THE STABILISING EFFECT OF METYLSULFONYLMETHANE (MSM) ON UREA CONTAINING FORMULATIONS

Johanna Margaretha Botha

B. Pharm

Dissertation submitted for partial fulfilment of the requirements for the degree

Magister Scientiae (Pharmaceutics) in the Department of Pharmaceutics, School of Pharmacy at the North-West University,Potchefstroom Campus.

Supervisor: Dr. E. Swanepoel

Co-supervisor: Prof. A.P. Lötter

POTCHEFSTROOM

2004

To all the people who helped me to become the person I am today

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to many people who have assisted me in various ways throughout my research.

Above all, I thank our loving Father in Heaven for giving me strength and wonderful opportunities in life, and for guiding me every step of the way. Thank you Lord for the endurance and ability to complete may study.

- Dr. Erna Swanepoel, for being a supervisor who taught me a lot, for her guidance and support. She was and is a great role model to me.
- Prof. A.P. Lötter, for his genius help and support throughout this study, and especially with the formulation of my products.
- Prof. Wilna Liebenberg, for believing in me from the start, and for her encouragement, interest and friendship.
- Mrs. Rianda Ganz, for her assistance with the GC, as well as her interest and support in my project.
- Mrs. Susan May, for her help with the viscosity.
- Personnel at the Research Institute for Industrial Pharmacy, for the chance to gain experience and for the kindness and laughter while working in the laboratories.
- My mom, for believing so much in me to give me the opportunity to study, for encouragement, love, support and a lot of prayers.
- My sister, for all her love and support.
- My soul mate, Dewald, for his continuous love, inspiration, encouragement, support and help throughout my study.

ABSTRACT

The skin is the largest area of the body that interfaces with bacteria and "problems" from the environment and is the first line of defence for the body. There are, of course, immune system functions that take place within the skin area and it would be nourished only by the very small arteries (capillaries). So the blood supply at the surface of the skin is not as great as it is further inside the body. When you can apply something on the skin, which nourishes the immune system cells, such as MSM (methylsulfonylmethane) and urea you are doing wonders in improving the health of the skin. The use of urea in dermatological therapy and cosmetics has become more and more important in recent years. However, urea is unstable in formulations, decomposing into carbon dioxide and ammonia. Products containing urea can be stabilised by including MSM (methylsulfonylmethane) in the formulation (Herschler, 1981:1).

The aim of this study was to determine the stabilising effect of MSM on urea in cosmetic products. Cosmetic products that were formulated with MSM and urea as actives, comprised of a moisturising lotion, massage cream, foot and heel balm, hair gel, hair spray and lip ice. The formulations were subjected to stability studies for three months under ICH conditions.

The formulated products were stored at 5°C, 25°C/60%RH and 40°C/75%RH. The strength of MSM and urea in each formulation was 5% m/m, except for the hair spray and lip ice where the concentrations were 10% m/m and 2.5% m/m respectively. HPLC analysis was used to determine the urea concentrations in all the formulated products, as well as the concentration released during membrane release studies. GC analysis was used to determine the MSM concentrations in the products. Other stability tests included appearance, spreadability, pH, relative density, penetration, viscosity, preservative content and preservative efficacy.

Chapter 1 gives a literature overview of the uses and importance of cosmetics. Chapter 2 is a literature overview of the skin and the properties and uses of urea and MSM. Chapter 3 deals with the formulation of urea and MSM products and the chemicals that have been used. Chapter 4 described the methods used for accelerated stability testing. Chapter 5-10 finally deals with the results obtained and the conclusions that were made.

The test results showed the following: There was a prominent change in the pH of all the formulated products; this can be due to decomposition of urea into carbon dioxide and ammonia (Beiersdorf, 2003:1). Higher temperature and moisture increase this decomposition and cause the pH to increase to values as high as 11 (Anon, 2003:20).

The viscosity, spreadability, penetration, relative density and appearance of the products remained more or less the same over three months, except for the hair spray where crystals formed in the product that was stored at 5°C for 1 month due to the menthol in the formulation. The urea and MSM content decreased with time. The lip ice formulation showed concentrations as low as 30% due to poor solubility of the urea and MSM crystals in the waxes of the lip ice formulation. HPLC analysis of the preservatives confirmed their stability in the formulated products. The preservative efficacy results proved that the products were sufficiently protected from microbial contamination.

The release study indicated that urea is released at a steady rate from the preparations tested. Urea was released from the moisturising lotion after 120 minutes. In general, the release is influenced by the viscosity of the medium and should be faster from the gel than from the creams or lotion (Shah *et al.*, 1991:55).

In conclusion it can be said that urea and MSM were successfully formulated into the six cosmetic products except for the lip ice, which showed low, unstable concentrations of the actives due to poor solubility of the actives in the waxes of the lip ice formulation. By comparing the results of Claasen, (2003:63) and the results optained in this study for the hair gel and foot and heel balm, it looks as if MSM had a stabilising effect on urea, by lowering the decrease of urea concentrations over time.

UITTREKSEL

Die vel is die grootste oppervlak van die liggaam wat in aanraking is met bakterieë en omgewingsfaktore, en dien dus as die grootste vorm van weerstand vir die liggaam teen hierdie gevare. Dit word immuunsisteemfunksies genoem, wat in die veloppervlak plaasvind. Bloedvoorsiening na die veloppervak is baie minder as in die res van die liggaam, omdat die vel deur klein kappillere vaatjies van bloed voorsien word. Wanneer die bloedvoorsiening na die vel verhoog word, deur die aanwending van metielsulfonielmetaan (MSM) en ureum, word die immuunsisteemselle gestimuleer en verbeter dit die veltoestand. Die gebruik van ureum in dermatologiese behandelings en kosmetiek word deesdae al meer belangrik. Ureum is baie onstabiel in formulerings, a.g.v die afbraak na koolstofdioksied en ammoniak, maar dit kan gekombinneer word met metielsulfonielmetaan (MSM) om die stabiliteit te verhoog (Herschler, 1981:1).

Die doel van die studie was om die stabiliseringseffek van MSM op ureum in kosmetiese produkte te bepaal. Kosmetiese produkte wat geformuleer is met MSM en ureum as aktiewe bestandele was: masseringsroom, haarjel, haarsproei, voet- en hakbalsem, lipsalf en 'n bevogtigingsroom. Die formulerings was onderhewig aan 'n stabiliteitstudie vir 'n periode van 3 maande, onder ICH (versnelde stabiliteitstoetse) kondisies by verskillende temperature nl. 5°C, 25°C/60%RH en 40°C/75%RH.

Die konsentrasiesterkte van MSM en ureum in elke formulering was 5% m/m, behalwe in die haarsproei en lipsalf, waar dit 10% m/m en 2.5% m/m respektiewelik was. HPLC - analise was gebruik om die konsentrasie ureum in die formulerings te bepaal, asook die vrystelling gedurende membraanvrystellingstudies. GC - analise was gebruik om die konsentrasie MSM in die formulerings te bepaal. Die res van die stabiliteitstoetse wat op die formulerings van toepassing was sluit in: pH, relatiewe digtheid, viskositeit, fisiese voorkoms, penetrasie, spreibaarheid, analise van preserveermiddels en preserveermiddeleffektiwiteit.

Hoofstuk 1 gee 'n literatuuroorsig van die gebruike en belangrikheid van kosmetiese produkte. Hoofstuk 2 is 'n literatuuroorsig van die vel en die eienskappe en gebruike van ureum en MSM. Hoofstuk 3 handel oor die formulering van die ureum en MSM bevattende produkte en die chemikalieë wat gebruik was. Hoofstuk 4 verduidelik die metodes wat gebruik was vir die versnelde stabiliteitstoetse. Hoofstuk 5-10 handel oor die resultate wat verkry is en die gevolgtrekkings wat gemaak is.

Die resultate van die toetse was soos volg: Daar was 'n prominente verandering in die pH van al die produkte wat geformuleer was, as gevolg van die afbraak van ureum na koolstofdioksied en ammoniak (Beiersdorf, 2003:1). Hoër temperature en humiditeit verhoog die afbraak en veroorsaak dat die pH na waardes so hoog as 11 kan toeneem (Anon, 2003:20).

Die viskositeit, relatiewe digtheid, penetrasie, spreibaarheid en voorkoms van die produkte het min of meer dieselfde gebly oor die drie maande, behalwe vir die haarsproei waar kristalle gevorm het in die monster wat vir 1 maand by 5°C gestoor was. Dit kan toegeskryf word aan die mentol in die formulering wat uitkristalleer. Die MSM en ureum konsentrasies het met tyd afgeneem, veral in die produkte wat by 40°C/75%RH gestoor was. Die lipsalf formulering het MSM konsentrasies so laag as 30% getoon a.g.v. swak oplosbaarheid in die was van die lipsalf formulering. HPLC analise van die preserveermiddels het hul stabiliteit in die geformuleerde produkte bevestig. Die preserveermiddeleffektiwiteit resultate het bewys dat die produkte genoegsaam beskerm was teen mikrobiologiese kontaminasie.

Die vrystellingstudie het getoon dat ureum teen 'n konstante tempo uit die produkte wat getoets was, vrygestel is. Ureumvrystelling uit die bevogtigingsroom was eers na 120 minute waargeneem. In die algemeen word die vrystelling geaffekteer deur die viskositeit van die medium en dit behoort vinniger vanuit die jel as vanuit die rome te wees (Shah *et al.*, 1991:55).

Die gevolgtrekking kan dus gemaak word dat ureum en MSM suksesvol in die ses kosmetiese produkte geformuleer was, behalwe vir die lipsalf wat lae onstabiele konsentrasies van die aktiewe getoon het. Dit wil voorkom asof MSM wel 'n stabiliserende effek op ureum gehad

het as die resultate van die haarjel en voet- en hak balsem in Claasen, (2003:63) met die resultate in die studie vergelyk word. Daar was 'n vermindering in die konsentrasie afname van ureum oor 'n drie maande tydperk.

AIM AND OBJECTIVES

The aim of this study was to develop different stable urea and MSM containing topical formulations for use as cosmetic products. The stability of urea and is somewhat of a problem in water-containing formulas that are stored for a long time because urea can decompose into carbon dioxide and ammonia (Beiersdorf, 2003:1). However, urea compositions can be stabilized when they contain Methylsulfonylmethane, MSM (Herschler, 1981:1).

The main objectives of this study included:

- To formulate a foot and heel balm, massage cream, moisturising lotion, hair gel, hair spray and lip ice, containing urea and MSM.
- To subject the urea and MSM containing formulations to accelerated stability indicating studies for three months under ICH conditions.
- To analyse urea by means of a stability indicating HPLC method.
- To analyse MSM by means of a stability indicating GC method.
- The physical and chemical evaluation of these products as required by the South African Medicines Control Council (2003:21-23).
- To determine the release of urea by means of membrane release studies.
- The preservative efficacy testing of these products.
- To analyse the preservative content of the products by means of a stability indicating HPLC method.

CHAPTER 1

PURPOSE, MEANING AND FUNCTION OF COSMETICS

1 PURPOSE OF COSMETICS

Cosmetics are becoming of more importance in daily life; they are used regularly by increasing numbers of people and very large quantities are consumed each year. When did people first use cosmetics? Even if we examine the history of cosmetics, it is extremely difficult to say when cosmetics were first used. Archaeological excavations confirm that they were used in the early Stone Age and we can safely assume that cosmetics have a very long history (Mitsui, 1997:3).

Why did early societies use cosmetics? If we examine the purpose of cosmetics, the most obvious is protection of the body from elements of nature, such as heat and sunlight. Early people painted themselves with oils or mixtures of oils, clays and plant materials to protect themselves against dryness from cold, burns from strong sunlight, and irritation from insect bites. Additionally, cosmetics were used for religious purposes. Fragrant woods for example were burnt to produce smoke and incense that would ward off evil spirits. Further protection was afforded to an individual by painting the body to guard against evil (Mitsui, 1997:3).

As societies came into the age of enlightenment, however, most of these purposes of cosmetics disappeared. The main purposes for using cosmetics in modern society are for personal hygiene, to enhance attractiveness through use of makeup, to improve selfesteem and promote tranquillity, to protect skin and hair from damaging ultraviolet light, pollutants, and other environmental factors, to prevent aging, and in general to help people enjoy a more full and rewarding life (Mitsui, 1997:3).

1.1 MEANING OF COSMETICS

How do we define cosmetics? The definition of cosmetics under the law varies slightly between countries but in general terms "cosmetics" means any article intended to be used by means of rubbing, sprinkling or by similar application to the human body for cleaning, beautifying, promoting attractiveness, altering the appearance of the human body, and for maintaining health of the skin and hair, provided that the action of the article on the human body is mild (Mitsui, 1997:4).

The current Pharmaceutical Affairs Law makes a clear distinction between cosmetics, and pharmaceuticals. Cosmetics are used by healthy people to maintain personal hygiene and to maintain a favourable personal appearance. Consequently the physiological activities of cosmetics must be mild. In contrast, pharmaceutical drugs are used for treatment and prevention of illness and they have an effect on the structure and functions of the body (Mitsui, 1997:4).

Since cosmetics are often used on a daily basis over long periods of time, safety without side effects is of paramount importance. By contrast, pharmaceutical drugs for medical use are used only over short time periods to treat medical conditions. Their primary purpose is to cure illness, and they must therefore be therapeutically effective. Sometimes slight side effects of these drugs cannot be avoided (Mitsui, 1997:4).

1.2 CLASSIFICATION

Cosmetics can be classified according to their use and area of application. In addition, they can be classified by composition and structure. However, based on usage, cosmetics can be classified into skin care cosmetics, makeup cosmetics, body cosmetics, hair care cosmetics, oral cosmetics and fragrances.

Skin care cosmetics are called facial cosmetics and they are mainly used on the face. There are three main usage purposes: cleansing, skin balance, and protection. Makeup cosmetics are mainly used on the face. Other makeup cosmetics include nail enamel. Face makeup are divided into base makeup (foundations) and point makeup (lipstick) (Mitsui, 1997:4).

Body cosmetics include sun care and suntan cosmetics, antiperspirants, deodorants, hair remover, bleaches, depilatories, soaps, hand care products and bath preparations. A special product in the body cosmetics group is insect repellents. Hair care cosmetics include shampoos, treatments, and hair styling preparations as well as permanent wave agents and hair dyes. Other products in the group include hair growth promoters and scalp treatment.

Oral care cosmetics primarily include toothpastes and products in the group such as mouthwashes. Fragrances are mainly used on the body but sometimes on the scalp hair and earlobes. Typical fragrance cosmetics are perfumes, but there are also eau de colognes made by varying the amount of fragrance used (Mitsui, 1997:4).

1.3 COSMETICS AND DELIVERY SYSTEMS

In the formulation of cosmetic products, active ingredients are combined with a variety of other compounds that give the product its physical form and may control the delivery of the active ingredient. By far the most conventional and widely used cosmetic delivery system is the oil – water emulsion. Most cosmetic creams and lotions on the market today are emulsions.

The carrier of the system can affect the delivery of active compounds by a number of different means, such as interacting with the active agent, controlling the rate of release from the vehicle, altering stratum corneum resistance, or enhancing stratum corneum hydration. Permeation enhancers may be incorporated in the system to increase the skin delivery of the active agent (Magdassi & Touitou, 1999:1).

1.4 QUALITY CHARACTERISTICS AND QUALITY ASSURANCE OF COSMETICS

1.4.1 Quality characteristics

Generally, "quality" is determined by the satisfaction of the user. In the industrial situation, quality is determined at three points: 1) design, 2) manufacture and 3) sales. From each point, there are requirements necessary to satisfy the high quality characteristics. Economics and market timing are also important factors to consider. When designing, manufacturing and marketing cosmetics, the basic quality requirements are safety, stability, efficacy, and usability; usability includes preference factors such as smell, colour, and package design, which are determined by the user's personal taste (Mitsui, 1997:5).

1.4.2 Quality assurance

The definition of quality assurance is given as: guaranteeing product quality to ensure full customer confidence and satisfaction when using the product. In addition, the quality must permit long-term usage. This definition indicates the importance of quality assurance in quality control systems for economically manufacturing products of a quality that meets user requirements. In addition, the definition shows that the cosmetics industry has a responsibility to protect the safety of the consumer (Mitsui, 1997:7).

1.5 SCIENTIFIC BACKGROUND, TECHNOLOGY AND ITS FUTURE

Until about 1970, the main interest of research was upon aspects of the product. The focus was on product stability, fine texture, and good after feel, manufacturing technology and quality control. Colloid science, rheology and statistics were the major areas of research. The first oil crisis in 1973 led to a period of low growth of cosmetics, and compatibility between user and product and the safety of cosmetic products became the primary interests of research.

In the 1980's, this trend became more evident. In addition to the problem of safety, product usefulness, that is what the product can do for a human, became the main research goal. In addition to scientific disciplines centred on products, research is increasingly being conducted into a wider range of human - related subjects including dermatology, physiology, biology, biochemistry, and pharmacology. It is evident that the development of cosmetics is a human science that combines both the technological aspects of producing a product, and physiological (peacefulness, happiness, relaxation) and psychological needs of the user. In the future, even more cutting-edge technology will be brought to bear upon the technological aspects of producing products, so that products with even better performance will be developed (Mitsui, 1997:9)

1.6 CONCLUSION

This chapter was a breath background of the meaning, function and purpose of cosmetics in our modern world. Cosmetic science covers the field from natural science to human and social science, making it an important element to research.

CHAPTER 2

PHYSICO-CHEMICAL PROPERTIES AND USES OF UREA AND MSM

2 INTRODUCTION

In order to formulate effective and safe skin care products, it is important to gain knowledge about the target area for intended use, the active ingredient that is used, and the factors that are applicable and that must be considered in the planned type(s) of formulations in this study. This chapter therefore focuses on the relevant literature, on which the rest of this study is founded.

In order to formulate a product that will be successful in the treatment of dry skin, it is important to understand causes of dry skin. Dry skin is caused by a lack of natural urea needed to maintain healthy hydrated skin. Carbamide (urea) compositions are stabilised when they contain methylsulfonylmethane (MSM), by inhibiting spontaneous carbamide decomposition. The effectiveness of MSM compositions is enhanced when such compositions contain carbamide. Therefore, MSM and carbamide are exceptionally well suited for use together (Herschler, 1981:18).

This chapter will start off with the structure and function of the skin, the properties and uses of urea and MSM, followed by a conclusion.

2.1 STRUCTURE OF THE SKIN

The skin is the largest organ of the human body and acts as a protective barrier with sensory and immunological functions (Foldvari, 2000:417). The skin of an average human is approximately four kilograms in weight and has a surface area of about 1,8 m^2 (Bronaugh & Collier, 1993:98).

Human skin is made up of four main layers: the stratum corneum (sc), epidermis, dermis and the subcutaneous fat layer (hypodermis). On average human skin is 0,5 mm thick but ranges in thickness from 0,05 mm-2 mm in different parts of the body (Foldvari, 2000:417).

The skin forms a virtually impenetrable barrier to the penetration of microorganisms and chemicals into the body. The principle barrier to penetration and transdermal drug delivery in human skin is the stratum corneum.

Transdermal drug delivery involves the application of a drug to the skin to treat systemic disease and is aimed at achieving systemically active levels of the drug (Flynn & Weiner, 1993:36). While topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of general disease, with the intent of confining the pharmacological or other effect of the drug to the surface of the skin or within the skin (Flynn & Weiner, 1993:35). Regional delivery, by contrast, involves the application of a drug to the skin for the purpose of treating disease or alleviating disease symptoms in deep tissue beneath the application site (Flynn & Weiner, 1993:35).

The skin is generally described in terms of these major multilaminate layers: the epidermis, the dermis and the subcutaneous fat layer or the hypodermis. Microscopically, the skin is a multi-layered organ composed of many histological layers, divided into five anatomical layers, with the outermost layer, the stratum corneum or the horny layer, exposed to the external environment (Chien, 1987:2).

Because of its highly organised structure and hydrophobic nature the stratum corneum is widely regarded as the rate-limiting factor in the penetration of therapeutic agents through the skin (Foldvari, 2000:418). For most practical purposes, removal of the stratum corneum by stripping it away with tape or other mechanical means eliminates the barrier properties of the skin and allows entry of foreign substances into the living tissue (Rieger, 1993:34).

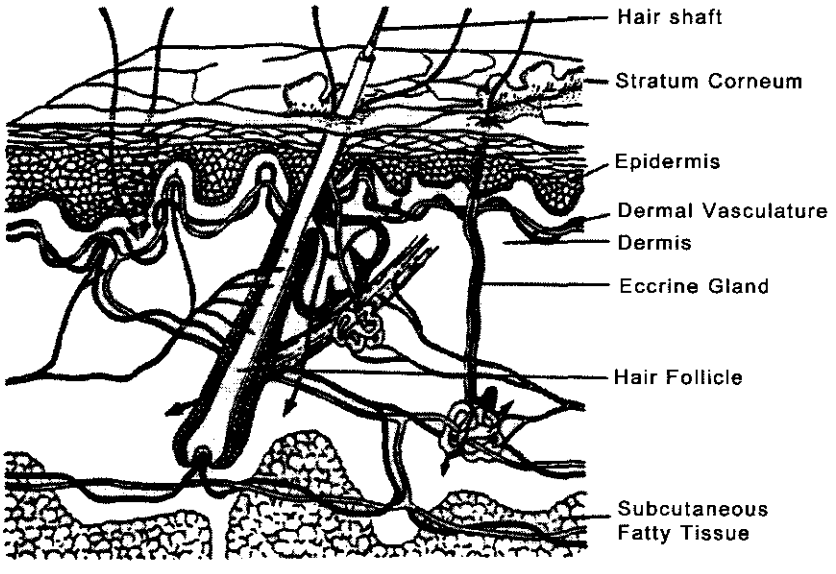


Figure 2.1 Structure of the skin (Roy, 1997:141).

2.1.1 The Stratum Corneum

The barrier function of the skin is accomplished entirely, and quite remarkably, by the highly hydrophobic outermost 10 μm to 20 μm of the skin, the stratum corneum (sc), a compositionally and morphologically unique bio membrane (Naik *et al.*, 2000:318). This extremely thin, approximately one hundredth of a millimetre, least permeable of all the skin layers is the ultimate stage in the epidermal differentiation process, forming a laminate of compressed keratin filled corneocytes (terminally differentiated keratinocytes), anchored in a lipophilic matrix (Naik *et al.*, 2000:318). The keratin deposited within the corneocytes provides strength and chemical resistance (Zats, 1993:12).

The stratum corneum lacks phospholipids, but is enriched in ceramides and neutral lipids like cholesterol, fatty acids and cholesteryl esters that are arranged in a bilayer format and form lipid channels (Foldvari, 2000:418). These lipid channels provide the only continuous phase and diffusion pathway from the surface of the skin to the base of the stratum corneum (Naik *et al.*, 2000:318).

2.1.2 The Viable Epidermis

The epidermis comprises of the viable epidermis and the stratum corneum (Walters, 1989:198). The viable epidermis is a layer of cells that undergo continuous differentiation to produce the stratum corneum, which is the outermost skin layer and principle barrier to penetration through the skin (Walters, 1989:198).

Ordinarily the viable tissue is not much of a diffusion impediment, and net drug passes by way of gradients through the living tissue towards the closest capillary bed, where it is taken up into systemic circulation (Flynn & Weiner, 1993:42).

2.1.3 The Dermis

Below the epidermis is the dermis or cornium. Convolutions in the boundary between the epidermis and dermis with its numerous blood vessels, nerves, and lymphatic increase the area of contact between these two layers and bring the blood supply closer to the skin surface (Lund, 1994:137). The dermis provides physiological support for the epidermis and because the blood vessels approach the interface between the two layers very closely, the dermis cannot be considered as a significant barrier in vivo (Walters, 1989:198).

2.1.4 The Hypodermis

The final layer of skin, the hypodermis or subcutaneous fat layer contains adipose cells, which serves primarily as an energy source. Additionally, the tissue cushions the outer skin layers from impact and its insulation properties contribute to the temperature regulation function of the skin (Lund, 1994:137).

2.1.5 Skin Appendages

The stratum corneum is breached by hair follicles and sweat ducts (Walters, 1989:198).

2.1.5.1 Hair Follicles

Hair follicles are sebum-filled openings from which keratinous hair filaments protrude. Follicles occupy about 0,1% of the skin surface area. They are however absent from plantar and palmer surfaces. Ducts into each hair follicle transport sebum secreted by one or more sebaceous glands, collectively the follicle and gland make up a pilosebaceous unit. About 100 sebaceous glands per square centimeter is the usual level of distribution, but on more hairy regions of the body they number between 400 and 900 per square centimeter (Lund, 1994:137).

2.1.5.2 Sweat Glands

Sweat glands are coiled tubules in the dermis, which open into the skin surface. They can be subdivided in two classes; eccrine glands and the larger apocrine glands (Lund, 1994:137).

