposed to the quadrant stretching of DBP [126].

For both DBP and DEHT the position of substitution on the benzene ring could be determined from IR bands observed in the fingerprint region, e.g. the *ortho*-phthalate, DBP, occurs as a doublet band at 650 $cm^{-1}$ and 738 $cm^{-1}$ (Figure 5.2) as opposed to the *p*-terephthalate, DEHT, which has a single band at 728 $cm^{-1}$ (Figure 5.3). Any ester, apart from having a carbonyl (C=O) spectral band, exhibits an ether (C-O) band assignment [119, 127].

PMD-citronellal acetal lacks a carbonyl group in its molecular structure, instead, it consists of a non-aromatic cyclohexane ring, attached to a 1,3-dioxane ring associated with an alkyl side chain. This 1,3-dioxane ring exhibits the O-C-O (ether) vibrational band at ~1107 $cm^{-1}$ as well as a non-aromatic alkene group (C=C) at ~1649 $cm^{-1}$, as can be seen from Figure 5.4. The C-H stretch band observed at ~3080 $cm^{-1}$ is associated as an overtone of the non-aromatic alkene (C=C) band of Acetal at 1649 $cm^{-1}$. At ~1377 $cm^{-1}$ and at ~1477 $cm^{-1}$ an 'umbrella band' shows as a double band for isopropyl groups [121].

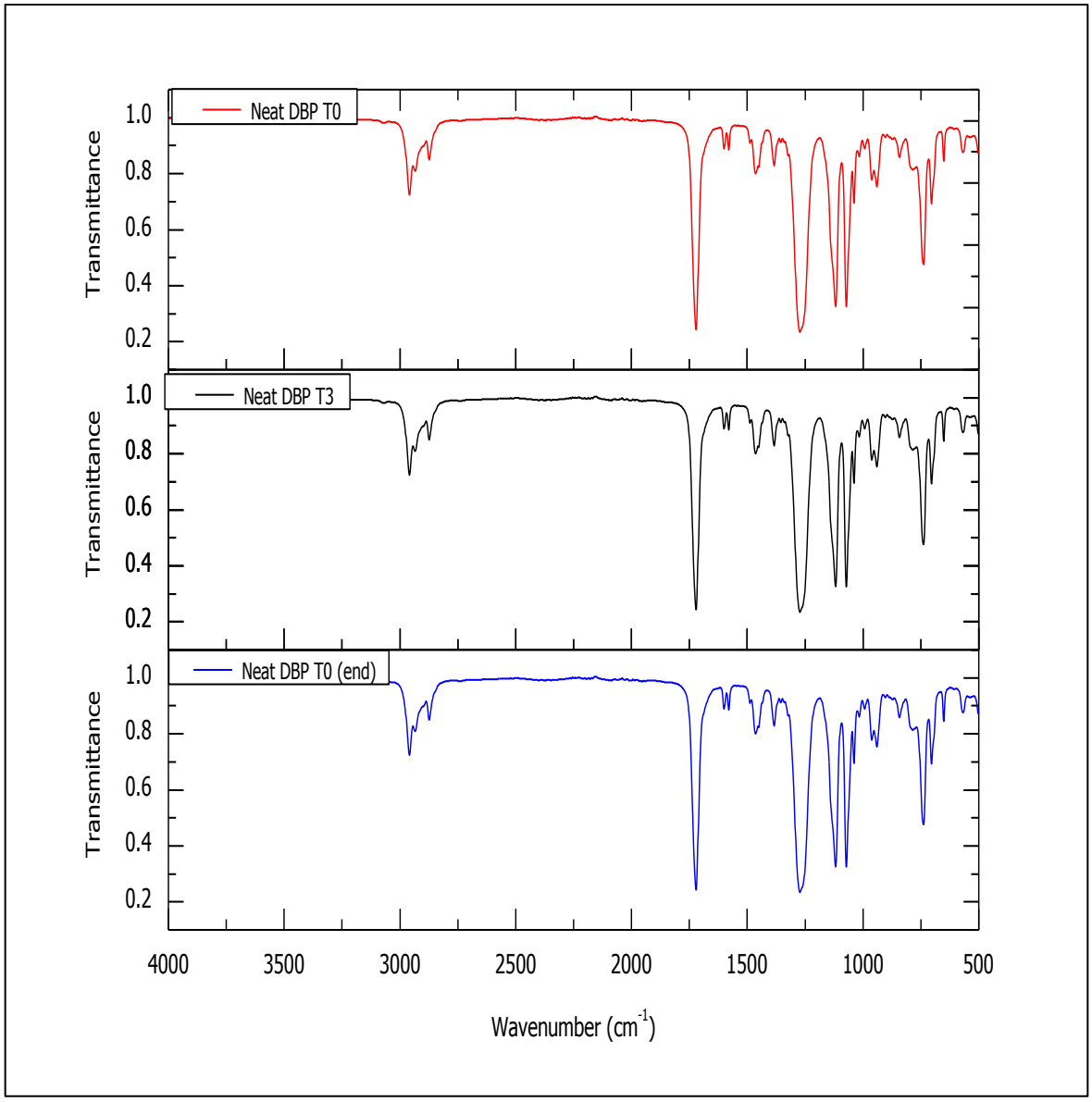



**Figure 5.5 FTIR-ATR spectra of neat DBP at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

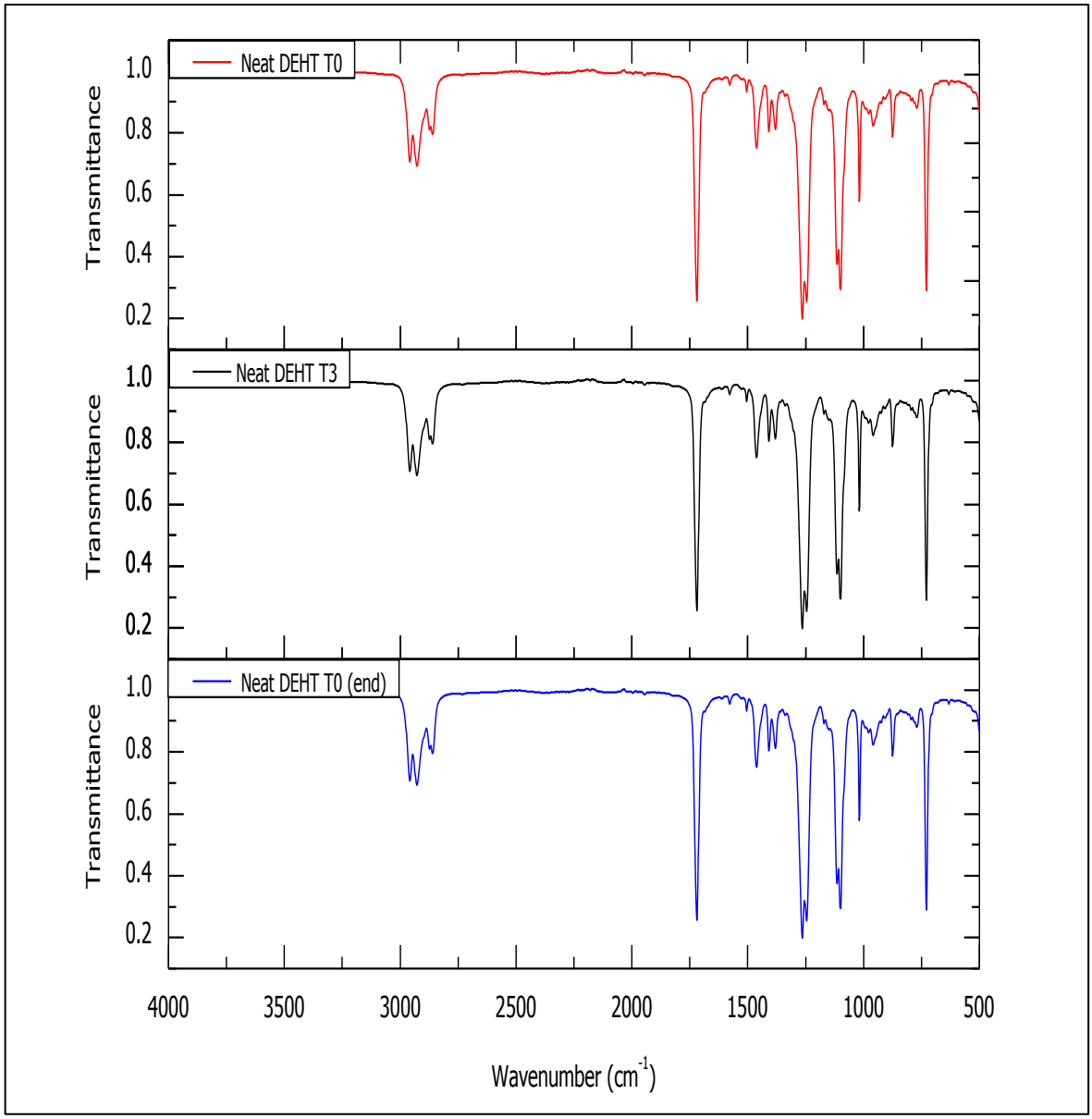



**Figure 5.6 FTIR-ATR spectra of neat DEHT at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

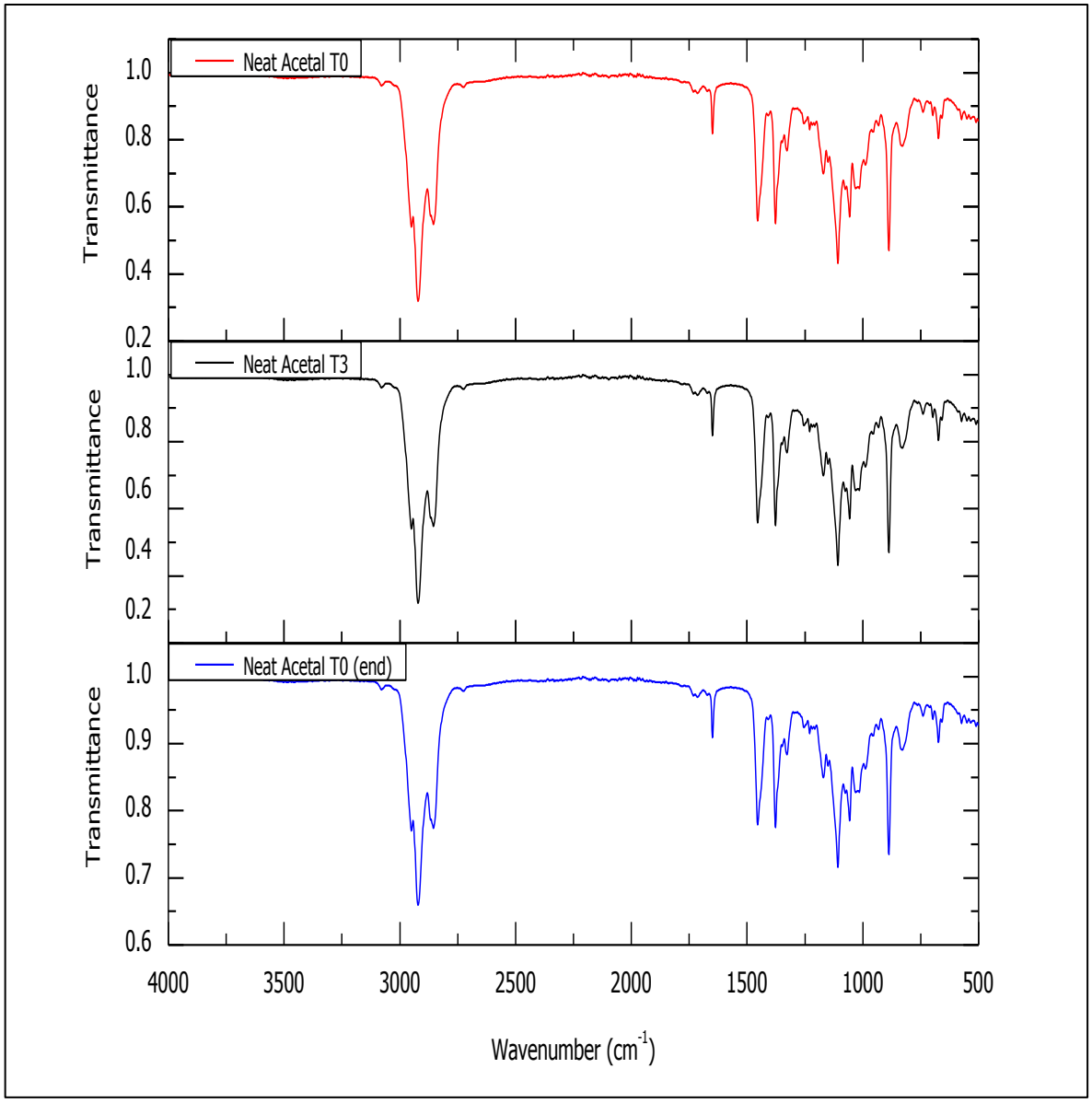



**Figure 5.7 FTIR-ATR spectra of neat PMD-citronellal acetal at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

As can be seen from Figures 5.5, 5.6 and 5.7, no shift of any spectral bands have taken place neither over RT nor at elevated temperature incubation storage periods, rendering the neat plasticisers as chemically stable.

## 5.4 Conclusion

Each of the FTIR spectra of the neat plasticisers, DBP, DEHT and Acetal displayed perfect overlays over the three month incubation period at RT Stage T0 to T3 versus RT Stage T0 (end), rendering all the neat plasticisers as chemically stable. It can, therefore, be concluded that elevated temperature had no effect on the chemical stability of any of the above neat plasticisers since no shift or change in band intensity occurred at any stage of the incubation period.

DPB, DEHT and Acetal were chemically stable at RT and elevated temperature over a three month incubation period.

FTIR-ATR spectra could be utilised as a tool to distinguish between aromatic diester molecules (DBP and DEHT), i.e. the carbonyl group (C=O) observed at ~1720 $cm^{-1}$ and the absence thereof in the non-aromatic cyclohexane-1,3-dioxane ring (PMD-citronellal acetal). FTIR-ATR spectra can also be used to distinguish between an *o*-diester phthalate and a *p*–terephthalate. The latter molecule (DEHT) showed a single spectral band at ~1580 $cm^{-1}$ whereas the FTIR spectrum for DBP exhibited a doublet at ~1580 $cm^{-1}$ and at ~1600 $cm^{-1}$ indicative of the quadrant stretch ring mode of the aromatic ring.

# CHAPTER 6

# FTIR-ATR ANALYSIS TO DETERMINE CHEMICAL STABILITY OF PLASTICISED COSMETIC FORMULATIONS

## 6.1 Introduction

The spectra observed in this Chapter will be discussed under the respective cosmetic headings, viz. a nail lacquer and a lip coat. If any chemical interactions had taken place over the three month incubation period, changes in functional groups would be evident from shifts in spectral bands using the FTIR-ATR technique. A few ways of achieving interactions between polymer chains and plasticisers could be via hydrogen-bonding, van der Waals interactions and others. Spectra obtained from polymers that are miscible with each other, should show a band shift or broadening when compared to the infrared spectra of their homopolymers [128].

Overlays of spectra are displayed between wavenumbers 4000 $cm^{-1}$ and 400 $cm^{-1}$ to detect any shift or change in intensity of spectral bands over the three month incubation period of the abovementioned cosmetic formulations.

## 6.2 Methodology

### 6.2.1 Nail lacquer formulations

The Blank formulation consists of the two film formers i.e. nitrocellulose and melamine formaldehyde, with the added solvent mixture as per nail formulation described in Chapter 3. However, this Blank formulation does not contain plasticiser. Furthermore, the hydroxyl groups of nitrocellulose form a cross-link with the primary amine groups of melamine formaldehyde in order to form ether, methylene and ester linkages [129]. Each plasticised, as well as a Blank cosmetic formulation, was incubated at 40 °C and tested at monthly intervals and stored at RT as a control in order to compare the chemical stability of samples stored at elevated temperature.

### 6.2.2 Lip coat formulations

The Blank lip coat formulation consists of PVP, HPC and solvent as described in Chapter 3. However, this Blank formulation does not contain a plasticiser. Intermolecular hydrogen-bonding interactions occur between hydroxyl groups (OH) groups of HPC and the carbonyl amide C=O group of PVP [130].

The lip coat formulations (Blank and plasticised) were incubated at elevated temperature (40 °C) and tested at monthly intervals. The lip formulations stored at RT, however, were tested as a control at the start (Stage T0) and at the end of the three month storage period [Stage T0 (end)] in order to compare with the chemical stability of samples stored at elevated temperature.

## 6.3 Results and discussion

### 6.3.1 Nail lacquer formulations

FTIR spectra of the Blank nail lacquer and DBP, DEHT and PMD-citronellal acetal plasticised nail lacquer formulations are shown in Figures 6.1 to 6.7.

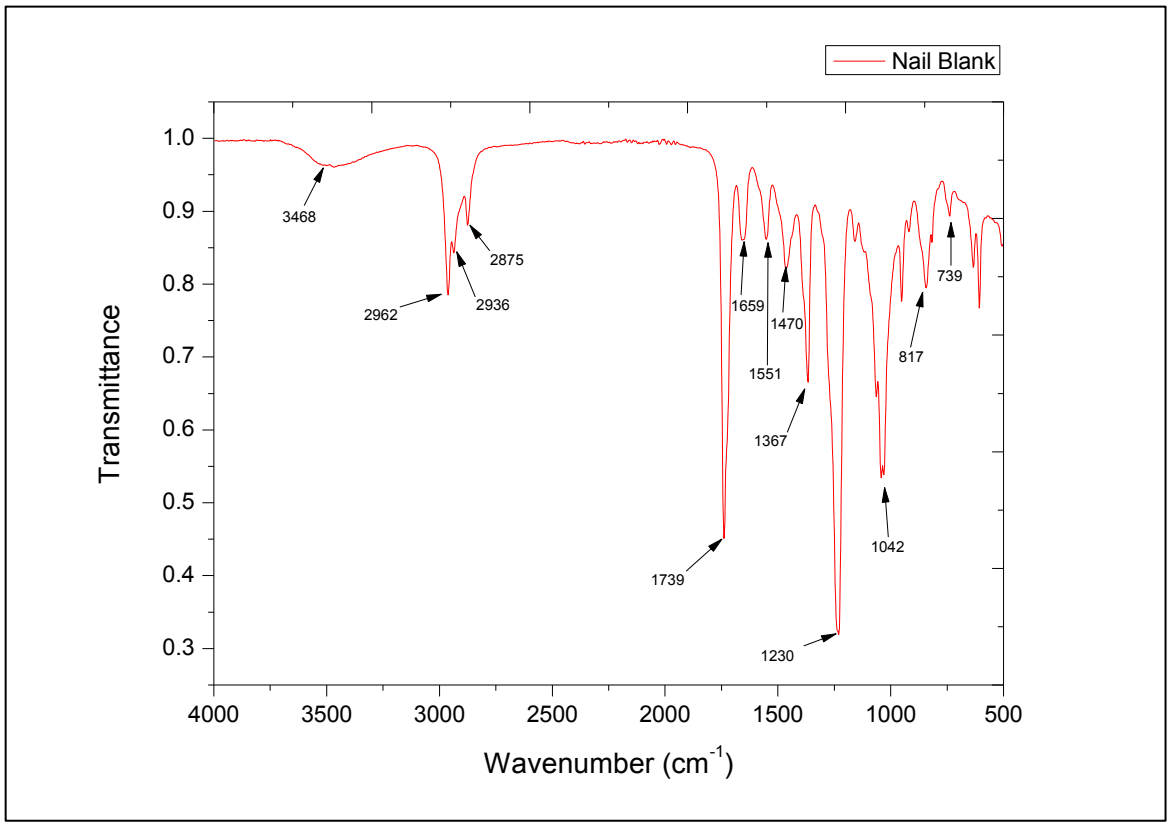



**Figure 6.1 FTIR-ATR spectrum of Blank nail lacquer formulation**

Figure 6.1 depicts the IR spectrum of the Blank nail lacquer formulation. It shows OH and NH bands at ~3468 $cm^{-1}$, CH stretches at ~2962 $cm^{-1}$, ~2936 $cm^{-1}$ and ~2875 $cm^{-1}$, C=O carbonyl ester peak at ~1739 $cm^{-1}$, C=O primary amide peak at

~1659 $cm^{-1}$, NH secondary amide peak at ~1551 $cm^{-1}$, CH methyl band at ~1470 $cm^{-1}$, CH rock vibration at ~1367 $cm^{-1}$, O-C-O ether bands at ~1230 $cm^{-1}$ and ~1042 $cm^{-1}$, amino-substituted triazine ring peak at 817 $cm^{-1}$ and a C-H bending band at 739 $cm^{-1}$. Table 6.1 summarises the main spectral bands of the Blank nail lacquer formulation [119, 131-134].

**Table 6.1 Summary of functional groups of Blank nail lacquer spectrum [119, 131-134]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3468 | OH, NH |
| 2875, 2936, 2962 | CH stretches |
| 1739 | C=O carbonyl ester |
| 1659 | C=O primary amide |
| 1551 | NH secondary amide |
| 1470 | CH methyl band |
| 1367 | CH rock |
| 1230, 1042 | O-C-O ether |
| 817 | amino-substituted triazine ring (2,4,6-triamino-1,3,5-triazine) |
| 739 | C-H bending band |

A spectral band in the region of ~1367 $cm^{-1}$, representative of melamine formaldehyde, can be seen from Figure 6.1 [134]. The latter band is diagnostic of an amino-substituted triazine ring. The band occurring at ~817 $cm^{-1}$ has proved to be the identifying characteristic band of an amino-substituted-triazine ring, showing interaction between the nail formulation film former and another molecule (polymer-plasticiser interaction) [119, 131-134].

Both hydroxyl (OH) and amine (NH) groups exhibit spectral band assignments in the same region, i.e. ~3500 $cm^{-1}$ to 3200 $cm^{-1}$. If the amine group has two hydrogens attached to it two peaks attributable to this primary amine group are visible [133].

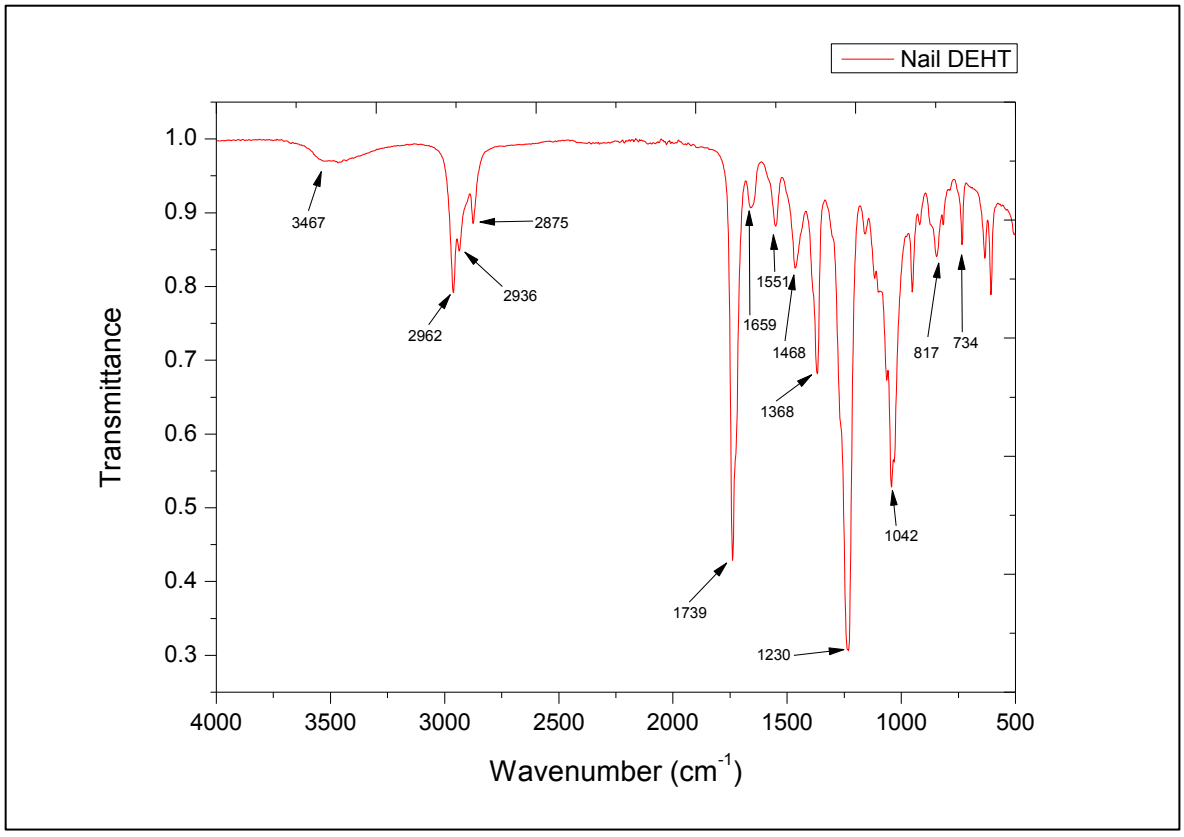



**Figure 6.2 FTIR-ATR spectrum of DEHT plasticised nail lacquer formulation**

Figure 6.2 depicts the IR spectrum of DEHT plasticised nail lacquer formulation. OH and NH bands can be observed at ~3467 $cm^{-1}$, CH stretches at ~2962 $cm^{-1}$, ~2936 $cm^{-1}$ and ~2875 $cm^{-1}$, C=O carbonyl ester peak at ~1739 $cm^{-1}$, C=O primary amide peak at ~1659 $cm^{-1}$, NH secondary amide peak at ~1551 $cm^{-1}$, CH methyl group at ~1468 $cm^{-1}$, CH rock vibration at ~1368 $cm^{-1}$, O-C-O ether bands

at ~1230 $cm^{-1}$ and ~1042 $cm^{-1}$, amino-substituted triazine ring peak at 817 $cm^{-1}$ and a C-H bending band at 734 $cm^{-1}$. Table 6.2 summarises the main spectral bands of the DEHT plasticised nail lacquer formulation [119, 131-134].

**Table 6.2 Summary of functional groups of DEHT plasticised nail lacquer formulation [119, 131-134]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3467 | OH, NH |
| 2875, 2936, 2962 | CH stretches |
| 1739 | C=O carbonyl ester |
| 1659 | C=O primary amide |
| 1551 | NH secondary amide |
| 1468 | CH methyl band |
| 1368 | CH rock |
| 1230, 1042 | O-C-O ether |
| 817 | amino-substituted triazine ring (2,4,6-triamino-1,3,5-triazine) |
| 734 | C-H bending band |

Figure 6.3 shows the Blank nail lacquer formulation and the DEHT plasticised formulation overlapped with each other.

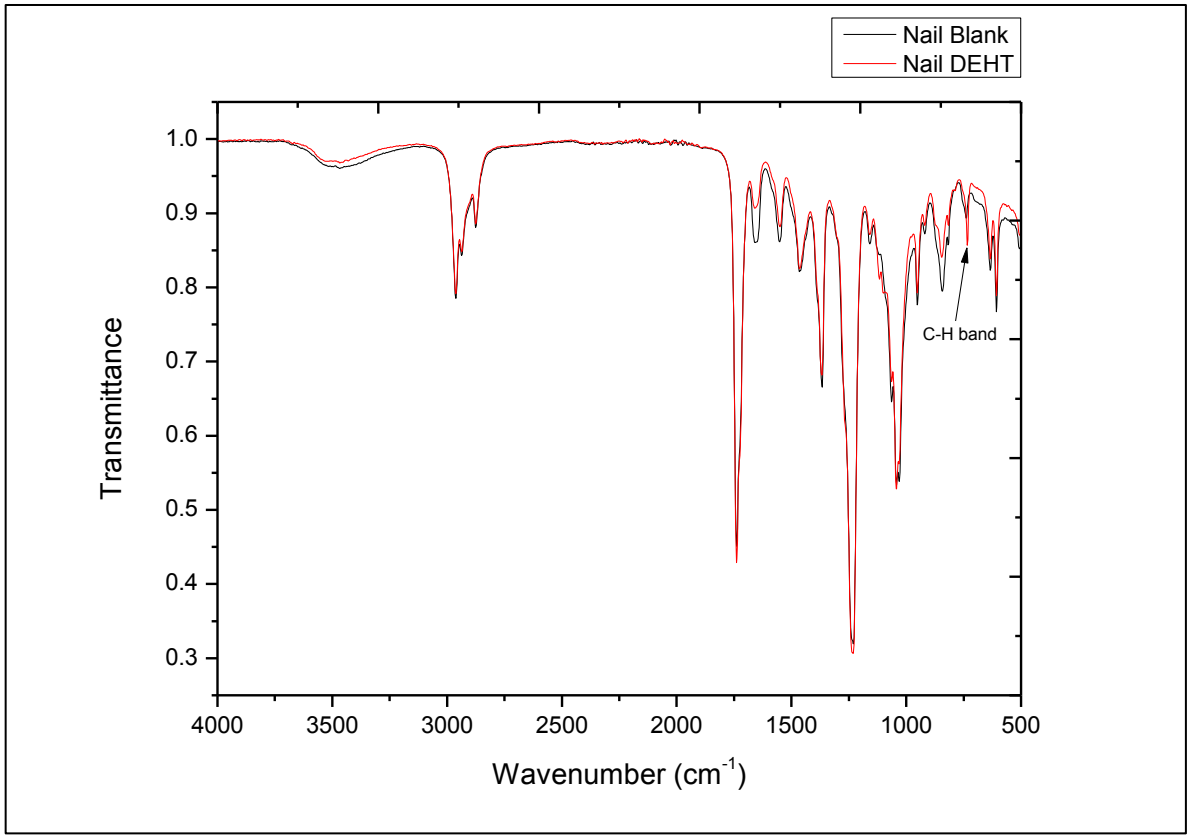



**Figure 6.3 FTIR-ATR spectra overlay of Blank and DEHT plasticised nail lacquer formulations**

The spectra show a decreased primary and secondary amide band, at ~1659 $cm^{-1}$ and ~1551 $cm^{-1}$, respectively, as well as a decreased triazine band at ~817 $cm^{-1}$ for DEHT formulation in comparison to the unplasticised Blank formulation. DEHT plasticised nail lacquer formulation shows a narrower and more intense CH bending band at a lower wavenumber (i.e. at ~734 $cm^{-1}$) versus that of the Blank formulation at ~739 $cm^{-1}$ (see Figure 6.9).

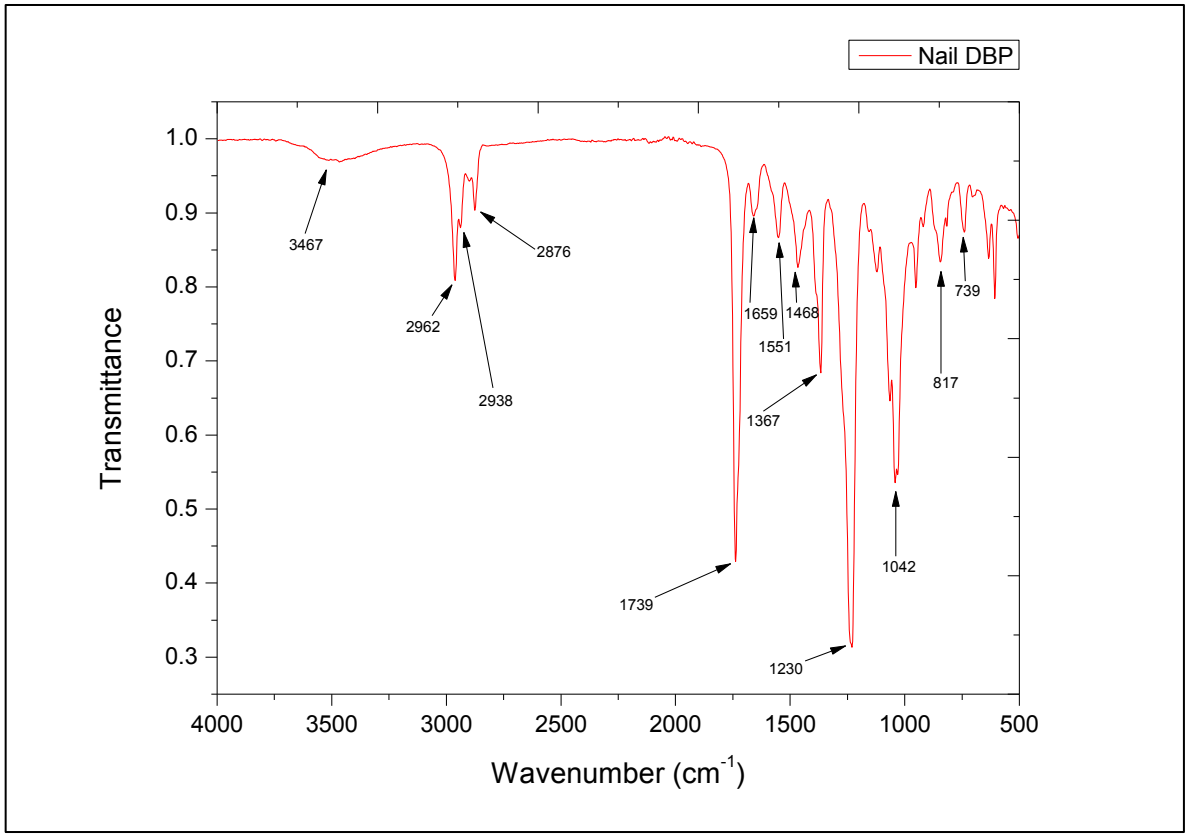



**Figure 6.4 FTIR-ATR spectrum of DBP plasticised nail lacquer formulation**

Figure 6.4 depicts the IR spectrum of DBP plasticised nail lacquer formulation. It shows OH and NH bands ~3467 $cm^{-1}$, CH stretches at ~2962 $cm^{-1}$, ~2938 $cm^{-1}$ and ~2876 $cm^{-1}$, C=O carbonyl ester peak at ~1739 $cm^{-1}$, C=O primary amide peak at ~1659 $cm^{-1}$, NH secondary amide peak at ~1551 $cm^{-1}$, CH methyl band at ~1468 $cm^{-1}$, CH rock band at ~1367 $cm^{-1}$, O-C-O ether bands at ~1230 $cm^{-1}$ and ~1042 $cm^{-1}$, amino-substituted triazine ring band at 817 $cm^{-1}$ and a C-H bending band at 739 $cm^{-1}$. Table 6.3 summarises the main spectral bands of the DBP nail lacquer formulation [119, 131-134].

**Table 6.3 Summary of functional groups of DBP nail lacquer [119, 131-134]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3467 | OH, NH |
| 2876, 2938, 2962 | CH stretches |
| 1739 | C=O carbonyl ester |
| 1659 | C=O primary amide |
| 1551 | NH secondary amide |
| 1468 | CH methyl band |
| 1367 | CH rock |
| 1230, 1042 | O-C-O ether |
| 817 | amino-substituted triazine ring (2,4,6-triamino-1,3,5-triazine) |
| 739 | C-H bending band |

The FTIR spectrum obtained with the DBP plasticised nail lacquer formulation shows that, converse to the DEHT nail lacquer formulation, the CH bending band remains at more or less the same wavenumber as the band obtained with the unplasticised Blank formulation, i.e. at ~740 $cm^{-1}$. However, the DBP nail lacquer spectrum displays an increase in band intensity at this wavenumber in comparison to the Blank nail lacquer.

Figure 6.5 shows the overlayed spectra of the Blank nail lacquer formulation and the DBP plasticised nail lacquer formulation.

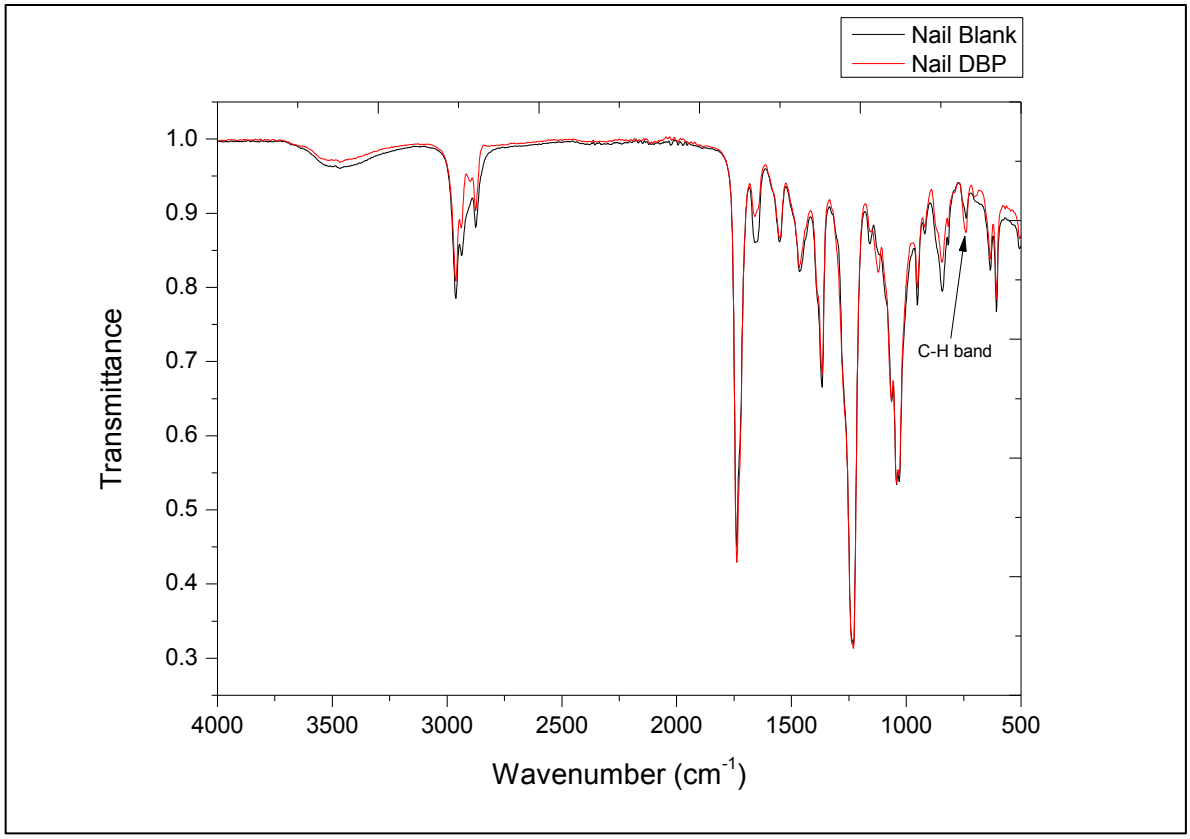



**Figure 6.5 FTIR-ATR spectra overlays of Blank nail lacquer and DBP plasticised nail lacquer formulations**

There is a decreased primary amide band at ~1659 $cm^{-1}$ and a decreased triazine band at ~817 $cm^{-1}$ for DBP nail lacquer formulation in comparison to the unplasticised Blank nail lacquer formulation. A perfect secondary amide band overlay is observed at ~1551 $cm^{-1}$ for the DBP formulation and the Blank formulation. The DBP nail lacquer spectrum displays an increase in the CH bending band at ~739 $cm^{-1}$ compared to the Blank nail lacquer formulation (refer to Figure 6. 9).

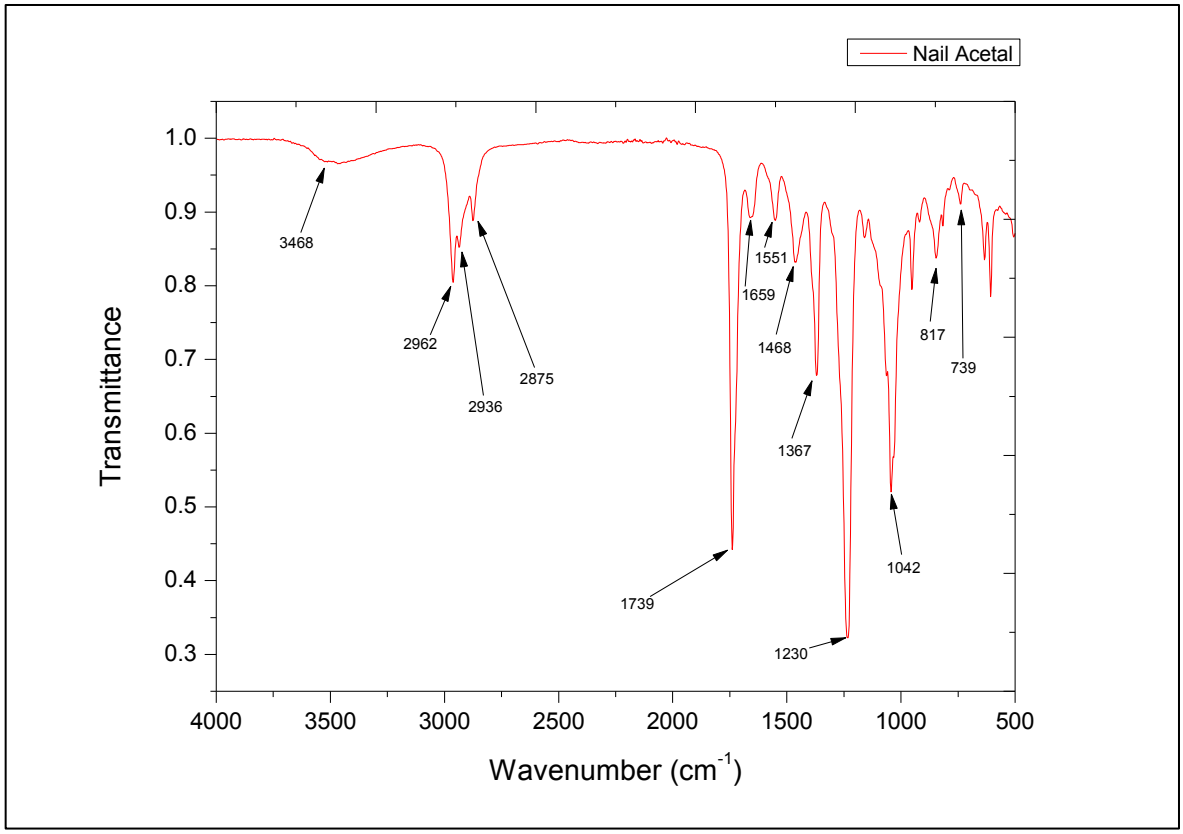



**Figure 6.6 FTIR-ATR spectrum of Acetal plasticised nail lacquer formulation**

Figure 6.6 depicts the IR spectrum of Acetal nail lacquer formulation. It shows OH and NH bands ~3468 $cm^{-1}$, CH stretches at ~2962 $cm^{-1}$, ~2936 $cm^{-1}$ and ~2875 $cm^{-1}$, C=O carbonyl ester peak at ~1739 $cm^{-1}$, C=O primary amide peak at ~1659 $cm^{-1}$, NH secondary amide peak at ~1551 $cm^{-1}$, CH methyl band at ~1468 $cm^{-1}$, CH rock at ~1367 $cm^{-1}$, O-C-O ether bands at ~1230 $cm^{-1}$ and ~1042 $cm^{-1}$, amino-substituted triazine ring peak at ~817 $cm^{-1}$ and a C-H bending band at ~739 $cm^{-1}$. Table 6.4 summarises the main spectral bands of the DBP plasticised nail formulation [119, 131-134].

**Table 6.4 Summary of functional groups of Acetal plasticised nail lacquer [119, 131-134]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3468 | OH, NH |
| 2875, 2936, 2962 | CH stretches |
| 1739 | C=O carbonyl ester |
| 1659 | C=O primary amide |
| 1551 | NH secondary amide |
| 1468 | CH methyl band |
| 1367 | CH rock band |
| 1230, 1042 | O-C-O ether |
| 817 | amino-substituted triazine ring (2,4,6-triamino-1,3,5-triazine) |
| 739 | C-H bending band |

Figure 6.7 shows the overlayed spectra of the Blank nail formulation and the Acetal plasticised nail formulation.

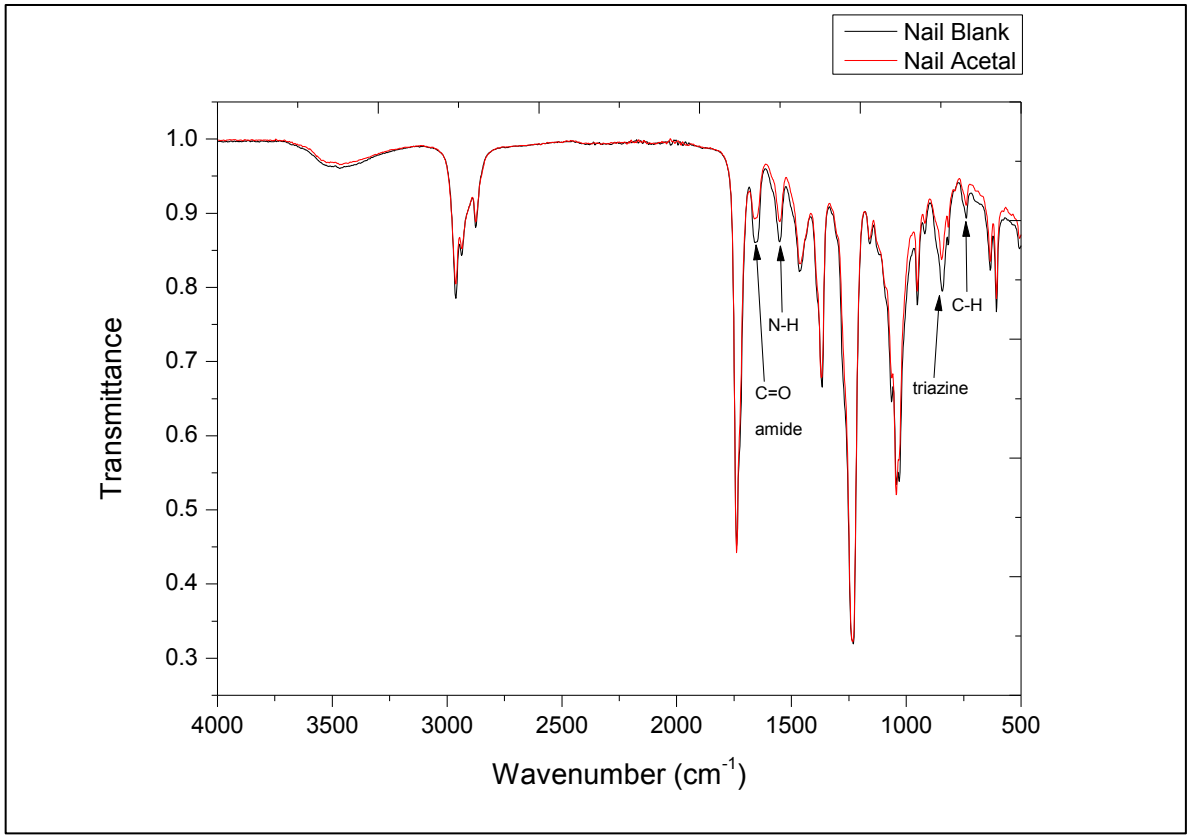



**Figure 6.7 FTIR-ATR spectra overlay of Blank nail lacquer formulation and Acetal plasticised nail formulations**

There is a decreased primary and secondary amide band, at ~1659 $cm^{-1}$ and ~1551 $cm^{-1}$, respectively, as well as a decreased triazine band at ~817 $cm^{-1}$ for Acetal nail lacquer formulation in comparison to the unplasticised Blank nail lacquer formulation. Acetal plasticised nail formulation shows a less intense CH bending band at the same wavenumber as that of the Blank nail lacquer (at ~739 $cm^{-1}$).

Figure 6.8 shows the differences in primary amide bands at ~1659 $cm^{-1}$ for the various formulations while Figure 6.9 shows the difference in CH bend for Blank nail lacquer, Acetal, DBP and DEHT nail lacquer formulations.

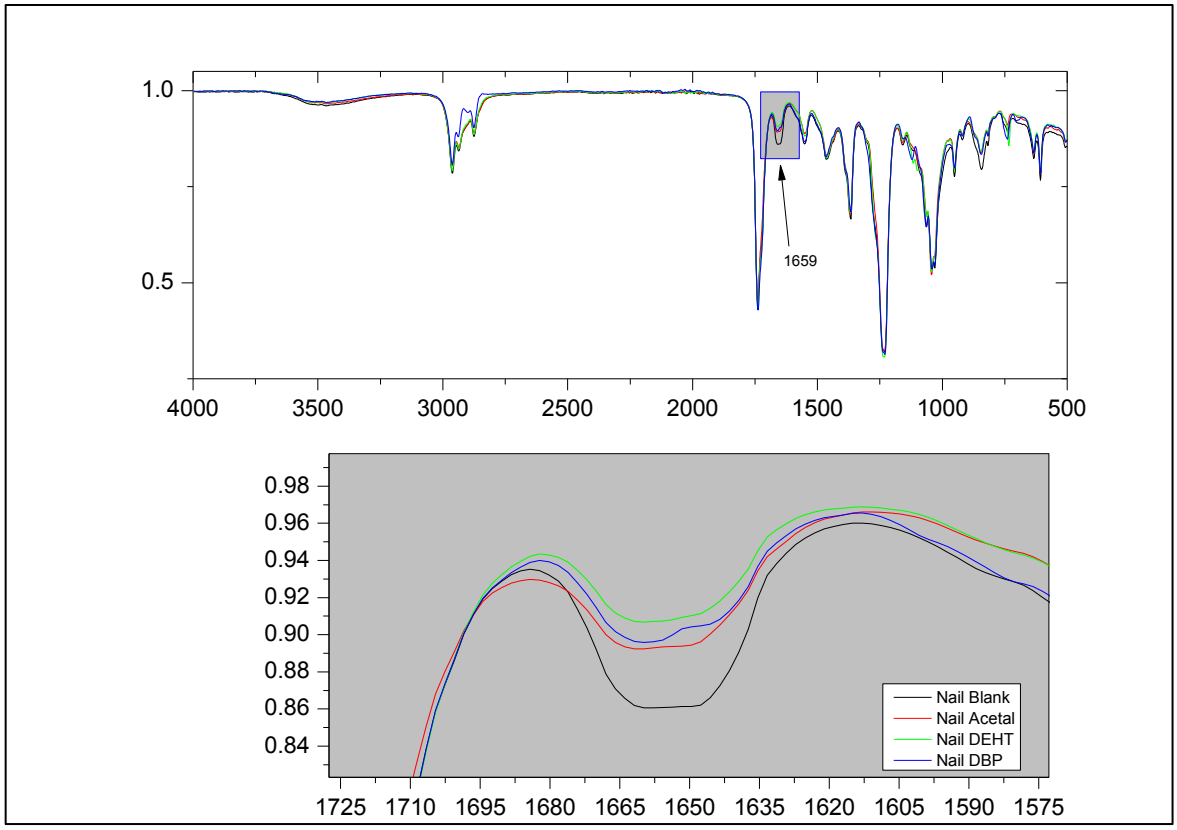



**Figure 6.8 FTIR-ATR spectra of primary amide band assignment at 1659 $cm^{-1}$ for Blank nail lacquer, Acetal, DBP and DEHT nail lacquer formulations (zoomed in)**

As can be seen from Figure 6.8, a decrease in the primary amide band at ~1659 $cm^{-1}$ is observed for all nail lacquer formulations relative to the Blank containing no plasticiser. This could be indicative of the loss of a proton from the primary amide of the cross-linked Blank film formers into the polymer matrix [117].

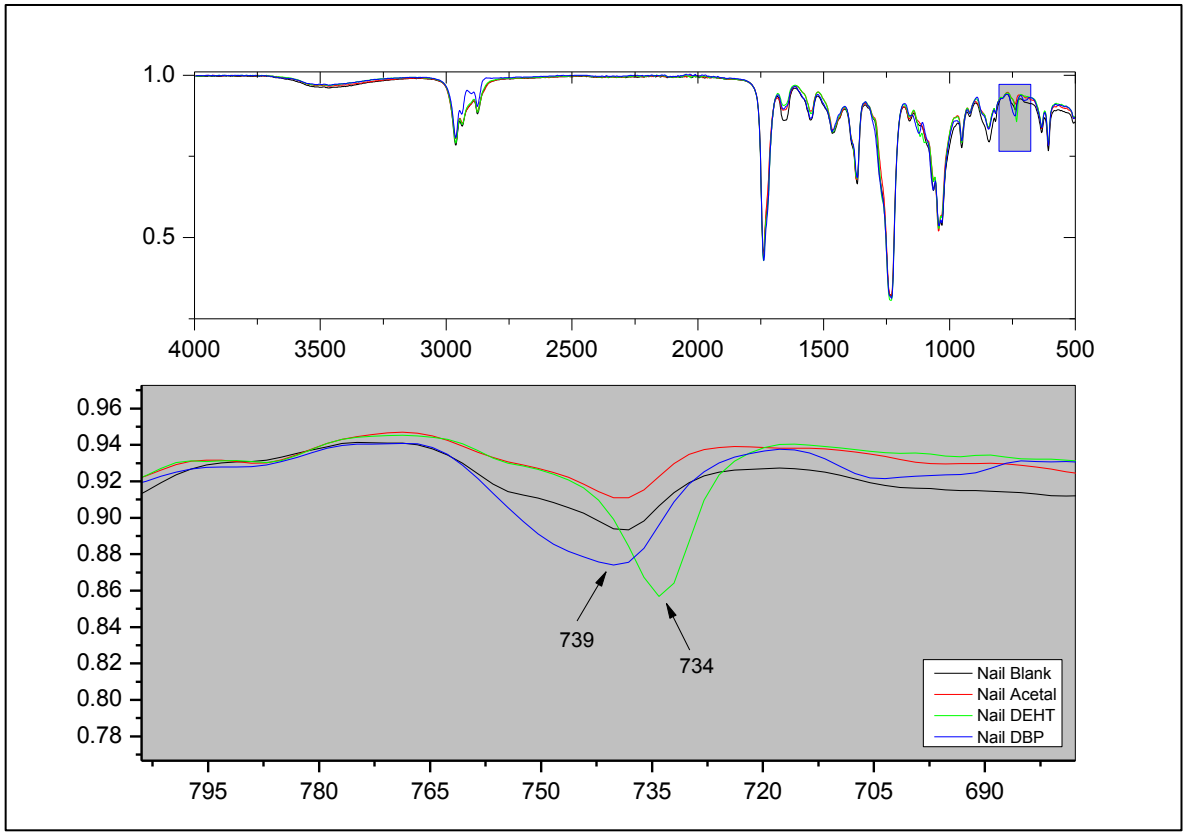



**Figure 6.9 FTIR-ATR spectra of shifting CH bending band for Blank nail lacquer, Acetal, DBP and DEHT nail lacquer formulations (zoomed in)**

It is noted from Figure 6.9 that a broader band is observed for DBP nail lacquer formulation with respect to the other band assignments at wavenumber ~740 $cm^{-1}$, rendering proof of the addition of DBP to the formulation, as it is the most polar plasticiser of the three studied. The zoomed in area displays this broader area obtained for Nail DBP clearly in Figure 6.9. It is also evident from Figure 6.9 that DEHT nail formulation reveals the narrowest band of the nail formulations and is shifted to a lower wavenumber. The narrow and more intense band observed is due to the DEHT plasticiser molecule exhibiting the longest carbon tail emanating from the diester group attached to the aromatic benzene ring. The peak for the Acetal formulation, similar in shape to that of the Blank nail lacquer formulation, displays a slightly smaller CH bending band at ~740 $cm^{-1}$. It is postulated that

interaction of the Acetal molecule is hindered by its 1,3-dioxane ring, associated with the hydrocarbon chain attached to it.

