Formulation of cosmetic products for the treatment of acne containing tea tree oil and salicylic acid

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TABLE OF CONTENTS

ABSTRACT i

UITSTREKSEL ii

RESEARCH OBJECTIVES iii

CHAPTER 1 1
ACNE TREATMENTS
1.1 Introduction 1
1.2 Pathogenesis of acne 1
1.3 Systemic agents used in the treatment of acne 3
1.4 Topical agents used in the treatment of acne 6
1.5 Salicylic acid 7
1.6 Tea tree oil 10

CHAPTER 2 12
FORMULATION OF ACNE PRODUCTS CONTAINING TEA TREE OIL AND SALICYLIC ACID
2.1 Introduction 12
2.2 Formulation of a cream 12
2.3 Formulation of a gel 16
2.4 Formulation of a ointment 19
2.5 Formulation of a cover stick 20
2.6 Formulation of a soap bar 21

CHAPTER 3 24
METHODS FOR STABILITY TESTING
3.1 Introduction 24
3.2 pH 25
3.3 Relative density 25
3.4 Viscosity 26
3.5 Spreadability 26
3.6 Penetration 27
3.7 Foamability 27
3.8 High performance liquid chromatography (HPLC) 27
3.9 Release studies with enhancer cell (Dissolution testing) 29

CHAPTER 4 31
STABILITY TEST RESULTS: CREAM
4.1 pH 31
4.2 Relative density 31
4.3 Viscosity 32
4.4 Spreadability 33
4.5 Penetration 34
4.6 Visual assessment 35
4.7 Assay 35
4.8 Release studies (Dissolution tests) 36
4.9 Preservative efficacy 38

CHAPTER 5 39
STABILITY TEST RESULTS: GEL
5.1 pH 39
5.2 Relative density 39
5.3 Viscosity 40
5.4 Visual assessment 41
5.5 Assay 42
5.6 Release studies (Dissolution tests) 42
5.7 Preservative efficacy 44

CHAPTER 6 45
STABILITY TEST RESULTS: OINTMENT
6.1 pH 45
6.2 Relative density 45
6.3 Viscosity 46
6.4 Spreadability 47
6.5 Penetration 48
6.6 Visual assessment 49
6.7 Assay 49
6.8 Release studies (Dissolution tests) 50
6.9 Preservative efficacy 52

CHAPTER 7 53
STABILITY TEST RESULTS: SOAP BAR
7.1 pH 53
7.2 Visual assessment 53
7.3 Foamability 54
7.4 Assay 55

CHAPTER 8 57
STABILITY TEST RESULTS: COVER STICK
8.1 Visual assessment 57
8.2 Assay 58

CHAPTER 9 59
CONCLUSION
9.1 Comparison between the five formulations 59
9.2 Conclusion 60

BIBLIOGRAPHY 61

APPENDIX A 65
GLOSSARY

APPENDIX B 71
VALIDATION

APPENDIX C 90
DISSOLUTION RESULTS

APPENDIX D 95
POSSIBLE PUBLICATION

ABSTRACT

Acne is a skin disease that affects most adolescents and young adults. There are four abnormalities in acne namely, sebum production, inflammation, hyperkeratosis and the presence of *Propionobacterium acnes*. To treat acne effectively it has been proved that combinational therapy is essential to be able to eliminate all four abnormalities. Both salicylic acid and tea tree oil have the properties to eliminate the four abnormalities of acne. These active ingredients were therefore chosen to be formulated into one cosmetic acne product.

These two active ingredients were formulated into five different acne products, i.e. a cream, gel, ointment, soap bar and a cover stick. All of these products contained 2% salicylic acid and 3% tea tree oil. The formulations had to be of such nature that they would not irritate the skin or worsen the acne.

After formulation, the products were placed under a three-month accelerated stability testing procedure. The products were stored at different temperatures and humidities. Stability indicating tests were carried out on all of the products throughout the three months.

All five products proved to be stable over the three-month stability testing period. During release rate studies with the enhancer cell dissolution technique, the gel showed the highest amount of salicylic acid released in comparison to the other products, whereas the ointment proved to release the highest amount of tea tree oil. Zone inhibition studies were not conducted as two previous studies have already proved that a correlation exists between zone inhibition and the release rate.

This study produced five new cosmetic acne formulations that remained stable throughout the study and therefore they can be used to treat acne effectively.

UITTREKSEL

Aknee is 'n abnormaliteit van die vel waarmee die meeste tieners en jong volwassenes sukkkel. Daar bestaan vier abnormaliteite in aknee naamlik, sebum produksie, inflammasie, hiperkeratose, en die teenwoordigheid van *Probionobacterium acnes*. Daar is bewys dat kombinasie terapie die mees effektiewe manier is om aknee te behandel en sodoende al vier die abnormaliteite te elimineer. Daarom is daar besluit om salisielsuur en teeboomolie in dieselfde kosmetiese aknee produk te formuleer. Beide hierdie aktiewe bestanddele is in staat om die vier abnormaliteite van aknee te elimineer.

Hierdie twee aktiewe bestanddele is in vyf verskillende aknee produkte geformuleer naamlik, 'n room, gel, salf, seep en 'n maskeer stiffie. Al vyf hierdie produkte het 2% salisielsuur en 3% teeboomolie bevat. Die formules moes van so 'n aard wees dat dit nie die vel sal irriteer of aknee vererger nie.

Na die formuleering is die produkte vir drie maande onder versnelde stabiliteitskondisies geplaas. Die produkte is by verskillende temperature en humiditeite geberg. Stabiliteits-aanduidende toetse is oor die drie maande op die produkte uitgevoer.

Al vyf die produkte was stabiel oor die drie maande stabiliteitsperiode. Die gel het die hoogste hoeveelheid salisielsuur vrygestel tydens "enhancer sel" dissolusie vrystellingsstudies, in vergelyking met die ander produkte, terwyl die salf die hoogste hoeveelheid teeboomolie vrygestel het. Sone-inhibisie toetse is nie uitgevoer nie aangesien daar alreeds op twee verskillende geleenthede bewys is dat daar 'n positiewe korrelasie bestaan tussen sone- inhibisie en die hoeveelheid vrygestel met die "enhancer cell" dissolusie tegniek.

Die studie het vyf nuwe kosmetiese aknee produkte opgelewer wat stabiel gebly het deur die hele studie en daarom kan hierdie produkte effektief gebruik word vir die behandeling van aknee.

CHAPTER 1

ACNE TREATMENTS

1.1 Introduction

Acne is a disease that 80% of adolescents and young adults have. For most of the people acne causes associated problems with self-esteem and social inhibition (De Souza *et al.*, 2005:40). The objective of this study is to formulate acne products containing tea tree oil and salicylic acid. A number of patents confirmed that salicylic acid and tea tree oil could be combined in one product. Both tea tree oil and salicylic acid have comedolytic (refer to appendix A) properties and research have shown that combinational therapies is the most effective in acne treatment.

1.2 Pathogenesis of acne

The pathogenesis of acne vulgaris is due to many factors. Acne vulgaris is a medical condition that begins in pilosebaceous units. These units consist of sebaceous glands and a single hair follicle. The sebaceous glands are continuously producing a clear, oily liquid called sebum, which finds its way through the hair follicle to the surface of the skin. Sebum has two major roles namely to lubricate the skin and to get rid of the old cells within the follicle called debris. Sebum synthesis and secretion is promoted by testosterone and therefore during puberty the sebaceous glands become very active (Mitsui, 1997:29).

Subsequently keratinisation with hyperkeratosis of the epithelium in the follicle leads to obstruction by a horny plug. The blocked duct consists of sebum and keratinous debris, forming non-inflammatory lesions (Berson & Shalita, 1995:531).

A lesion becomes inflamed because the excess sebum provides an anaerobic growth medium for *Propionobacterium acnes* (a Gram-positive bacteria), which is responsible for the metabolism of fatty acids from triglycerides that is present in the

sebum (Berson & Shalita 1995:531; Mitsui 1997:30; Johnson 2000:1823). *P. acnes* attract neutrophils through certain chemotactic factors. These neutrophils releases lysosomal enzymes, which rupture the follicle wall, releasing mediators like keratin and lipids into the surrounding area. Inflammatory lesions result (Berson & Shalita, 1995:531).

There are consequently four abnormalities found in acne, namely sebum production, keratinisation of the follicle, presence of *P.acnes* populations and inflammation (Berson & Shalita, 1995:531).

To effectively manage acne these four factors must be addressed. A diversity of acne treatments is available, each with a different mechanism of action. Therefore it is important when treating acne to make use of combinational therapies. Topical treatment is usually for mild-to-moderate inflammatory acne. The advantage of topical treatment is that the side effects of systemic treatment are eliminated (Berson & Shalita, 1995:531).

The focus of this study is on the different topical treatment pathways. If one can formulate a product which will be able to act on all four pathways then one can eliminate acne. Figure 1.2.1 shows the different treatment pathways.

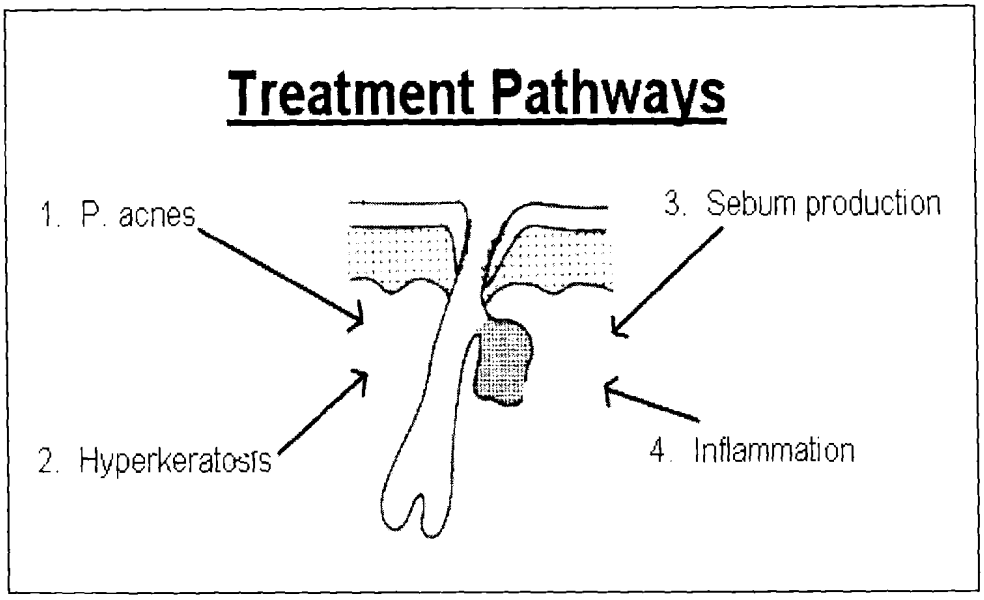

Figure 1.2.1: Treatment pathways (Berson & Shalita, 1995:533)

In order to formulate the most effective acne product, it is important to gain knowledge about the mechanism of action of different types of acne drugs, topical and systemic.

Topical tretinoin and adapalene, as well as the topical antibacterials, like clindamycin and erythromycin, are regulated by prescription, whereas benzoyl peroxide, salicylic acid and tea tree oil are available in over-the counter acne treatments. Systemic acne treatment, for example hormonal agents like contraceptives and drugs like spironolactone, act as anti-androgens, which helps dealing with acne (Akhavan & Bershad, 2003:474).

1.3 Systemic agents used in treatment of acne

Systemic therapy in acne treatment is usually for severe inflammatory lesions. Systemic treatment includes retinoids, antibacterials and hormones. Most of the time oral therapy is considered when topical treatment fails (Akhavan & Bershad, 2003:475).

Retinoids include natural compounds and synthetic derivatives of retinol that exhibit vitamin A activity. Several mechanisms of action are responsible for the usefulness of retinoid agents as acne therapy. These include modulation of keratinocyte proliferation, induction of orthokeratosis, comedoysis, and inhibition of inflammation (Akhavan & Bershad, 2003:477). From a pharmacological point the retinoids interact with a retinoic acid receptor (RAR) and regulates gene transcription through activation of nuclear receptors. The ligand-receptor complex formed then binds to the promoter region of a target gene. This initiates protein syntheses, which are responsible for the pharmacological effects and unwanted side effects (Wyatt *et al.*, 2001:1801). Isotretinoin (13-*cis* retinoic acid) is a natural isomer of vitamin A found in small amounts in the body. It reduces sebaceous gland size, decreases sebum production and practically corrects all four abnormalities found in acne (Berson & Shalita, 1995:537). Approximately 40% of patients have a relapse after isotretinoin therapy. The most common side effects of isotretinoin are dry eyes, dry skin, nosebleed, hair loss and headaches (Berson & Shalita, 1995:537). These adverse effects are dose related and depent on the duration of isotretinoin therapy. After the drug is discontinued the side effects will disappear (Jensen *et al.*, 1991:426). Less common adverse effects include difficulties with night vision, elevated blood lipids and abnormal liver function (Berson & Shalita, 1995:537).

The FDA has designated oral isotretinoin a Pregnancy Category X drug so it is very teratogenic and therefore female patients must be on oral contraceptives and have a negative pregnancy test before starting treatment (Akhavan & Bershad, 2003:480).

Systemic antimicrobials decrease inflammation and reduce *P. acnes* colonisation. They are usually added to the regimen when moderate-to-severe inflammatory lesions do not react to topical combinations. The oral antibiotics include tetracycline, erythromycin, minocycline, doxycycline, and if these antibiotics don't work, treatment with trimethoprim-sulfamethoxazole can be attempted (Berson & Shalita, 1995:536).

Table 1.3.1 shows the different systemic antibiotics together with their benefits and side effects.

Table 1.3.1: Systemic antibiotics (Berson & Shalita, 1995:536)

ANTIBIOTIC	BENEFITS	SIDE EFFECTS
Tetracycline	50% reduction of *P. acnes*	Low compliance; GI upset; dairy products and iron limit efficacy
Erythromycin	Reduces *P. acnes*	GI upset
Minocycline	Penetrates sebaceous gland; high *P. acnes* reduction at low dose; reduces potential for yeast infections	Vestibular involvement; true vertigo-like symptoms
Doxycycline	Effective, low cost	Photosensitivity; GI upset
Trimethoprim-sulfamethoxazole	Lipid solubility; very effective in severe acne	Bone marrow suppression; severe drug eruption

Hormonal therapy reduces sebum production by counteracting androgenous effects on the sebaceous gland. There are in general three choices namely, estrogens, glucocorticoids, such as prednisone and dexamethasone and systemic antiandrogens such as spironolactone (Berson & Shalita, 1995:539).

Table 1.3.2 indicates the different hormonal therapies.

Table1.3.2: Hormonal therapy (Berson & Shalita, 1995:539)

HORMONAL THERAPY	BENEFITS	SIDE EFFECTS
Estrogens	Suppress ovarian androgen; Decrease sebum production	Nausea; Cancer; Hypertention; breast tenderness; headache
Glucocorticoids	Suppress adrenal androgen; Anti-inflammatory	Peptic ulcer; myopathy; pshycosis
Systemic antiandrogens	Suppress sebum production; Inhibits androgen production in ovaries and adrenals	Menstrual irregularities; breast tenderness; headache; fatigue; possible hyperkalemia

1.4 Topical agents used in treatment of acne

Topical acne treatment is used for patients with noninflammatory comedones (refer to appendix A) or mild-to-moderate inflammatory lesions. Some advantages of topical treatment are that it minimises unwanted side-effects of systemic drugs and secondly, direct application to the affected skin area promotes maximum drug delivery (Berson & Shalita, 1995:531 and Akhavan & Bershad, 2003:476). Typical acne products that are used for topical treatment are benzoyl peroxide, antibiotics, tretinoin, salicylic acid and tea tree oil.

Benzoyl peroxide acts as an antibacterial by effectively reducing *P. acnes* populations (Berson & Shalita, 1995:533). The mechanism of action is the degradation of bacterial proteins via release of free-radical oxygen (Akhavan & Bershad, 2003:482). Products containing benzoyl peroxide are available in concentrations ranging from 2.5% to 10%. These products are available in creams, lotions, gels and cleansers (Berson & Shalita, 1995:534). Benzoyl peroxide can initially irritate the skin and the frequency of application and concentration can be controlled to minimize adverse effects. The most familiar adverse effects of benzoyl peroxide are skin dryness, erythema and peeling (Berson & Shalita, 1995:534 and Akhavan & Bershad, 2003:482).

Topical antibiotics like erythromycin and clindamycin are the most common antibiotics used for controlling inflamed acne (Berson & Shalita, 1995:534). Clindamycin is a lincosamide antimicrobial, which inhibits bacterial protein synthesis by attaching to the 50S subunit of the bacterial ribosome, which disables *P. acnes* to grow. Erythromycin is a macrolide antibacterial that also binds on the 50S subunit of the bacterial ribosomes, and therefore also inhibits protein synthesis (Wyatt *et al.*, 2001:1809). Through inhibiting protein synthesis it decreases the concentration of comedogenic free fatty acids (Berson & Shalita, 1995:535). A very important factor when using antibactarials is that resistance of the bacteria should be taken into consideration and if improvement diminished treatment should be discontinued for 1 month.

Tretinoin is the single most effective comedolytic agent because it promotes drainage of comedones that already exists and inhibits the formation of new comedones. It reduces the growth of *P. acnes* (Berson & Shalita, 1995:532). The most frequent adverse effects are peeling, erythema, dryness, burning and itching (Akhavan & Bershad, 2003:478).

For the treatment of acne a variety of products can be used. For eliminating the four pathways of acne, combination therapy is the most effective. In this study the focus is on formulating a product containing salicylic acid and tea tree oil in order to treat acne. Therefore a more in depth study concerning salicylic acid and tea tree oil was made.

1.5 Salicylic acid

Salicylic acid is comedolytic because of its lipophilic nature and because the ancestor of all acne lesions is the microcomedo, it is obvious to begin therapy with a comedolytic agent (Berson & Shalita, 1995:536 and Ho-Sup & IL-Hwan, 2003:1196). It promotes desquamation and accelerates the resolution of inflammatory lesions (Berson & Shalita, 1995:536), through dissolving the intercellular cement that holds epithelial cells together (Akhavan & Bershad, 2003:484). Salicylic acid causes stomach irritation when taken orally (Akhavan & Bershad, 2003:485). Salicylic acid is a β-hydroxy acid and therefore are also keratolytic.