2.1.5.3 Eccrine Sweat Glands

Eccrine sweat glands are involved in the regulation of body temperature by water elimination. There are about two million eccrine sweat glands on the average human body. Sweat secreted by eccrine sweat glands varies in composition with the stimulus, the rate of sweating and the site. It is a clear watery liquid of acid pH and electrolytes to help with the prevention of microbial infection of the skin (Lund, 1994:137).

2.1.5.4 Apocrine Sweat Glands

Apocrine sweat glands are larger than eccrine but fewer in number; they are mainly located in the hairier regions of the axillae and around the nipples. Apocrine sweat differs in composition from eccrine and may be cloudy and coloured (Lund, 1994:137).

2.2 FUNCTIONS OF THE SKIN

Human skin is a dynamic organ with a myriad of biological functions. The most obvious is its barrier property, which is of primary relevance to percutaneous absorption. Another major function of human skin is thermoregulation, since maintenance of body temperature is one of the defining characteristics, which distinguishes mammals from lower vertebrates (Riviere, 1993:113). Control of water evaporation is perhaps the most important function of the skin.

Other functions include excretion of wastes, receiving sensory stimuli and to separate and protect the sensitive protoplasmic jelly of the body's interior from the environment. The skin also prevents the intrusion of microbes, chemicals and various forms of radiation (Zats, 1993:12).

2.3 UREA

Although experts in the field of skin care are still battling with what causes dry skin and eczema, it is understood that a lack of urea plays a vital part. Extensive research carried out by experts has proved that very dry skin sufferers lack the natural urea needed to maintain healthy hydrated skin. Urea prevents, cares and treats extreme dry skin conditions and eczema by replacing and maintaining urea levels in the skin.

2.3.1 What is Urea?

Urea is a natural substance and a final metabolite of proteins in the body. One of its most remarkable properties is an increase in the water-holding capacity of the SC in its presence (Wolfram, 2001:768). It has little effect on the epidermal water barrier, but reduces the thickness of the epidermis by approximately 20 percent (Wolfram 2001:768). Percutaneous absorption enhancement by urea is strongly dependent on cosolvents used. For

example, the penetration of ketoprofen was enhanced in the presence of urea in aqueous solutions (Buyuktimkin *et al.*, 1997:365).

Along with epidermal lipids and proteins, human skin contains three natural moisturising factors: urea, lactic acid and amino acids. These are produced during the keratinisation process which occurs as skin cells become flatter and eventually die. One of the most effective natural moisturisers is urea, which the skin makes from protein and represents 7% of the natural moisture balance and the suppleness of the skin. Urea is non-toxic, non-allergenic, colourless and odourless (Buyuktimkin *et al.*, 1997:365).

Urea is naturally present in healthy skin, but when the skin is dry, and in some skin conditions, such as eczema, psoriasis and dermatitis, the level of urea in the skin is reduced. In the epidermis of healthy skin there is approximately 28 micrograms of urea per 2.5 square centimeters. In dry skin urea concentration is diminished by 50%, in skin affected by psoriasis urea concentration is reduced by 40% and in skin affected by atopic eczema urea concentration is reduced by 85% (Buyuktimkin *et al.*, 1997:365).

As a result of the reduced levels of urea, the water binding capacity of the skin is decreased and moisture is lost. This leads to roughness, tightness, scaly or flaky skin and irritation (Buyuktimkin *et al.*, 1997:365).

2.3.2 Physical and chemical properties of urea

A model of the structure of the urea molecule is given in Figure 2.2. Table 2.1 summarises the physical and chemical properties of urea.

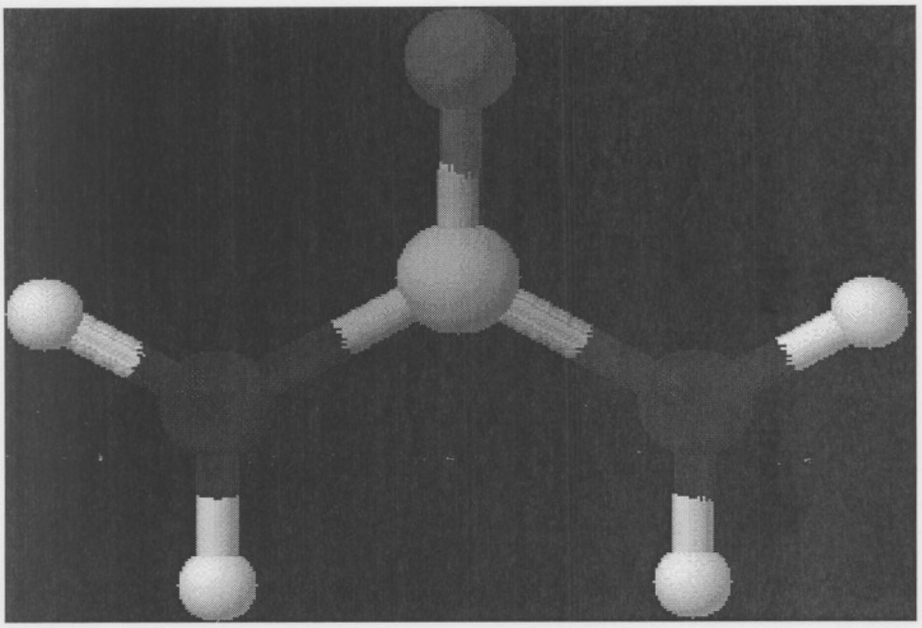


Figure 2.2 Model of the urea molecule (ACD / ChemSketch – Freeware Version 5.12, 2003).

Table 2.1 Physical and chemical properties of urea (Chimco ad., 1997).

Chemical name	Carbamide
Commonly used synonyms	Urea
Molecular Formula	$CO(NH_2)_2$
Appearance	Colourless to white, prismatic crystals or as a white crystalline powder.
Odour	Odourless but may gradually develop a slight ammoniac odour on long standing (McEUOY, 2002:3457).
pH water solution (conc. 10%)	9-10
Melting point	133°C (decomposes)
Oxidising properties	None
Bulk density	700-800 kg/m^3
Solubility in water	1080 g/l at 20°C

2.3.3 Main advantages of urea application to the skin

Applying urea directly to the skin increases the moisture binding capacity of the skin, thus rehydrating the skin, softening it and reducing cracking and roughness. It also helps reduce the cycle of itching and irritation, producing a localised anesthetic effect, as well as reduces the likelihood of flare-ups.

Urea penetrates and rehydrates the corneum. Also, the addition of urea to dermatological preparations increases the penetration of other substances, such as corticosteroids, which is attributed to urea's ability to increase skin hydration after application (Beiersdorf, 2003:1).

The proteolytic characteristics of urea are well recognised, where, depending on the concentration, urea modifies the structure of amino-chains as well as of polypeptides. This is significant for skin moisturising since a correlation exists between water content and amino acid content in skin – the dryer the skin, the lower the share of dissolved amino acids (Beiersdorf, 2003:1).

Urea's incorporation into a product can also result in a lower use of other preservatives since urea can act as an antimicrobial given its ability to inhibit the growth of microorganisms (Friedler, 1977:1).

2.3.4 How does applied urea penetrate into the skin?

Penetration of urea is dependent on the vehicle in which it is contained. It has been shown that the penetration is much deeper in layers of the stratum corneum (which contains around 30 layers of flattened cells) when the urea is applied in a water and oil emulsion, such as creams, lotions and gels. When the urea is applied to the skin in a water/oil emulsion the stratum corneum is able to retain water for longer, plus water loss through evaporation is also slowed down (Beiersdorf, 2003:1).

2.3.5 Uses of urea.

Urea is used topically in the treatment of dry skin. At concentrations of 5-30%, urea promotes hydration of keratin and keratolysis in dry and hyperkeratotic skin. Urea increases the uptake of water by the stratum corneum, giving it a high water-binding capacity. Topically applied urea may also have an antipruritic effect. At high concentrations (e.g., 40%), urea is a protein denaturant (McEUOY, 2002:3457).

Parima Inc (2003) reported use of urea as treatment for:

- Direct diuretic - meaning it can increase diuresis by boosting the function of the renal epithelia (Robert & Fils, 2000).
- Wounds – wounds can be treated by spraying urea or a 2% solution of same (Robert & Fils, 2000).
- Athlete's foot – in Russia more concentrated solutions of urea are used to treat athlete's foot and certain related pathologies (Robert & Fils, 2000).
- Perfusions used in neurosurgery – before, during, or after eye surgery and to treat brain swelling (Robert & Fils, 2000).
- Water retention – such as swollen face, headaches, premenstrual water retention and enuresis (Robert & Fils, 2000).
- Urinary infection without renal lesions.
- Cancer – it seems that urea modifies the tumor's support and exposes its peripheral characteristics to the immune system (Robert & Fils, 2000).
- Hyperkeratotic conditions such as:
    - ❖ Dry skin
    - ❖ Rough skin
    - ❖ Dermatitis
    - ❖ Psoriasis

- ❖ Xerosis
- ❖ Ichthyosis
- ❖ Eczema
- ❖ Keratosis
- ❖ Keratoderma
- ❖ Cons
- ❖ Calluses

If urea, a physiologic substance, is applied locally, it has a favourable effect on the skin; it keeps the horny layer moist. However, high concentrations might impair the function of the skin, for urea penetrates easily into the skin, exerts mucolytic and keratolytic effects, changes the keratin structure, and promotes just like dimethylsulfoxide, the permeation and the resorption of active ingredients. Moreover, urea causes a thinning of the epidermis by influencing the epidermal proliferation. Therefore, high concentrations of urea should only be applied for long periods of time, and on large surfaces of the skin, for instance in the treatment of dry skin, in the form of cosmetics, under medical supervision (It works marketing, 2003:2).

2.3.6 Urea in cosmetic formulations

Urea is of significance for the hydration of the stratum corneum. Normal skin contains approximately one percent urea. Furthermore, urea has keratolytic and pruritus-easing properties and may be incorporated as an active ingredient in moisturisers due to its humectant properties.

The amount of urea, in extracts from the stratum corneum of normal skin, in comparison to extracts from skin after cleansing, or from skin after a prolonged topical application of urea-supplemented emulsions, was measured. Skin cleansing with sodium lauryl sulphate solution (SDS, 4 percent), a standard cleansing product and water as a control led to a dramatic decrease in the amount of extractable urea from the stratum corneum. If a cleansing product was supplemented with 10% urea, a measurable positive effect on extractable urea was achieved. A lasting effect for at least 24 hours after final application of the urea containing cream was observed (Anon, 2003:3).

As a conclusion, the urea content in the stratum corneum varies. After skin cleansing, its status is reduced, whereas after a prolonged application of urea containing emulsions, its level is increased. In pathological skin diseases, these results may be of importance to compensate for urea deficiencies. In dry skin, a lack of water-retaining substances may be compensated by urea containing cosmetics (Anon, 2003:3).

2.4 MSM (Methylsulfonylmethane)

The skin is the largest area of the body that interfaces with bacteria and "problems" from the environment and is the first line of defence for the body. There are, of course, immune system functions that take place within the skin area which would be nourished, only by the very small arteries (capillaries), so the blood supply at the surface of the skin is not as great as it is further inside the body. When something is applied to the skin, which nourishes the immune system cells, such as MSM (methylsulfonylmethane), it helps to improve the health of the skin (curing or preventing a disease) through the natural function of the body (the immune system) (Loren, 2000:1).

2.4.1 What is MSM?

Methylsulfonylmethane (MSM) is an organic sulfur compound that is a metabolite of dimethyl-sulfoxide (DMSO). It is a white, odourless, slightly bitter tasting, crystalline substance, which contains 34% elemental sulfur. It is easy soluble in water.

The cycle of these naturally occurring compounds begins in the ocean where microscopic plankton release sulfur compounds called dimethyl-sulfonium salts. These salts are transformed in the ocean into the very volatile compound DMS that escapes from the water as a gas, which rises into the upper atmosphere. Exposed to ozone and high-energy ultraviolet light the DMS is converted to DMSO and MSM. Both the DMSO and MSM are very soluble in water and they return to the surface of the earth in rainwater. Plants then take up the two compounds into the plant structure. Through the process of plant metabolism the MSM, along with the other sulfur compounds it has spawned, are ultimately mineralised and transported back to the ocean and the sulfur cycle begins again (Lawrence, 2002:1).

MSM is found naturally in the human body. It occurs in the blood and in other organs and has been detected in normal human urine. The levels of MSM in the circulatory system of an adult human male is about 0.2 parts per million. Normal human adults excrete from four to eleven milligrams MSM per day in their urine. MSM is rated as one of the least toxic substances in biology, similar in toxicity to water (Lawrence, 2002:1).

2.4.2 Physical and chemical properties of MSM

Figure 2.3 depicts a model of the structure of the MSM molecule. Table 2.2 summarises the physical and chemical properties of MSM.

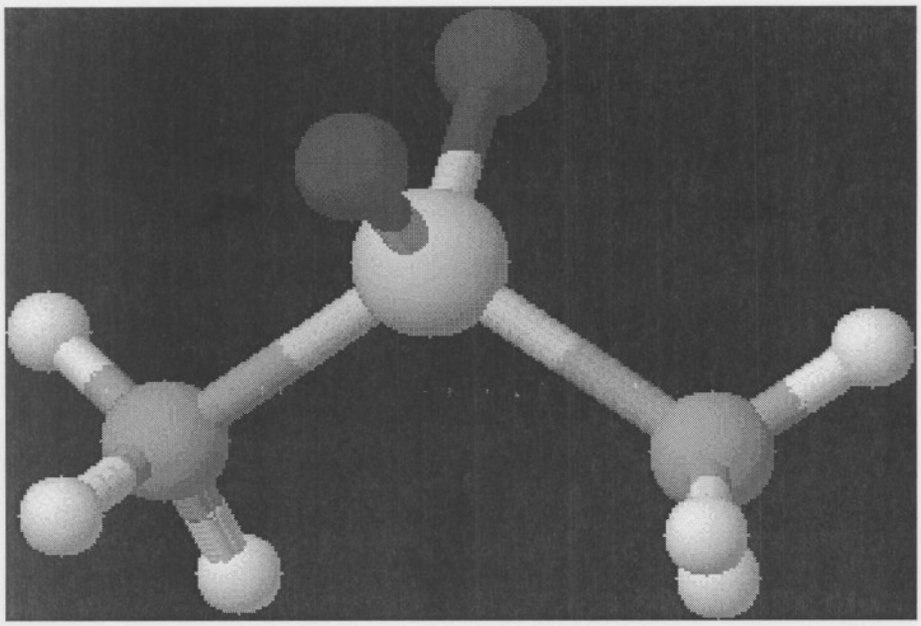


Figure 2.3 Model of the methylsulfonylmethane molecule (ACD / ChemSketch – Freeware Version 5.12, 2003).

Table 2.2 Physical and chemical properties of MSM (Lawrence, 2002:1).

Chemical name	Methylsulfonylmethane
Commonly used synonyms	MSM
Molecular formula	$(DH_3)_2SO_2$
Appearance	White, slightly bitter tasting, crystalline substance, which contains 34% elemental sulfur.
Odour	Odourless
pH water solution (conc. 10%)	9-10
Melting point	100°C (decomposes)
Oxidising properties	None
Bulk density	800 -2400 kg/m^3
Solubility in water	Easily soluble

2.4.3 Main advantages of MSM

MSM, and many precursor compounds which are readily converted to MSM in the body, are found in most natural, unprocessed foods. Because of its volatility, it is readily lost when fresh food is processed and/or stored. Unless the diet consists largely of raw, unprocessed foods, it is unlikely that sufficient MSM will be ingested to contribute significantly to the daily nutritional sulfur requirements, thus furthering the high impact of sulfur-deficient diets that exist globally (Vita-flex nutrition, 2001:1).

Sulfur plays an indispensable role in human nutrition, which is often overlooked. It is responsible for the conformation of body proteins through formation of disulfide bonds, thereby holding connective tissue together. Thiol (i.e. sulfhydryl) groups are vital for the catalytic function of the body enzymes. To perform these roles, constant intake of assimilable sulfur is needed by the body.

Although MSM has not yet been established to be a vitamin, it does have a vitamin-like moderating or normalising activity for various body functions, as there appears to be a high correlation between abnormal physiological symptoms and low MSM blood levels in human beings. Although MSM is found as a natural constituent of foodstuffs, like vitamin D, the principle supply is believed to be syntheses in the body using one of its naturally occurring precursor compounds. Also, MSM occurs in lower concentrations with increasing age. Too low a body concentration of MSM may potentially result in adverse physical and psychological stress, tissue malfunction, and increased susceptibility to disease (Vita-flex nutrition. 2001:1).

MSM is a penetrant. That means that this substance can and does penetrate through the skin into the inner cells more quickly and easily than almost any other substance in the world. Without MSM, various ingredients have a hard time penetrating the skin, to get inside the body and often sit on the skin, or even clog the pores.

MSM is also a great detoxifier and a great source of methyl groups, which are needed for the immune system to function. A methyl group consists of one atom of carbon with three

associated atoms of hydrogen. MSM consists of methyl groups connected to sulfur and oxygen (Loren, 2000:1).

2.4.4 Uses of MSM (Methylsulfonylmethane)

2.4.4.1 Health benefits of MSM

- Aging process

The body's content of MSM declines in tandem with the progression of the aging process.

- Cardiovascular system

MSM improves blood circulation.

- Digestive system

MSM (100-500 mg per day) prevent and treats constipation (especially in elderly persons).

MSM prevents intestinal parasites from adhering to the gastrointestinal tract.

- Eyes

MSM (applied topically as eye drops) reduces intraocular pressure in persons afflicted with glaucoma.

- Immune system

MSM helps to prevent allergies.

MSM helps to prevent breast cancer.

MSM helps to prevent colon cancer.

MSM (lotion applied topically) destroys many types of detrimental fungi that reside on the skin.

MSM reduces inflammation.

- Metabolism

MSM reduces the toxic accumulation of lactic acid following exercise

MSM is claimed to alleviate fatigue.

- Musculoskeletal system

MSM (lotion applied topically) reduces the inflammation associated with Carpal Tunnel Syndrome.

MSM (lotion applied topically) alleviates dry skin.

MSM (administered orally) may facilitate hair growth (due to its sulfur content).

MSM (lotion applied topically) alleviates insect bites/stings.

MSM (administered orally) may facilitate the growth of nails (due to its sulfur content).

MSM (lotion applied topically) alleviates psoriasis and rashes.

MSM (lotion applied topically) alleviates sunburn.

MSM accelerates the healing of wounds.

- Respiratory system

MSM is claimed to reverse emphysema.

- Dietary source of MSM

MSM is a content of some plants.

- MSM enhances the function of the following substances

  - ENDOGENOUS SUBSTANCES

Amino acids: MSM contributes its sulfur component to methionine and cysteine.

Carbohydrates-Glycosaminoglycans: MSM contributes its sulfur component to chondrotin sulfate (CSA).

Proteins: MSM contributes its sulfur component to keratin sulfate.

  - EXOGENOUS SUBSTANCES

Minerals: MSM provides a highly bioavailable source of sulfur to the body (sulfur is utilized in the production of, and is a component of, many endogenous compounds of the body).

Sulfuric compounds: MSM is believed to be the active therapeutic constituent of dimethyl sulfoxide (DMSO).

- MSM reduces the toxic effects of the following substances

Organic acids: MSM reduces the accumulation of lactic acid following exercise (Super vitamin outlet.com, 2002:1).

2.4.5 The benefits of MSM in the horse industry

Veterinary interest in dimethyl-sulfoxide (DMSO) and its derivative methylsulfonylmethane (MSM) has increased over the years. Topical use of DMSO is FDA approved, but as internal use of DMSO increases (such as IV injections), practitioners are turning to MSM because of the absence of the objectionable taste/smell (Jones, 1987:1).

Using radiolabeled S35, quite a lot has been learned about MSM movement and metabolism in the body. Orally taken MSM binds to the sites of the muscosal membranes. MSM is absorbed, passing to the blood, and then to the unit body structure, or cells. It quickly crosses membranes and can thereafter be found in the inner or subcellular fractions including nuclear, mitochondrial, lysosomal and microsomal structures. The sulfur is biotransformed into the multitude of organo-sulfur molecules required for the horse's health. In mammals, MSM is not metabolised and is eventually excreted by the renal pathway, and through perspiration and respiration (Jones, 1987:1).

MSM donates sulfur for the biosynthesis of methionine and cysteine, important protein-building substances. Sulfur derived from MSM is also found in keratin, (hoof, horn and hair protein), serum albumins, connective tissue, and immunoglobulin and transferring. Sulfur bonds, derivable from MSM, also sustain proper conformation of specific enzyme molecules; an absolute requirement for metabolic function. This means MSM is the most stable and convenient source of the macronutrient supplement for horses (Jones, 1987:1).

It has been suggested that most mammals are chronically deficient in bio-available sulfur, as young horses fed a ration of MSM seem huskier and simply look better.

Classified as a drug, MSM falls under jurisdiction of the FDA. But, as a food, it does not. There are substances, such as sodium chloride that can be considered both a food and a drug. Sprinkled on your eggs in the morning, salt is a food. Dissolved in sterile water and given IV, it is a drug. Currently the daily amount of MSM given as a food (on grain) is two heaped

teaspoonfuls morning and night. It works best if the powder is dissolved first in warm water and then poured over the grain. Used orally as a drug, the dosage of MSM may be as high as 100 grams or more per week (Jones, 1987:1).

2.4.6 MSM in cosmetic formulations

It has been discovered that compositions containing methylsulfonylmethane (MSM) can be used effectively to soften skin, to dilute blood, and for a variety of other useful purposes. MSM compositions are stable and safe for administration to human or other animal subjects and therefore eliminate many problems of the prior art. In particular, topically applied MSM compositions benefit the skin to a far greater degree than lanolin or carbamide. MSM can be combined with lanolin and/or carbamide to form skin preparations, which are exceptionally effective (Royal body care, 2002:1).

MSM has proved to have a variety of useful properties when applied to any animal tissue. It has been observed to beautify the complexion, to enhance scalp and hair, and generally to help make the body of the user more flexible and comfortable. Various MSM formulations are also exceedingly useful as bland vehicles for pharmaceuticals (Royal body care, 2002:1).

Manicuring preparations can advantageously include MSM. Such preparations can increase nail toughness by reducing brittleness and can be used for softening cuticles for easy removal.

Depending on its intended use, a preparation can contain MSM in solution or in dispersion. It may take the form of a cream, lotion, gel or paste for topical administration or a liquid, solid or vapour for administration by other routes such as injection, inhalation, oral ingestion and the like.

2.5 CONCLUSION

In conclusion it can be said that urea and MSM possess all the properties that is necessary to treat dryness of the skin, therapy in allergic eczema, and for a lot of other medical conditions. Therefore, if it is incorporated into dermatological vehicles and properly analysed and evaluated, there is a possibility that these products could be even more effective in the treatment of certain medical conditions. This is because of the stabilising effect of these two actives on one another.

CHAPTER 3

FORMULATION OF SKIN CARE PRODUCTS CONTAINING UREA AND MSM

3 WHY SKIN CARE COSMETICS?

The skin can be said to be one of the body's organs. But, because we see it every day, it is not usually thought of as being very important. However, as is clear from the evolutionary history of living organisms, the skin has the very important function of preventing them and their cells from drying out by keeping the water, which is so vital to life inside them. Some of the functions skin has are protecting the body from ultraviolet radiation using its melanin, regulating body temperature, and mitigating external stimuli through its neutralising capability. The skin is the interface between the body and the external environment and it protects the body by responding to various changes in it. It is thus a very important organ for the body (Mitsui, 1997:319).

The functioning of the skin and its mechanisms are upset by changes in the environment, and aging. It is the purpose of skin care cosmetics to keep the skin functioning properly and its mechanisms working well. So, ideal skin care cosmetics will protect the skin from the harmful effects of drying, ultraviolet radiation and oxidation; back up the skin's homeostasis function and keep it looking beautiful and healthy (Mitsui, 1997:319).

3.1 PURPOSE, FUNCTION AND ROLE OF SKIN CARE COSMETICS

3.1.1 Purpose of skin care cosmetics

In our modern world, people benefit both from nature and the highly sophisticated environment they live in, but also adversely affect them. For instance, an air conditioner, by keeping the temperature under control, provides a comfortable environment to live in, but it can cool the skin too much and cause it to dry out. Ultraviolet radiation can affect the skin badly and cause it to dry out. For people living in such a sophisticated environment, the purpose of skin care cosmetics is considered to be as follows:

- clean the skin;
- preserve the skin's moisture balance;
- stimulate skin metabolism;
- protect the skin from harmful ultraviolet radiation.

Formerly, skin care cosmetics were considered to just have three purposes, but now it is normal to include the fourth because the idea of photo aging has become accepted as the result of research that has been carried out in recent years. It goes without saying that skin care cosmetics must be designed to be excellent in terms of safety, stability, texture and usability based on thorough research and understanding of the physiological functions of the skin (Mitsui, 1997:319).