Figures 6.10 to 6.13 depict the FTIR-ATR spectra of the Blank, DEHT, DBP and Acetal nail lacquer formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods. The spectra obtained at all the monthly intervals for each nail lacquer formulation are shown in Appendix B: Figures 36 to 55.

It is evident from Figures 6.10 to 6.13 that neither RT nor elevated temperature has an influence on the size or intensity of any spectral bands. No shift in band intensity is observed and therefore all formulations remain chemically stable over the three month incubation period.

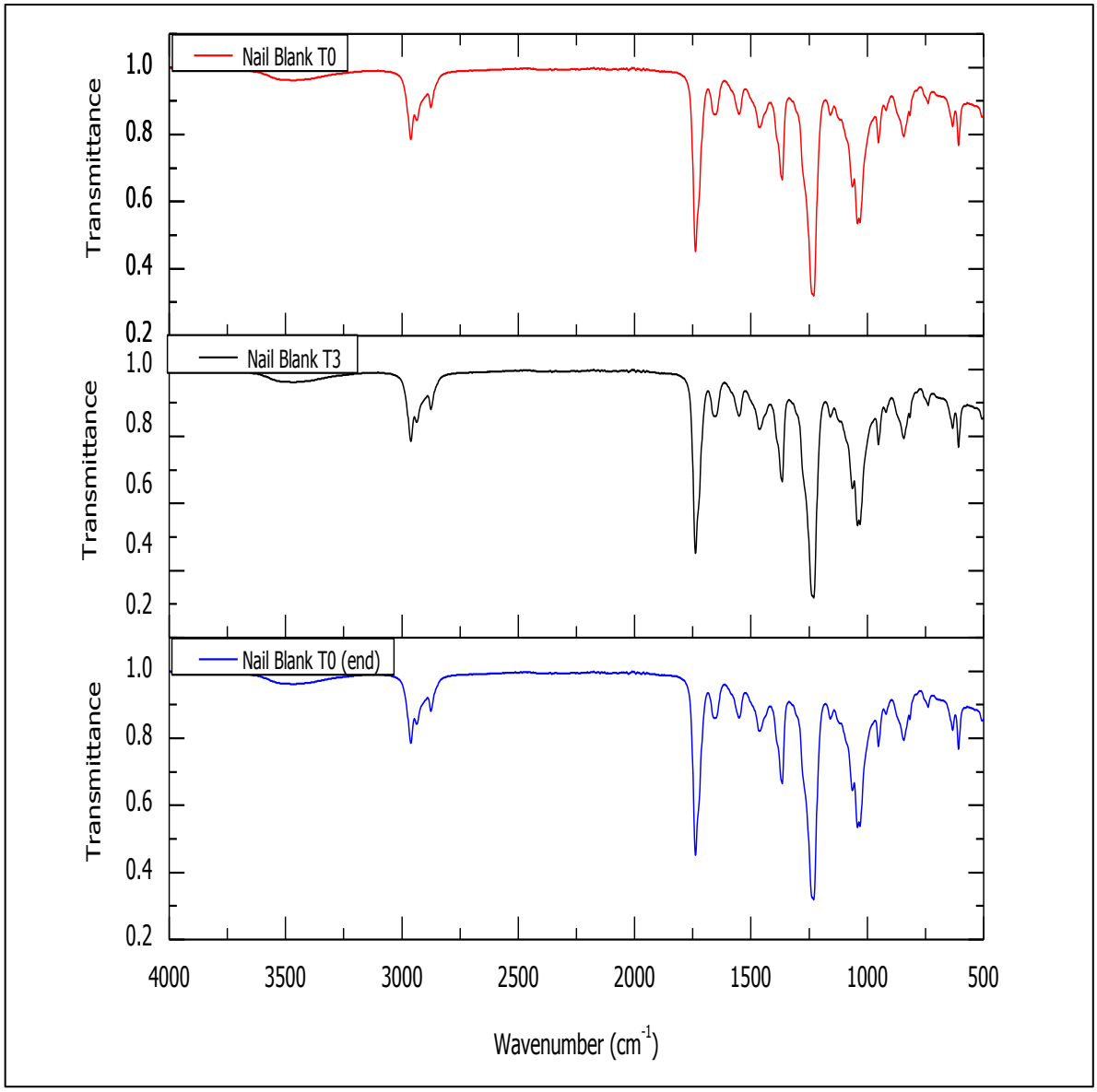



**Figure 6.10 FTIR-ATR spectra of Blank nail lacquer formulation at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

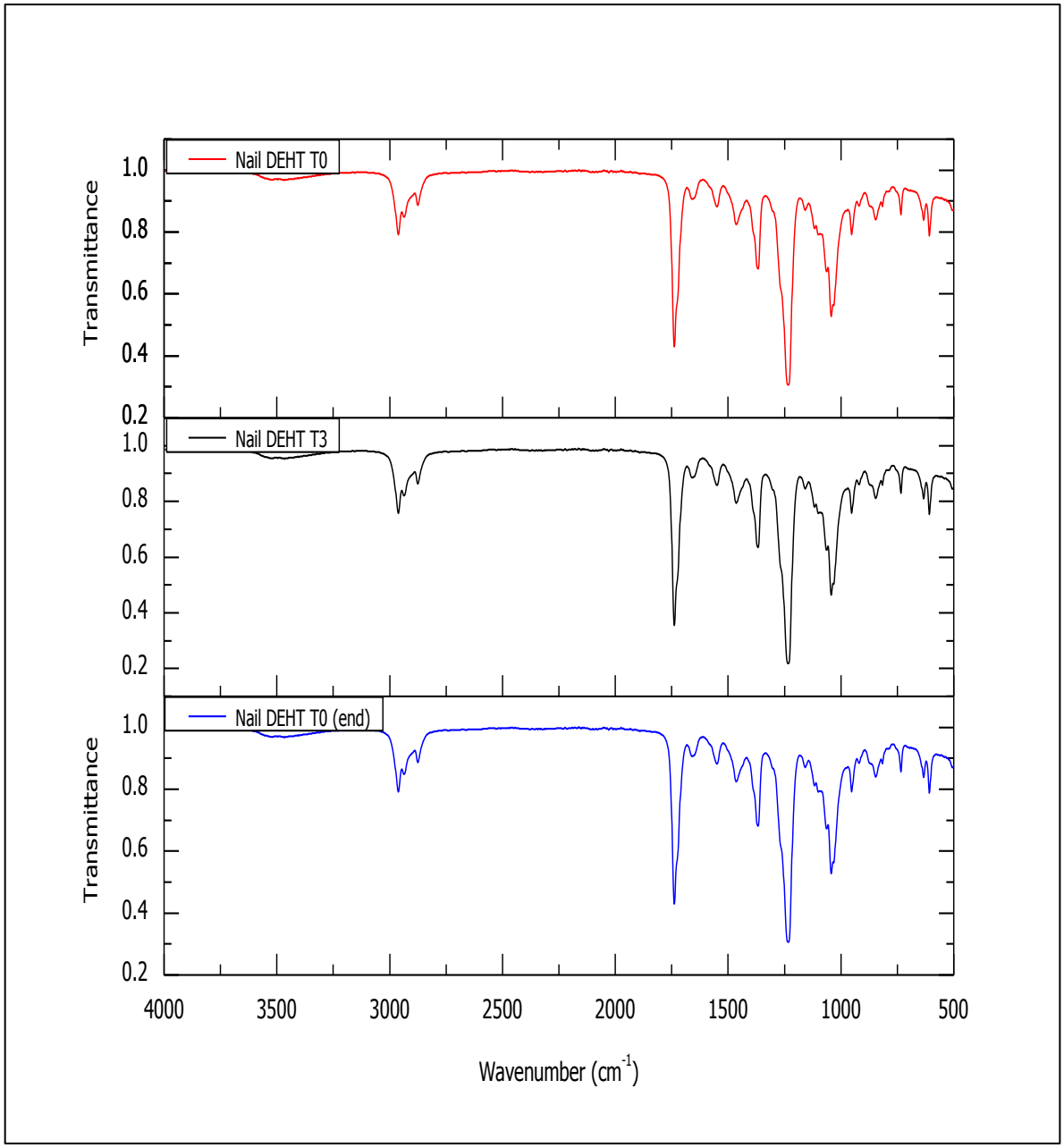



**Figure 6.11 FTIR-ATR spectra of DEHT plasticised nail lacquer formulation at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

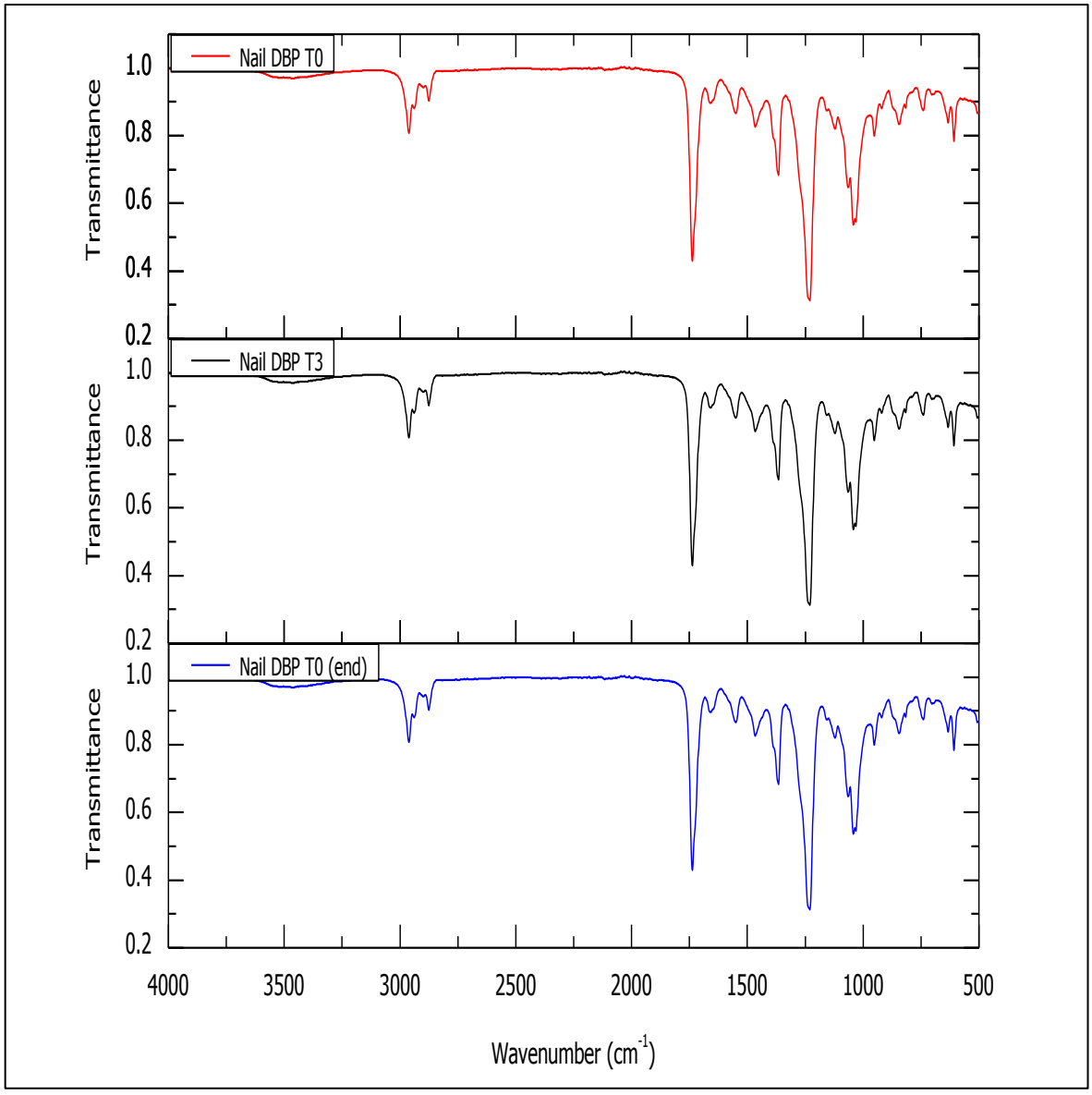



**Figure 6.12 FTIR-ATR spectra of DBP plasticised nail lacquer formulation at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

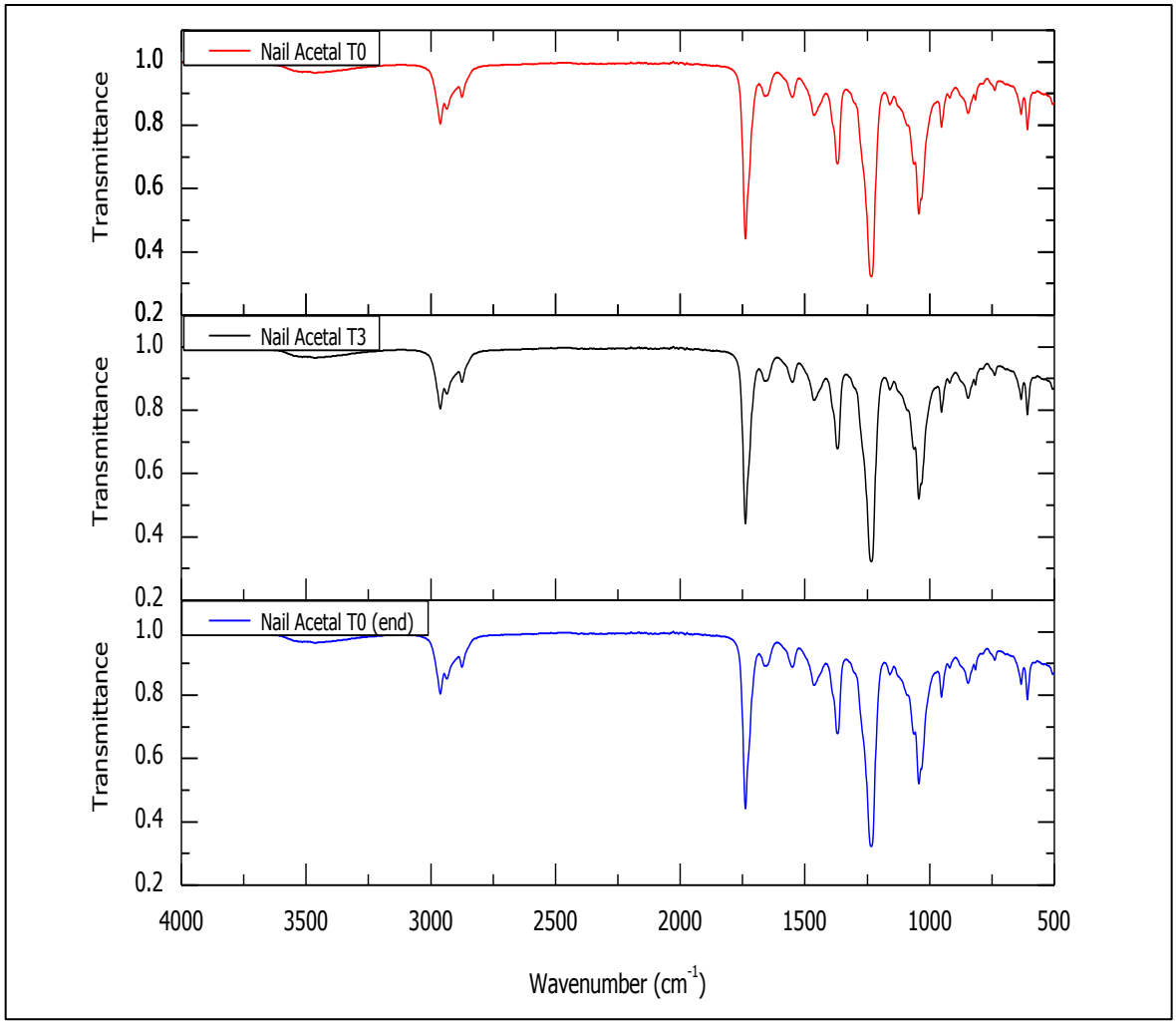



**Figure 6.13 FTIR-ATR spectra of Nail Acetal formulation at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

6.3.2 Lip coat formulations

FTIR spectra of the Blank, DBP, DEHT and PMD-citronellal acetal plasticised lip coat formulations are shown in Figures 6.14 to 6.20 with the band assignments summarised in Table 6.5 to 6.8.

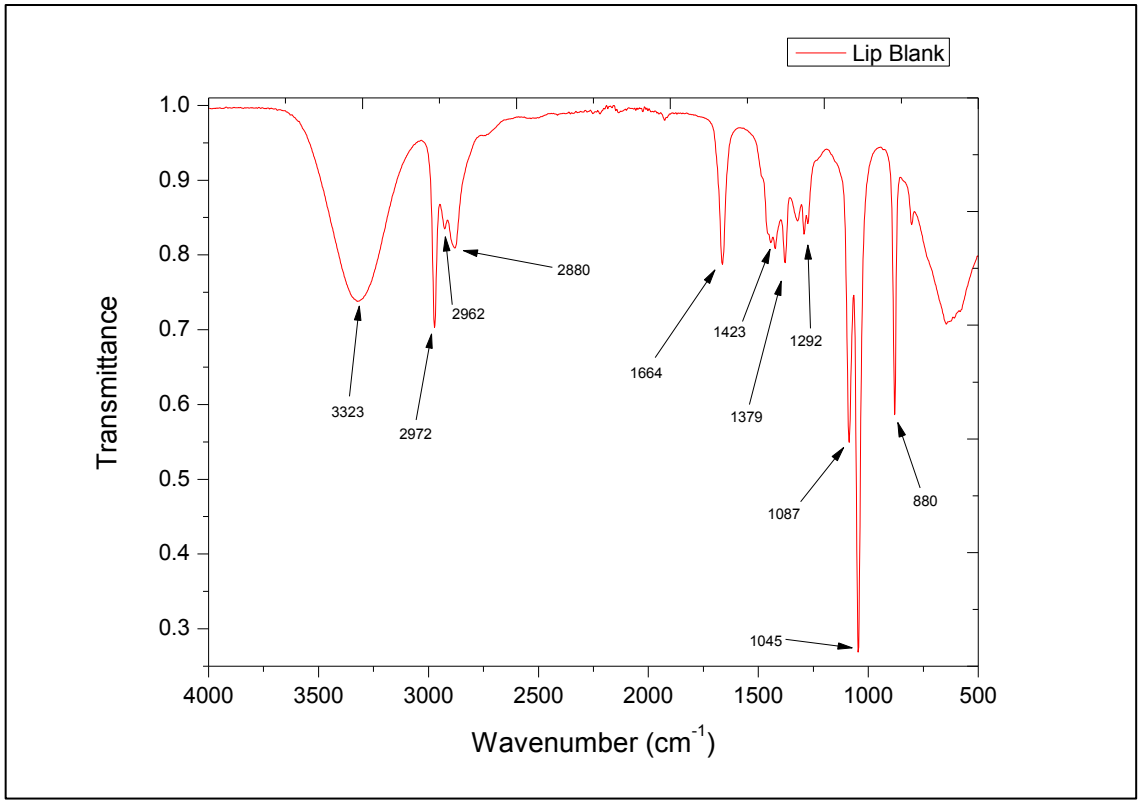



**Figure 6.14 FTIR-ATR spectrum of the Blank lip coat formulation**

Figure 6.14 depicts the IR spectrum of the Blank lip coat formulation. It illustrates an OH band at ~3323 $cm^{-1}$, CH stretches at ~2972 $cm^{-1}$, ~2926 $cm^{-1}$ and ~2880 $cm^{-1}$, C=O carbonyl amide peak at ~1664 $cm^{-1}$, $CH_2$ bending vibrations at ~1423 $cm^{-1}$, CH rock band at ~1379 $cm^{-1}$, C-N vibration at ~1292 $cm^{-1}$, C-O ether groups at ~1087 $cm^{-1}$ and ~1045 $cm^{-1}$ and C-C bending at ~880 $cm^{-1}$. Table 6.5 summarises the main spectral bands of the Blank lip coat formulation [121, 135].

**Table 6.5 Summary of functional groups of the Blank lip coat [121, 135]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3323 | OH stretch |
| 2972, 2926, 2880 | CH stretches |
| 1664 | C=O carbonyl amide |
| 1423 | $CH_2$ bending vibration |
| 1379 | CH rock band |
| 1292 | C-N vibration |
| 1087, 1045 | C-O ether groups |
| 880 | C-C bending |

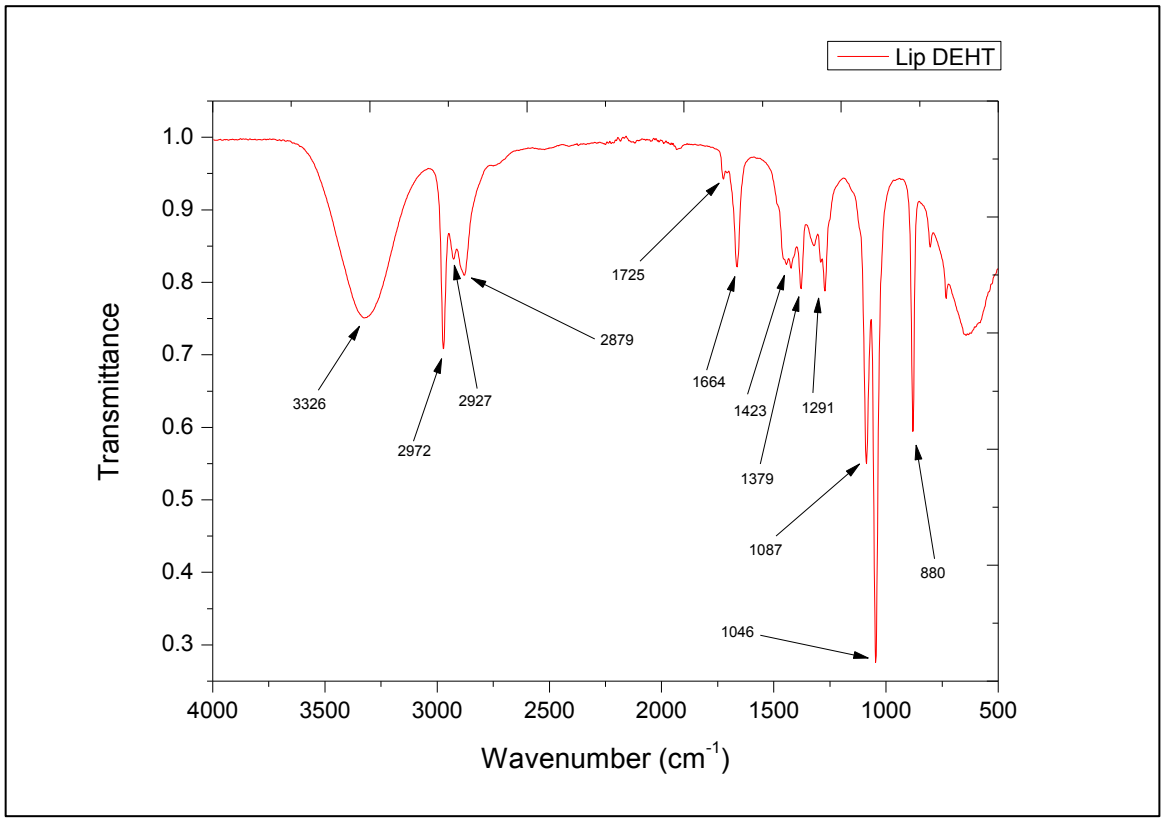



**Figure 6.15 FTIR-ATR spectrum of DEHT plasticised lip coat formulation**

Figure 6.15 depicts the IR spectrum of the DEHT plasticised lip coat formulation. It shows an OH stretch band at ~3326 $cm^{-1}$, CH stretches at ~2972 $cm^{-1}$, ~2927 $cm^{-1}$ and ~2879 $cm^{-1}$, C=O ester carbonyl peak at ~1725 $cm^{-1}$, C=O carbonyl amide peak at ~1664 $cm^{-1}$, $CH_2$ bending vibration at ~1423 $cm^{-1}$, CH rock band at ~1379 $cm^{-1}$, C-N vibration at ~1292 $cm^{-1}$, C-O ether groups at ~1087 $cm^{-1}$ and ~1046 $cm^{-1}$ and C-C bending at ~880 $cm^{-1}$. Table 6.6 summarises the main spectral bands of the DEHT plasticised lip coat formulation [121, 122, 135].

**Table 6.6 Summary of functional groups of DEHT plasticised lip coat [121, 122, 135]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3326 | OH stretch |
| 2972, 2927, 2879 | CH stretches |
| 1725 | C=O carbonyl ester |
| 1664 | C=O carbonyl amide |
| 1423 | $CH_2$ bending vibration |
| 1379 | CH rock band |
| 1291 | C-N stretch |
| 1087, 1046 | C-O ether groups |
| 880 | C-C bending |

Figure 6.16 shows an overlay of the FTIR spectra of the Blank lip coat and DEHT lip coat formulations for comparison.

Figure 6.16 shows the appearance of a 'shoulder' (at ~1725 $cm^{-1}$) on the left side of the carbonyl amide band (at ~1664 $cm^{-1}$) of the unplasticised Blank lip coat formulation, showing some interaction with the ester plasticiser, DEHT. The addition of 5% DEHT therefore resulted in the ester carbonyl band of neat DEHT (see Figure 5.3) to move from a lower to higher wavenumber (~1718 $cm^{-1}$ to

~1725 $cm^{-1}$). The unplasticised Blank lip coat (Figure 6.14) lacks this shoulder due to the absence of plasticiser.

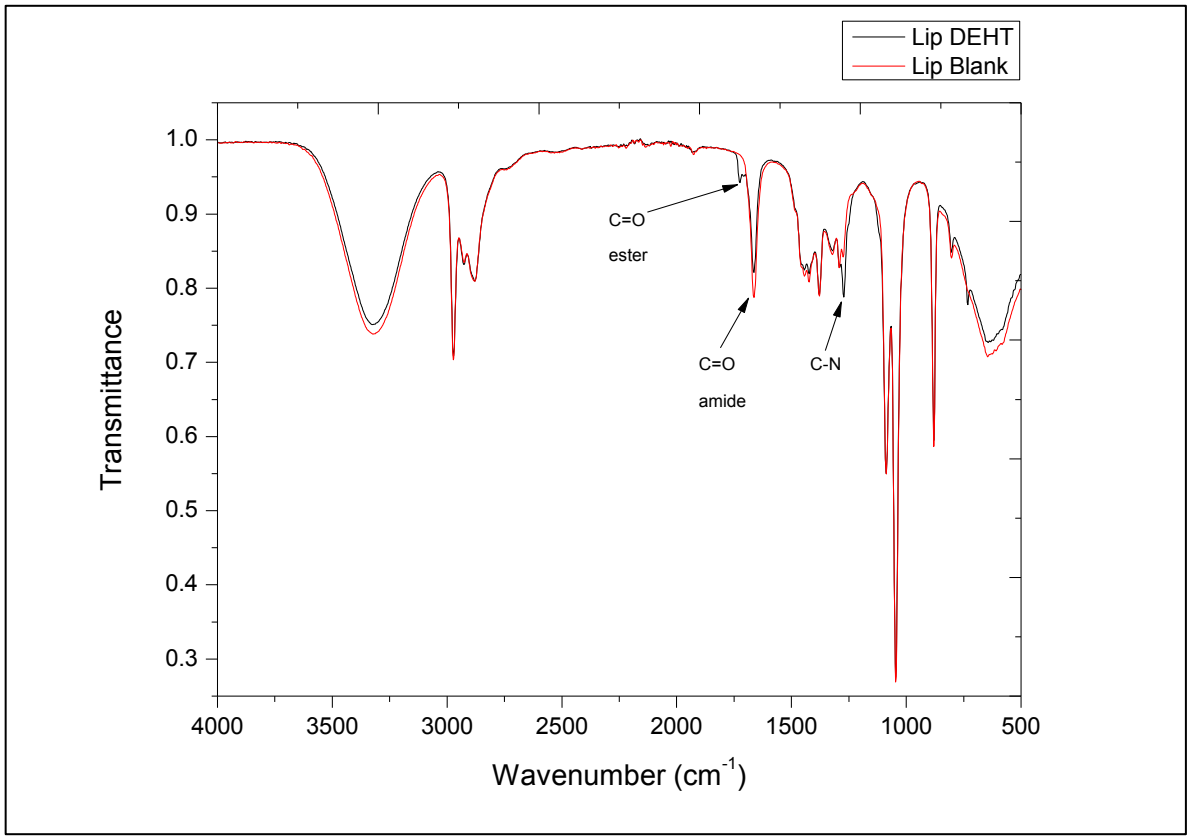



**Figure 6.16 FTIR-ATR spectra overlay of Blank lip coat and DEHT lip coat formulations**

From Figure 6.16 an increase in the C-N band intensity is observed at ~1291 $cm^{-1}$ [135] for the DEHT lip coat formulation typical of an amide carbonyl interaction. A similar observation was made by Sivaiah *et al.* [136] upon interaction of an ester carbonyl plasticiser with the nitrogen of the PVP molecule, probably causing vibrational intensity to increase. Hence, the intensity of the spectral band at ~1291 $cm^{-1}$ increased in comparison to the Blank lip coat (Figure 6.16).

The carbonyl (C=O) amide group of the Blank lip coat appears at ~1664 $cm^{-1}$ which is in agreement with literature that states that the carbonyl (C=O) group of an amide absorbs at a lower wavenumber (~1640 $cm^{-1}$ to ~1680 $cm^{-1}$) than an ester (C=O) carbonyl group (~1720 $cm^{-1}$) [137].

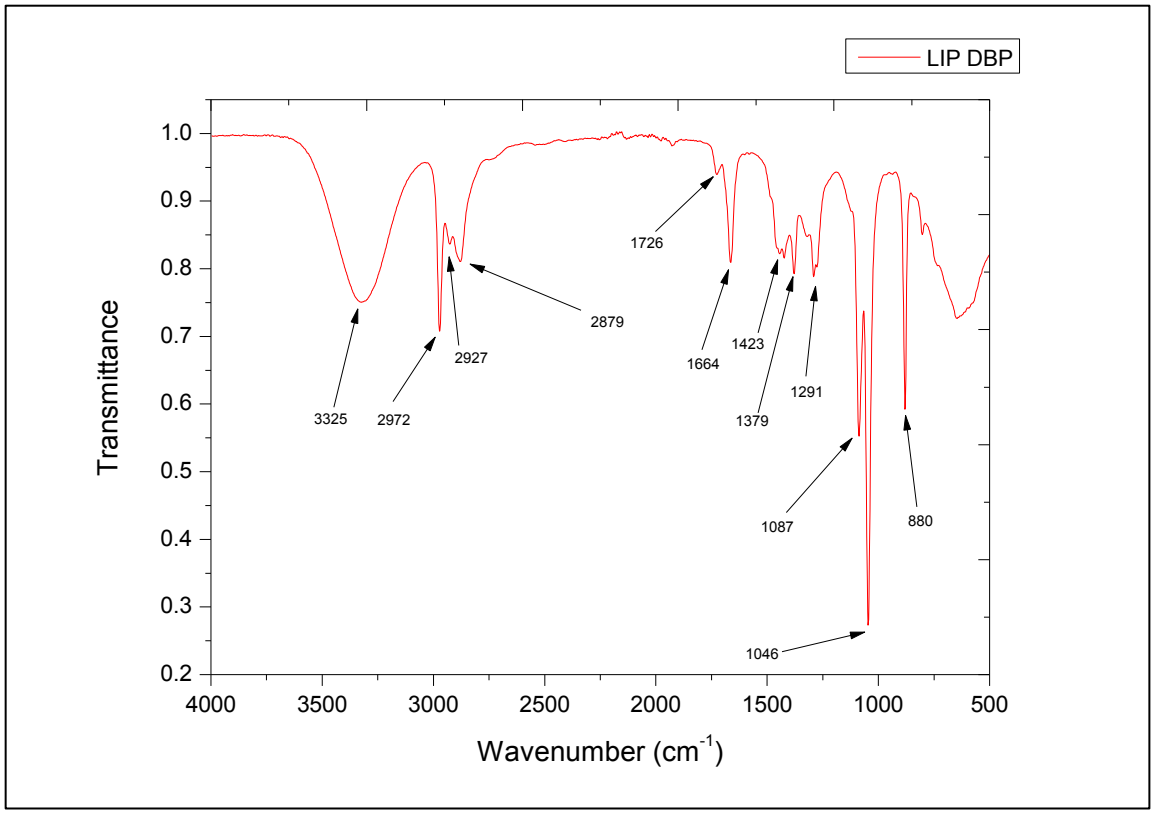



**Figure 6.17 FTIR-ATR spectrum of DBP lip coat formulation**

Figure 6.17 illustrates the IR spectrum of the DBP lip coat formulation. It shows an OH stretch band at ~3325 $cm^{-1}$, CH stretches at ~2972 $cm^{-1}$, ~2927 $cm^{-1}$ and ~2879 $cm^{-1}$, C=O ester carbonyl peak at ~1726 $cm^{-1}$, C=O carbonyl amide peak at ~1664 $cm^{-1}$, $CH_2$ bending vibrations at ~1423 $cm^{-1}$, CH rock peak at ~1379 $cm^{-1}$, C-N vibration at ~1291 $cm^{-1}$, C-O ether groups at ~1087 $cm^{-1}$ and ~1046 $cm^{-1}$ and C-C bending at ~880 $cm^{-1}$. Table 6.7 summarises the main spectral bands of the DBP lip coat formulation [121, 122, 135].

**Table 6.7 Summary of functional groups of DBP lip coat [121, 122, 135]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3325 | OH stretch |
| 2972, 2927, 2879 | CH stretches |
| 1726 | C=O carbonyl ester |
| 1664 | C=O carbonyl amide |
| 1423 | $CH_2$ bending vibration |
| 1379 | CH rock band |
| 1291 | C-N stretch |
| 1087, 1046 | C-O ether groups |
| 880 | C-C bending |

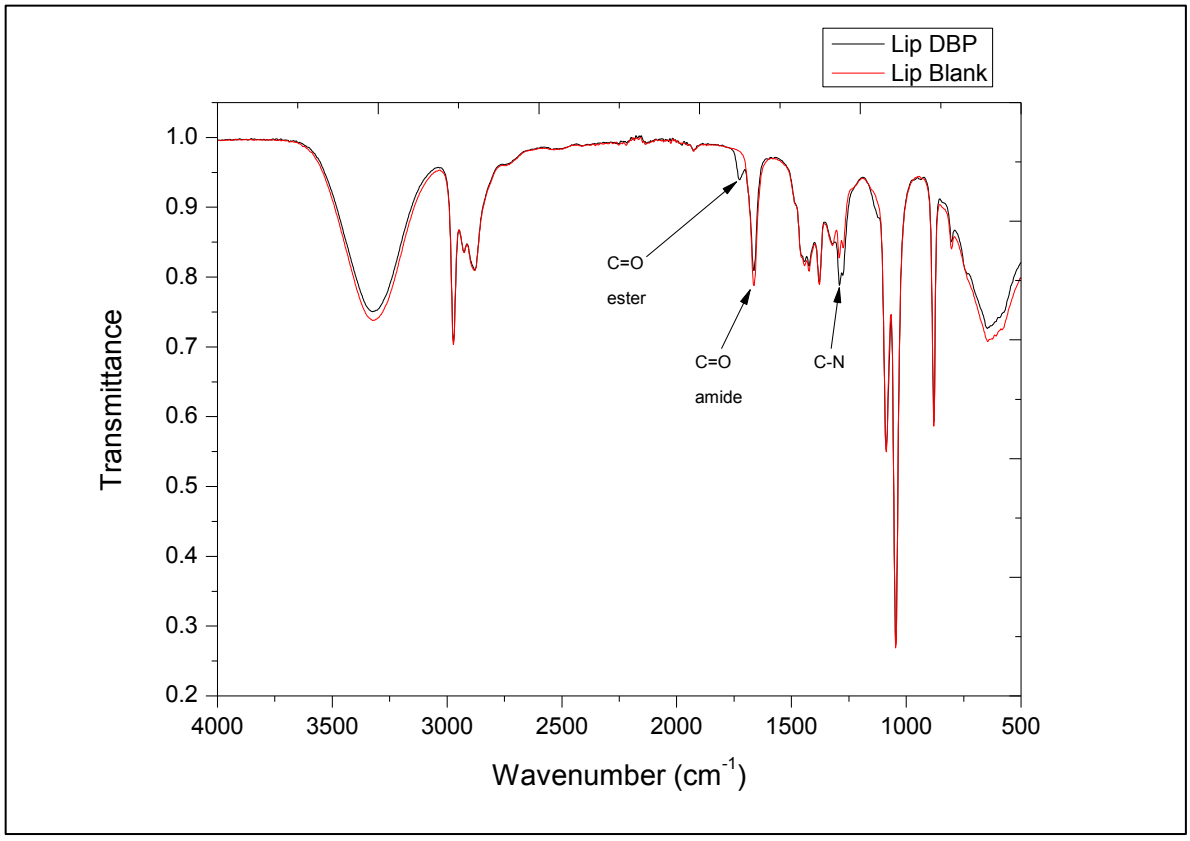



**Figure 6.18 FTIR-ATR spectra overlay of Blank lip coat and DBP lip coat formulations**

Figure 6.18 shows an overlay of the Blank lip coat and the DBP plasticised lip coat formulations for comparison.

Figure 6.18 displays the appearance of a 'shoulder' (~1726 $cm^{-1}$) on the left side of the carbonyl amide band at ~1664 $cm^{-1}$ of the unplasticised Blank lip coat formulation, showing some interaction with the ester plasticiser, DBP. Hence, the addition of 5% DBP (as per formulation, see Chapter 3) resulted in the ester carbonyl band of neat DBP (see Figure 5.2) shifting from ~1720 $cm^{-1}$ to ~1726 $cm^{-1}$, i.e. from a lower to a higher wavenumber.

From Figure 6.18 it is observed that the increase in band intensity for the C-N band at ~1291 $cm^{-1}$ for DBP lip coat is more intense than that of the Blank lip coat [135]. This is due to amide carbonyl interaction, where the lone pairs of electrons on the carbonyl ester plasticiser, DBP, are donated to the nitrogen of the PVP molecule [136]. Therefore it is postulated that these donated electrons to the nitrogen of the PVP molecule caused the vibrational intensity for the C-N band (at ~1291 $cm^{-1}$) to increase for the DBP lip coat formulation (Figure 6.18).

Figure 6.19 displays the IR spectrum of the Acetal plasticised lip coat formulation. It shows an OH stretch band at ~3322 $cm^{-1}$, CH stretches at ~2972 $cm^{-1}$, ~2926 $cm^{-1}$ and ~2879 $cm^{-1}$, C=O carbonyl amide peak at ~1664 $cm^{-1}$, $CH_2$ bending vibrations at ~1423 $cm^{-1}$, CH rock band at ~1379 $cm^{-1}$, C-N vibration at ~1292 $cm^{-1}$, C-O ether groups at ~1087 $cm^{-1}$ and ~1046 $cm^{-1}$ and a C-C bending band at ~880 $cm^{-1}$. Table 6.8 summarises the main spectral bands of the Acetal lip coat formulation [121, 135].

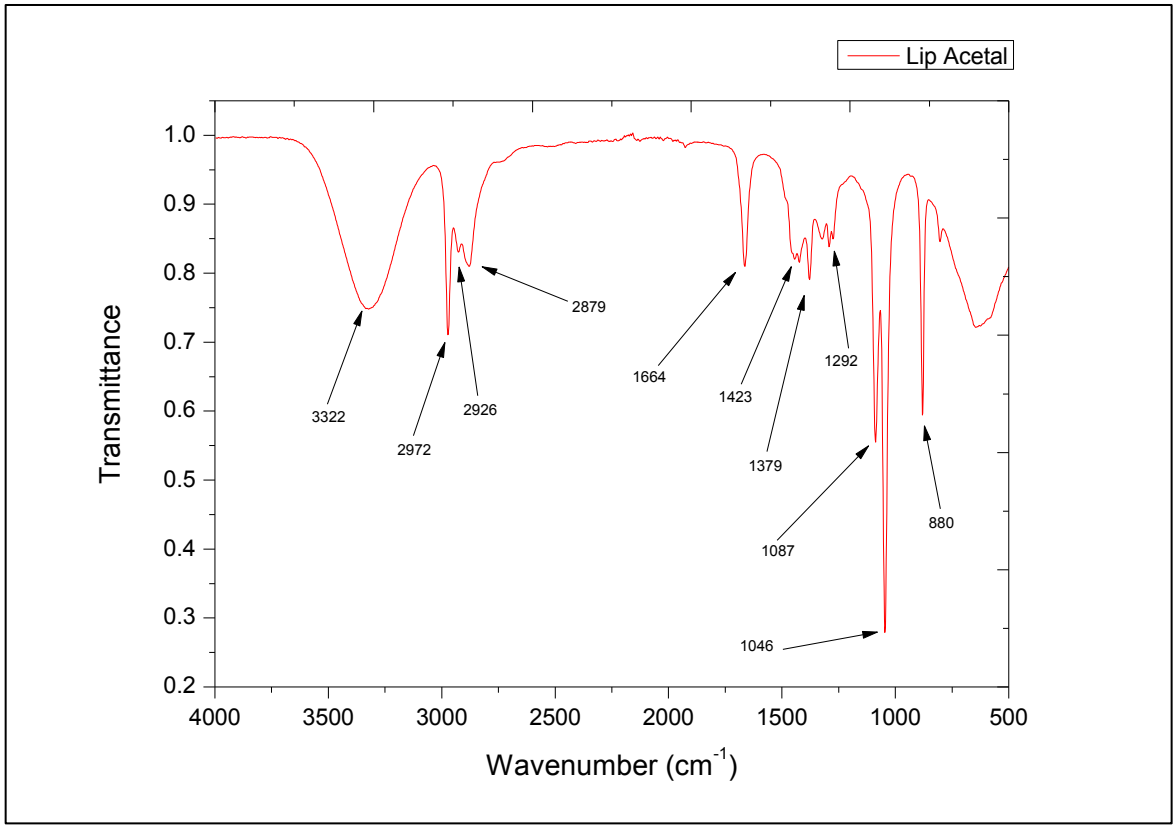



**Figure 6.19 FTIR-ATR spectrum of Acetal plasticised lip coat formulation**

**Table 6.8 Summary of functional groups of Acetal lip coat [121, 135]**

| Wavenumber ($cm^{-1}$) | Functional group |
|---|---|
| 3322 | OH stretch |
| 2972, 2926, 2879 | CH stretches |
| 1664 | C=O carbonyl amide |
| 1423 | $CH_2$ bending vibration |
| 1379 | CH rock band |
| 1292 | C-N stretch |
| 1087, 1046 | C-O ether groups |
| 880 | C-C bending band |

Figure 6.20 shows an overlay of the Blank lip coat and the Acetal lip coat formulations for comparison.

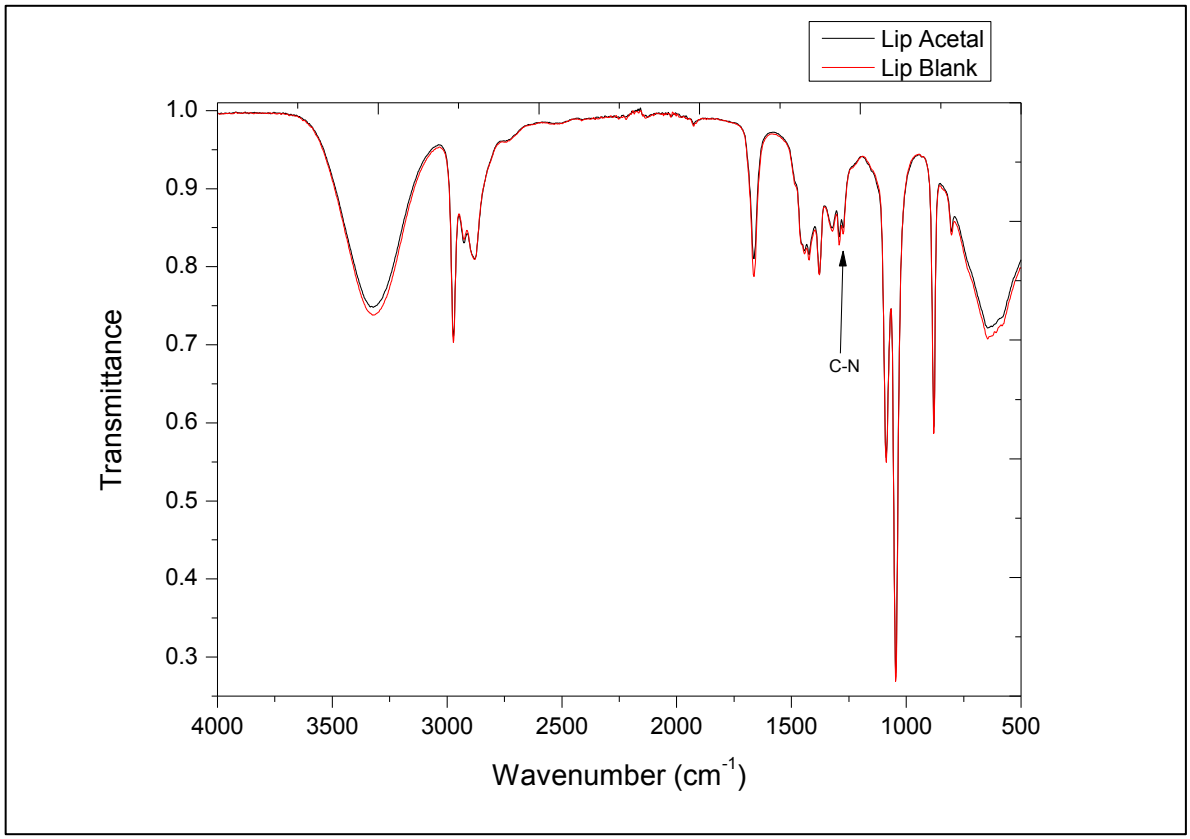



**Figure 6.20 FTIR-ATR spectra overlay of Blank lip coat and Acetal lip coat formulation**

It is evident from Figure 6.20 that the Blank lip coat and Acetal lip coat spectra overlaid spectra look similar. No change in intensity for the C-N band for Acetal lip coat is observed at ~1292 $cm^{-1}$ in comparison to the Blank lip coat. Sivaiah *et al.* [136] explained in a previous study that the lone pair of electrons from an ester carbonyl plasticiser group was donated to the nitrogen of the PVP molecule resulting in an increase in the (C-N) spectral band at ~1290 $cm^{-1}$. However, due to the absence of a carbonyl group in the molecular structure of Acetal, this type of interaction was not possible and therefore no change in band size was observed at

this wavenumber. It is postulated that non-polar interactions with the alkyl groups of Acetal and the PVP molecule of the Blank formulation occurred. However, DBP and DEHT molecules both exhibit carbonyl diester groups attached to their aromatic rings, forming secondary bonds with the polar groups of the PVP of the Blank.

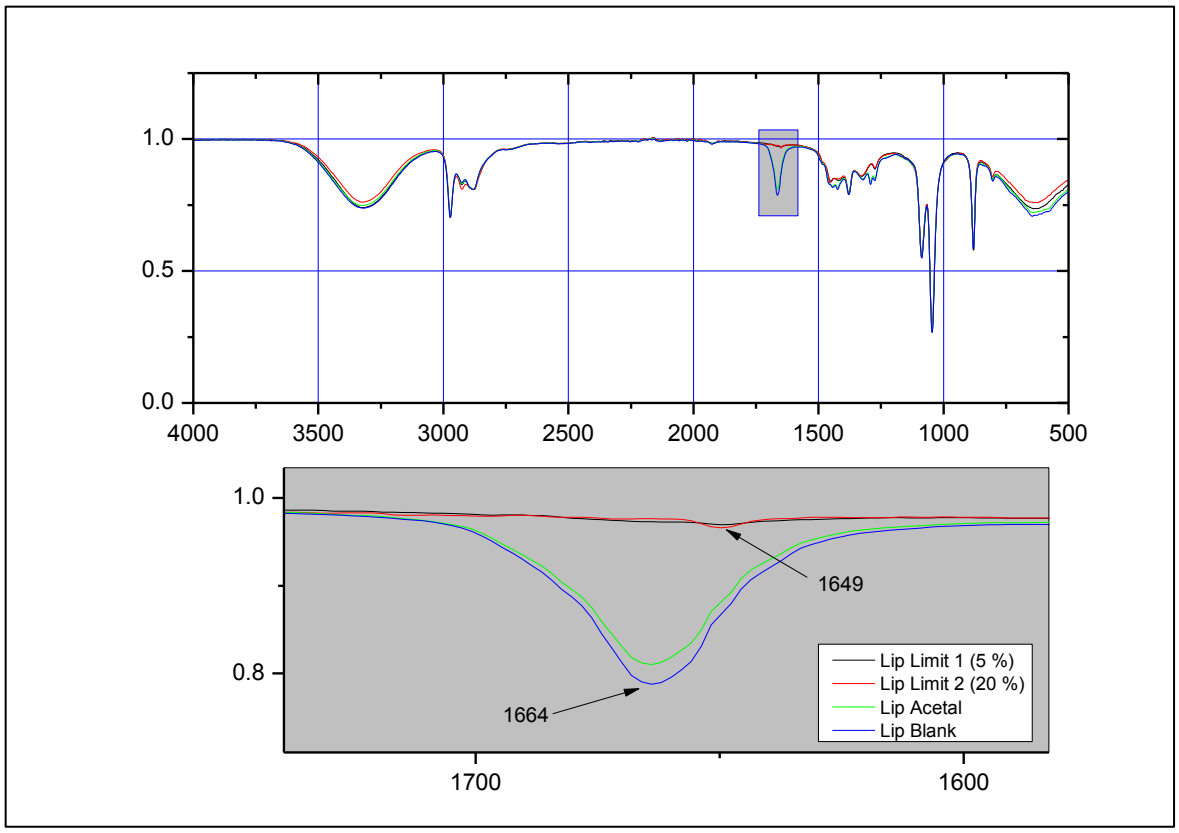



**Figure 6.21 FTIR-ATR illustrating neat Acetal lip coat limit solutions (zoomed in) at 1649 $cm^{-1}$ compared to Acetal lip coat and Blank lip coat at 1664 $cm^{-1}$**

Somashekarappa *et al.* [120] showed in a previous study that a change in band intensity is indicative of polymer-plasticiser interaction. A similar observation is evident (Figure 6.21) due to a change in band intensity between the Blank lip coat and Acetal Lip coat formulations at ~1664 $cm^{-1}$. In order to prove the presence of

added Acetal to the lip coat formulation, neat Acetal limit solutions were prepared as a 5 and 20% in formulation solvent (ethanol). These limit solutions are illustrated as an overlay in Figure 6.21, in conjunction with an overlay of the Acetal lip coat and the Blank lip coat formulations. Both Acetal limit solutions show minute non-aromatic alkene (C=C) bands at ~1649 $cm^{-1}$ unique to Acetal (Figure 5.4). These bands are obscured from the 'broad' spectral band obtained by the overlayed spectral bands of Acetal lip coat and Blank lip coat formulations at ~1664 $cm^{-1}$ (Figure 6.21). Hence, it cannot be observed that 5% Acetal (as per formulation) was added to the Acetal lip coat formulation.

Figures 6.22 to 6.25 depict the FTIR-ATR spectra of the Blank, DEHT and Acetal lip coat formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods. The spectra obtained at all the monthly intervals are shown in Appendix B: Figures 16 to 35.

No limit solutions were required to substantiate the addition of DBP and DEHT to the lip coat formulations since their presence was verified by the ester carbonyl 'shoulder' band obtained at ~1726 $cm^{-1}$ in their respective lip coat formulations (Figures 6.16 and 6.18).

Perfect FTIR spectra overlays were obtained for all Blank lip coat formulations and plasticised lip coat formulations over the three month incubation period at RT and elevated temperature (Figure 6.22 to 6.25).