Classification

Salicylic acid is a beta-hyddroxy acid (Figure 1.5.1). Salicylic acid can be obtained in nature from willow bark, wintergreen leaves and sweet birch (Vedamurthy, 2004:136).

Mechanism of action

Salicylic acid function through dissolving the intercellular cement that holds the epithelial cells together. The dermatopharmacological effect is associated with the reaction on the stratum corneum affecting intercorneocyte cohesion and desquamation (Bashir *et al.*, 2005:187). Because of its lipophilic nature it has a powerful comedolytic effect. Salicylic acid is also a keratolytic agent and is used for peeling in products containing 3-6% salicylic acid (Vedamurthy, 2004:136 and Akhavan & Bershad, 2003:484).

Indications

Some of the indications of salicylic acid include:

Acne vulgaris, enlarged pores, rough and oily skin, melasma, post-inflammatory hyperpigmentation and photoaging (Vedamurthy, 2004:137). Due to salicylic acid's keratolytic properties, hyperkeratotic skin conditions like callus, corns, ichthyosis and psoriasis can also be treated (Behnam *et al.*, 2005:812).

Toxicology

Toxicity depends on the dosage of the product. With high doses taken orally salicylism could occur. The early symptoms of salicylism are CNS stimulation, vomiting, hypernea, hyperactivity and convulsions, which then lead to respiratory failure and collapse (Abounassif *et al.*, 1994:444). Salicylic acid has a relatively low toxicity when administerd topically. No gastointestinal tract, kidney or liver effects have been reported on topical aplication. Tests proved that salicylates move across the placental barrier so should be avoided in pregnant and breast-feeding women (Dollery, 1999:s1).

Physico-Chemical Characteristics

The physico-chemical characteristics of salicylic acid are listed in Table 1.5.1 and the chemical structure of salicylic acid are given in Figure 1.5.1.

Table 1.5.1: Physico-chemical characteristics of Salicylic acid (Abounassif *et al.*, 1994:424)

SALICYLIC ACID PROPERTY	PHYSICO-CHEMICAL CHARACTERISTICS
Chemical name	2-Hydroxybenzoic acid
Generic name	Salicylic acid
Molecular weight	138.12 g/ml
pKa	2.98
Melting point	158°C-161°C
Appearance	Fluffy, white crystalline powder
Odour	Odourless
Taste	Sweetish acrid taste
Density	$1.443^{20}{}_{4}$
pH	Saturated solution- 2.4

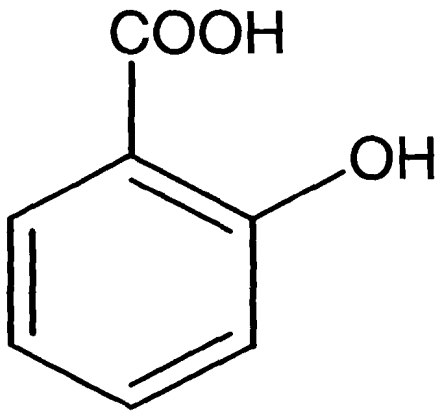

Figure 1.5.1: Chemical structure of salicylic acid

Clinical studies has proven that a 2 % salicylic acid acne treatment in contrast with a 10% benzoyl peroxide cream showed better results. The 2% salicylic acid treatment showed the most significant reduction in inflammatory lesions, while being gentle and mild to facial skin at the same time (Miller *et al.*, 2005:105). Therefore, the formulation of the acne products in this study contains 2% salicylic acid, which will consequently lead to reducing acne while still being gentle on the skin.

1.6 Tea tree oil

Tea tree oil originate from the Australian tree Melaleuca alternifolia, a member of the Myrtaceae family and is obtained from the leaves and terminal branchlets of the plant (Merck Index:9174). The oil was retrieved by distilling oil from the leaves (Carson *et al,*. 1998:175). Tea tree oil consists of a complex mixture of hydrocarbons and terpenes (Merck Index:9174). The oil consists of approximately 100 components, including terpinen-4-ol, 1,8-cineole, α-terpineol, terpinolene and α- and γ-terpinene, making up 90% of the oil (Carson *et al.,* 1998:176). Tea tree oil does undergo photo-oxidation under the influence of light, moisture, warmth and oxygen (Hausen *et al.,* 1999:69).

In one specific study they compared the antimicrobial activity of essential oils for therapeutic use. Tea tree oil had the best combination of useful properties and showed high antimicrobial activity (Williams *et al.,* 1998:30). Another study on acne treatment proved that a 5% tea tree oil gel and a 5% benzoyl peroxide lotion had the same outcome on 124 patients. Although the onset of action in the case of tea tree oil was slower, fewer side effects were experienced (Smith & Navilliat, 1997:21-24).

Physico-Chemical Characteristics

The physico-chemical characteristics of tea tree oil are listed in Table 1.6.1.

Table 1.6.1: Physico-chemical characteristics of Tea tree oil (British Pharmacopiae, 2002:1650; and Budavari, 2001:9174)

TEA TREE OIL PROPERTY	**PHYSICO-CHEMICAL CHARACTERISTICS**
Chemical name	Melaleuca alternifolia oil
Generic name	Tea tree oil
Appearance	Clear, mobile, colourless to pale yellow liquid
Odour	Fresh terpene type odour with nutmeg associations and possibly with citrus or floral undertones

Indications:

Tea tree oil is a broad spectrum antimicrobial, antiseptic, a mild anti-inflammatory and analgesic. Therefore it is widely used as treatment for conditions like acne, arthritis, burns, vaginal thrush, tinea and dandruff (Williams *et al.,* 1998:30).

Figure 1.6.1 gives an illustration of the tea tree oil plant.

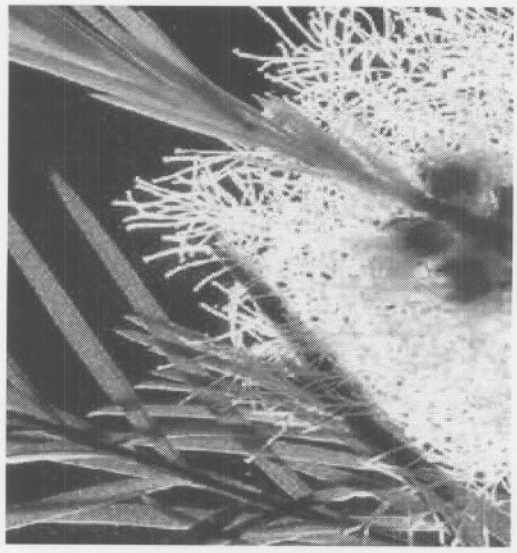

Figure 1.6.1: Tea tree oil plant

In this study the formulation of the acne products contain 3% tea tree oil.

Five acne formulations were made, namely a cream, ointment, gel, cover stick and a soap bar, to be used for acne on the facial area. Chapter 2 introduces a thorough discussion on the formulation of these products.

CHAPTER 2

FORMULATION OF ACNE PRODUCTS CONTAINING TEA TREE OIL AND SALICYLIC ACID

2.1 Introduction

In this study five formulated products, combining tea tree oil and salicylic acid was made. A cream, gel, ointment, cover stick, and soap bar was formulated. Each contained 2% salicylic acid and 3% tea tree oil. In the treatment of acne the vehicle (refer to appendix A) are just as important as the active ingredient. Creams are appropriate for patients with a sensitive or dry skin whereas a gel will be profitable in patients with an oily skin because the gel will have a drying effect on the skin (Russell, 2000:359). During the formulation of the products the trade names of the substances was used. Refer to appendix A for the generic names and properties of the substances.

2.2 Formulation of a cream

A cream consists of two immiscible liquids like for example water and oil, which makes it some type of emulsion. These two liquids are then made into a dispersion by making one the dispersed phase and the other the dispersion medium. Keeping the skin moist and maintaining the moisture balance is most likely the main function of a cream (Mitsui, 1997:341).

A cream usually shows a plastic flow behavior and does not flow at low shear stress. It contains >20% water and volatiles and/or <50% of hydrocarbons, waxes or polyethylene glycols as the vehicle. The appearance and feel of a cream is non-greasy to mildly greasy, viscous and is likely to evaporate or be absorbed when rubbed onto the skin (Buhse *et al.*, 2005:110).

Creams are either a W/O or O/W emulsion depending on the amount of oil and water in the cream. Tea tree oil dissolves in the oil phase and the salicylic acid was

dispersed in the emulsion. The cream should not be too oily since this could increase acne. An O/W emulsion would then be more appropriate.

Cream formula 1:

	Composition	%m/m
A	Cetyl alcohol	7%
	Cremophor A6*	1.5%
	Cremophor A25*	1.5%
	Liquid paraffin	12%
B	Propylene glycol	8%
	Tween 80*	4%
	Tea tree oil	3%
C	Salicylic acid	2%
D	Distilled water	to 100%

Method

Combine the ingredients in A and heat the mixture and the water separately to approximately 80°C.

Add A to the obtained solution D with rigorous stirring.

Combine the ingredients in B and mix with A and D and continue to stir while cooling to room temperature.

When finished mix in C.

Outcomes

The salicylic acid did not dissolve completely and caused the emulsion to break. The pH was very low.

A second formulation was subsequently tried.

Cream formula 2:

	Composition	%m/m
A	Emulsifing wax	9%
	Soft paraffin	15%
	Liquid paraffin	6%
	Tea tree oil	3%
B	Distilled water	to 100%
C	Salicylic acid	2%

Method

Combine the ingredients in A and heat the mixture until it melts. (Approximately 60°C)

Add A to B with rigorous stirring.

Cool the cream whilst stirring.

Add C through a sieve and mix thoroughly

Outcomes

The cream had a homogeneous white texture and was not too oily, nor too hydrous. The cream applied easily.

2.3 Formulation of a Gel

Gels present a uniform external appearance and range from transparent to semitransparent. The most important functions of an aqueous gel are to moisturize the skin through water-supply and to stimulate skin circulation. This is therefore very effective for oily skin, which is in most cases the problem with acne (Mitsui, 1997:351).

A gel is a semisolid that contains a gelling agent to supply stiffness to a solution and exhibits plastic flow behavior. Mainly a gel contains an aqueous or alcoholic vehicle and a gelling agent to form a gel, which is transparent, non-greasy and provides a cooling sensation when administered to the skin (Buhse *et al.*, 2005:110).

Formula 1 was an existing tea tree oil formula to which salicylic acid was added (Coetzee, 2002:15)

Gel formula 1:

	Composition	%m/m
A	Distilled water	73.1%
	Carbopol (Ultrez 10)™	0.6%
B	Disodium EDTA	0.1%
	Propylene glycol	5.0%
C	Triethanolamine	0.6%
D	Propylene glycol	0.6%
	Tween 80*	10%
	Ethanol 99.98%	5.0%
	Tea tree oil	3.0%
	Salicylic acid	2.0%

Method

Combine the ingredients in A and mix until the Carbapol is completely dissolved.

Add B whilst mixing.

Combine the ingredients in D in the order given and add to A and B.

Finally add C whilst mixing until a clear gel is formed.

Outcomes

No gel was formed and it had a white colour thus was not transparent. Addition of sodium hyroxide and more triethanolamine did not improve matters.

A second formulation was subsequently tried.

Gel formula 2:

	Composition	%m/m
A	Sodium dihydrogen orthophosphate dihydrate	0.1%
B	Propylene glycol	62%
	Ethanol 99.98%	16%
C	Salicylic acid	2%
	Tea tree oil	3%
D	HPMC (65 HG)	2%
E	Distilled water	to 100%

Method

Dissolve A in 2% distilled water in a 100ml flask.

Add B to A and mix.

Add C and shake till everything is dissolved.

Add D and shake to a homogeny mixture.

Fill the flask to 100ml with distilled water.

Outcomes

No gel was formed and it had a white colour. A third formulation was initiated.

Gel formula 3:

	Composition	%m/m
A	Propan-2-ol	35%
	Propylene glycol	20%
B	Salicylic acid	2.0%
	Tea tree oil	3.0%
C	HPMC (65 HG)	2.0%
	Glycerin	10%
D	Distilled water	28%

Method

Mix A and dissolve B in A.

Wet the HPMC with the glycerine.

Add C and D with A and B and stir (not too fast) to form the gel.

Outcomes

A clear gel was formed and had a pleasant tea tree oil smell.

2.4 Formulation of an ointment:

An ointment is a suspension or emulsion semisolid which contains <20% water and volatiles and >50% of hydrocarbons, waxes, or polyethylene glycols acting as the vehicle (refer to appendix A), and from there its very viscous property (Buhse *et al.*, 2005:110).

	Composition	%m/m
A	Polyethylene glycol 400	57%
	Polyethylene glycol 4000	38%
	Salicylic acid	2%
	Tea tree oil	3%

Method

Combine all ingredients in A and melt.

Stir until the ointment cools down to room temperature.

Outcomes

The ointment had a homogeneous texture and applied easily.

2.5 Formulation of a cover stick

This solid-type of foundation was developed to act against acne and at the same time to serve as a blemish stick to cover up the red spots that acne causes.

Cover stick formula:

	Composition	%m/m
A	Polyethylene glycol 4000	57%
	Polyethylene glycol 400	38%
	Salicylic acid	2%
	Tea tree oil	3%
	Colour	qs

Method

Combine all ingredients in A and melt.

Mould into a compact stick.

Outcomes

The cover stick applied easily and the colour was suitable for dark skin. It was not too oily, nor too hydrous.

2.6 Formulation of a soap bar

In Savona, Italy soap was made for the first time in the 8th century. The English word soap was derived from the italian word "savon". A soap industry was then started in Marseilles and so it developed to the rest of the world (Mitsui, 1997:447).

Soap formula:

	Composition	%m/m
A	Sunflower oil	27.5%
	Stearic acid	10%
	Propylene glycol	20%
	Glycerin	6.0%
B	Sucrose	12.5%
C	Distilled water	14.1%
D	NaOH in water (1:1)	4.95%
E	Tea tree oil	3.0%
	Salicylic acid	2.0%
F	Colour	qs

Method

Combine the ingredients in A and heat it too approximately 70°C.

Dissolve B in C and combine with A.

Dissolve D in water and mix with A,B and C when the temperature is also at 70°C.

Mix till everything is dissolved.

Add E and F

Mould and let it cool down to form soap.

Outcomes

The soap was semitransparent and had a nice tee tree oil smell with an appropriate yellow colour.

These products were transferred into appropriate containers which are not transparent protecting the products, for both the active ingredients are unstable in direct sunlight.

Chapter 3 describes the conditions and methods that were used in testing the stability (refer to appendix A) of these products.

CHAPTER 3

METHODS FOR STABILITY TESTING

3.1 Introduction

The successful formulation of cosmetic products requires that the products should be placed under certain conditions and tested to determine their stability over a fixed time period. The geographic climate of the target market should be kept in mind when stability studies are performed. The climate in South-Africa is seldom below 5°C and above 40°C. Therefore in this study, all five of the formulated products were stored at 5°C, 25°C + 60%RH and 40°C + 75%RH for three months.

The stability tests that were conducted on all five products are given in table 3.1.1. All the tests were done every month except for the release studies and the preservative efficacy tests were done on the initial product and the product stored at the different temperatures after three months.

Table 3.1.1 Stability tests conducted on the cream

TEST	CREAM	GEL	OINTMENT	COVER STICK	SOAP BAR
Salicylic acid concentration assay	✓	✓	✓	✓	✓
Tea tree oil concentration assay	✓	✓	✓	✓	✓
Release tests (dissolution)	✓	✓	✓		
pH	✓	✓	✓		✓
Density	✓	✓	✓		
Viscosity	✓	✓	✓		

Spreadability	✓	✓	✓		
Penetration	✓	✓	✓		
Visual assessment	✓	✓	✓	✓	✓
Foamability					✓
Preservative afficacy test	✓	✓	✓		

3.2 pH

The apparatus that was used to measure the pH (refer to appendix A) of the products (excluding the soap and cover stick) was a Mettler Toledo pH meter. A glass calomel electrode was used and the apparatus was calibrated (refer to appendix A) each time before use with a buffer pH 2 and buffer pH 7 (RIIP SOPPH05).

The pH of the soap bar was measured with non-bleeding pH-indicator strips with a pH range of 7.5 – 14 (Akalit®). The soap bar was wetted and the strip was placed on top of the bar. The pH was taken while the strip was still moist. This process was repeated twice.

3.3 Relative density

The polytop method was used to measure the density (refer to appendix A) of the cream, ointment and gel. First the empty polytop was weighed and the mass recorded. Then 10ml distilled water was pipetted into the polytop and weighed again. The water level was then indicated on the polytop before the polytop was emptied. The polytop was then filled with the product to the indicated line and that mass was then also recorded. Density was then calculated by using the following formula:

$$Density = \frac{(Polytop + Sample) - (Empty\ Polytop)}{(Polytop + Water) - (Empty\ Polytop)}$$

3.4 Viscosity

A viscometer is an instrument used to measure the viscosity (refer to appendix A) of a fluid, semi-solid or solid suspension. A rotary viscometer measures the viscosity of a sample by determining the resistance to a rotating spindle immersed in the sample medium. The spindle turns at a specified rate, measured in rpm. A Brookfield Model DV II+ viscometer was used. The viscosity of the cream and gel was measured with a small sample adapter that consisted of a spindle, a sample chamber, a flow jacket and a mounting bracket. The spindle that was used in the small sample adapter was SC4-25. The sample was transferred into the chamber and covered with parafilm and left to stand overnight which allowed all the air bubbles to escape. The sample was then attached to the viscometer and left to stand for approximately 45 minutes to reach 25°C. After that a viscosity reading was measured every two minutes at a certain sequence interval until ten readings was conducted.

The ointment was transferred into 200 ml glass beakers and the same conditions as the gel and cream were reached. Thereafter one viscosity reading was measured at a speed of 10 rpm (RIIP SOPVIS 01).

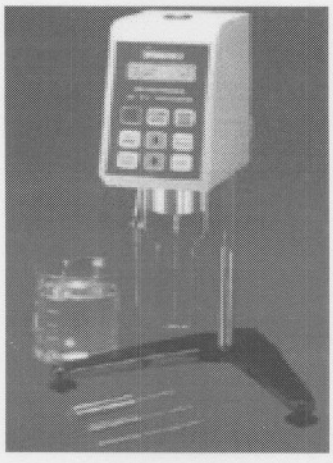

Figure 3.4.1: Brookfield Model DV II

3.5 Spreadability

To measure the spreadability of the cream and ointment two glass plates was used. The one glass plate was clear and the other had a scaled 1 mm incremented grid fixed underneath. The sample was transferred into a syringe and a clear plastic tube was fitted to the syringe. The scaled glass plate was then put on the scale and tarred. Approximately 0.25 g of sample was then squeezed onto the plate. The clear glass plate was then placed on top of the other plate and a 100 g brass weight was

placed on top of that. It was taken off after 60 seconds elapsed. The diameter of the sample was then measured with a Vernier caliper. The experiment was done in duplicate.