3.1.2 Function of skin care cosmetics

Skin care cosmetics contain substances that enable the skin to function properly. They support its homeostasis function so that it is maintained in a beautiful and healthy condition or regains such a state if it is not. So, skin care cosmetics have many different functions. The basic ones are cleansing, anti-drying, ultraviolet damage prevention, antioxidation and invigoration, but they can also clear up skin problems, have a whitening effect to combat skin aging-associated troubles (liver spots and freckles due to the sun's rays), prevent wrinkles, sagging skin and acne (Mitsui, 1997:320).

However, these functions will only be manifested if the skin care cosmetics having them are used in an appropriate manner. It is therefore necessary to make a thorough consideration of their order of usage (beauty treatment system), season, the living environment, age, experience of using cosmetics and skin type of the user, preference regarding utility and occasion on use. Of the many types of beauty treatment systems on the market, the following are the major ones:

- *Basic care.* Face cleansers (makeup removal, face washing) + lotions (moisture retention, humectant effect) + emulsions (moisture retention, humectant effect, provide oil, promote skin metabolism) and
- *Additional care.* Essences, massage creams, packs, powders, etc. (special care). Additional care system products are designed to cover aspects not included in basic care products (Mitsui, 1997:320).

3.1.3 Role of skin care cosmetics

Skin care cosmetics may be defined fundamentally as a product that cleans the skin and moisturise the horny layer of the skin. The moisturising mechanism of the skin can be broadly considered in terms of the horny layer and the dermis. In the horny layer, the natural moisturising factor (NMF) that consists of amino acids, etc., sebum and skin surface lipids derived from epidermal oil components, are very important. But in the dermis, it is

hydrophobic substances such as phospholipids and macromolecular hydrophilic substances like hyaluronic acid, collagen and elastin that are considered to be important (Mitsui, 1997:321).

If something goes wrong with the moisturising mechanism at the horny layer level, it is essential to lighten the burden of the skin in order make it healthy and beautiful again and this can be done by giving it a good balance of high quality oils and hydrophilic substances with great moisturising capacity. So, when something goes wrong with the skin as a result of its homeostasis function not working properly, this must be corrected through the use of skin care cosmetics, which are designed to redress the moisture balance (Mitsui, 1997:321).

At the horny level, NMF and lipid amounts decrease as the skin ages, resulting in a reduction in its moisture retention capability, leading to hardening of the horny layer. The reduction in moisture, NMF and lipid occurring through aging can be compensated by supplying equivalent substances (water, humectants and oils) in cosmetics to effect biological changes which will serve to regain homeostasis in the skin's moisture balance.

To summarise the roles played by skin care cosmetics: they maintain homeostasis in the skin and restore it if it has been lost, delay aging in the skin, and provide a solution to skin problems. They must also be safe when used constantly over long periods (Mitsui, 1997:321).

3.2 DEVELOPMENT PROGRAM FOR SKIN CARE COSMETICS

To develop a new product, a formulator must identify raw materials with the desired functionality, and combine these materials in the proper ratios to yield an acceptable finished product that performs as intended, and that remains stable (Schueller & Romanowski, 1999:35).

Prior to the development of a skin care product in which an existing active ingredient is used, it is essential to perform a preformulation study. Such a study can be defined as an investigation to establish the necessary physico-chemical parameters, kinetic rate profile and physical characteristics of an existing active; alone and in combination with commonly used excipients, in order to establish compatibility. Preformulation studies and product development are finely interweaved and cannot be seen as sole entities, since different aspects are investigated throughout the entire product development process.

Before embarking upon a formal development program, one must consider the following (Shaw, 1998:5,6):

- The available physico-chemical data (including chemical structure and different salt available);
- The therapeutic class of the compound and anticipated dose;
- The intended dosage forms to be developed;
- The supply situation and the development schedule;
- The availability of a stability indicating assay;
- The nature of the information that the formulator should have.

Since product development is so extensive, certain aspects of the process that are applicable to this study will be discussed next.

3.2.1 Preformulation

The preformulation study comprised of a literature study, since all the necessary information was available at the time.

3.2.2 Early formulation

During early formulation a trial-and-error approach was used. The main reason for this approach was its cost-effectiveness. Existing formulas were taken and urea and MSM were incorporated into these formulas. These formulas were then changed as necessary. Compatibility tests, as well as accelerated stability were performed. Assay methods had to be developed.

3.2.3 Final formulation

After the formulation of the final formulas was complete, the products were prepared in bulk for storage and testing purposes. A stability program according to the International Conference on Harmonisation (ICH), Tripartite Guideline (2000:4) was followed at three storage temperatures for three months.

3.2.4 Preservation of skin care formulations

It is essential to add preservatives to skin formulations for long-term protection against putrefaction and the development of unpleasant smells due to bacterial contamination from the fingers during use, and other sources.

Characteristics of preservatives include (Mitsui, 1997:202):

- Efficacy against many species of microorganisms;

- Solubility in the respective solvent;
- High safety, no irritation;
- No effect on product pH;
- No reduction of the active ingredient effectiveness;
- No adverse effects on product appearance;
- Stability over a wide temperature and pH range;
- Readily available;
- Low in price, and economical to use.

3.3 FORMULATION OF A CREAM

3.3.1 Purpose and function of a cream

Creams are a type of emulsion in which two liquids that do not mix together, like water and oil, are made into a stable dispersion by making one the dispersion phase and dispersing it through the other which acts as the dispersion medium. Being semi-solid, creams are stable over a wider range of conditions than milky lotions and oils, and humectants and water can be included in a greater range of proportions. Because of this characteristic, creams occupy a very important position among skin care cosmetics (Mitsui, 1997:342).

The main functions of creams are to maintain the moisture balance and keep the skin moist and supple through the supply of water, humectants and oils. In addition to creams which moisturise the skin and make it suppler, there are many others that have the additional functions of stimulating the circulation, cleansing the skin and removing makeup (Table 3.1).

Table 3.1 Purpose and function of different creams (Mitsui, 1997:341).

Purpose/function	Product type
Moisturising and softening action	Emollient creams (nutrient cream, nourishing cream, moisture cream, vanishing cream, night cream, etc. in which the emulsion type, amount of oil and humectant are varied to cater to the season, skin type, preference, etc.)
Stimulate circulation, soften skin	Massage cream
Cleansing, makeup removal, etc,	Cleansing cream
Under makeup cream, makeup base	Makeup cream, base cream, pre-makeup cream
Other specific purposes	
E.g.: UV protection	Sunscreen cream, suntan cream
Depilatory action	Hair removal
Hairstyling	Hair cream
Shaving	Shaving cream
Horny layer softening	Horny layer softening cream

As creams are so easy to use and apply to the skin, and such a wide range of formulations is possible for them, they can be made to feel light on use, slightly oily, hard or soft, give a moist feeling, spread well, easily penetrate the skin, be easy to wipe off and be capable of being rinsed off with water. It is easy to vary the amount of water, and the amount and type of humectant and oil in the formulation to suit different purposes of use in order to cater to different skin types, skin conditions, cosmetic routines and preferences which vary with the season, age of the user and living environment (Mitsui, 1997:342).

3.3.2 Main ingredients of creams

Some of the main ingredients of creams are oily ingredients, aqueous ingredients, surfactants, preservatives, chelating agents, perfumes and pharmaceutical agents. Creams are either O/W (oil in water) or W/O (water in oil) emulsions whose special features are provided by the surfactants and oil ingredients used in them. In the case of the O/W type, hydrophilic surfactants are used. The oily ingredients used can vary widely from those with no polarity to those with very high polarity. In the case of creams with a high internal phase proportion, the cream state is achieved when the structural fluidity disappears as a result of a rise in the density of the emulsion particles (Mitsui, 1997:341).

When the internal phase ratio is low, in order to achieve a degree of firmness, it is necessary to add such amphiphilic substances as higher alcohols and higher fatty acids so that the fluidity of the external phase disappears and the stability of the cream is enhanced. Higher alcohols are the amphiphilic substances normally used. It has been shown that if they are used in combination with non-ionic surfactants, lamellar liquid crystals form in the external phase (water phase), which makes a gel structure in it. To make this type of cream become more stable with time, it is very effective to use cetyl alcohol together with stearyl alcohol (Mitsui, 1997:341).

For the W/O emulsion cream, such as the massage cream, the lipophilic surfactant is the main type used. Oily ingredients used comprise mainly of the non-polar type. In order to raise stability, it is important to prevent sedimentation in the internal phase (water phase) and to do this it is necessary to take care in the selection and combination of oils. In the case of creams which have a lot of oil phase, the W/O emulsion has been used to increase the oily nature and the O/W type when a light feeling is desired. The emulsion type may be freely selected in this way only when there is a lot of oil component, as in the case of massage creams. As a result, virtually all creams which have little oil component have been limited to the O/W emulsion (Mitsui, 1997:341).

3.3.3 Massage cream formula

The formula for the massage cream is given in Table 3.2.

Table 3.2 Cream formula.

INGREDIENTS	% m/m	ACTIVITY
A. Cremophor A6®	1.5%	Emulsifying agent
Cremophor A25®	1.5%	Emulsifying agent
Luvitol EHO™	4%	Oil phase of emulsion
Cetyl alcohol	2%	Thickening agent
GMS A/S™	2%	Co-emulsifying agent
Liquid Paraffin™	8%	Oil phase of emulsion
B. Glycerine	5%	Moisturiser
Urea	5%	Active
Methylsulfonylmethane	5%	Active
Methylparaben	0.3%	Preservative
Propylparaben	0.2%	Preservative
Distilled water	to 100%	Solvent

3.3.3.1 Procedure to prepare the formulation

- Mix A together and melt at 80°C in a glass beaker.
- Mix B together and melt at 80°C in a glass beaker.
- Mix B into A with a homogeniser (Silverson mixer) and cool down to room temperature whilst stirring.

3.3.3.2 Outcomes / Findings

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

3.4 FORMULATION OF A LOTION

3.4.1 Purpose and function of a lotion

Moisturising lotions have a character that is intermediate to that of lotions and creams. They are emulsions, which contain little oil and have high fluidity. It is important to maintain homeostasis in the skin and help it to recover from skin deceases. Moisturising lotions play a role in the maintaining of the skin moisture balance mainly by supplying it with water, humectants and oils to keep it moist and supple. The purpose and function of moisturising lotions are listed in Table 3.3.

Moisturising lotions employ dispersions of liquids, which, like oil and water, are mutually insoluble in each other. Moisturising lotions are thermodynamically unstable emulsion systems. Stokes law expresses the relationship between the separation of the dispersion phase (emulsion particles) from the dispersion medium (continuous phase). Examples of measures which should be taken to maintain the stability of the system are the following: 1) make the emulsion particles very fine; 2) reduce the difference between the specific gravities of the internal and external phases; or 3) raise the viscosity of the external phase (Mitsui, 1997:335).

Table 3.3 Purpose and functions of different lotions (Mitsui, 1997:336).

Purpose / Function	Product category
Moisturising / softening the skin	Moisturising lotion (The emulsion type, oil and humectant amounts and other factors are adjusted to suit the season, different skin types and user preferences)
Stimulate skin circulation / increase softness	Massage lotion
Cleansing / makeup removal	Cleansing lotion
UV protection (daily use sun protection products)	Sun protect (Called protect emulsion, sun protector UV care milk)
Others:	
Ultraviolet protection	Sunscreen
Under makeup cream	Makeup lotion
Soften horny layer	Horny layer smoother Elbow lotion
Protect hair	Hair milk
For body and hands	Body lotion

Such methods as thickening the dispersion medium using water-soluble polymers and clay minerals or giving the emulsion particles a protective colloid character, are often used to enhance stability as well as improve texture. The pH of most moisturising lotions is made weakly acidic to neutral to fit in with the pH range of the skin's surface. The pH of a special type for softening the horny layer on the elbows and heels is alkaline (Mitsui, 1997:335).

3.4.2 Main ingredients of moisturising lotions

Many of the ingredients of moisturising lotions are similar to those of creams but the proportion of solid oils and waxes is much less in moisturising lotions. Virtually all employ the O/W type emulsion, but the W/O type is also used for certain special products and applications. The surfactants used for emulsification are mostly the non-ionic and anionic types, which are highly safe. There are also biological emulsifiers in the form of protein-based surfactants. Oily ingredients used comprise of hydrocarbons, oils, waxes, higher fatty acids, higher alcohols and esters, as well as straight chain and cyclic silicone oils. The types and quantities of such oily ingredients to be used are determined in consideration of desired texture and stability characteristics (Mitsui, 1997:335).

Examples of aqueous ingredients used are purified water, ethanol, polyhydric alcohols and water-soluble polymers. Other ingredients used include preservatives, pharmaceutical agents, chelating agents, ultraviolet absorbents, anti-oxidants, dispersants, anti-fading agents, buffers, colourings agents, utility enhancers and perfumes. Formulae for moisturising lotions should be designed with consideration for features desired in the product, as well as on the basis of a good understanding of a wide range of characteristics for each ingredient, such as stability, safety, preservation and texture (Mitui, 1997:335).

3.4.3 Moisturising lotion formula

The formula of the moisturising lotion is given in Table 3.4.

Table 3.4 Formula of lotion.

INGREDIENTS	% m/m	ACTIVITY
A. Cremophor A6®	2%	Emulsifying agent
Cremophor A25®	2%	Emulsifying agent
Luvitol EHO™	6%	Oil phase of emulsion
Cetyl alcohol	0.5%	Thickening agent
GMS A/S™	6%	Co-emulsifying agent
Liquid Paraffin™	6%	Oil phase of emulsion
B. Propylene Glycol	3%	Moisturiser
Vitamin E Acetate	3%	Anti-oxidant
Urea	5%	Active
Methylsulfonylmethane	5%	Active
Methylparaben	0.3%	Preservative
Propylparaben	0.2%	Preservative
Distilled water	To 100%	Solvent

3.4.3.1 Procedure to prepare the formulation

- Mix A together and melt at 80°C in a glass beaker.
- Mix B together and melt at 80°C in a glass beaker.

- Mix B into A with a homogeniser (Silverson mixer) and cool down to room temperature whilst stirring.

3.4.3.2 Outcomes / Findings

The lotion applied easily, it was not too oily or too hydrous, it had a homogeneous white texture and it had a pleasant floral odour.

3.5 FORMULATION OF A HAIR GEL

3.5.1 Purpose and function of a hair gel

This type of cosmetic product is used every day, particularly after washing the hair, to add the finishing touches to hair care. Functionally speaking, they are divided into two types, one mainly for setting and adjusting the hairstyle and the other, known as the hair treatment type, for enhancing the hair's gloss and feeling to the touch, giving it a nice quality and making it easier to manage. There are also combinations of these two types (Mitsui, 1997:418).

3.5.2 Main ingredients in a hair gel

Hair gel and water grease are jelly-form transparent preparations, which contain styling ingredients and are thickened using water-soluble polymers. The styling ingredients added are resins and they produce a dry effect (Mitsui, 1997:420).

The ingredients for hair gel include water-soluble polymers (carboxyvinyl polymer, methylcellulose, carrageenan, etc.), setting agents (polyvinyl pyrrolidone, polyvinyl pyrolidone/vinyl polymer acetate, etc.), humectants, alkalising agents, surfactants and chelating agents. The major ingredients of gels are water soluble polymers, humectants

(glycerine), setting agents, alkalising agents, surfactants and chelating agents (Mitsui, 1997:420).

3.5.3 Hair Gel Formula

The formula of the hair gel is given in Table 3.5.

Table 3.5 Formula of gel.

INGREDIENTS	% m/m	ACTIVITY
A. Carbopol (Ultrez 10)™	0.5%	Gel forming agent
B.Tris(hyroxymethyl) aminomethane	0.5%	pH-adjustment for gelling
Disodium EDTA	0.1%	Completing agent
C. Urea	5%	Active
Methylsulfonylmethane	5%	Active
Propylene glycol	10%	Moisturiser / Preservative
Glycerine	25%	Moisturiser
Distilled Water	To 100%	Solvent

3.5.3.1 Procedure to prepare the formulation

- Dissolve urea and methylsulfonylmethane in 50 ml water from C in a glass beaker and add the propylene glycol and glycerine.
- No heating is required because of the high solubility of urea and methylsulfonylmethane in water.
- Add A to C and homogenise thoroughly with a homogeniser (Silverson mixer).
- Remove the foam manually.
- Mix B and dissolve in 3.0 ml of water and add B to C.

3.5.3.2 Outcomes / Findings

A clear gel was obtained, which indicated that all substances dissolved. The product had a good smell and applied easily.

3.6 FORMULATION OF A HAIR SPRAY

The purpose of this hair spray is to promote hair growth, prevent hair loss, and to improve hair condition, and not for setting or styling of the hair.

3.6.1 Purpose and function of hair growth promoters

Hair growth promoters are preparations made by adding various pharmaceutical agents to an alcohol-water solution, which is applied to the scalp to normalise its functions. By increasing the circulation in the scalp, they improve hair follicle function, which promotes hair growth and prevents hair loss. They also help prevent dandruff and itchiness.

With the current aging of society, an increase in the demand for hair growth promoters is expected, and in keeping pace with this, new pharmaceutical agents for promoting hair growth and preventing hair loss should be continually developed (Mitsui, 1997:413).

3.6.1.1 Causes of hair loss

The factors currently considered to cause hair loss are (Mitsui, 1997:414):

- **Reduced hair follicle function due to male hormones.**

The following inferences have been made regarding the relationship between male hormones in the hair follicles and metabolism in the hair root cells. The male hormone testosterone is converted to 5α-dihyrotesterone (DHT), a substance with higher biological activity, at the hair

follicles through the action of 5α-reductase. It is currently thought that the action of DHT is the principal cause of hair loss.

- **Reduction in metabolic functions of hair follicles and hair bulbs**

It is the division, proliferation and differentiation of the hair matrix at the hair roots, which form hair and make it grow up to the epidermis. The hair matrix receives the supply of nutrients, which it requires for cell division from the capillaries to help the hair to grow by supplying blood to them. The development of the network of capillaries surrounding the hair follicles and dermal papilla has thus been of great importance to hair growth. If the flow of blood in the capillaries surrounding the hair follicles and dermal papilla is reduced, the supply of nutrients to the dermal papilla and hair matrix will not be sufficient, hence impairing cell metabolism and having an adverse effect on hair growth.

- **Reduction in scalp physiological function**

Excessive build-up of dandruff flakes will block the pores of the scalp through which hairs exit the epidermis. This will have an adverse effect on the hair production at the hair root, and the substances formed when bacteria decompose the dandruff will irritate the scalp-giving rise to such conditions as pityriasis accompanied by itching and inflammation. Leaving this untreated will cause the hair loss to spread giving rise to the condition known as pityriasis type hair loss.

- **Local impairment of circulation due to tension in the scalp**

A loss in flexibility in the scalp will cause a reduction in the flow of blood in the peripheral blood vessels in the subcutaneous tissue of the scalp, adversely affecting hair growth.

3.6.2 Main ingredients in hair growth promoters

Among pharmaceutical agents, which dilate the capillaries, there are those which act on the vascular nervous system such as vitamin E and its derivatives, and those which invigorate the circulation by local stimulation, such as vanilyl amide nonylate.

Some of the pharmaceutical agents used to enhance hair follicle function are hinokitiol and placenta extract. Having a sterilising action, hinokitiol also prevents dandruff. Pharmaceutical agents having an anti-male hormone action are estradiol and estrone, while sulfur, thioxorone and vitamin B6 are used for their anti-seborrhea effect. Having horny layer dissolution and sterilising action, salicylic acid prevents dandruff. Glycyrrhetic acid and its derivatives and menthol are used to prevent inflammation in the scalp. Amino acids, vitamins and crude drug extracts are included in hair growth promoters to supplement the supply of nutrients to the follicles and invigorate enzyme activity (Mitsui, 1997:415).

3.6.3 Formula of the hair spray

The formula of the hair spray is given in Table 3.6.

Table 3.6 Formula of hair spray.

INGREDIENTS	% m/m	ACTIVITY
A. Menthol	6%	Coolant / Preservative
Propylene glycol	10%	Moisturiser / Preservative
Isopropanol	32.5%	Solvent
Eucalyptus oil	1%	Flavouring agent
Peppermint oil	1%	Flavouring agent/Coolant
Urea	10%	Active
Methylsulfonylmethane	10%	Active
Distilled water	To 100%	Solvent

3.6.3.1 Procedure to prepare the formulation

- Dissolve the urea and methylsulfonylmethane in the isopropanol.
- Add the propylene glycol.
- Add the rest of the substances.

3.6.3.2 Outcomes / Findings

A clear solution was obtained, which indicated that all substances dissolved completely. The perfume was smelled and oil drops formed on the surface of the solution. It had a slight smell of menthol, but it was acceptable, since the menthol also served as the preservative and anti-inflammatory agent.

3.7 FORMULATION OF A LIP ICE

3.7.1 Purpose and function of a lip ice

Lip products used to protect, rather than decorate, the lips are known as lip balms, salves or lip ice. They consist of an adherent, flexible, moisture-resistant film of oily substances and no dye. Chap sticks are used to lubricate and smooth dry (chapped) lips (Hunting, 1983:115).

3.7.2 Quality requirements for lip ice

From the point of view of quality, lip ice should satisfy the following requirements:

- It should not cause irritation or harm to the lips;
- It should not have unpleasant taste or odour;

- It should go on smoothly, not smear;
- It should retain their form with no breakage, deforming or softening during storage or use;
- It should neither sweat nor bloom.

3.7.3 Main ingredients in a lip ice

Lip ice consists mainly of an oily base material, such as waxes, that are solid at ordinary temperatures to provide the stick form of the lip ice. The waxes used are natural waxes such as carnauba wax, beeswax and candelilla wax, and mineral waxes such as solid paraffin, microcrystalline wax and other hydrocarbon waxes. Oils used are those that are liquid at ordinary temperatures or whose melting points are around body temperature. Examples are such natural oils as cocoa butter, castor oil and lanolin oil. Castor oil, which has been in use for a very long time, gives the moulded lip ice a proper viscosity. It is also important because it acts as the solvent for the active solid ingredients.

The natural ingredients used have a certain amount of polarity so they help to ensure the stability. Among the often-used oils, the natural waxes have particularly great hardening capacity. Because of such excellent characteristics, natural ingredients are used a lot in lip ice but it is necessary to pay attention to sweating of the lip ice, due to the absorption of moisture by the raw materials, as well as rancidity. Lip ice is mainly composed of oily ingredients like the ones described above and recently, various kinds having a humectant function, have started to be used in consideration of the lips moisture balance. In some lip ice, an emulsion, which directly incorporates water and a humectant in stable proportions, is used in the base formula. In some others, ultraviolet absorbers and reflectors, and other pharmaceutical agents are added to protect the lips against ultraviolet radiation and from drying out (Mitsui, 1997:385).

3.7.4 Formula of a lip ice

The formula of the lip ice is given in Table 3.7.

Table 3.7 Formula of lip ice.

INGREDIENTS	% m/m	ACTIVITY
A. Beeswax	15%	Stiffening agent
Cocoa butter	8%	Emollient
Shea butter	15%	Emollient
B. Propylene glycol	25%	Solvent / Preservative
Vitamin E	25%	Anti-oxidant
Perfume (Strawberry)	10%	Flavouring agent
Urea	1%	Active
Methylsulfonylmethane	1%	Active

3.7.4.1 Procedure to prepare the formulation

- Melt all the substances in A together.
- Dissolve the urea, methylsulfonylmethane and vitamin E in the propylene glycol.
- Mix the two solutions together until homogeneous and add the perfume.
- Pour into moulds.

3.7.4.2 Outcomes / Finding

The lip ice applied easily, it was not too oily nor too hydrous, it had a homogeneous texture and a suitable colour. The lip ice was not too soft or too hard.

3.8 FORMULATION OF A FOOT AND HEEL BALM

3.8.1 Purpose and function of foot care products

Foot care products are for cleaning and treating the feet. One of their purposes is to prevent roughness in the feet. There are two types: a cream type with a strong moisturising effect to prevent the skin drying out; and a roughness preventing, water repellent type (Mitsui, 1997:464).

3.8.2 Main ingredients of foot care products

In order to give the moisturising cream a humectant action, various types of humectants and petrolatum are used in the formula. Silicone oils and silicone polymers are used to give the roughness preventing type its water repellence. Suitable emulsions are the lotion type and the W/O type. In addition to humectants and petrolatum, pharmaceutical agents like urea, anti-inflammatory agents and vitamins should be included in the formula for products used to alleviate conditions in which the roughness has become so bad that the skin is cracking (Mitsui, 1997:465).

3.8.3 Formula of the foot and heel balm

The formula for the foot and heel balm is given in Table 3.8.