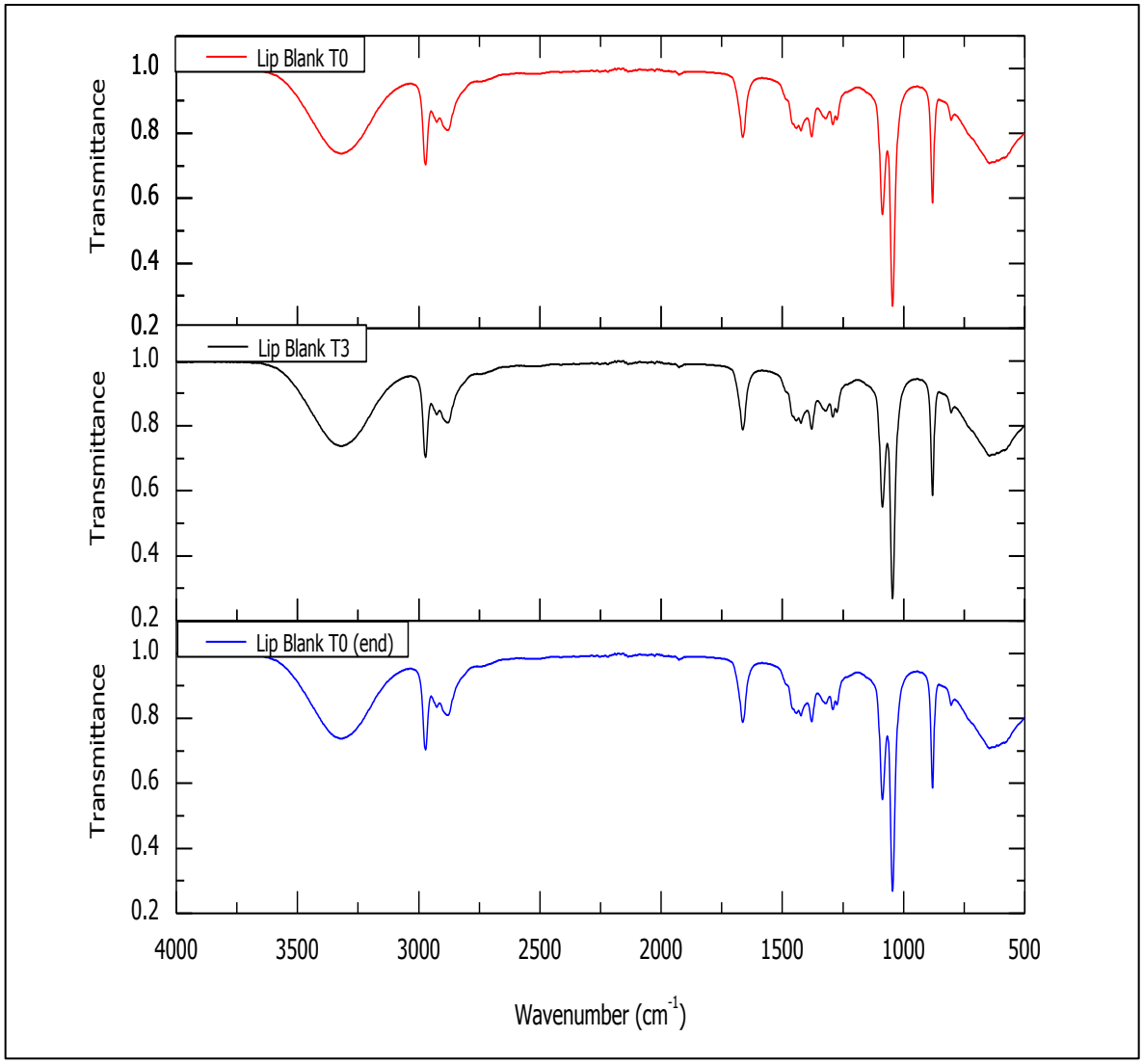



**Figure 6.22 FTIR-ATR spectra of Blank lip coat formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

Therefore it is evident that elevated temperature has no effect on the chemical stability of lip coat formulations. The control samples at Stage T0 and Stage T0 (end), as well as the Lip coat formulations, tested at Stage T3, show no shift in spectral bands. No change in band size or intensity over the incubation periods is noticed.

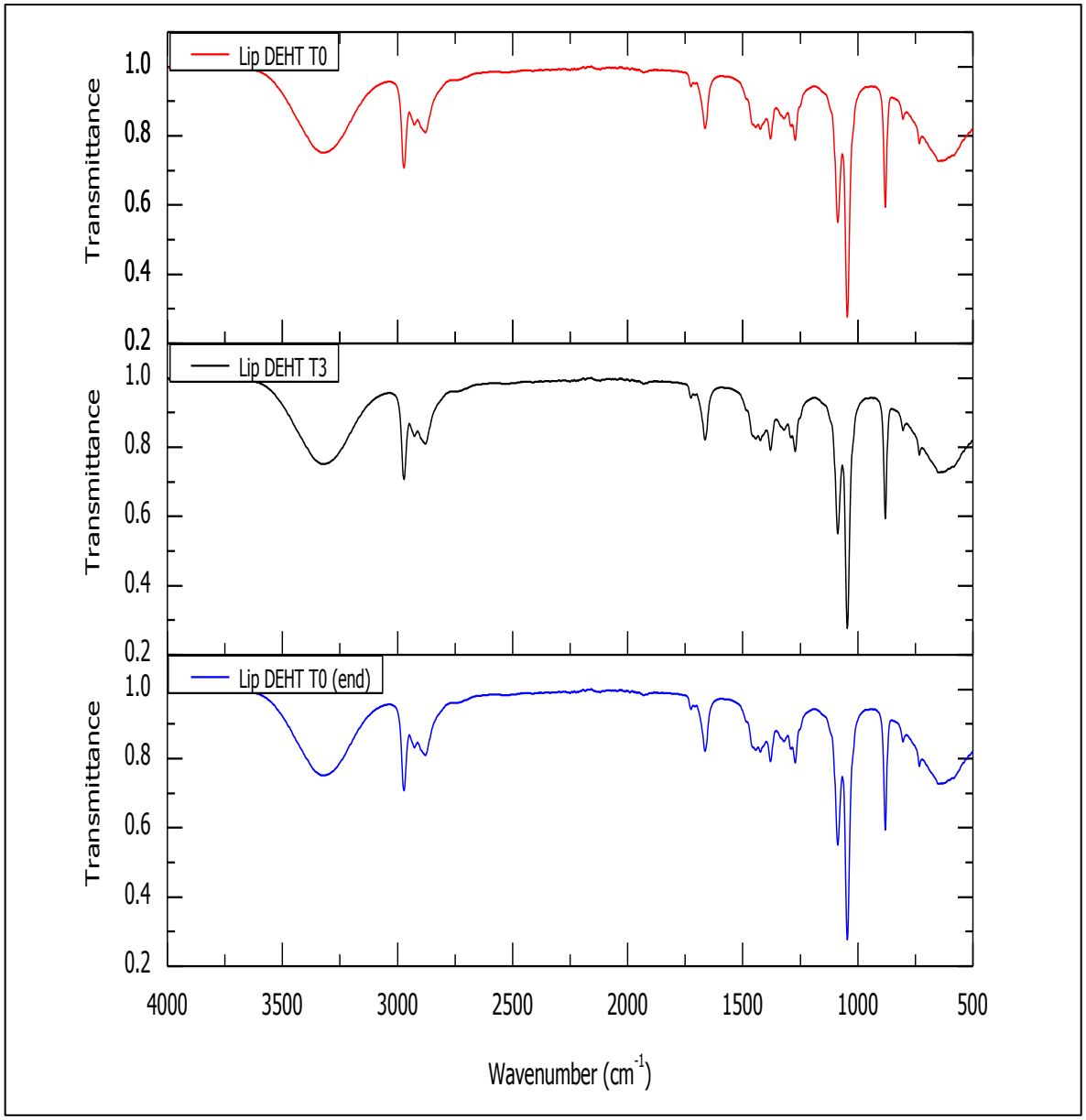



**Figure 6.23 FTIR-ATR spectra of DEHT lip coat formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

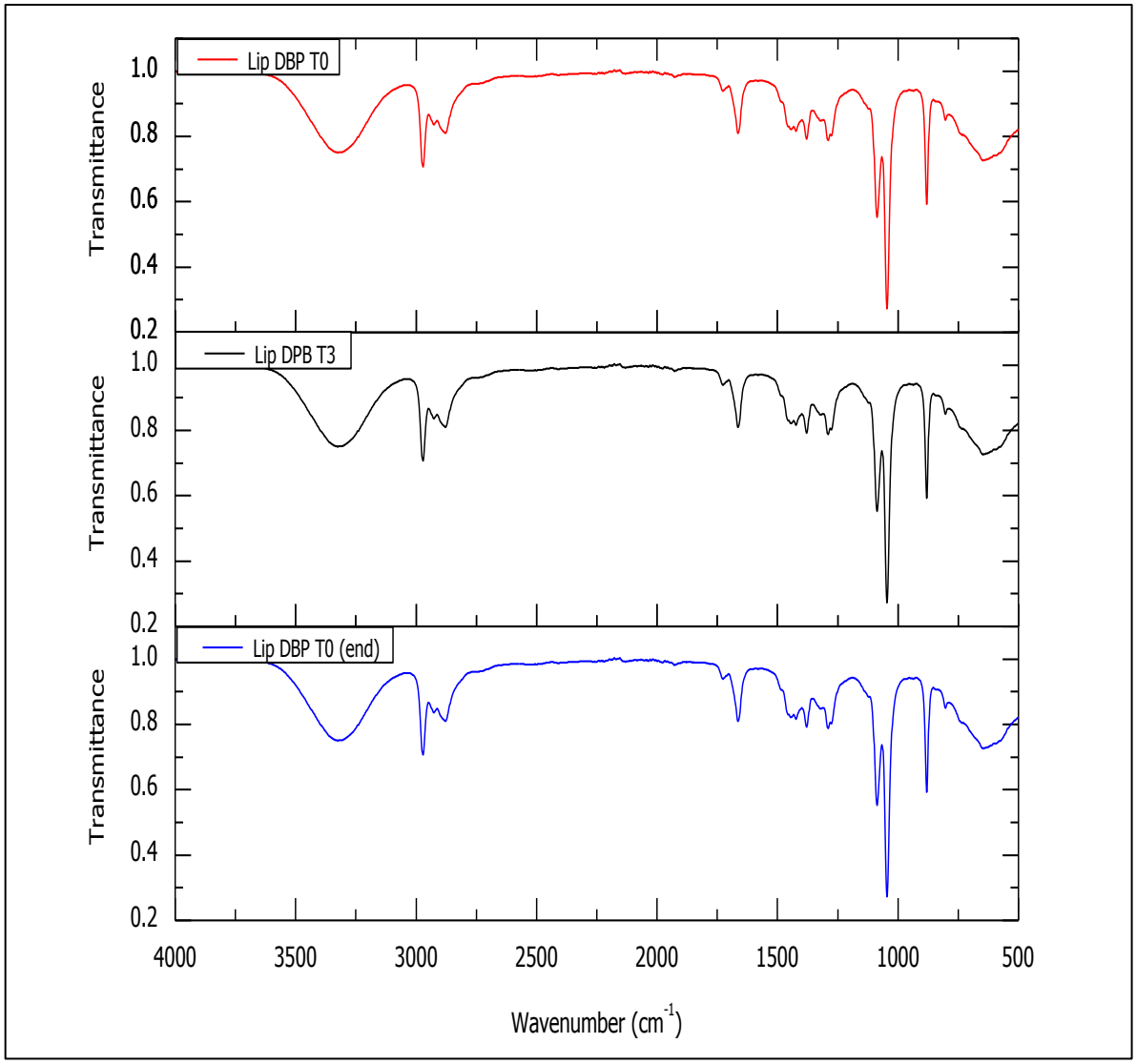



**Figure 6.24 FTIR-ATR spectra of DBP lip coat formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

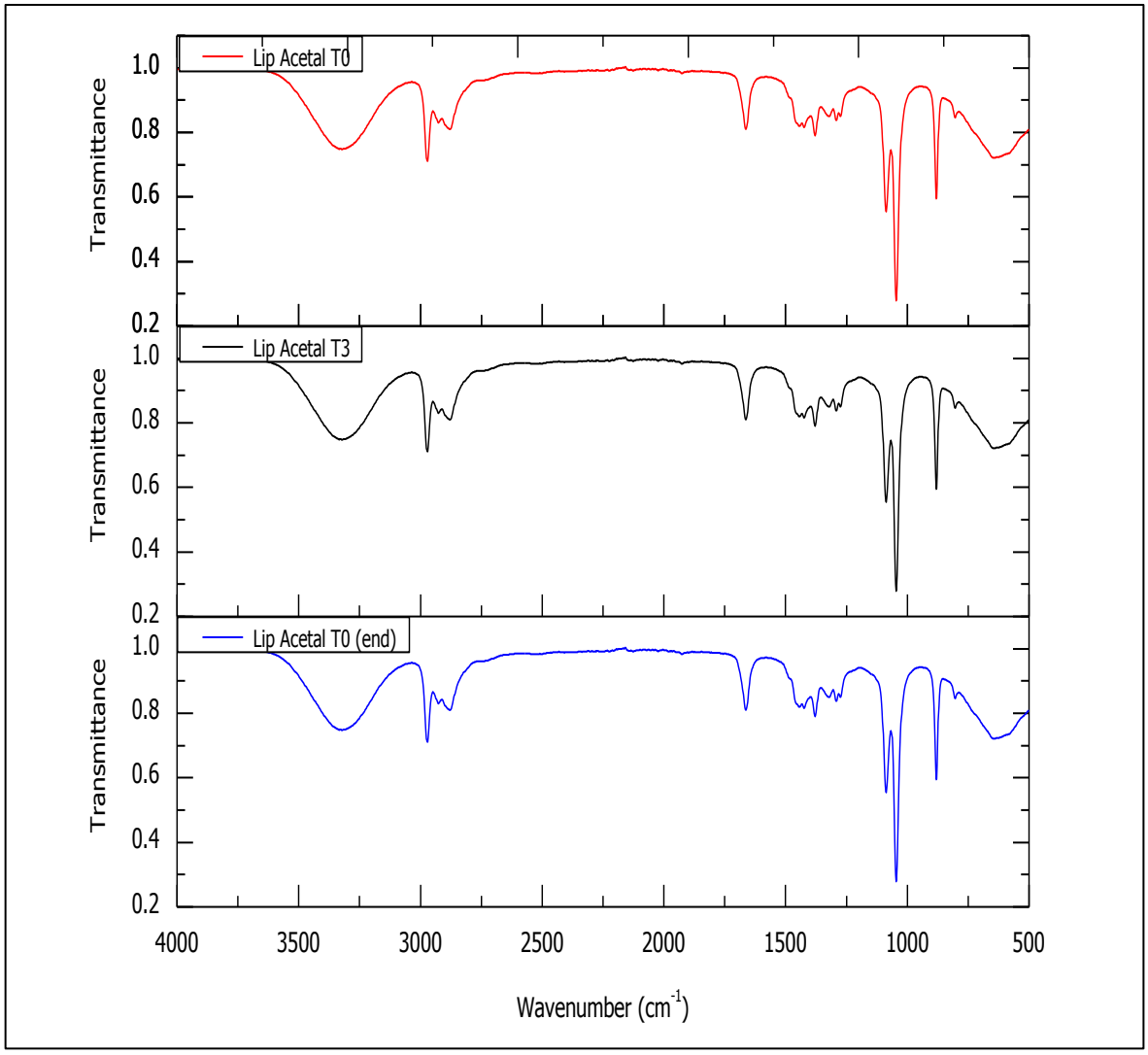



**Figure 6.25 FTIR-ATR spectra of Acetal lip coat formulations at Stage T0, Stage T3 and Stage T0 (end) incubation periods**

## 6.4 Conclusions

In comparison to the Blank nail lacquer formulation, the spectral band changes observed within each respective plasticised formulation remained consistent over

the incubation period. These changes were the result of polymer-plasticiser interaction. The changes that occurred were as follows:

A decrease in secondary amide band at ~1551 $cm^{-1}$ was observed for DEHT and Acetal (both being more hydrophobic than DBP, respectively) plasticised nail formulations. On the contrary, the Blank and DBP nail lacquer formulations displayed a perfect overlay at this same wavenumber (~1551 $cm^{-1}$). A decrease in the primary amide band at ~1659 $cm^{-1}$ was observed for all nail lacquer formulations relative to the blank, possibly due to a loss of a proton from the primary amide [117]. The change in the band intensity refers to a type of polymer-plasticiser interaction, also found in previous studies by Somashekarappa *et al.* [120].

The band occurring at ~817 $cm^{-1}$, being the identifying characteristic band of an amino-substituted-triazine ring, also showed a decrease in size for all plasticised nail lacquer formulations relative to the unplasticised nail lacquer formulation. This is indicative of interaction between the nail lacquer formulation film former and the plasticiser (polymer-plasticiser interaction) [119, 131-134].

The spectrum obtained for DEHT nail lacquer was the only nail lacquer formulation which revealed a CH bending band shift to a lower wavenumber (~739 $cm^{-1}$ to ~734 $cm^{-1}$). This CH spectral band observed was the narrowest and most intense due to the DEHT plasticiser molecule exhibiting the longest hydrocarbon alkyl chains emanating from the ester groups, occupying both *p*-positions on the benzene ring. The Acetal nail lacquer formulation, similar in shape to the Blank nail lacquer, displayed a slightly smaller CH bending band at ~740 $cm^{-1}$. It is postulated that interaction of the Acetal molecule is hindered by its 1,3-dioxane ring, associated with the hydrocarbon chain attached to it.

Upon overlaying each plasticised lip coat formulation with the Blank unplasticised lip coat formulation, it was clear that each plasticiser interacted with the Blank in a similar fashion and to the same extent, at both RT and elevated temperature. Hence, from Stage T0, this interaction resulted in similar spectral band size and intensity at each elevated temperature testing interval until Stage T3, as well as over the three month incubation period at RT [Stage T0 (end)].

Upon addition of DBP and DEHT to their respective lip coat formulations, a shift was revealed in their carbonyl spectral bands from lower to higher wavenumbers, respectively, in comparison to the carbonyl groups of neat DBP and neat DEHT in Chapter 5. This shift in carbonyl band is indicative of polymer-plasticiser interaction [138].

The 5% addition of each DBP and DEHT plasticisers, revealed their ester carbonyl spectral band as a 'shoulder' (~1726 $cm^{-1}$) on the left side of the carbonyl amide band (~1664 $cm^{-1}$) of the Blank lip coat formulation. Both the unplasticised lip coat and Acetal lip coat formulations do not exhibit this 'shoulder', due to the lack of a carbonyl functional group in their molecular structures.

An increase in (C-N) band intensity at ~1290 $cm^{-1}$ was observed for DEHT lip coat and DBP lip coat. However, no change in band intensity was found for Acetal lip coat at ~1290 $cm^{-1}$ due to the absence of carbonyl amide interaction. The Acetal molecule was therefore unable to donate electrons to the nitrogen atom of the PVP molecule.

All three plasticised lip coat formulations showed a slight decrease in the carbonyl amide band at ~1664 $cm^{-1}$ in comparison to the unplasticised Blank lip coat, possibly due to some interaction. However, the Acetal lip coat formulation displayed the least interaction presumably due to its molecular structure lacking

the carbonyl ester groups being part of the molecular structure of both DBP and DEHT.

No shift of spectral bands has taken place for any unplasticised and plasticised nail lacquer and lip coat formulation during incubation period intervals at either RT or elevated temperature.

It can therefore be concluded from the FTIR-ATR spectra that all plasticised nail lacquer and lip coat formulations remained chemically stable over the three month incubation period at RT and elevated temperature.

# CHAPTER 7
# SPE AND UPLC ANALYSES TO EVALUATE LEACHING OF PLASTICISERS FROM COSMETIC FILMS

## 7.1 Background

Leaching is one of the most difficult challenges to overcome within the field of plasticiser applications. If the plasticiser leaches out into, for example, a liquid, the polymer loses its flexibility and is no longer appropriate for its intended application [81]. This is the major cause in alteration and even failure of film properties [82]. Film thickness and concentration and MW of a plasticiser are a few factors which influence leaching [139-141].

Many cosmetic products contain phthalate esters which create problems when these phthalates leach from the formulations. Little or no exposure occurs from products such as soaps, shampoos, and conditioners that are used frequently but then washed off the skin. Exposure can occur from cosmetics that are left on the skin for extended periods of time, with actual exposure being a function of the area of skin exposed to the product, the frequency of application and length of time left on the skin, and the absorption rate through the skin [142, 143].

A lip coat formulation may leach plasticiser from the lips into mouth saliva, and by oral ingestion, subsequently travels into the bloodstream, causing adverse effects of phthalate 'build up' inside the human body. Phthalates can also be hydrolysed

by saliva upon intake so that monoesters of the released phthalate could possibly be ingested [65, 144]. In the case of a nail lacquer, dermal contact and absorption of phthalates via the skin are also possible.

This chapter investigates leaching of the three studied plasticisers from the nail lacquer and lip coat formulations into an aqueous environment. Water and artificial saliva simulant are used to simulate these environmental conditions. Two temperatures were selected for the experiment, namely 31 °C (used as body temperature in a previous study [67]) and 50 °C in order to mimic accelerated leaching conditions (elevated temperature) and for observations to be made in a shorter time period, as per research done by Rahman and Brazel [28].

## 7.2 Quantification of leaching by UPLC

Several methods have been used before to quantitate leaching of phthalate esters from cosmetic products. Shen *et al.* [145] described a method, in which phthalates were extracted by means of sonication, followed by SPE, and high-performance liquid chromatography (HPLC) analysis using a gradient elution programme with methanol/water as mobile phase. Detection of phthalates took place by means of a diode array (PDA) detector at 230 nm [142].

For this study the protocol followed by Shen *et al.* [104] was used and adapted, e.g. mechanical agitation was applied to sample test tubes by means of vortexing and sonication in order to mimic possible leaching of nail lacquer in water upon washing of hands. Licking of lips and the consequent ingestion of (phthalate) plasticiser would probably require a less 'harsh' protocol as mechanical agitation but it was chosen to standardise the method.

The main advantage of using the UPLC method is that it is more than seven times faster than HPLC and GC methods due to shorter run times. The use of UPLC as analytical chromatographic method therefore results in plasticisers exhibiting shorter retention times. More than 90% less solvent is used in UPLC than existing HPLC methods, resulting in UPLC being a cost-saving effective method.

A validated UPLC method from Waters was used for this purpose, but it necessitated the determination of UV-VIS compatibility of DBP, DEHT and Acetal prior to the execution of UPLC analyses.

## 7.3 Solid Phase Extraction

### 7.3.1 Introduction

Methods, such as liquid-liquid extraction, SPE and solid-phase micro-extraction have been used as sample pre-treatment procedures for phthalate ester determination. Of these methods SPE is by far the most widely used and has proven useful and successful in simplifying sample preparation prior to UPLC for the quantification of very low concentration phthalate esters [146]. SPE is a viable alternative to liquid-liquid extraction owing to its simplicity and low cost. It is an extraction method that uses a solid phase based on silica adsorbents and a liquid phase to isolate one analyte from a solution before using a chromatographic method to quantitate the amount of analyte in the sample. Advantages of using SPE in comparison to the more traditional sample preparation techniques include rapid and selective sample preparation, high analyte recoveries, highly purified extracts, simultaneously extracting analytes of wide polarity range and low volumes of solvents required. A wide range of phases based on silica are also

available from suppliers, including reversed phase, normal phase, ion exchange, and mixed-mode phases [32, 147].

### 7.3.2 Methodology of SPE

The procedure for SPE involves a liquid sample being introduced into a cartridge containing a bed of chromatographic packing material (stationary phase) at the bottom. Solvent, acting as the mobile phase, flows via this bed at the bottom of the cartridge. SPE, therefore, could be considered as having the same fundamental basis as an HPLC column. By using the appropriate combination of stationary and mobile phases, sample components can be either selectively retained in this column bed or they can pass directly through. The strategy employed for isolating a component of interest is to adsorb the component of interest while matrix interferences pass through the cartridge unretained. Compounds present at extremely low levels are retained by this strategy. A summary of the method described above in a complete SPE procedure is as follows and is illustrated in Figure 7.1 below:

- Conditioning of the cartridge
  Solvent is passed through the SPE material to wet the chromatographic bed in order to ensure the consistent interaction of bonded functional groups
- Loading the sample
- Removing or retaining analytes of interest with a solvent
- Elution of the fractions [147, 148]

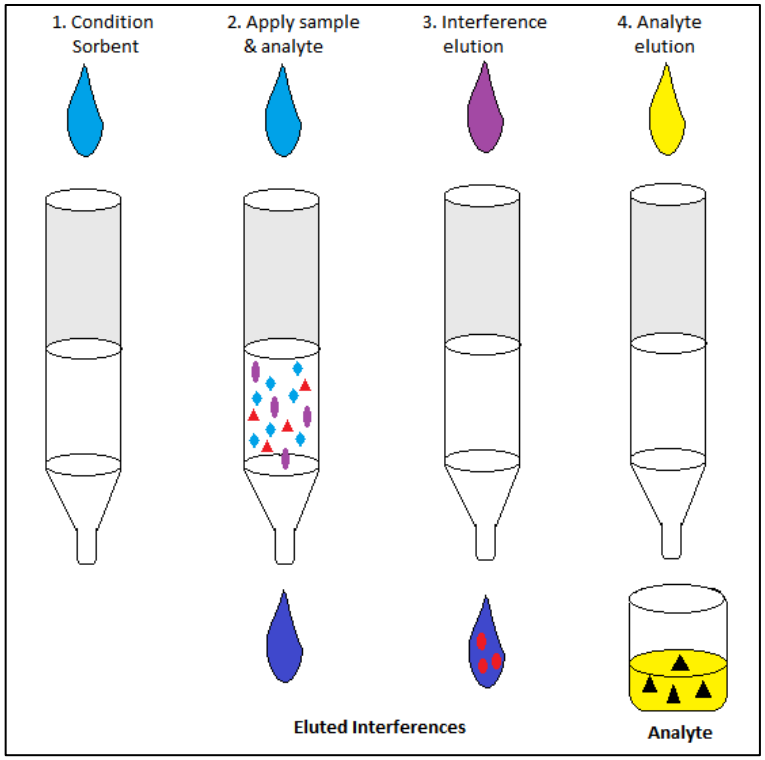



**Figure 7.1 Summary of steps involved in following the SPE procedure**

In order to identify a suitable SPE retention mechanism a reversed phase C18-E sorbent (chromatographic bed) was selected due to its ability to retain hydrophobic analytes (plasticisers), such as Acetal, DBP and DEHT.

### 7.3.3 SPE efficiency by using UPLC

The efficiency of the SPE method can be illustrated by determining the percentage recovery rate of each plasticiser, i.e. passing a known concentration of plasticiser through the cartridge (chromatographic bed) at a constant rate of 2 ml/minute, eluting the target compounds, then determining the amount of analyte recovered using a validated UPLC method [142]. See Figure 7.2 for a schematically illustration of the SPE procedure.

The calculation used was as follows [142]:

$$\%\, recovery = \frac{Average\ peak\ area\ of\ plasticiser\ with\ SPE\ x\ 100}{Average\ peak\ area\ of\ plasticiser\ without\ SPE} \quad \text{....equation 7.1}$$

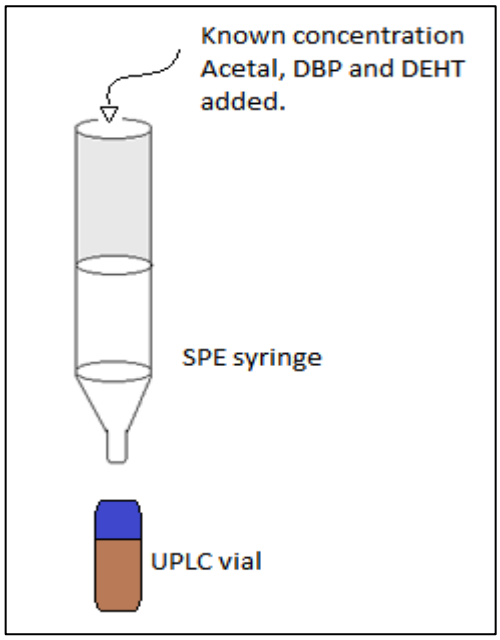



**Figure 7.2 Illustration of SPE procedure**

SPE efficiency is performed by UPLC since each plasticiser can be detected by this chromatographic method. The peak area obtained at a specific retention time using SPE, should equal the area obtained at the same retention time without using SPE, in order to use SPE for further experimental purposes.

UPLC/HPLC, equipped with PDA has a great advantage of running multiple samples simultaneously, obtaining peak responses capable of absorbing energy at different wavelengths. When it is necessary to quantitate minute or trace amounts of

leachate, solid phase extraction [57] in conjunction with HPLC/UPLC is a very useful method [27].

## 7.4 Preliminary Experiments

### 7.4.1 UV-VIS screening

A Schimadzu 1700 series UV-visible spectrophotomer was used to determine the wavelength of the maximum absorbance for each individual plasticiser. The instrument was blanked with HPLC grade methanol, thus correcting for any background responses. A 1% (v/v) solution of each plasticiser was transferred into a quartz cuvette and screened between 196 and 798 nm. From each spectrum a maximum absorbance was obtained i.e. at 234 nm for Acetal, 275 nm for DBP and 285 nm for DEHT. This would be indicative of the wavelength to be used for detection of each plasticiser upon injection by UPLC (see 7.4.3).

### 7.4.2 SPE efficiency by using UPLC

The efficiency of SPE was determined for each neat plasticiser, namely DBP, DEHT and Acetal, respectively, by conducting an experiment for the recovery rate of each of the three plasticisers with and without following the SPE experiment. UPLC was used as the chromatographic analytical method for this purpose. The procedure was as follows: a pure standard solution was made containing 1 µg/ml of neat plasticiser. A volume of 1 ml of this stock standard solution was added accurately to 1 ml of methanol, well mixed and capped in a UPLC vial. Following the SPE method, 1 ml of the neat plasticiser stock standard solution was added to 1 ml of Milli-Q water and mixed well. This mixture was passed through the SPE syringe, while the plasticiser was being retained within the SPE chromatographic

bed. About 10 ml of air was then passed through the cartridge with an empty syringe to drive out any possible excess water. Theoretically, the SPE bed should contain 1 µg of plasticiser. A volume of 2 ml HPLC grade methanol was accurately flushed via the SPE cartridge containing the 1 µg of retained plasticiser, (in order to dissolve it) and then meticulously transferred into a UPLC vial, as can be seen in Figure 7.2. Air flow was forced through the cartridge by means of a plunger in order to force the entire hydrophobic matrix through the chromatographic bed into the UPLC vial. The final concentration of the resultant solution (the leachate) obtained from the SPE experiment should be similar to the standard containing 0.5 µg/ml, thus UPLC chromatography should reveal the same peak areas. For each neat plasticiser, the same procedure was followed in order to investigate the efficiency of the SPE method with regard to the recovery rate of each plasticiser. SPE extraction recoveries were calculated by comparing peak areas of these extracts with those areas of the standard solutions prepared in the same solvent.

An UPLC chromatograph, equipped with a PDA was used in this study to determine the peak area obtained for each respective plasticiser and used as detector response.

### 7.4.3 Determination of LOD and LOQ: UPLC

A limit of detection (LOD) and limit of quantification (LOQ) was performed in order to ensure that trace amounts of leaching plasticisers could be quantified.

The LOD is used to determine the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified. The LOQ refers to the lowest concentration of an analyte that can be determined with acceptable precision and accuracy.

The LOD and LOQ limits were determined by injecting a series of standard dilutions into the chromatographic system (UPLC). A signal to noise ratio of approximately 3 should be obtained for the limit of detection, similarly, this ratio should be more or less 10 for the limit of quantification [149]. These calculations were not performed manually but automatically calculated by Empower 3 chromatography data software, which is part of the UPLC instrument used in this experiment.

The following UPLC instrument method settings were used:

| | |
|---|---|
| LC system: | Acquity UPLC H-Class with attached PDA detector |
| Run time: | 5.00 min |
| Column: | Acquity UPLC BEH C18 |
| Column Temp: | 50 °C |
| Sample temp.: | 10 °C |
| Mobile phase A: | Water + 0.1% Formic acid |
| Mobile phase B: | Methanol + 0.1% Formic acid |
| Flow rate: | 0.6 ml/min |
| Injection volume: | 5.0 µl |
| Detection: | PDA detector with wavelengths set at 234 nm for Acetal, 275 nm for DBP and 285 nm for DEHT |
| Mobile phase gradient programme: | Detailed in Table 7.1 |

**Table 7.1 Mobile phase gradient programme**

| | Time (min) | Flow rate (mL/min) | %A | %B |
|---|---|---|---|---|
| 1 | Initial | 0.60 | 30 | 70 |
| 2 | 1.00 | 0.60 | 30 | 70 |
| 3 | 1.50 | 0.60 | 10 | 90 |
| 4 | 4.00 | 0.60 | 10 | 80 |
| 5 | 4.01 | 0.60 | 30 | 70 |
| 6 | 5.00 | 0.60 | 30 | 70 |

Note: Methanol vial washes were inserted in between sample stages in order to prevent possible carry-over of peaks between injections.

7.4.4 Calibration curve determination

In order to create a calibration curve, the validated UPLC method for leaching of plasticiser from cosmetics by Waters [150] was used. A series of dilutions were made from each of the neat plasticisers ranging from 0.2 µg/ml to 4.0 µg/ml. This forms part of a validation test referred to as 'linearity'. The amount of leaching can be obtained from this curve [102, 107]. The calibration curve was used to calculate the amount of leaching analyte, if any, and the calibration range was 5 mg/kg to 100 mg/kg of the respective analyte in the extracted sample. The NOAEL amount for toxic DBP is 50 mg/kg/day [15] was considered and was therefore included in this range, if any leaching was to be detected [107].

### 7.4.5 Results of SPE efficiency determination

Table 7.2 shows a summary of the average peak areas obtained in absorbance units (AU) for the efficiency experiment for neat plasticisers with and without the use of SPE. The experiment was performed in triplicate.

**Table 7.2 Summary of peak areas obtained (in triplicate) with and without SPE**

| Plasticiser | Mean peak area without SPE (AU) | Mean peak area with SPE (AU) |
|---|---|---|
| Acetal | 47193 | 46008 |
| DBP | 39807 | 39455 |
| DEHT | 302619 | 298951 |

The full set of data with peak height, mean value and % RSD are shown in Appendix C (Tables 1 to 9). Appendix C (Figures 1 to 12) shows the corresponding UPLC chromatograms obtained for each plasticiser for the first injection of the sample with and without SPE.

The % recovery calculation (equation 7.1) was used to calculate the efficiency of each studied plasticiser from the data obtained in Table 7.2. The results of each plasticiser efficiency are summarised in Table 7.3.

**Table 7.3 SPE efficiency (mean) for each plasticiser**

| | SPE Plasticiser efficiency (%) | | |
|---|---|---|---|
| **Sample Repeats** | **PMD Acetal** | **DBP** | **DEHT** |
| Sample 1 | 98.20 | 99.10 | 98.60 |
| Sample 2 | 97.50 | 98.00 | 98.80 |
| Sample 3 | 96.70 | 98.30 | 98.90 |
| Average | 97.47 | 98.47 | 98.77 |
| *SD* | 0.75 | 0.57 | 0.15 |

Table 7.3 shows that SPE technology resulted in high recovery rates with low standard deviation values. From the above results it is evident that SPE is an efficient method to be used for determining trace amounts of analyte (plasticiser) in a sample matrix. Figure 7.3 graphically displays the % SPE recovery in triplicate for each plasticiser (series 1 to 3 denote triplicate sample treatment).

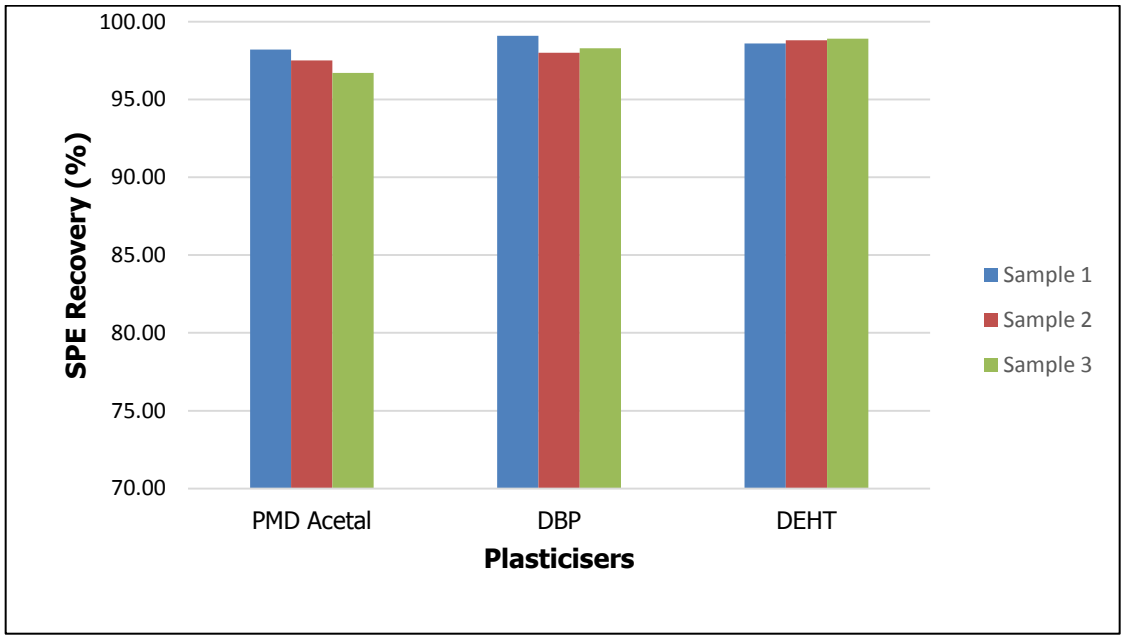



**Figure 7.3 SPE recovery (%) for Acetal, DBP and DEHT**

## 7.5 Leaching of plasticisers from nail lacquer and lip coat formulations

After determination of the LOQ and LOD (Section 7.5.3.1), possible leaching of nail and lip formulations was determined by means of SPE in conjunction with UPLC methods.

A Milli-Q purification system from Millipore was used to de-ionise purified water in the laboratory prior to use, onwards being referred to as Milli-Q water. Extreme care was taken to prevent glassware contamination from sources of phthalates, the latter being ubiquitous contaminants to the environment [142, 151]. Glassware was therefore thoroughly cleaned, rinsed with Milli-Q water and ethanol, and dried in an oven before use.

### 7.5.1 Lip coat experimental procedure

After application of a lip coat onto the lips the plasticiser could possibly leach into the mouth environment, hence artificial saliva was prepared to mimic the aqueous environment of the inside of the mouth. Table 7.4 illustrates the constituents used for the saliva simulant [152].

**Table 7.4 Preparation of saliva simulant**

| | |
|---|---|
| Sodium chloride | 4.5 g |
| Potassium chloride | 0.3 g |
| Sodium sulfate | 0.3 g |
| Ammonium chloride | 0.4 g |
| Urea | 0.2 g |
| Lactic acid | 3.0 g |

The constituents in Table 7.4 were dissolved in 1000 ml of distilled water and the pH adjusted to 7.03 with 5M NaOH [153].

The preliminary sample preparation sequence for 5 replicates of each plasticised lip formulation and a blank formulation was performed as follows:

- A mass of 0.40 g of each lip coat formulation and the blank were accurately and meticulously weighed into a flat-bottomed cylindrical glass tube, covering the entire inner, bottom surface of the tube, without any sample touching the sides of the tube
- samples were left to air dry for 48 hours at RT (23 ± 1 °C)
- saliva simulant (2.5 ml) was pipetted onto each dried sample inside the glass tube, stoppered and sealed with an aluminium foil-bound screw cap

Upon addition of the saliva simulant it was noticed that all the lip coat and blank films started to disintegrate.

### 7.5.2 Nail lacquer experimental procedure

The preliminary sample preparation sequence for 5 replicate nail lacquer formulations and a blank formulation was performed in a similar fashion as the lip coat and blank formulations. The experimental procedure was adapted from Cooper [150]:

- A mass of 0.40 g of each nail lacquer formulation and a blank were accurately and meticulously weighed into a flat cylindrical glass tube, covering the entire inner, bottom surface of the tube, without any sample touching the sides of the tube
- Samples were left to air dry for 48 hours at RT (23 ± 1 °C)
- Milli-Q water (2.5 ml) was pipetted onto each dried sample inside the glass tube, stoppered and sealed with an aluminium foil bound cap
- Mechanical agitation was used by vortexing each glass tube at maximum speed for 2 minutes
- Glass tubes were then transferred to an ultrasonic bath for 30 minutes to maximize further extraction of plasticiser [150]. This step was taken in order to mimic possible leaching from the nail lacquer while washing hands

The preliminary experimental procedure for the nail lacquer was deemed successful as the films did not disintegrate and therefore it was decided to continue with the experimental procedure as follows:

- A mass of 0.40 g of each nail lacquer formulation was accurately and meticulously weighed into a flat cylindrical glass tube, covering the entire

inner, bottom surface of the tube, without any sample touching the sides of the tube

- Samples were left to air dry for 48 hours at RT (23 ± 1 °C)
- Film thicknesses were recorded by means of digital callipers and average readings of 1.22 ± 0.22 mm were obtained

  The surface area of the dried film inside the glass tube was determined as follows: area = $\pi r^2$ = 486.95 $mm^2$ or 4.8695 $cm^2$, inside diameter = 24.90 mm
- Milli-Q water (2.5 ml) was pipetted onto each dried sample inside the glass tube, stoppered and sealed with an aluminium foil bound cap
- Mechanical agitation was used by vortexing each glass tube at maximum speed for 2 minutes
- Glass tubes were then transferred to an ultrasonic bath for 30 minutes to maximise further extraction of plasticiser [150]. This step was taken in order to mimic possible leaching from the nail lacquer while washing hands
- Glass tubes were transferred to the pre-calibrated 31 °C and 50 °C ovens representing body temperature and 'accelerated' leaching, respectively [28, 67]

After predetermined intervals, i.e. 24, 48 and 72 hours, the plasticised samples (as well as the blank samples) were removed from the 31 °C and 50 °C ovens and each tube inverted gently ten times and cooled down to RT as previously performed by Yu *et al.* [154]. Each sample vial was treated as explained in the SPE procedure. Conditioning of the chromatographic bed was firstly carried out with methanol, followed by Milli-Q water. Air was forced via the SPE syringe with another empty syringe action in order to make sure all the Milli-Q water was run through the syringe. The analyte of interest (plasticiser) was retained on the chromatographic bed. Methanol (1 ml) was pipetted into the syringe and eluted via the chromatographic bed at a constant flow rate of 2 ml/minute [155].

UPLC vials were filled accordingly with the eluting analyte, sealed and transferred to be injected by the auto-injector of the UPLC instrument. The UPLC instrument conditions settings remained the same as for the determination of LOD and LOQ experiment (see Section 7.4.3).

In order to prevent the carryover of possible leaching analytes, solvent (methanol) and blank formulations (containing no plasticiser) were run to confirm the absence of Acetal, DBP and DEHT peaks respectively and no chromatographic responses were observed at the retention time of any plasticiser.

### 7.5.3 Results and Discussion

#### 7.5.3.1 LOD and LOQ: UPLC

The LOD and LOQ obtained for Acetal was 0.025 µg/ml and 0.081 µg/ml, respectively. For DBP the LOD was determined as 0.01 µg/ml and the LOQ as 0.05 µg/ml. The LOD and LOQ values for DEHT were established as 0.01 µg/ml and LOQ 0.04 µg/ml, respectively. The dilutions for above data can be seen in Appendix C (Figures 7 to 12).

#### 7.5.3.2 Calibration curves

A calibration curve for each plasticiser was constructed from calibration data. Reference standards of each plasticiser at various concentrations are shown as 'response' in the Tables 7.5, 7.8 and 7.11.

A linear regression was fitted to the calibration data as shown below

$$y = Bx + A \qquad \text{.....equation 7.2}$$

where:

$B = slope$

$A = intercept$

$x = plasticiser\ concentration$

Residual data (Tables 7.6, 7.9 and 7.12) was calculated from the calibration data and graphs were constructed for each plasticiser (Figures 7.4, 7.5 and 7.6). From the graphs, the correlation coefficients ($R^2$) and *y*-intercepts of the linear curves were calculated.

Furthermore, the y-intercept value was expressed as a percentage of the detector response at 100% analyte value using the formula below to determine the assessment value (*z*):

$$z = \left(\frac{y - intercept}{100\ \%\ detector\ response}\right) x\ 100 \qquad \text{.....equation 7.3}$$

The assessment value (*z*) falls within the specified limits only when +10 > *z* > -10. From the data obtained a residual standard deviation (*SD*) was calculated for each plasticiser. The residual *SD* is a statistical term used to describe the *SD* of points formed around a linear function and is an estimate of the accuracy of the dependent variable being measured.

**Table 7.5 Calibration data for PMD-citronellal acetal used to construct the calibration curve**

| | Peak area (AU) |
|---|---|
| **Concentration (µg/ml)** | **Response** |
| 0.2455 | 28833 |
| 0.4910 | 49679 |
| 0.9820 | 99832 |
| 1.9640 | 190878 |
| 2.9460 | 287222 |
| 3.9280 | 382263 |

Figure 7.4 is the analytical calibration using a simple linear curve fit, with error estimation obtained for PMD-citronellal acetal from Tables 7.5 and 7.6 (calculated PMD-citronellal acetal assessment value). PMD-citronellal acetal had an $R^2$ value of 0.999, z value of 3.99 and a residual *SD* value of 0.4% (Table 7.7).

**Table 7.6 Calculated residuals for PMD-citronellal acetal peak areas**

| **Gradient (of regression line, from graph)** | | 96132.22 |
|---|---|---|
| **Intercept (of regression line, from graph)** | | 3981.20 |
| **Predicted Y (peak area)** | **Residuals** | **Standard Residuals** |
| 27581.66 | 1251.34 | 0.88 |
| 51182.12 | -1503.12 | -1.06 |
| 98383.04 | 1448.96 | 1.02 |
| 192784.88 | -1906.88 | -1.35 |
| 287186.73 | 35.27 | 0.03 |
| 381588.57 | 674.43 | 0.48 |
| *SD* | 1415.42 | |

**Table 7.7 Calculated PMD-citronellal acetal assessment value**

| | |
|---|---|
| *y*-Intercept | 3981.20 |
| 100% response | 99832.00 |
| *z* value | 3.99 |

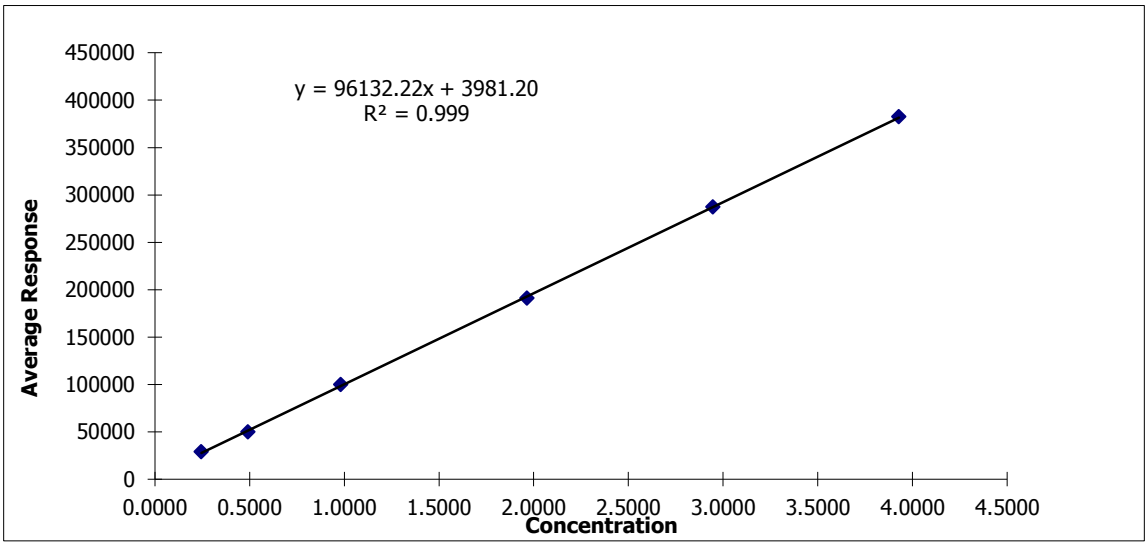



**Figure 7.4 Calibration curve and best fit line for PMD-citronellal acetal**

Figure 7.5 is the analytical calibration using a simple linear curve fit, with error estimation obtained for DBP from Tables 7.8 and 7.9 (calculated DBP assessment value). DBP had a $R^2$ value of 0.997, *z* value of -1.14 and a residual *SD* value of 1.7% (Table 7.10).

**Table 7.8 Calibration data used to construct the calibration curve for DBP**

| | **Peak area (AU)** |
|---|---|
| **Concentration (µg/ml)** | **Response** |
| 0.2620 | 23496 |
| 0.5240 | 40588 |
| 1.0480 | 82645 |
| 2.0960 | 152292 |
| 3.1440 | 248209 |
| 4.1920 | 331141 |

**Table 7.9 Calculated residuals for DBP**

| **Gradient (of regression line, from graph)** | | 78466.08 |
|---|---|---|
| **Intercept (of regression line, from graph)** | | -937.98 |
| **Predicted Y** | **Residuals** | **Standard Residuals** |
| 19620.13 | 3875.87 | 0.69 |
| 40178.24 | 409.76 | 0.07 |
| 81294.47 | 1350.53 | 0.24 |
| 163526.93 | 11234.93 | -1.99 |
| 245759.39 | 2449.62 | 0.43 |
| 327991.84 | 3149.16 | 0.56 |
| *SD* | 5641.91 | |

**Table 7.10 Calculated DBP assessment value**

| | |
|---|---|
| *y*-Intercept | -937.98 |
| 100% response | 82645.00 |
| *z* | -1.14 |

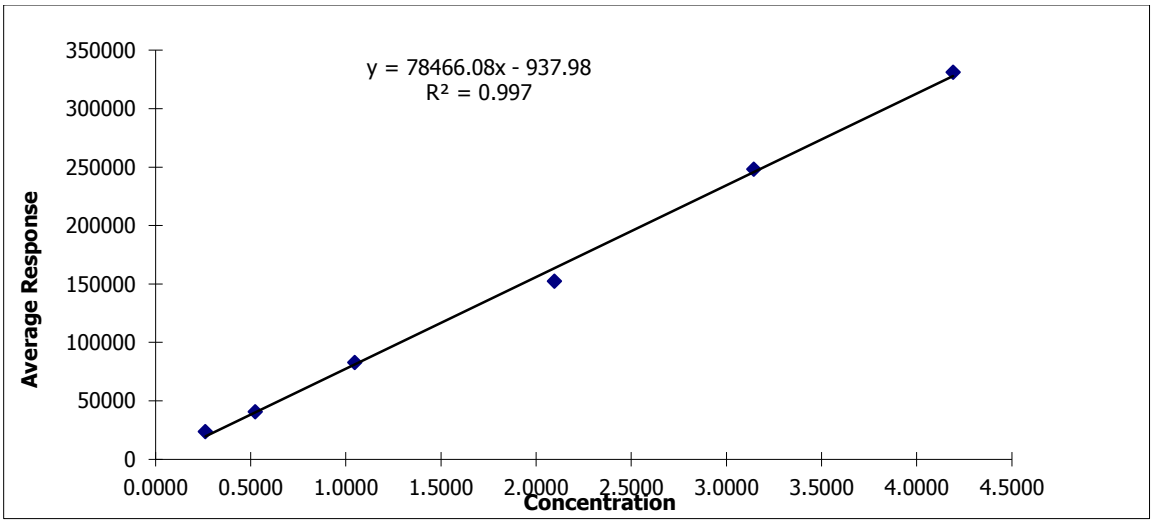



**Figure 7.5 Calibration curve and best fit line for DBP**

Figure 7.6 is the analytical calibration using a simple linear curve fit, with error estimation obtained for DEHT from Tables 7.11 and 7.12 (calculated DEHT assessment value). DEHT had a $R^2$ value of 0.996, $z$ value of 8.21 and a residual $SD$ value of 2.2% (Table 7.13).

**Table 7.11 Calibration data used to construct the calibration curve for DEHT**

| | Peak area (AU) |
|---|---|
| **Concentration (µg/ml)** | **Response** |
| 0.2460 | 151175 |
| 0.4920 | 303178 |
| 0.9840 | 615782 |
| 1.9680 | 1158673 |
| 2.9520 | 1742119 |
| 4.1920 | 331141 |

**Table 7.12 Calculated residuals for DEHT**

| **Gradient (of regression line, from graph)** | | 549890.15 |
|---|---|---|
| **Intercept (of regression line, from graph)** | | 50546.16 |
| **Predicted Y** | **Residuals** | **Standard Residuals** |
| 185819.14 | 34644.14 | -0.74 |
| 321092.12 | 17914.12 | -0.37 |
| 591638.07 | 24143.93 | 0.50 |
| 1132729.98 | 25943.02 | 0.54 |
| 1673821.89 | 68297.11 | 1.41 |
| 2214913.80 | 65825.80 | -1.36 |
| *SD* | 48527.59 | |

**Table 7.13 Calculated DEHT assessment value**

| | |
|---|---|
| y-Intercept | 50546.16 |
| 100% response | 615782.00 |
| z | 8.21 |

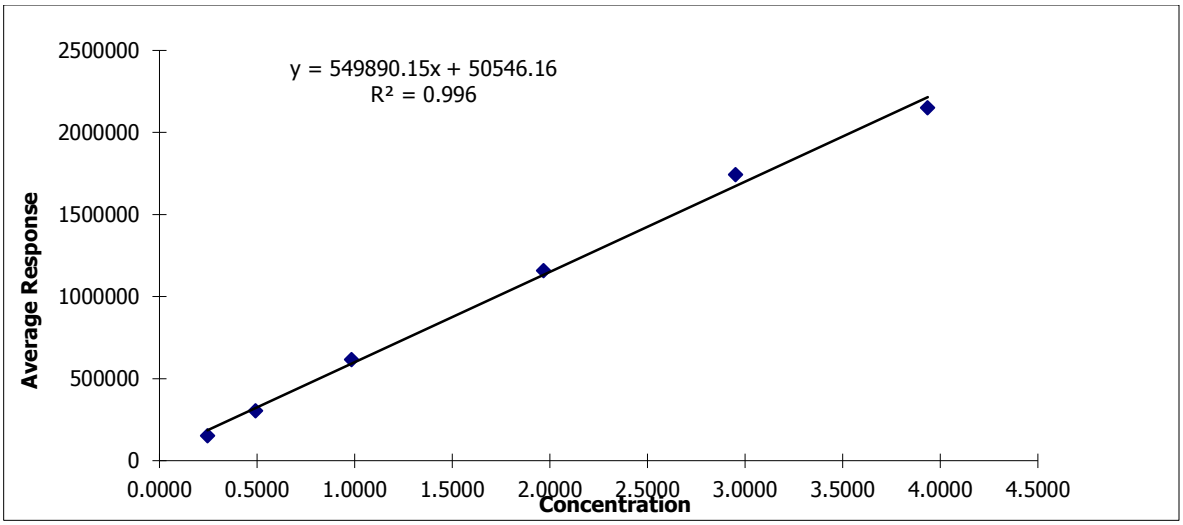



**Figure 7.6 Calibration curve for DEHT, average response versus concentration**

UPLC calibration curves obtained for Acetal, DBP and DEHT were found to be linear over the concentration range of 0.2 µg/ml to 4 µg/ml. All regression correlation coefficients ($R^2$) were ≥ 0.99. Good linearity was therefore obtained for the three plasticisers.