3.6 Penetration

A penetrometer was used to measure the penetration of the cream and ointment. The apparatus was placed on a level surface. The sample was transferred to a 500 g container and left to stand for 24 hours. The penetrating object was placed 1cm above the sample. The penetrating object was then released and the depth of the penetration object was measured. The sample was left to stand for another 24 hours and another two readings was conducted.

3.7 Foamability

To measure the foamability of the soap bar, a 250 ml stopperd measuring cylinder was used. Two grams of the soap bar was dissolved in 100 ml distilled water at 20°C and at 40°C. The measuring cylinder was shaken for 15 seconds. The foam that formed was measured at the beginning and after 30 minutes.

3.8 High performance liquid chromatography (HPLC)

Salicylic acid and tea tree oil concentrations in the formulated products was determined with HPLC (refer to appendix A) analysis.

CHROMATOGRAPHIC CONDITIONS:

Analytical instrument:	Agilent 1100 series HPLC equipped with a gradient pump, autosampler, UV detector and chemstation Rev. A.06.02 data acquisition and analysis software or equivalent. (Agilent, Palo alts, CA)
Column:	Luna C18(2) 150x4.6 mm, 5 µm (Phenomenex, Torrance, CA)

Mobile phase:	Acetonitrile/water pH adjusted to 2.5 with phosphoric acid
Gradient:	45% acetonitrile to 1.5 minutes, then to 100% after 4 minutes
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Detection:	UV at 220 nm
Retention time:	Approximately 3.5 and 9.9 minutes for salicylic acid and tea tree oil respectively
Stop time:	15 minutes
Solvent:	30% THF and 70% Methanol

SAMPLE PREPARATION:

Weigh approximately 1 g of the sample accurately into a tared 100 ml volumetric flask. Add solvent (refer to appendix A) and sonicate for 10 minutes. Allow to cool to room temperature and make up to volume with solvent. Transfer the solution into vials and inject into the chromatograph in duplicate.

STANDARD SOLUTION:

Weigh approximately 20 mg salicylic acid and 30 mg tea tree oil accurately and dissolve in 100 ml of solvent with sonication.
Allow to cool to room temperature and make up to volume with solvent.
Inject into the chromatograph in duplicate.

Figure 3.8.1: Agilent 1100 series HPLC equipment.

3.9 Release studies with enhancer cell (Dissolution testing)

DISSOLUTION APPARATUS: VanKel 7000 with 200 ml enhancer cells and small paddles

DISSOLUTION MEDIUM: 85% Ethanol solution

STIRRING SPEED: 200 rpm

TEMPERATURE: 32 ±0.5°C

MEMBRANE: Cellulose acetate membrane (refer to Appendix A), 0.45 µm pore size. Membrane surface area: 3.977679 cm^2.

METHOD: The samples were each carefully transferred into six enhancer cells per dissolution, which were then each covered with the cellulose acetate membrane. The enhancer cells were dropped into the dissolution medium at 30 seconds intervals in between. The paddles were then adjusted to rotate 25 mm above the membrane. 200 µl of test samples were withdrawn at 30, 60, 90, 120, 180, 240, 300,

360 minutes and transferred to HPLC vials for HPLC analysis.

TIME: Each dissolution took 6 hours

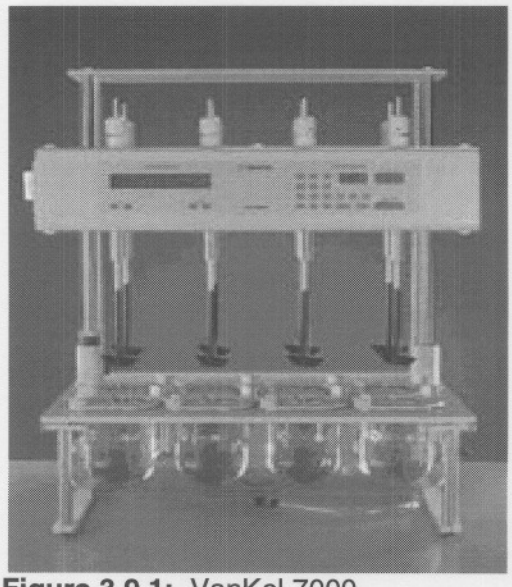

Figure 3.9.1: VanKel 7000

CHAPTER 4

STABILITY TEST RESULTS: CREAM

4.1 pH

The pH was measured on the initial cream, as well as the cream stored at 5°C, 25°C+60%RH and 40°C+75%RH every month as described in section 3.2.

Results

The pH of the cream over a three months period are given in table 4.1.1.

Table 4.1.1: The pH of the cream over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	2.46	-
1 Month	2.45	2.68	2.72
2 Months	-	2.43	2.41
3 Months	-	2.77	2.41

Discussion

The pH of the cream remained relatively stable over the three months. The pH of the cream is low due to the acidity of salicylic acid.

4.2 Relative Density

The relative density of the cream was measured once a month for a period of three months as described in section 3.3.

Results

The relative density results of the cream are given in table 4.2.1.

Table 4.2.1: Relative Density (g/cm^3) of the cream over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	0.9817	-
1 Month	0.9612	0.9485	0.9418
2 Months	-	0.9736	1.0162
3 Months	-	1.0115	0.9534

Discussion

There was no significant change in the relative density of the cream. This indicated on a stable product.

4.3 Viscosity

The viscosity of the cream was measured once a month for three months as described in section 3.4.

Results

The viscosity results of the cream over the stability period of three months are given in table 4.3.1.

Table 4.3.1: Viscosity (cP) of the cream over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	8875	-
1 Month	10155	8534	9387
2 Months	-	11008	12630
3 Months	-	14933	11350

Discussion

The viscosity of the cream increased slightly over the three months. There would then be expected that the spreadability and the penetration of the cream would decrease over the three months.

4.4 Spreadability

The spreadability of the cream was tested each month, for three months on the creams stored at the different temperatures. The spreadability were tested as described in section 3.5.

Results

The spreadability of the cream is given in table 4.4.1.

Table 4.4.1: Spreadability (mm) of the cream over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Init. Sample 1	-	48.56	-
Init. Sample 2	-	48.82	-
1 Mnth. Sample 1	48.28	51.18	51.52
1 Mnth. Sample 2	49.22	48.42	47.62
2 Mnth. Sample 1	-	49.34	48.44
2 Mnth. Sample 2	-	48.58	48.78
3 Mnth. Sample 1	-	47.02	48.68
3 Mnth. Sample 2	-	46.68	47.48

Discussion

The spreadability of the cream remained virtually unchanged over the three months. When the viscosity of the cream increases the spreadability is due to decrease. In other words there persist an inverse relationship between spreadability and viscosity.

4.5 Penetration

The penetration of the cream were measured once a month for a period of three months for samples stored at 5°C, 25°C+60%RH and 40°C+75%RH as described in section 3.6.

Results

The penetration results of the cream is given in table 4.5.1.

Table 4.5.1: Penetration (mm) of the cream over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Init. Day 1	-	48.58	-
Init. Day 2		52.50	
Init. Day 3	-	52.54	-
1 Mnth. Day 1	42.06	42.10	42.16
1 Mnth. Day 2	42.18	42.12	42.16
1 Mnth. Day 3	42.48	44.38	42.46
2 Mnth. Day 1	-	40.38	39.86
2 Mnth. Day 2	-	41.04	40.24
2 Mnth. Day 3	-	41.22	40.64
3 Mnth. Day 1	-	39.86	39.28
3 Mnth. Day 2	-	39.66	39.44
3 Mnth. Day 3	-	39.82	39.34

Discussion

The penetration indicated a "stiffening" effect of the cream after the initial tests, where after the penetration remained almost constant. This could be due to the fact that the cream took a while to settle after formulation. Such settling is often observed with cosmetic products.

4.6 Visual assessment

The cream was visually assessed every month, for three months on the creams stored at different temperatures. For the consumer the visual appearance is the most important factor of a cosmetic product. Therefore special attention was paid to assessing the product in good visible light.

Results

The cream was visually inspected each month and the results are given in table 4.6.1

Table 4.6.1: Visual assessment of the cream over three months

INITIAL	1 MONTH	2 MONTHS		3 MONTHS	
		25°C+60%RH	40°C+75%RH	25°C+60%RH	40°C+75%RH
Color: White Tea tree oil odor Applies easily Slightly fluent Smooth Not too oily Not too hydrous Homogeneous	No Change	No Change	Colour: Off white	No Change	Colour: Beige

Discussion

The cream appeared to have stayed the same over the three months except for the change in colour over the last two months at 40°C.

4.7 Assay

The concentration salicylic acid and tea tree oil in the cream over a period of three months at 5°C, 25°C+60%RH and 40°C+75%RH were determined by HPLC as described in section 3.8.

Results

The results of the salicylic acid and the tea tree oil concentration assay (refer to appendix A) are given in table 4.7.1, and table 4.7.2, respectively.

Table 4.7.1: Concentration (%) salicylic acid in the cream over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	108.7	-	-
25°C+60%RH	105.9	103.2	105.3	104.5
40°C+75%RH	-	100.1	107.2	106.2

Table 4.7.2: Concentration (%) tea tree oil in the cream over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	101.5	-	-
25°C+60%RH	105.5	102.6	103.5	100.2
40°C+75%RH	-	100.5	100.4	101.2

Discussion

The concentration of salicylic acid and tea tree oil, did not show any significant change and remained within the acceptable limits (90-110%). This indicated good stability of the cream.

4.8 Release studies (Dissolution tests)

Dissolution tests were done on the initial cream as well as the cream stored at 25°C+60%RH and 40°C+75%RH after three months, as described in section 3.8.

Results

The amount of salicylic acid and tea tree oil released from the initial cream and the cream stored at 25°C+60%RH and 40°C+75%RH after three months are given in graphically in figure 4.8.1 and 4.8.2. For the real values of the dissolution tests, refer to appendix C.

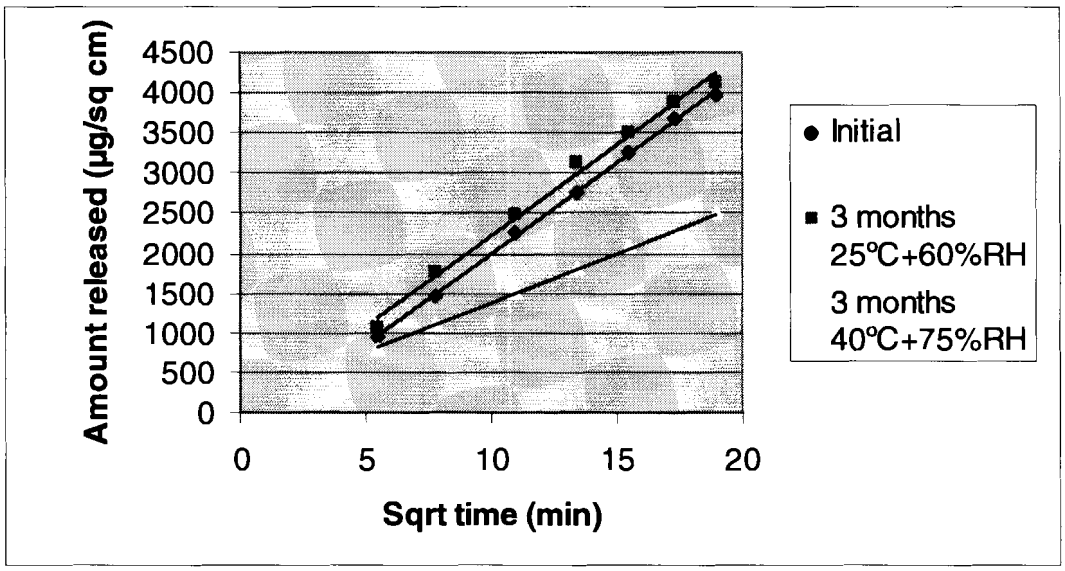

Figure 4.8.1: Amount of salicylic acid released from cream

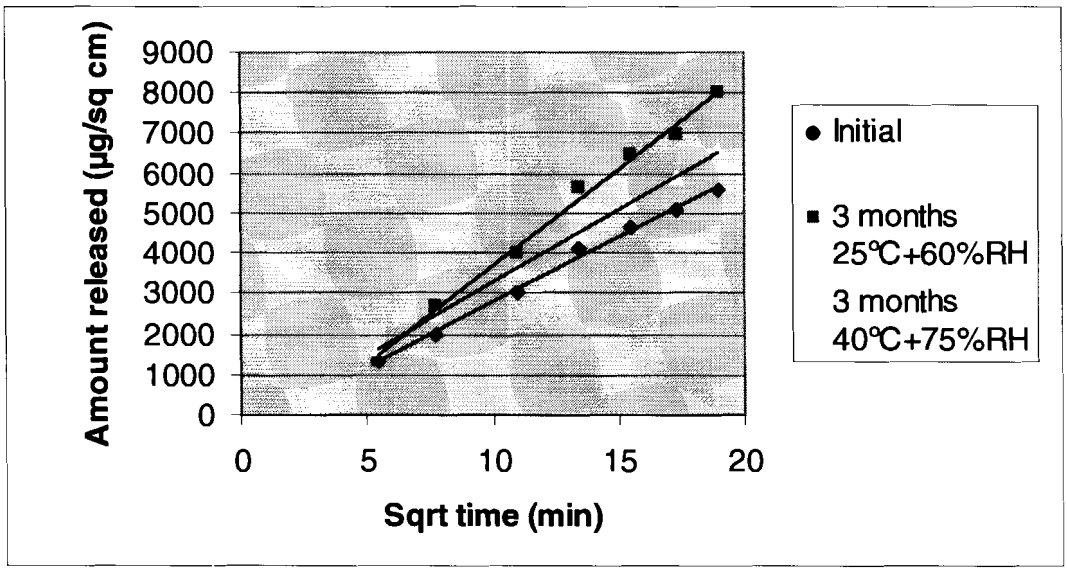

Figure 4.8.2: Amount of tea tree oil released from the cream

Discussion

The release rate of the tea tree oil decreased over the time period. The release of the salicylic acid decreased and then increased again slightly.

4.9 Preservative efficacy

The preservative efficacy of the cream was done by Cosi pharmaceuticals and the test was carried out according to the USP 28 and this was a category 2 product. The initial cream and the cream after three months was tested but unfortunately only the initial cream results was received.

Results

The results for the preservative efficacy of the initial cream are given in table 4.9.1

Table 4.9.1: The preservative efficacy results of the initial cream

TEST ORGANISM	INITIAL INOCULUM	LOG UNIT REDUCTION			SPECIFIED LIMITS FOR CATEGORY 2 PRODUCTS
		DAY 7	DAY 14	DAY 28	
E.coli	3.1×10^5	>3.0	>3.0	>3.0	Not less than 2,0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days
P.aeruginosa	2.0×10^5	>3.0	>3.0	>3.0	
S.aureus	3.6×10^5	>3.0	>3.0	>3.0	
A.niger	1.0×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
C.albicans	1.9×10^5	>3.0	>3.0	>3.0	

Conclusion

It can be concluded that the cream complied with the requirement of the USP 24, and therefore tea tree oil has shown to be an effective preservative in cosmetic products.

CHAPTER 5

STABILITY TEST RESULTS: GEL

5.1 pH

The pH of the gel was measured once a month for three months on the initial gel, as well as the gel stored at 5°C, 25°C+60%RH and 40°C+75%RH as described in section 3.2.

Results

The pH of the gel over a three months period are given in table 5.1.1.

Table 5.1.1: pH of gel over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	2.72	-
1 Month	2.76	2.89	2.98
2 Months	-	2.72	2.94
3 Months	-	2.9	3.02

Discussion

The pH of the gel remained stable over the three months.

5.2 Relative density

The relative density of the cream was measured once a month for a period of three months as described in section 3.3.

Results

The density measured over three months is given in table 5.2.1.

Table 5.2.1: Relative density of the gel over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	1.0132	-
1 Month	1.0049	0.9605	0.9232
2 Months	-	1.0206	0.9699
3 Months	-	1.0427	0.9711

Discussion

The relative density of the gel did not show any significant change over the three month stability period.

5.3 Viscosity

The viscosity of the gel was measured once a month for three months as described in section 3.4.

Results

The measured viscosity concerning the gel is given in table 5.3.1.

Table 5.3.1: Viscosity (cP) of the gel over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	9557	-
1 Month	9998	9557	6656
2 Months	-	6059	3670
3 Months	-	6059	2475

Discussion

The viscosity of the gel decreased slightly over the three months. If the viscosity decrease the spreadability are expected to increase. The decrease in viscosity can be a result of reaction between some of the ingredients.

5.4 Visual assessment

The gel was visually assessed every month, for three months on the gel stored at different temperatures.

Results

The visual results are given in table 5.4.1

Table 5.4.1: Visual assessment of the gel over three months

INITIAL	1 MONTH	2 MONTHS		3 MONTHS	
		25°C+60%RH	40°C+75%RH	25°C+60%RH	40°C+75%RH
Colourless Transparent Fluent and smooth Applies easily Tea tree oil odor Homogeneous	No Change	No Change	Colour: Light yellow	No Change	Colour: Yellow

Discussion

The gel remained the same over the three months, accept for the colour that changed. The gel also became a bit more fluent towards the end of the three months stability period and this could also be seen in the viscosity results.

5.5 Assay

The concentration salicylic acid and tea tree oil in the gel over a period of three months at 5°C, 25°C+60%RH and 40°C+75%RH were determined by HPLC described in section 3.8.

Results

The results of the salicylic acid and tea tree oil concentration assay's are given in table 5.5.1 and table 5.5.2 respectively.

Table 5.5.1: Concentration (%) salicylic acid in the gel over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	109.5	-	-
25°C+60%RH	102.4	102.8	99.6	102.1
40°C+75%RH	-	101.6	102.2	101.2

Table 5.5.2: Concentration (%) tea tree oil in the gel over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	107.33	-	-
25°C+60%RH	102.18	100.60	100.37	99.59
40°C+75%RH	-	99.11	101.35	102.15

Discussion

The concentration of salicylic acid and tea tree oil remained stable.