Table 3.8 Formula of foot and heel balm

INGREDIENTS	% m/m	ACTIVITY
A. Cremophor A6®	2%	Emulsifying agent
Cremophor A25®	2%	Emulsifying agent
Liquid Paraffin™	2%	Oil phase of emulsion
Sweet oil	3%	Oil phase of emulsion
Cetyl alcohol	7%	Thickening agent
GMS A/S™	4%	Co-emulsifying agent
B. Glicerine	5%	Moisturiser
Urea	5%	Active
Methylsulfonylmethane	5%	Active
Methylparaben	0.3%	Preservative
Propylparaben	0.2%	Preservative
Distilled water	To 100%	Solvent

3.8.3.1 Procedure to prepare the formulation

- Mix A together and melt at 80°C in a glass beaker.
- Mix B together and melt at 80°C in a glass beaker.
- Mix B into A with a homogeniser (Silverson mixer) and cool down to room temperature whilst stirring.

3.8.3.2 Outcomes / Findings

The balm applied easily, it had a homogeneous white/yellow texture, and a pleasant odour.

3.9 MATERIALS USED IN THE FORMULATIONS

The materials used in this study are discussed under the following classifications: active ingredients, solvents, preservatives and others.

3.9.1 Active ingredients

Table 3.9 Active ingredients used in formulations.

ACTIVE INGREDIENTS	SUPPLIER	BATCH NUMBER
Urea	Saarchem (UNIVAR®)	1023102
Methylsulfonylmethane	Brunel Laboratoria	02443

3.9.2 Solvents

A solvent must allow the optimum solubility of the solute. Table 3.10 lists all the solvents that were used in the formulations in this study.

Table 3.10 Solvents used in formulations.

SOLVENT	SUPPLIER	BATCH NUMBER
Distilled water	RIIP	-
Propylene glycol	ACE-Company	15719/5050
Luvitol EHO	BASF	-
Liquid paraffin	ACE-Company	11241/1741
Glycerine	Saarchem (UNIVAR®)	1020133
Isopropanol	Saarchem (UNIVAR®)	-

3.9.3 Preservatives

Table 3.11 lists the preservatives that were used in the formulations in this study.

Table 3.11 Preservatives used in formulations.

PRESERVATIVES	SUPPLIER	BATCH NUMBER
Methylparaben	Galderma	JA 240178
Propylparaben	Galderma	P 14992

3.9.4 Others

Table 3.12 lists all the materials that were used in the formulations in this study, which are neither preservatives nor solvents.

Table 3.12 Other materials used in formulations.

MATERIALS	SUPPLIER	BATCH NUMBER
Cremophor A6®	BASF	-
Cremophor A25®	BASF	-
Carbopol (Ultrez 10)™	BASF	-
Cetyl Alcohol	CRODA	-
GMS A/S™	Link Care	190825
Sweet oil	Riedel-de Haien	402
Tris(hydroxymethyl) aminomethane	Saarchem (UNIVAR®)	81550
Vitamin E acetate	BASF	-
Beeswax	Rebound chemicals	-
Shea butter	CRODA	-
Cocao butter	CRODA	-
Menthol	Saarchem (UNIVAR®)	104567
Peppermint oil	Link Care	13245
Eucalyptus oil	Link Care	5678

3.10 CONCLUSION

Each of the final formulations was prepared in sufficient quantities and stored at different temperatures during stability testing. The appearance and texture of the formulated formulations were acceptable before storage at different temperatures.

Chapter 4 discusses the stability testing that was performed on these newly formulated products. The goal of the stability testing is the selection of the most stable dosage form. Formulators will attempt different formulas, and comparing their stability is one criterion for formula selection (Carstensen, 1990:12).

CHAPTER 4

METHODS FOR STABILITY TESTING

4 INTRODUCTION

The formulation and manufacturing of a new product are not complete without an evaluation of its stability. The Medicines Control Council (MCC) of South Africa requires a written testing program designed to assess stability characteristics of dosage forms. The stability of the active substance and dosage form should be determined during this study (Medicines Control Council, 2003:21).

The goal of a stability program is not uniquely defined, but it depends on the stage of the formulated product in question. In the preclinical formulation phase, the choice of formula is the primary goal (Carstensen, 1990:12).

Stability information from accelerated and long-term testing is to be provided for at least two batches of the same formulation and dosage form, in the containers and closure, proposed for marketing. Long-term and accelerated testing should at least cover three months at the time of submission. The manufacturing process of trial batches should meaningfully simulate that which would be applied to large-scale batches for marketing. The long-term testing will be continued for a sufficient time beyond three months to cover shelf-life at appropriate test periods (Medicines Control Council, 2003:21).

The three months accelerated testing should be carried out at a temperature of at least 15°C above its designated long-term storage temperature (together with appropriate relative humidity conditions for that temperature) (Medicines Control Council, 2003:23).

Both physical and chemical characteristics of the product should be monitored during storage. The following tests must be included in application for registration of new products for all dosage forms (Medicines Control Council, 2003:22).

- Appearance;
- Assay of all actives;
- Degradation, if relevant.

These MCC requirements were followed in the manufacturing and testing of the new formulations, which were developed in this study (see Chapters 5-10).

4.1 STABILITY PROGRAM

Six products, a massage cream, foot and heel balm, moisturising lotion, hair gel, hair spray and lip ice, were formulated during this study, as discussed in Chapter 3. These formulations were stored at three storage temperatures during the three months stability-testing period.

4.1.1 Concentrations

Each of the six formulated products comprised of 5% m/m urea and 5% m/m MSM, except for the hair spray that comprised of 10% m/m urea and 10% m/m MSM and the lip ice that comprised of 2.5% m/m urea and 2.5% m/m MSM.

4.1.2 Storage Temperatures

The physical stability of semi-solid preparations should be determined over a wide range of temperatures. All formulations were stored at three temperatures.

- 5°C – To determine if these formulated products would require keeping in the refrigerator, during winter or summer climate, once on the market (ICH Tripartite Guideline, 2000:4);
- 25°C + 60% RH – To determine the stability of these formulations at room temperature (ICH Tripartite Guideline, 2000:4);
- 40°C + 75% RH – To determine the stability of these formulations, in a tropical climate (ICH Tripartite Guideline, 2000:4).

4.1.3 Stability Tests Conducted

All tests, as required by the South African MCC, were conducted on the formulations of the six dosage forms (see Tables 4.1-4.6). All tests were done using calibrated and / or validated test apparatus, where appropriate.

Table 4.1 Stability tests conducted on the massage cream containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
Urea release (dissolution)	√			√
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
Preservative assay	√	√	√	√
Penetration	√	√	√	√
pH	√	√	√	√
Relative density	√	√	√	√
Visual assessment	√	√	√	√
Spreadability	√	√	√	√
Viscosity	√	√	√	√
Preservative efficacy	√			√

Table 4.2 Stability tests conducted on the foot and heel balm containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
Urea release (dissolution)	√			√
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
Preservative assay	√	√	√	√
Penetration	√	√	√	√
pH	√	√	√	√
Visual assessment	√	√	√	√
Spreadability	√	√	√	√
Preservative efficacy	√			√

Table 4.3 Stability tests conducted on the moisturising lotion containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
Urea release (dissolution)	√			√
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
Preservative assay	√	√	√	√
pH	√	√	√	√
Relative density	√	√	√	√
Visual assessment	√	√	√	√
Viscosity	√	√	√	√
Preservative efficacy	√			√

Table 4.4 Stability tests conducted on the hair gel containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
Urea release (dissolution)	√			√
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
pH	√	√	√	√
Relative density	√	√	√	√
Visual assessment	√	√	√	√
Viscosity	√	√	√	√
Preservative efficacy	√			√

Table 4.5 Stability tests conducted on the hair spray containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
pH	√	√	√	√
Relative density	√	√	√	√
Visual assessment	√	√	√	√
Viscosity	√	√	√	√
Preservative efficacy	√			√

Table 4.6 Stability tests conducted on the lip ice containing urea and MSM.

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Urea concentration assay	√	√	√	√
MSM concentration assay	√	√	√	√
Preservative efficacy	√			√

4.2 TEST METHODS

All tests were done under Good Laboratory Practice (GLP) conditions, in order to ensure the accuracy of the test results being generated over the stability period. The following test methods were used during stability testing of the formulated products:

4.2.1 Urea release with enhancer cell (Dissolution testing)

The release of the active ingredient, MSM was not carried out, because for the purpose of this study the main role of MSM in the formulation is to stabilise urea. The release of the active ingredient, urea, was carried out with the enhancer cell unit, that was used on the VanKel 700 dissolution apparatus (see Figure 4.1). The apparatus used is calibrated semi-annually using USP calibrator tablets, and is checked monthly according to standard operating procedures. The test samples were each carefully transferred into six enhancer cells per dissolution, which were then each covered with a cellulose acetate membrane, with 0.45 μm pore size. The enhancer cells were dropped at fixed intervals in-between, into 200 ml vessels, containing 100 ml of dissolution medium, as soon as the dissolution medium stabilised at 32 ± 0.5°C (see Figure 4.2).

The dissolution medium used was distilled water, the temperature of the dissolution medium was set at 32 ± 0.5°C and the paddles were rotated at 100 rotations per minute (rpm).

1 ml of test sample was withdrawn at 30, 60, 120, 240, and 360 minutes and transferred to HPLC vials and 1 ml ethanol was added to each vial for HPLC analysis.

Standard solutions containing urea were also prepared. The 50 / 50 dissolution medium and ethanol were used as the solvent. 80 mg of urea was weighed, transferred to a 100 ml volumetric flask and filled up to volume with the dissolution medium and ethanol (50 / 50). Aliquots of this solution were further diluted using the dissolution medium to yield

concentrations of 80, 133.3, 200, 400 and 800 μg/ml. These standards were analysed using the HPLC method as described in 4.2.3.1.

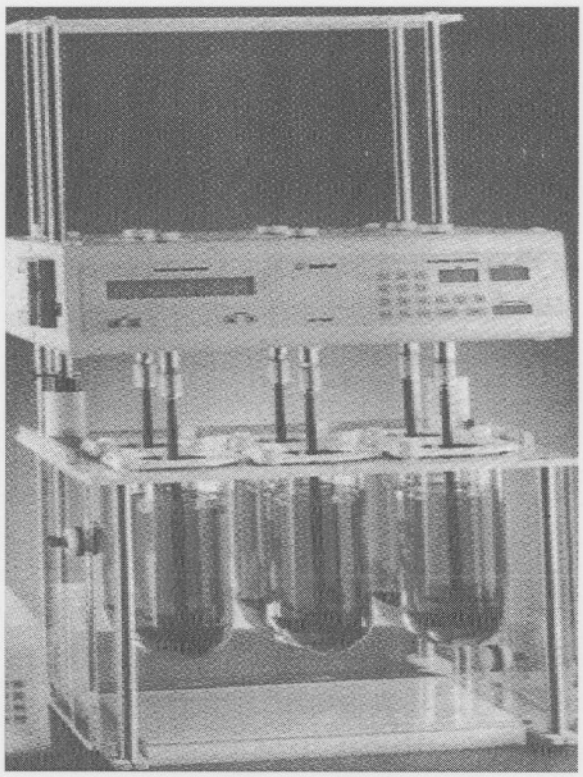
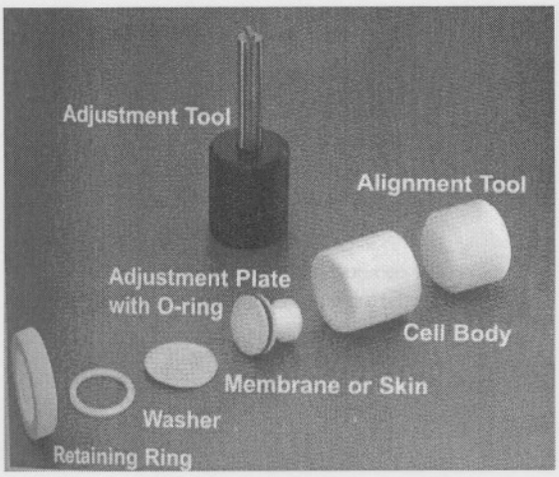
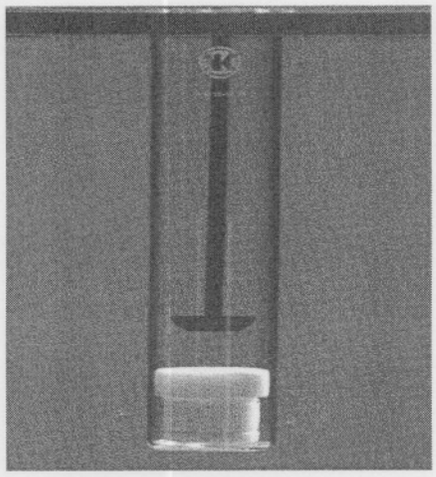


Figure 4.1 VanKel 700 dissolution tester.





A B

Figure 4.2 A represents the enhancer cell and B represents the vessel with a small paddle and enhancer cell.

4.2.2 Urea concentration assay

Methanol was used as solvent for the massage cream, foot and heel balm, hair gel, moisturising lotion and hair spray. The lip ice was dissolved in 40/60 tetrahydrofuran and methanol.

Standard preparation: For the preparation of the standard 50 mg of urea was accurately weighed and transferred to a 50 ml volumetric flask and filled with methanol up to volume. These samples were filtered; 1 ml of each sample was transferred into a HPLC vial and analysed using the HPLC method described in 4.2.3.1.

Sample preparation: Samples were prepared by weighing 1 g of the formulated massage cream, foot and heel balm, moisturising lotion and hair gel containing 5% m/m urea, and 2 g of the formulated lip ice containing 2.5% m/m urea, and 0.5 g of the formulated hair spray containing 10% m/m urea. The samples were transferred to a 50 ml volumetric flask and diluted to 50 ml. All diluted samples were filtered and 1 ml was transferred to HPLC vials.

HPLC analysis was performed (see section 4.2.3.1) to determine the concentration of urea in the formulated product.

Calculation:

$$\frac{Sample\ peak\ area \times Mass\ of\ std.(mg) \times \%\ potency \times 100 \times SG}{Std.\ peak\ area \times 100 \times Mass\ of\ sample(g)} = \%\ \text{m/m}$$

NOTE:RETAIN SAMPLE PREPARATION FOR ANALYSIS OF PRESERVATIVE CONCENTRATION.

4.2.3 High performance liquid chromatography (HPLC)

HPLC analysis was used to determine the urea concentrations in all the formulated products, as well as the concentration released during dissolution testing. The concentrations of the preservatives of the massage cream, foot and heel balm and moisturising lotion were also determined with HPLC.

4.2.3.1 HPLC analysis of urea concentrations

The HPLC parameters used were as follows.

HPLC employing reagents and equipment used:

COLUMN:	Nucleosil NH_2 (Macherey-Nagel), – 4.6×150 mm.
MOBILE PHASE:	Pre-mixed Mix 100 ml orthophosphoric acid solution (0.1% v/v) with 900 ml acetonitrile. For on line solvent mixing instruments **Mobile phase A:** To 1000 ml water, add 1.0 ml phosphoric acid 85% and mix. 10% **Mobile phase B:** Acetonitrile. 90%
FLOW RATE:	0.7 ml/minute
INJECTION VOLUME:	50 µl
TEMPERATURE:	15°C to 25°C
DETECTION:	UV at 200 nm
RETENTION TIME:	7 minutes
RUN TIME:	12 minutes
APPARATUS:	Hewlett Packard 1050 HPLC, equipped with a variable wavelength UV detector, pump, injection device and integrator or recorder, or similar equipment that meets the United States Pharmacopoeia (USP 27, 1659) standards for system suitability.
SOLVENT:	Methanol

The standard and sample solutions containing urea were injected into the chromatograph. Peak area was measured for each standard and sample.

4.2.3.2 HPLC analysis of preservative concentrations

The HPLC parameters used were as follows.

HPLC employing reagents and equipment:

COLUMN:	Nova-pak C18 (Macherey-Nagel), 150×3.9mm
MOBILE PHASE:	Acetonitrile/water 50/50
FLOW RATE:	1.5 ml/min
INJECTION VOLUME:	20 µl
DETECTION:	UV at 254 nm
RETENTION TIME:	±1.4 and 2.3 minutes for methyl- and propylparaben respectively
RUN TIME:	6 minutes
APPARATUS:	Hewlett Packard 1050 HPLC, equipped with a variable wavelength UV detector, pump, injection device and integrator or recorder, or similar equipment that meets the United States Pharmacopoeia (USP 27, 2004:1659) standards for system suitability.
SOLVENT:	Methanol

Standard preparation: 60 mg of the methylparaben was weighed and transferred into a 100 ml volumetric flask . 40 mg of the propylparaben was weighed and transferred into the same 100 ml volemetric flask as the methylparaben and diluted to volume with methanol. 5ml of the diluted sample above were diluted to 50 ml. These samples were filtered, 1 ml of each sample was transferred into a HPLC vial and analysed using the HPLC method described above.

Sample preparation: The sample preparation of massage cream, foot and heel balm and moisturising lotion were used as prepared for urea assay.

Calculation:

$$\frac{Sample\ peak\ area \times Mass\ of\ std.(mg) \times \%\ potency \times 100 \times SG \times 50}{Std.\ peak\ area \times 100 \times Mass\ of\ sample(g) \times 100} = \%\ \text{m/m}$$

4.2.4 Gas chromatography (GC)

GC was used to obtain the MSM concentrations of the formulated products.

The following conditions applied:

COLUMN:	ZB-wax 100% propyleneglycol-30m×0.32mm ID×0.25µm FT
COLUMN TEMPERATURE:	160°C
INJECTION TEMPERATURE:	220°C
DETECTOR TEMPERATURE:	220°C
INJECTION VOLUME:	0.5ml

Standard preparation: 50 mg of the MSM was weighed and transferred into a 50 ml volumetric flask and diluted to volume with chloroform.

Internal Standard preparation: 3 g of decanol was weighed and transferred into a 50 ml volumetric flask and diluted to volume with chloroform. 10 ml of the diluted sample was then diluted to 50 ml with chloroform. 1 ml of the internal standard was transferred to each of the sample preparations.

Sample preparation: Samples were prepared by weighing 1 g of the formulated massage cream, foot and heel balm, moisturising lotion and hair gel containing 5% m/m MSM, and 2 g of the formulated lip ice containing 2.5% m/m MSM, and 0.5 g of the formulated hair spray containing 10% m/m MSM. The samples were transferred to a 50 ml volumetric flask; 1 ml

of internal standard was added and diluted to 50 ml with chloroform. All diluted samples were filtered and 1 ml was transferred to GC vials. GC analysis was performed (see section 4.2.4) to determine the concentration of MSM in the formulated product.

Calculation:

$$\frac{Sample\ peak\ area \times Mass\ of\ std.(mg) \times Theoretical\ mass\ of\ sample \times 5}{Std.\ peak\ area \times Theoretical\ mass\ of\ std. \times Mass\ of\ sample(g)} = \%\ \text{m/m}$$

4.2.5 pH

The pH meter used was calibrated with buffer solutions of pH 4 and 7. The pH was measured using the Metrohm Autotitrator 785 DMP Titrino (see Figure 4.3) for the massage cream, foot and heel balm and moisturising lotion. The Mettler Toledo MP 220 pH meter was used to measure the pH of the hair spray and hair gel.

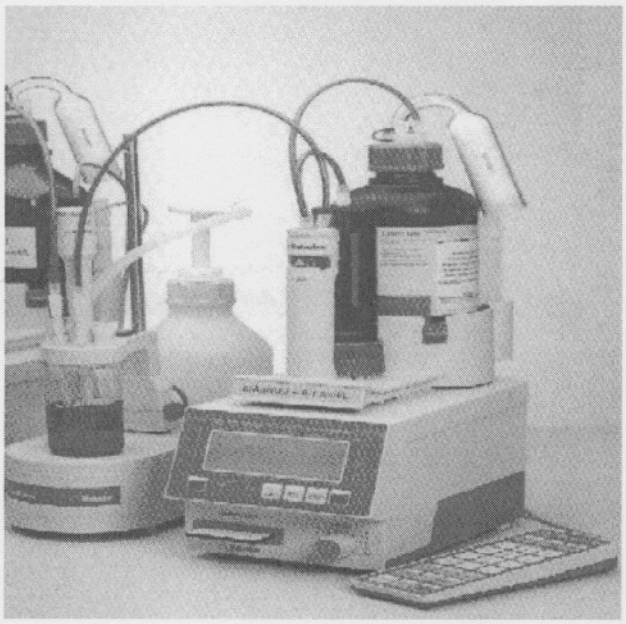


Figure 4.3 Metrohm Autotitrator 785 DMP Titrino.

4.2.6 Relative Density

The relative density of a substance is the ratio of the mass of a given volume of the substance to the mass of an equal volume of water, both weighed at 20°C (BP, 2003:2079).

The polytop method was used to measure the relative density of the massage cream, foot and heel balm and moisturising lotion.

A clean dry polytop was used and weighed; 5 ml of water was transferred to the polytop and weighed. A mark was made at the meniscus of the water. Each ploytop was filled with the product to volume, and weighed.

Calculation:

$$\frac{Mass\ of\ polytop + water - Mass\ of\ emty\ polytop}{Mass\ of\ polytop + sample - Mass\ of\ emty\ polytop} = Density\ (g/ml)$$

The apparatus used to measure the relative density of the hair spray and hair gel was the Anton Paar DMA 38 Density/Specific Gravity/Concentration meter. This meter was calibrated before testing.

4.2.7 Viscosity

Rheology is the science of the flow of matter and its study begins with gathering data on fluid's viscosity – its resistance to flow caused by its internal friction. Knowledge of a material's rheological characteristics is valuable to predict its pourability, its performance in a dipping or coating operation, or the ease with which it may be handled, processed or used.

Viscosity data provides an accurate reference point in the formulation of materials, facilitating the achievement of consistency from batch to batch (Brookfield, 1998:2).

The viscosity was determined on a Brookfield Model DV –II+ viscometer (Figure 4.4) with Brookfield small sample adapter. The Brookfield small sample adapter (Figure 4.5) was used for its rheological evaluation of materials where sample volume is limited, the sample chamber can be easily changed, and the flow jacket allows temperature control and simultaneous sample temperature measurement (Brookfield, 1998:22).

Figure 4.4 Brookfield Model DV –II+ viscometer.

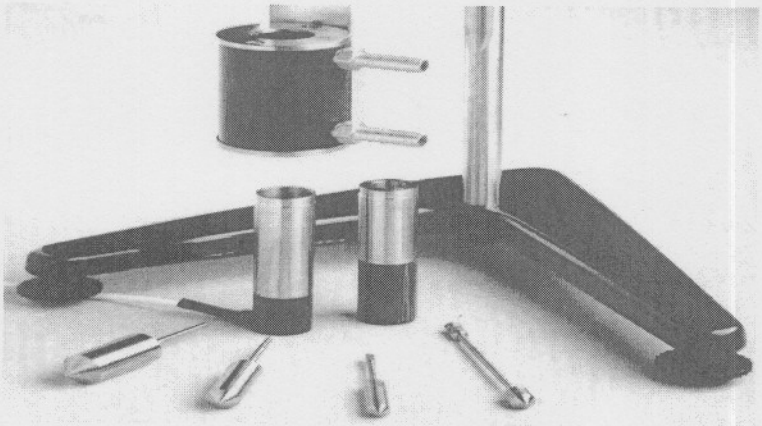


Figure 4.5 Brookfield small sample adapter.

The operator calibrated the viscometer every three months. Spindle number 25 was used. The sample was transferred into the sample chamber which fitted into a flow jacket so that precise temperature control could be achieved, the sample was left to stand for 1 hour to assure that no air bubbles were present. The stirring action of the rotating spindle, plus the small sample volume helped to keep the temperature gradient across the sample to a minimum. Direct readout of sample temperature was provided using sample chambers with embedded RTD sensors connected to the Brookfield Digital Temperature Indicator (DTI). All the experiments were conducted at a temperature of 25°C at initial, 1, 2 and 3 months.

4.2.8 Spreadability

The apparatus used consisted of two glass plates, of which the one plate was clear, whereas the other had a scaled 1 mm incremented grid fixed underneath. The sample was transferred into a syringe, fitted with a clean rubber tube (6 mm in diameter) to the syringe tip. The scaled glass plate was put on a balance and tarred (reading zero), after which a 10 mm long sample was carefully squeezed onto the plate, and the mass recorded. The glass plate was then placed on a level and secure surface, and the clear glass plate was then put on the sample, with a 100 g brass weight on top of that. It was left for 60 seconds and the longest sample diameter was measured with a Vernier caliper.

4.2.9 Appearance

A visual assessment of each stored trial batch was carried out once a month. Colour, odour and texture were examined.