7.5.3.3 Leaching results of lip coat formulations

All air-dried lip coat formulations disintegrated in the saliva simulant before the start of oven incubation. The 5-membered PVP ring contains a proton accepting carbonyl group whereas HPC (being water-soluble) has hydroxyl groups, as a result hydrogen-bonding prevailed between these molecules [130, 156]. Strong hydrogen-bonding between hydroxyl groups of HPC and carbonyl groups of PVP therefore led to the misicibility of HPC and PVP in aqueous solution (saliva simulant). This is in agreement with studies done by Reddy *et al.* [130]. Subsequently, upon addition of the saliva simulant to the dried films in the glass tubes, all formulations disintegrated, rendering them unsuitable for leaching tests to be performed.

7.5.3.4 Leaching results of nail lacquer formulations

No leaching data was produced for plasticised nail lacquer and Blank nail formulations at 31 °C over a three day incubation period since corresponding chromatographic peak areas for DBP were below the LOQ, whereas no corresponding peak areas were detected for neither Acetal, DEHT or blank formulations

Leaching data of all plasticised nail lacquer and blank formulations observed over a three day incubation period at 50 °C is shown in Table 7.14. A one-way (single factor) ANOVA was performed on the data (using Statsoft® Statistica software) for DPB nail lacquer and results are shown in Table 7.14 with plots of the means and confidence intervals in Figure 7.7.

There was a statistically significant difference in leaching of DPB at the $p$ <.05 level for the three time conditions [$F$ (2, 12) = 5.454, p = 0.02]. From Figure 7.7 it is observed that the amount of DPB leaching reduced significantly over the studied time period.

A post hoc Tukey test showed that leaching between 48 and 72 H and leaching between 24 and 72 H of DBP nail lacquer differ significantly at $p$ <.05 (Table 7.16).

**Table 7.14 Leaching results of plasticised nail lacquers and blank**

| | **Formulation: 24 hours – 50 ˚C (µg/ml Leaching)** | | | |
|---|---|---|---|---|
| Sample Replication (n=5) | **Nail DBP** | **Nail Acetal** | **Nail DEHT** | **Nail Blank** |
| 1 | 1.0818 | Not detected | Not detected | Not detected |
| 2 | 1.0636 | Not detected | Not detected | Not detected |
| 3 | 0.9793 | Not detected | Not detected | Not detected |
| 4 | 1.0197 | Not detected | Not detected | Not detected |
| 5 | 1.0532 | Not detected | Not detected | Not detected |
| | **48 hrs – 50 ˚C (µg/ml Leaching)** | | | |
| 1 | 0.9899 | Not detected | Not detected | Not detected |
| 2 | 1.0286 | Not detected | Not detected | Not detected |
| 3 | 1.019 | Not detected | Not detected | Not detected |
| 4 | 1.0673 | Not detected | Not detected | Not detected |
| 5 | 1.0425 | Not detected | Not detected | Not detected |
| | **72 hours – 50 ˚C (µg/ml Leaching)** | | | |
| 1 | 0.9722 | Not detected | Not detected | Not detected |
| 2 | 1.0105 | Not detected | Not detected | Not detected |
| 3 | 0.9797 | Not detected | Not detected | Not detected |
| 4 | 0.9196 | Not detected | Not detected | Not detected |
| 5 | 0.982 | Not detected | Not detected | Not detected |

**Table 7.15 Analysis of variance of DBP nail lacquer formulation leaching results at 50 ˚C for 24, 48 and 72 hours**

**ANOVA: Single Factor**

| SUMMARY | | | | |
|---|---|---|---|---|
| ***Groups*** | ***Count*** | ***Sum*** | ***Average*** | ***Variance*** |
| 24 | 5 | 5.20 | 1.040 | 0.002 |
| 48 | 5 | 5.15 | 1.030 | 0.001 |
| 72 | 5 | 4.86 | 0.973 | 0.001 |

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***p-value*** | ***F crit*** |
| **Between Groups** | 0.013 | 2 | 0.006 | 5.454 | 0.020 | 3.885 |
| **Within Groups** | 0.014 | 12 | 0.001 | | | |
| **Total** | 0.027 | 14 | | | | |

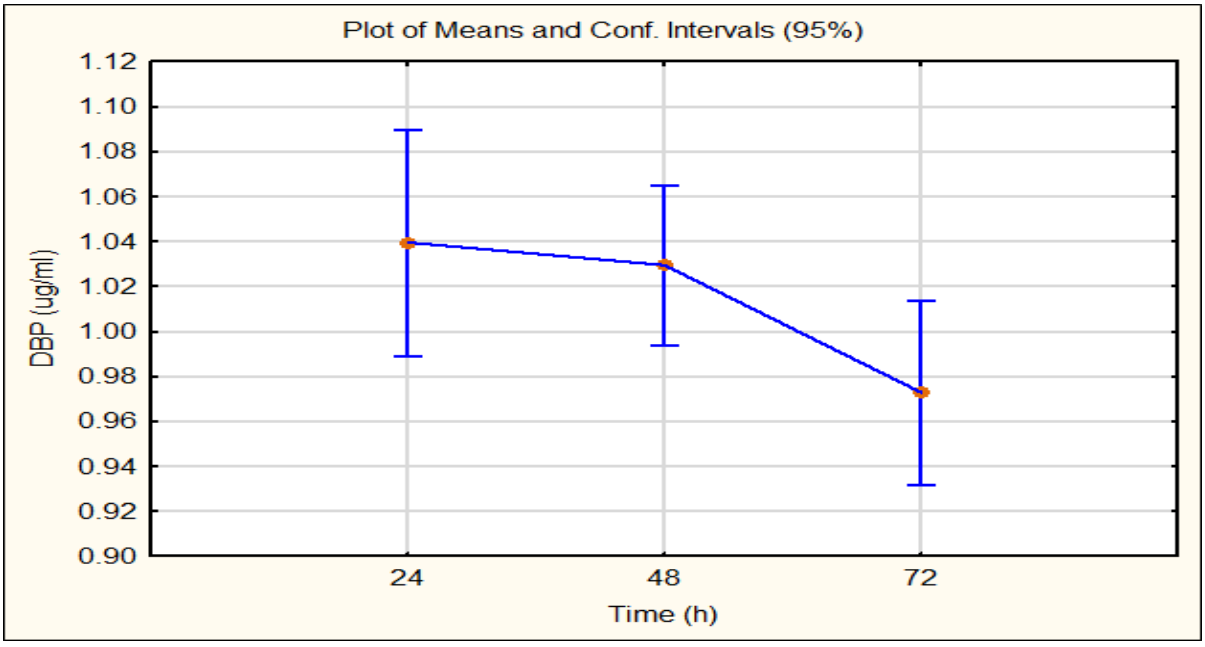



**Figure 7.7 Graph of means of DBP plasticised nail lacquer formulation leached over a three day time period**

**Table 7.16 Tukey test for DBP nail lacquer formulation leaching showing *p*-values obtained between variables**

| Time | | Tukey HSD test, Variable: DBP | | |
|---|---|---|---|---|
| | | {1} | {2} | {3} |
| 24 | {1} | | 0.890 | 0.025 |
| 48 | {2} | 0.890 | | 0.056 |
| 72 | {3} | 0.025 | 0.056 | |

After 24 hours, 1.0395 µg/ml (*SD* = .041) DBP leached. 1.0294 µg/ml DBP (*SD* = .029) leached after 48 hours and 0.9728 µg/ml (*SD* = .0331) DPB leached after 72 hours. From the data obtained it can be observed that leaching gradually slowed down over time. Similar trends where observed by Rahman and Brazel [28], Brydson [157] and Zhang and Chen [158]. The reason for this leaching trend is that the polymer and plasticiser molecules are continuously undergoing association and segregation. Therefore there is a high tendency for the plasticiser to be removed from the polymer matrix through leaching. Over time leaching of the plasticiser is expected to diffuse down the concentration gradient to the interface between the polymer surface and the external medium [10, 28].

At lower plasticiser concentrations, the diffusion of plasticisers through the polymer matrix is thought to be the rate limiting step [28]. It is therefore postulated that leaching of plasticiser is due to diffusing of the DBP molecules through the polymer matrix. See Appendix C (Tables 10 to 12) for Nail DBP leaching peak areas and retention times. Figures 13 to 36 in Appendix C illustrate the chromatograms obtained for the leaching of Acetal, DBP and DEHT nail lacquer formulations at the different time and temperature intervals.

No statistics could be done for Acetal and DEHT nail lacquer formulations as no leaching was detected.

## 7.6 Conclusions

Maximum absorbance levels were established for Acetal, DBP and DEHT by means of UV-VIS spectroscopy. It was further established that the SPE method is an efficient method to be used to determine trace amount of analyte (plasticiser) in a sample matrix.

UPLC calibration curves obtained for Acetal, DBP and DEHT were found to be linear over the experiment concentration range of 0.2 µg/ml to 4 µg/ml. All regression correlation coefficients ($R^2$) were ≥ 0.99. Good linearity was therefore obtained for the three plasticisers.

A gradual decrease in leaching of DBP from nail lacquer was observed over the three day incubation period at 50 °C. It was found that there is a statistically significant difference in leaching of DBP over the 24, 48 and 72 H time conditions at elevated temperature. It was observed that leaching of DBP from the nail formulations gradually slowed down over the studied time period. Samples incubated at normal body temperature, i.e. 31 °C, showed peak areas below the LOQ for the DBP nail lacquer formulation. Results obtained at 50 °C incubation temperature were almost negligible. Results found were in good agreement with previous studies done, showing evidence of DBP being sensitive to temperature changes and that elevated temperature enhances the leaching process [157, 159].

No leaching out of the nail formulation for Acetal and DEHT could be detected over 24, 48 or 72 H at either 31 or 50 °C.

Previous studies by Patuska and Shashoua *et al.* [140, 160] show that lower MW plasticisers have a higher tendency of leaching into an aqueous environment. This

is in agreement with leaching results obtained for DBP, being the lowest MW plasticiser of the three investigated.

On the contrary, the bigger the MW of the plasticiser the more difficult exudation of plasticiser becomes. This implies that the resistance of extraction is being increased as the MW of the plasticiser is increased. The longer the aliphatic hydrocarbon chain emanating from the diester group attached to the benzene ring, the less likely is the plasticiser to leach out of the formulation, as in the case of DEHT [116, 157]. Acetal also exhibits a long hydrocarbon chain and therefore is also less likely to leach into an aqueous environment.

DEHT exhibits a higher MW than Acetal which furthermore contributes to the retardation of leaching. Results obtained are evidence thereof since no leaching was detected at either 31 °C nor at 50 °C for DEHT nail lacquer formulation. It was also confirmed that DEHT, being the highest MW plasticiser of the three investigated, has a higher resistance of extraction into an aqueous environment [161]. DEHT has the longest hydrocarbon chains in the *p*-positions of the benzene ring when compared to DBP and PMD-citronellal acetal and therefore, theoretically, should reflect the lowest leaching results. On the contrary, DBP, despite of it exhibiting shorter carbon chains emanating from the diester groups of the benzene ring, could leach more easily from the formulation due to its lower MW compared to that of DEHT. Elevated temperature also enhances the leaching process [158], as found in the case of DBP leaching results at 50 °C.

Since the MW of Acetal and DEHT is higher than that of DBP, the likelihood of leaching into an aqueous environment is further reduced.

Research done by Kastner *et al.* [162] found that DINCH, having structural similarities to PMD-citronellal acetal showed no leaching after three weeks. DINCH plasticiser exhibits long hydrocarbon chains attached to each of the diester groups.

At the end of each of these carbon chains, this molecule has methyl groups contributing to the hydrophobicity of the plasticiser, resulting in a decrease in leaching into an aqueous environment. Therefore, methyl end groups add to the stabilising effect towards hydrolysis. These 'hydrolysis-protecting' methyl end groups, as well as the added advantage of combining a slightly branched polymer structure, could have a synergistic effect of 'prevention' or 'protection' against hydrolysis [163]. Similarly, the PMD-citronellal acetal molecule also exhibits methyl groups at the end of its hydrocarbon chain. Furthermore, two methyl groups are attached to the 1,3-dioxane ring and another methyl group attached to the cyclohexane ring. It is postulated that these methyl groups contributed to the hydrophobicity of the Acetal molecule and therefore leaching was hindered from the nail lacquer formulation.

The finding in this thesis that no leaching was found from any of the PMD-citronellal acetal plasticised nail formulations is therefore in good agreement with research done by Kastner *et al*. [162].

All air-dried lip coat formulations disintegrated in the saliva simulant, before leaching studies could be performed.

# CHAPTER 8
# CONCLUSION

The use of certain cosmetic products containing phthalate plasticisers poses a possible health risk to humans.

Previous research done at InnoVenton has shown that PMD-citronellal acetal could be a suitable replacement for dibutyl phthalate as a bio-plasticizer since it exhibits similar plasticising properties to that of DBP in selected cosmetic products as well as in PVC e.g. imparting flexibility in these products.

In this thesis, the chemical stability of PMD-citronellal acetal over a three month period at elevated temperature was evaluated and compared with two other commonly used plasticisers viz. DBP (phthalate) and DEHT (non-phthalate) in two different cosmetic formulations, viz. a nail lacquer and a lip coat.

The following objectives of the dissertation that were identified at the start of the thesis, are listed below:

- compare the physical performance of PMD-citronellal acetal, DBP and DEHT within nail and lip formulations
- determine the chemical stability of the neat plasticisers and cosmetic formulations by means of FTIR-ATR
- evaluate leaching rates of the three plasticisers from nail lacquer and lip coat formulations by means of SPE and UPLC analyses

## 8.1 Physical performance

Two cosmetic formulations, viz. a nail lacquer and a lip coat formulation were prepared with the three different plasticisers in order to investigate and compare their physical performance properties. A blank formulation without plasticiser was also prepared.

### 8.1.1 Flexibility

Flexibility of the Nail Blank formulation and Lip Blank formulations failed at all 5 stages compared to the plasticised formulations. As expected, the Blank nail lacquer formulation cracked and peeled because of the absence of plasticiser in this formulation. The DEHT molecule has the longest carbon chains emanating from each side of the ester in the *p*-positions and therefore imparts additional free volume in formulation 3. Theoretically, DEHT resulted in the most flexible film, in comparison to the films obtained from DBP and Acetal, respectively. It can be concluded that flexibility for all plasticised formulations remained stable at RT and elevated temperature, as no cracking of any films was observed using the conical mandrel test.

Therefore, the longer the alkyl chain of the plasticiser (as in the case of DEHT) the more free volume it will occupy inside the polymer matrix, and the more efficient the plasticised polymer should behave. However, in the case of cross-linking in the nail lacquers, where the plasticiser is being 'trapped' between the polymer chains, the opposite effect takes place. Therefore it is hypothesised that cross-linking decreases the free volume in which the plasticiser resides, causing less mobility thereof, resulting in a 'stiffer' (less flexible) film. However, an increase in temperature causes free volume to increase, which compensates for the reduced mobility of the plasticiser caused by cross-linking. It is therefore postulated that

elevated temperature outperformed the effect of cross-linking. As a result, all plasticised nail lacquer films remained flexible over the three month elevated incubation period. It can therefore be deducted that elevated temperature and plasticisation efficiency are competing for 'free volume' and that cross-linking impedes the desired plasticisation function.

### 8.1.2 Adhesion

By investigating adhesion, it was found that the Blank formulation failed adhesion. DBP, DEHT and Acetal formulations were considered to have very strong levels of adhesion over the first two months elevated incubation period. At Stage T2 and Stage T3 incubation at elevated temperature, the adhesion performance of Nail DBP deteriorated. DEHT and Acetal formulations remained stable as the control sample was again evaluated after the three month RT storage condition, as well as at elevated temperature.

It is postulated that this performance characteristic is related to the leaching of DBP plasticiser from the nail lacquer at elevated temperature. Furthermore, it was found that the Nail Blank formulation failed adhesion after the three month elevated incubation period, since cross-linking increased with elevated temperature and as a result, the entire film cracked due to brittleness (and the absence of plasticiser).

### 8.1.3 Homogeneity, colour and odour

The homogeneity performed at all stages did not show any settlement for any lip nor nail formulations.

The Nail and Lip Blank, as well as the nail and lip plasticised formulations remained unchanged with regard to colour and odour over the entire incubation period. Elevated temperature and storage time therefore had no influence on the organoleptic properties of any formulation.

### 8.1.4 Hardness

Acetal plasticised nail formulations outperformed both DEHT and DBP formulations with regard to hardness throughout the three month elevated and RT incubation period. This could be due to cross-linking of the film formers, nitrocellulose and melamine formaldehyde, which prevented segmental mobility of the plasticiser in the polymer matrix, leading to harder films. Conversely, Nail DEHT resulted in a 'softer' plasticised nail lacquer, compared to Acetal nail lacquer. This could be attributed to the DEHT molecule exhibiting the longest hydrocarbon chains emanating from the ester carbonyl group occupying the *p*-positions on the benzene ring, hence imparting a higher degree of plasticising efficiency to the formulation. The latter explanation demonstrates the importance of selecting a plasticiser with the appropriate molecular structure for its intended function in a certain cosmetic formulation.

Lip coat formulations lack cross-linking between PVP and HPC, instead, hydrogen-bonding takes place between these film formers. The mobility of plasticiser within the lip formulations is, therefore, less restricted than in the case of cross-linked nail lacquers. The absence of cross-linking in all lip coat formulations resulted in excellent adhesion and flexibility throughout the three month incubation period at RT and elevated temperature. Films were all 'softer' than those of plasticised nail lacquers, with Acetal lip coat formulation showing the softest films obtained. This is in agreement with the molecular structure of Acetal, i.e. the ether (O-C-O) moiety of the 1,3-dioxane ring attached to the cyclohexane ring of PMD-citronellal

acetal, exhibits electronegativity due to the lone pairs of electrons surrounding the oxygen molecules, leading to a 'softer' film, in comparison to the DBP and DEHT lacking the 1,3-dioxane ring as part of their molecular structures.

### 8.1.5 NVC

Both DEHT nail and lip formulations showed stable but slightly higher non-volatile content results than the other formulations throughout RT and elevated incubation periods. This could be attributable to the fact that DEHT, being the highest molecular weight plasticiser of the three pertaining to this thesis, exhibits the highest viscosity and boiling point and as a result has a higher surface tension, hindering intermolecular bonds to break. A higher percentage of non-volatile content (solid matter) was therefore retained for DEHT lip coat and DEHT nail lacquer formulations. The Blank lip coat and Blank nail lacquer showed the lowest NVC due to the absence of plasticiser in their formulations.

### 8.1.6 Viscosity

From the observed results for viscosity, both Blank lip coat and Blank nail lacquer showed the highest values compared to the corresponding plasticised formulations. This correlates well with theory that plasticisers impart viscosity.

Acetal plasticised lip coat formulation exhibited the lowest viscosity of all the lip coat formulations. This lower viscosity compared well with the lowest hardness results obtained for this formulation. The molecular structure of the Acetal molecule exhibits an ether moiety (O-C-O) as part of its 1,3-dioxane ring. This contributes to more electronegativity due to the lone pairs of electrons on the oxygen atoms, resulting in a higher fluidity formulation. The Acetal formulation therefore imparts a more flexible and 'softer' formulation for the lip coat.

### 8.1.7 pH

Neither RT nor elevated temperature had an effect on the pH of any cosmetic formulation tested at the different time interval periods. Addition of all plasticisers caused a lowering of the Blank pH. Plasticised nail formulations exhibited lower pH values than the lip formulations and could, therefore, be considered to be more acidic.

## 8.2 Chemical stability

### 8.2.1 Chemical stability of neat plasticisers

FTIR-ATR analysis was used to determine the chemical stability of the neat plasticisers in their liquid form over a three month period. These samples, in conjunction with their respective plasticised cosmetic formulations, viz. a nail lacquer and a lip coat, were also analysed in their liquid form for their chemical stability over the same time period. Blank formulations (formulations without plasticiser) of each were stored at RT and elevated temperature (the latter referred to as 'accelerated ageing') and tested at the respective time intervals.

FTIR overlays of the three neat plasticisers were done in order to detect any shift in spectral bands at elevated temperature at each monthly interval. The control samples stored at RT were used as a control at the start (Stage T0) and end of the three month period Stage T0 (end).

Each of the control neat plasticisers, DBP, DEHT and Acetal displayed perfect overlays over the three month incubation period at RT Stage T0 versus RT Stage T0 (end), rendering all the control neat plasticisers as chemically stable. These

spectra also displayed perfect overlays with their respective neat plasticisers incubated at elevated temperature over the three month period. It can, therefore, be concluded that elevated temperature has got no influence on the chemical stability of any of the above neat plasticisers since no shift or change in band intensity occurred at any stage of incubation temperature.

DPB, DEHT and Acetal were chemically stable at RT and elevated temperature over a three month incubation period.

FTIR-ATR was utilised as analytical tool to distinguish between aromatic diester molecules (DBP and DEHT), i.e. the carbonyl group (C=O) observed at ~1720 $cm^{-1}$ and the absence thereof in the non-aromatic cyclohexane-1,3-dioxane ring (PMD-citronellal acetal). FTIR-ATR spectra were used to distinguish between an *o*-diester phthalate and a *p*–terephthalate. The latter molecule (DEHT) showed a single spectral band at ~1580 $cm^{-1}$, whereas the FTIR spectrum for DBP exhibited a doublet at ~1580 $cm^{-1}$ and at ~1600 $cm^{-1}$, indicative of the quadrant stretch ring mode of the aromatic ring.

### 8.2.2 Chemical stability of formulations

#### 8.2.2.1 Nail lacquer

Perfect FTIR spectra overlays were obtained for all nail lacquer formulations over the three month incubation period at RT and elevated temperature.

Elevated temperature had no effect on the chemical stability of the three plasticised nail lacquer formulations over the three month incubation period. The control of each formulation stored at RT also remained chemical stable, since no shift of any spectral band was observed at any test stage interval.

In addition to FTIR spectra showing evidence that an 'interactive molecule' (plasticiser) was added to each cosmetic formulation, physical performance results indicated secondary proof thereof, as each physical performance result reflected a specific plasticiser function being executed, in comparison to the Blank without added plasticiser.

No band shift has taken place for any plasticised nail formulation during incubation period intervals at neither RT nor elevated temperature, rendering them as chemical stable. In comparison to the Nail Blank, the spectral band changes within each respective plasticised formulation remained consistent over the incubation period. These changes were the result of polymer-plasticiser interaction.

DBP, DEHT and Acetal nail lacquer formulations remained chemically stable over the three month incubation period since the control of each plasticised formulation at Stage T0 displayed a perfect spectral overlay with the control at Stage T0 (end) at RT, as well as at Stage T3 at elevated temperature. No shift of spectral bands was observed. Temperature and the three month incubation period, therefore, had no influence on the chemical stability of any of the three formulations. However, the Blank nail lacquer formulation showed interaction with each plasticiser, namely DBP, DEHT and Acetal due to changes in spectral intensities of functional groups. For each plasticised nail formulation, these interactions remained stable throughout the incubation period at RT and elevated temperature. All plasticisers showed interaction with the Blank nail lacquer since spectral band intensity differences were evident from their respective spectral overlays. This was found to be indicative of polymer-plasticiser (blank and plasticiser) interaction and subsequently, proof of the addition of plasticiser to its respective formulation. A decrease in secondary amide band at ~1551 $cm^{-1}$ was observed for DEHT and Acetal (both being more hydrophobic than DBP, respectively) plasticised nail formulations. On the contrary, the Blank nail lacquer and DBP nail lacquer displayed a perfect overlay at this same wavenumber (~1551 $cm^{-1}$).

A decrease in the primary amide band at ~1659 $cm^{-1}$ was observed for all nail lacquer formulations relative to the Blank, possibly due to a loss of a proton from the primary amide. The spectrum obtained for DEHT nail lacquer was the only nail formulation which revealed a CH bending band shift to a lower wavenumber (~739 $cm^{-1}$ to ~734 $cm^{-1}$). This CH spectral band observed was the narrowest and most intense due to the DEHT plasticiser molecule exhibiting the longest hydrocarbon alkyl chains emanating from the ester groups, each occupying the *p*-position on the benzene ring. The Acetal nail lacquer formulation, similar in shape to the Nail Blank, displayed a slightly smaller CH bending band at ~740 $cm^{-1}$. It is postulated that interaction of the Acetal molecule is hindered by its 1,3-dioxane ring, associated with the hydrocarbon chain attached to it.

The band occurring at ~817 $cm^{-1}$, being the identifying characteristic band of an amino-substituted-triazine ring, showed a decrease in band size relative to the Nail Blank for all plasticised nail lacquers, indicative of polymer-plasticiser interaction.

8.2.2.2 Lip coat

FTIR-ATR has shown that the Blank lip coat, Acetal lip coat, DBP lip coat and DEHT lip coat displayed perfect overlays at elevated temperature with their respective formulations stored at RT as control samples, rendering all lip formulations chemically stable.

However, upon overlaying each plasticised lip coat formulation with the Blank lip coat formulation, it was clear that each plasticiser interacted with the Blank lip coat (polymer-plasticiser interaction) in a similar fashion and to the same extent, at both RT and elevated temperature.

Both DBP lip coat and DEHT lip coat formulations revealed a shift in their carbonyl spectral bands from lower to higher wavenumbers, respectively, in comparison to the carbonyl groups of neat DBP and neat DEHT.

The 5% addition of each DBP and DEHT plasticisers, revealed its ester carbonyl spectral band as a 'shoulder' on the left side of the carbonyl amide band of the Blank lip coat at ~1664 $cm^{-1}$. On the contrary, the 5% addition of Acetal plasticiser to its respective Lip formulation did not exhibit this 'shoulder', due to the lack of a carbonyl functional group in its molecular structure. This 'shoulder' was also absent in the unplasticised Lip Blank.

An increase in (C-N) band intensity at ~1290 $cm^{-1}$ was observed for DEHT lip coat and DBP lip coat. However, no change in band intensity was found for Acetal lip coat at ~1290 $cm^{-1}$ due to the absence of carbonyl amide interaction. The Acetal molecule is therefore hindered to donate electrons to the PVP molecule of the Lip Blank.

All three Lip formulations showed a slight decrease in the carbonyl amide band at ~1664 $cm^{-1}$ in comparison to the unplasticised Lip Blank, possibly due to some interaction. However, the Acetal lip coat formulation displayed the least interaction presumably due to its molecular structure lacking the carbonyl ester groups being part of the molecular structure of both DBP and DEHT.

It can therefore be concluded that all Lip formulations remained chemically stable over the three month incubation period at elevated temperature. Only in comparison to the Lip Blank, polymer-plasticiser interaction was noticeable, the least interaction between Lip Acetal and Lip Blank, as explained above.

## 8.3 Leaching studies

Chemical stability was evaluated by means of leaching tests at two temperatures 31 °C and 50 °C, and three time intervals, 24, 48 and 72 hours. Analytical techniques, namely SPE and UPLC, were used to establish leaching of plasticiser from the plasticised nail lacquer films.

UPLC calibration curves obtained for Acetal, DBP and DEHT were found to be linear over the experimental concentration range of 0.2 µg/ml to 4.0 µg/ml. All regression correlation coefficients ($R^2$) were ≥ 0.99. Good linearity was therefore obtained for the three plasticisers.

Acetal displayed LOD and LOQ values of 0.025 µg/ml and 0.081 µg/ml, respectively. For DBP the LOD was determined as 0.01 µg/ml and the LOQ as 0.05 µg/ml. The LOD value for DEHT was 0.01 µg/ml and the LOQ value 0.04 µg/ml, respectively.

It was observed that leaching of the DBP from the nail lacquer formulation gradually slowed down over the studied time period. It was found that there is a statistical significant difference in leaching of DBP over the 24, 48 and 72 hour's time conditions at elevated temperature. A gradual decrease in leaching of DBP from nail lacquer is observed over the three day incubation period at 50 °C. Samples incubated at normal body temperature, i.e. 31 °C, showed peak areas below the LOQ for the DBP nail lacquer formulation.

No leaching out of the nail formulation for Acetal and DEHT could be detected over 24, 48 or 72 hours at either 31 °C or 50 °C. DEHT exhibits long hydrocarbons chains emanating from the diester groups situated at both *p*-positions of the benzene ring of the molecule, contributing to its hydrophobicity, hence no leaching

took place into an aqueous environment. The high MW of DEHT could also be a contributing factor to this finding. Acetal also exhibits a long hydrocarbon chain and therefore is also less likely to leach into an aqueous environment. As a result, the DEHT nail lacquer and Acetal nail lacquer formulations both preserved their plasticising properties by remaining inside their polymer film, hence prolonging the lifespan of these nail lacquers that come into contact with water upon washing of hands.

On the contrary, DBP, despite of it exhibiting shorter carbon chains emanating from the diester groups on the benzene ring, leached from the formulation due to its lower MW in comparison to that of DEHT. Furthermore, elevated temperature enhanced the leaching process, as found in the case of DBP leaching at 50 °C.

Leaching tests for lip coat formulations could not be executed and subsequently different applications for this formulation were proposed as part of 'future work to be done'. The water solubility properties of the lip coat formulation could lay the foundation for future pharmaceutical formulations in combination with an active pharmaceutical ingredient for rapid sublingual administration and release of the drug. This is to the advantage of the elderly and bedridden patient to whom swallowing a tablet with water can be difficult.

Both DEHT as well as PMD-citronellal acetal can be incorporated as bio-plasticisers (substituting DBP) into a nail lacquer formulation.

From the research findings, the research hypothesis can be answered. FTIR analyses showed that all the plasticised lip and nail formulations remained chemically stable over the studied incubation period at elevated temperature. PMD-citronellal acetal, DEHT and DBP remained chemically stable. However, elevated temperature did have an influence on the physical performance of the cosmetic formulations. SPE and UPLC analyses revealed that only DBP leached

from the Nail DBP formulation. Since PMD-citronellal acetal and DEHT did not leach from the nail lacquer, they preserved their plasticising properties. Therefore, it can be concluded that PMD-citronellal acetal and DEHT can be selected as bio-plasticisers based on their performance stability and non-leaching criteria, and aid in the selection of alternative plasticisers to the toxic DBP.

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# APPENDIX A: PHYSICAL PERFORMANCE TESTS

## Appendix A: Table 1 Flexibility test values for three formulations

| Stage: TO | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 4** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 5** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 6** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

| Stage: T1 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 4** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 5** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 6** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

| T2 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 4** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 5** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 6** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

| T3 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 4** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 5** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 6** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

| T0 (end) | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 2** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 3** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 4** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 5** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |
| **Replicate 6** | Fails | Fails | Pass | Pass | Pass | Pass | Pass | Pass |

## Appendix A: Table 2 Adhesion test values for three formulations

| Stage: TO | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 2** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 3** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 4** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 5** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 6** | Fails - 2 B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

| Stage: T1 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 2** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 3** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 4** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 5** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 6** | Fail - 2 B | Fail - 2 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

| Stage: T2 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 2** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 3** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 4** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 5** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 6** | Fail - 2 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

| Stage: T3 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fail - 1 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Replicate 2** | Fail - 1 B | Fail - 2 B | Pass - 4B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 3** | Fail - 1 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 4** | Fail - 1 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 5** | Fail - 1 B | Fail - 2 B | Pass - 4 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 6** | Fail - 1 B | Fail - 2 B | Pass - 4B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

| **Stage: T0 (end)** | **Blank FORMULATION 1** | | **DBP FORMULATION 2** | | **DEHT FORMULATION 3** | | **Acetal FORMULATION 4** | |
|---|---|---|---|---|---|---|---|---|
| **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **Replicate 1** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 2** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 3** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 4** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 5** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |
| **Replicate 6** | Fail - 1B | Fails – 2B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B | Pass - 5 B |

## Appendix A: Table 3 Hardness test values for three formulations

| TEST | Stage: TO | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| HARDNESS | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| | Replicate 1 | 52 | 40 | 30 | 24 | 26 | 21 | 34 | 20 |
| | Replicate 2 | 52 | 41 | 30 | 24 | 25 | 22 | 34 | 19 |
| | Replicate 3 | 53 | 41 | 31 | 24 | 26 | 21 | 35 | 20 |
| | Replicate 4 | 53 | 41 | 30 | 24 | 26 | 21 | 35 | 19 |
| | Replicate 5 | 52 | 41 | 31 | 23 | 26 | 22 | 34 | 20 |
| | Replicate 6 | 53 | 41 | 31 | 23 | 26 | 22 | 35 | 20 |
| MEAN | | 53 | 41 | 31 | 24 | 26 | 22 | 35 | 20 |
| SD | | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |

| TEST | Stage: T1 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| HARDNESS | Replicate 1 | 63 | 42 | 31 | 24 | 25 | 20 | 35 | 18 |
| | Replicate 2 | 62 | 42 | 32 | 24 | 26 | 20 | 35 | 18 |
| | Replicate 3 | 63 | 43 | 32 | 23 | 26 | 19 | 34 | 18 |
| | Replicate 4 | 63 | 44 | 31 | 22 | 25 | 20 | 33 | 19 |
| | Replicate 5 | 62 | 44 | 31 | 23 | 26 | 21 | 34 | 18 |
| | Replicate 6 | 62 | 44 | 31 | 23 | 26 | 20 | 33 | 19 |
| MEAN | | 63 | 43 | 31 | 23 | 26 | 20 | 34 | 18 |
| SD | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| TEST | T2 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| HARDNESS | Replicate 1 | 67 | 42 | 34 | 23 | 28 | 21 | 36 | 18 |
| | Replicate 2 | 67 | 42 | 34 | 23 | 28 | 21 | 37 | 18 |
| | Replicate 3 | 67 | 43 | 35 | 22 | 28 | 21 | 37 | 18 |
| | Replicate 4 | 66 | 43 | 35 | 23 | 27 | 20 | 36 | 17 |
| | Replicate 5 | 66 | 43 | 35 | 23 | 28 | 20 | 36 | 17 |
| | Replicate 6 | 66 | 43 | 35 | 23 | 28 | 20 | 36 | 17 |
| MEAN | | 67 | 43 | 35 | 23 | 28 | 21 | 36 | 18 |
| SD | | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |

| TEST | T3 | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| HARDNESS | Replicate 1 | 72 | 43 | 35 | 24 | 30 | 21 | 38 | 18 |
| | Replicate 2 | 73 | 43 | 35 | 24 | 31 | 21 | 37 | 18 |
| | Replicate 3 | 73 | 43 | 36 | 23 | 31 | 21 | 38 | 17 |
| | Replicate 4 | 72 | 42 | 35 | 24 | 31 | 20 | 38 | 18 |
| | Replicate 5 | 72 | 43 | 35 | 24 | 30 | 21 | 38 | 18 |
| | Replicate 6 | 72 | 42 | 35 | 24 | 31 | 21 | 37 | 18 |
| MEAN | | 72 | 43 | 35 | 24 | 31 | 21 | 38 | 18 |
| SD | | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |

| TEST | T0 [100] | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| HARDNESS | Replicate 1 | 53 | 40 | 32 | 23 | 26 | 21 | 35 | 19 |
| | Replicate 2 | 52 | 41 | 32 | 23 | 26 | 22 | 35 | 20 |
| | Replicate 3 | 52 | 41 | 33 | 23 | 26 | 22 | 36 | 19 |
| | Replicate 4 | 54 | 42 | 31 | 24 | 26 | 23 | 36 | 20 |
| | Replicate 5 | 54 | 42 | 31 | 23 | 26 | 22 | 36 | 20 |
| | Replicate 6 | 53 | 42 | 31 | 23 | 26 | 22 | 36 | 20 |
| MEAN | | 53 | 41 | 32 | 23 | 26 | 22 | 36 | 20 |
| SD | | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |

## Appendix A: Table 4 NVC test values for three formulations

| TEST | | Blank | | DBP | | DEHT | | Acetal | |
|---|---|---|---|---|---|---|---|---|---|
| | Stage: TO | FORMULATION 1 | | FORMULATION 2 | | FORMULATION 3 | | FORMULATION 4 | |
| NVC (%) | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| | Replicate 1 | 10.81 | 11.04 | 17.75 | 16.02 | 18.25 | 16.05 | 17.48 | 16.02 |
| | Replicate 2 | 10.76 | 11.06 | 17.70 | 16.03 | 18.20 | 16.06 | 17.50 | 16.03 |
| | Replicate 3 | 10.81 | 10.98 | 17.75 | 16.06 | 18.34 | 16.11 | 17.60 | 16.05 |
| | Replicate 4 | 10.82 | 10.99 | 17.78 | 16.05 | 18.28 | 16.12 | 17.62 | 16.06 |
| | Replicate 5 | 10.78 | 10.97 | 17.74 | 16.04 | 18.22 | 16.05 | 17.55 | 16.00 |
| | Replicate 6 | 10.81 | 10.99 | 17.89 | 16.05 | 18.26 | 16.07 | 17.58 | 16.01 |
| MEAN | | 10.80 | 11.01 | 17.77 | 16.04 | 18.26 | 16.08 | 17.56 | 16.03 |
| SD | | 0.02 | 0.04 | 0.06 | 0.01 | 0.05 | 0.03 | 0.06 | 0.02 |

| T1 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
|---|---|---|---|---|---|---|---|---|---|
| NVC (%) | Replicate 1 | 10.75 | 10.99 | 17.88 | 16.04 | 18.22 | 16.11 | 17.51 | 15.99 |
| | Replicate 2 | 10.79 | 10.97 | 17.80 | 16.04 | 18.24 | 16.09 | 17.52 | 15.97 |
| | Replicate 3 | 10.81 | 11.02 | 17.77 | 16.06 | 18.30 | 16.08 | 17.62 | 15.95 |
| | Replicate 4 | 10.84 | 11.04 | 17.78 | 16.01 | 18.26 | 16.03 | 17.64 | 15.98 |
| | Replicate 5 | 10.81 | 11.01 | 17.85 | 16.02 | 18.20 | 16.06 | 17.58 | 16.02 |
| | Replicate 6 | 10.82 | 11.01 | 17.82 | 16.05 | 18.25 | 16.01 | 17.57 | 16.03 |
| MEAN | | 10.80 | 11.01 | 17.82 | 16.04 | 18.25 | 16.06 | 17.57 | 15.99 |
| SD | | 0.03 | 0.02 | 0.04 | 0.02 | 0.03 | 0.04 | 0.05 | 0.03 |

| T2 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
|---|---|---|---|---|---|---|---|---|---|
| NVC (%) | Replicate 1 | 10.80 | 11.02 | 17.88 | 16.03 | 18.21 | 16.05 | 17.50 | 16.06 |
| | Replicate 2 | 10.79 | 11.04 | 17.85 | 15.97 | 18.22 | 16.01 | 17.54 | 16.01 |
| | Replicate 3 | 10.81 | 11.05 | 17.75 | 15.96 | 18.18 | 16.06 | 17.60 | 16.03 |
| | Replicate 4 | 10.84 | 10.99 | 17.77 | 16.02 | 18.17 | 16.01 | 17.62 | 16.01 |
| | Replicate 5 | 10.83 | 10.97 | 17.85 | 16.05 | 18.22 | 15.98 | 17.58 | 16.02 |
| | Replicate 6 | 10.85 | 11.03 | 17.81 | 16.01 | 18.21 | 15.97 | 17.66 | 15.98 |
| MEAN | | 10.82 | 11.02 | 17.82 | 16.01 | 18.20 | 16.01 | 17.58 | 16.02 |
| SD | | 0.02 | 0.03 | 0.05 | 0.04 | 0.02 | 0.04 | 0.06 | 0.03 |

| T3 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
|---|---|---|---|---|---|---|---|---|---|
| NVC (%) | Replicate 1 | 10.76 | 10.99 | 17.81 | 16.02 | 18.22 | 16.12 | 17.63 | 15.95 |
| | Replicate 2 | 10.72 | 11.05 | 17.86 | 15.99 | 18.26 | 16.09 | 17.59 | 15.99 |
| | Replicate 3 | 10.79 | 11.03 | 17.78 | 15.94 | 18.19 | 16.05 | 17.55 | 15.94 |
| | Replicate 4 | 10.89 | 11.01 | 17.72 | 16.04 | 18.15 | 16.03 | 17.61 | 16.02 |
| | Replicate 5 | 10.91 | 11.05 | 17.75 | 16.01 | 18.21 | 16.08 | 17.65 | 15.97 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Replicate 6** | 10.84 | 11.06 | 17.79 | 16.02 | 18.20 | 16.05 | 17.67 | 15.95 |
| **MEAN** | | 10.82 | 11.03 | 17.79 | 16.00 | 18.21 | 16.07 | 17.62 | 15.97 |
| **SD** | | 0.07 | 0.03 | 0.05 | 0.04 | 0.04 | 0.03 | 0.04 | 0.03 |

| **T0 (end)** | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
|---|---|---|---|---|---|---|---|---|---|
| **NVC** | **Replicate 1** | 10.75 | 11.01 | 17.85 | 16.06 | 18.25 | 16.11 | 17.48 | 16.01 |
| **(%)** | **Replicate 2** | 10.82 | 11.05 | 17.70 | 16.01 | 18.22 | 16.12 | 17.50 | 16.03 |
| | **Replicate 3** | 10.83 | 11.02 | 17.75 | 16.02 | 18.31 | 16.14 | 17.60 | 15.99 |
| | **Replicate 4** | 10.74 | 11.03 | 17.78 | 16.04 | 18.28 | 16.08 | 17.62 | 15.95 |
| | **Replicate 5** | 10.71 | 11.07 | 17.82 | 16.07 | 18.27 | 16.12 | 17.58 | 15.96 |
| | **Replicate 6** | 10.78 | 11.08 | 17.85 | 16.02 | 18.29 | 16.15 | 17.57 | 15.99 |
| **MEAN** | | 10.77 | 11.04 | 17.79 | 16.04 | 18.27 | 16.12 | 17.56 | 15.99 |
| **SD** | | 0.05 | 0.03 | 0.06 | 0.02 | 0.03 | 0.02 | 0.06 | 0.03 |

## Appendix A: Table 5 Viscosity test values for three formulations

| TEST | Stage: TO | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **[164]** | **Replicate 1** | 68.80 | 48.02 | 51.10 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 2** | 68.80 | 51.10 | 48.02 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 3** | 65.94 | 51.10 | 51.10 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 4** | 68.80 | 51.10 | 51.10 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 5** | 68.80 | 51.10 | 51.10 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 6** | 65.94 | 51.10 | 51.10 | 31.69 | 44.87 | 38.42 | 57.15 | 24.64 |
| Stage T1 | **MEAN** | **67.85** | **50.59** | **50.59** | **31.69** | **44.87** | **38.42** | **57.15** | **24.64** |
| | **SD** | **1.48** | **1.26** | **1.26** | **0.00** | **0.00** | **0.00** | **0.00** | **0.00** |
| | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **VISCOCITY** | **Replicate 1** | 68.80 | 51.10 | 51.10 | 44.87 | 44.87 | 38.42 | 57.15 | 24.64 |
| **[164]** | **Replicate 2** | 68.80 | 51.10 | 51.10 | 44.87 | 44.87 | 38.42 | 57.15 | 28.21 |
| | **Replicate 3** | 68.80 | 54.15 | 51.10 | 44.87 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 4** | 65.94 | 51.10 | 51.10 | 44.87 | 44.87 | 38.42 | 57.15 | 24.64 |
| | **Replicate 5** | 68.80 | 51.10 | 51.10 | 41.67 | 48.02 | 35.09 | 60.12 | 24.64 |
| | **Replicate 6** | 68.80 | 51.10 | 51.10 | 41.67 | 48.02 | 38.42 | 57.15 | 24.64 |
| Stage T2 | **MEAN** | **68.32** | **51.61** | **51.10** | **43.80** | **45.92** | **37.87** | **57.65** | **25.24** |
| | **SD** | **1.17** | **1.25** | **0.00** | **1.65** | **1.63** | **1.36** | **1.21** | **1.46** |
| | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **VISCOCITY** | **Replicate 1** | 71.41 | 51.10 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| **[164]** | **Replicate 2** | 68.80 | 51.10 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 28.21 |
| | **Replicate 3** | 68.80 | 51.10 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **Replicate 4** | 68.80 | 51.10 | 60.12 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **Replicate 5** | 71.41 | 54.15 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **Replicate 6** | 68.80 | 51.10 | 57.15 | 41.67 | 51.10 | 38.42 | 65.94 | 28.21 |
| Stage T3 | **MEAN** | **69.67** | **51.61** | **57.65** | **44.34** | **51.10** | **38.42** | **63.52** | **25.83** |
| | **SD** | **1.35** | **1.25** | **1.21** | **1.31** | **0.00** | **0.00** | **1.18** | **1.84** |
| | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
| **VISCOCITY** | **Replicate 1** | 79.99 | 57.15 | 57.15 | 44.87 | 57.15 | 38.42 | 68.80 | 28.21 |
| **[164]** | **Replicate 2** | 77.23 | 57.15 | 60.12 | 44.87 | 54.15 | 38.42 | 71.41 | 24.64 |
| | **Replicate 3** | 79.99 | 57.15 | 60.12 | 44.87 | 57.15 | 38.42 | 71.41 | 24.64 |
| | **Replicate 4** | 79.99 | 57.15 | 60.12 | 44.87 | 57.15 | 38.42 | 68.80 | 28.21 |
| | **Replicate 5** | 79.99 | 57.15 | 60.12 | 44.87 | 57.15 | 38.42 | 68.80 | 28.21 |
| | **Replicate 6** | 79.99 | 60.12 | 60.12 | 44.87 | 54.15 | 38.42 | 68.80 | 24.64 |
| Stage Blank | **MEAN** | **79.53** | **57.65** | **59.63** | **44.87** | **56.15** | **38.42** | **69.67** | **26.43** |
| | **SD** | **1.13** | **1.21** | **1.21** | **0.00** | **1.55** | **0.00** | **1.35** | **1.96** |

| | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
|---|---|---|---|---|---|---|---|---|---|
| **VISCOCITY [164]** | **Replicate 1** | 74.45 | 51.10 | 57.15 | 44.87 | 54.15 | 38.42 | 63.04 | 24.64 |
| | **Replicate 2** | 74.45 | 51.10 | 57.15 | 44.87 | 51.10 | 41.67 | 63.04 | 24.64 |
| | **Replicate 3** | 74.45 | 51.10 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **Replicate 4** | 74.45 | 51.10 | 57.15 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **Replicate 5** | 79.99 | 51.10 | 57.15 | 41.67 | 51.10 | 38.42 | 65.94 | 24.64 |
| | **Replicate 6** | 74.45 | 51.10 | 60.12 | 44.87 | 51.10 | 38.42 | 63.04 | 24.64 |
| | **MEAN** | **75.37** | **51.10** | **57.65** | **44.34** | **51.61** | **38.96** | **63.52** | **24.64** |
| | **SD** | **2.26** | **0.00** | **1.21** | **1.31** | **1.25** | **1.33** | **1.18** | **0.00** |

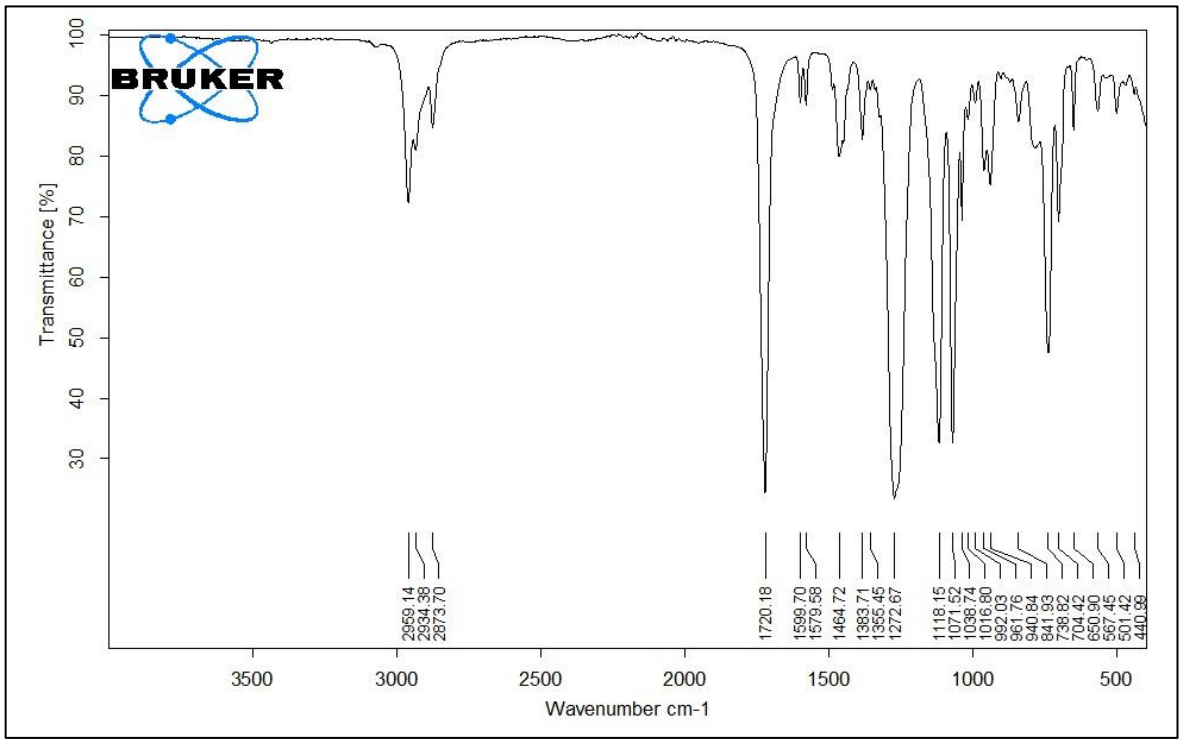
## Appendix A: Table 6 pH test values for three formulations

| TEST | | Blank FORMULATION 1 | | DBP FORMULATION 2 | | DEHT FORMULATION 3 | | Acetal FORMULATION 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Stage T0 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| pH | Replicate 1 | 5.6 | 6.2 | 3.9 | 5.8 | 4 | 5.9 | 3.8 | 5.7 |
| | Replicate 2 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 3 | 5.6 | 6.2 | 3.9 | 5.8 | 4 | 5.9 | 3.8 | 5.7 |
| | Replicate 4 | 5.6 | 6.2 | 3.9 | 5.8 | 4 | 5.9 | 3.8 | 5.7 |
| | Replicate 5 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 6 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| MEAN | | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| SD | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| T1 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| pH | Replicate 1 | 5.6 | **6.2** | 3.9 | 5.8 | 4.00 | 5.9 | 3.8 | 5.7 |
| | Replicate 2 | 5.6 | **6.2** | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 3 | 5.6 | **6.2** | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 4 | 5.6 | **6.2** | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 5 | 5.6 | **6.2** | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 6 | 5.6 | **6.2** | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| MEAN | | 5.60 | 6.20 | 3.90 | 5.80 | 4.00 | 5.90 | 3.80 | 5.70 |
| SD | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| T2 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| pH | Replicate 1 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 2 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 3 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 4 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 5 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 6 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| MEAN | | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| T3 | BATCH | NAIL | LIP | NAIL | LIP | NAIL | LIP | NAIL | LIP |
| pH | Replicate 1 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 2 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 3 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 4 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | Replicate 5 | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |

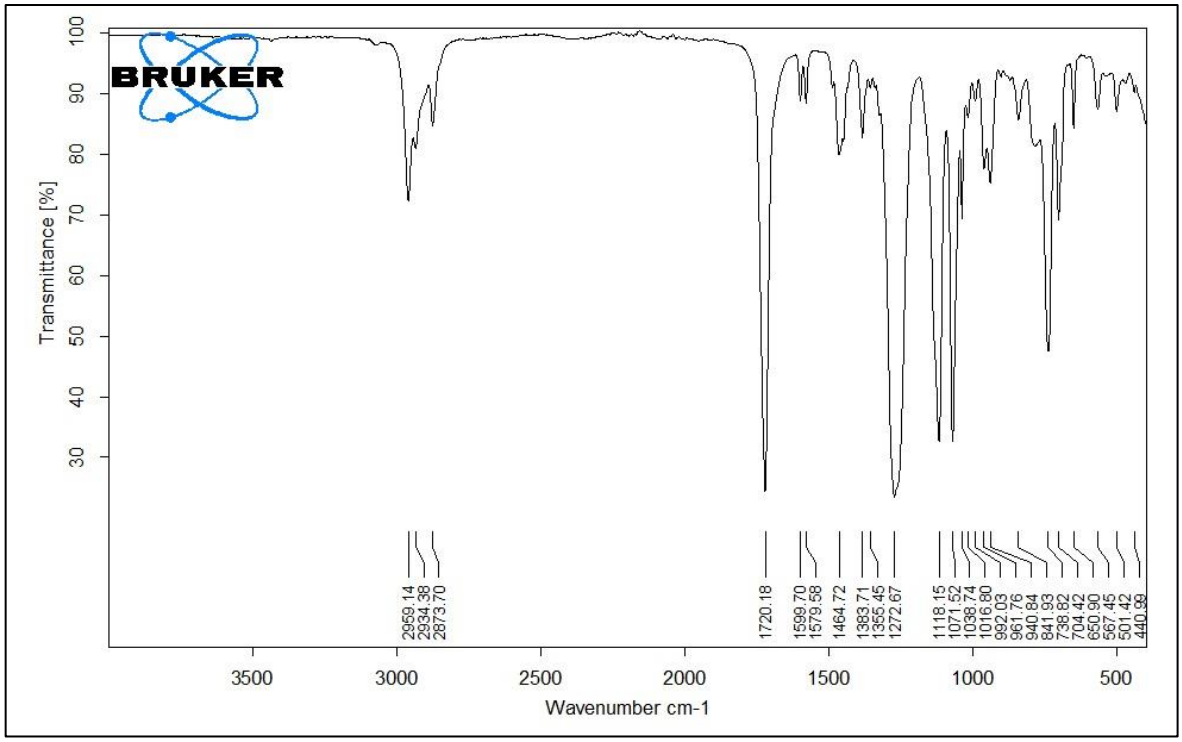
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Replicate 6** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| **MEAN** | | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |

| **T0 (end)** | **BATCH** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** | **NAIL** | **LIP** |
|---|---|---|---|---|---|---|---|---|---|
| **pH** | **Replicate 1** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | **Replicate 2** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | **Replicate 3** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | **Replicate 4** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | **Replicate 5** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| | **Replicate 6** | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |
| **MEAN** | | 5.6 | 6.2 | 3.9 | 5.8 | 4.0 | 5.9 | 3.8 | 5.7 |