5.6 Release studies (Dissolution tests)

Dissolution tests were done on the initial gel as well as the gel stored at 25°C+60%RH and 40°C+75%RH after 3 months.

Results:

The amount salicylic acid and tea tree oil released after three months are given in graphically in figure 5.6.1 and 5.6.2. For the real values of the dissolution tests, refer to appendix C.

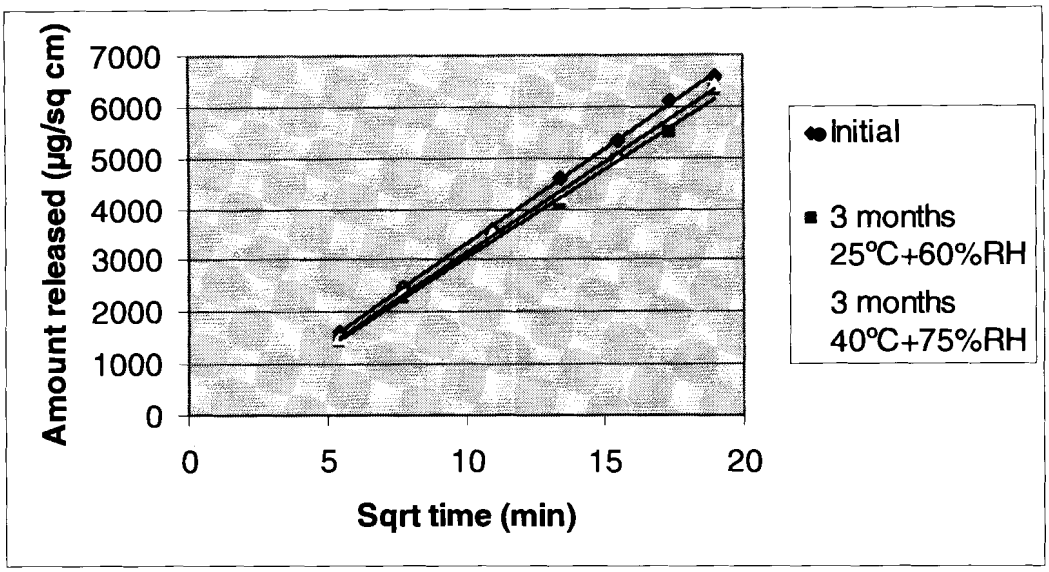

Figure 5.6.1: Amount of salicylic acid released from the gel

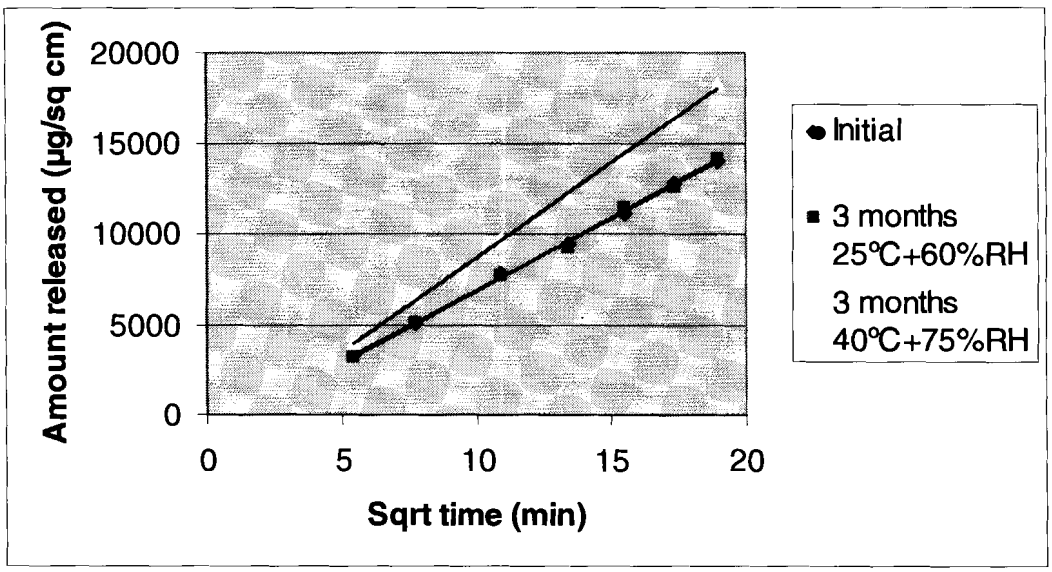

Figure 5.6.2: Amount of tea tree oil released from the gel.

Discussion

The amount tea tree oil released from the gel is the same for the initial gel and the gel stored at 25°C+60%RH after three months. The gel stored at 40°C+75%RH showed a small increase in tea tree oil release. The amount salicylic acid released stayed the same throughout.

5.7 Preservative efficacy

The preservative efficacy of the gel was done by Cosi pharmaceuticals and the test was carried out according to the USP 28 and this was a category 2 product. The initial gel and the gel after three months were tested but unfortunately only the initial gel results was received.

Results

The results for the preservative efficacy of the initial gel are given in table 5.7.1

Table 5.7.1: The preservative efficacy of the initial gel

TEST ORGANISM	INITIAL INOCULUM	LOG UNIT REDUCTION			SPECIFIED LIMITS FOR CATEGORY 2 PRODUCTS
		DAY 7	DAY 14	DAY 28	
E.coli	3.1×10^5	>3.0	>3.0	>3.0	Not less than 2,0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days
P.aeruginosa	2.0×10^5	>3.0	>3.0	>3.0	
S.aureus	3.6×10^5	>3.0	>3.0	>3.0	
A.niger	1.0×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
C.albicans	1.9×10^5	>3.0	>3.0	>3.0	

Conclusion

The gel complied with the requirement of the USP 24, and therefore tea tree oil has shown to be an effective preservative in cosmetic products.

CHAPTER 6

STABILITY TEST RESULTS: OINTMENT

6.1 pH

The pH was measured on the initial ointment, as well as the ointment stored at 5°C, 25°C+60%RH and 40°C+75%RH every month as described in section 3.2.

Results

The pH of the ointment over a stability period of three months are given in table 6.1.1.

Table 6.1.1: pH of the ointment over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	4.06	-
1 Month	4.07	4.12	4.07
2 Months	-	3.95	4.02
3 Months	-	4.07	4.28

Discussion

There was no significant change in the pH over the three months.

6.2 Relative Density

The relative density of the ointment was measured once a month for a period of three months as described in section 3.3.

Results

The relative density of the ointment over the three months are given in table 6.2.1.

Table 6.2.1: Relative density of the ointment over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	1.1421	-
1 Month	1.1661	1.156	1.1334
2 Months	-	1.2206	1.1952
3 Months	-	1.1483	1.1433

Discussion

The density of the ointment showed no serious changes in the three months.

6.3 Viscosity

The viscosity of the ointment was measured once a month for three months as described in section 3.4.

Results

The viscosity of the ointment stored at different temperatures are given in table 6.3.1.

Table 6.3.1: Viscosity (cP) of the ointment over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	225000	-
1 Month	188000	205000	215000
2 Months	-	174000	188000
3 Months	-	218000	171000

Discussion

The vicosity of the ointment decreased a little over the three months.

6.4 Spreadability

The spreadability of the ointment was tested each month, for three months on the creams stored at the different temperatures as described in section 3.5.

Results

The spreadability results of the ointment are given in table 6.4.1.

Table 6.4.1: Spreadability (mm) of the ointment over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Init. Sample 1	-	23.12	-
Init. Sample 2	-	23.32	-
1 Mnth. Sample 1	23.46	24.88	25.48
1 Mnth. Sample 2	22.60	24.12	25.18
2 Mnth. Sample 1	-	24.98	25.46
2 Mnth. Sample 2	-	25.25	25.88
3 Mnth. Sample 1	-	25.82	25.62
3 Mnth. Sample 2	-	26.42	25.78

Discussion

The spreadability of the ointment was expected to increase due to the decrease in viscosity.

6.5 Penetration

The penetration of the ointment were measured once a month for a period of three months for samples stored at 5°C, 25°C+60%RH and 40°C+75%RH as described in section 3.6.

Results

The penetration results of the ointment are given in table 6.5.1

Table 6.5.1: Penetration (mm) of the ointment over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Init. Day 1	-	47.14	-
Init. Day 2		40.02	
Init. Day 3	-	35.84	-
1 Mnth. Day 1	39.32	38.24	38.38
1 Mnth. Day 2	35.86	39.48	34.00
1 Mnth. Day 3	41.10	37.92	38.28
2 Mnth. Day 1		41.44	42.74
2 Mnth. Day 2		41.86	42.88
2 Mnth. Day 3		42.02	43.36
3 Mnth. Day 1		43.18	44.36
3 Mnth. Day 2		43.66	44.82
3 Mnth. Day 3		43.82	44.88

Discussion

The penetration of the ointment was expected to increased over the three months stability period due to the decrease in viscosity.

6.6 Visual assessment

The ointment was visually assessed every month, for three months on the products stored at different temperatures.

Results

The visual results of the ointment over the three months are given in table 6.6.1.

Table 6.6.1: Visual assessment of the ointment over three months

INITIAL	1 MONTH	2 MONTHS	3 MONTHS	
			25°C+60%RH	40°C+75%RH
Color: White Tea tree oil odor Applies easily Non-fluent Not too oily Not too hydrous Homogeneous	No Change	No Change	No Change	Color: Cream

Discussion

The ointment, visually stayed the same over the three months. The colour of the ointment at 40°C+75%RH changed from white to cream.

6.7 Assay

The concentration salicylic acid and tea tree oil in the ointment over a period of three months at 5°C, 25°C+60%RH and 40°C+75%RH were determined by HPLC described in section 3.8.

Results

The results of the concentration salicylic acid and tea tree oil in the ointment over the three months are given in table 6.7.1 and table 6.7.2.

Table 6.7.1: Concentration (%) salicylic acid in the ointment over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	104.2	-	-
25°C+60%RH	107.0	102.3	101.5	104.4
40°C+75%RH	-	106.8	101.3	101.7

Table 6.7.2: Concentration (%) tea tree oil in the ointment over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	101.9	-	-
25°C+60%RH	102.4	99.0	101.1	96.9
40°C+75%RH	-	101.5	100.9	100.9

Discussion

The concentration of the salicylic acid and tea tree oil in the ointment remained relatively stable throughout the three months.

6.8 Release studies (Dissolution tests)

Dissolution tests were done on the initial ointment as well as the ointment stored at 25°C+60%RH and 40°C+75%RH after three months.

Results

The amount salicylic acid and tea tree oil released from the ointment after three months are given in graphically in figure 6.8.1 and 6.8.2. For the real values of the dissolution tests, refer to appendix C.

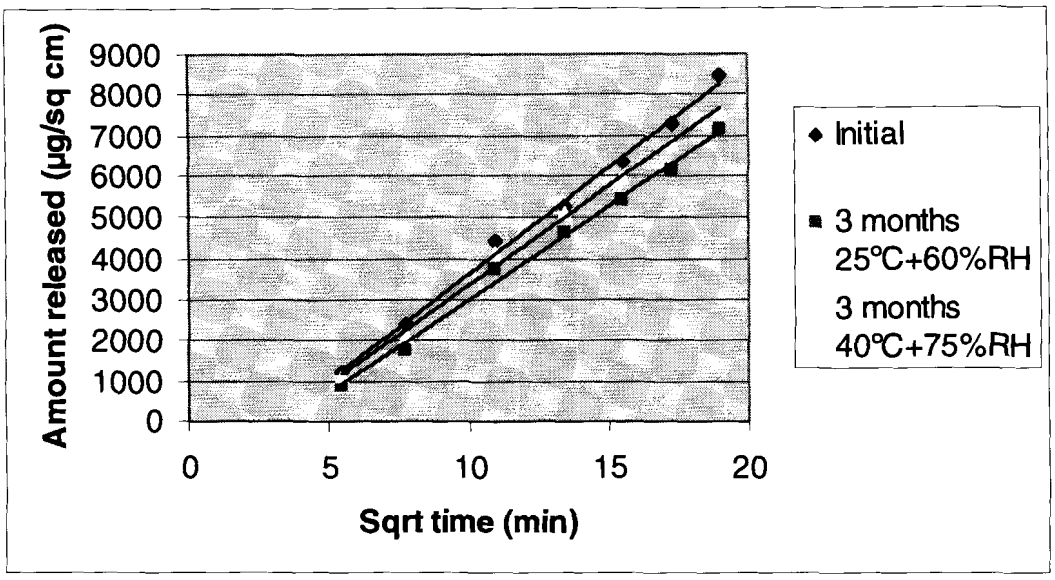

Figure 6.8.1: Amount of salicylic acid released from the ointment

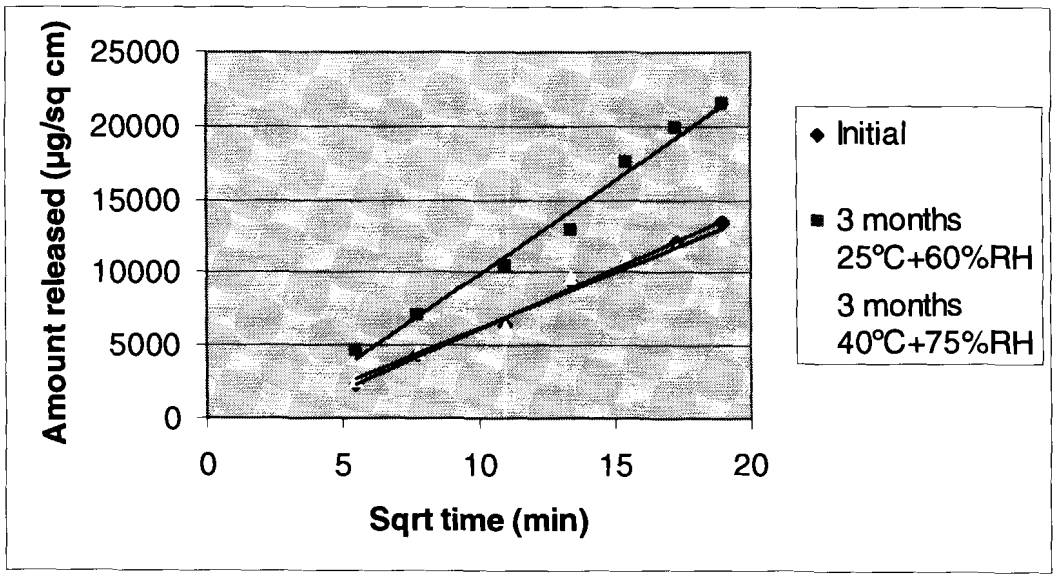

Figure 6.8.2: Amount of tea tree oil released from the ointment

Discussion

The amount tea tree oil released increased from the initial ointment to the ointment stored at 25°C+60%RH for three months. Then the amount decreased again with the ointment that was stored at 40°C+75%RH for three months. The amount of the salicylic acid that was released stayed the same throughout the three months.

6.9 Preservative efficacy

The preservative efficacy of the ointment was also done by Cosi pharmaceuticals and the test was carried out according to the USP 28 and this was a category 2 product. The initial ointment and the ointment after three months was tested but unfortunately only the initial ointment results was received.

Results

The results for the preservative efficacy of the initial ointment are given in table 6.9.1

Table 6.9.1: The preservative efficacy results of the initial ointment

TEST ORGANISM	INITIAL INOCULUM	LOG UNIT REDUCTION			SPECIFIED LIMITS FOR CATEGORY 2 PRODUCTS
		DAY 7	DAY 14	DAY 28	
E.coli	3.1×10^5	>3.0	>3.0	>3.0	Not less than 2,0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days
P.aeruginosa	2.0×10^5	>3.0	>3.0	>3.0	
S.aureus	3.6×10^5	>3.0	>3.0	>3.0	
A.niger	1.0×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
C.albicans	1.9×10^5	>3.0	>3.0	>3.0	

Conclusion

The ointment complied with the requirement of the USP 24, and therefore tea tree oil has shown to be an effective preservative in cosmetic products.

CHAPTER 7

STABILITY TEST RESULTS: SOAP BAR

7.1 pH

Results

The pH of the soap bar is given in table 7.1.1.

Table 7.1.1: pH of the soap bar over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	9	-
1 Month	9	9	9
2 Months	-	9	8.5
3 Months	-	9	8.5

Discussion

The pH of the soap bar showed no significant changes over the three months stability period.

7.2 Visual assessment

Results

The soap bar visual results are given in table 7.2.1

Table 7.2.1: Visual assessment of the soap bar over three months

INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Colour: Yellow Semi-transparent Foams easily No cracking Tea tree oil odour Uniform and solid	No Change	No Change	No Change

Discussion

The soap bar had really good characteristics and showed no visual changes over the three months stability period.

7.3 Foamability

The foamability of the soap bar was tested once a month for three months, on the initial soap bar as well as the soap bar stored at 5°C, 25°C+60%RH and 40°C+75%RH. The foamability was done as described in section 3.7.

Results

The foamability of the soap bar tested in 20°C distilled water is given in table 7.3.1, and the foamability of the soap bar tested in 40°C distilled water is given in table 7.3.2.

Table 7.3.1: Foambility of the soap bar over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	13.92	-
1 Month	12.64	13.66	12.94
2 Months	-	13.24	13.04
3 Months	-	13.48	13.36

Table 7.3.2: Foamability of the soap bar over three months

Time interval	5°C	25°C+60%RH	40°C+75%RH
Initial	-	23.88	-
1 Month	23.18	23.24	23.42
2 Months	-	23.76	23.16
3 Months	-	23.82	23.28

Discussion

There was no significant change in the foamability of the soap bar over the three months.

7.4 Assay

The concentration salicylic acid and tea tree oil in the soap bar over a period of three months at 5°C, 25°C+60%RH and 40°C+75%RH were determined through HPLC chromatography described in section 3.8.

Results

The results of the concentration salicylic acid and tea tree oil in the soap bar are given in table 7.4.1 and table 7.4.2 respectively.

Table 7.4.1: Concentration (%) salicylic acid in the soap bar over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	107.7	-	-
25°C+60%RH	104.9	101.1	103.0	103.2
40°C+75%RH	-	102.6	101.3	100.5

Table 7.4.2: Concentration (%) of tea tree oil in the soap bar over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.2	-	-
25°C+60%RH	100.9	102.7	99.2	98.3
40°C+75%RH	-	100.2	104.6	97.1

Discussion

The concentrations of both the active ingredients did not show any significant change over the three months.