4.2.10 Penetration

Penetration is a measurement of consistency and was done using a penetrometer. The penetrometer consists of a stand and penetrating object, a device to check that the base is horizontal and a scale showing the depth of penetration at 0.1 mm increments. The samples were transferred into 500 g containers and left to stand for 24 hours to allow air bubbles to escape. Three measurements were taken with a twenty-four hour lapse between measurements.

4.2.11 Preservative efficacy

Methylparaben and propylparaben were used as preservatives in the massage cream, moisturising lotion and foot and heel balm. Other preservatives, like propylene glycol, were used in the other formulations. The samples were sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2. This test determined the ability of these preservatives to work individually against preventing contamination of the formulated product.

Sources of contamination can be divided into three groups (Devleeschouwer & Siguet, 2001:781):

- The microbiological quality of raw materials, including water;
- The manufacturing process; and
- The galenical form of the product.

In this study microorganisms with the ideal growth and temperature characteristics as well as pathogenic nature were used. These organisms were also chosen according to the stipulations given by the USP (USP 27, 2004:1853) in conjunction with the methods of testing.

Growth characteristics and requirements:

1. Fungi used:

- ***Aspergillius niger***

Family: Deutromycetes

Morphology: Black hyphae

Temperature requirements: 20°C-28°C (optimum)

pH requirements: 4.4 – 4.6

- ***Candida albicans 354***

Family: Cryptococcus

Morphology: Cocci

Temperature requirements: 20°C - 35°C (optimum of 25°C)

pH requirements: 4.4 – 4.6

2. Bacteria used:

- ***Escherichia coli 20***

Family: Enterobacteriaceae

Morphology: Straight rod

Temperature requirements: 28°C - 37°C (optimum)

pH requirements: 6.0 – 7.0

- ***Pseudomonas auruginosa 13***

Family: Pseudomona daceae (Prokaryote)

Morphology: Bacillius

Temperature requirements: 4°C - 43°C (optimum)

pH requirements: 6.6 – 7.0

- ***Staphylococcus aureus 10***

Family: Micrococcaceae (Prokaryote)

Morphology: Cocci

Temperature requirements: 6.5°C - 46°C (optimum of 37°C)

pH requirements: 4.2 – 9.3

Identification of growth

The following colour characteristics were used for the detection of the colonies on the TS agar plates:

- *Aspergillus niger* – Black, furry-like colonies or mats;
- *Candida albicans* – Small, white, slightly raised colonies;
- *Escherichia coli* – Rigid, cream colonies;
- *Pseudomonas auriginosa* – Light greenish, rounded colonies
- *Staphylococcus aureus* – White, rounded colonies.

It is essential to determine which colony growth on the agar plate is true growth and which is contamination. Note that this colour identification is applicable only to TS agar, used in preservative efficacy tests.

It is generally accepted that adequate preservation of a finished product, with preservatives or based on active preservation of a formulation, implies that the product remains stable and safe during storage (shelf-life) and consumer use. From a public-health point of view, preservation must avoid infection of the consumer, and for product-quality reasons it must prevent a deterioration of the preparation. It is especially important to point out that the use of preservatives must not mask a lack of hygiene during manufacturing. It is thus imperious to manufacture any cosmetic product according to Good Manufacturing Practices (GMPs), (Devleeschouwer & Siquet, 2001:782).

The use of the word "antimicrobial" preservatives raises the need to define exactly what kind of activity is needed for a preservative. The organisms of concern are: bacteria, fungi, viruses, and even spores. The scale of the activity spectrum is based on three parameters: (1) the survival, or even multiplication, of particular organisms in a wide range of products; (2) the pathogenicity of these organisms by the route of administration; and (3) the possibility to find effective chemicals at non-toxic concentrations (Devleeschouwer & Siquet, 2001:783).

For the purpose of testing, compendial articles have been divided into four categories. The criteria of antimicrobial effectiveness for these products are a function of the route of administration. Topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes are classified as category 2 (USP 27, 2004:1899).

The requirements for antimicrobial effectiveness are met if the criteria for category 2 products are met. The products were sent to the University of the Witwatersrand and tests were carried out according to guidelines given by the USP 27 for Category 2 products (USP 27, 2004:1899) (Table 4.7).

Table 4.7 Criteria for tested microorganisms (Category 2) (USP 27, 2004:1899).

Bacteria	Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days.
Yeast and moulds	No increase from the initial calculated count at 7, 14, 28 days.

4.3 CONCLUSION

The outcomes of the test procedures, described in this chapter, are discussed and represented graphically in Chapters 5 – 10. The data that was generated during testing for each of the six formulations will be dealt with separately in these chapters. In each chapter conclusions and possible relationships will be drawn from the data generated.

CHAPTER 5

RESULTS AND DISCUSSION

MASSAGE CREAM

5 INTRODUCTION

Body moisturisers have two primary functions. The traditional view of the function of moisturisers is that they alleviate pre-existing dry skin and prevent its return (Epstein & Simon, 2001:518).

The following parameters of the formulated massage cream were investigated: pH, relative density, appearance, viscosity, urea assay, MSM assay, preservative content, spreadability, penetration and preservative efficacy. All of the tests that are discussed in 5.1 - 5.11 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 4.

5.1 pH

The pH of the massage cream, measured over three months, is given in Table 5.1.

Table 5.1 pH of the massage cream measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.79		
25°C/60%RH	7.36	7.40	7.62	7.14
40°C/75%RH		11.16	11.14	11.12

5.1.1 Discussion

The pH of the massage cream stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. Urea can decompose into carbon dioxide and ammonia. This can cause the pH to increase to values as high as 11 (Beiersdorf, 2003:1). This volatilisation is affected by temperature and moisture. Higher temperature and moisture increase volatilisation (Anon, 2003:20). Urea compositions can be stabilized when they contain methylsulfonylmethane (MSM) (Herschler, 1981:1).

5.2 RELATIVE DENSITY

The relative density of the massage cream, measured over three months, is given in Table 5.2.

Table 5.2 Relative density (g/ml) of the massage cream measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		0.982		
25°C/60%RH	1.003	1.006	1.000	1.005
40°C/75%RH		0.932	0.998	0.989

5.2.1 Discussion

There were no significant changes in the relative density of the massage cream (Table 5.2). Temperature, moisture and pH didn't have any influence on the relative density.

5.3 SPREADABILITY

Spreadability test results show how easily the cream is applied to the skin. The spreadability of the cream was determined once a month for three months as described in Chapter 4 and given in Table 5.3.

Table 5.3 Spreadability (mm) of the massage cream measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		50.48		
25°C/60%RH	47.18	47.33	45.13	47.83
40°C/75%RH		43.13	48.91	47.16

5.3.1 Discussion

There were no significant changes in spreadability over the three months test intervals.

5.4 PENETRATION

Penetration is a useful way to determine if phase separation has occurred. The penetration of the cream was determined once a month for three months as described in Chapter 4 and given in Table 5.4.

Table 5.4 Penetration (mm) of the cream measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		29.34		
25°C/60%RH	29.87	30.89	29.36	29.69
40°C/75%RH		31.03	29.09	29.36

5.4.1 Discussion

There was no significant change in the penetration over the three months test intervals.

5.5 APPEARANCE

The massage cream is a white cream with no odour. It spreads smoothly and no grittiness was observed. There was no change in the odour or colour of the massage cream after the three months.

5.6 VISCOSITY

The higher the viscosity, the better the droplets of the dispersed phase are held in place. Viscosity is a measurement of "thickness" and flow properties and any changes in viscosity will directly influence the formulated product (Schueller & Romanowski, 2000:149). The viscosity results are given in Table 5.5.

Table 5.5 The viscosity (in cP) of the massage cream measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		2150		
25°C/60%RH	2254	2048	1979	1897
40°C/75%RH		2150	1973	1509

5.6.1 Discussion

The viscosity of the cream decreased over the three months stability test intervals (see table 5.5). The decrease in viscosity can be explained as a reaction between some ingredients or perhaps the cream was still settling before it went into the final resting stage. A large decrease in viscosity is a warning of instability.

5.7 UREA ASSAY

The concentration of urea in the massage cream was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 5.6 and graphically in Figure 5.1.

Table 5.6 The concentration (%) of urea in the massage cream measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.32%		
25°C/60%RH	100.45%	100.41%	99.36%	98.59%
40°C/75%RH		102.36%	99.22%	98.32%

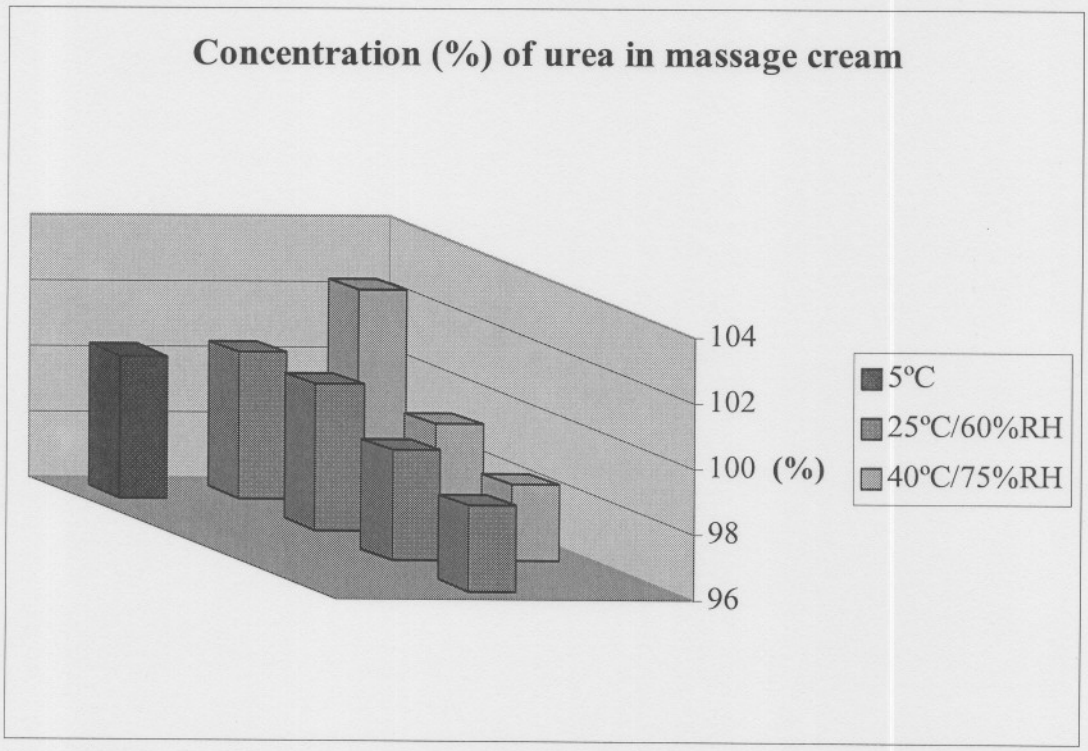


Figure 5.1 Concentration (%) of urea in the massage cream measured over three months.

5.7.1 Discussion

The concentration of urea in the formulated massage cream decreased with time, especially in the sample that was stored at 40°C/75%RH. This decrease however was not significant.

5.8 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the massage cream was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 5.7 and graphically in Figure 5.2.

Table 5.7 The concentration (%) of MSM in the massage cream measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.16%		
25°C/60%RH	103.73%	103.54%	102.19%	100.45%
40°C/75%RH		103.75%	100.82%	100.34%

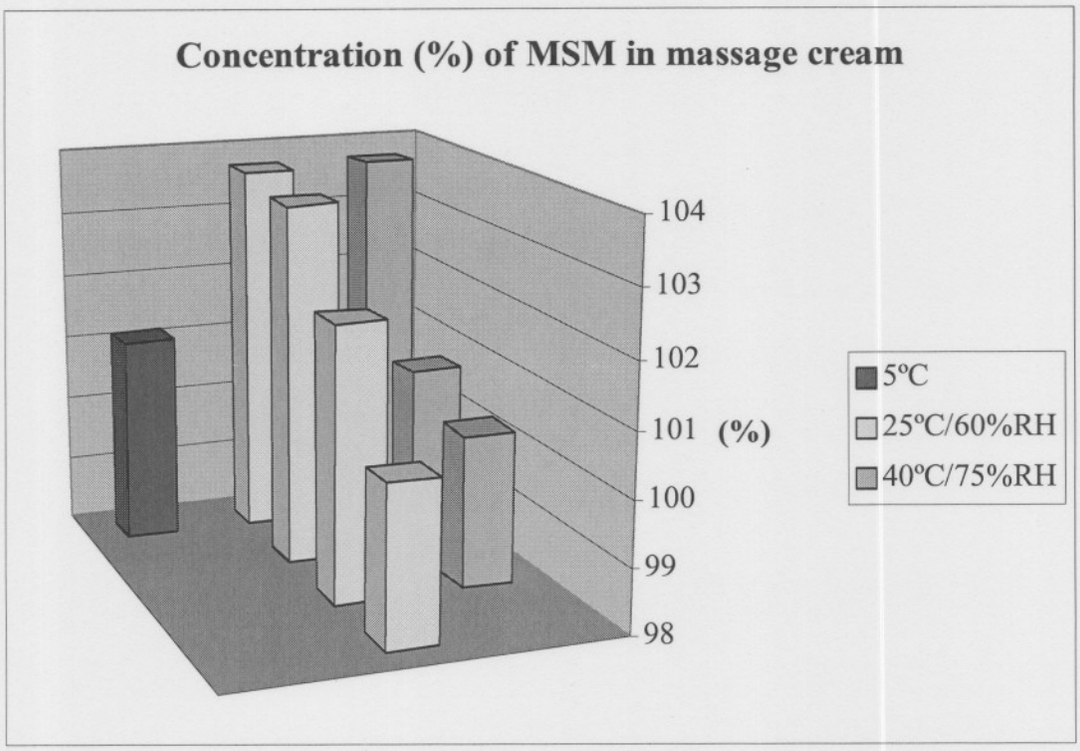


Figure 5.2 Concentration of MSM measured over the three months intervals in the massage cream.

5.8.1 Discussion

The concentration of MSM in the formulated massage cream did not show any significant change.

5.9 METHYL- AND PROPYLPARABEN ASSAY

Preservatives are added to cosmetics to suppress the proliferation of microorganisms, which have contaminated them, and to kill them in time, thereby preventing deterioration of the product (Mitsui, 1997:201). The methylparaben and propylparaben concentrations of the massage cream were determined at the initial and then monthly for 3 months, using HPLC chromatography as described in Chapter 4. The results of the methyl- and propylparaben assay are given in Table 5.8 and 5.9 and graphically in Figure 5.3 and 5.4.

Table 5.8 The concentration (%) of methylparaben in the massage cream measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		99.85%		
25°C/60%RH	103.73%	103.00%	102.45%	101.27%
40°C/75%RH		102.64%	102.17%	101.20%

Table 5.9 The concentration (%) of propylparaben in the massage cream measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.57%		
25°C/60%RH	101.52%	101.41%	101.48%	101.60%
40°C/75%RH		99.49%	100.12%	100.59%

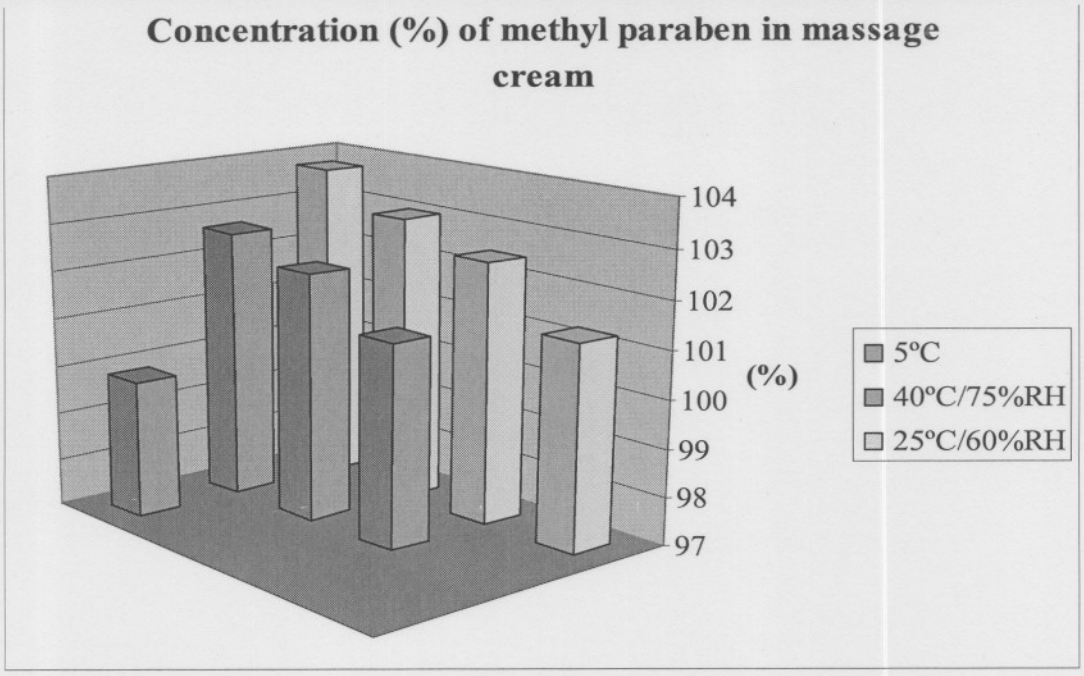


Figure 5.3 Concentration (%) of methylparaben in the massage cream.

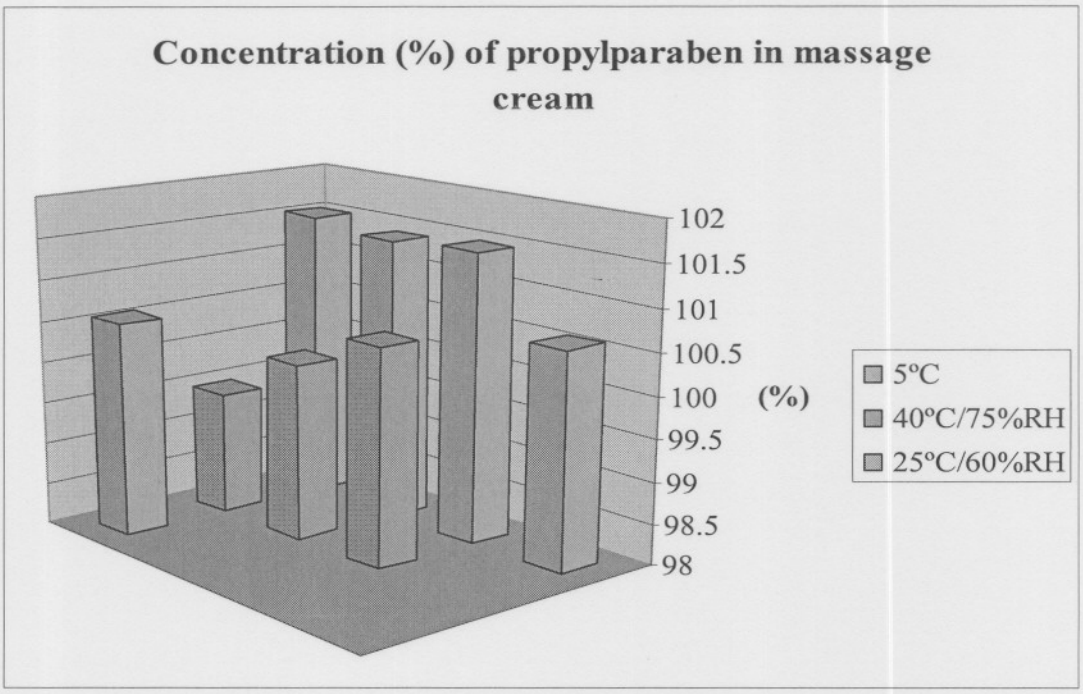


Figure 5.4 Concentration of propylparaben measured over the three months interval in the massage cream.

5.9.1 Discussion

The concentrations of methylparaben and propylparaben in the formulated massage cream did not show any significant change. The inconsistency of the results could be due to experimental variation and sample manipulation.

5.10 PRESERVATIVE EFFICACY

The massage cream was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 5.10 and Table 5.11).

Table 5.10 Preservative efficacy results of the massage cream (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 5.11 Preservative efficacy results of the massage cream (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

5.10.1 Discussion

The preservative efficacy of the massage cream complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the massage cream against microbial contamination.

5.11 UREA RELEASE

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved.

5.11.1 Concentration of urea released from the massage cream

This dissolution test was performed to prove that urea was released from the massage cream formulation. The concentration of urea released from the massage cream was determined at initially and after three months storage at 25°C/60%RH and 40°C/75%RH.

The concentration of urea released from the massage cream (initial) as a function of time is given in Table 5.12 and Figure 5.5.

Table 5.12 The concentration (μg/sqcm) of urea released from the massage cream (initial) as a function of time.

Time (minutes)	Concentration (μg/sqcm)
30	3347.12
60	3743.52
120	4349.94
240	4991.18
360	5452.49

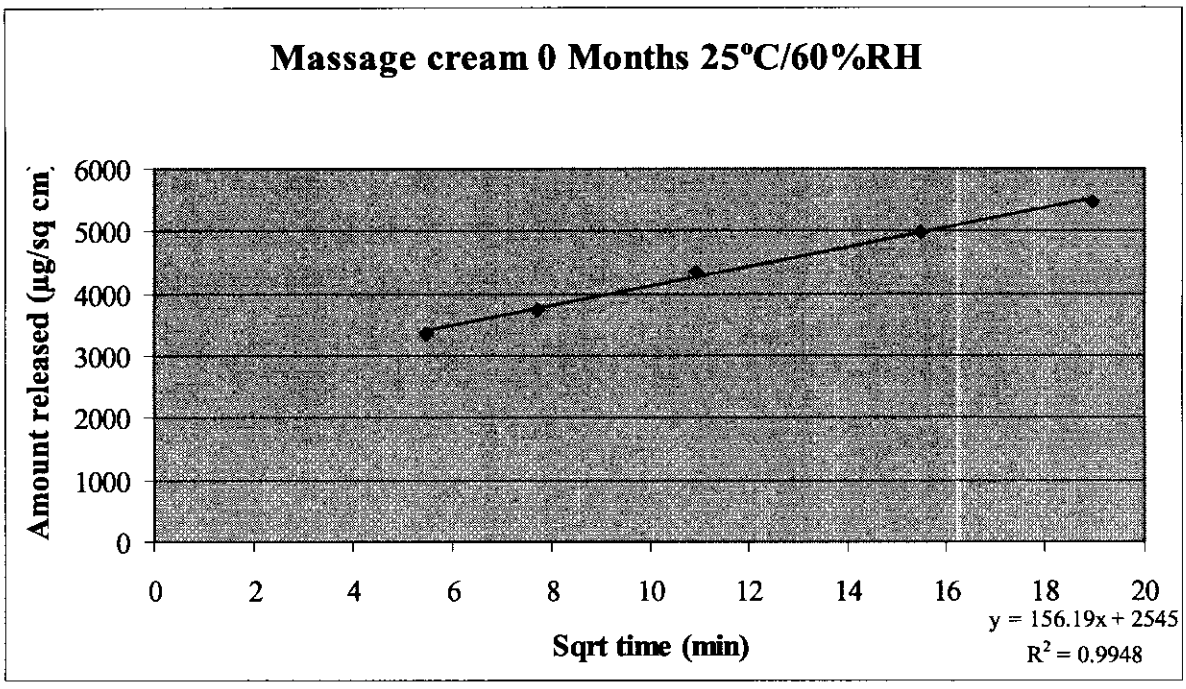


Figure 5.5 The concentration of urea released from the massage cream at initial.

The concentration of urea that was released from the massage cream after three months at 25°C/60%RH and 40°C/75%RH as a function of time is given in Table 5.13 and Figure 5.6 and Table 5.14 and figure 5.7 respectively.