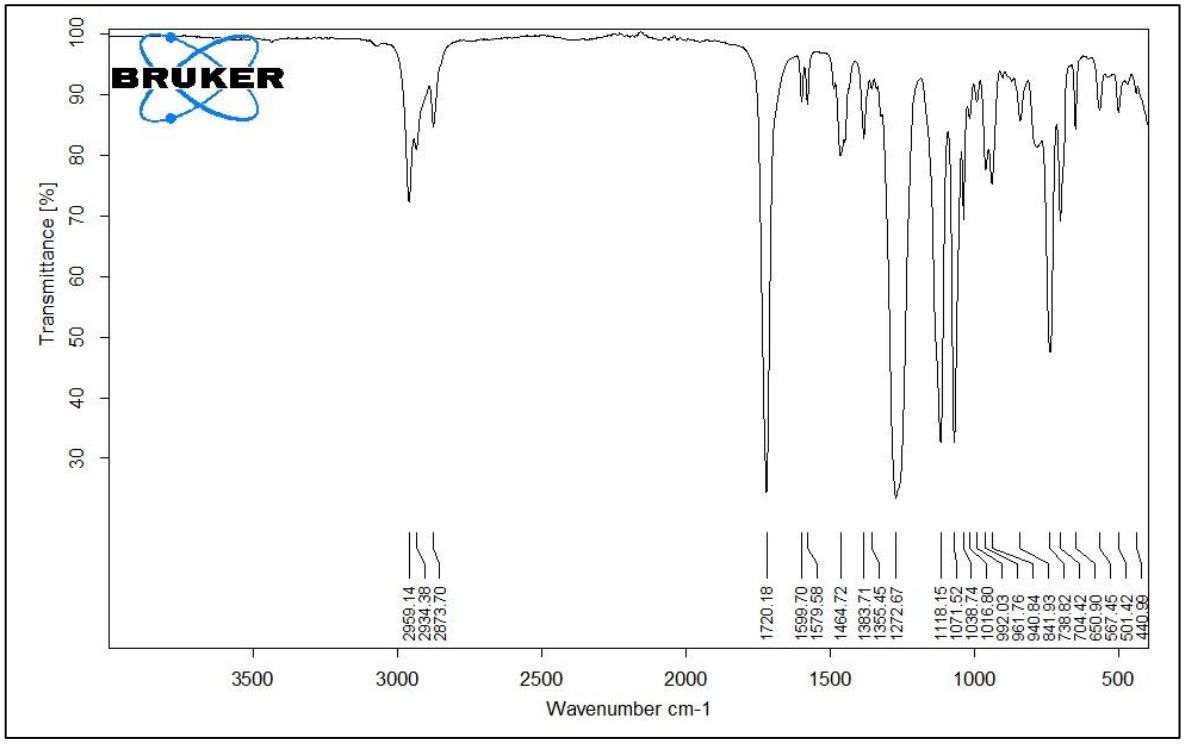
# APPENDIX B: FTIR-ATR ANALYSIS



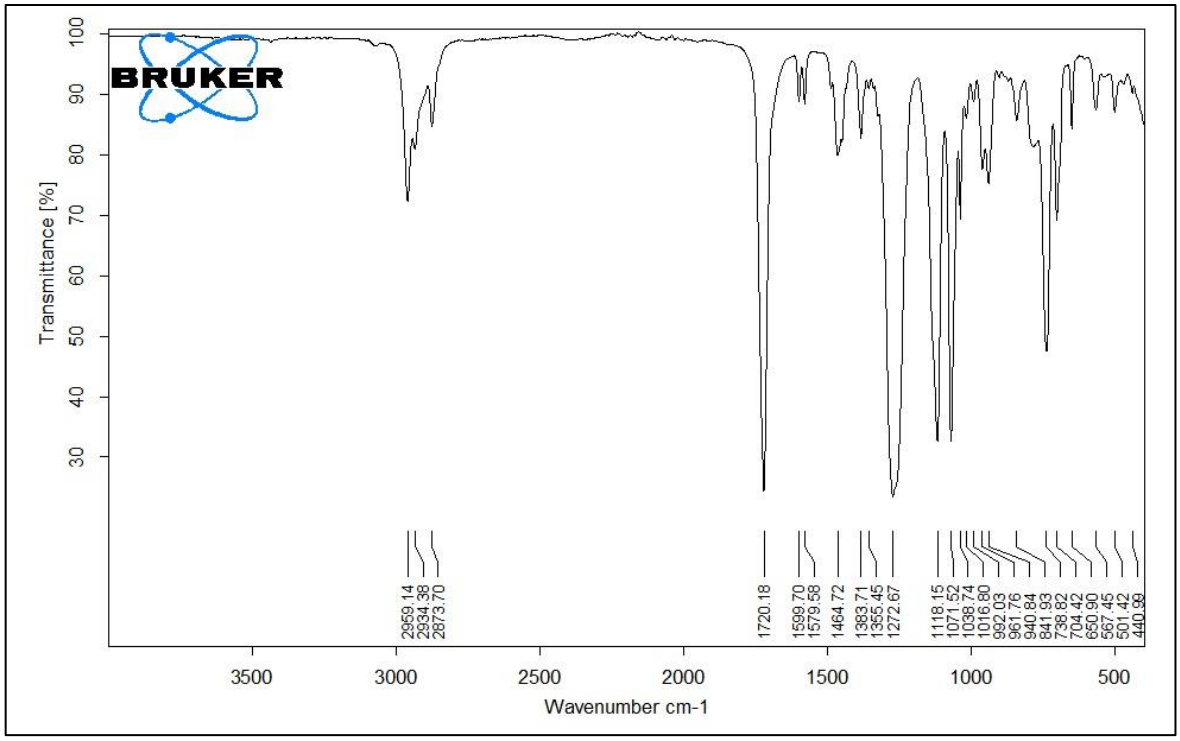

**Appendix B: Figure 1 FTIR spectrum of neat DBP control at T0**



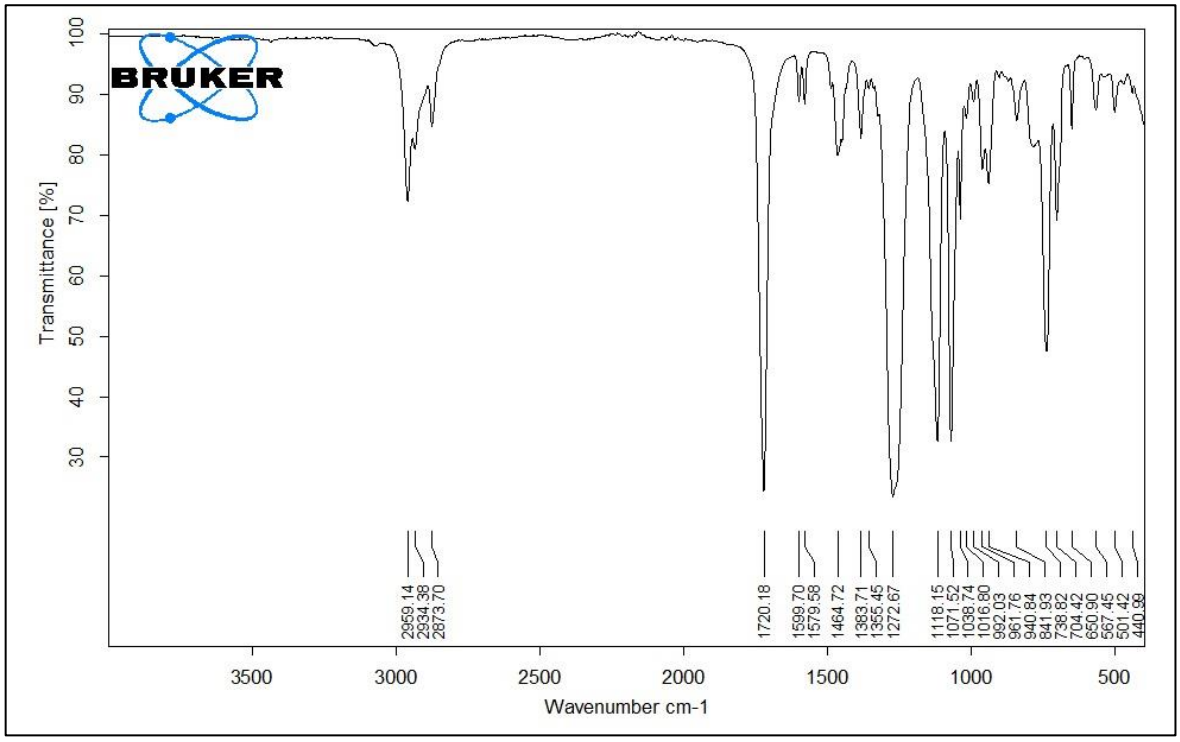

**Appendix B: Figure 2 FTIR spectrum of neat DBP elevated at T1**



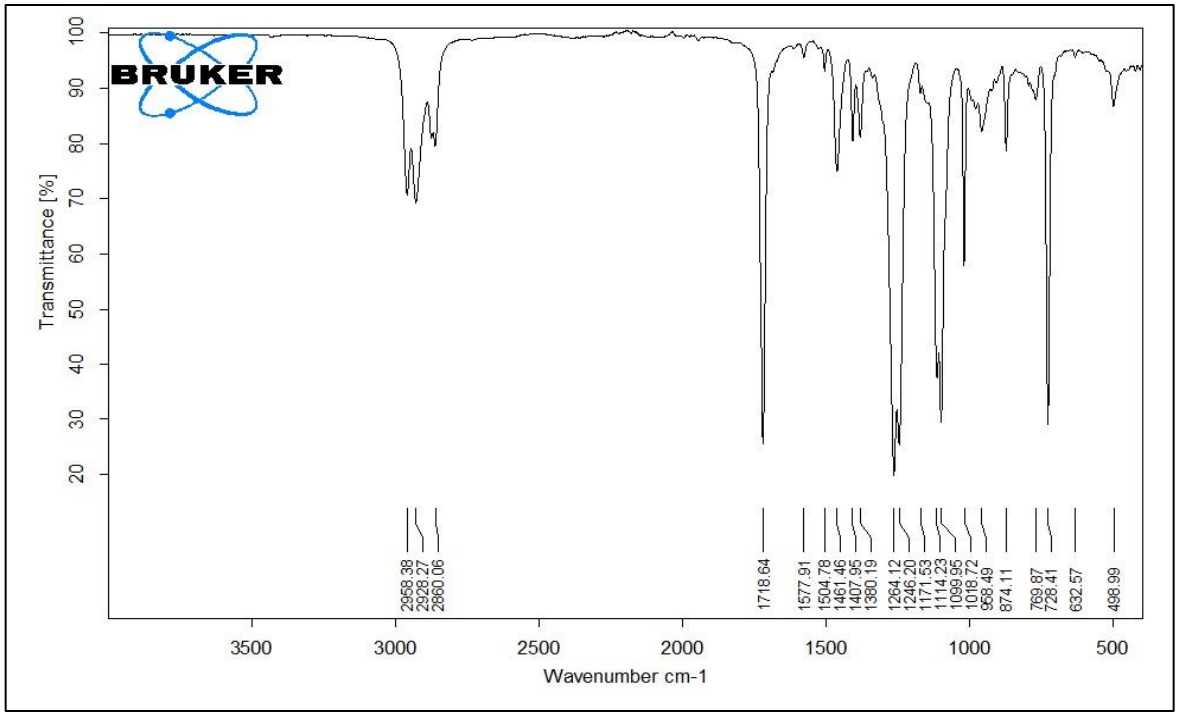

**Appendix B: Figure 3 FTIR spectrum of neat DBP elevated at T2**



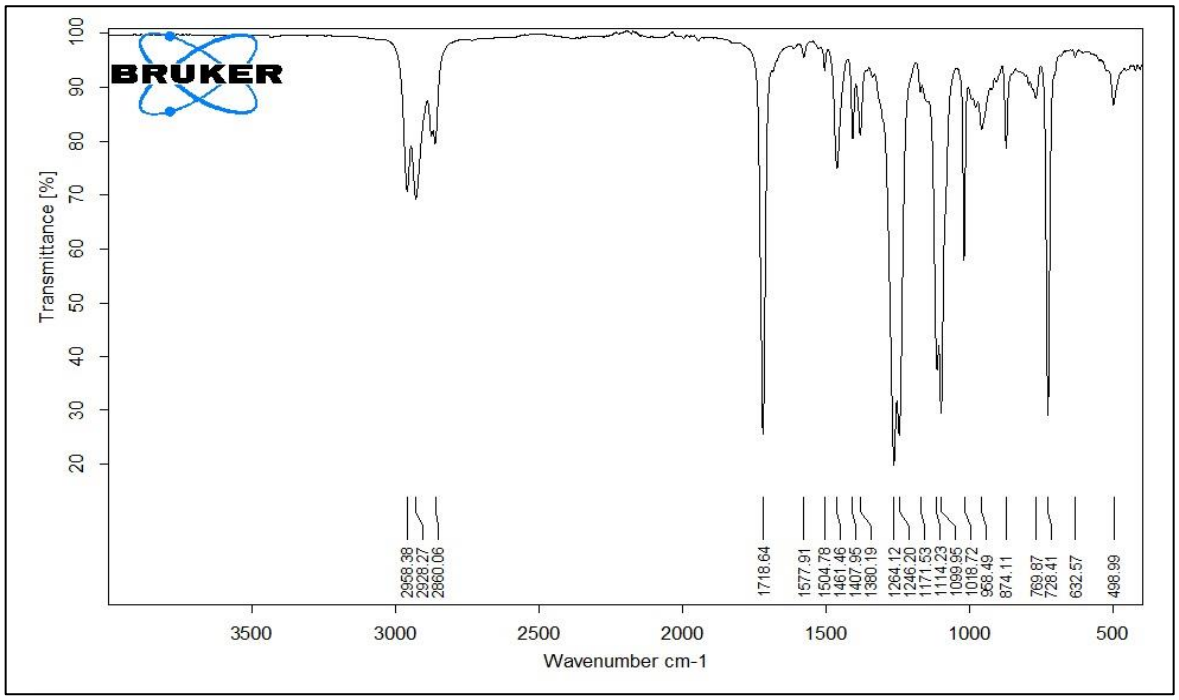

**Appendix B: Figure 4 FTIR spectrum of neat DBP elevated at T3**



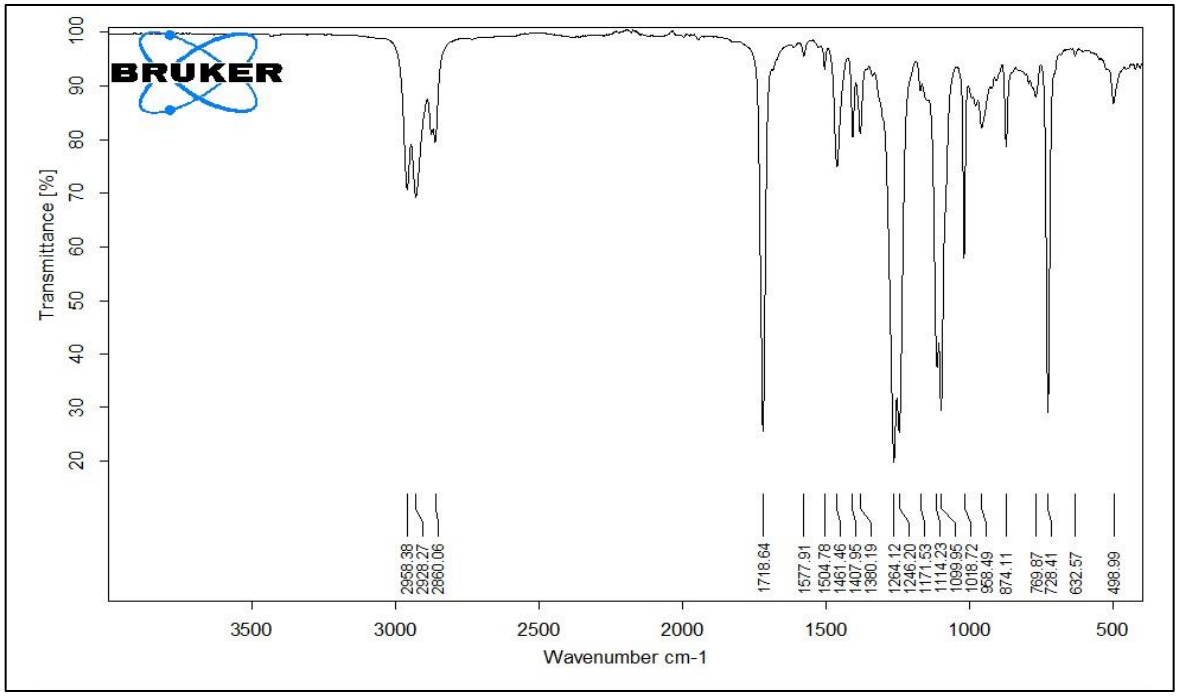

**Appendix B: Figure 5 FTIR spectrum of neat DBP control at T0 (end)**



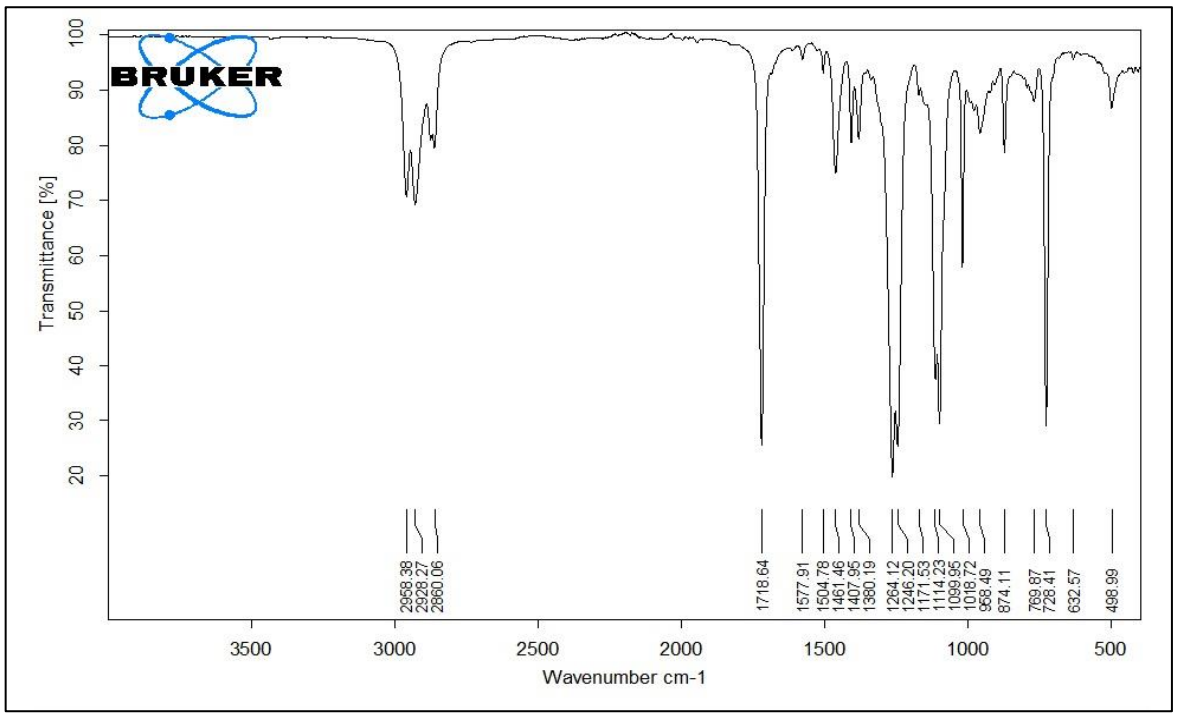

**Appendix B: Figure 6 FTIR spectrum of neat DEHT control at T0**



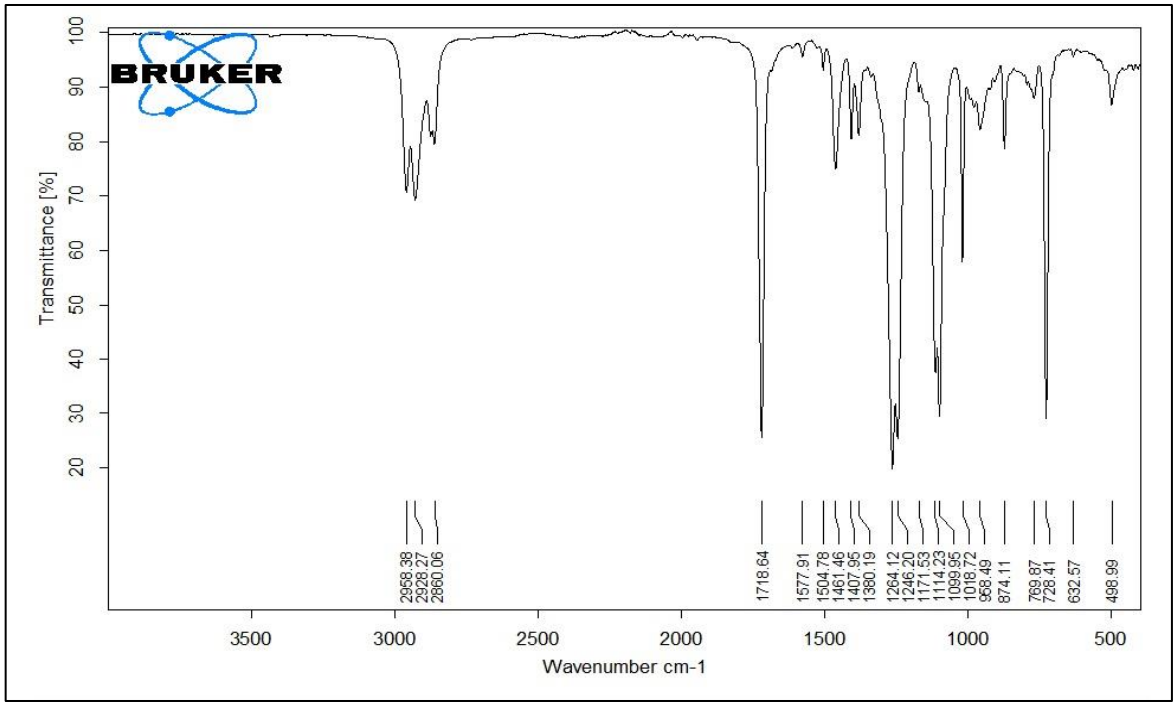

**Appendix B: Figure 7 FTIR spectrum of neat DEHT elevated at T1**



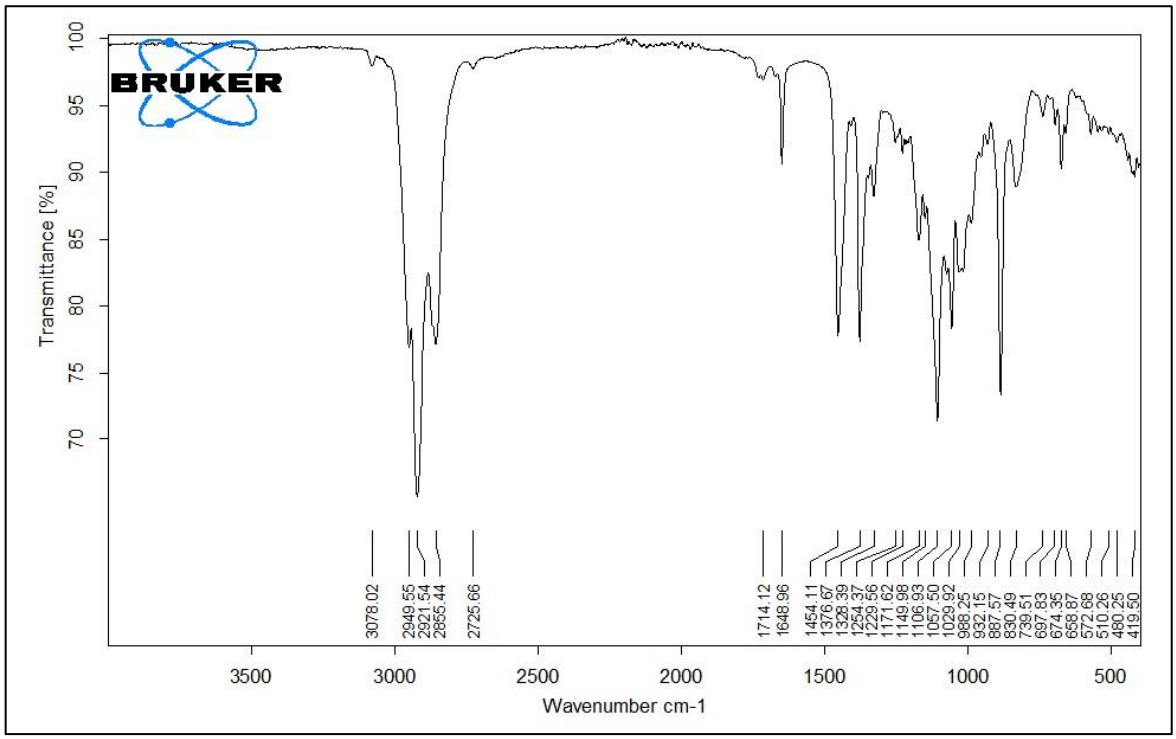

**Appendix B: Figure 8 FTIR spectrum of neat DEHT elevated at T2**



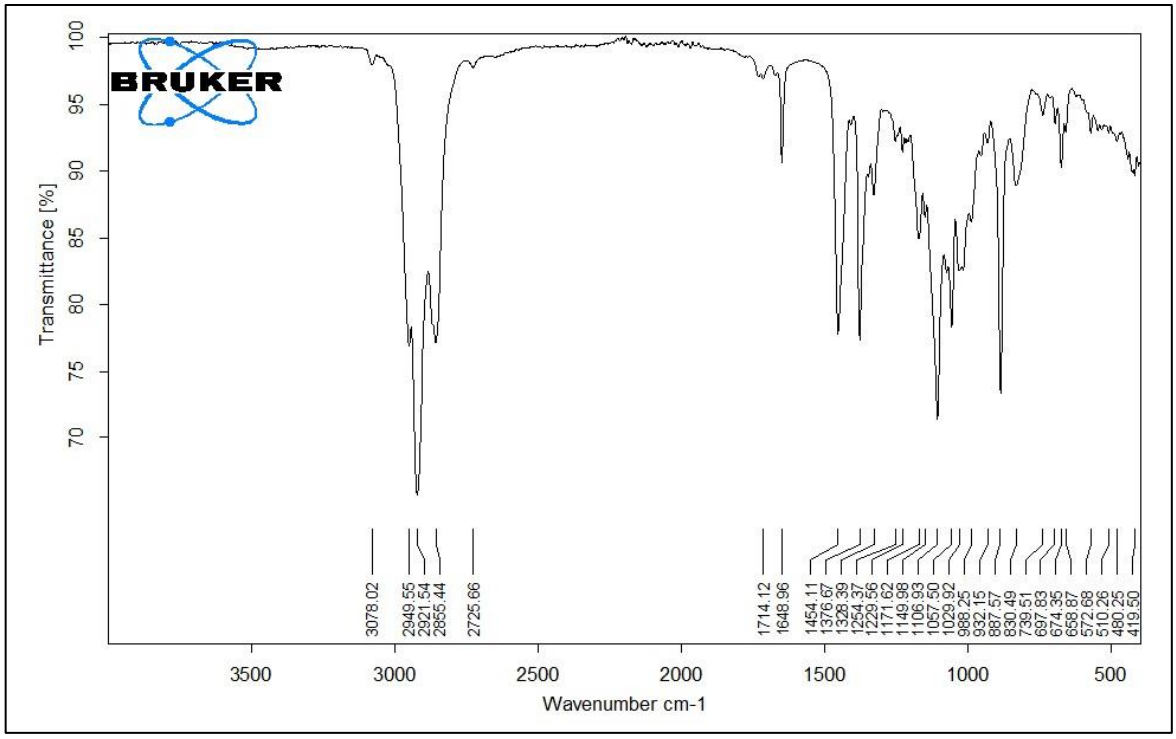

**Appendix B: Figure 9 FTIR spectrum of neat DEHT elevated at T3**



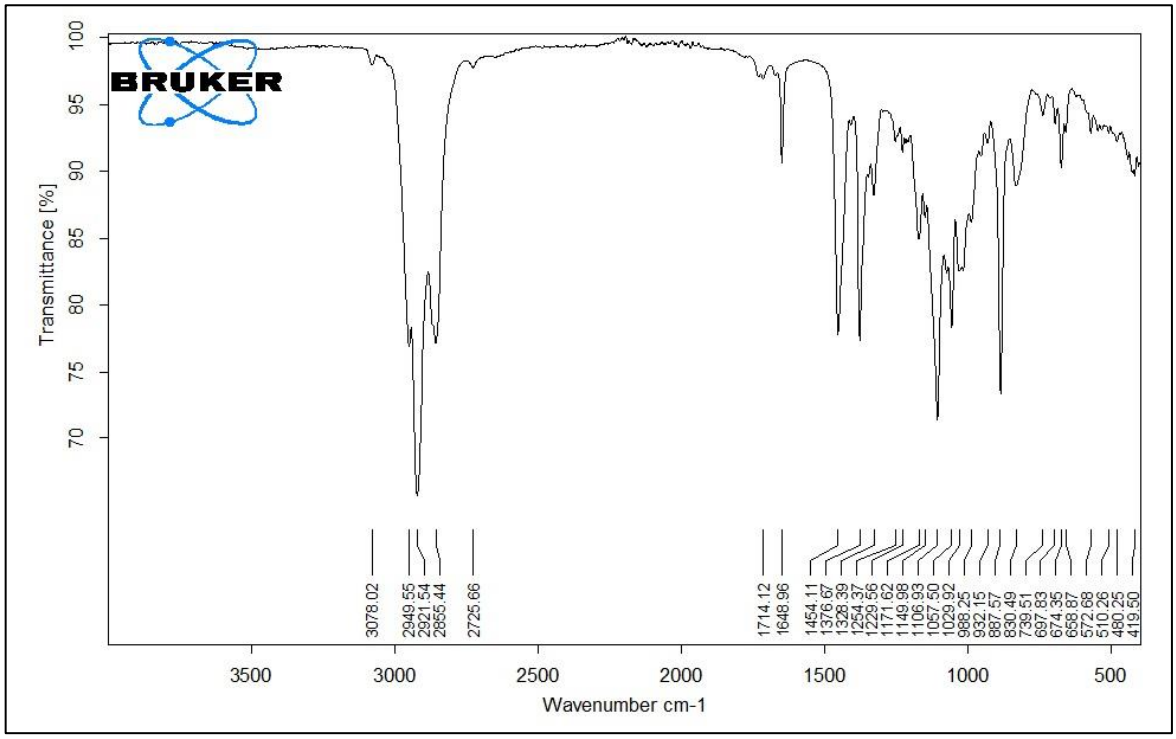

**Appendix B: Figure 10 FTIR spectrum of neat DEHT control at T0**



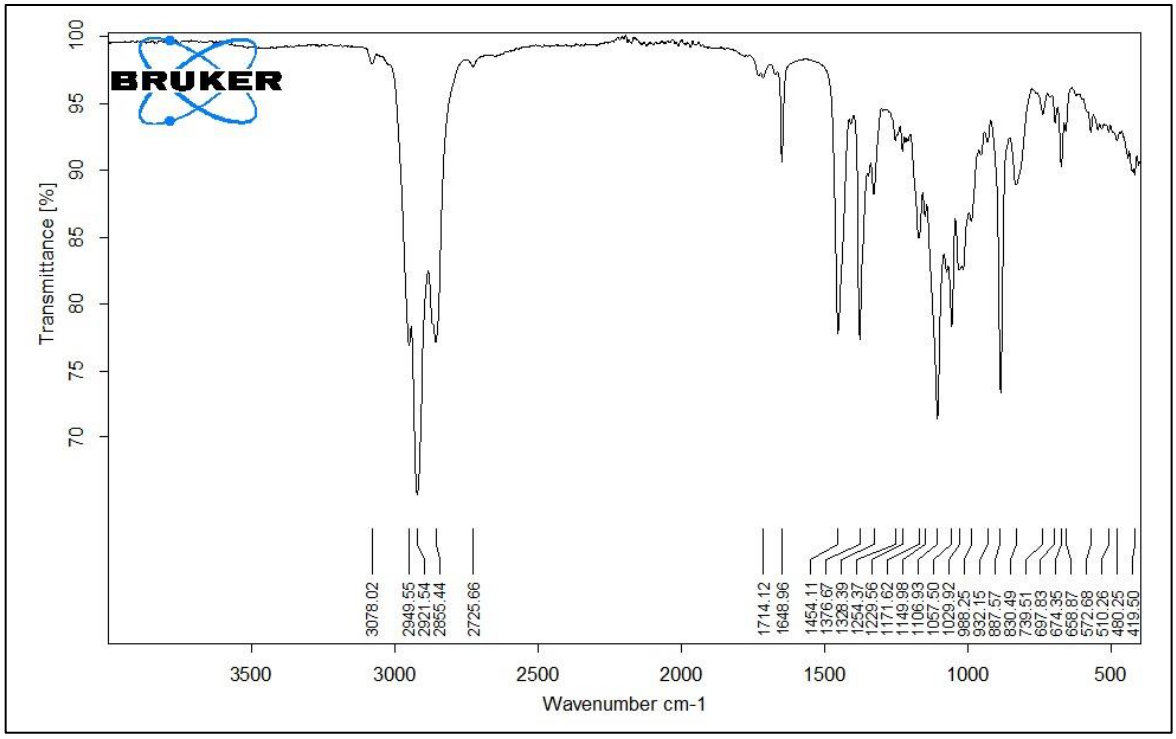

**Appendix B: Figure 11 FTIR spectrum of neat PMD control at T0**



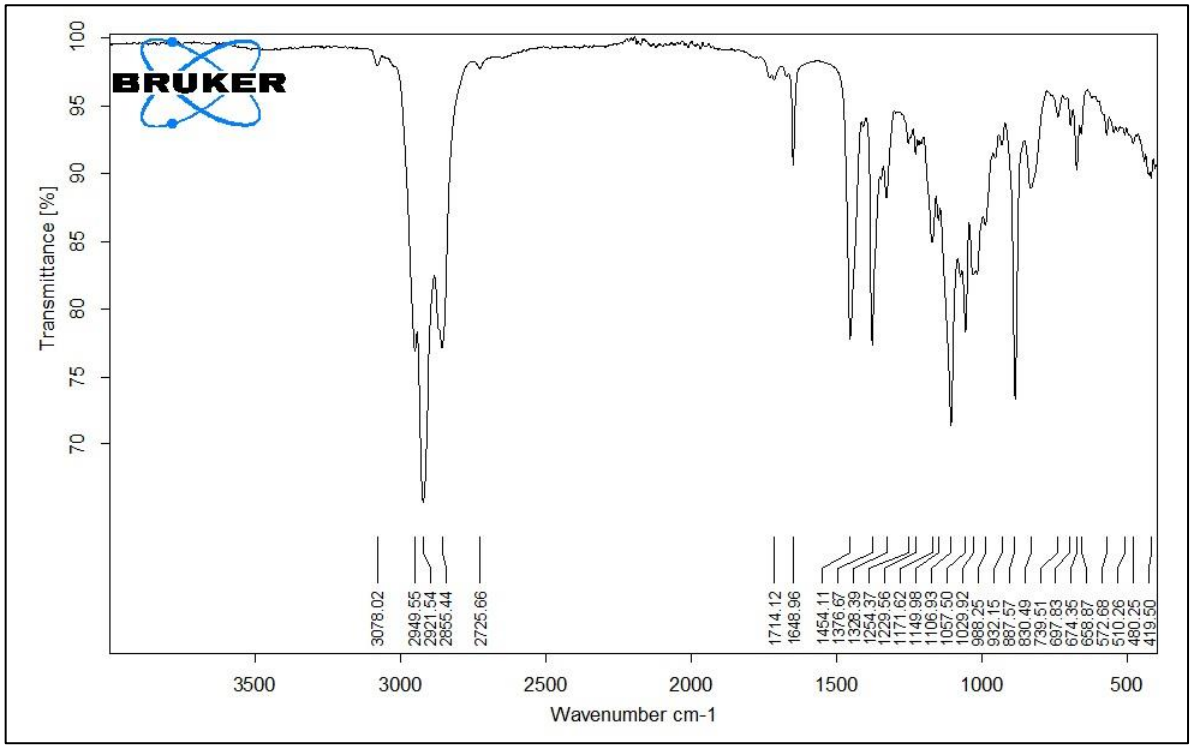

**Appendix B: Figure 12 FTIR spectrum of neat PMD elevated at T1**



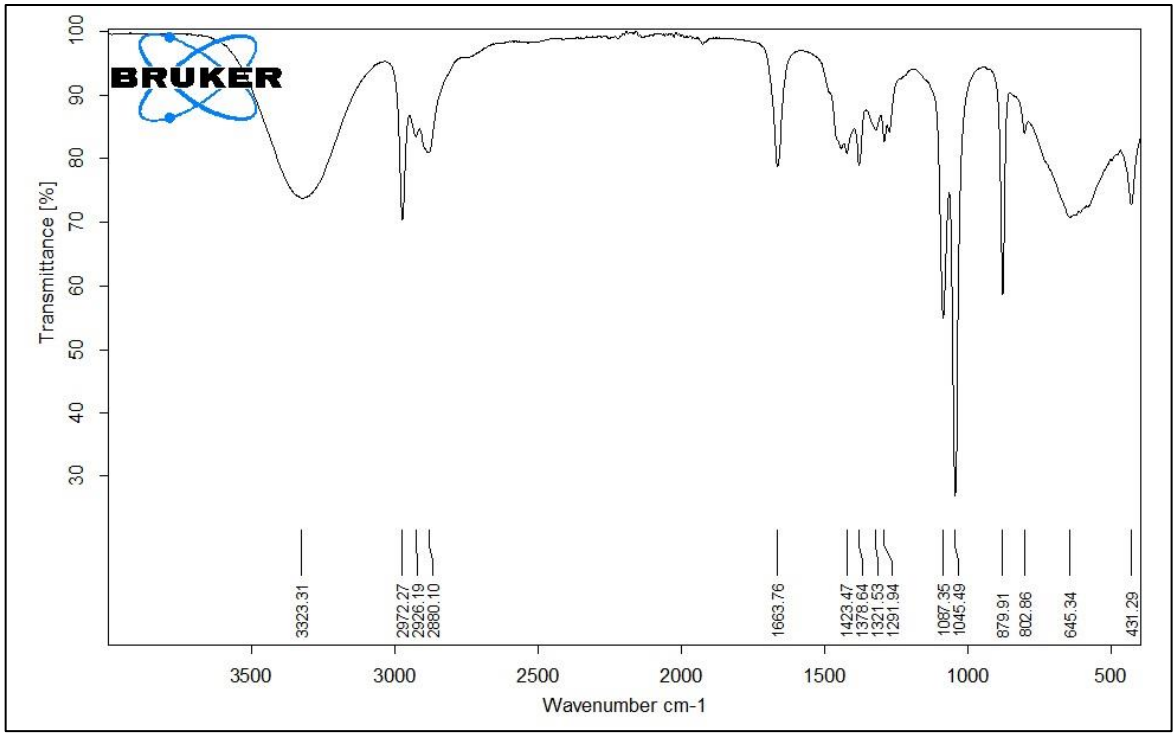

**Appendix B: Figure 13 FTIR spectrum of neat PMD elevated at T2**



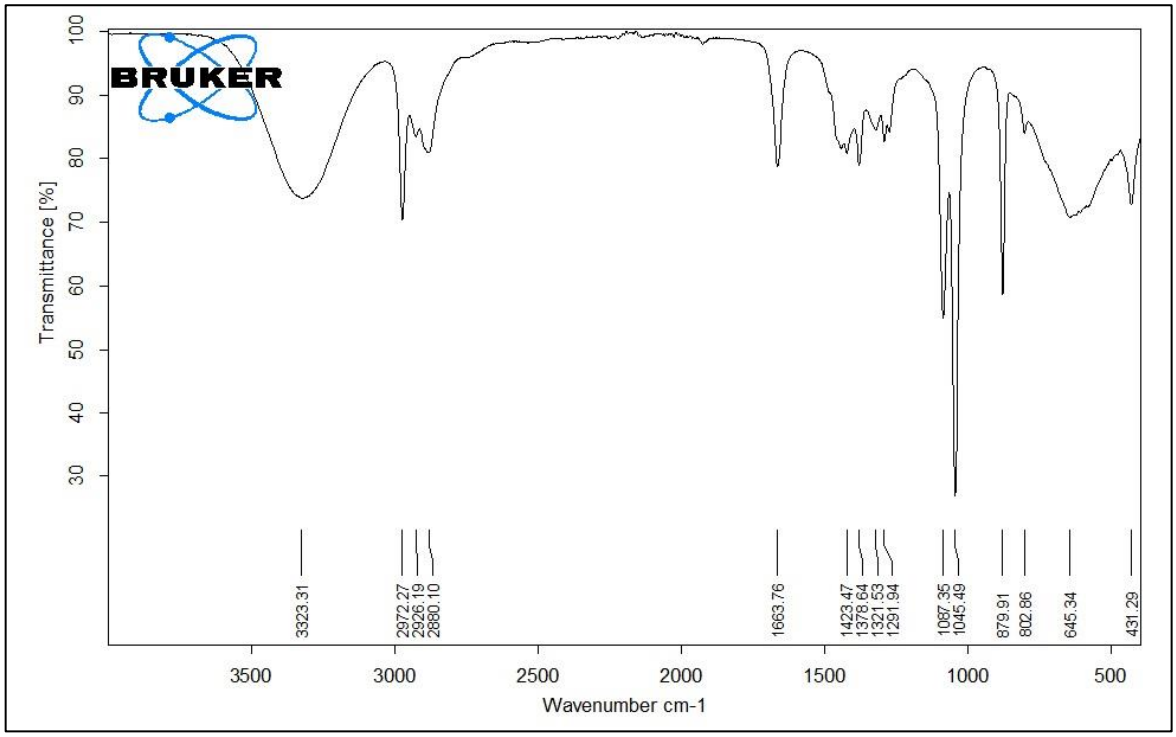

**Appendix B: Figure 14 FTIR spectrum of neat PMD elevated at T3**



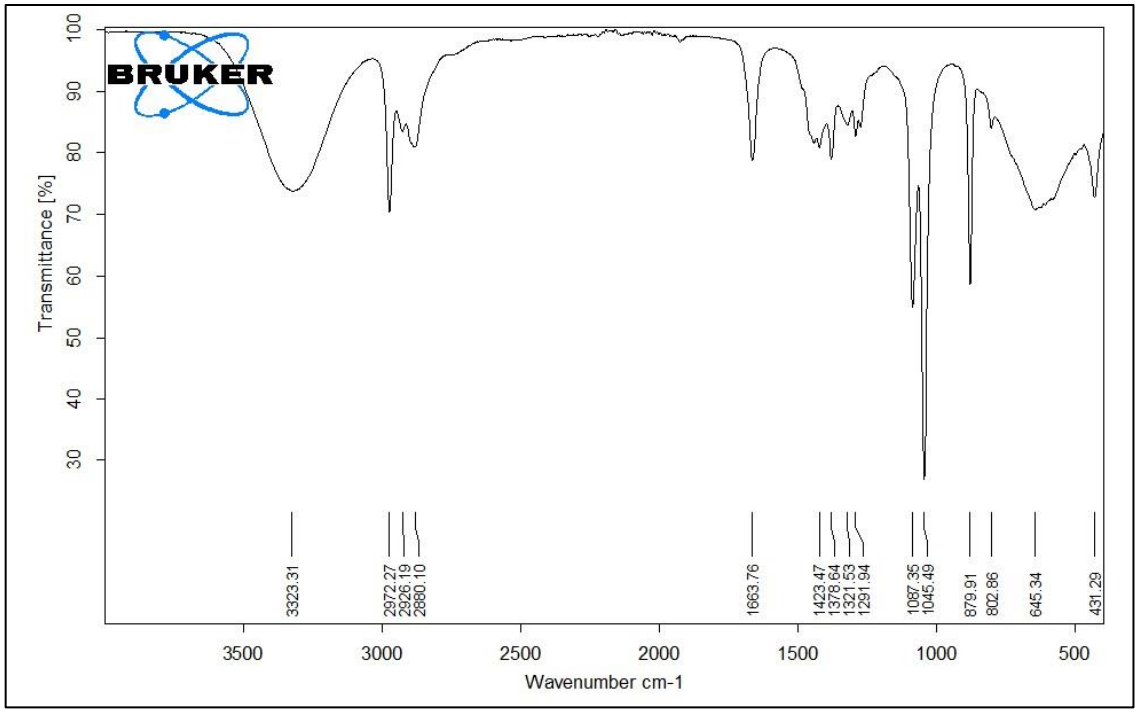

**Appendix B: Figure 15 FTIR spectrum of neat PMD control at T0 (end)**



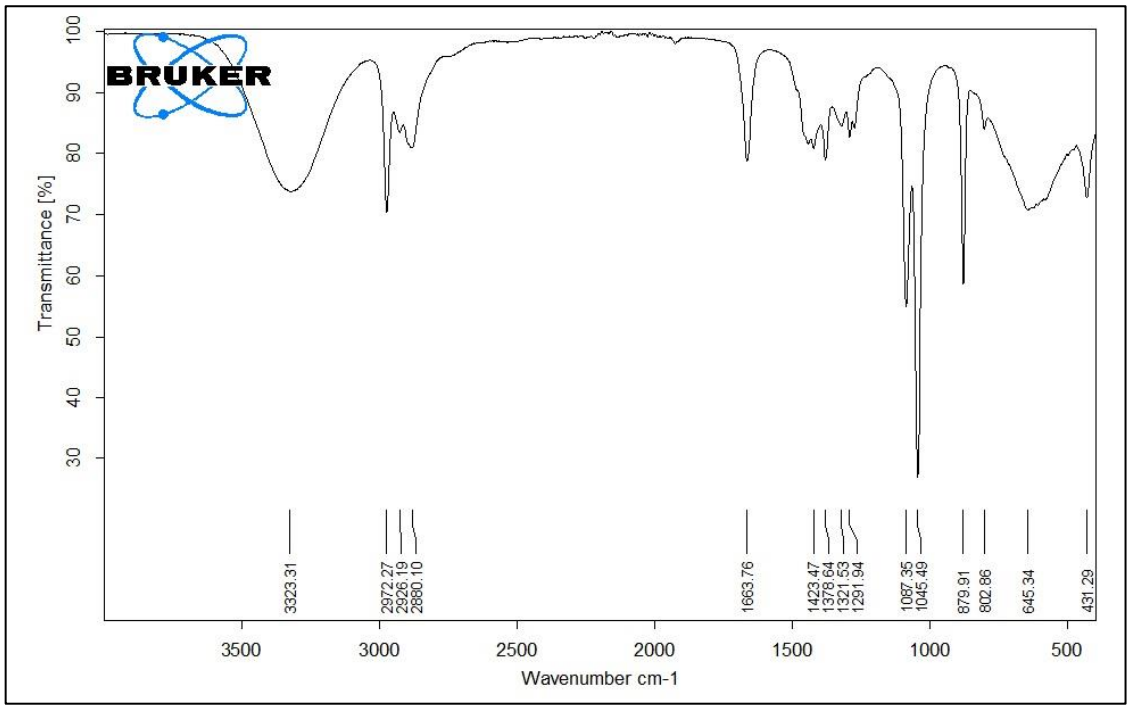

**Appendix B: Figure 16 FTIR spectrum of Blank lip coat Stage T0**



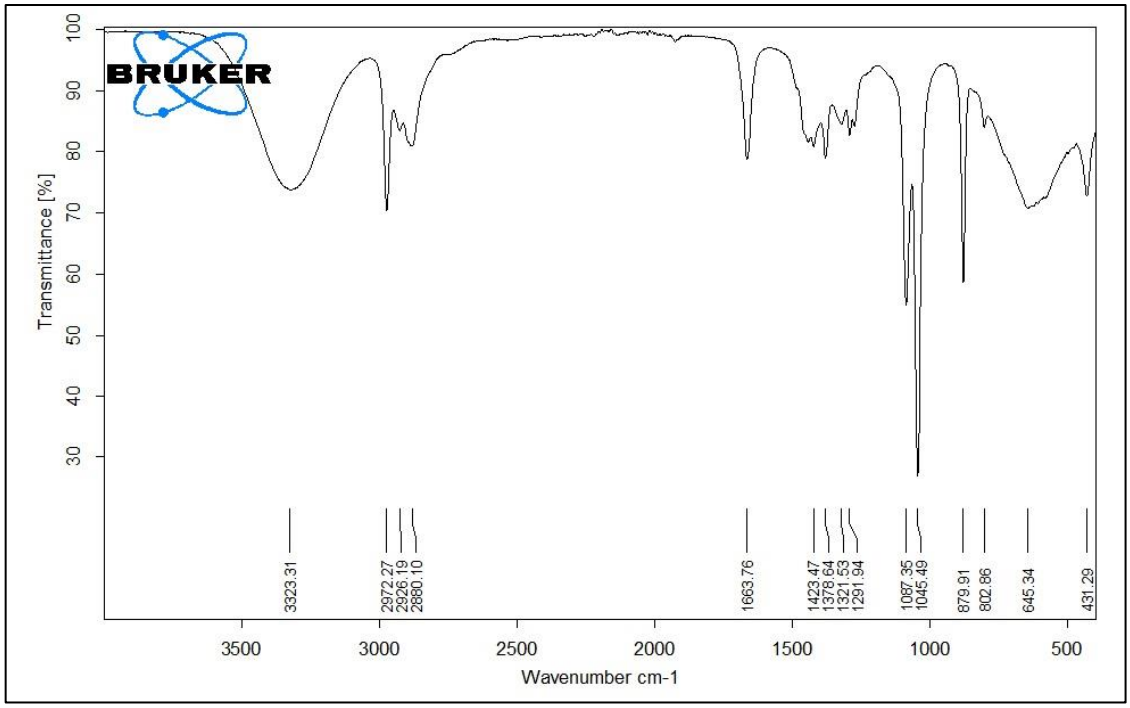

**Appendix B: Figure 17 FTIR spectrum of Blank lip coat Stage T1**



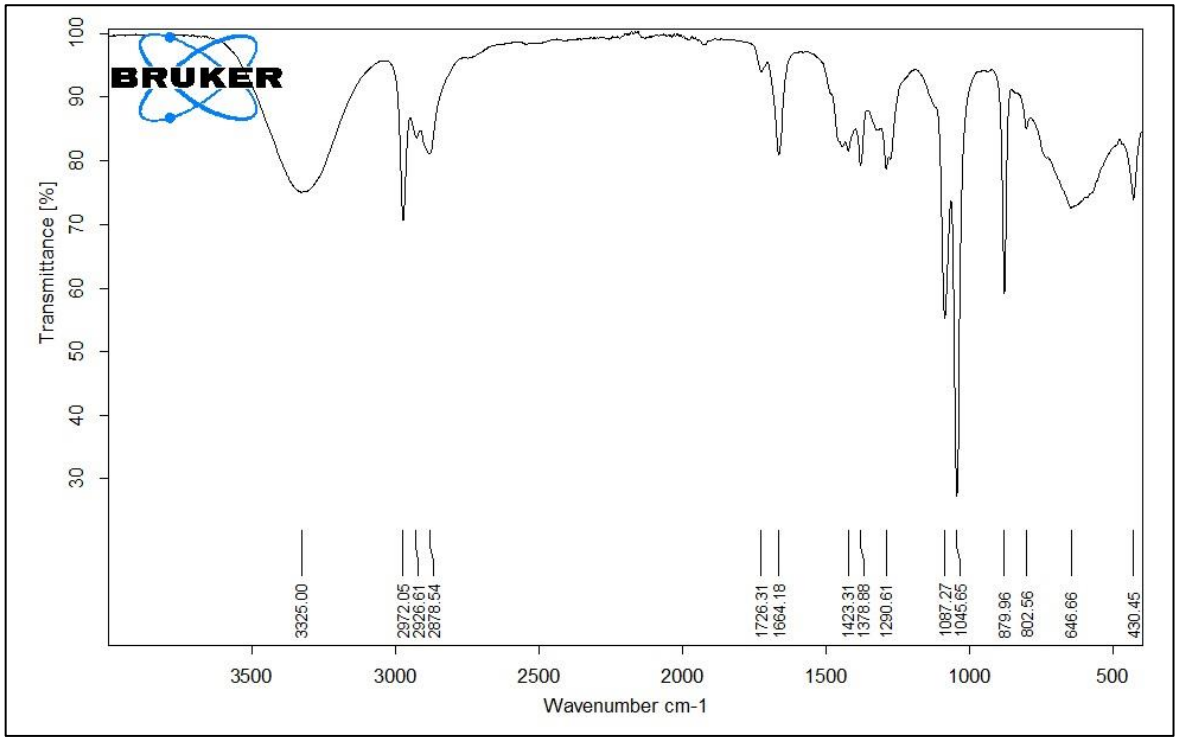

**Appendix B: Figure 18 FTIR spectrum of Blank lip coat Stage T2**



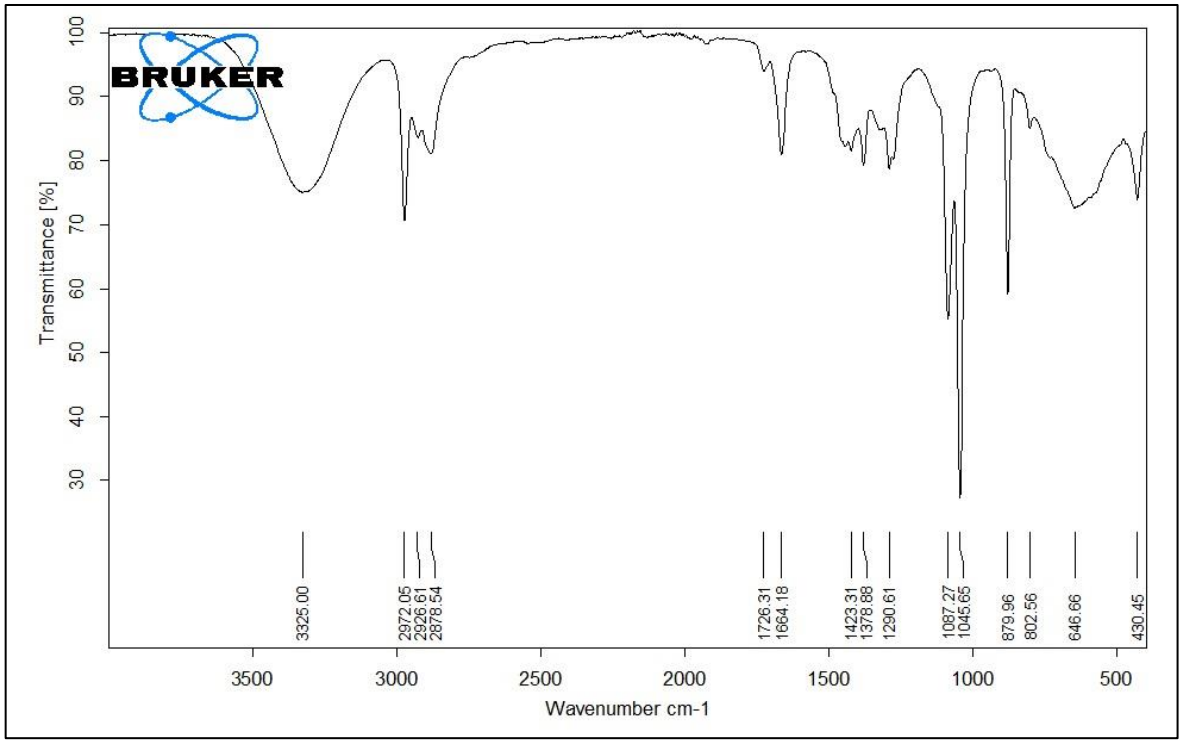

**Appendix B: Figure 19 FTIR spectrum of Blank lip coat Stage T3**



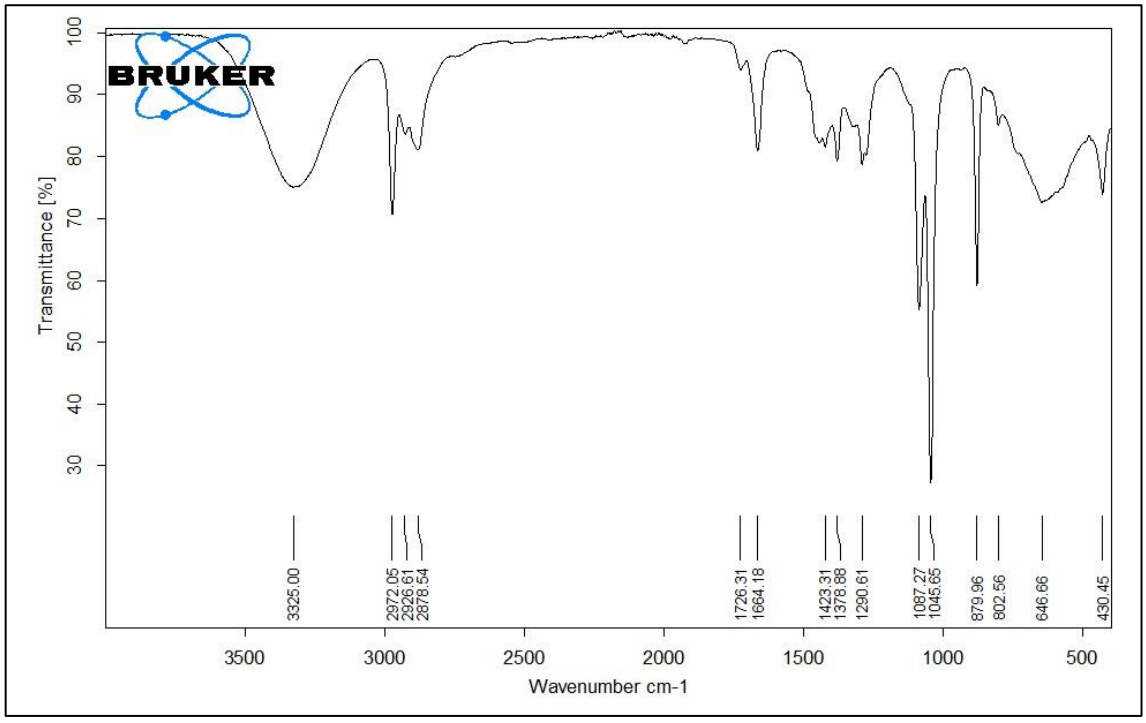

**Appendix B: Figure 20 FTIR spectrum of Blank lip coat Stage T0 (end)**



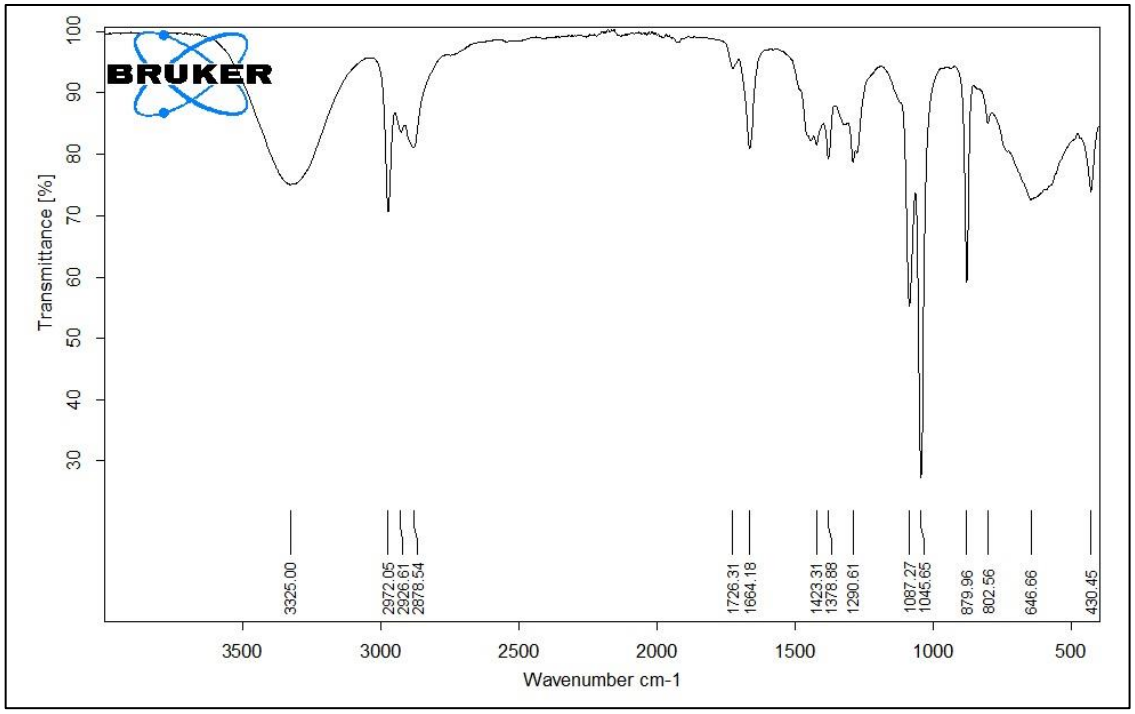

**Appendix B: Figure 21 FTIR spectrum of DBP lip coat Stage T0**



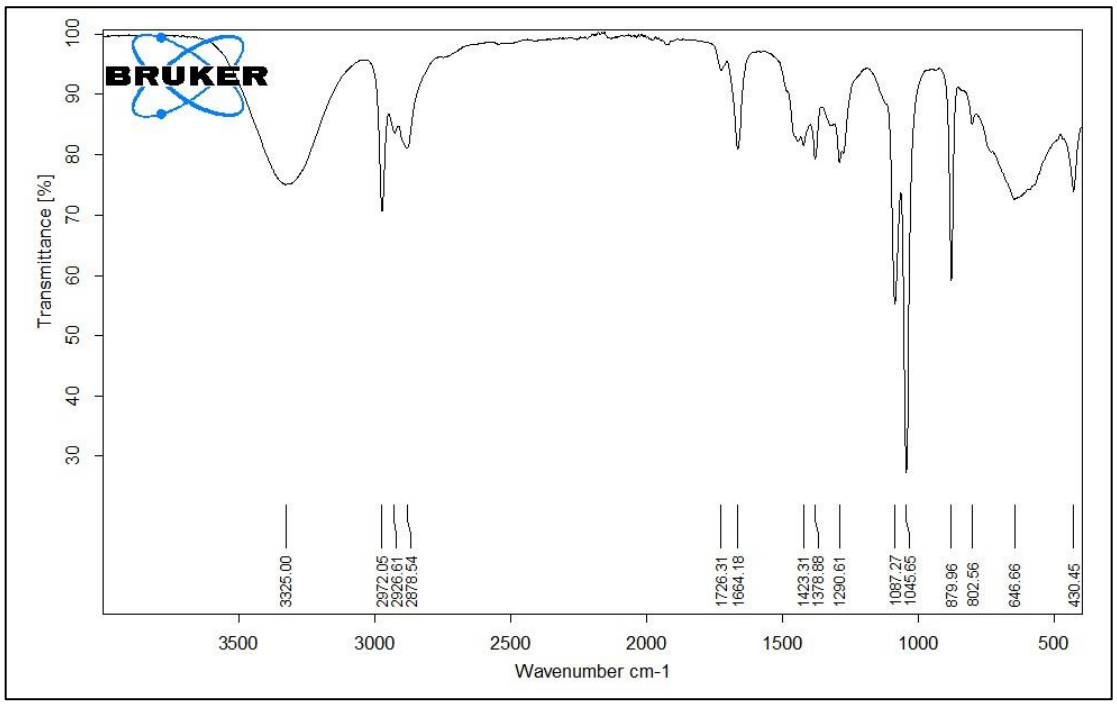

**Appendix B: Figure 22 FTIR spectrum of DBP lip coat Stage T1**



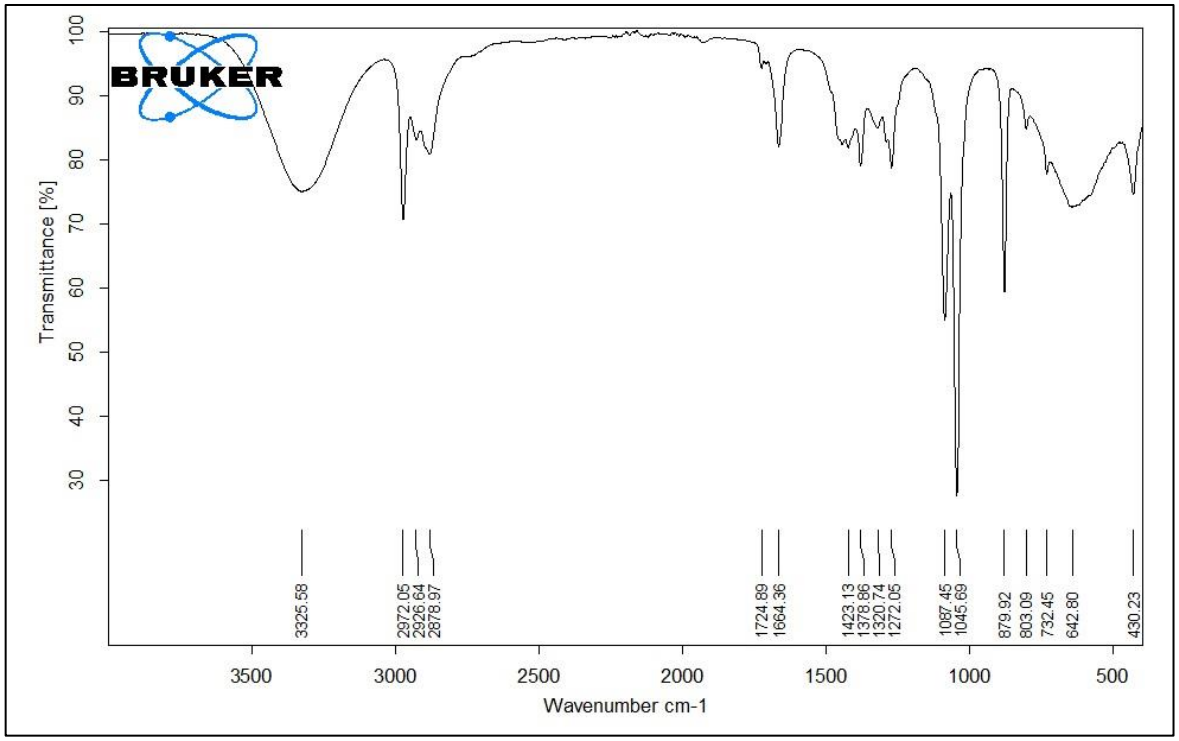

**Appendix B: Figure 23 FTIR spectrum of DBP lip coat Stage T2**



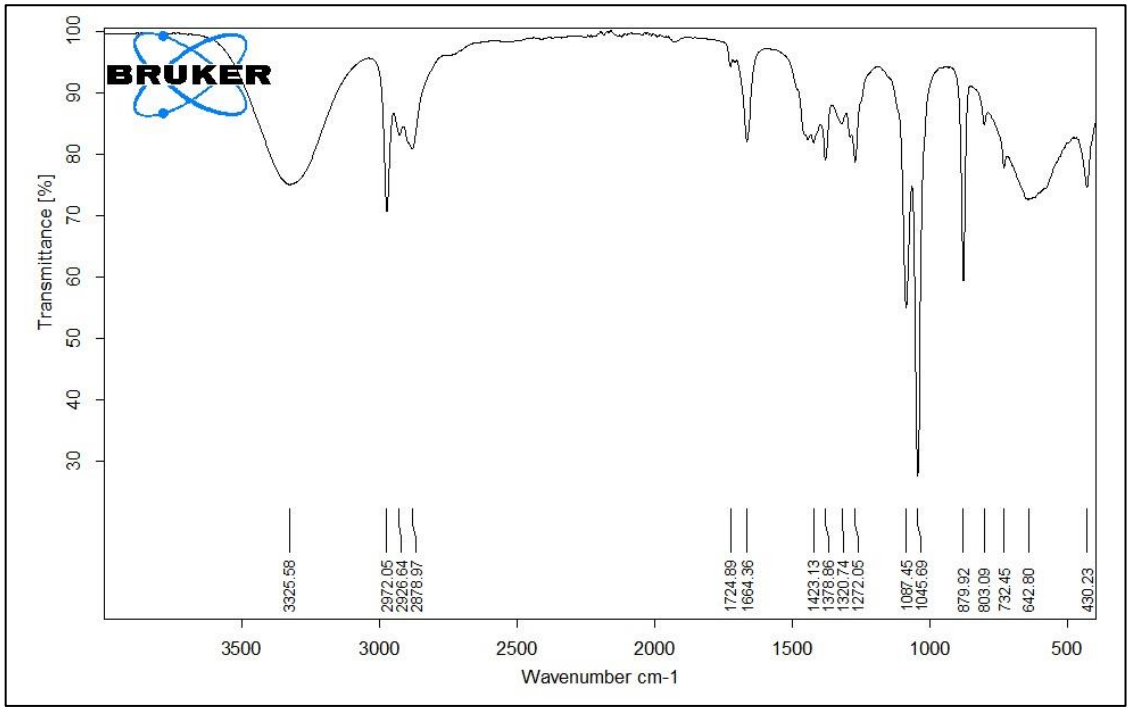

**Appendix B: Figure 24 FTIR spectrum of DBP lip coat Stage T3**



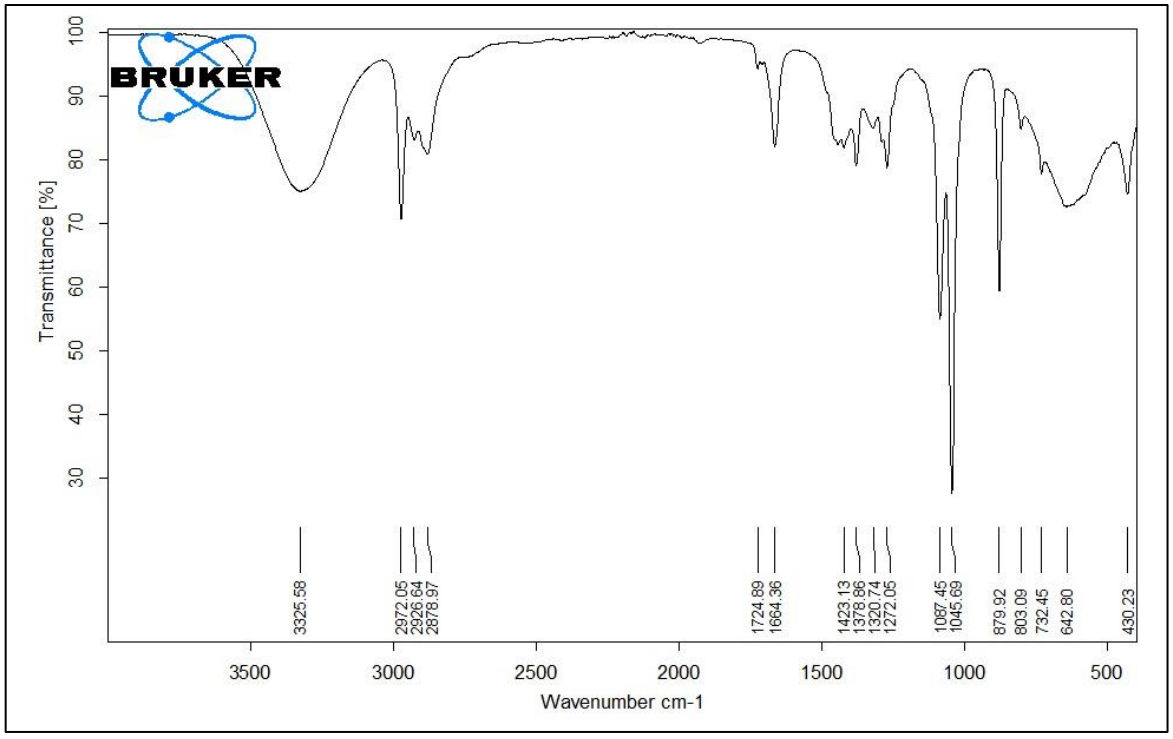

**Appendix B: Figure 25 FTIR spectrum of DBP lip coat Stage T0 (end)**



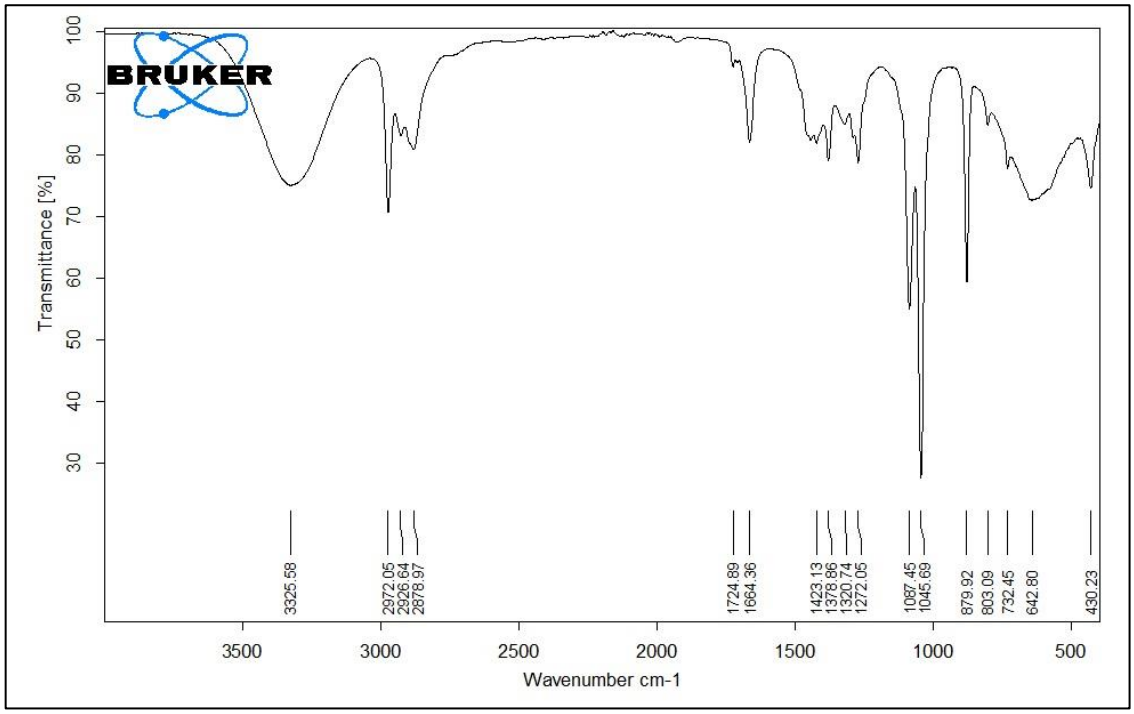

**Appendix B: Figure 26 FTIR spectrum of DEHT lip coat Stage T0**



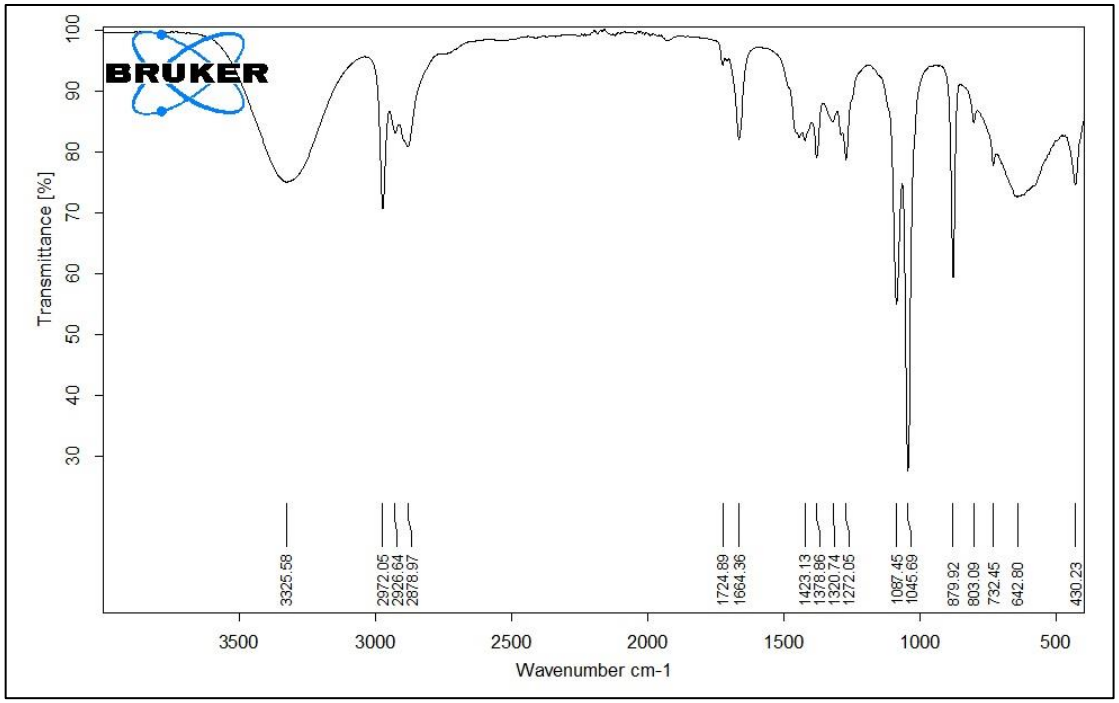

**Appendix B: Figure 27 FTIR spectrum of DEHT lip coat Stage T1**



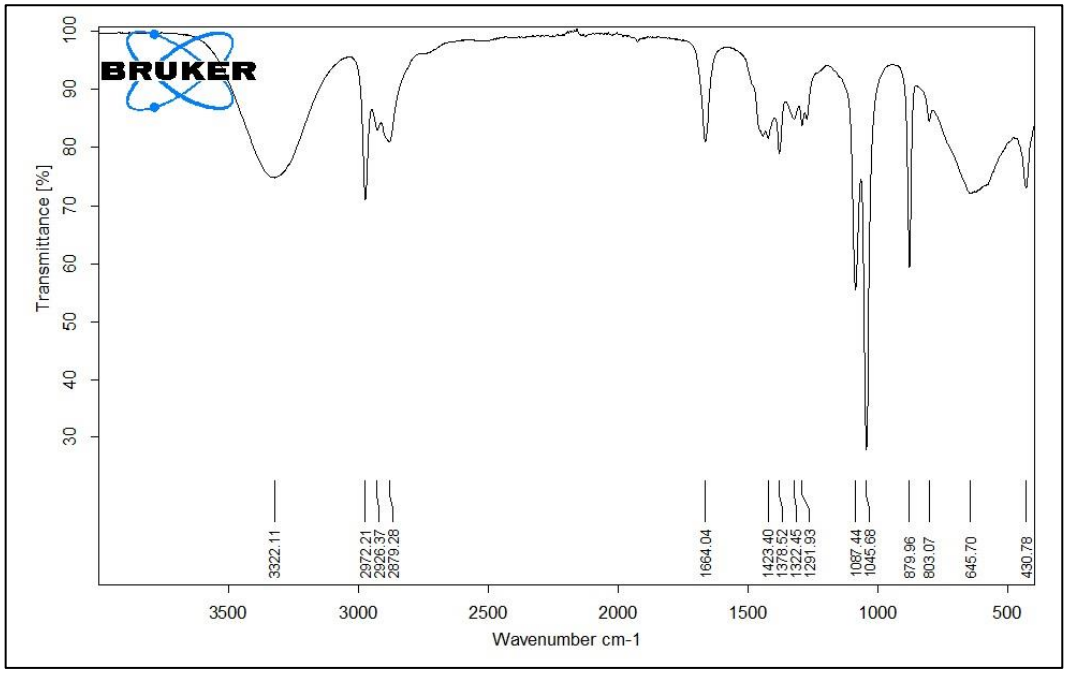

**Appendix B: Figure 28 FTIR spectrum of DEHT lip coat Stage T2**



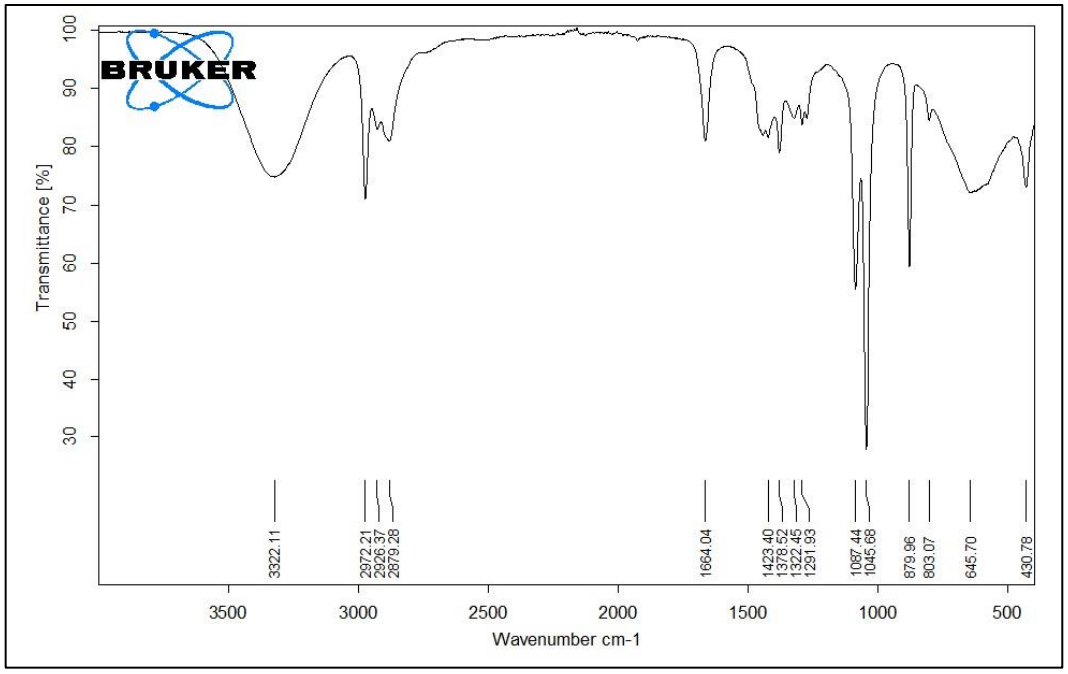

**Appendix B: Figure 29 FTIR spectrum of DEHT lip coat Stage T3**



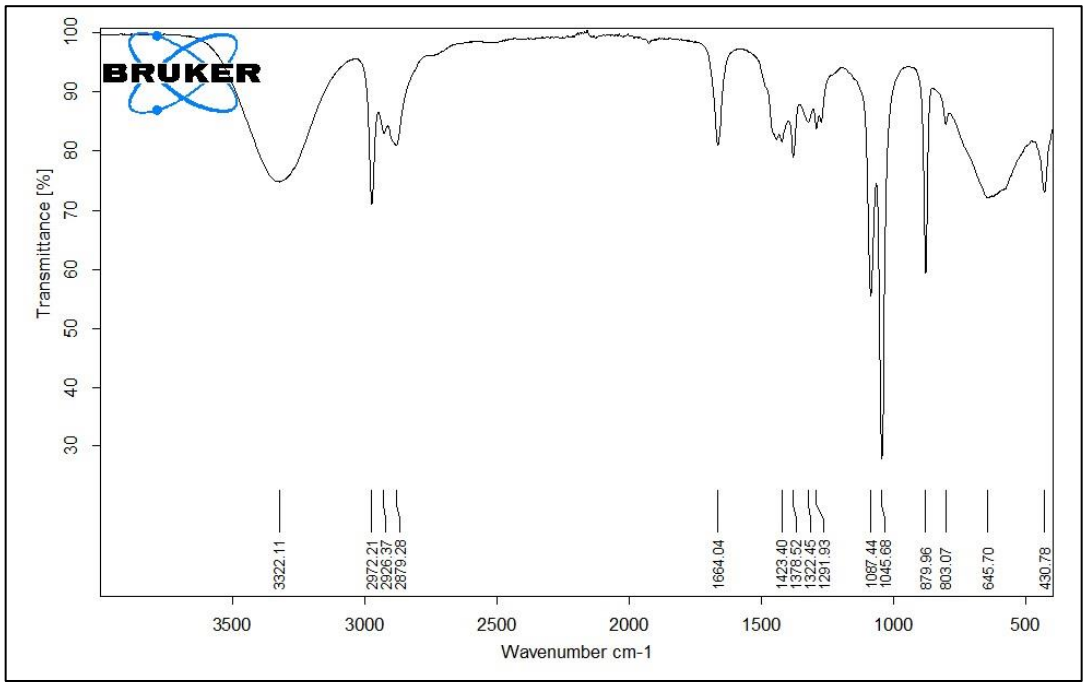

**Appendix B: Figure 30 FTIR spectrum of DEHT lip coat Stage T0 (end)**



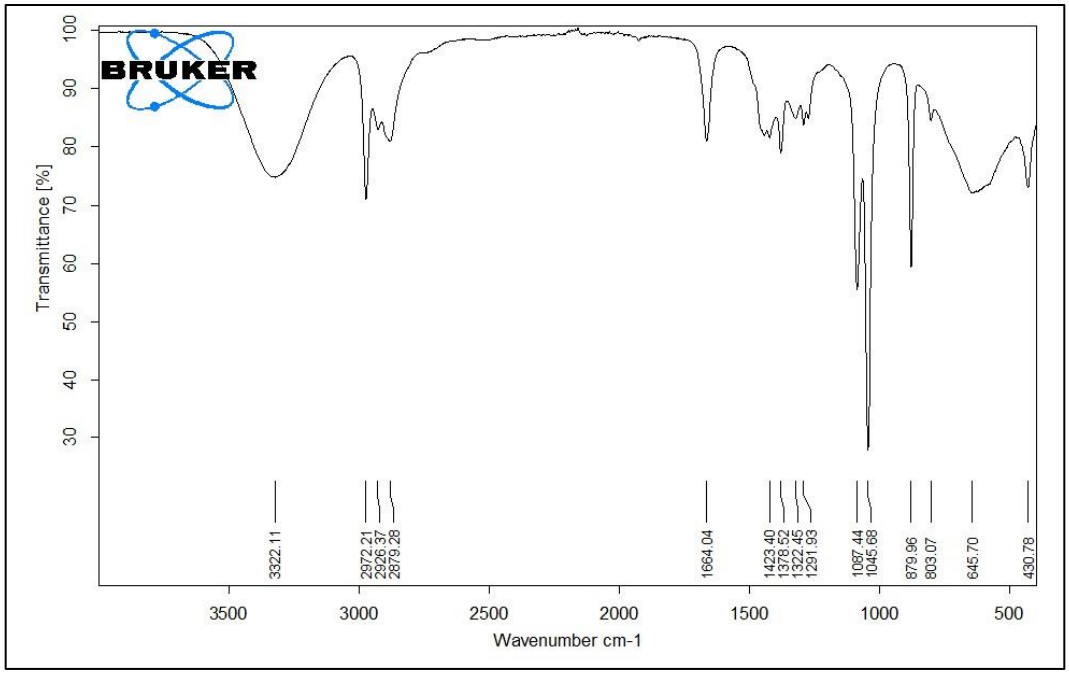

**Appendix B: Figure 31 FTIR spectrum of PMD-citronellal acetal lip coat Stage T0**



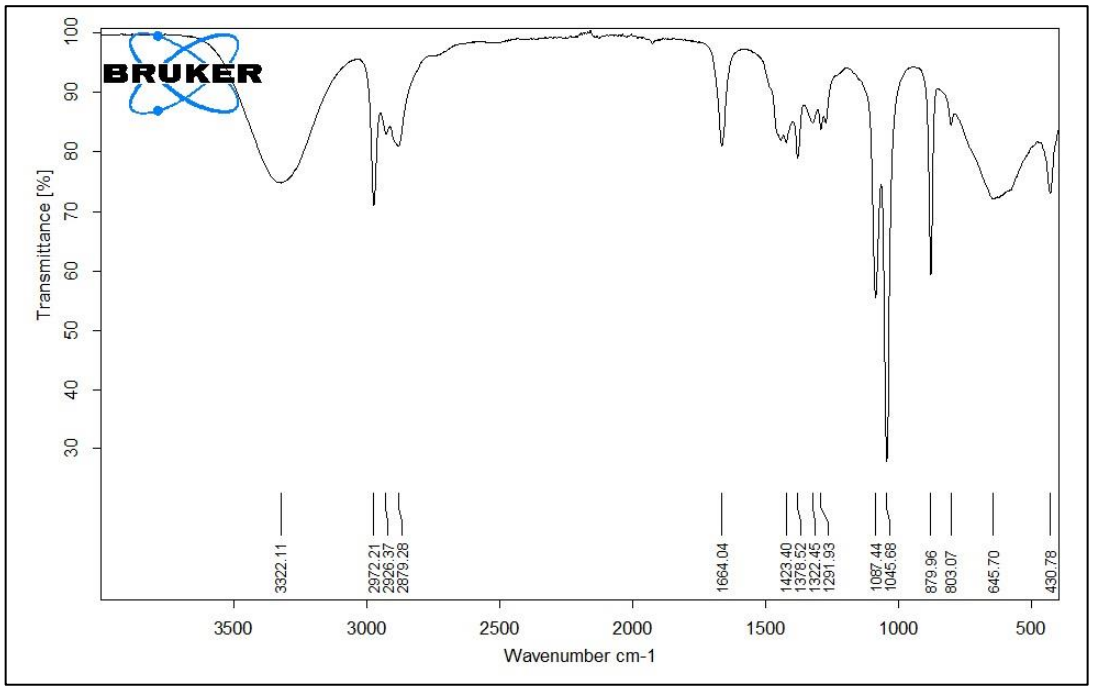

**Appendix B: Figure 32 FTIR spectrum of PMD-citronellal acetal lip coat Stage T1**



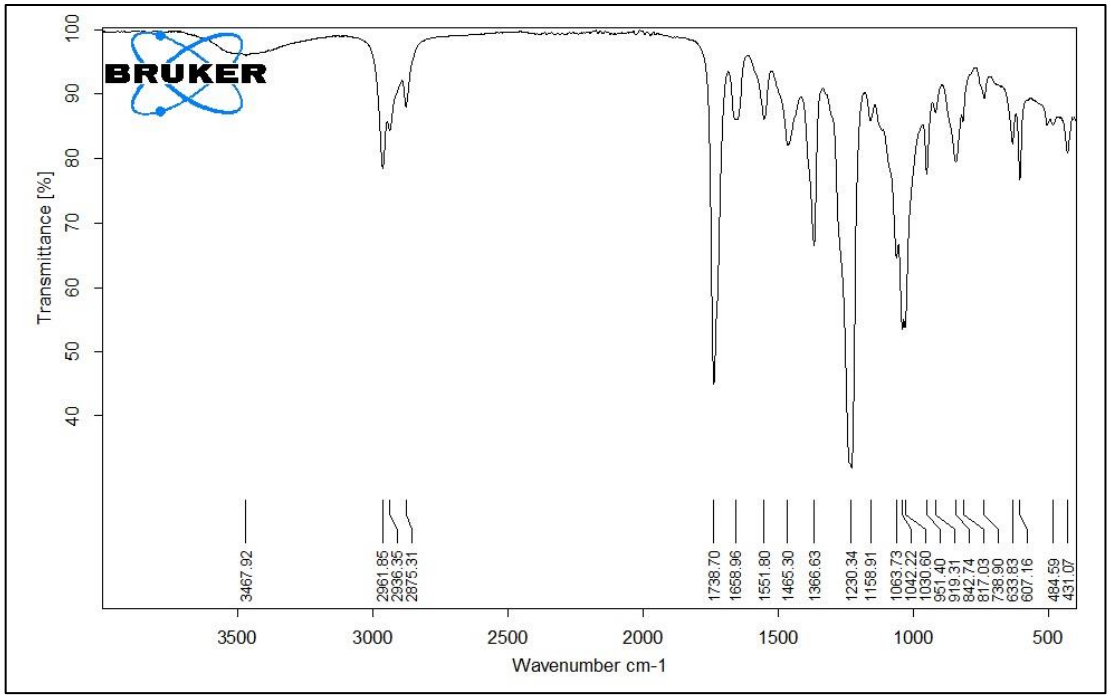

**Appendix B: Figure 33 FTIR spectrum of PMD-citronellal acetal lip coat Stage T2**



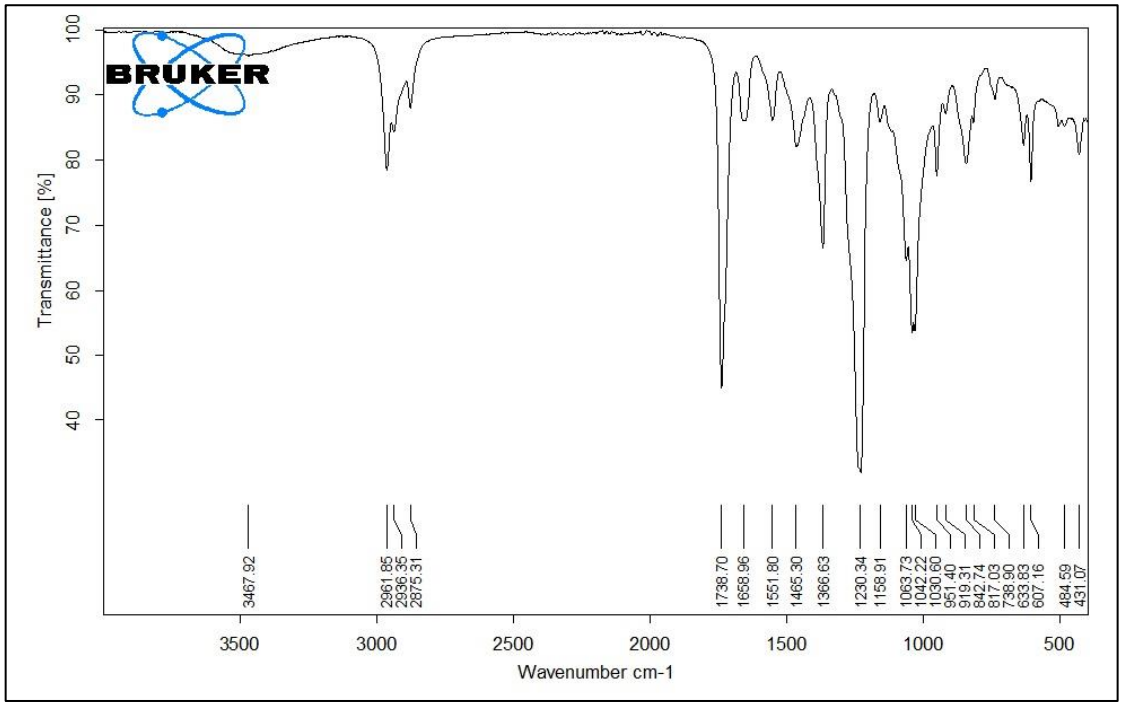

**Appendix B: Figure 34 FTIR spectrum of PMD-citronellal acetal lip coat Stage T3**



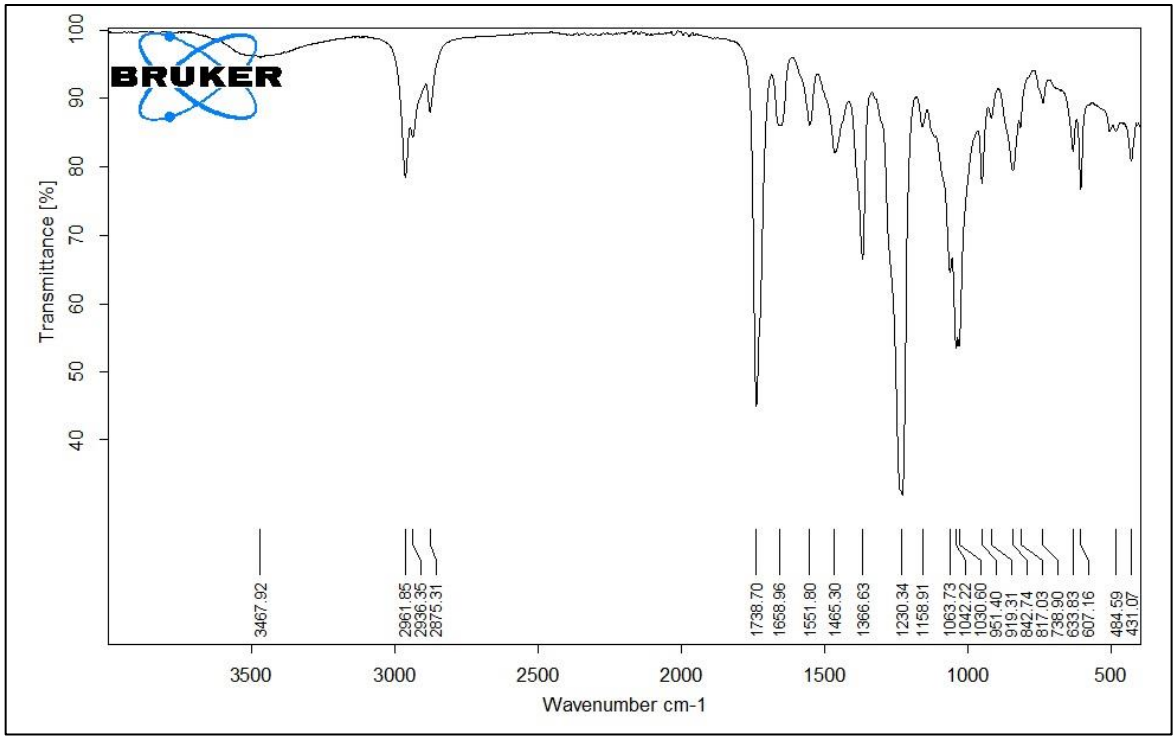