CHAPTER 8

STABILITY TEST RESULTS: COVER STICK

8.1 Visual assessment

Results

The cover stick was visually inspected each month and the results are given in table 8.1.1.

Table 8.1.1: Visual assessment of the cover stick over three months.

INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Colour: Brown Applies easily Tea tree oil odour Slightly oily Hard	No Change	No Change	Change: Softer

Discussion

Visually the cover stick remained the same over the three months. The cover stick stored at 40°C+75%RH after three months, did appear to be a little softer than the initial cover stick.

8.2 Assay

Results

The concentration salicylic acid and tea tree oil in the cover stick is given in table 8.2.1 and table 8.2.2 respectively.

Table 8.2.1: Concentration (%) salicylic acid in the cover stick over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.5	-	-
25°C+60%RH	102.1	98.7	102.1	98.4
40°C+75%RH	-	101.9	103.3	98.3

Table 8.2.2: Concentration (%) tea tree oil in the cover stick over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.5	-	-
25°C+60%RH	102.1	98.7	102.1	98.4
40°C+75%RH	-	101.9	103.3	98.3

Discussion

Both the active ingredients in the cover stick did not show any significant change over the three months stability period.

CHAPTER 9

CONCLUSION

9.1 Comparison between the five formulations

The pH and the relative density of the cream, gel and ointment stayed the same over the three months stability period. The viscosity of the cream increased slightly over the three months, whereas the viscosity of the gel and the ointment decreased slightly. Due to the inverse relationship between spreadability, penetration and viscosity, the spreadability and penetration of the cream decreased over the three months. The spreadability and penetration of the gel and ointment increased over time. But these changes are very small and therefore all the products remained stable over the three months stability period.

Considering the assays of the five formulated products, the cream, gel and ointment proved to have stayed the same over the three months. The soap bar and cover stick showed a small decrease in concentration of the two active ingredients, but remained within the acceptable limits of 90-110%.

Visually the cream, gel and ointment showed a difference in colour at the end of the three months stability period. Tea tree oil does undergo photo-oxidation under the influence of light, moisture, warmth, and oxygen (Hausen *et al.,* 1999:67). If light resistant and tight sealing containers are used in the future it may improve these results. Degradation of some of the ingredients in those products could be the reason for the change in colour.

According to the release studies, the gel released the highest amount of salicylic acid in comparison to the cream and the ointment. A reason for this phenomenon could be that during the formulation the salicylic acid dissolved completely in the alcohol of the gel formulation and therefore could be released easier than in a product that contained no alcohol for example the cream and the ointment.

The ointment released the highest amount of tea tree oil. Again this could be explained by the solubility properties of tea tree oil. The formulation of the ointment contained no water with high concentrations of lipophilic ingredients and therefore the incorporation of tea tree oil was appropriate. This allowed the tea tree oil to be released easier than in a formulation that contained water like for example the cream and the gel.

All five the products showed excellent stability properties.

9.2 Conclusion

The aims of this study were to formulate five acne products that consisted of high quality and remained stable in accelerated stability testing procedures. Combinational therapy is the key factor when treating acne successfully. Therefore the formulations consisted of two active ingredients each with its own mechanism of action for the elimination of acne. The concentration of these active ingredients was of such a nature that it would not cause irritations but still act as a medicament. Because of the scars and redness that acne can cause, the cover stick formulation was intended to beautify and act as a medicament simultaneously.

The acne products was of high quality and remained stable throughout the study, thus the aims were reached and these products can successfully be implemented for acne treatment.

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APPENDIX A

GLOSSARY

Active ingredient

Any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and are present in the drug product in a modified form intended to furnish the specified activity or effect.

Assay

A technique (test) for measuring a biological response or for determining characteristics such as composition, purity, activity, and weight.

Calibration

A comparison of a measurement standard or instrument of unknown accuracy to detect, correlate, report, or eliminate by adjustment of any variation in the accuracy of the unknown standard or instrument.

Carbapol (Ultrez 10)™

Noveon, Cleveland

Cetyl alcohol

Merck chemicals, South Africa

Cremophor A 6

Ceteareth-6 (and) Stearyl alcohol, a white waxy substance. (BASF, South Africa)

Cremophor A 25

Ceteareth-25, free-flowing, non-dusting microbeads. (BASF, South Africa)

Comedo

A plug of keratin and sebum within the dilated orifice of a hair follicle, frequently containing the bacteria Propionibacterium acnes, Staphylococcus albus and Pityrosporon ovale, also called a blackhead.

Density

Density is defined as the ratio of the mass of an object to its volume.

Dilution

Lowering the concentration of a solution by adding more solvent.

EDTA

Ethylenediamine tetra-acetic acid.

Properties and Uses:

EDTA is a chelating agent (complexone) used to inactivate traces of heavy metals in hair and skin care products because heavy metals accelerate the decomposition of many active ingredients, e.g. hydrogen peroxide, and the rancidness of natural oils. It is also a synergist (substance which enhances the effectiveness) of antioxidants and preservatives. (Merck chemicals, South Africa)

Emulsifying wax

Croda chemicals, South Africa

Ethanol 99.98%

Merck chemicals, South Africa

Gel

A colloid, where the dispersed phase is liquid and the dispersion medium is solid.

Glycerin

Natural, sweet-tasting, colourless, thick liquid which freezes to a gummy paste and which has a high boiling point. The pure chemical product is called Glycerol (which shows that it is an alcohol). (Merck chemicals, South Africa)

Hormone

A type of chemical messenger, occurring both in plants and animals, that acts to inhibit or excite metabolic activities. Its site of production is distant from the site of biological activity.

HPLC (High Pressure Liquid Chromatography)

Sometimes called high-performance liquid chromatography, it is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations (into distinct bands) are achieved by partition, adsorption, or ion-exchange processes, depending upon the type of stationary phase used. Each band is then profiled as the solvent flows through a UV detector, or by fluorescence, or refractive index detectors

HPMC

Hydroxypropylmethylcellulose 65 HG (Fluka, South Africa)

Membrane

A barrier, usually thin, that only permits the passage of particles of a certain size or special nature. Filtration membranes are thin polymer films that are permeable to water and other fluids:

$NaH_2PO_4 \cdot 2H_2O$

Sodium dihydrogen orthophosphate dihydrate

Liquid paraffin

Merck chemicals, South Africa

pH

The pH value of an aqueous solution is a number describing its acidity or alkalinity. A pH is the negative logarithm (base 10) of the concentration of hydrogen ions (equivalent per litre). The pH value of a neutral solution is 7. An acidic solution has a pH less than 7, while a basic solution has a pH greater than 7, up to 14.

Preservative

A bacteriostatic or bactericidal agent added to some multiple dose parenterals and most cosmetics.

Polyethylene glycol 400

Merck chemicals, South Africa

Polyethylene glycol 4000

Merck chemicals, South Africa

Propan-2-ol

Merck chemicals, South Africa

Propylene glycol

A very versatile propylene oxide derivative. Synonyms: 1,2-Propanediol; 1,2-Dihydroxypropane; Methylethylene glycol; Trimethylglycol; 1,2-Propylene glycol; monopropylene glycol

MF=$C_3H_8O_2$

Properties and Uses:

Propane 1,2-diol is a viscous, colourless, almost odourless liquid that is miscible with water, ethanol and ethereal oils, and insoluble in hydrocarbons, fats and oils. It acts as a solubilizing agent for ethereal oils and humectant for emulsions, and also improves the efficacy of some preservatives. (Merck chemicals, South Africa)

Reagent

A substance used (as in detecting or measuring a component, in preparing a product, or in developing photographs) because of its chemical or biological activity.

Relative Humidity (%RH)

The ratio (measured in percent) of actual water vapour pressure in air to the pressure of saturated water vapour in air at the same temperature and pressure.

Salicylic acid

Croda chemicals, South Africa

Sebaceous Gland

Small, sacculated organs found in the corium of the dermis. Each gland has a single duct that emerges from a cluster of oval alveoli. Each alveolus consists of a transparent basement membrane enclosing epithelial cells. The ducts from most sebaceous glands open into hair follicles, but some open on the general surface of the skin. Sebaceous glands secrete SEBUM.

Soft paraffin

Croda chemicals, South Africa

Solvent

An inorganic or organic liquid used as a vehicle for the preparation of solutions or suspensions in the manufacture of an intermediate or API (Active Pharmaceutical Ingredient).

Stearic acid

Stearic acid is a waxy solid, and its chemical formula is $CH_3(CH_2)15COOH$. Its name comes from the Greek word, stear, which means tallow. Its IUPAC name is octadeanoic acid.

Stearic acid is a typical example of a fatty acid, which is essentially a long hydrocarbon chain containing a carboxyl group at one end and a methyl group at the other. The chain lengths can vary from 3 (propionic acid) to 24 (lignoceric acid) but the majority of fatty acids found in hydrogenated vegetable or animal oils are around C_{16}-C_{20} in length. Stearic acid is a *saturated* acid, since there are no double bonds between neighbouring carbon atoms. This means that the hydrocarbon chain is flexible and can roll up into a ball or stretch out into a long zigzag. (Merck chemicals, South Africa)

Sodium hydroxide

Merck chemicals, South Africa

Stability

Generally, stability refers to the physico-chemical condition of a parenteral, biological, or to the shelf life of labile drugs. Certain drugs must pass U.S.P. or CFR

stability tests. Manufacturers must have documentation of the potency of labile products under labelled storage conditions.

Topical product

A pharmaceutical product meant to be applied to the skin or soft tissue in the form of liquid, cream, or ointment, and therefore needs not be aseptic. Sterile ophthalmic products throughout are manufactured aseptically.

Triethanolamine

Properties and Uses:

Triethanolamine is an organic base that does not irritate or corrode the skin. It is used as a neutralising agent for stearic or oleic acid, resulting in soaps that make good emulsifiers. (Merck chemicals, South Africa)

Tea tree oil

Croda chemicals, South Africa

Tween 80

Polyoxyethylenesorbitan monooleate . (Merck chemicals, South Africa)

Validation

A documented programme that provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting pre-determined acceptance criteria.

Vehicle

Any solvent or carrier fluid in a pharmaceutical product that has no pharmacological role. For example, water is the vehicle for xilocaine and propylene glycol is the vehicle for many antibiotics.

Viscosity

The tendency of a fluid to resist flowing because of molecular attraction (cohesion). The SI physical unit of dynamic viscosity is pascal-second, which is identical to 1 $N \bullet s/m^2$.

APPENDIX B

VALIDATION

Test	Result
Specificity	Complies
Range	Salicylic acid 6.4-304.8 µg/ml Tea tree oil 9.2-442.9 µg/ml
Linearity	Salicylic acid R^2 = 0.9998 Tea tree oil R^2 = 0.9995
Accuracy	Salicylic acid 102.4% Tea tree oil 101.7%
Precision	Salicylic acid RSD 1.70% Tea tree oil RSD 0.72%

1. CHROMATOGRAPHIC CONDITIONS.

Analytical instrument: Agilent 1100 series HPLC equipped with a gradient pump, autosampler, UV detector and chemstation Rev. A.06.02 data acquisition and analysis software or equivalent. (Agilent, Palo alts, CA)

Column: Luna C18(2) 150x4.6 mm, 5 µm (Phenomenex, Torrance, CA)
Mobile phase: Acetonitrile/water pH adjusted to 2.5 with phosphoric acid
Flow rate: 1.0 ml/min.
Injection volume: 10 µl.
Detection: UV at 220 nm.
Retention time: Approximately 3.5 and 9.9 minutes for salicylic acid and tea tree oil respectively.
Solvent: 30 % THF and 70 % Methanol

2. SAMPLE PREPARATION.

1. Remove the plunger from a 10 ml disposable syringe and fill it with cream.
2. Attach about 15 cm of plastic tubing to the syringe, and refit the plunger.
3. Place a 100 ml volumetric flask on an analytical balance and tare.
4. Put the tubing into the volumetric flask and squeeze about 1 g of sample into the flask.
5. Note the mass of sample used.
6. Add about 90 ml of mobile phase to the flask and put the flask in an ultrasonic bath for 15 minutes in lukewarm water.
7. Check that all the sample has dissolved. If solid pieces of sample are still visible, shake the flask and sonicate it again until it is completely dissolved.
8. Allow the flask to cool to room temperature and fill to volume with solvent.
9. Filter the solution into an autosampler vial and analyse.

3. STANDARD SOLUTION.

Weigh approximately 20 mg of salicylic acid and 30 mg tea tree oil accurately.
Transfer into a 100 ml volumetric flask and dissolve and make up to volume with solvent.
Transfer this solution to an autosampler vial and inject into the chromatograph.

CALCULATIONS.

$$\frac{\text{Sample area} \times \text{mass of standard (g)} \times \text{potency of standard}}{\text{Standard area} \times \text{Sample mass} \times 50} = \%\ \text{w/w}$$

4. SYSTEM SUITABILITY PARAMETERS.

- Make duplicate injections of a standard solution.
- Calculate the relative standard deviation of the peak areas obtained.

5. VALIDATION TEST PROCEDURE AND ACCEPTANCE CRITERIA:

5.1 Linearity.

Preparation of standards.

Prepare a standard solution as described under standard preparation.
Inject variable volumes into the chromatograph to obtain standards from 75-125 % of the expected sample concentration.

5.2 Accuracy.

Measure 3 times 0.8 g, 3 x 1 g and 3 x 1.2 g of placebo into 100 ml volumetric flasks.
Spike with known amounts of active at concentrations of approximately 80, 100 and 120% of the expected sample concentration.
Inject into the chromatograph in duplicate.

5.3 Precision.

5.3.1 Intra-day precision (repeatability).

Measure approximately 3 x 0.8 g, 3 x 1 g and 3 x 1.2 g of sample into 100 ml volumetric flasks and fill to volume with solvent.
Inject into the chromatograph in duplicate.

5.3.2 Inter-day precision.

Analyse the same homogenous sample in triplicate as described above for intra-day precision (at 100% of the sample concentration) on two more occasions to determine the between-day variability of the method. On one occasion (day 3) a different analyst should perform the analysis on a different set of equipment.

5.4 Ruggedness.

5.4.1 Stability of sample solutions.

Prepare a sample as described under sample preparation in the method.
Inject the sample into the chromatograph.
Leave the sample in the autosampler tray and reanalyse over a period to determine the stability of the sample.

5.4.2 System repeatability.

Inject a sample six times consecutively in order to test the repeatability of the peak area as well as the retention time.

5.5 System and method performance characteristics (system suitability).

Calculate the chromatographic performance characteristics of the separation, like retention time, USP peak tailing factor, capacity factor and resolution between peaks and repeatability of multiple injections.
Use the data obtained to set realistic performance limits that should be met before the analysis can be performed.

6 VALIDATION RESULTS.

6.1 SPECIFICITY:

A sample prepared from the placebo product is shown in Figure 6.1. Figure 6.2 is a chromatogram of a standard solution, while figures 6.3-6.5 are chromatograms of samples that have been stressed for 4 hours. The analyte peaks that remained in the water sample after being stressed were analysed by means of diode array peak purity testing to determine whether the peaks are still pure.

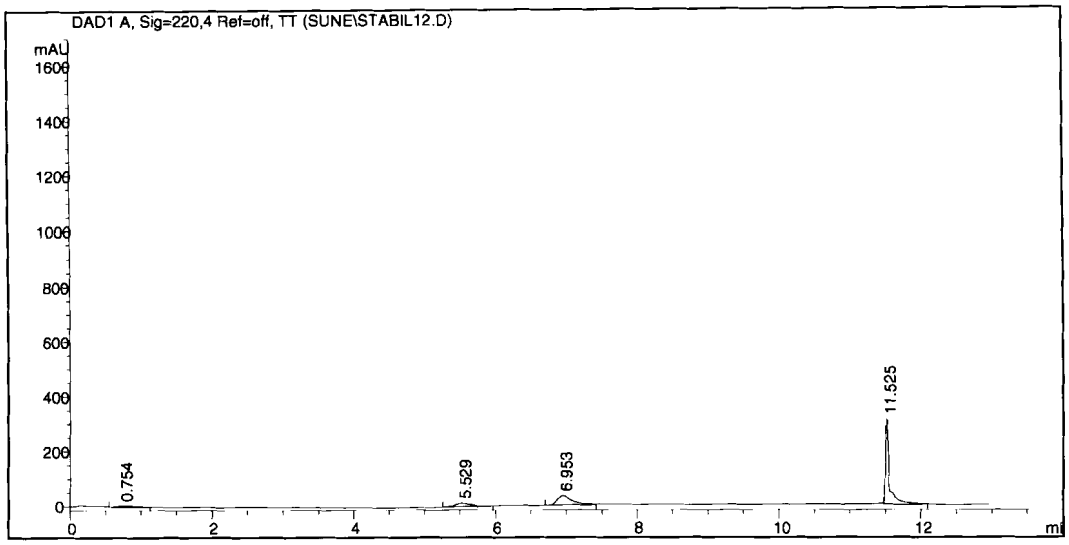

Figure 6.1: Placebo of the sample

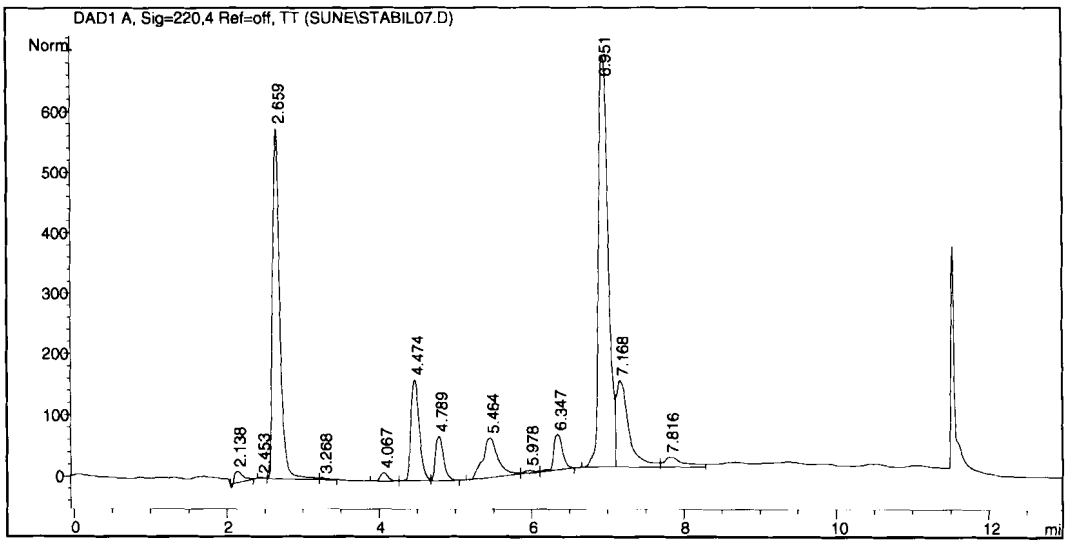

Figure 6.2: Chromatogram of the standard solution

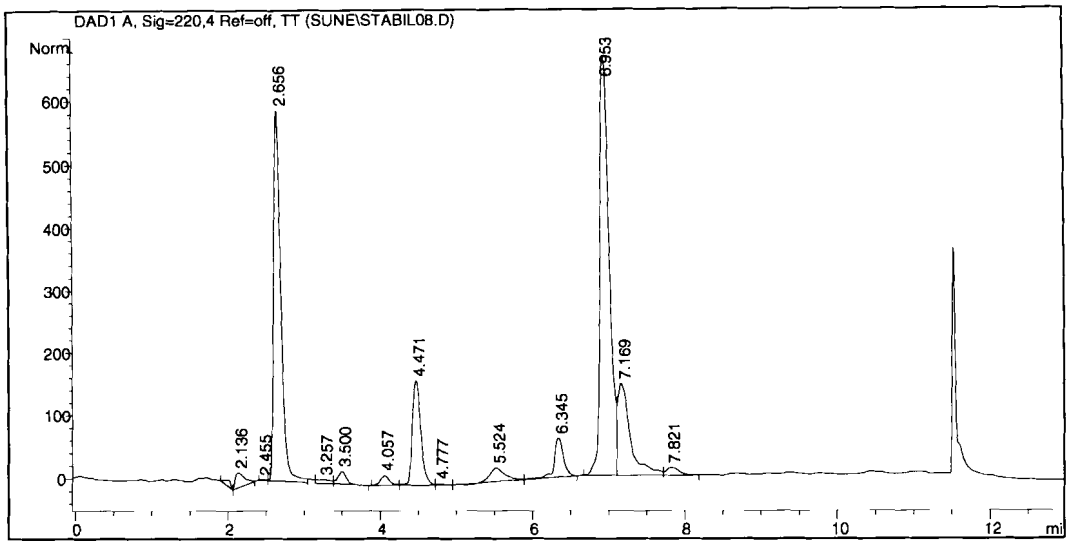

Figure 6.3: Sample stressed in water at 40 °C for 4 hours.