Table 5.13 The concentration (µg/sqcm) of urea released from the massage cream (three months) at 25°C/60%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	2646.73
60	3329.59
120	4132.01
240	5323.66
360	6440.99

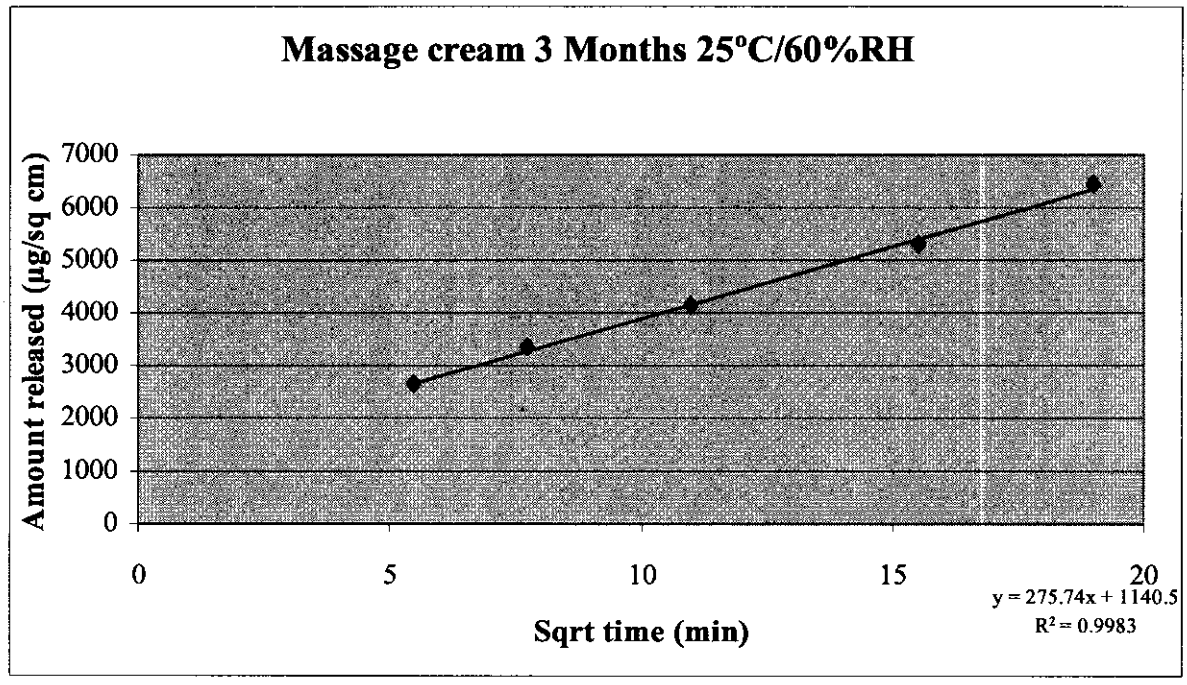


Figure 5.6 The concentration of urea released from the massage cream at 3 months 25°C/60%RH.

Table 5.14 The concentration (µg/sqcm) of urea released from the massage cream (three months) at 40°C/75%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	2929.67
60	4214.07
120	6776.10
240	9585.38
360	12295.34

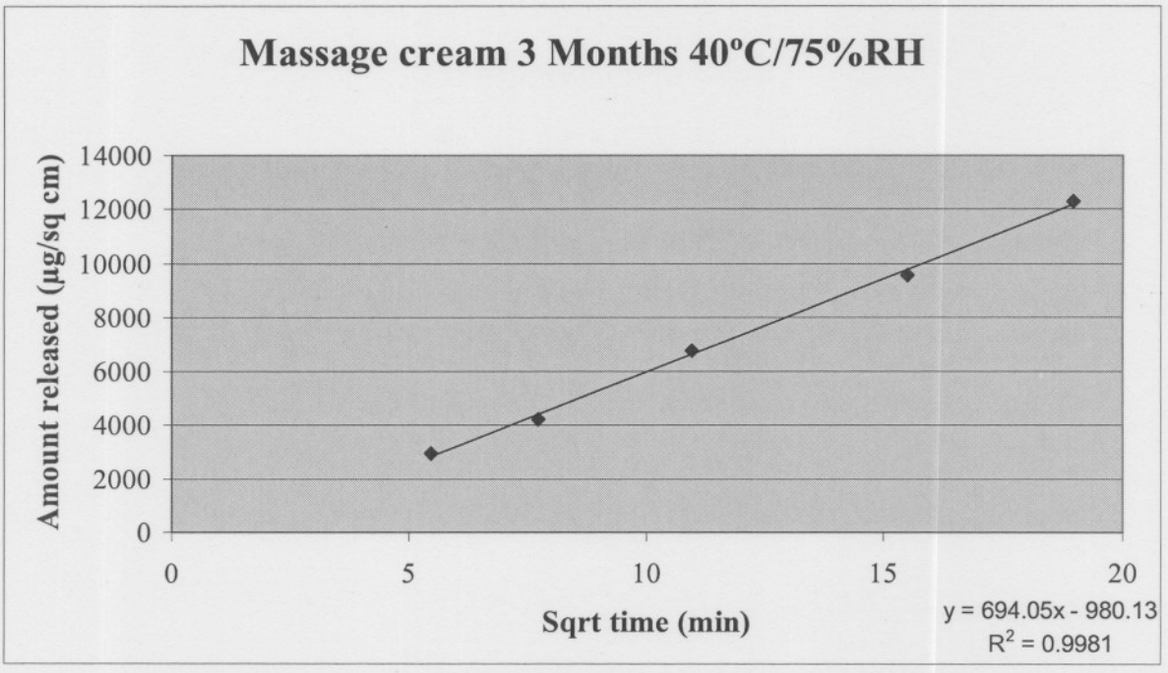


Figure 5.7 The concentration of urea released from the massage cream at 3 months 40°C/75%RH.

The release of urea from the massage cream is graphically given in Figure 5.8.

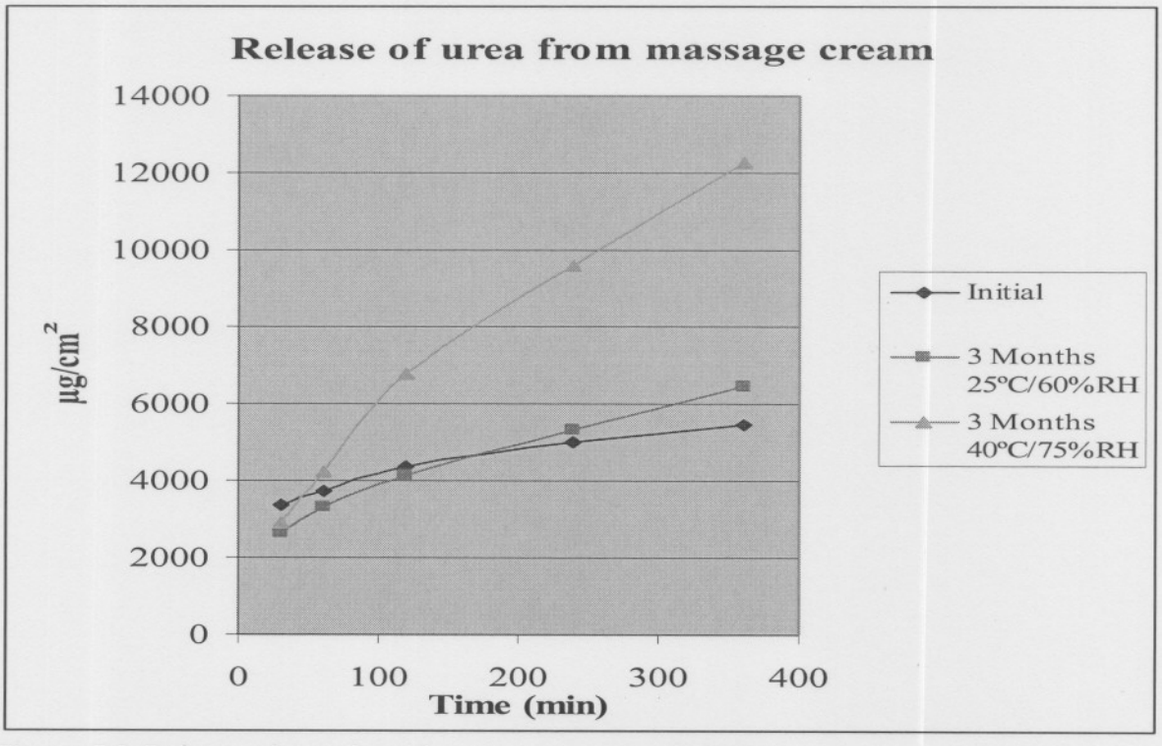


Figure 5.8 Release of urea from the massage cream against time.

5.11.2 Discussion

According to theory a plot of μg urea released per cm^2 membrane against the square root of time in minutes should produce a straight line. This is indeed the case for the massage cream. The release of urea over time was much higher in the sample stored at 40°C/75%RH for three months than the initial sample and the sample stored at 25°C/60%RH. This could be due to a decrease in the viscosity of the massage cream over the three months period at 40°C/75%RH.

5.12 CONCLUSION

In this Chapter, the formulated massage cream was tested over a three month period, using an extensive range of stability indicative tests. All the tests showed good results. A comparison of the different test results generated during the test procedures indicates that this formulated massage cream has good marketing potential.

CHAPTER 6

RESULTS AND DISCUSSION

FOOT AND HEEL BALM

6 INTRODUCTION

The following parameters of the formulated foot and heel balm were investigated: pH, relative density, spreadability, penetration, appearance, urea assay, MSM assay, preservative content, urea release rate (dissolution), and preservative efficacy. All the tests were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 4.

6.1 pH

The pH of the foot and heel balm, measured over three months, is given in Table 6.1.

Table 6.1 pH of the foot and heel balm measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.10		
25°C/60%RH	7.32	8.37	7.85	8.86
40°C/75%RH		7.75	10.35	11.31

6.1.1 Discussion

The pH of the foot and heel balm stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. Refer to discussion in 5.1.1.

6.2 RELATIVE DENSITY

The relative density of the foot and heel balm, measured over three months, is given in Table 6.2

Table 6.2 Relative density (g/ml) of the foot and heel balm measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		0.9002		
25°C/60%RH	0.9718	1.0263	0.9163	0.9082
40°C/75%RH		1.0799	0.8991	0.8589

6.2.1 Discussion

According to the results in Table 6.2, there was a slight decrease in the relative density with time.

6.3 SPREADABILITY

Spreadability test results show how easily the foot and heel balm is applied to the feet. The spreadability of the balm was determined once a month for three months as described in Chapter 4 and given in Table 6.3.

Table 6.3 Spreadability (mm) of the foot and heel balm measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		39.96		
25°C/60%RH	43.18	41.02	37.32	35.45
40°C/75%RH		39.17	36.41	37.76

6.3.1 Discussion

According to the results in Table 6.3, there was a slight decrease in spreadability with time.

6.4 PENETRATION

Penetration is a useful way to determine if phase separation has occurred. The penetration of the foot and heel balm was determined once a month for three months as described in Chapter 4 and given in Table 6.4.

Table 6.4 Penetration (mm) of the foot and heel balm measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		17.19		
25°C/60%RH	16.76	16.99	17.32	18.20
40°C/75%RH		16.75	16.26	16.55

6.4.1 Discussion

There was no significant change in the penetration over the three months test intervals.

6.5 APPEARANCE

The foot and heel balm is a white cream with no odour. It spreads smoothly and no grittiness was observed. A light yellow colour appeared after three months at 40°C/75%RH.

6.6 UREA ASSAY

The concentration of urea in the foot and heel balm was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 6.5 and graphically in Figure 6.1.

Table 6.5 The concentration (%) of urea in the foot and heel balm measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		99.82%		
25°C/60%RH	101.97%	101.16%	101.15%	100.03%
40°C/75%RH		104.33%	100.24%	100.85%

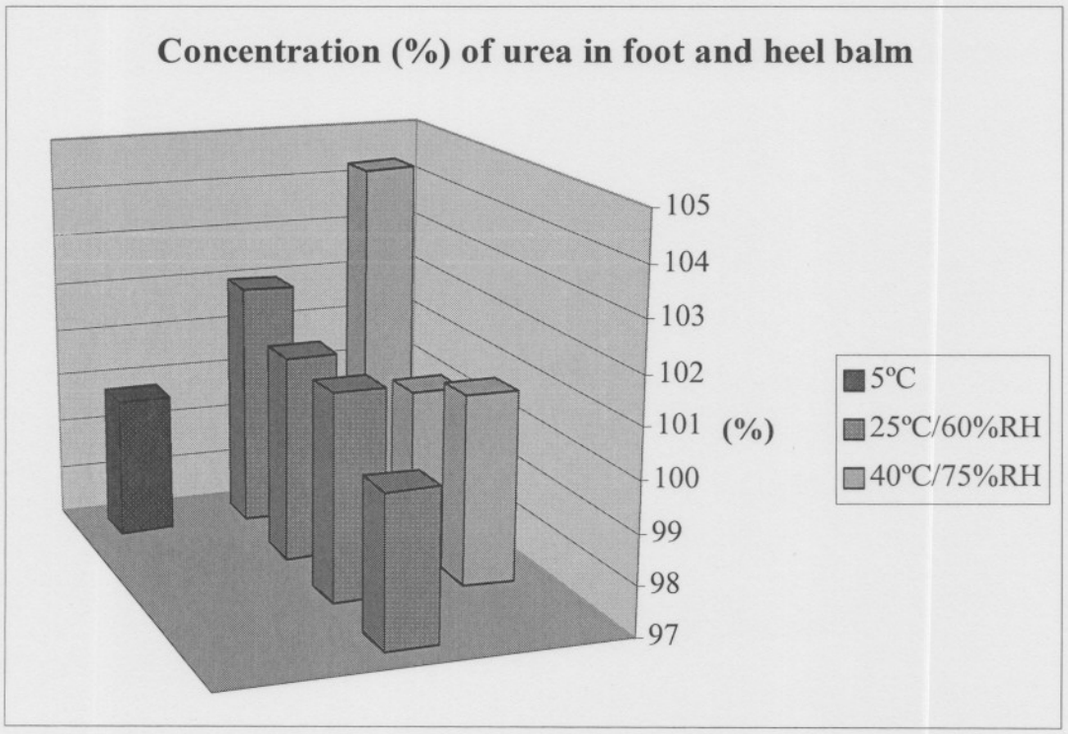


Figure 6.1 Concentration of urea measured over the three month interval in the foot and heel balm.

6.6.1 Discussion

There were no significant changes in the urea concentration over time in the foot and heel balm. The high value at 1 month 40°C/75%RH could be due to experimental variations.

6.7 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the foot and heel balm was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in table 6.6 and graphically in Figure 6.2.

Table 6.6 The concentration (%) of MSM in the foot and heel balm measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		103.54%		
25°C/60%RH	104.23%	104.49%	103.52%	100.54%
40°C/75%RH		102.36%	100.42%	100.20%

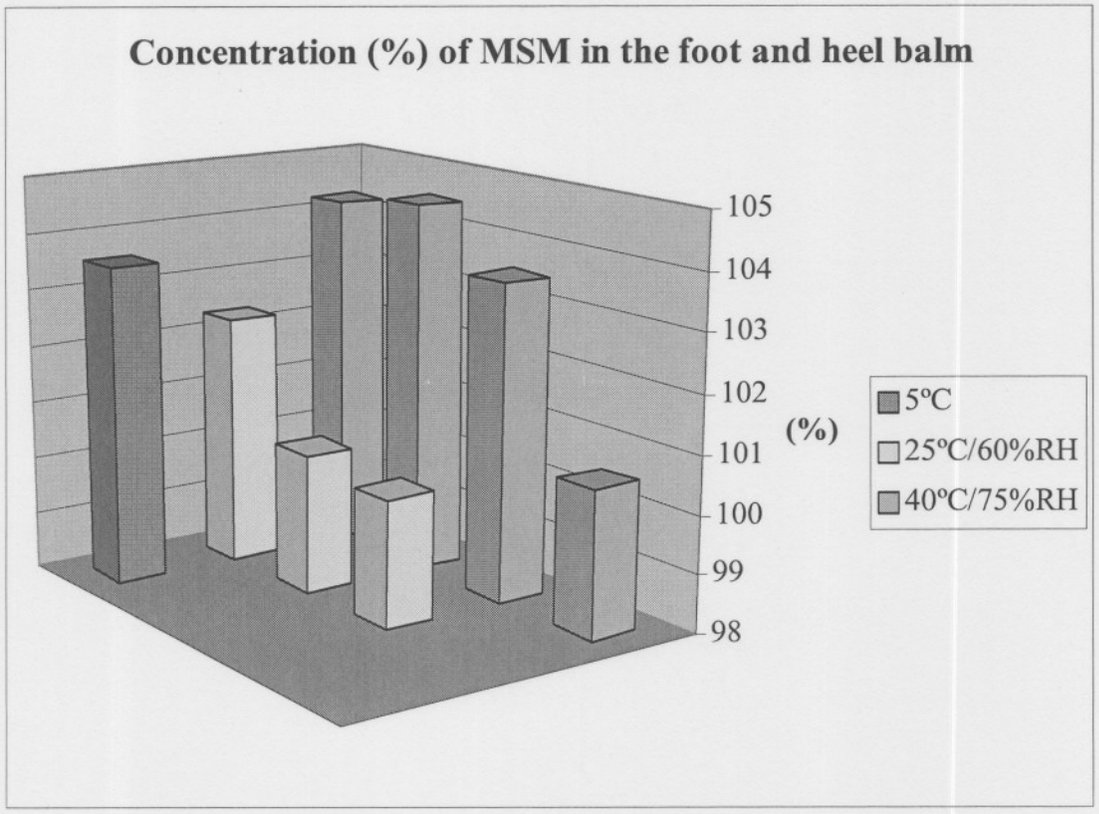


Figure 6.2 Concentration of MSM measured over the three months intervals in the foot and heel balm.

6.7.1 Discussion

The concentration of MSM in the formulated foot and heel balm did not show any significant change.

6.8 METHYL- AND PROPYLPARABEN ASSAY

Preservatives are added to cosmetics to suppress the proliferation of microorganisms, which have contaminated them, and to kill them in time, thereby preventing deterioration of the product (Mitsui, 1997:201). The methylparaben and propylparaben concentrations of the foot and heel balm were determined at the initial and then monthly for 3 months, using HPLC

chromatography as described in Chapter 4. The results of the methyl- and propylparaben assay are given in Table 6.7 and 6.8 and graphically in Figure 6.3 and 6.4.

Table 6.7 The concentration (%) of methylparaben in the foot and heel balm measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.63%		
25°C/60%RH	99.65%	100.11%	101.17%	101.62%
40°C/75%RH		101.07%	101.01%	100.67%

Table 6.8 The concentration (%) of propylparaben in the foot and heel balm measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.48%		
25°C/60%RH	101.38%	102.45%	100.49%	101.18%
40°C/75%RH		103.80%	102.16%	100.50%

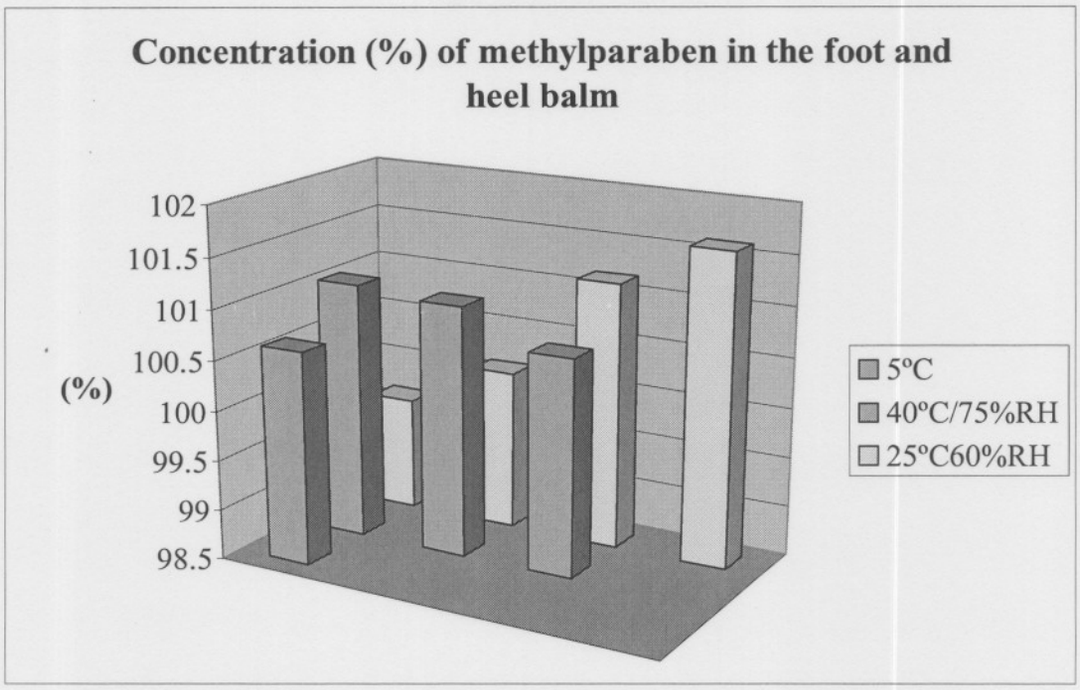


Figure 6.3 Concentration (%) of methylparaben in the foot and heel balm.

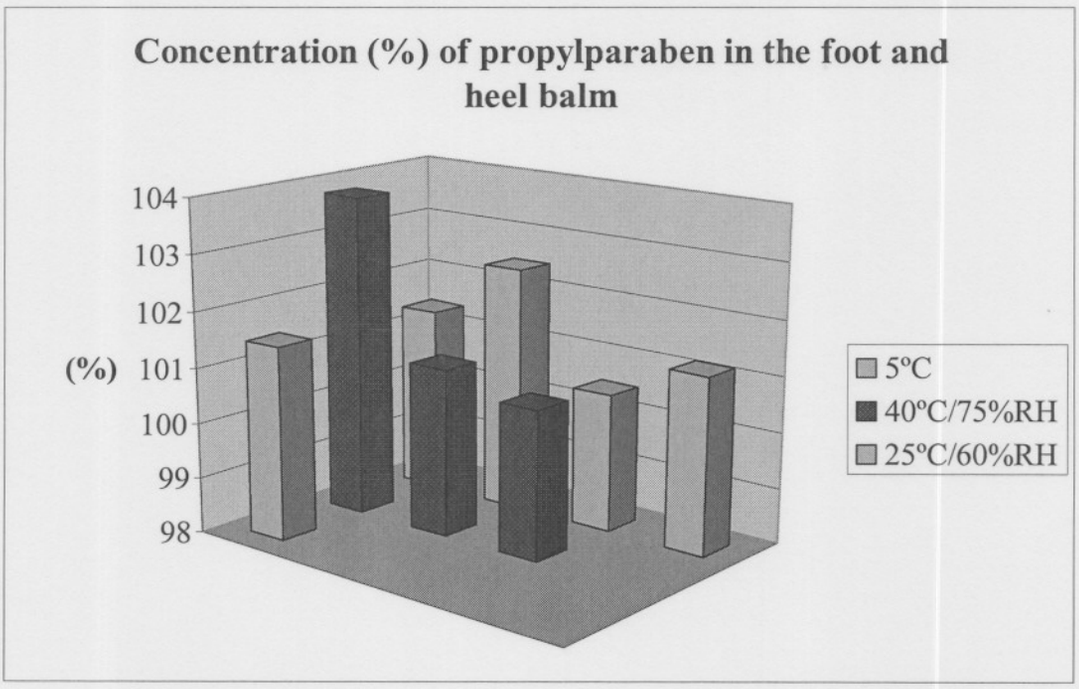


Figure 6.4 Concentration (%) of propylparaben in the foot and heel balm.

6.8.1 Discussion

The concentrations of methylparaben and propylparaben in the formulated foot and heel balm did not show any significant change. The inconsistency of the results could be due to experimental variation and sample manipulation.

6.9 PRESERVATIVE EFFICACY

The foot and heel balm was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 6.9 and Table 6.10).

Table 6.9 Preservative efficacy results of the foot and heel balm (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 6.10 Preservative efficacy results of the foot and heel balm (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

6.9.1 Discussion

The preservative efficacy of the foot and heel balm complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the foot and heel balm against microbial contamination.

6.10 UREA RELEASE

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved.

6.10.1 Concentration of urea released from the foot and heel balm

This dissolution test was performed to prove that urea was released from the foot and heel balm formulation. The concentration of urea that was released from the foot and heel balm was determined initial and after three months storage at 25°C/60%RH and 40°C/75%RH.

The concentration of urea, released from the foot and heel balm (initial), as function of time is given in Table 6.11 and Figure 6.5.

Table 6.11 The concentration (μg/sqcm) of urea released from the foot and heel balm (initial) as a function of time.