**Appendix B: Figure 35 FTIR spectrum of PMD-citronellal acetal lip coat Stage T0 (end)**



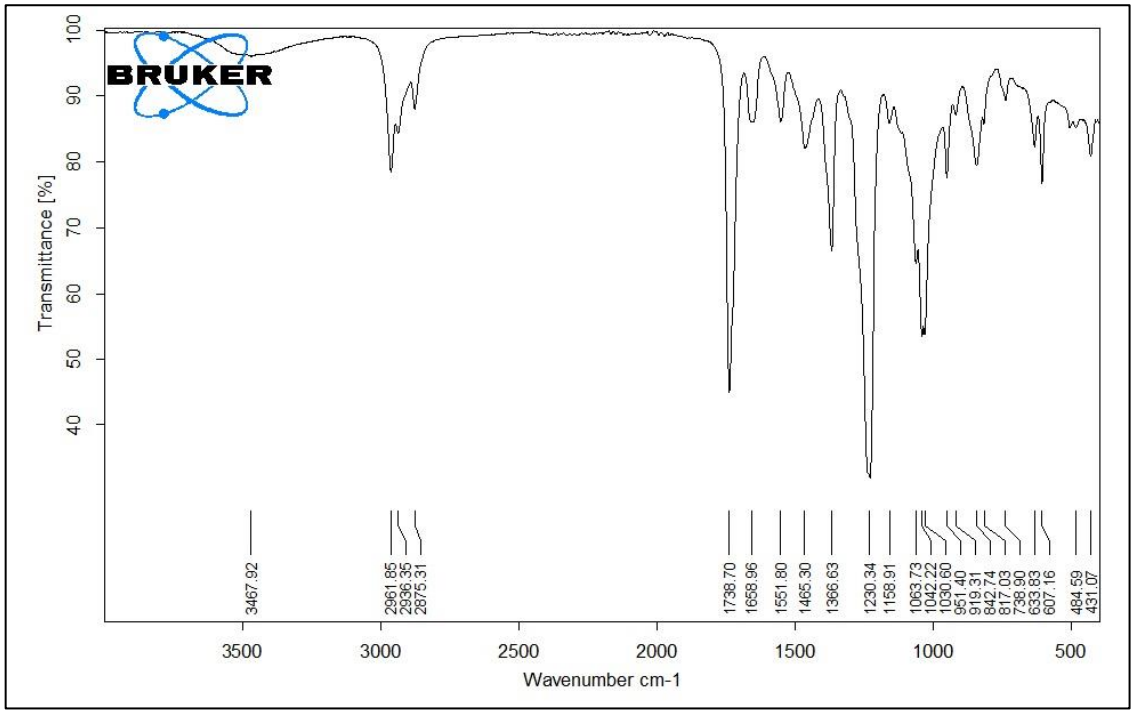

**Appendix B: Figure 36 FTIR spectrum of Blank nail lacquer Stage T0**



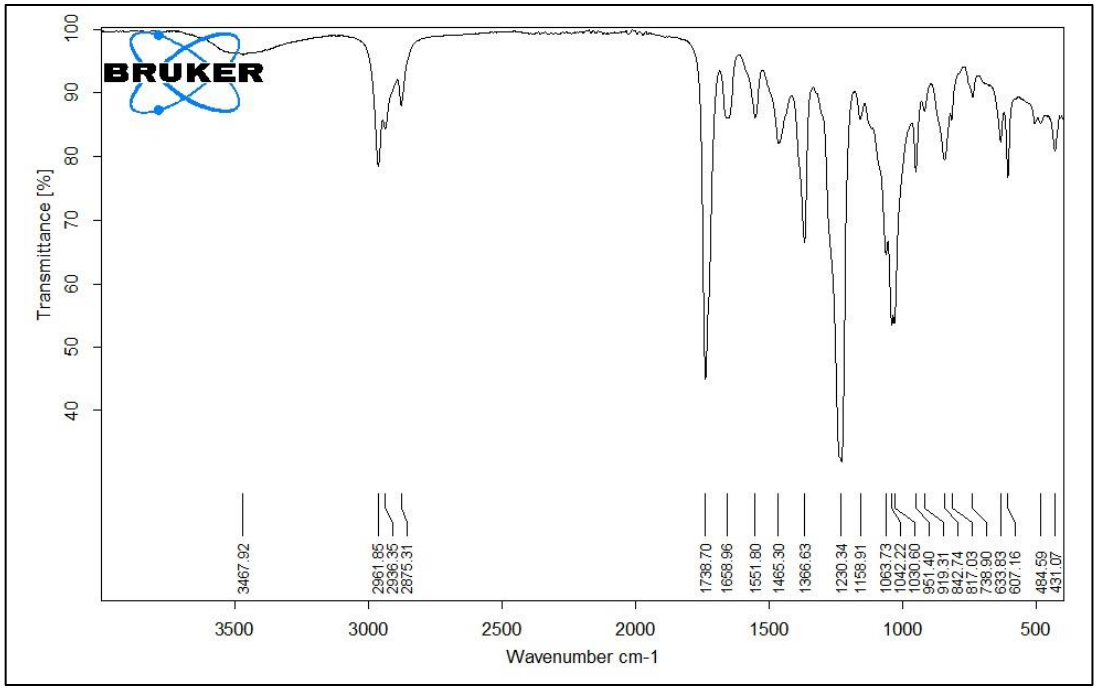

**Appendix B: Figure 37 FTIR spectrum of Blank nail lacquer Stage T1**



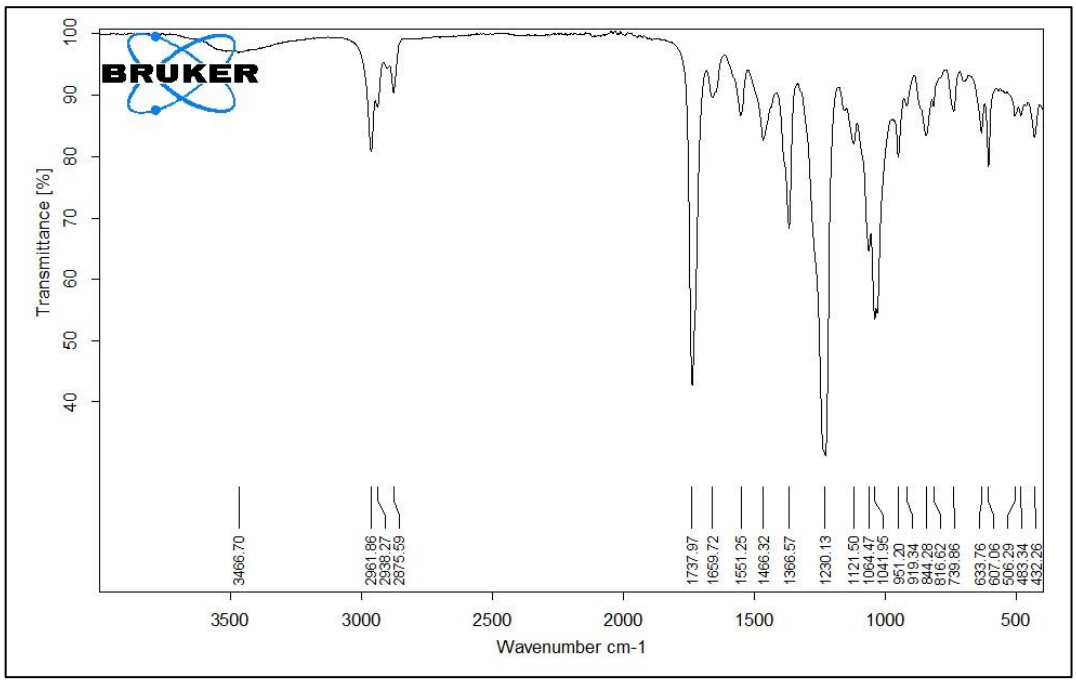

**Appendix B: Figure 38 FTIR spectrum of Blank nail lacquer Stage T2**



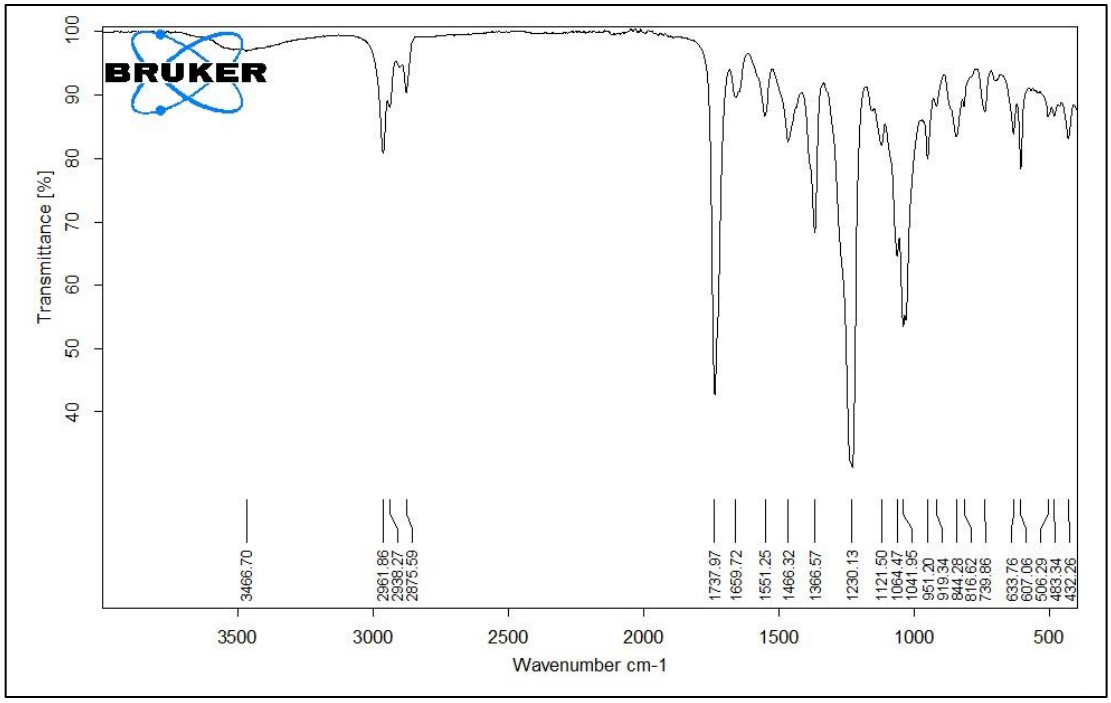

**Appendix B: Figure 39 FTIR spectrum of Blank nail lacquer Stage T3**



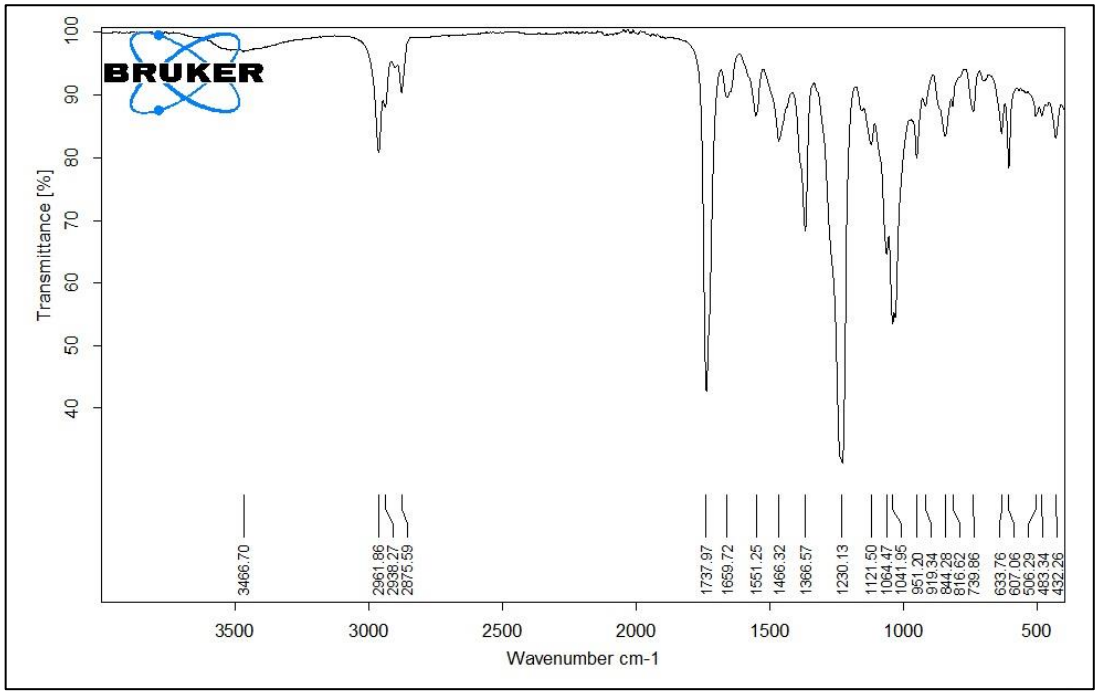

**Appendix B: Figure 40 FTIR spectrum of Blank nail lacquer Stage T0 (end)**



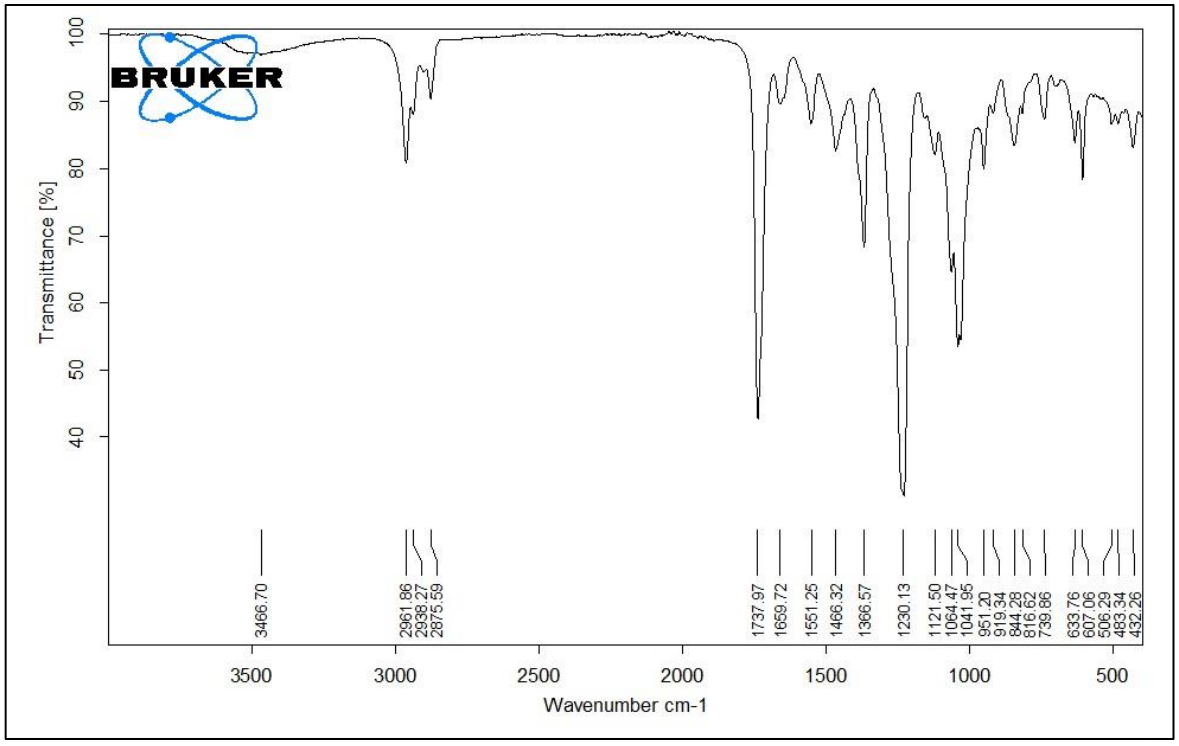

**Appendix B: Figure 41 FTIR spectrum of DBP nail lacquer Stage T0**



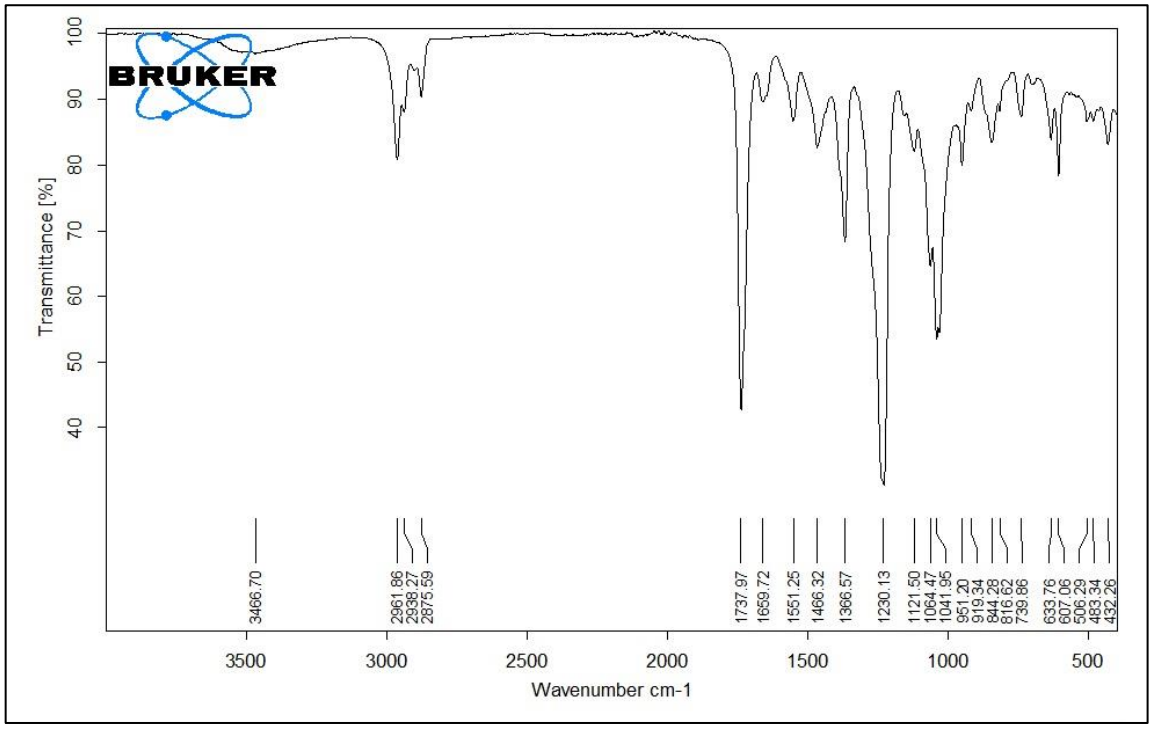

**Appendix B: Figure 42 FTIR spectrum of DBP nail lacquer Stage T1**



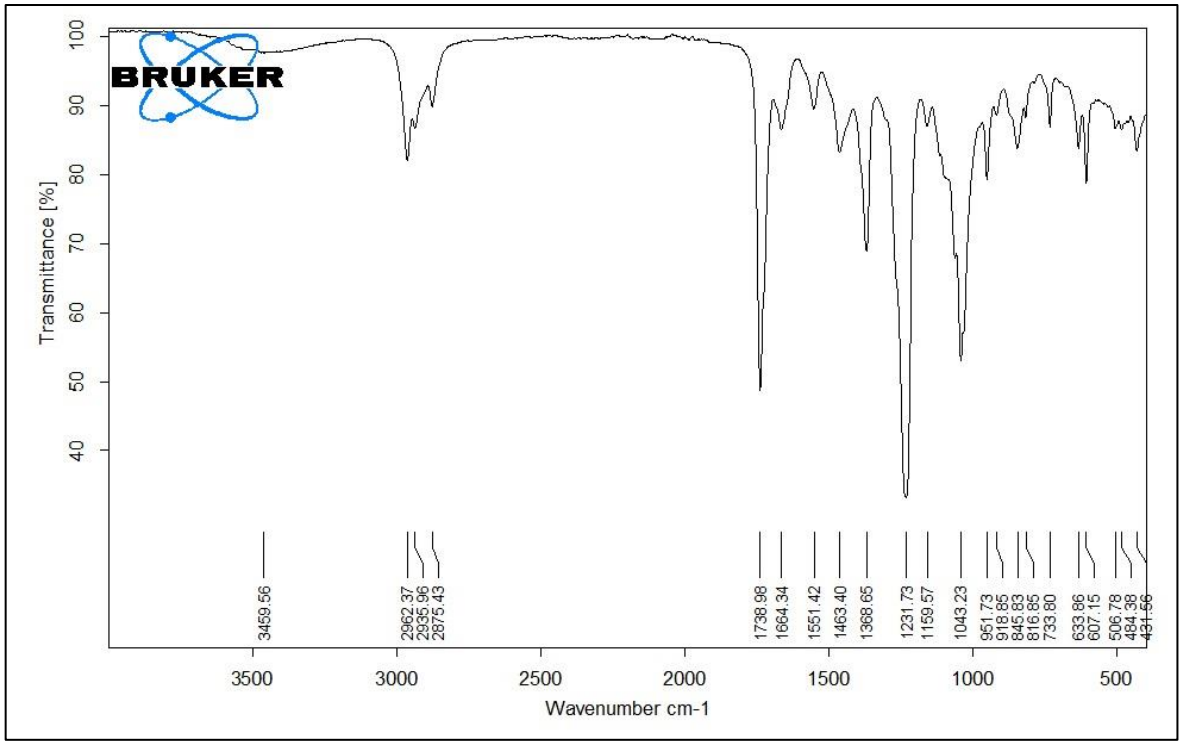

**Appendix B: Figure 43 FTIR spectrum of DBP nail lacquer Stage T2**



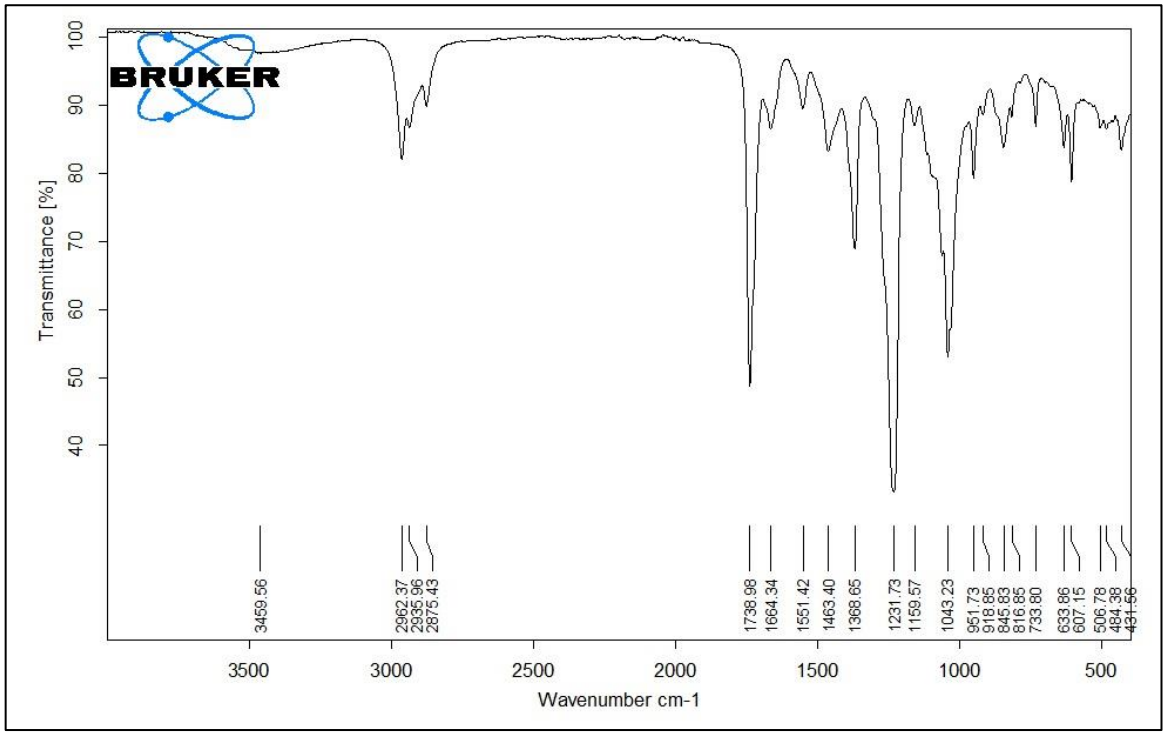

**Appendix B: Figure 44 FTIR spectrum of DBP nail lacquer Stage T3**



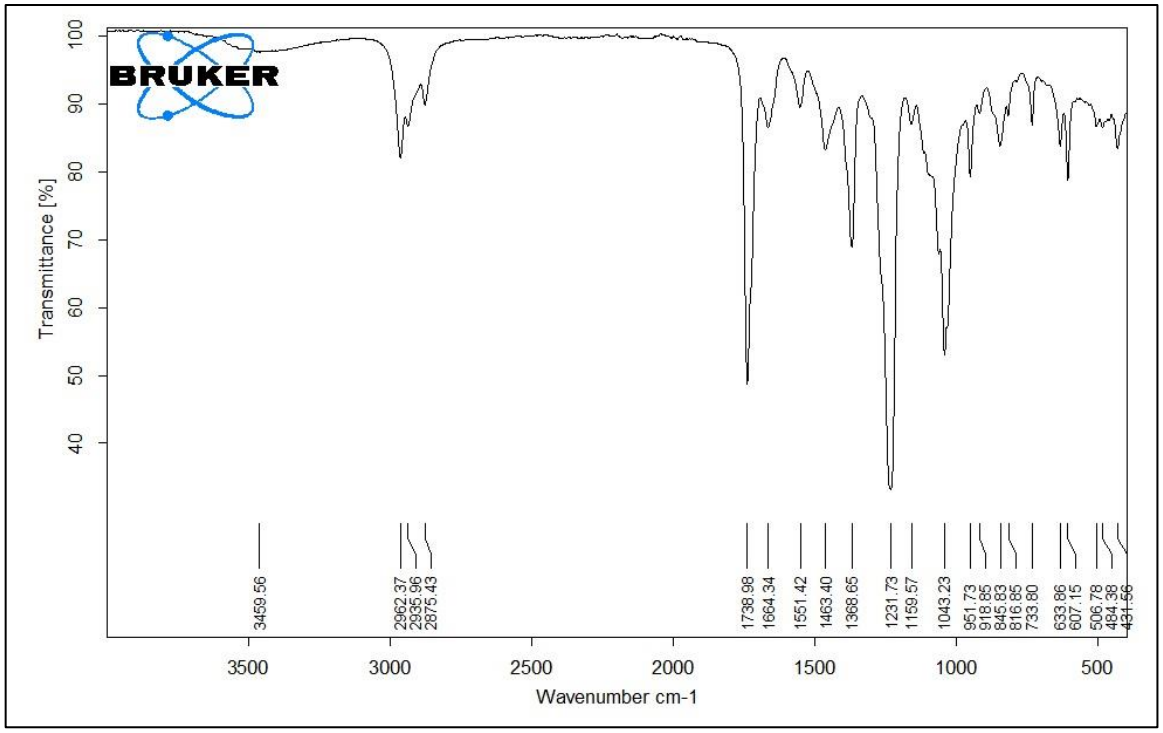

**Appendix B: Figure 45 FTIR spectrum of DBP nail lacquer Stage T0 (end)**



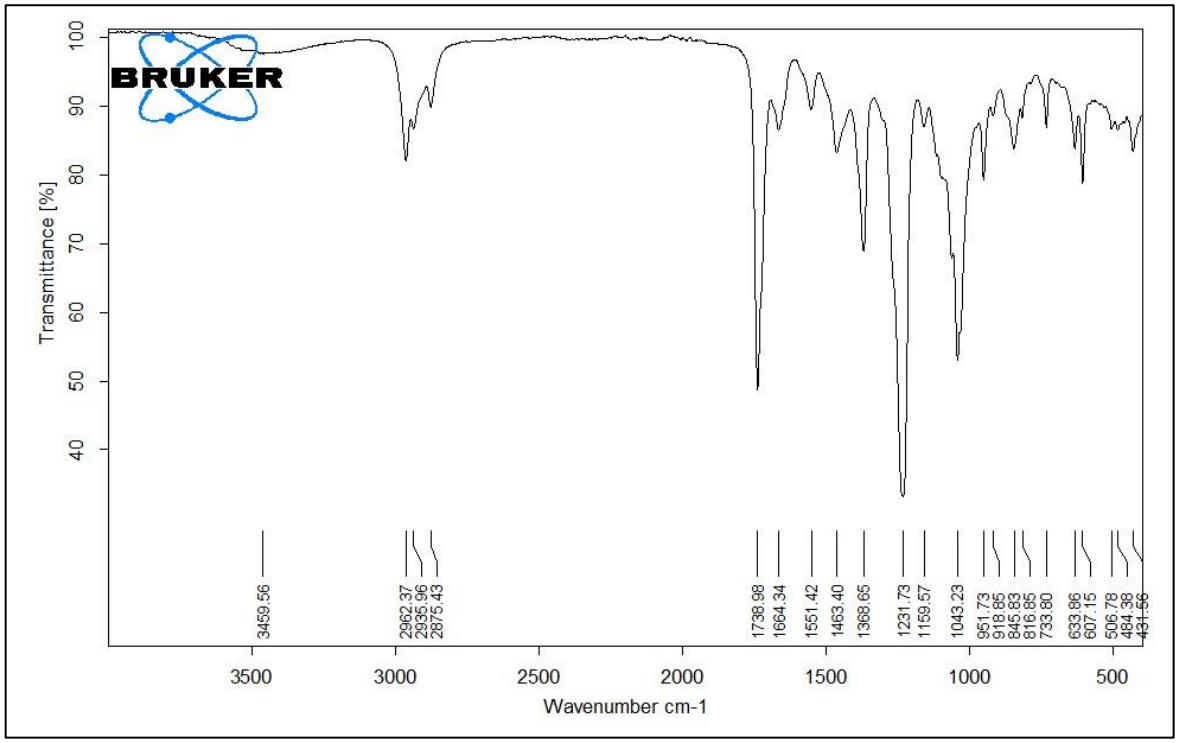

**Appendix B: Figure 46 FTIR spectrum of DEHT nail lacquer Stage T0**



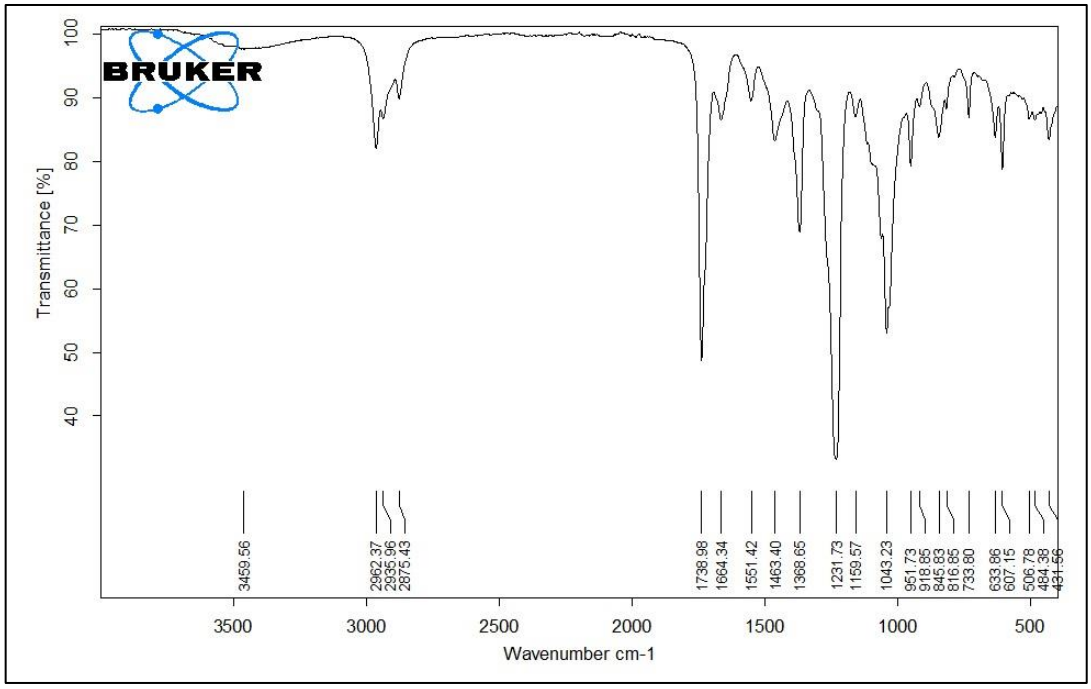

**Appendix B: Figure 47 FTIR spectrum of DEHT nail lacquer Stage T1**



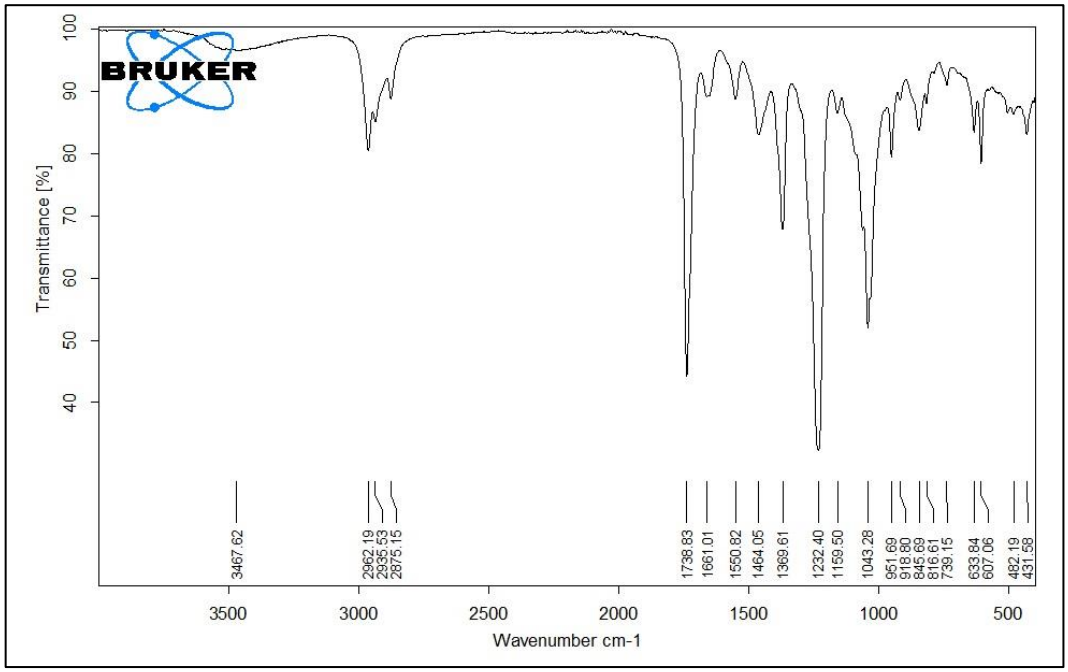

**Appendix B: Figure 48 FTIR spectrum of DEHT nail lacquer Stage T2**



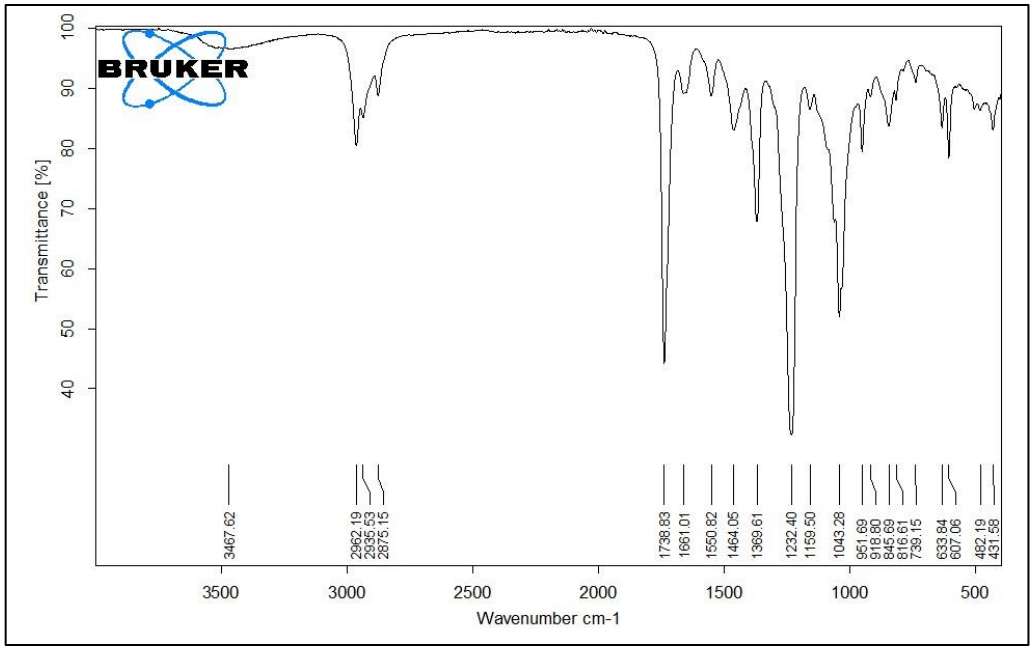

**Appendix B: Figure 49 FTIR spectrum of DEHT nail lacquer Stage T3**



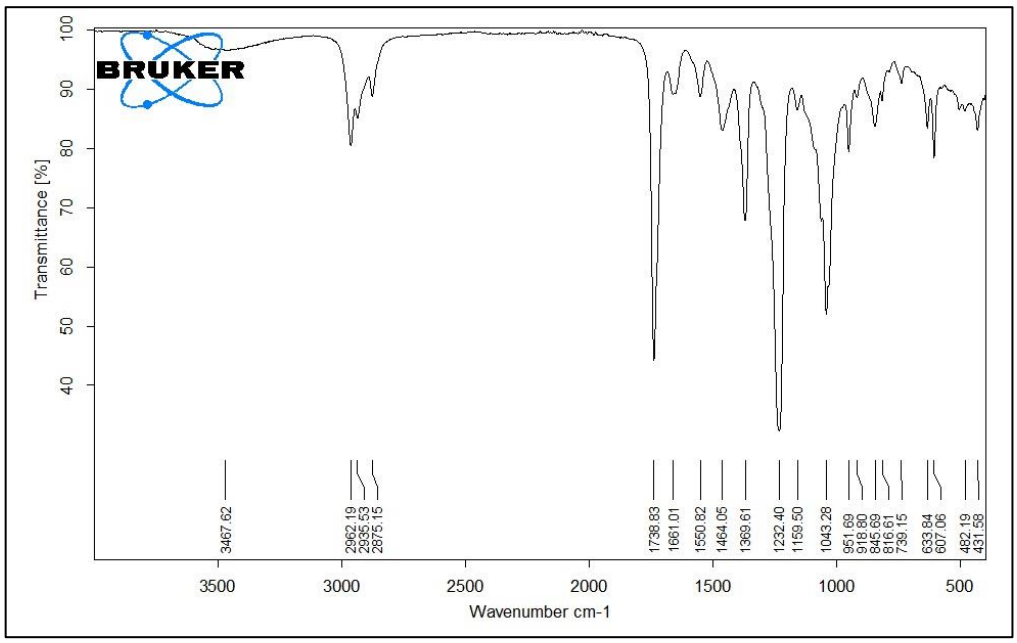

**Appendix B: Figure 50 FTIR spectrum of DEHT nail lacquer Stage T0 (end)**



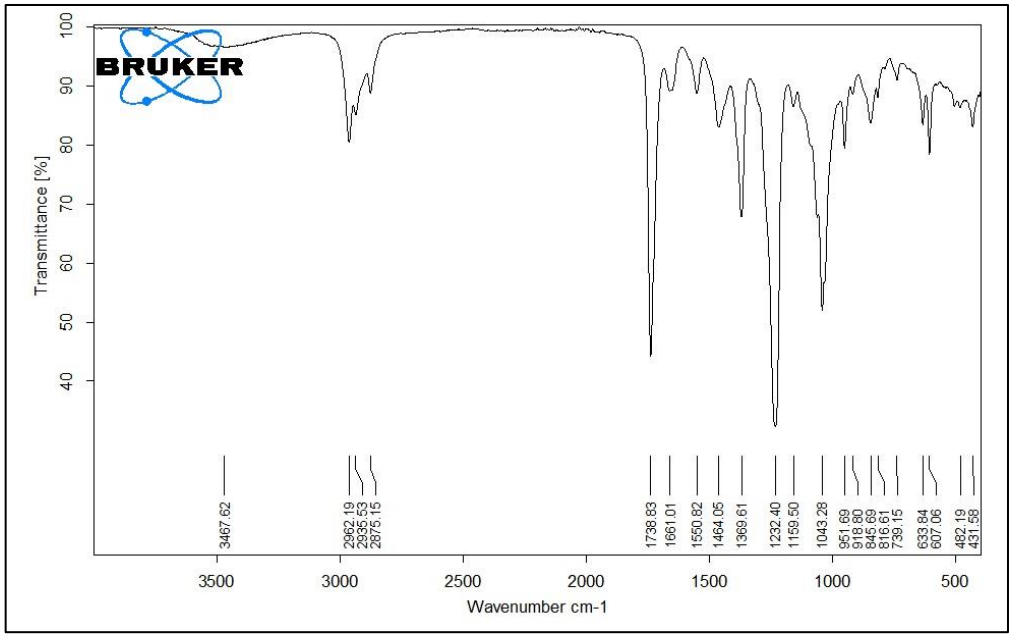

**Appendix B: Figure 51 FTIR spectrum of PMD-citronellal acetal nail lacquer Stage T0**



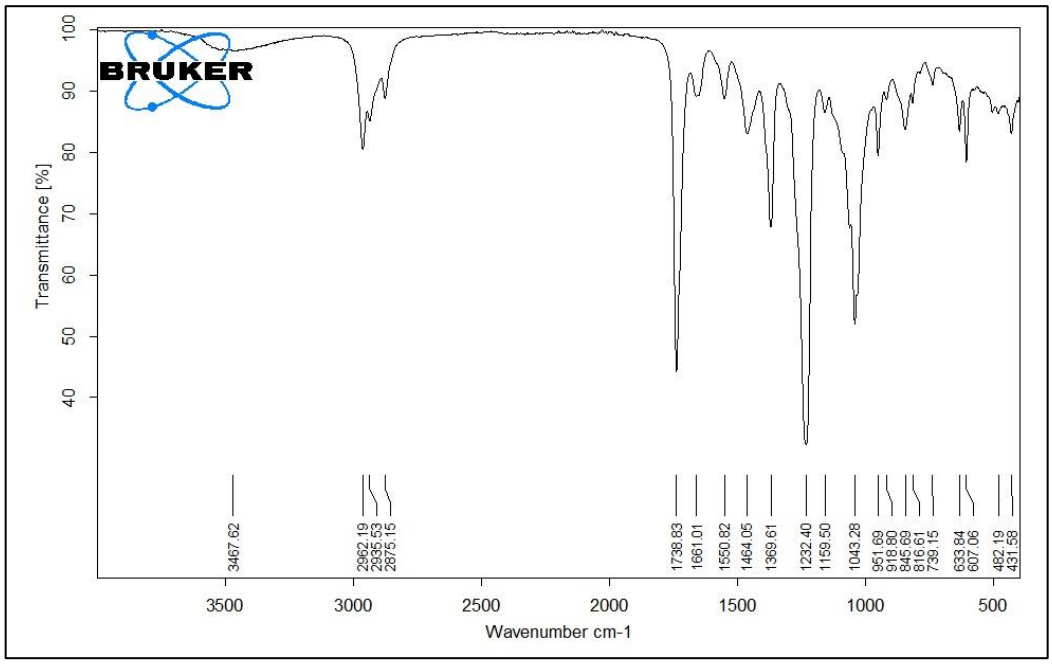

**Appendix B: Figure 52 FTIR spectrum of PMD-citronellal acetal nail lacquer Stage T1**




**Appendix B: Figure 53 FTIR spectrum of PMD-citronellal acetal nail lacquer Stage T2**




**Appendix B: Figure 54 FTIR spectrum of PMD-citronellal acetal nail lacquer Stage T3**




**Appendix B: Figure 55 FTIR spectrum of PMD-citronellal acetal nail lacquer Stage T0 (end)**

# APPENDIX C: LEACHING

**Appendix C: Table 1 Table showing peak areas obtained for Acetal without use of SPE**

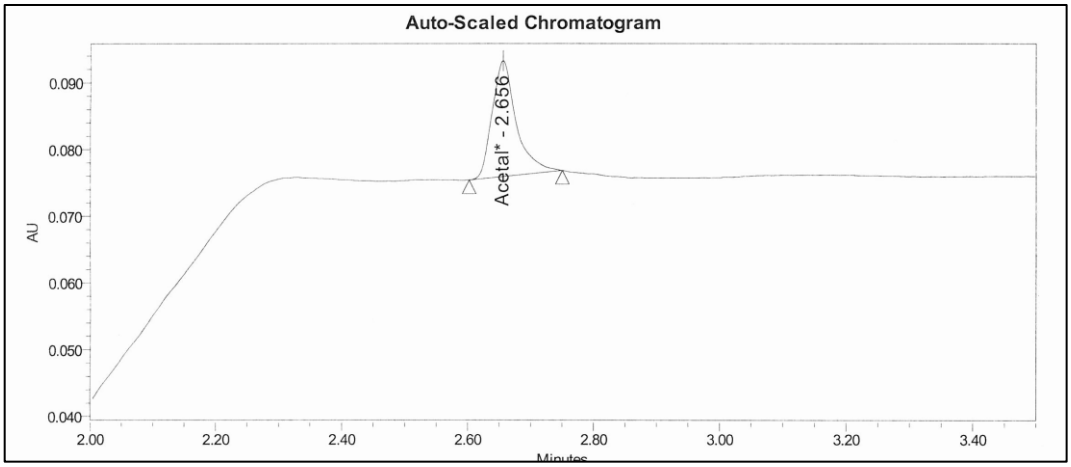
**Summary All No Stats Table**
**Name: Acetal***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | Acetal* | 1 | 2.7 | 46954 | 17431 | 1.36 | 23240 |
| 2 | Acetal* | 1 | 2.7 | 46724 | 17413 | 1.36 | 23289 |
| 3 | Acetal* | 1 | 2.7 | 47901 | 17498 | 1.40 | 23051 |
| Mean | | | | 47193 | 17447 | 1.4 | 23193 |
| % RSD | | | | 1.32 | 0.26 | | |

**Appendix C: Table 2 Table showing peak areas obtained for Acetal with use of SPE**

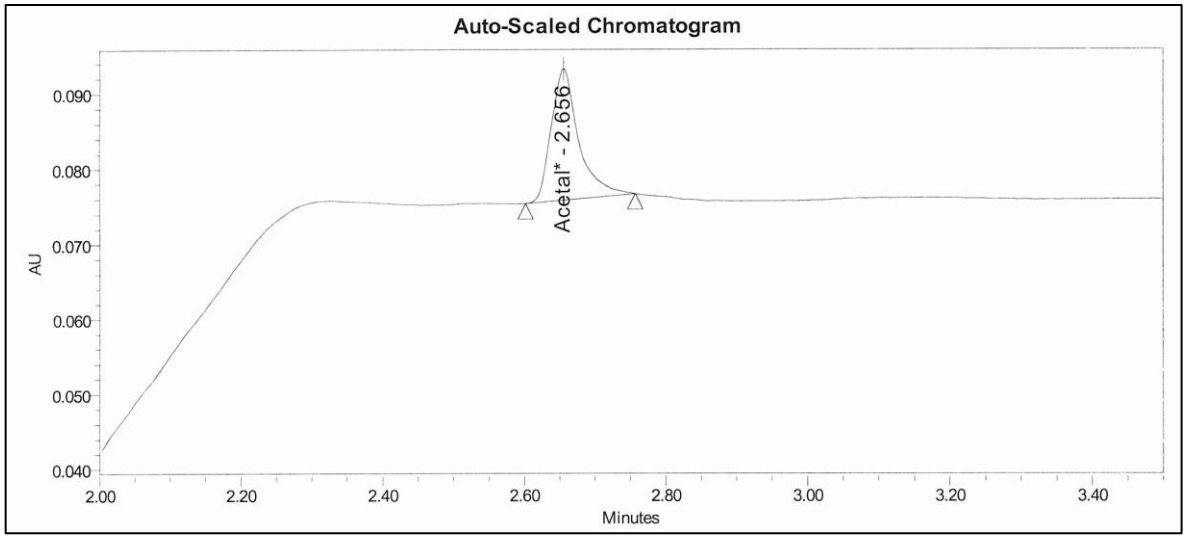
**Summary All No Stats Table**
**Name: Acetal***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | Acetal* | 1 | 2.7 | 46147 | 17367 | 1.34 | 23416 |
| 2 | Acetal* | 1 | 2.7 | 45537 | 17316 | 1.32 | 23558 |
| 3 | Acetal* | 1 | 2.7 | 46341 | 17383 | 1.34 | 23373 |
| Mean | | | | 46008 | 17355 | 1.3 | 23449 |
| % RSD | | | | 0.91 | 0.20 | | |




| | Name | RT | Area |
|---|---|---|---|
| 1 | Acetal* | 2.656 | 46147 |

**Appendix C: Figure 1 UPLC peak results for Acetal chromatogram (1st injection) without use of SPE**




| | Name | RT | Area |
|---|---|---|---|
| 1 | Acetal* | 2.656 | 46954 |

**Appendix C: Figure 2 UPLC peak results for Acetal chromatogram (1st injection) with use of SPE**

**Appendix C: Table 3 Table showing peak areas obtained for DPB without use of SPE**

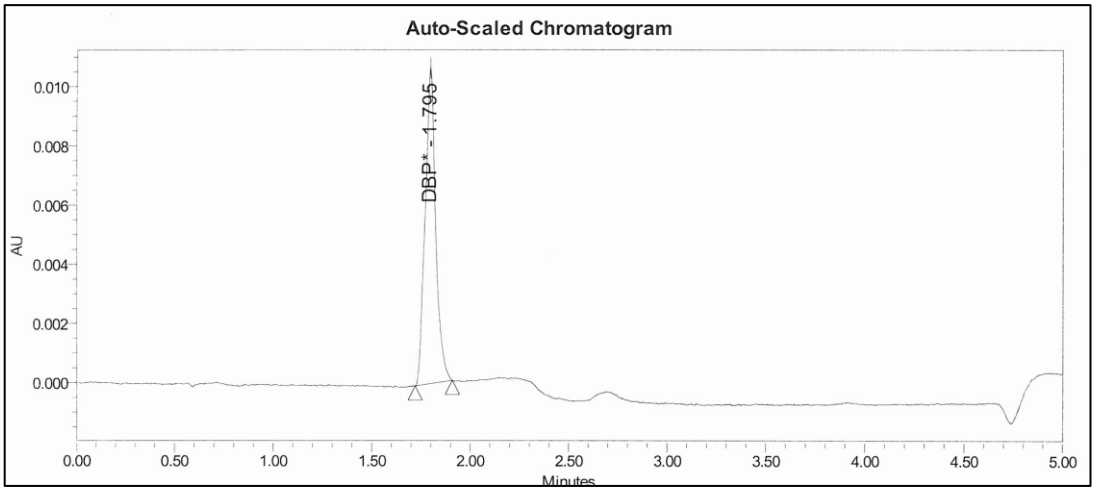