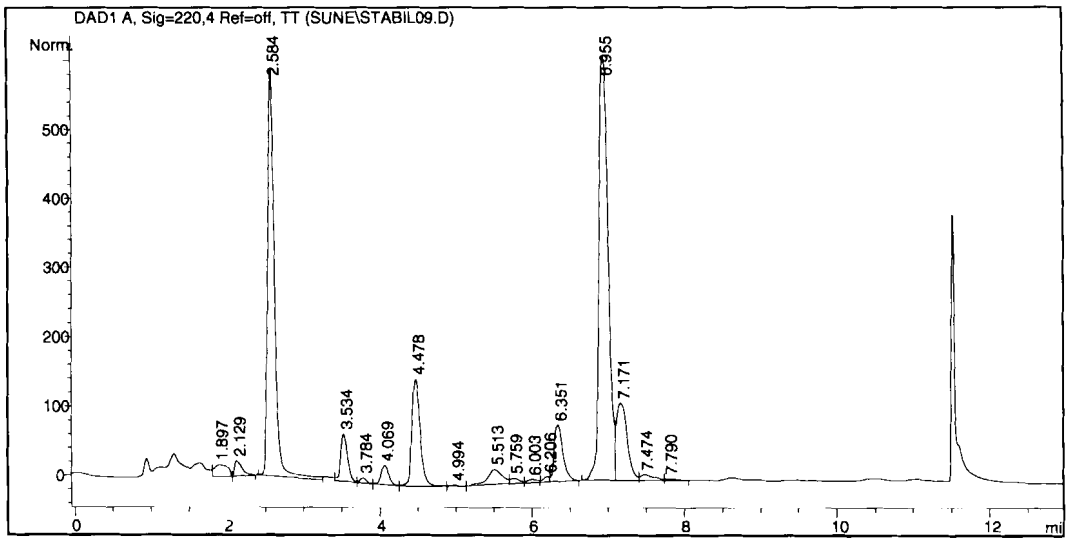

Figure 6.4: Sample stressed in 0.1 M hydrochloric acid at 40 °C for 4 hours.

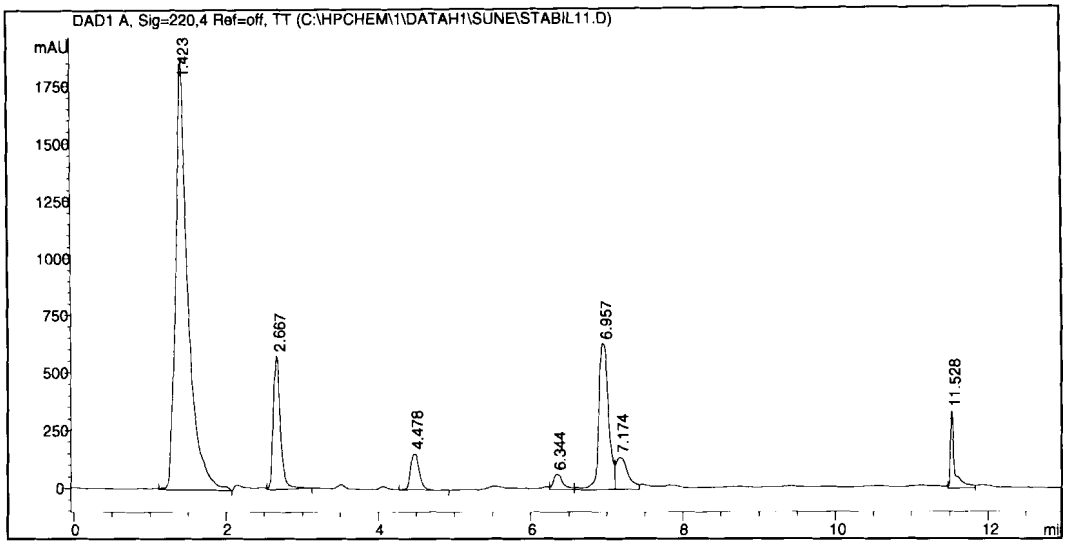

Figure 6.5: Sample stressed in 0.1 M sodium hydroxide at 40 °C for 4 hours.

Conclusion:

None of the ingredients in the placebo interfered with the analyte peaks. Extra peaks formed during forced degradation did not interfere with the remainder of the analyte peaks. Peak purity testing of the remaining peaks after forced degradation in water showed that the peaks were still pure, thus proving that the method is stability-indicating.

7. SALICYLIC ACID:

7.1 LINEARITY AND RANGE:

Results:

µg/ml	Area 1	Area 2	Mean
6.4	49	66	57
12.7	131	128	130
25.4	269	266	267
38.1	402	408	405
50.8	533	536	534
63.5	687	678	683
101.6	1053	1053	1053
152.4	1610	1569	1590
203.2	2094	2157	2126
254.0	2693	2698	2696
304.8	3181	3192	3187

Regression statistics:

R Squared	0.99985	Lower 95%	Upper 95%
Intercept	-1.36745	-15.6248	12.88994
Slope	10.50465	10.40836	10.60094

Conclusion:

The method is linear over the concentration range 30-48 µg/ml. The method is suitable for single point calibration.

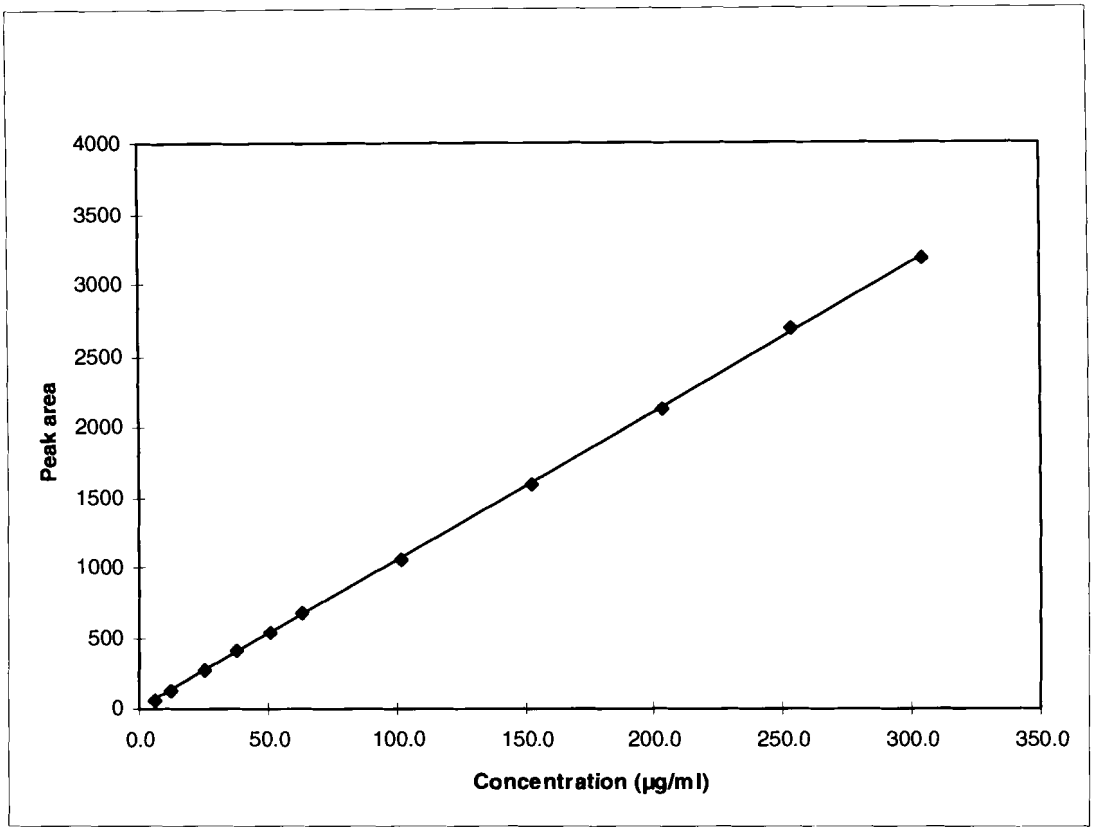

Figure 7.1: Linear regression graph for salicylic acid.

7.2 ACCURACY:

Conc. spiked			Mean	Recovery	
µg/ml	Area 1	Area 2		µg/ml	%
161.3	1756.4	1776.1	1766	168.3	104.3
161.3	1710.6	1799.1	1755	167.2	103.6
161.3	1702.5	1702.9	1703	162.2	100.6
201.6	2167.2	2257.3	2212	210.7	104.5
201.6	2268.6	2120.4	2195	209.0	103.7
201.6	2198.2	2254.3	2226	212.1	105.2
241.9	2546.2	2503.4	2525	240.5	99.4
241.9	2525.7	2536.4	2531	241.1	99.7
241.9	2567.4	2554.2	2561	243.9	100.8

Statistical analysis	
Mean	102.4
SD	2.1
% RSD	2.1
95% confidence intervals	
Lower limit	99.0
Upper limit	100.8
Estimated median	99.8
Confidence Level(95.0%)	0.9

Conclusion:

Over the range of 80-120% of the sample concentration, the method yielded a mean recovery of 102.4 %.

7.3 PRECISION:

7.3.1 Intermediate (intra-day) precision:

Mass (g)	Area 1	Area 2	Mean	Conc.µg/ml	%
0.8817	1859.5	1971.25	1915	208.4	104.2
0.8381	1842.2	1786.8	1815	207.7	103.8
0.8315	1806.6	1758.9	1783	205.7	102.8
1.0871	2137.1	2382.1	2260	199.4	99.7
1.0462	2143	2191	2167	198.7	99.3
1.0755	2183.7	2298.4	2241	199.9	99.9
1.2752	2710.3	2734.9	2723	204.8	102.4
1.2484	2645.2	2723.1	2684	206.2	103.1
1.1910	2587.8	2478.2	2533	204.0	102.0

Mean	203.85	101.92
SD	3.46	1.73
RSD %	1.70	1.70

Conclusion:

Precision was satisfactory with a RSD of 1.70 %.

7.3.2 Inter-day precision:

	DAY 1 %	DAY 2 %	DAY 3 %	Inter day
	99.7	103.0	100.6	
	99.3	102.7	103.9	
	99.9	102.5	103.2	
Mean	99.65	102.73	102.56	101.65
SD	0.24	0.23	1.41	1.41
RSD %	0.25	0.22	1.38	1.39

ANOVA single factor statistics:

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	287.199	95.733	0.797
Day 2	3	282.380	94.127	2.551
Day 3	3	286.451	95.484	0.367

ANOVA					
Source of	SS	df	MS	F	P-value
Inter day	4.484	2.0	2.242	1.811	0.242
Intra day	7.428	6.0	1.238		
Total	11.913	8.0			

SS = sum of squares

df = degrees of freedom

MS = mean squares

F = F ratio

Conclusion:

The inter-day variance was higher than the intra-day variance. The repeatability is, however, still within acceptable limits, and the assay should perform well, even when executed by other personnel in a different laboratory.

7.4 RUGGEDNESS:

7.4.1 STABILITY OF SAMPLE SOLUTIONS:

A sample was left on the autosampler tray and re-analyzed over several time intervals to determine the sample stability.

Results:

Time (hours)	Peak Area	% Remaining
0	2149.7	100.0
1	2130.3	99.1
2	2136.7	99.4
3	2100.1	97.7
4	2092.4	97.3
5	2040.6	94.9
6	2041.3	95.0
7	2082.2	96.9
8	2039.7	94.9
9	2068.8	96.2
10	2130.4	99.1
11	2079.5	96.7
Mean	2097	97.5
SD	38.72	1.80
RSD %	1.85	1.85

Conclusion:

The salicylic acid is stable over a period of 11 hours.

7.4.2 SYSTEM REPEATABILITY:

A sample was injected six times in order to test the repeatability of the peak area as well as the retention time.

Results.

	Area	Retention time (minutes)
	1920	3.605
	1982	3.547
	1936	3.546
	1988	3.543
	1962	3.563
	1943	3.585
Mean	1955	3.565
SD	24.57	0.023
RSD %	1.26	0.645

Conclusion:

System performance proved well with RSD values of 1.26 % for peak area and 0.645 % for retention time respectively.

8. TEA TREE OIL:

8.1 LINEARITY AND RANGE:

Results:

µg/ml	Area 1	Area 2	Mean
9.2	27	23	25
18.5	22	24	23
36.9	50	43	46
55.4	64	61	62
73.8	80	81	80
92.3	103	102	102
147.6	149	152	151
221.5	226	217	222
295.3	294	300	297
369.1	367	375	371
442.9	440	443	442

Regression statistics:

R Squared	0.999529	Lower 95%	Upper 95%
Intercept	9.511633	6.081714	12.94155
Slope	0.973705	0.957764	0.989645

Conclusion:

The method is linear over the concentration range 15-25 µg/ml. The method is suitable for single point calibration.

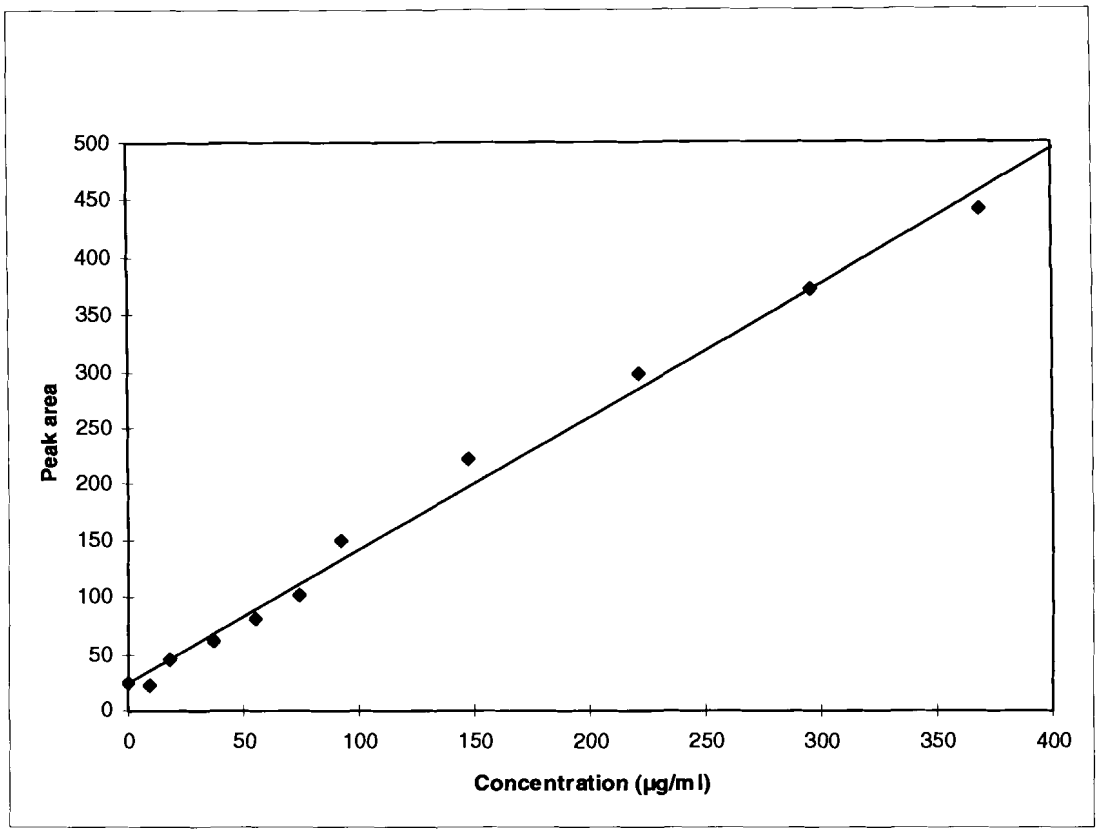

Figure 8.1: Linear regression graph for propyl paraben.