Time (minutes)	Concentration (μg/sqcm)
30	-799.88
60	-446.35
120	228.15
240	1190.03
360	2061.55

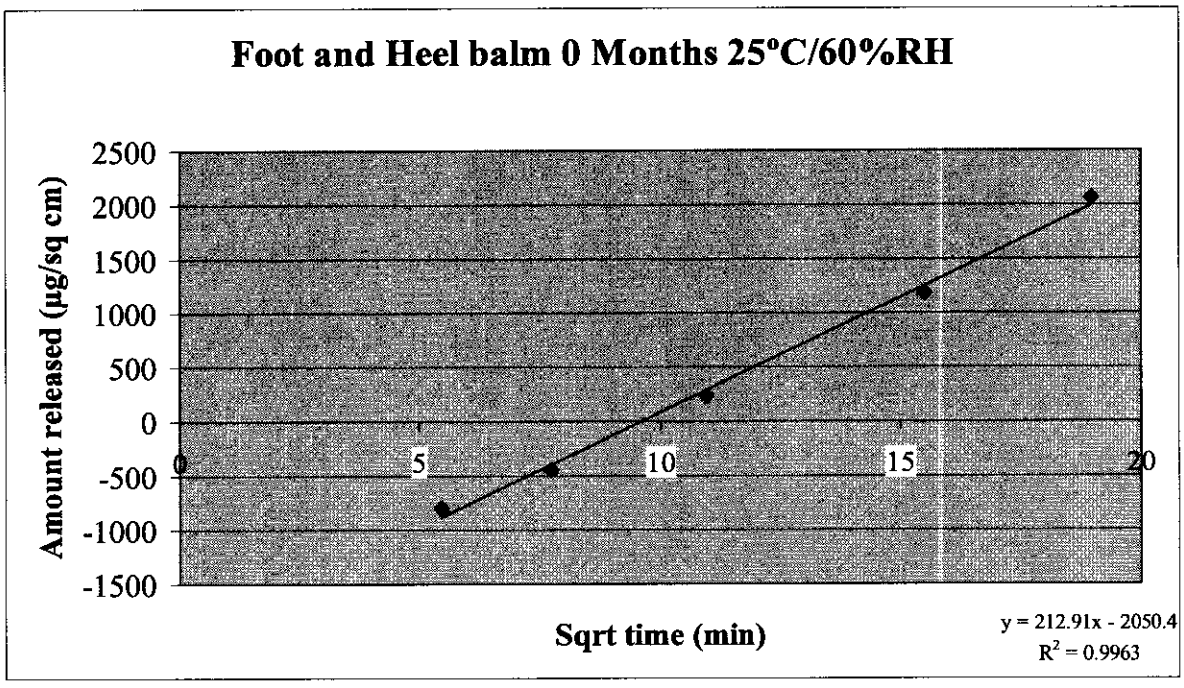


Figure 6.5 The concentration release of urea in the foot and heel balm (initial) at 25°C/60%RH.

The concentration of urea released from the foot and heel balm after three months at 25°C/60%RH and 40°C/75%RH as function of time is given in Table 6.12

and Figure 6.6 and Table 6.13 and Figure 6 .7 respectively.

Table 6.12 The concentration (µg/sqcm) of urea released from the foot and heel balm (three months) at 25°C/60%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	1945.04
60	2374.68
120	3335.58
240	4463.03
360	5358.85

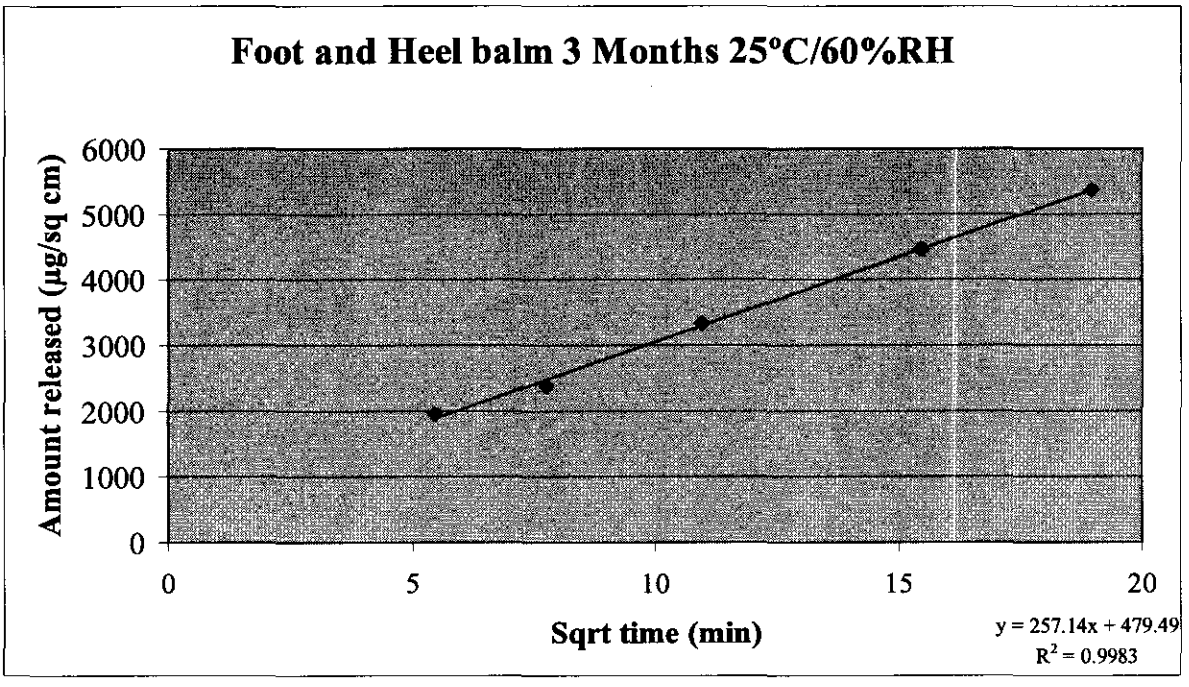


Figure 6.6 The concentration of urea released from the foot and heel balm at 3 months 25°C/60%RH.

Table 6.13 The concentration (µg/sqcm) of urea released from the foot and heel balm (three months) at 40°C/75%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	1524.51
60	1790.65
120	2504.70
240	3224.70
360	3947.48

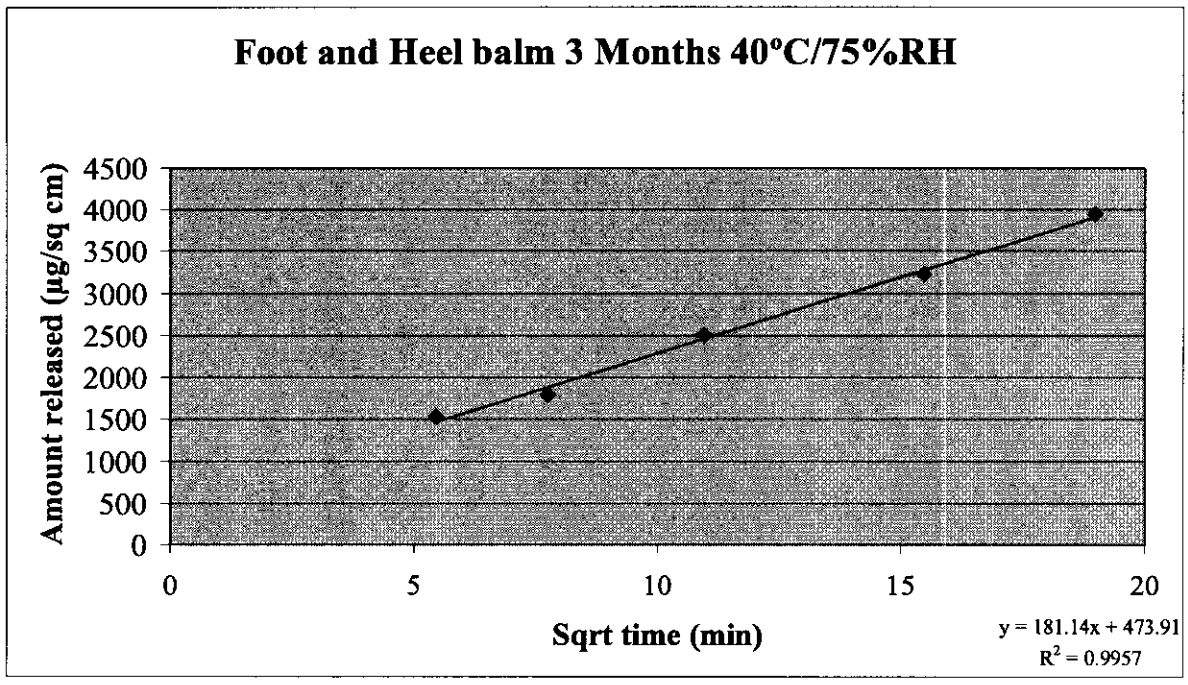


Figure 6.7 The concentration of urea released from the foot and heel balm at 3 months 40°C/75%RH.

The release of urea from the foot and heel balm is graphically given in Figure 6.8.

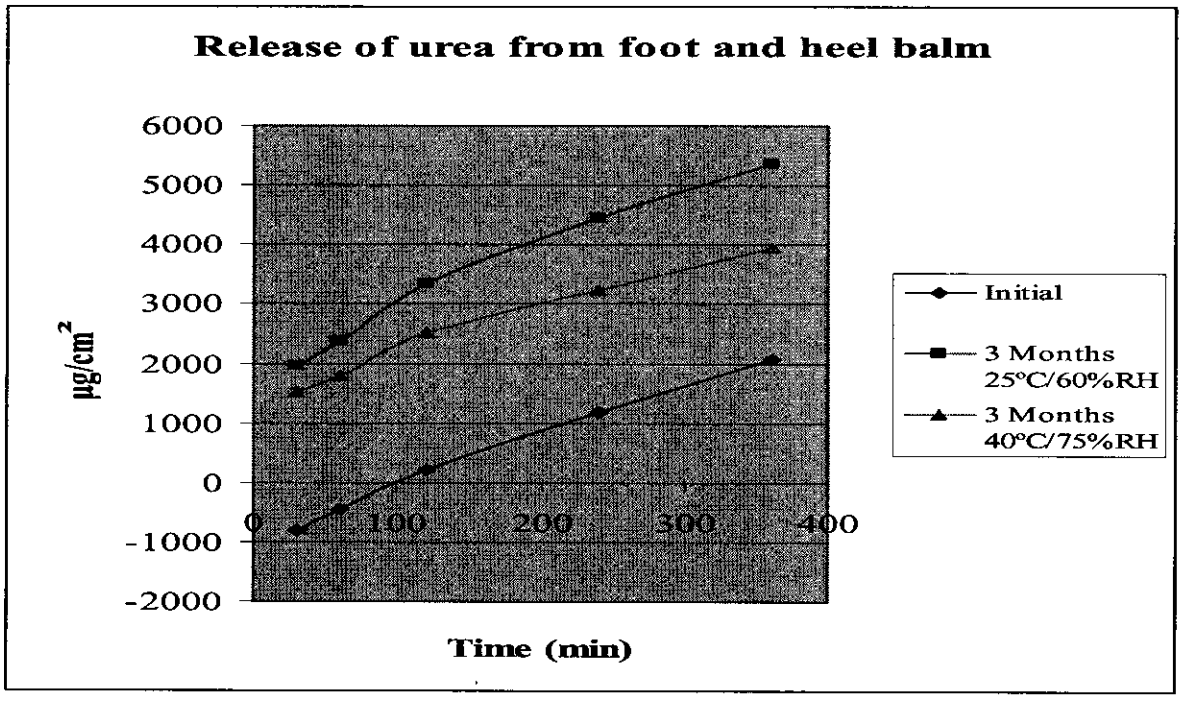


Figure 6.8 Release of urea from foot and heel balm against time.

6.10.2 Discussion

According to theory a plot of μg urea released per cm^2 membrane against the square root of time in minutes should produce a straight line. This is indeed the case for the foot and heel balm. The release of urea from the foot and heel balm was much higher after three months than initially, this could be due to a decrease in the viscosity.

6.11 CONCLUSION

In this chapter the formulated foot and heel balm was tested over a three-month period, using an extensive range of stability indicative tests. According to Claasen, C.I.: the concentration of urea in the foot and heel balm decreased drastically over time especially in the sample stored at 40°C/75%RH. In this study, where MSM was included in the same formulation there was no significant change in the urea concentation over the three months. This could be due to the stabilising effect of MSM on urea containing products (Herschler, 1981:1) by inhibiting spontaneous carbamide decomposition. A comparison of the different test results generated during the test procedures indicated that this formulated foot and heel balm has good marketing potential.

CHAPTER 7

RESULTS AND DISCUSSION

MOISTURISING LOTION

7 INTRODUCTION

The following parameters of the formulated moisturising lotion were investigated: pH, relative density, appearance, viscosity, urea assay, MSM assay, preservative content, urea release rate (dissolution), and preservative efficacy. All the tests were performed according to the requirements of the MCC under GLP conditions, as discussed in Chapter 4.

7.1 pH

The pH of the moisturising lotion, measured over three months, is given in Table 7.1.

Table 7.1 pH of the moisturising lotion measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.27		
25°C/60%RH	7.19	7.40	9.75	9.50
40°C/75%RH		8.14	10.66	11.16

7.1.1 Discussion

The pH of the moisturising lotion stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. See discussion in section 5.1.1.

7.2 RELATIVE DENSITY

The relative density of the moisturising lotion, measured over three months, is given in Table 7.2.

Table 7.2 Relative density of the moisturising lotion, measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		0.9414		
25°C/60%RH	0.9215	1.0355	0.9270	1.0426
40°C/75%RH		0.9328	0.9447	0.9994

7.2.1 Discussion

The big variation in the relative density results could be due to experimental variation.

7.3 APPEARANCE

The moisturising lotion is a white lotion with no odour. It spreads smoothly and no grittiness was observed. There was no change in colour, texture and odour after the three months.

7.4 VISCOSITY

Viscosity is a measurement of "thickness" and flow properties and any changes in viscosity will directly influence the formulated product (Schueller & Romanowski, 2000:149). The viscosity results for the moisturising lotion are given in Table 7.3.

Table 7.3 The viscosity (in cP) of the moisturising lotion measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		20821		
25°C/60%RH	19968	18773	21163	10189
40°C/75%RH		18432	19285	10086

7.4.1 Discussion

The viscosity of the lotion remained stable up to 2 months, thereafter a sharp decrease (see Table 7.3). The decrease in viscosity can be explained as a reaction between some ingredients or perhaps the lotion was still settling before it went into the final resting stage. A large decrease in viscosity is a warning of instability.

7.5 UREA ASSAY

The concentration of urea in the moisturising lotion was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 7.4 and graphically in Figure 7.1.

Table 7.4 The concentration (%) of urea in the moisturising lotion measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		98.69%		
25°C/60%RH	105.76%	105.23%	103.34%	102.88%
40°C/75%RH		104.26%	104.02%	103.37%

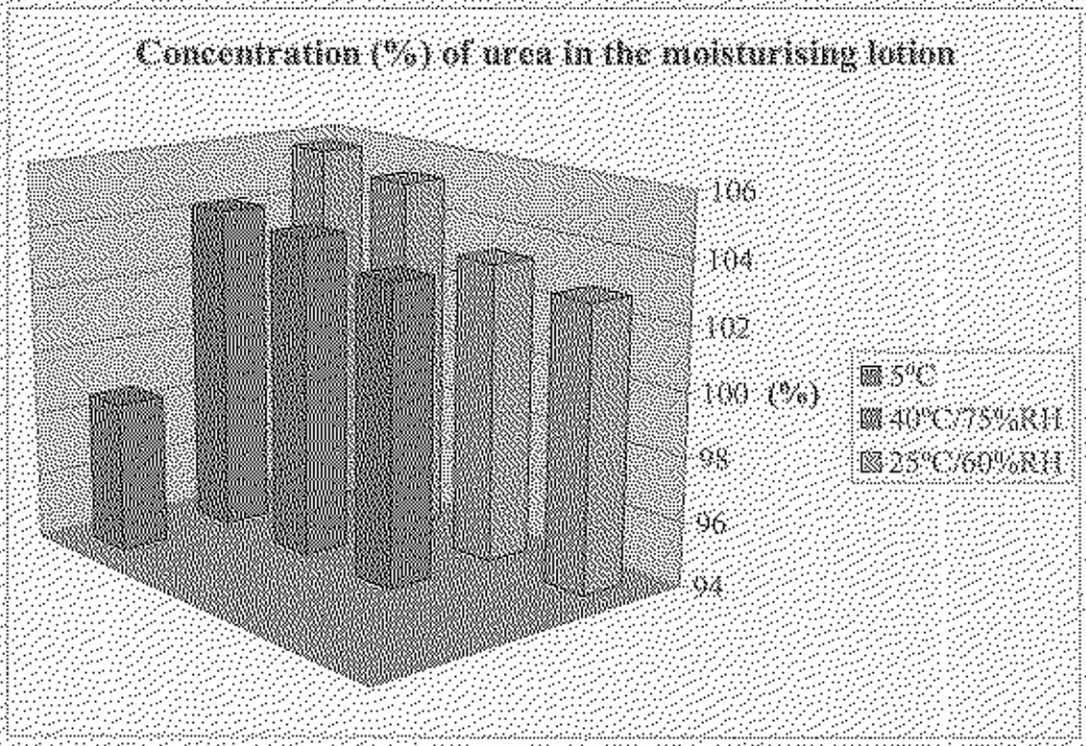


Figure 7.1 Concentration of urea measured over the three months interval in the moisturising lotion.

7.5.1 Discussion

There was no significant change in the urea concentration over time.

7.6 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the moisturising lotion was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 7.5 and graphically in Figure 7.2.

Table 7.5 The concentration (%) of MSM in the moisturising lotion measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		95.06%		
25°C/60%RH	100.73%	100.43%	99.53%	98.03%
40°C/75%RH		99.99%	98.59%	98.77%

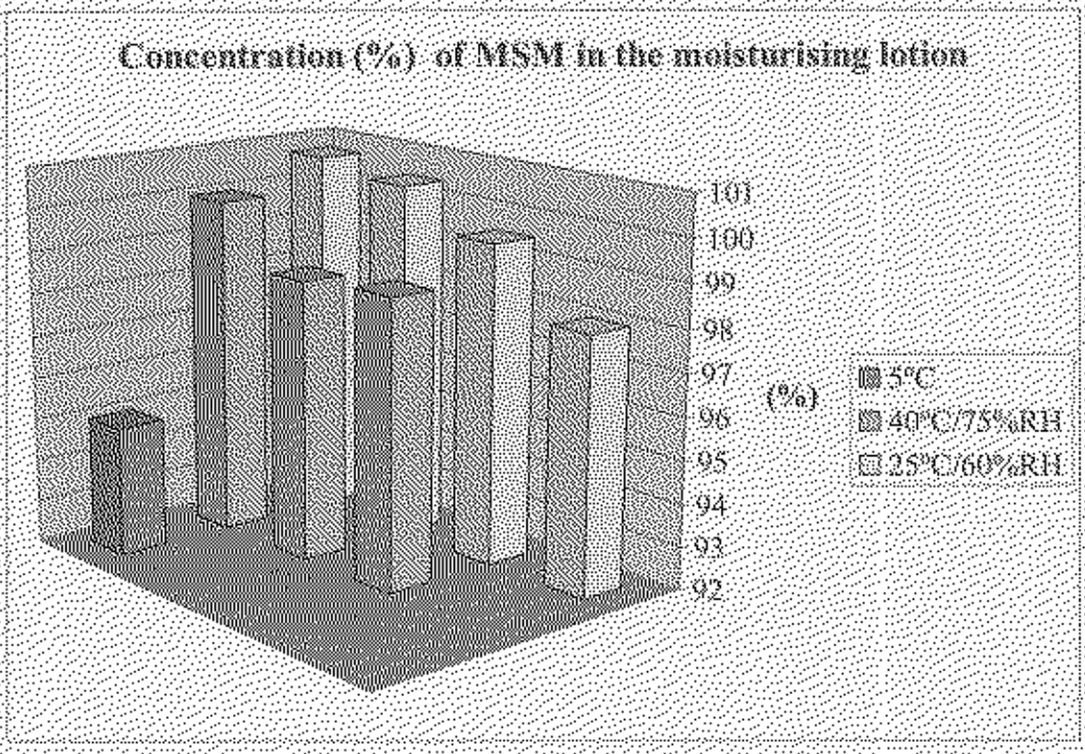


Figure 7.2 Concentration of MSM measured over the three months intervals in the moisturising lotion.

7.6.1 Discussion

The concentration of MSM in the formulated moisturising lotion did not show any significant change with time.

7.7 METHYL- AND PROPYLPARABEN ASSAY

Preservatives are added to cosmetics to suppress the proliferation of microorganisms, which have contaminated them, and to kill them in time, thereby preventing deterioration of the product (Mitsui, 1997:201). The methylparaben and propylparaben concentrations of the moisturising lotion were determined at the initial and then monthly for 3 months, using HPLC chromatography as described in Chapter 4. The results of the methyl- and propylparaben assay are given in Table 7.6 and 7.7 and graphically in Figure 7.3 and 7.4.

Table 7.6 The concentration (%) of methylparaben in the moisturising lotion measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.35%		
25°C/60%RH	98.70%	100.02%	99.98%	100.30%
40°C/75%RH		97.75%	97.61%	96.50%

Table 7.7 The concentration (%) of propylparaben in the moisturising lotion measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.80%		
25°C/60%RH	98.84%	98.59%	97.58%	97.06%
40°C/75%RH		96.95%	96.38%	97.50%

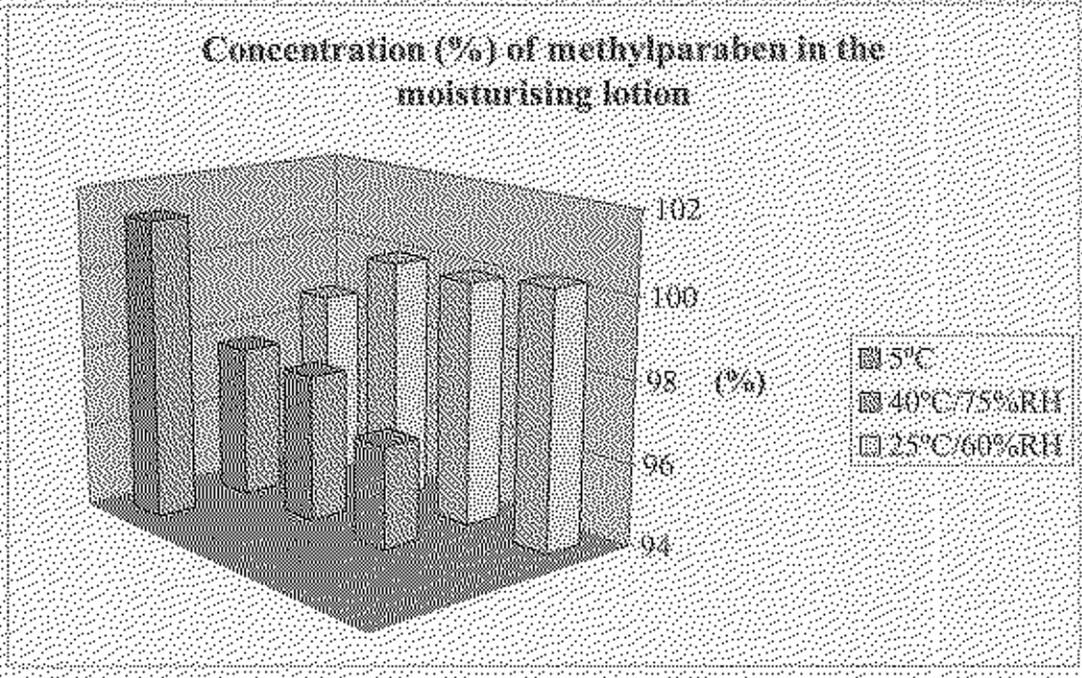


Figure 7.3 Concentration (%) of methylparaben in the moisturising lotion.

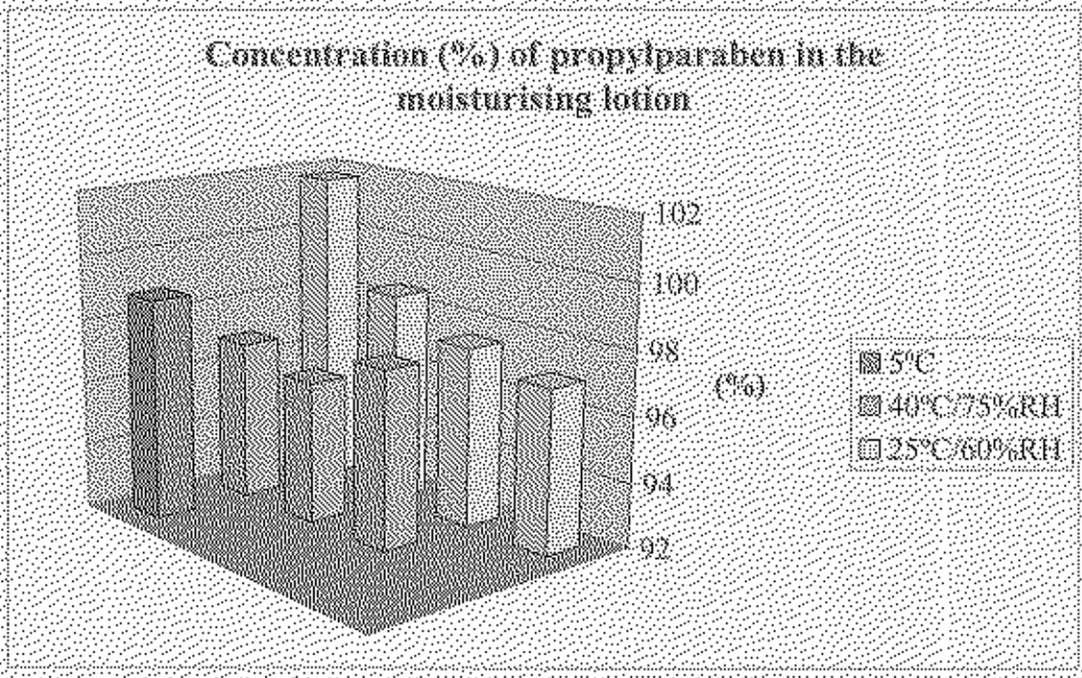


Figure 7.4 Concentration (%) of propylparben in moisturising lotion.