**Summary All No Stats Table**
**Name: DBP***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | DBP* | 1 | 1.8 | 39670 | 10682 | 1.16 | 4669 |
| 2 | DBP* | 1 | 1.8 | 39837 | 10693 | 1.17 | 4660 |
| 3 | DBP* | 1 | 1.8 | 39915 | 10698 | 1.17 | 4655 |
| Mean | | | | 39807 | 10691 | 1.2 | 4661 |
| % RSD | | | | 0.31 | 0.08 | | |

**Appendix C: Table 4 Table showing peak areas obtained for DPB with use of SPE**

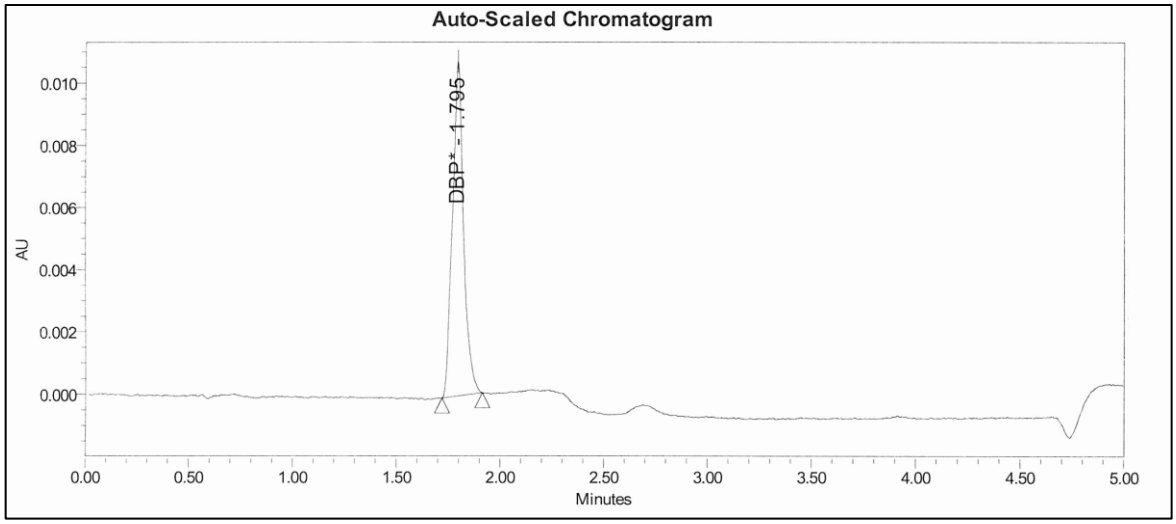
**Summary All No Stats Table**
**Name: DBP***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | DBP* | 1 | 1.8 | 40038 | 10759 | 1.16 | 4656 |
| 2 | DBP* | 1 | 1.8 | 39057 | 10635 | 1.14 | 4711 |
| 3 | DBP* | 1 | 1.8 | 39270 | 10652 | 1.15 | 4696 |
| Mean | | | | 39455 | 10682 | 1.1 | 4688 |
| % RSD | | | | 1.31 | 0.63 | | |




| | Name | RT | Area |
|---|---|---|---|
| 1 | DBP* | 1.795 | 39670 |

**Appendix C: Figure 3 UPLC peak results for DBP chromatogram (1st injection) without use of SPE**




| | Name | RT | Area |
|---|---|---|---|
| 1 | DBP* | 1.795 | 40038 |

**Appendix C: Figure 4 UPLC peak results for DBP chromatogram (1st injection) with use of SPE**

**Appendix C: Table 5 Table showing peak areas obtained for DEHT without use of SPE**

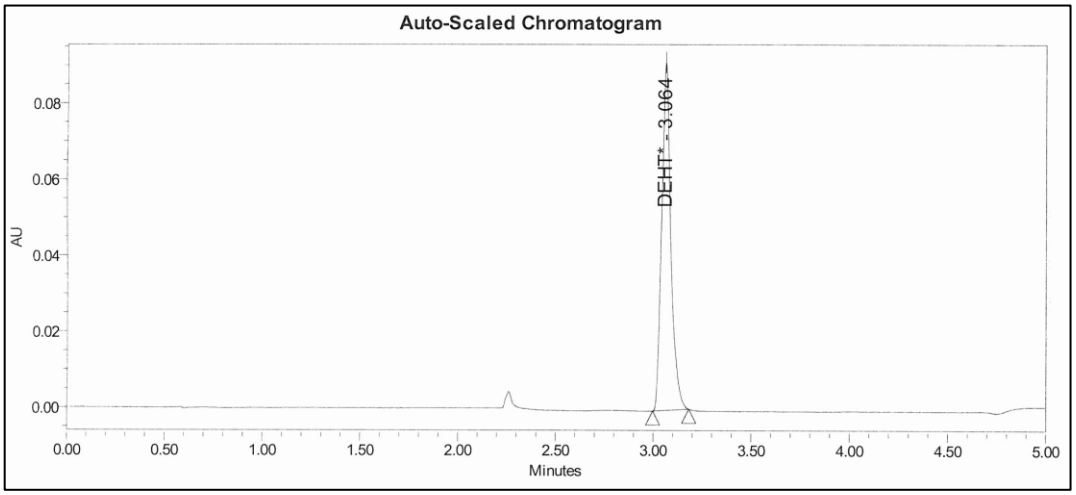
**Summary All No Stats Table**
**Name: DEHT***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | DEHT* | 1 | 3.1 | 302655 | 91777 | 1.30 | 19304 |
| 2 | DEHT* | 1 | 3.1 | 302574 | 91773 | 1.30 | 19306 |
| 3 | DEHT* | 1 | 3.1 | 302628 | 91776 | 1.30 | 19304 |
| Mean | | | | 302619 | 91776 | 1.3 | 19304 |
| % RSD | | | | 0.01 | 0.00 | | |

**Appendix C: Table 6 Table showing peak areas obtained for DEHT with use of SPE**

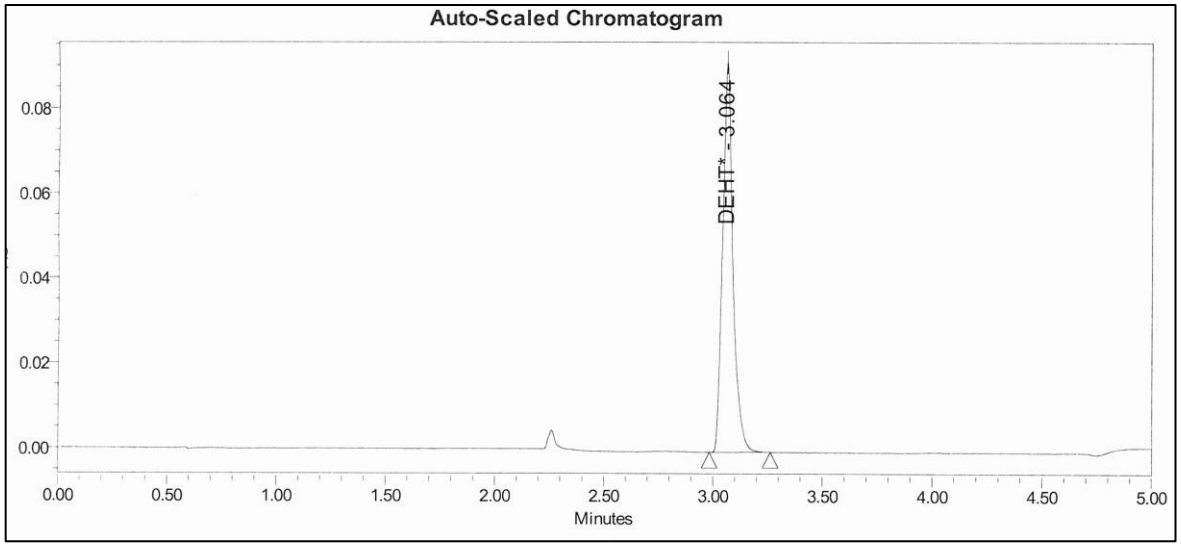
**Summary All No Stats Table**
**Name: DEHT***

| | Name | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | DEHT* | 1 | 3.1 | 298537 | 91535 | 1.28 | 19420 |
| 2 | DEHT* | 1 | 3.1 | 299037 | 91572 | 1.28 | 19402 |
| 3 | DEHT* | 1 | 3.1 | 299278 | 91591 | 1.28 | 19394 |
| Mean | | | | 298951 | 91566 | 1.3 | 19405 |
| % RSD | | | | 0.13 | 0.03 | | |




| | Name | RT | Area |
|---|---|---|---|
| 1 | DEHT* | 3.064 | 298537 |

**Appendix C: Figure 5 UPLC peak results for DEHT chromatogram (1st injection) with use of SPE**




| | Name | RT | Area |
|---|---|---|---|
| 1 | DEHT* | 3.064 | 302655 |

**Appendix C: Figure 6 UPLC peak results for DEHT chromatogram (1st injection) without use of SPE**

**Appendix C: Table 7 Standards injected for the Acetal calibration curve**

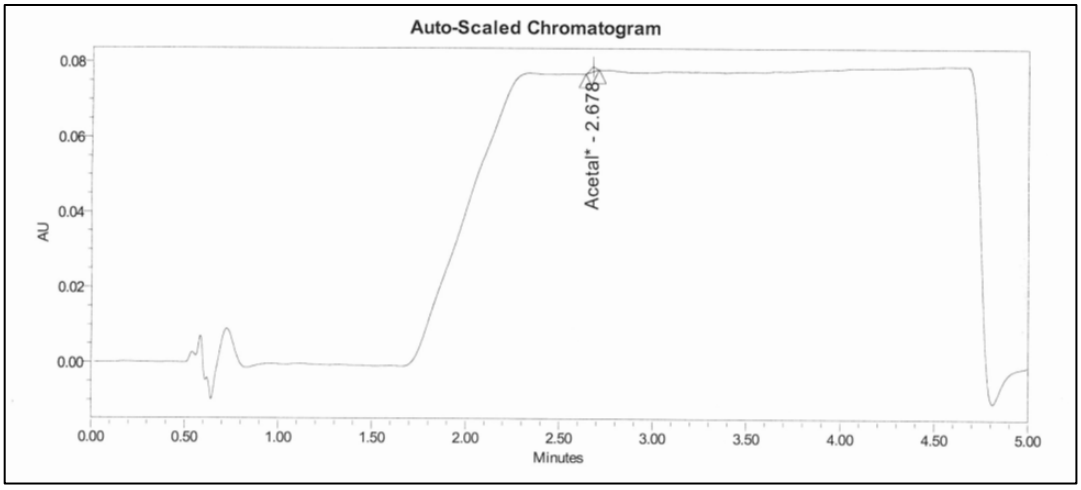
| | SampleName | Name | Vial | Inj | RT | Area |
|---|---|---|---|---|---|---|
| 1 | Std 1 | Acetal* | 2:A,2 | 1 | 2.7 | 28833 |
| 2 | Std 2 | Acetal* | 2:A,3 | 1 | 2.7 | 49679 |
| 3 | Std 3 | Acetal* | 2:A,4 | 1 | 2.7 | 99832 |
| 4 | Std 4 | Acetal* | 2:A,5 | 1 | 2.7 | 190878 |
| 5 | Std 5 | Acetal* | 2:A,6 | 1 | 2.7 | 287222 |
| 6 | Std 6 | Acetal* | 2:A,7 | 1 | 2.7 | 382263 |

## Appendix C: Table 8 Standards injected for the DBP calibration curve

| | SampleName | Name | Vial | Inj | RT | Area |
|---|---|---|---|---|---|---|
| 1 | Std 1 | DBP* | 1:A,2 | 1 | 1.8 | 23496 |
| 2 | Std 2 | DBP* | 1:A,3 | 1 | 1.8 | 40588 |
| 3 | Std 3 | DBP* | 1:A,4 | 1 | 1.8 | 82645 |
| 4 | Std 4 | DBP* | 1:A,5 | 1 | 1.8 | 152292 |
| 5 | Std 5 | DBP* | 1:A,6 | 1 | 1.8 | 248209 |
| 6 | Std 6 | DBP* | 1:A,7 | 1 | 1.8 | 331141 |

## Appendix C: Table 9 Standards injected for the DEHT calibration curve

| | SampleName | Name | Vial | Inj | RT | Area |
|---|---|---|---|---|---|---|
| 1 | Std 1 | DEHT* | 1:A,2 | 1 | 3.1 | 151175 |
| 2 | Std 2 | DEHT* | 1:A,3 | 1 | 3.1 | 303178 |
| 3 | Std 3 | DEHT* | 1:A,4 | 1 | 3.1 | 615782 |
| 4 | Std 4 | DEHT* | 1:A,5 | 1 | 3.1 | 1158673 |
| 5 | Std 5 | DEHT* | 1:A,6 | 1 | 3.1 | 1742119 |
| 6 | Std 6 | DEHT* | 1:A,7 | 1 | 3.1 | 2149088 |



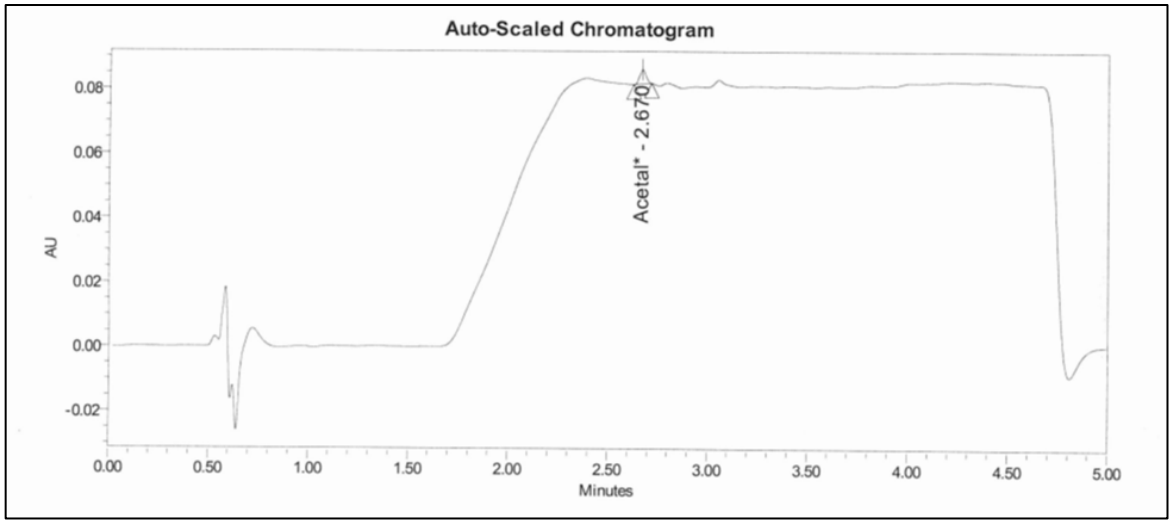

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Acetal* | 2.678 | 2702 | 1289 |

The 25% solution from the calibration curve was used to prepare the limit of detection solution.