8.2 ACCURACY:

Conc. spiked			Mean	Recovery	
µg/ml	Area 1	Area 2		µg/ml	%
245.0	224.6	237.9	231	227.7	92.9
245.0	225.9	233.1	230	225.9	92.2
245.0	232.1	229.5	231	227.3	92.8
306.2	279.1	286.4	283	280.6	91.6
306.2	288.6	272.7	281	278.5	90.9
306.2	277.3	287.9	283	280.5	91.6
367.4	324.2	344.2	334	333.5	90.8
367.4	335.9	342.5	339	338.6	92.2
367.4	347.6	323.8	336	335.0	91.2

Statistical analysis	
Mean	91.8
SD	0.7
% RSD	0.8
95% confidence intervals	
Lower limit	99.9
Upper limit	101.1
Estimated median	100.4
Confidence Level(95.0%)	0.6

Conclusion:

Over the range of 80-120% of the sample concentration, the method yielded a mean recovery of 91.8 %.

8.3 PRECISION:

8.3.1 Intermediate (intra-day) precision:

Mass (g)	Area		Mean	Conc.µg/ml	%
0.8358	194.5	195.2	195	323.5	107.8
0.8466	203.3	190.4	197	322.7	107.6
0.8579	193.7	200.2	197	318.6	106.2
1.0428	238.4	237.9	238	316.9	105.6
1.0138	239.3	225.6	232	318.2	106.1
1.0157	230.6	235.8	233	318.6	106.2
1.2157	289.9	275.3	283	322.6	107.5
1.2235	253.3	308.2	281	318.5	106.2
1.2496	284.7	294.7	290	321.7	107.2

Mean	320.16	106.72
SD	2.31	0.77
RSD %	0.72	0.72

Conclusion:

Precision was satisfactory with a RSD of 0.72 %.

8.3.2 Inter-day precision:

	DAY 1 %	DAY 2 %	DAY 3 %	Inter day
	105.6	101.7	102.0	
	106.1	100.7	102.6	
	106.2	102.8	100.6	
Mean	105.98	101.72	101.73	103.14
SD	0.24	0.86	0.82	2.00
RSD %	0.23	0.85	0.81	1.94

ANOVA single factor statistics:

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	298.820	99.607	0.472
Day 2	3	294.532	98.177	0.105
Day 3	3	300.681	100.227	0.027

ANOVA					
Source of	SS	df	MS	F	P-value
Inter day	6.630	2.0	3.315	16.482	0.004
Intra day	1.207	6.0	0.201		
Total	7.836	8.0			

SS = sum of squares

df = degrees of freedom

MS = mean squares

F = F ratio

Conclusion:

The inter-day variance and the intra-day variance was of the same order as can be seen from the sums of squares. The repeatability is still within acceptable limits, and the assay should perform well, even when executed by other personnel in a different laboratory.

8.4 RUGGEDNESS:

8.4.1 STABILITY OF SAMPLE SOLUTIONS:

A sample was left on the autosampler tray and re-analyzed over several time intervals to determine the sample stability.

Results:

Time (hours)	Peak Area	% Remaining
0	224.6	100.0
1	203.8	90.7
2	207.2	92.3
3	203.3	90.5
4	204.1	90.9
5	221.8	98.8
6	223.3	99.4
7	189.1	84.2
8	198.7	88.5
9	187.5	83.5
10	186.9	83.2
11	222.7	99.2
Mean	210	93.3
SD	11.68	5.20
RSD %	5.57	5.57

Conclusion:

The tea tree oil is stable over a period of 11 hours.

8.4.2 SYSTEM REPEATABILITY:

A sample was injected six times in order to test the repeatability of the peak area as well as the retention time.

Results.

	Area	Retention time (minutes)
	262	7.416
	257	7.464
	258	7.469
	259	7.431
	261	7.402
	260	7.416
Mean	260	7.433
SD	1.71	0.025
RSD %	0.66	0.339

Conclusion:

System performance proved well with RSD values of 0.66 % for peak area and 0.339 % for retention time respectively.

9. CONCLUSION:

The method performed well and should be suitable to analyse salicylic acid and tea tree oil in the products for stability testing, quality control and batch release purposes. No interference were encountered from stressed samples or known related substances, thus the method can be regarded as being stability-indicating.

APPENDIX C

DISSOLUTION RESULTS

Table 1.1 Concentration and amount salicylic acid released from the cream at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	20.5	23.4	20.1	22.5	17.6	14.7	19.8	945.4
7.746	60	28.1	33.6	30.3	33.2	29.0	29.3	30.6	1461.5
10.954	120	35.6	52.5	43.9	48.3	50.5	53.9	47.4	2266.0
13.416	180	42.9	66.0	55.1	57.8	61.9	63.2	57.8	2761.1
15.492	240	49.2	77.2	64.3	68.7	73.9	75.1	68.1	3250.9
17.321	300	53.2	91.9	76.0	76.1	83.1	81.6	77.0	3677.5
18.974	360	57.2	98.8	83.0	82.4	90.9	88.1	83.4	3982.8

Table 1.2 Concentration and amount tea tree oil released from the cream at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	34.7	26.8	24.3	24.6	29.0	28.2	27.9	1334.4
7.746	60	36.9	41.6	35.0	38.5	47.8	45.7	40.9	1954.6
10.954	120	59.1	61.0	60.4	69.0	64.3	66.6	63.4	3028.6
13.416	180	82.2	84.0	89.5	90.4	90.2	79.4	86.0	4105.9
15.492	240	96.3	102.6	99.7	102.4	92.6	88.6	97.0	4635.5
17.321	300	97.1	109.6	109.0	118.4	114.1	90.5	106.4	5084.6
18.974	360	109.4	121.6	123.6	124.1	118.7	102.7	116.7	5574.1

Table 1.3 Concentration and amount salicylic acid released from the cream stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	19.1	22.3	22.2	27.3	24.7	20.1	22.6	1080.2
7.746	60	31.7	37.0	33.5	45.4	38.0	36.2	37.0	1765.1
10.954	120	45.0	50.8	49.6	58.7	54.5	52.7	51.9	2478.4
13.416	180	57.1	61.0	62.3	74.0	75.1	62.9	65.4	3124.3
15.492	240	65.6	69.4	71.4	82.2	79.6	72.9	73.5	3511.9
17.321	300	72.6	76.0	78.4	88.8	89.8	80.5	81.0	3870.2
18.974	360	75.5	82.9	83.5	99.0	94.5	82.4	86.3	4123.0

Table 1.4 Concentration and amount of tea tree oil released from the cream stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	22.9	24.8	28.8	28.0	30.1	34.5	28.2	1346.3
7.746	60	54.4	59.3	46.3	56.5	58.8	58.7	55.7	2658.2
10.954	120	82.0	89.0	79.6	84.3	84.3	86.2	84.2	4024.1
13.416	180	116.5	113.9	112.9	123.1	125.7	115.5	117.9	5633.3
15.492	240	143.7	138.0	132.6	133.9	136.7	128.0	135.5	6472.3
17.321	300	144.9	149.4	143.5	151.1	152.5	131.8	145.5	6950.9
18.974	360	159.2	171.1	174.3	173.7	168.2	158.9	167.6	8004.6

Table 1.5 Concentration and amount salicylic acid released from the cream stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	16.6	15.5	16.2	15.9	17.4	17.8	16.6	791.9
7.746	60	25.5	21.3	22.8	23.0	23.8	24.5	23.5	1122.1
10.954	120	34.8	26.5	33.4	30.3	32.2	33.1	31.7	1515.4
13.416	180	43.3	30.2	39.4	36.7	37.6	40.8	38.0	1815.1
15.492	240	53.5	35.7	46.1	41.4	41.1	44.3	43.7	2086.6
17.321	300	57.1	37.8	48.2	43.2	45.3	48.9	46.7	2232.3
18.974	360	72.6	42.4	51.6	45.6	49.7	54.0	52.7	2515.4

Table 1.6 Concentration and amount tea tree oil released from the cream stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	31.3	35.2	43.3	36.0	30.5	30.7	34.5	1647.0
7.746	60	53.4	53.1	61.5	50.4	46.3	43.0	51.3	2449.3
10.954	120	84.5	73.2	82.6	73.2	74.1	65.6	75.5	3606.9
13.416	180	105.5	99.9	102.9	92.1	82.3	84.4	94.5	4514.3
15.492	240	136.0	105.3	115.4	109.4	96.1	98.4	110.1	5258.7
17.321	300	146.8	119.9	130.1	126.9	103.8	109.3	122.8	5865.3
18.974	360	185.2	136.1	134.2	131.0	112.2	125.4	137.3	6560.1

Table 1.7 Concentration and amount salicylic acid released from the gel at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	32.3	30.1	31.7	30.6	36.1	45.6	34.4	1642.3
7.746	60	51.1	47.6	44.4	50.3	62.1	55.8	51.9	2479.1
10.954	120	75.2	77.7	68.5	67.4	79.2	81.3	74.9	3577.7
13.416	180	111.8	90.5	88.4	90.1	96.8	101.7	96.6	4611.9
15.492	240	113.7	109.2	103.5	109.2	117.2	118.7	111.9	5345.2
17.321	300	126.5	122.2	124.4	118.5	132.0	143.9	127.9	6110.3
18.974	360	136.7	134.1	130.7	129.9	147.6	146.3	137.6	6570.3

Table 1.8 Concentration and amount tea tree oil released from the gel at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	61.9	64.5	64.8	68.0	70.7	86.6	69.4	3316.1
7.746	60	105.5	103.6	97.1	104.7	111.6	113.7	106.0	5065.1
10.954	120	168.5	165.7	146.5	154.4	173.7	166.6	162.6	7764.5
13.416	180	203.5	195.7	184.4	193.2	205.9	209.1	198.6	9487.7
15.492	240	246.2	226.8	218.0	227.3	238.7	239.5	232.7	11117.6
17.321	300	267.8	266.0	255.3	262.3	268.8	277.8	266.3	12721.8
18.974	360	286.3	282.5	301.3	284.3	299.1	299.6	292.2	13956.3

Table 1.9 Concentration and amount salicylic acid released from the gel stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	28.7	27.2	30.6	30.7	28.9	35.4	30.2	1444.7
7.746	60	51.4	46.1	49.9	45.2	46.5	48.1	47.9	2286.0
10.954	120	71.6	80.5	77.2	68.2	70.5	69.8	72.9	3484.5
13.416	180	87.2	78.7	89.9	84.3	85.2	87.7	85.5	4084.6
15.492	240	102.7	96.2	115.6	99.0	100.6	106.7	103.5	4941.9
17.321	300	114.9	105.8	125.0	114.1	114.7	114.1	114.8	5482.3
18.974	360	137.0	123.6	138.8	129.5	130.8	131.8	131.9	6300.7

Table 1.10 Concentration and amount tea tree oil released from the gel stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	65.8	58.9	70.4	74.4	65.1	63.9	66.4	3172.0
7.746	60	109.3	92.2	110.7	114.0	104.0	102.7	105.5	5039.7
10.954	120	163.3	144.8	170.8	166.7	157.7	161.8	160.9	7683.4
13.416	180	201.1	179.7	207.9	194.5	192.5	189.9	194.3	9279.7
15.492	240	255.0	211.5	259.4	243.6	238.7	232.6	240.2	11471.2
17.321	300	268.9	241.3	276.3	265.9	251.7	264.9	261.5	12490.3
18.974	360	294.8	266.3	323.2	289.6	306.0	293.4	295.5	14117.2

Table 1.11 Concentration and amount salicylic acid released from the gel stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	29.0	30.1	29.8	29.2	37.7	32.1	31.3	1496.0
7.746	60	58.2	45.2	52.5	46.1	51.9	46.0	50.0	2387.4
10.954	120	66.7	72.8	67.2	72.7	81.6	78.9	73.3	3502.4
13.416	180	86.4	95.9	83.0	87.5	86.4	92.9	88.7	4236.0
15.492	240	101.1	100.2	99.8	101.8	109.6	111.6	104.0	4969.3
17.321	300	114.6	119.3	112.3	132.3	125.7	126.6	121.8	5817.2
18.974	360	129.9	130.5	138.1	137.6	134.6	134.3	134.2	6408.2

Table 1.12 Concentration and amount tea tree oil released from the gel stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	84.6	82.9	87.6	84.0	84.0	80.5	83.9	4010.0
7.746	60	140.7	131.1	134.2	145.5	145.5	127.2	137.4	6561.1
10.954	120	200.6	190.3	214.4	208.3	205.5	197.2	202.7	9682.6
13.416	180	252.3	240.7	246.0	263.6	254.7	249.8	251.2	11998.6
15.492	240	289.0	299.6	296.6	309.4	298.0	287.4	296.7	14170.5
17.321	300	324.0	345.4	342.1	366.9	346.6	344.9	345.0	16478.8
18.974	360	391.1	359.0	375.4	389.3	417.7	356.1	381.4	18219.7

Table 1.13 Concentration and amount of salicylic acid released from the ointment at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	23.9	32.7	20.5	21.6	18.0	36.2	25.5	1216.7
7.746	60	45.8	52.6	46.1	48.5	35.2	72.7	50.2	2396.3
10.954	120	79.0	82.3	68.0	55.2	51.5	92.2	92.8	4431.9
13.416	180	90.3	138.2	106.0	91.1	91.1	147.0	110.6	5283.8
15.492	240	104.3	157.6	126.4	96.9	99.4	152.2	133.3	6365.5
17.321	300	136.6	147.7	147.4	119.2	100.2	197.2	151.9	7255.0
18.974	360	182.5	197.5	149.2	148.1	166.9	217.7	177.0	8453.9

Table 1.14 Concentration and amount of tea tree oil released form the ointment at 0 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	56.2	63.1	37.9	47.7	33.3	58.0	49.4	2357.3
7.746	60	81.0	118.2	85.7	71.2	64.3	119.2	89.9	4296.3
10.954	120	129.8	154.2	104.2	127.8	89.5	144.6	133.2	6362.2
13.416	180	147.5	272.0	191.0	162.7	151.7	245.4	195.0	9316.8
15.492	240	173.6	292.7	241.2	199.5	196.6	238.5	223.7	10684.5
17.321	300	195.5	247.5	227.2	183.9	147.9	194.6	253.0	12083.9
18.974	360	212.9	304.1	218.2	264.2	254.8	440.0	282.4	13487.6

Table 1.15 Concentration and amount salicylic acid released from the ointment stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	19.3	17.8	19.5	20.5	20.5	16.5	19.0	908.6
7.746	60	40.3	40.4	40.3	32.2	27.7	41.1	37.0	1766.9
10.954	120	65.6	71.1	71.9	56.9	66.3	82.3	78.7	3758.9
13.416	180	84.6	66.6	102.6	83.8	79.5	79.5	96.6	4615.4
15.492	240	123.5	98.0	110.4	89.0	102.5	249.0	113.0	5398.7
17.321	300	121.3	110.6	138.7	155.6	127.2	151.3	128.8	6153.2
18.974	360	172.7	123.2	162.5	152.6	144.2	141.6	149.5	7140.3

Table 1.16 Concentration and amount tea tree oil released from the ointment stored at 25°C+60%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	87.7	160.5	98.2	85.0	83.0	67.7	97.0	4634.9
7.746	60	181.1	183.2	173.1	123.8	96.4	131.0	148.1	7074.7
10.954	120	235.1	250.1	250.1	191.6	200.5	265.0	219.3	10476.6
13.416	180	290.9	229.2	321.1	246.2	273.3	264.7	270.9	12940.1
15.492	240	422.5	327.9	366.1	299.9	370.9	410.6	366.3	17499.0
17.321	300	348.8	355.7	425.2	437.8	503.1	460.4	416.6	19898.4
18.974	360	547.0	358.1	485.2	459.8	413.0	425.2	448.1	21402.2

Table 1.17 Concentration and amount salicylic acid released from the ointment stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	19.5	14.2	25.9	20.0	31.9	30.2	23.6	1127.5
7.746	60	38.8	32.7	50.6	43.7	59.2	42.1	44.5	2125.0
10.954	120	73.3	50.8	90.4	65.0	103.2	72.6	86.9	4151.2
13.416	180	93.6	70.5	126.3	106.3	112.6	98.0	106.7	5097.2
15.492	240	133.6	97.1	137.9	123.0	129.0	130.6	124.8	5962.2
17.321	300	134.8	109.6	177.2	134.5	164.8	150.2	142.3	6795.4
18.974	360	157.2	134.6	168.6	154.7	167.0	171.8	159.0	7593.9

Table 1.18 Concentration and amount tea tree oil released from the ointment stored at 40°C+75%RH after 3 months

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	Avg µg/ml	Amount (µg/cm²)
5.477	30	50.9	34.0	55.8	48.3	66.9	73.6	54.9	2623.8
7.746	60	91.4	79.3	115.7	90.4	97.1	88.3	93.7	4475.0
10.954	120	176.2	122.6	196.6	185.1	262.3	157.1	129.5	6184.5
13.416	180	231.6	167.0	278.0	196.8	273.2	236.0	202.0	9651.1
15.492	240	316.9	223.4	299.3	246.3	275.2	294.0	231.5	11056.3
17.321	300	331.3	222.3	455.7	281.5	287.0	256.3	245.9	11746.3
18.974	360	247.7	184.8	274.4	254.0	294.7	290.5	257.7	12308.6

Cosmetic products for the treatment of acne containing Tea tree oil and Salicylic acid

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Abstract

Acne is a disease that 80% of adolescents and young adults have. For most of the people acne causes associated problems with self-esteem and social inhibition. There are four abnormalities in acne namely, sebum production, inflammation, hyperkeratosis and the presence of *Propionobacterium acnes*. To treat acne effectively it is proven that combinational therapy is essential to be able to eliminate all four abnormalities. Therefore salicylic acid (2%) and tea tree oil (3%) were chosen to be formulated in one cosmetic acne product. Both these active ingredients have the properties to eliminate the four abnormalities of acne. Five different acne products, i.e. a cream, gel, ointment, soap bar and a cover stick were formulated and then placed under three months accelerated stability conditions. Stability indicating tests proved that the products remained stable and could be used to treat acne effectively.

Introduction

The pathogenesis of acne vulgaris is due to many factors. Acne vulgaris is a medical condition that begins in pilosebaceous units. These units consist of sebaceous glands and a single hair follicle. The sebaceous glands are continuously producing a clear, oily liquid called sebum [1]. Subsequently keratinisation with hyperkeratosis of the epithelium in the follicle leads to obstruction by a horny plug. The blocked duct consists of sebum and keratinous debris, forming non-inflammatory lesions [2]. A lesion becomes inflamed because the excess sebum provides an anaerobic growth medium for *Propionobacterium acnes* (a Gram-positive bacteria), which is responsible for the metabolism of fatty acids from triglycerides that is present in the sebum [1], [2], [3].

There are consequently four abnormalities found in acne, namely sebum production, keratinisation of the follicle, presence of *P.acnes* populations and inflammation [2]. The formulation of a product that would be able to eliminate these four abnormalities, would eliminate acne.

Salicylic acid and tea tree oil consists of the properties to eliminate these four factors. Salicylic acid is comedolytic because of its lipophilic nature. It promotes desquamation and accelerates the resolution of inflammatory lesions [2]. Tea tree oil is a broad spectrum antimicrobial, antiseptic, a mild anti-inflammatory and analgesic [4].

In this study five formulated products, combining tea tree oil and salicylic acid was made. A cream, gel, ointment, cover stick, and soap bar was formulated. Each contained 2% salicylic acid and 3% tea tree oil.

Experimental

The five acne products were formulated and placed under different temperatures and relative humidities (RH). The products were placed at 5°C, 25°C + 60% RH and 40°C +75%RH for three months. Stability indicating tests were done in order to determine the products stability. Tests for example pH, relative density, viscosity, spreadability and penetration were done. Release studies and concentration assays were also submitted.

Assay

The concentration assays of the five formulated products were conducted on the initial products as well as the products stored at the different temperatures and at the different time periods.

Materials and equipment

A Agilent 1100 series HPLC equipped with a gradient pump, autosampler, UV detector and chemstation revision A.06.02 data acquisition and analysis software was used, with a Luna C18(2) 150x4.6 mm, 5 µm column and a mobile phase of acetonitrile/water with the pH adjusted to 2.5 with phosphoric acid, at a flow rate of 1.0 ml/min and injection volume of 10 µl, and UV detection at 220 nm. The gradient was set at 45% acetonitrile to 1.5 minutes, then to 100 % after 4 minutes. The retention time was approximately 3.5 and 9.9 minutes for salicylic acid and tea tree oil respectively.

Standard preparation

Standards were prepared by dissolving 20 mg salicylic acid and 30 mg tea tree oil in 100 ml of solvent. The solvent consisted of 30 % THF and 70% Methanol.

Release studies (Dissolution tests)

The dissolution tests of the five formulated products were conducted on the initial products as well as the products stored at the different temperatures for three months.

Materials and equipment

The release of the two active ingredients, salicylic acid and tea tree oil, was carried out with the enhancer cell using a VanKel 7000 with 200 ml flasks and small paddles. A cellulose acetate membrane with a 0.45 µm pore size and a surface area of 3.977679 cm^2 was used. The enhancer cells were dropped into the flasks containing a 85 % ethanol dissolution medium. The temperature was set at 32°C with a stirring speed of 200 rpm. A six hour dissolution were carried out and 200 µl of test sample were withdrawn at 30, 60, 90, 120, 180, 240,300 and 360 minutes and transferred to HPLC vials for analysis. Standards were prepared as for the assay.

Results and discussion

Cream: Assay Results

Table I: Concentration (%) salicylic acid in the cream over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	108.7	-	-
25°C+60%RH	105.9	103.2	105.3	104.5
40°C+75%RH	-	100.1	107.2	106.2

Table II: Concentration (%) tea tree oil in the cream over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	101.5	-	-
25°C+60%RH	105.5	102.6	103.5	100.2
40°C+75%RH	-	100.5	100.4	101.2

Cream: Release rate results

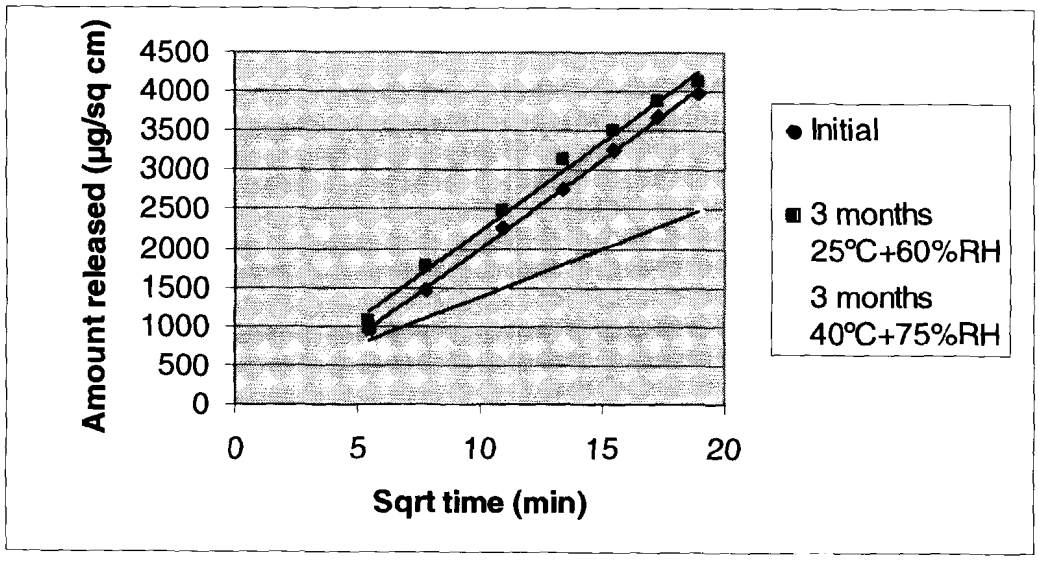

Figure 1: Amount of salicylic acid released from cream

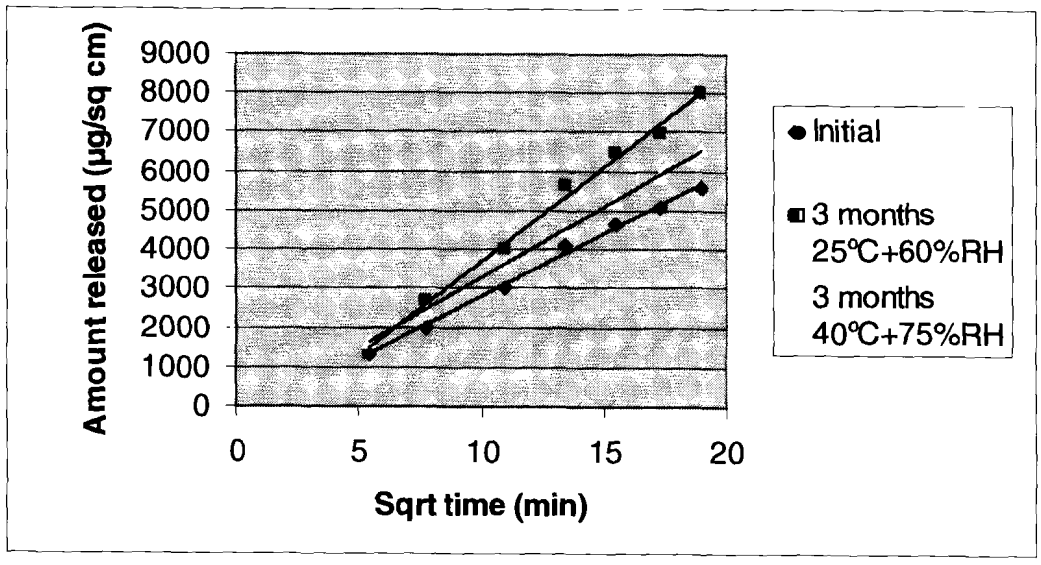

Figure 2: Amount of tea tree oil released from the cream

Gel: Assay results

Table III: Concentration (%) salicylic acid in the gel over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	109.5	-	-
25°C+60%RH	102.4	102.8	99.6	102.1
40°C+75%RH	-	101.6	102.2	101.2

Table IV: Concentration (%) tea tree oil in the gel over three months

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	107.33	-	-
25°C+60%RH	102.18	100.60	100.37	99.59
40°C+75%RH	-	99.11	101.35	102.15

Gel: Release rate results

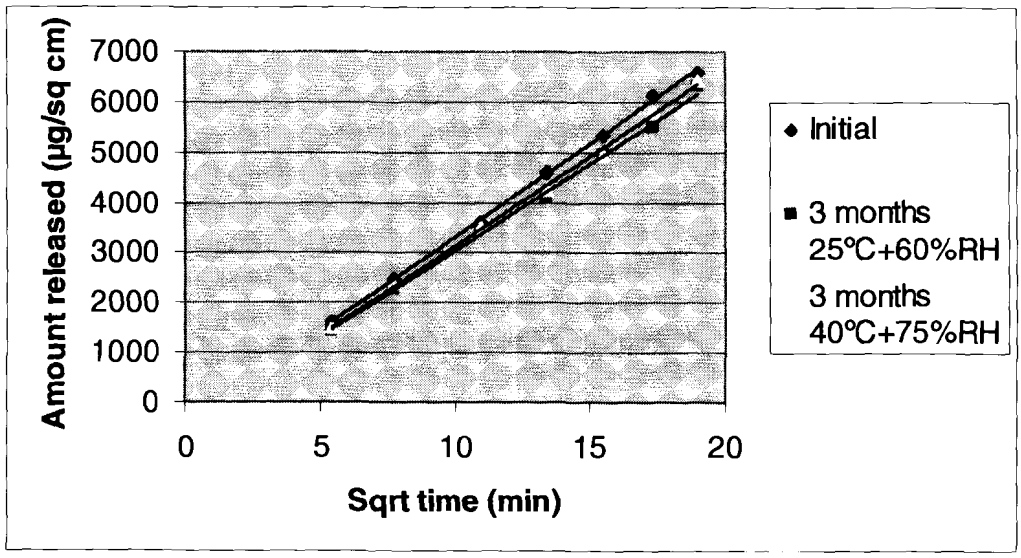

Figure 3: Amount of salicylic acid released from the gel

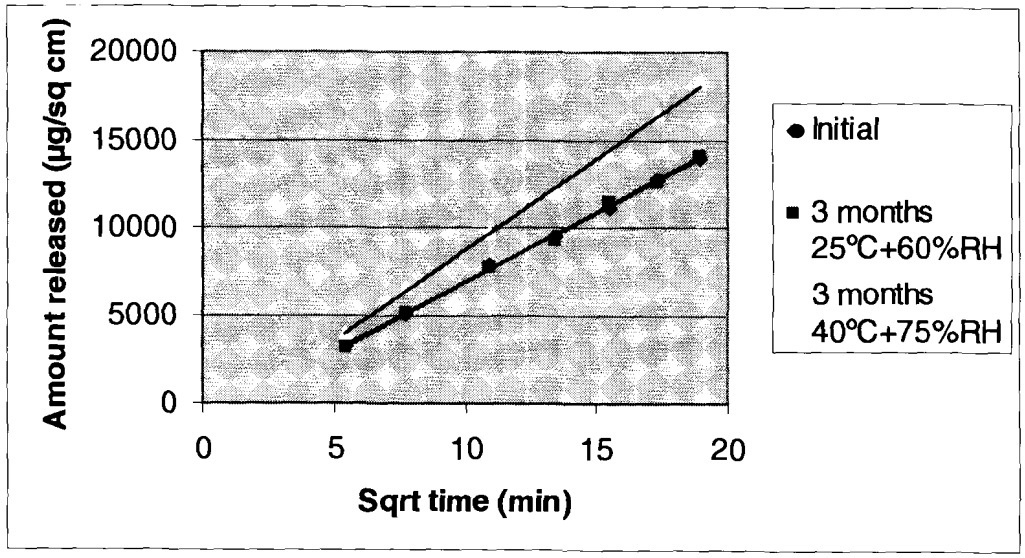

Figure 4: Amount of tea tree oil released from the gel.

Ointment: Assay results

Table V: Concentration (%) salicylic acid in the ointment

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	104.2	-	-
25°C+60%RH	107.0	102.3	101.5	104.4
40°C+75%RH	-	106.8	101.3	101.7

Table VI: Concentration (%) tea tree oil in the ointment

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	101.9	-	-
25°C+60%RH	102.4	99.0	101.1	96.9
40°C+75%RH	-	101.5	100.9	100.9

Ointment: Release rate results

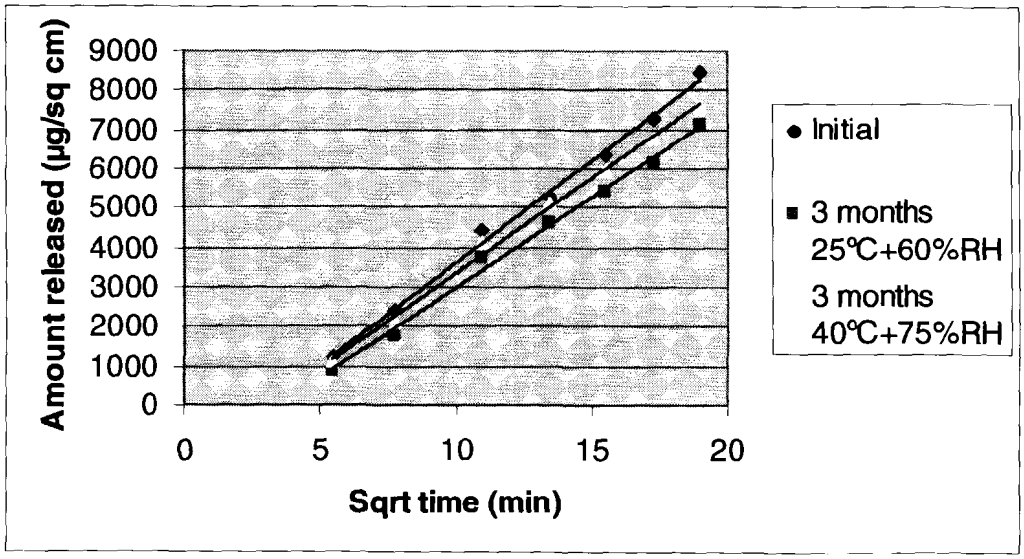

Figure 5: Amount of salicylic acid released from the ointment

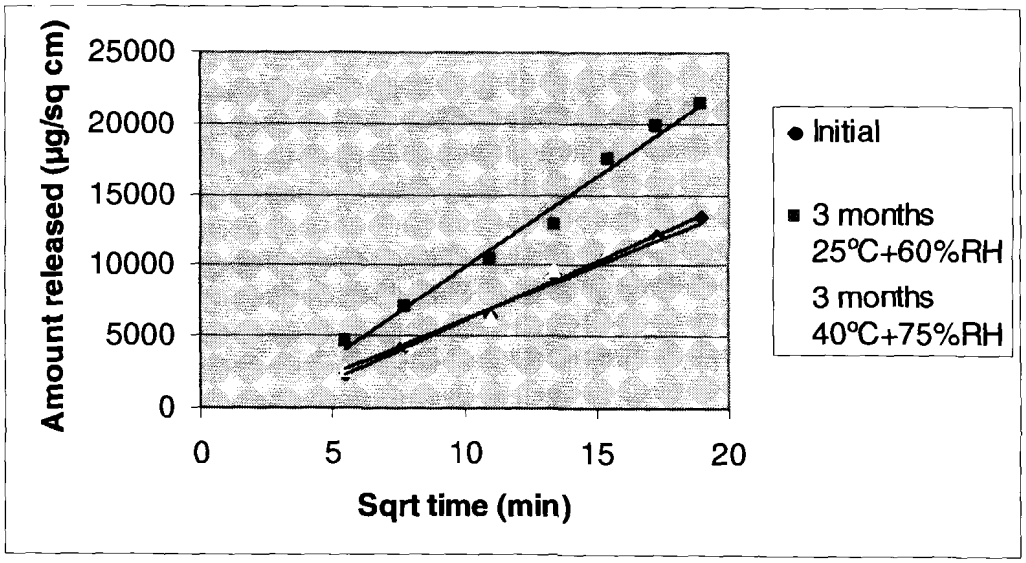

Figure 6: Amount of tea tree oil released from the ointment

Soap bar: Assay results

Table VII: Concentration (%) salicylic acid in the soap bar

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	107.7	-	-
25°C+60%RH	104.9	101.1	103.0	103.2
40°C+75%RH	-	102.6	101.3	100.5

Table VIII: Concentration (%) of tea tree oil in the soap bar

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.2	-	-
25°C+60%RH	100.9	102.7	99.2	98.3
40°C+75%RH	-	100.2	104.6	97.1

Cover stick: Assay results

Table IX: Concentration (%) salicylic acid in the cover stick

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.5	-	-
25°C+60%RH	102.1	98.7	102.1	98.4
40°C+75%RH	-	101.9	103.3	98.3

Table X: Concentration (%) tea tree oil in the cover stick

TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C	-	103.5	-	-
25°C+60%RH	102.1	98.7	102.1	98.4
40°C+75%RH	-	101.9	103.3	98.3

The concentration assays of the two active ingredients in all five products did not show any significant change and remained within the acceptable limits of 90-110%.

According to the release studies, the gel released the highest amount of salicylic acid in comparison to the cream and the ointment. A reason for this phenomenon could be that during the formulation the salicylic acid dissolved completely in the alcohol of the gel formulation and therefore could be released easier than in a product that contained no alcohol for example the cream and the ointment.

The ointment released the highest amount of tea tree oil. Again this could be explained by the solubility properties of tea tree oil. The formulation of the ointment contained no water with high concentrations of lipophilic ingredients and therefore the incorporation of tea tree

oil was appropriate. This allowed the tea tree oil to be released easier than in a formulation that contained water like for example the cream and the gel.

The pH and the relative density of the cream, gel and ointment stayed the same over the three months stability period. The viscosity of the cream increased slightly over the three months, whereas the viscosity of the gel and the ointment decreased slightly. Due to the inverse relationship between spreadability, penetration and viscosity, the spreadability and penetration of the cream decreased over the three months. The spreadability and penetration of the gel and ointment increased over time. But these changes are very small and therefore all the products remained stable over the three months stability period.

Conclusion

The aims of this study were to formulate five acne products that consisted of high quality and remained stable in accelerated stability testing procedures. Combinational therapy is the key factor when treating acne successfully. Therefore the formulations consisted of two active ingredients each with its own mechanism of action for the elimination of acne. The concentration of these active ingredients was of such a nature that it would not cause irritations but still act as a medicament. Because of the scars and redness that acne can cause, the cover stick formulation was intended to beautify and act as a medicament simultaneously.

The acne products was of high quality and remained stable throughout the study, thus the aims were reached and these products can successfully be implemented for acne treatment.

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