25% → 0.2455 µg/ml

1 ml of the 25% solution was accurately transferred to a 10 ml volumetric flask and made to volume with solvent (methanol), rendering the LOD solution concentration at approximately 0.025 µg/ml.

**Appendix C: Figure 7 Chromatogram and calculation of PMD-citronellal acetal for the LOD (0.025 ug/ml)**



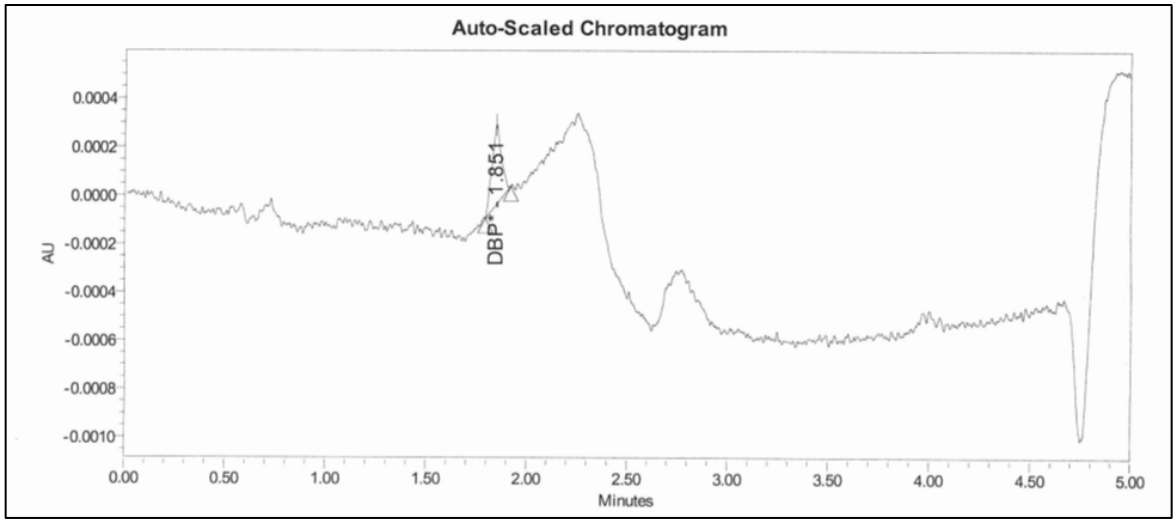

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Acetal* | 2.670 | 9849 | 4474 |

The 25% solution from the calibration curve was used to prepare the limit of quantification solution.

25% → 0.2455 µg/ml

A 3.3 ml of the 25% solution was transferred into a 10 ml volumetric flask and made to volume with solvent (methanol), rendering the LOQ solution concentration at approximately 0.081 µg/ml.

**Appendix C: Figure 8 Chromatogram and calculation of PMD-citronellal acetal for the LOQ**



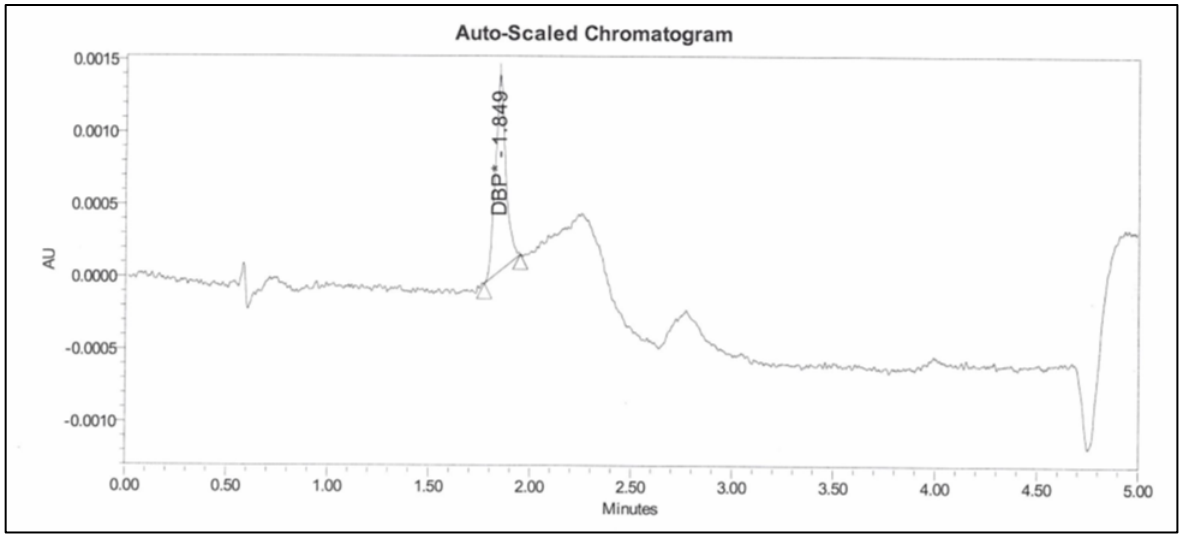

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | DBP* | 1.851 | 1061 | 326 |

The 25% solution from the calibration curve was used to make the limit of detection solution.

25% → 0.2620 µg/ml

A 4 ml of the 25% solution was transferred to a 100 ml volumetric flask and made to mark, rendering the LOD solution concentration at approximately 0.01 µg/ml.

**Appendix C: Figure 9 Chromatogram and calculation of DBP for the LOD**



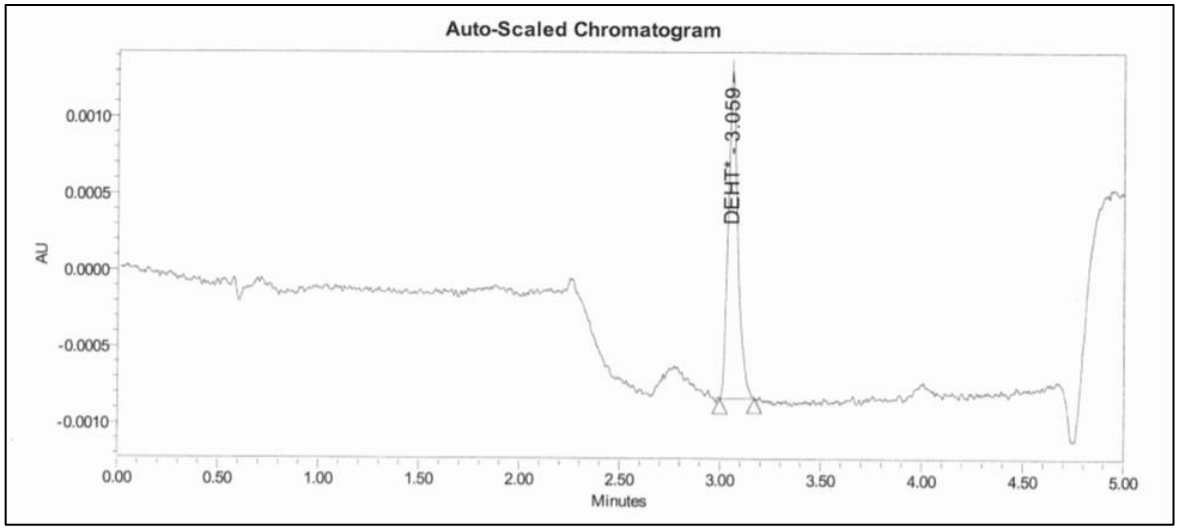

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | DBP* | 1.849 | 4846 | 1357 |

The 25% solution from the calibration curve was used to make the limit of quantification solution.

25% → 0.2620 µg/ml

19 ml of the 25% solution was transferred to a 100 ml volumetric flask and made to mark, rendering the LOQ solution concentration at approximately 0.05 µg/ml.

**Appendix C: Figure 10 Chromatogram and calculation of DBP for the LOQ**



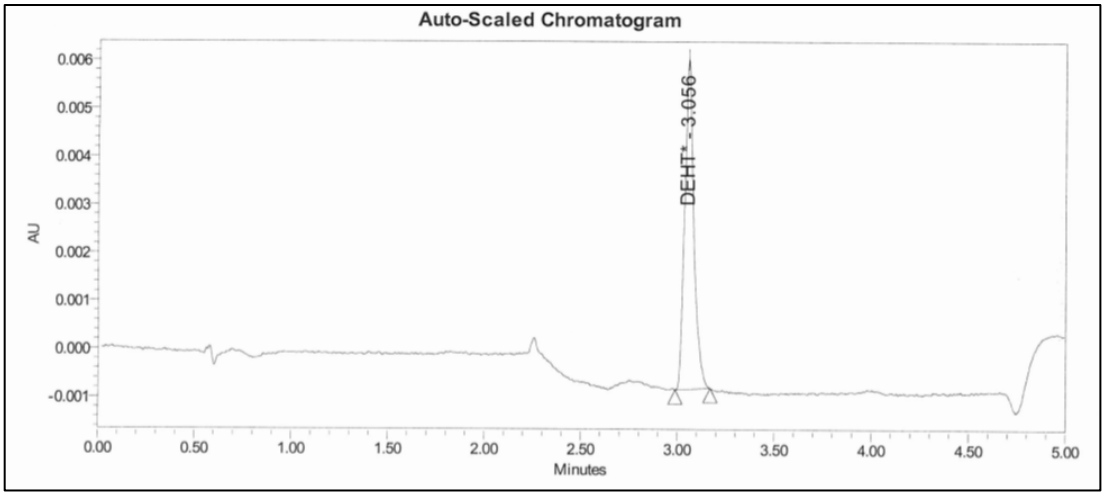

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | DEHT* | 3.059 | 7077 | 2138 |

The 25% solution from the calibration curve was used to make the limit of detection solution.

25% → 0.2460 µg/ml

A 4.8 ml of the 25% solution was dissolved in a 100 ml volumetric flask and made to mark, rendering the LOD solution concentration at approximately 0.01 µg/ml.

**Appendix C: Figure 11 Chromatogram and calculation of DEHT for the LOD**



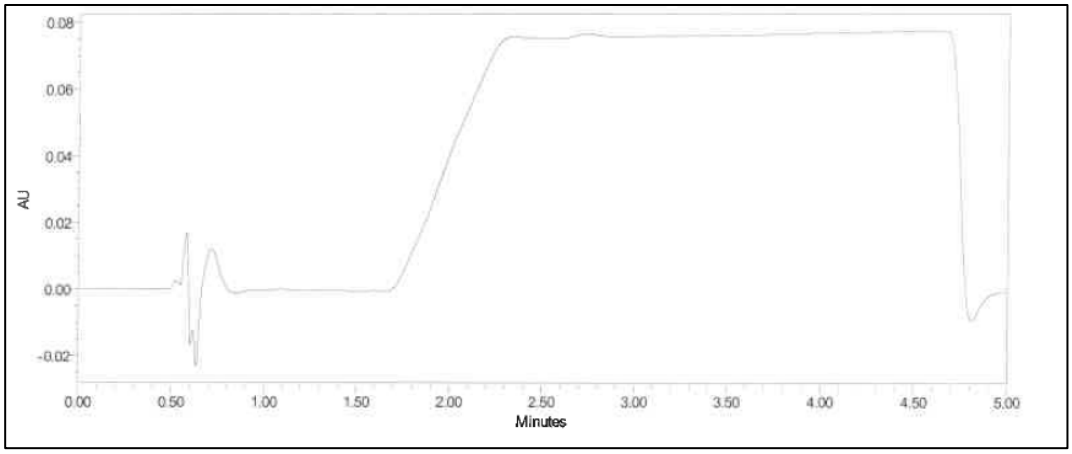

| | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | DEHT* | 3.056 | 22653 | 6856 |

The 25% solution from the calibration curve was used to make the limit of quantification solution.

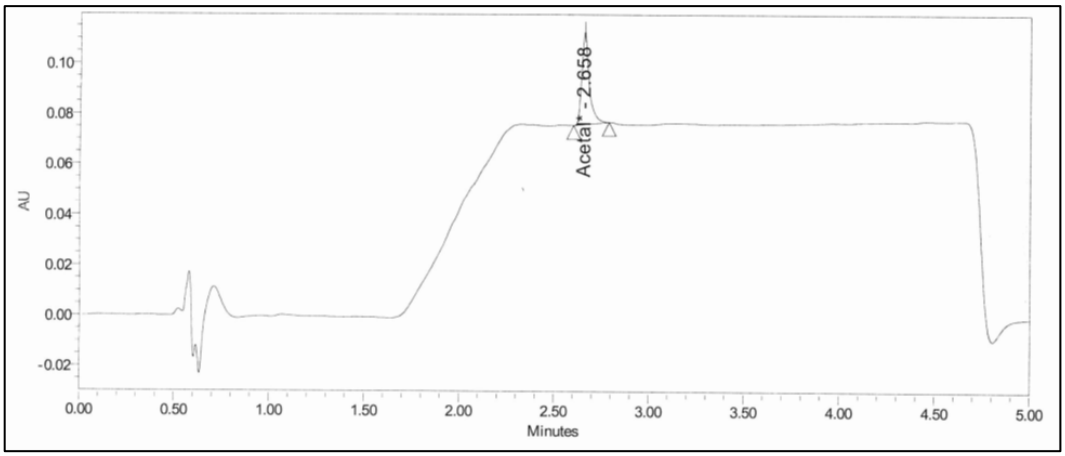
25% → 0.2460 µg/ml

A 16 ml of the 25% solution was dissolved into a 100 ml volumetric flask and made to mark, rendering the LOD solution concentration at approximately 0.04 µg/ml.

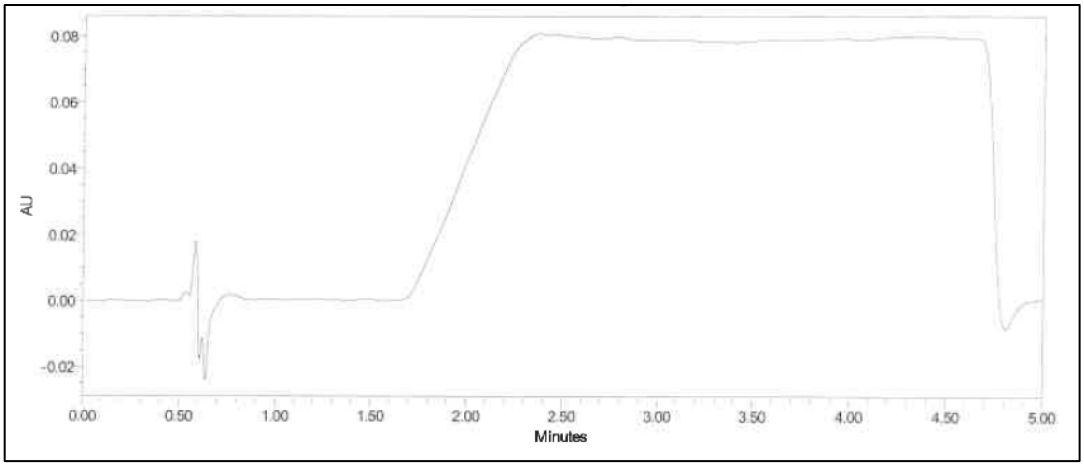
**Appendix C: Figure 12 Chromatogram and calculation of DEHT for the LOQ**



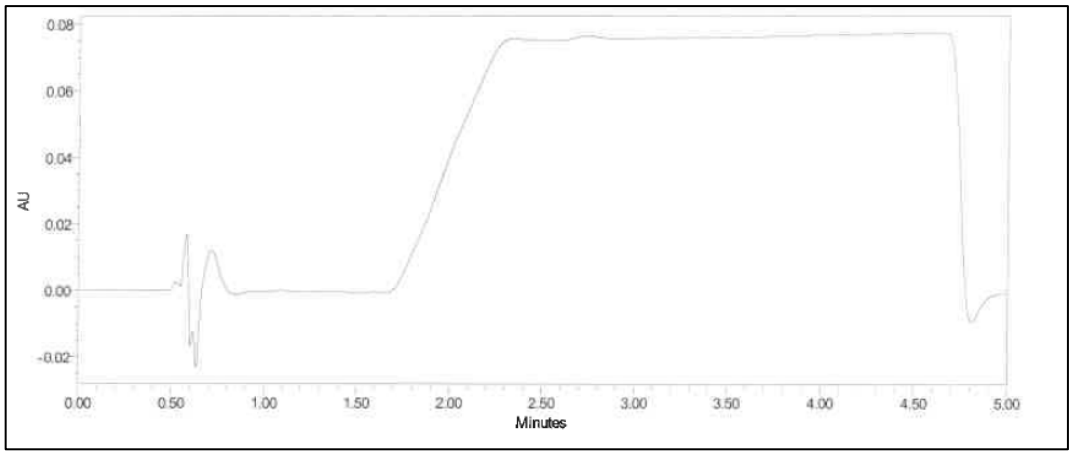

**Appendix C: Figure 13 Chromatogram of the Blank obtained for PMD-citronellal acetal**



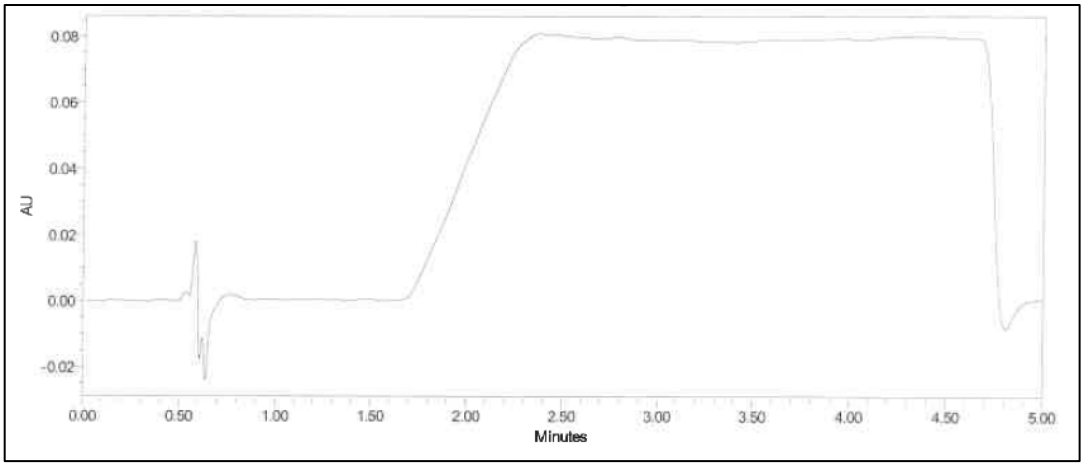

**Appendix C: Figure 14 Standard chromatogram obtained for PMD-citronellal acetal**



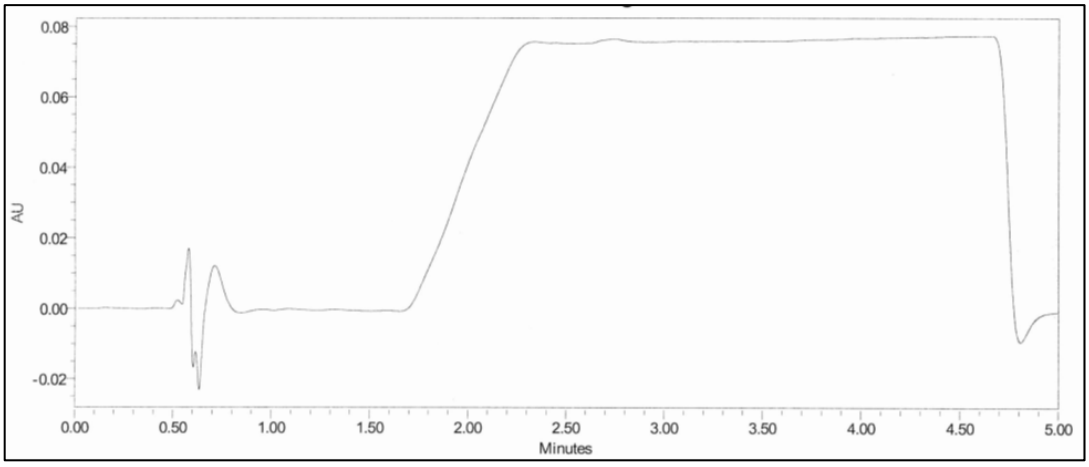

**Appendix C: Figure 15 Sample chromatogram obtained for PMD-citronellal acetal at 31 °C (24 H interval)**



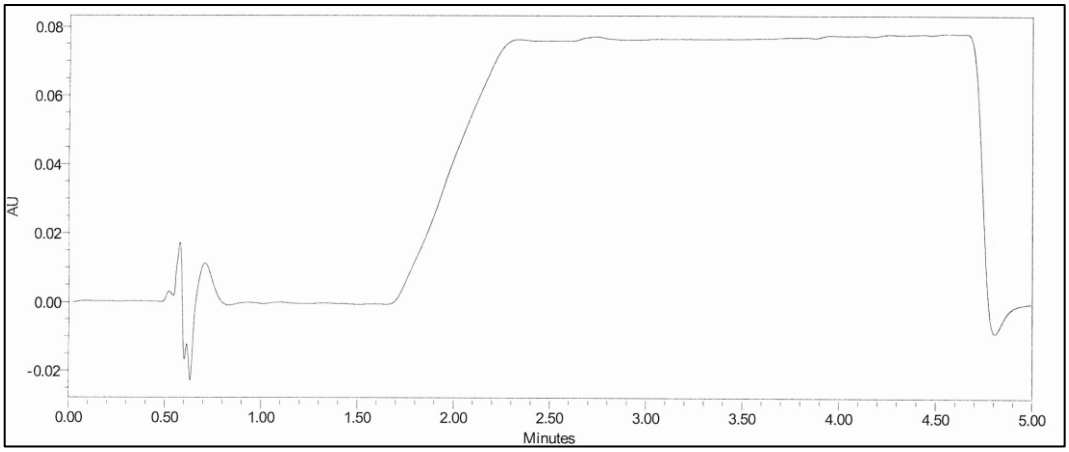

**Appendix C: Figure 16 Sample chromatogram obtained for PMD-citronellal acetal at 31 °C (48 H interval)**



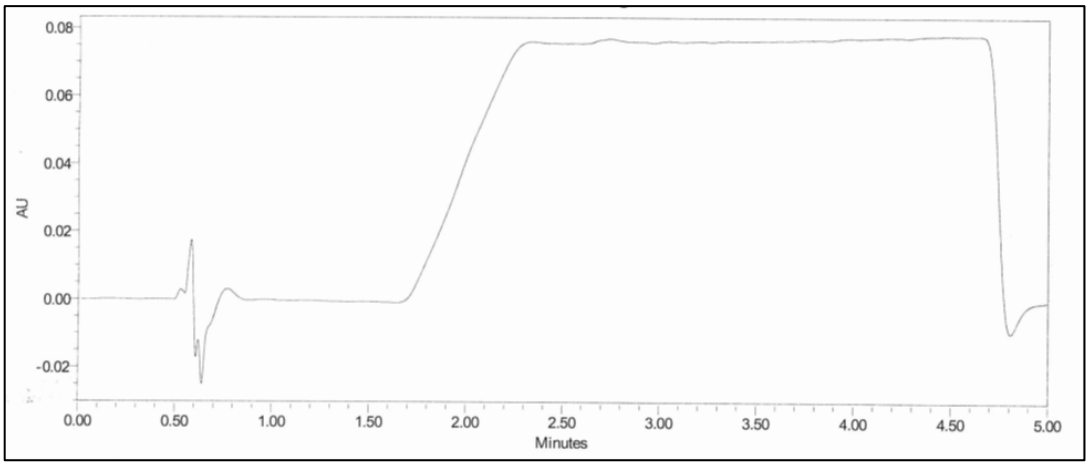

**Appendix C: Figure 17 Sample chromatogram obtained for PMD-citronellal acetal at 31 °C (72 H interval)**




**Appendix C: Figure 18 Sample chromatogram obtained for PMD-citronellal acetal at 50 °C (24 H interval)**




**Appendix C: Figure 19 Showing a Sample chromatogram obtained for PMD-citronellal acetal at 50 °C (48 H interval)**




**Appendix C: Figure 20 Showing a Sample chromatogram obtained for PMD-citronellal acetal at 50 °C (72 H interval)**

**Appendix C: Table 10 Showing samples injected after oven incubation at 50 °C for 24 H**

**Summary All No Stats Table**
**Name: DBP***

| | SampleName | Name | Vial | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|---|---|
| 1 | D1_50 | DBP* | 1:B,1 | 1 | 1.8 | 83944 | 22766 | 1.19 | 4934 |
| 2 | D2_50 | DBP* | 1:B,2 | 1 | 1.8 | 82520 | 22206 | 1.19 | 4802 |
| 3 | D3_50 | DBP* | 1:B,3 | 1 | 1.8 | 75904 | 20667 | 1.19 | 5061 |
| 4 | D4_50 | DBP* | 1:B,4 | 1 | 1.8 | 79074 | 21650 | 1.20 | 5300 |
| 5 | D5_50 | DBP* | 1:B,5 | 1 | 1.8 | 81700 | 22404 | 1.20 | 5342 |
| Mean | | | | | | 80628 | 21939 | 1.2 | 5088 |
| % RSD | | | | | | 3.94 | 3.73 | | |

**Appendix C: Table 11 Showing samples injected after being in the oven at 50 °C (48 H interval)**

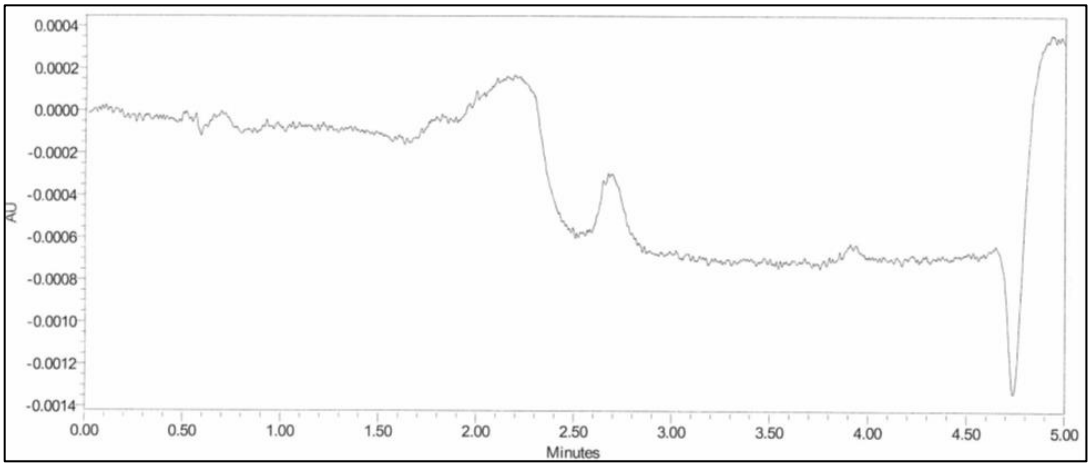
**Summary All No Stats Table**
**Name: DBP***

| | SampleName | Name | Vial | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|---|---|
| 1 | D6_50 | DBP* | 1:B,6 | 1 | 1.8 | 76733 | 21790 | 1.21 | 5896 |
| 2 | D7_50 | DBP* | 1:B,7 | 1 | 1.8 | 79770 | 22212 | 1.21 | 5574 |
| 3 | D8_50 | DBP* | 1:B,8 | 1 | 1.8 | 79018 | 21648 | 1.21 | 5309 |
| 4 | D9_50 | DBP* | 1:C,1 | 1 | 1.8 | 82812 | 22280 | 1.20 | 5023 |
| 5 | D10_50 | DBP* | 1:C,2 | 1 | 1.8 | 80859 | 21683 | 1.21 | 5022 |
| Mean | | | | | | 79838 | 21923 | 1.2 | 5365 |
| % RSD | | | | | | 2.81 | 1.37 | | |

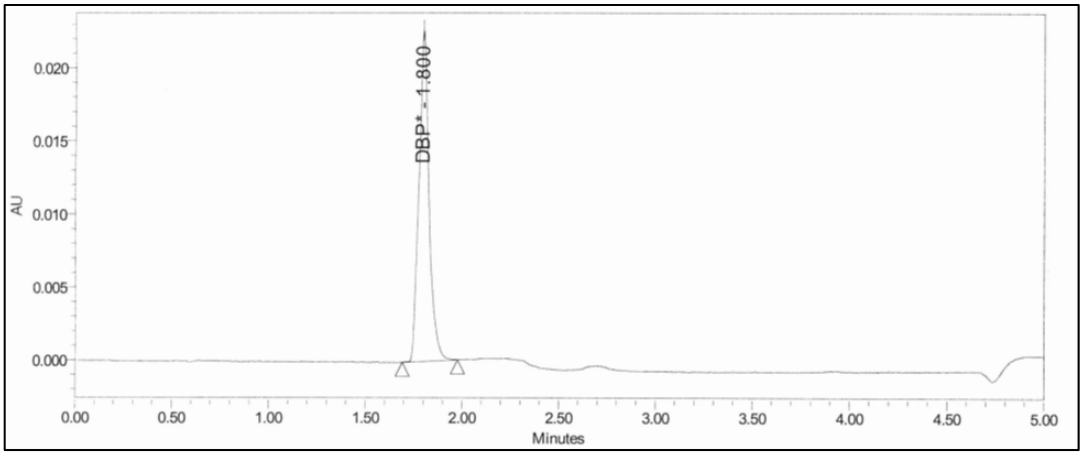
**Appendix C: Table 12 Showing samples injected after being in the oven at 50 °C (72 H interval)**

**Summary All No Stats Table**
**Name: DBP***

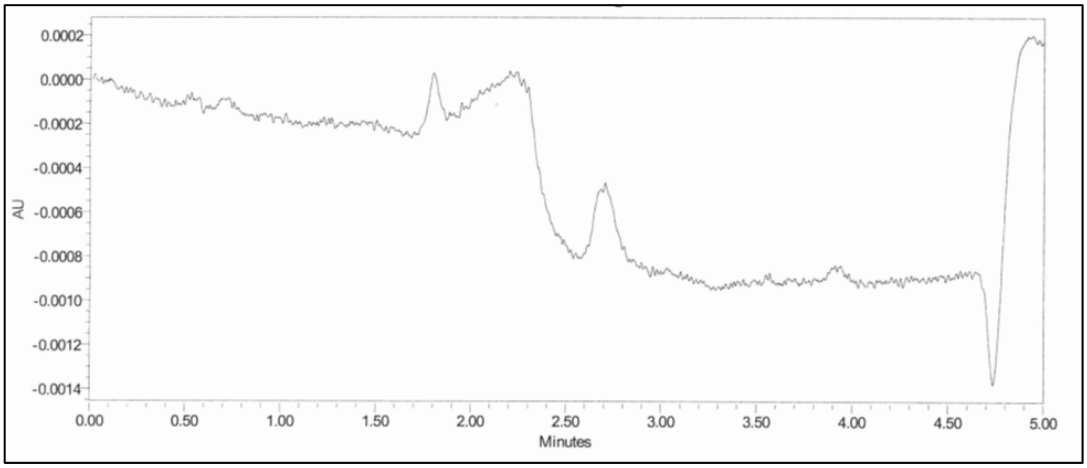
| | SampleName | Name | Vial | Inj | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|---|---|
| 1 | D11_50 | DBP* | 1:C,3 | 1 | 1.8 | 75346 | 20016 | 1.19 | 4741 |
| 2 | D12_50 | DBP* | 1:C,4 | 1 | 1.8 | 78352 | 20769 | 1.20 | 4742 |
| 3 | D13_50 | DBP* | 1:C,5 | 1 | 1.8 | 75937 | 20439 | 1.20 | 4984 |
| 4 | D14_50 | DBP* | 1:C,6 | 1 | 1.8 | 71218 | 19891 | 1.21 | 5654 |
| 5 | D15_50 | DBP* | 1:C,7 | 1 | 1.8 | 76116 | 21432 | 1.21 | 5877 |
| Mean | | | | | | 75394 | 20509 | 1.2 | 5200 |
| % RSD | | | | | | 3.45 | 3.03 | | |



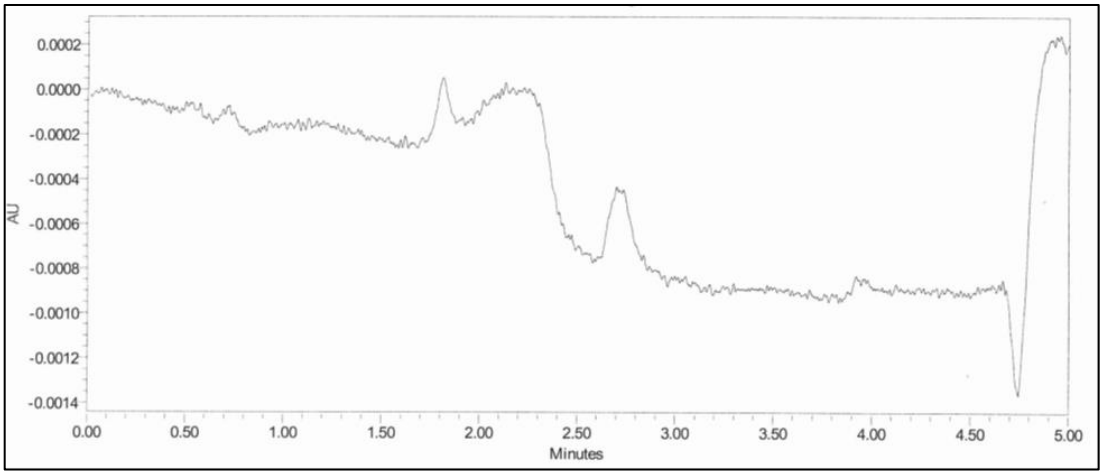

**Appendix C: Figure 21 Blank chromatogram obtained for DBP**



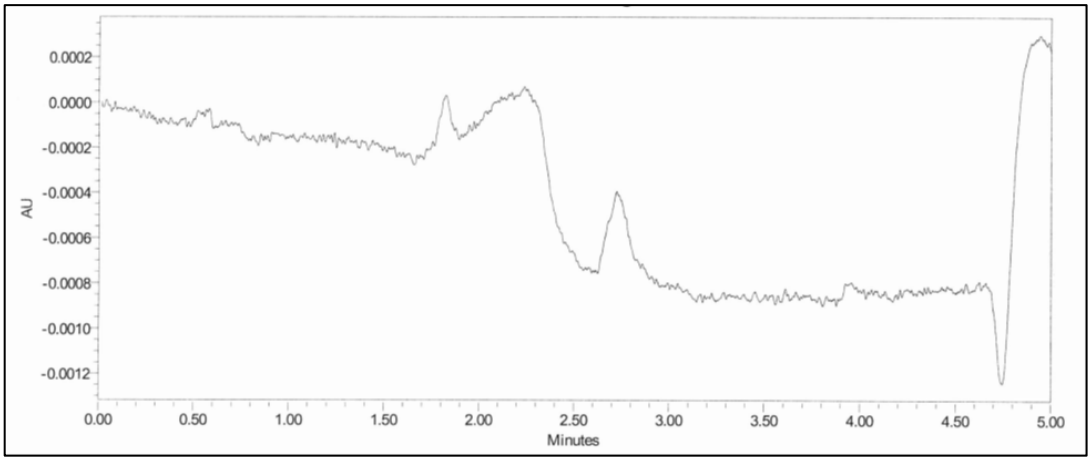

**Appendix C: Figure 22 Standard chromatogram obtained for DBP**



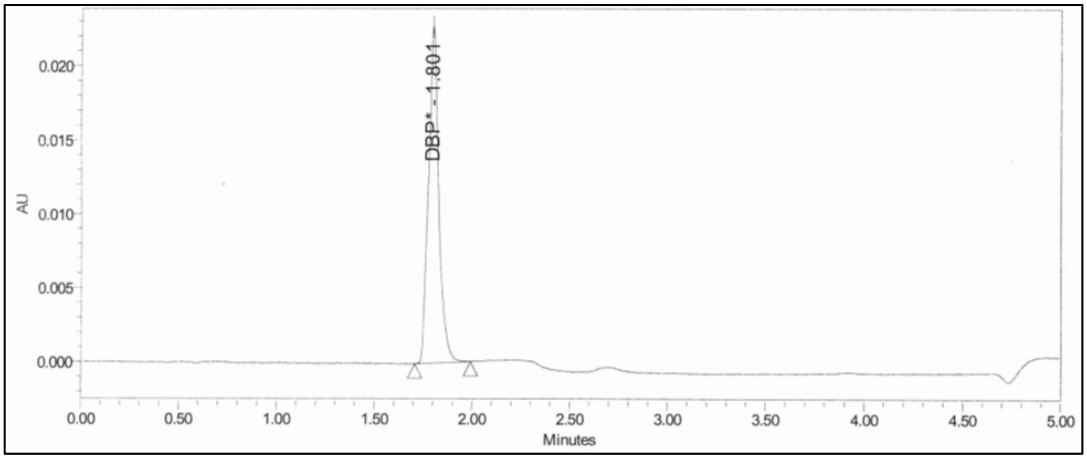

**Appendix C: Figure 23 Sample chromatogram obtained for DBP at 31 °C (24 H interval)**



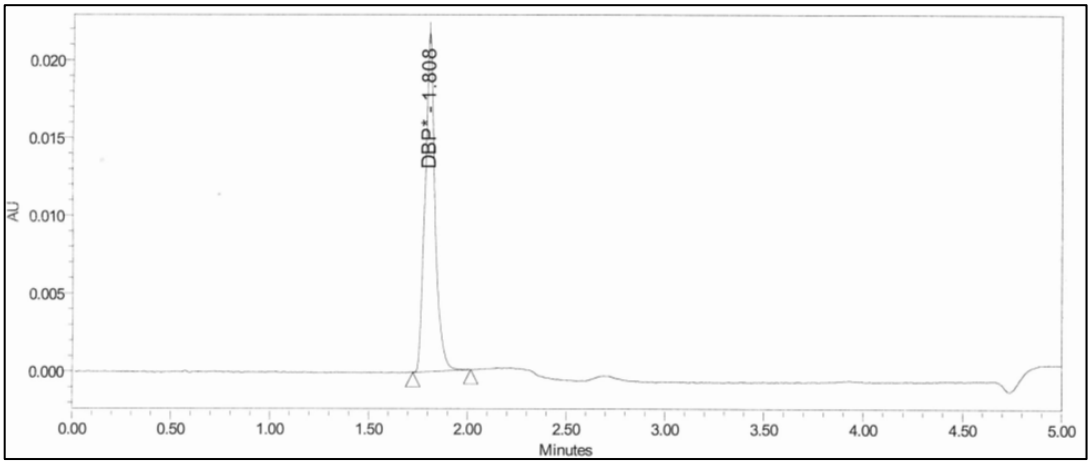

**Appendix C: Figure 24 Sample chromatogram obtained for DBP at 31 °C (48 H interval)**



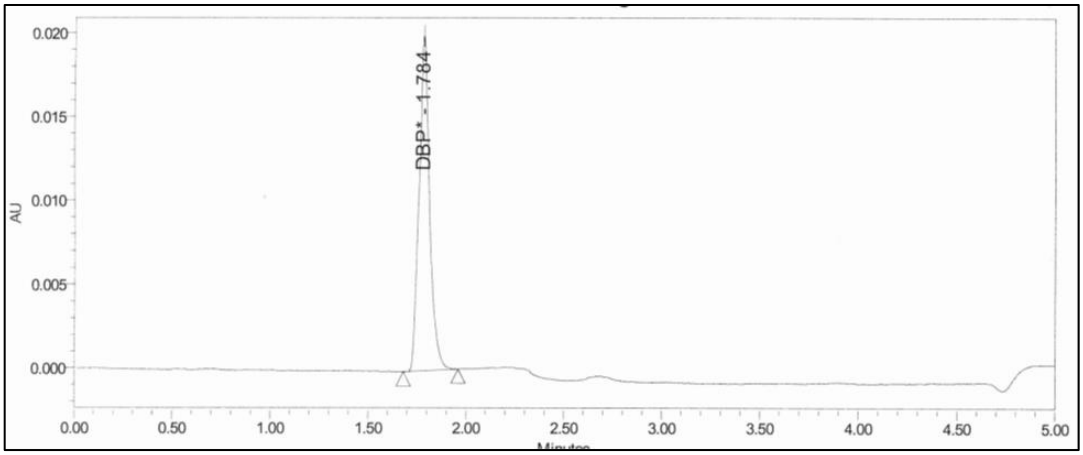

**Appendix C: Figure 25 Sample chromatogram obtained for DBP at 31 °C (72 H interval)**



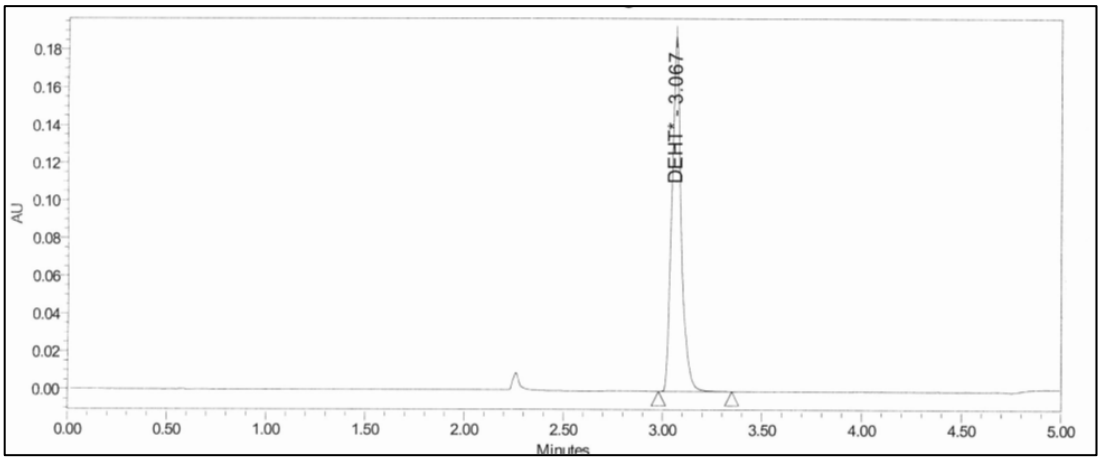

**Appendix C: Figure 26 Sample chromatogram obtained for DBP at 50 °C (24 H interval)**



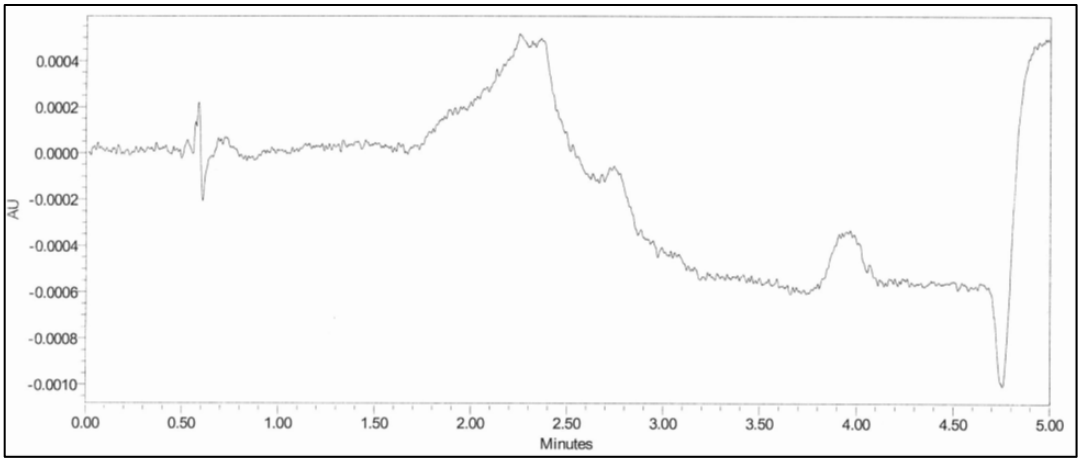

**Appendix C: Figure 27 Sample chromatogram obtained for DBP at 50 °C (48 H interval)**



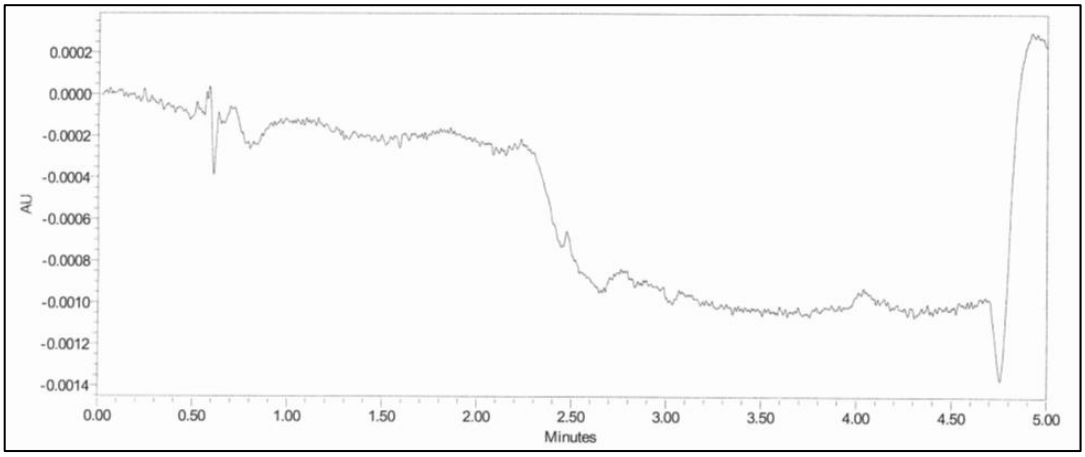

**Appendix C: Figure 28 Showing a Sample chromatogram obtained for DBP at 50 °C (72 H interval)**



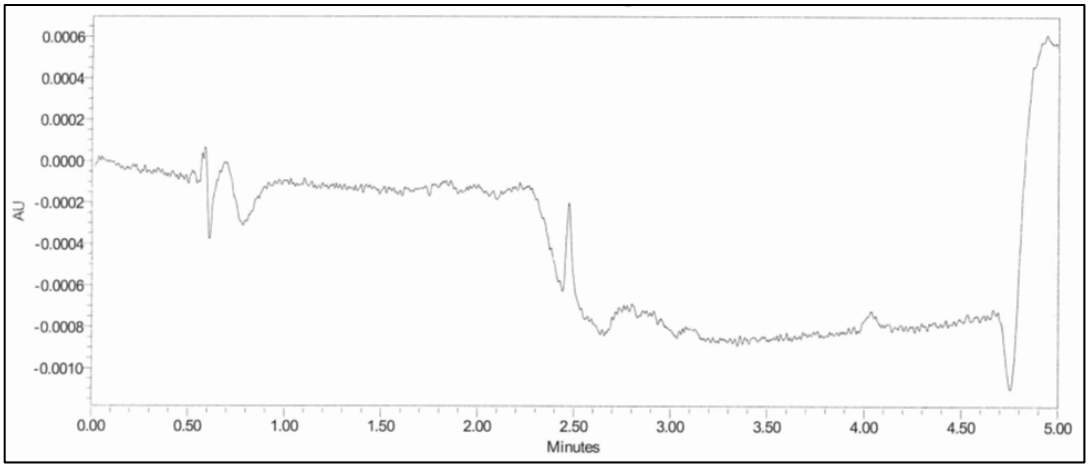

**Appendix C: Figure 29 Standard chromatogram obtained for DEHT**



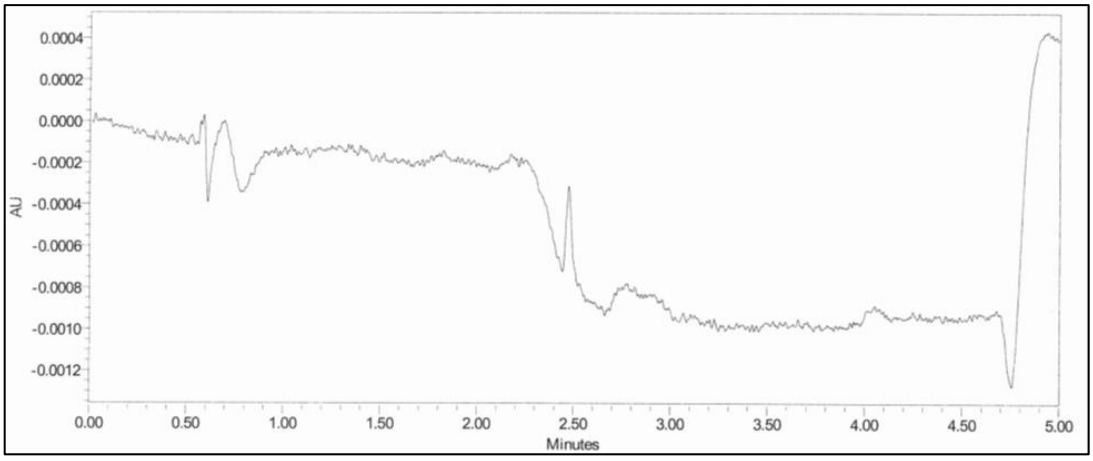

**Appendix C: Figure 30 Blank chromatogram obtained for DEHT**



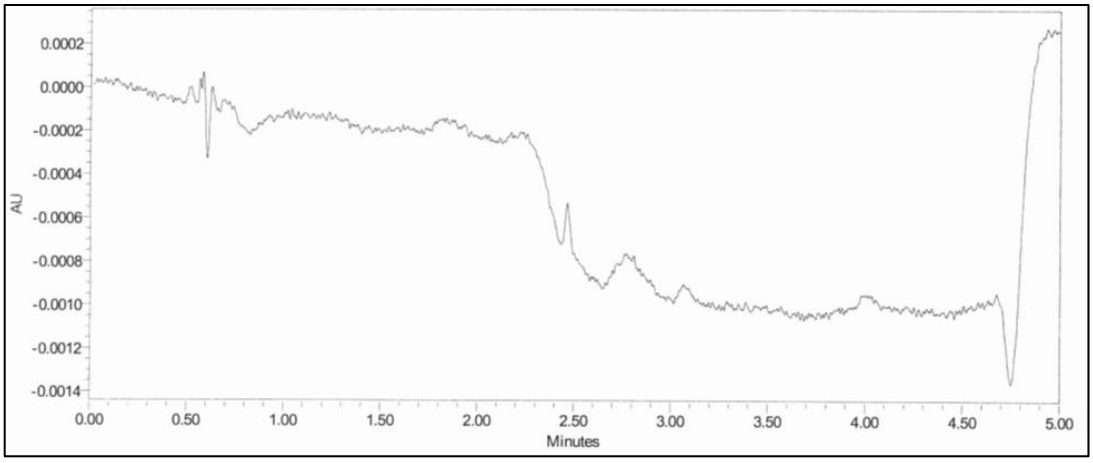

**Appendix C: Figure 31 Sample chromatogram obtained for DEHT at 31 °C (24 H interval)**



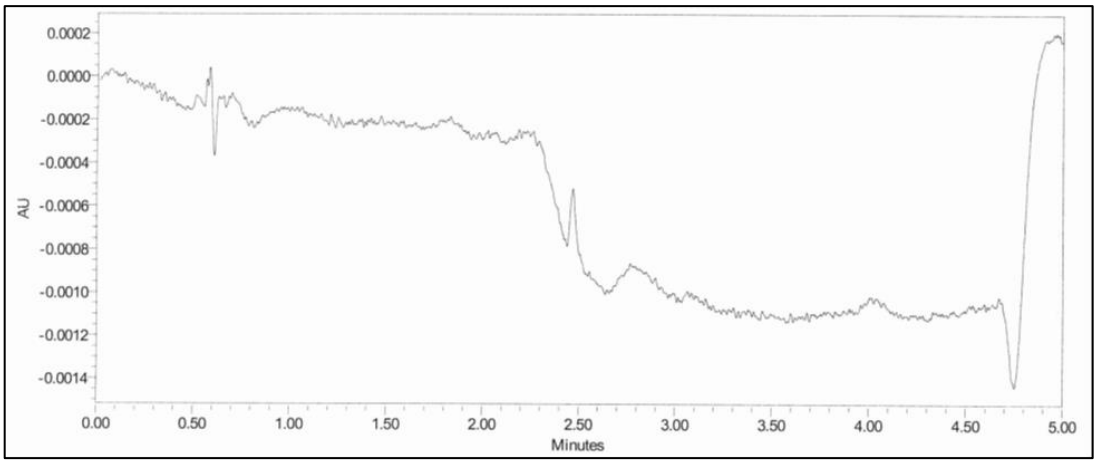

**Appendix C: Figure 32 Sample chromatogram obtained for DEHT at 31 °C (48 H interval)**



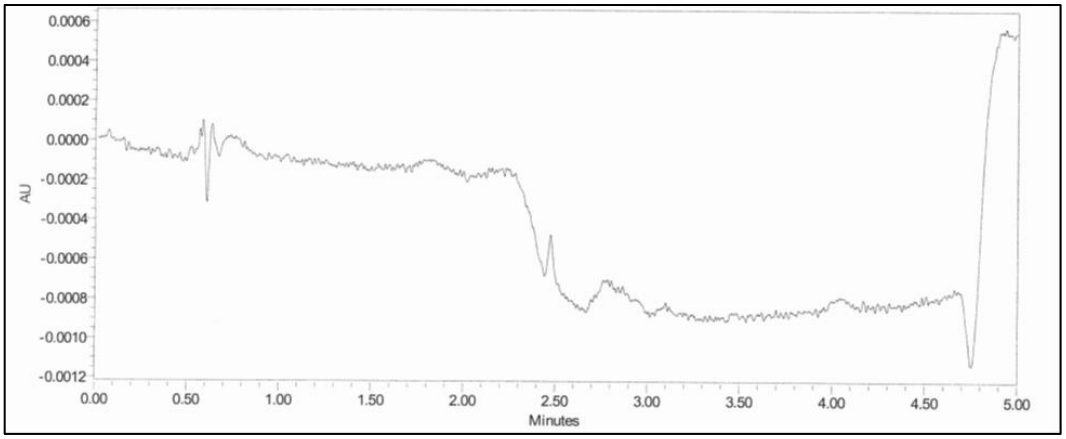

**Appendix C: Figure 33 Sample chromatogram obtained for DEHT at 31 °C (72 H interval)**




**Appendix C: Figure 34 Sample chromatogram obtained for DEHT at 50 °C (24 H interval)**




**Appendix C: Figure 35 Showing a Sample chromatogram obtained for DEHT at 50 °C for (48 H interval)**




**Appendix C: Figure 36 Sample chromatogram obtained for DEHT at 50 °C (72 H interval)**