7.7.1 Discussion

The concentrations of methylparaben and propylparaben in the formulated moisturising lotion did not show any significant change. The inconsistency of the results could be due to experimental variation and sample manipulation.

7.8 PRESERVATIVE EFFICACY

The moisturising lotion was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 7.8 and Table 7.9).

Table 7.8 Preservative efficacy results of the moisturising lotion (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 7.9 Preservative efficacy results of the moisturising lotion (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Samples complies with the requirements of USP 27.

7.8.1 Discussion

The preservative efficacy of the moisturising lotion complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the lotion against microbial contamination.

7.9 UREA RELEASE

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved.

7.9.1 Concentration of urea released from the moisturising lotion

This dissolution test was performed to prove that urea was released from the moisturising lotion formulation. The concentration of urea that was released from the moisturising lotion was determined at initial and after three months storage at 25°C/60%RH and 40°C/75%RH.

The concentration of urea, released from the moisturising lotion (initial), as function of time, is given in Table 7.10 and Figure 7.5.

Table 7.10 The concentration (μg/sqcm) of urea released from the moisturizing lotion (initials) as a function of time.

Time (minutes)	Concentration (μg/sqcm)
30	1921.74
60	1921.74
120	2476.17
240	3157.37
360	3802.49

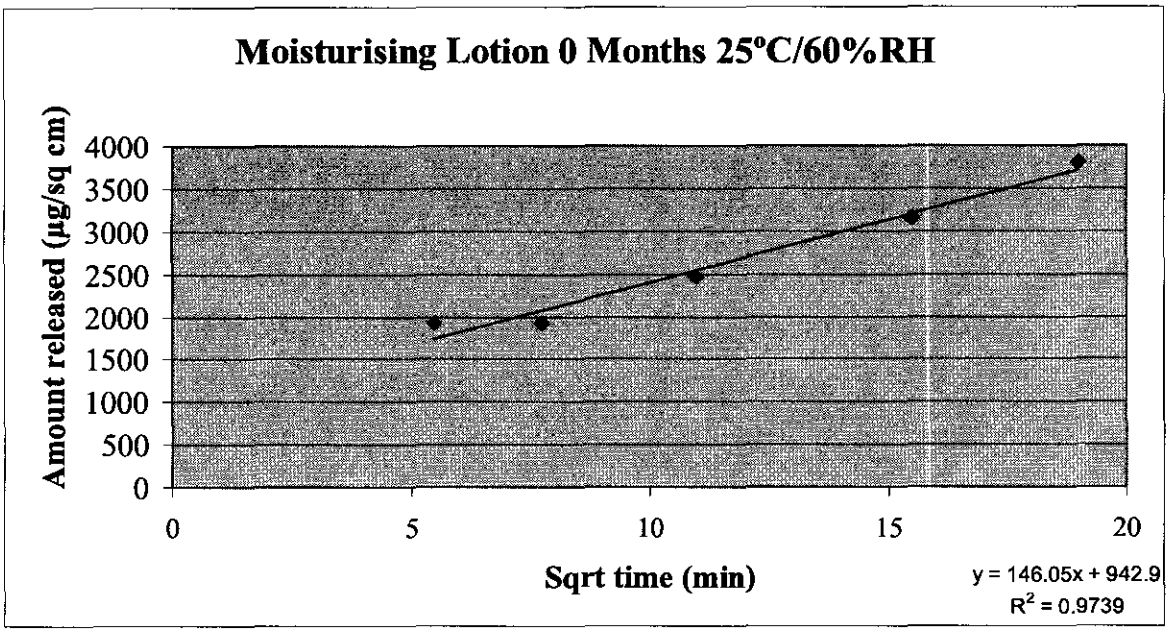


Figure 7.5 The release of urea in the moisturising lotion (initial) at 25°C/60%RH.

The concentration of urea released from the moisturising lotion after three months at 25°C/60%RH and 40°C/75%RH as function of time is given in Table 7.11 and Figure 7.6, and Table 7.12 and Figure 7 .7 respectively.

Table 7.11 The concentration (µg/sqcm) of urea released from the moisturising lotion (three months) at 25°C/60%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	1760.09
60	1760.09
120	2581.17
240	3550.17
360	4241.47

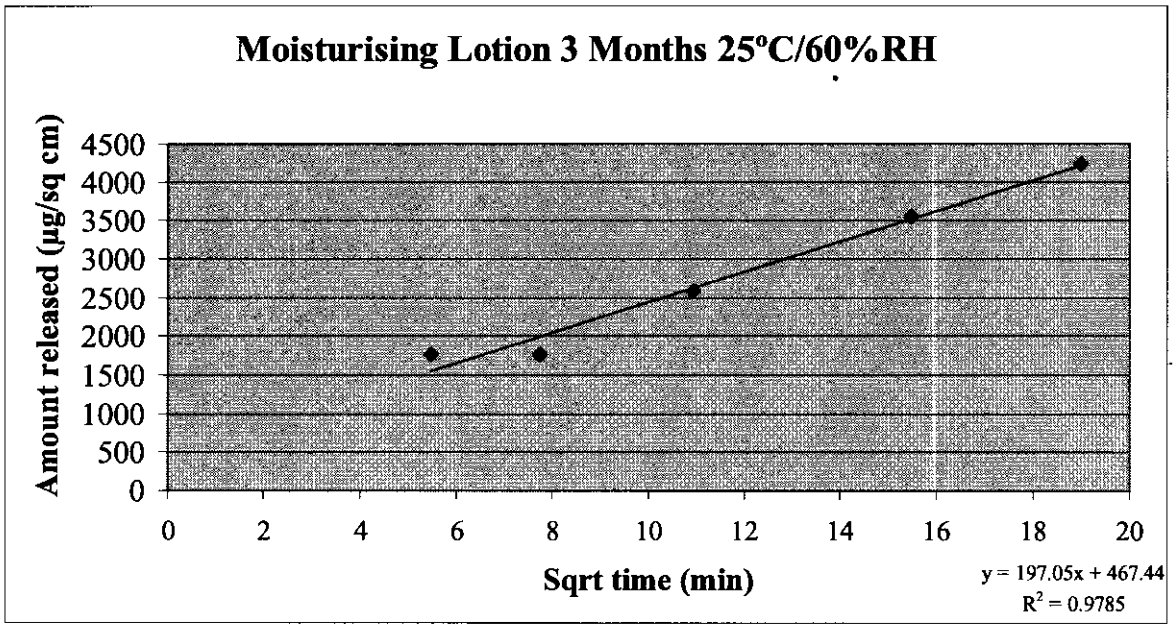


Figure 7.6 The concentration of urea released from the moisturising lotion at 3 months 25°C/60%RH.

Table 7.12 The concentration (µg/sqcm) of urea released from the moisturising lotion (three months) at 40°C/75%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	735.41
60	735.41
120	1336.15
240	2071.42
360	2710.89

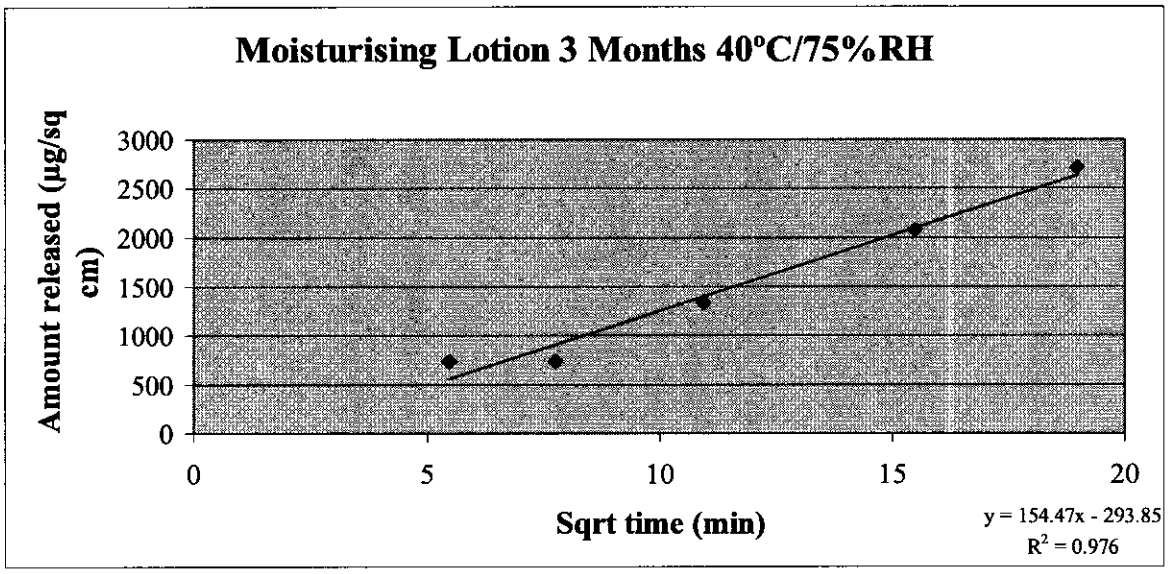


Figure 7.7 The concentration of urea released from the moisturising lotion at 3 months 40°C/75%RH.

The release of urea from the moisturising lotion is graphically given in Figure 7.8.

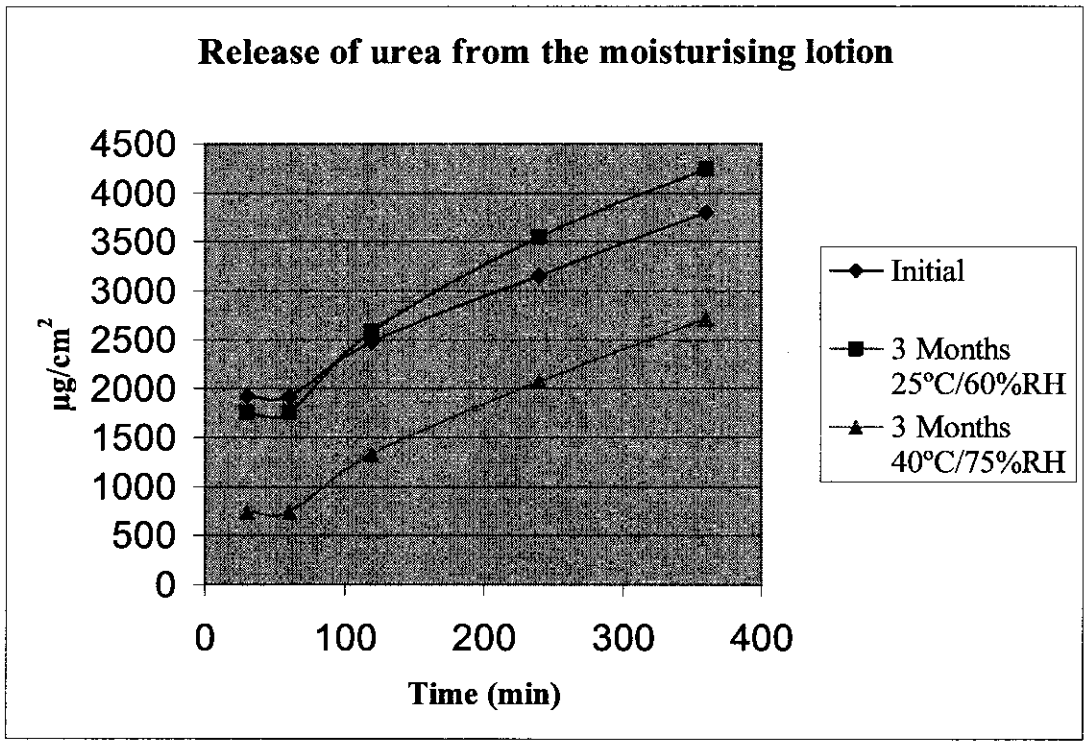


Figure 7.8 Release of urea from the moisturising lotion over the three-month period.

7.9.2 Discussion

According to theory a plot of μg urea released per cm^2 membrane against the square root of time in minutes should produce a straight line. This is not the case for the moisturising lotion. Urea was released from the moisturising lotion after 120 min. This could influence the marketing potential, because the active will be released after 120 min after application on the skin and this could affect the ability of the product. The release of urea from the moisturising lotion against time was much lower after three months stored at 40°C/75%RH, this could be due to a change in the viscosity over time.

7.10 CONCLUSION

In this chapter the formulated moisturising lotion was tested over a three-month period, using an extensive range of stability indicative tests. A comparison of the different test results generated during the test procedures indicated that this formulated moisturising lotion has good marketing potential.

CHAPTER 8

RESULTS AND DISCUSSION

HAIR GEL

8 INTRODUCTION

The following parameters of the formulated hair gel were investigated: pH, relative densities, appearance, viscosity, urea assay, MSM assay, urea release rate (dissolution), and preservative efficacy. All the tests were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 4.

8.1 pH

The pH of the hair gel, measured over three months, is given in Table 8.1.

Table 8.1 pH of the hair gel measured over three months at different storage condition.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.52		
25°C/60%RH	6.61	6.77	6.99	7.12
40°C/75%RH		7.97	8.47	8.64

8.1.1 Discussion

The pH of the hair gel stored at 40°C/75%RH was slightly higher than the pH of the samples stored at 25°C/60%RH. The rise in pH in the samples stored at 40°C/75%RH was not as dramatic as that observed for the massage cream, foot and heel balm and moisturising lotion.

8.2 RELATIVE DENSITY

The relative density of the hair gel, measured over three months, is given in Table 8.2.

Table 8.2 Relative density of the hair gel, measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1.0932		
25°C/60%RH	1.0976	1.0991	1.0983	1.0990
40°C/75%RH		1.0940	1.0941	1.1000

8.2.1 Discussion

There were no significant changes in the relative density of the hair gel (Table 8.2). Temperature, moisture and pH didn't have any influence on the relative density.

8.3 APPEARANCE

The hair gel is a clear gel with an orange flavour. It spreads smoothly and no grittiness was observed and it applied easily to hair. There was no change in colour, texture and odour after the three months.

8.4 VISCOSITY

The viscosity of the hair gel, measured over three months, is given in Table 8.3.

Table 8.3 The viscosity (in cP) of the hair gel measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		3738		
25°C/60%RH	9045	3994	7509	3482
40°C/75%RH		3482	6656	1587

8.4.1 Discussion

The viscosity of the hair gel decreased over the three months stability test intervals (see Table 8.3). The lower values at 1 month (all temperatures) could be due to experimental error. The decrease in viscosity can be explained as a reaction between some ingredients or perhaps the gel was still settling before it went into the final resting stage. A large decrease in viscosity is a warning of instability.

8.5 UREA ASSAY

The concentration of urea in the hair gel was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 8.4 and graphically in Figure 8.1.

Table 8.4 The concentration (%) of urea in the hair gel measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.19%		
25°C/60%RH	99.16%	105.37%	103.98%	100.61%
40°C/75%RH		104,65%	103.50%	100.82%

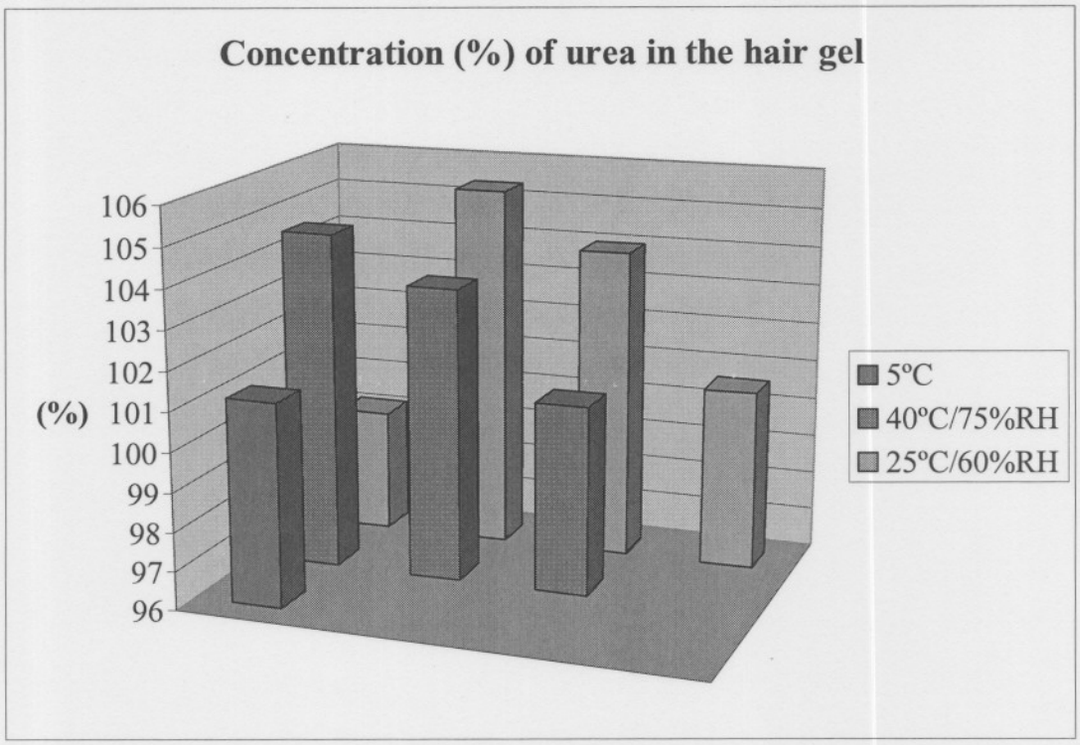


Figure 8.1 Concentration of urea measured over the three months intervals in the hair gel.

8.5.1 Discussion

There was no sighnificant change in the concentration of urea over the three-month stability period in the formulated hair gel.

8.6 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the hair gel was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 8.5 and graphically in Figure 8.2.

Table 8.5 The concentration (%) of MSM in the hair gel measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		104.63%		
25°C/60%RH	99.88%	105.28%	103.49%	103.61%
40°C/75%RH		103.12%	102.84%	100.72%

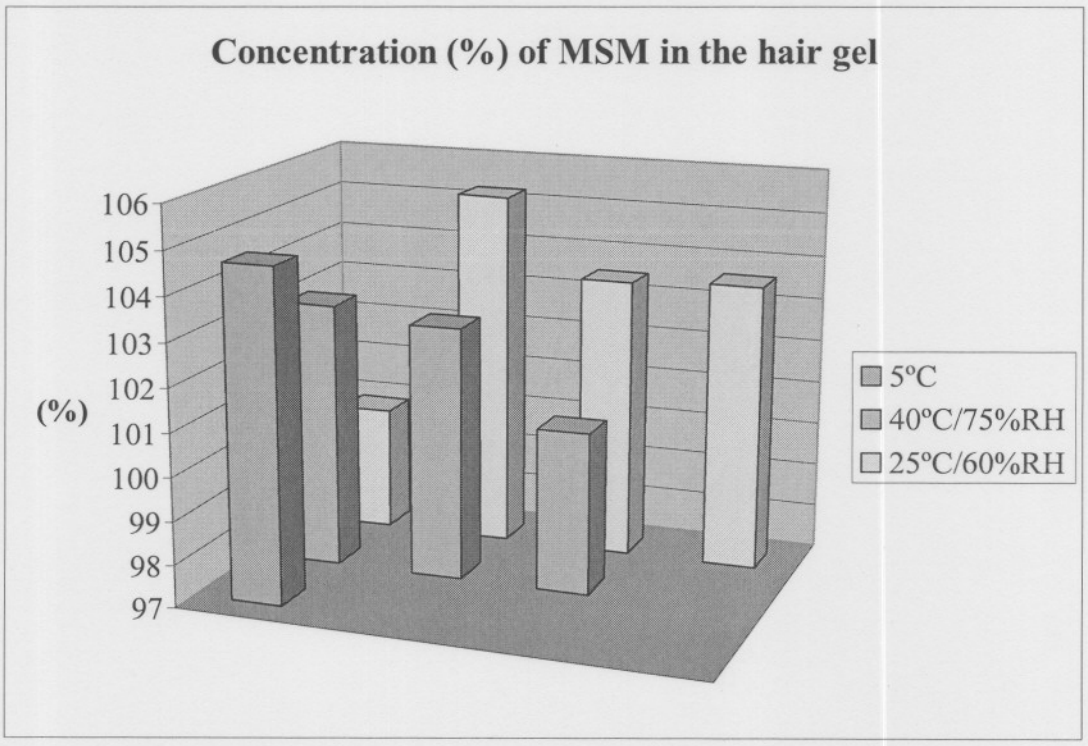


Figure 8.2 Concentration of MSM measured over the three months intervals in the hair gel.

8.6.1 Discussion

The concentration of MSM in the formulated hair gel did not show any significant change.

8.7 PRESERVATIVE EFFICACY

The hair gel was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 8.6 and Table 8.7).

Table 8.6 Preservative efficacy results of the hair gel (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 8.7 Preservative efficacy results of the hair gel (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

8.7.1 Discussion

The preservative efficacy of the hair gel complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the gel against microbial contamination.

8.8 UREA RELEASE

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved.

8.8.1 Concentration of urea released from the hair gel

This dissolution test was performed to prove that urea was released from the hair gel formulation. The concentration of urea that was released from the hair gel was determined initially and after three months storage at 25°C/60%RH and 40°C/75%RH.

The concentration of urea, released from the hair gel (initial), as function of time, is given in Table 8.8 and Figure 8.3.

Table 8.8 The concentration (μg/sqcm) of urea released from the hair gel (initials) as a function of time.

Time (minutes)	Concentration (μg/sqcm)
30	2652.25
60	5125.33
120	9413.73
240	14662.12
360	18562.47

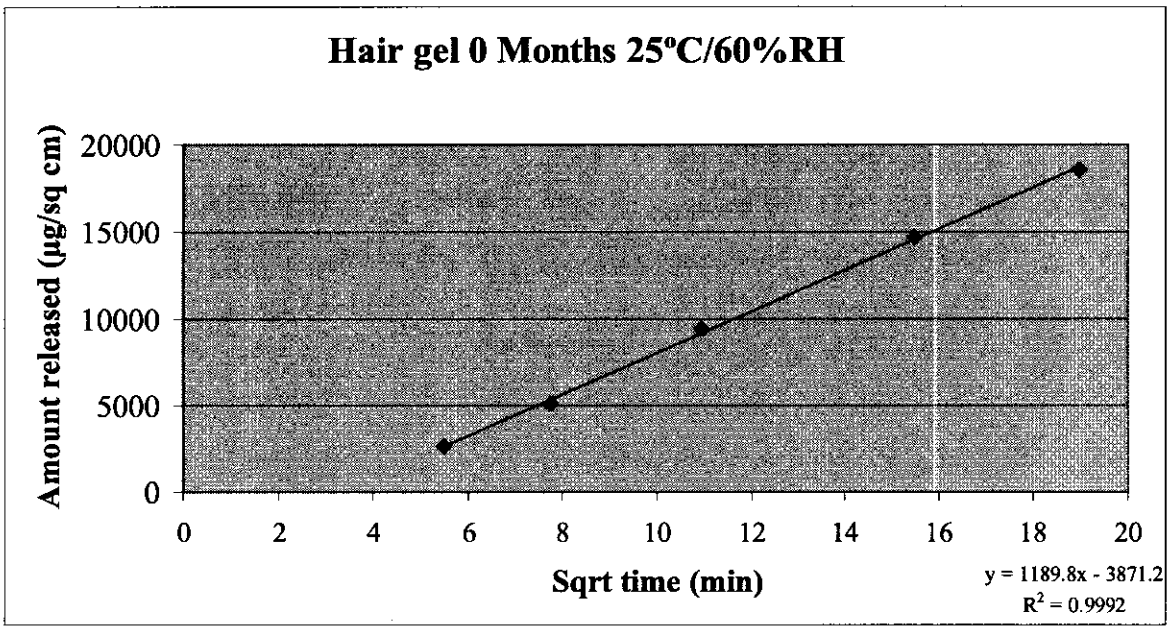


Figure 8.3 The concentration release of urea in the hair gel (initial) at 25°C/60%RH.

The concentration of urea that was released from the hair gel after three months at 25°C/60%RH and 40°C/75%RH as function of time is given in Table 8.9 and Figure 8.6, and Table 8.10 and Figure 8 .7 respectively.

Table 8.9 The concentration (µg/sqcm) of urea released from the hair gel (three months) at 25°C/60%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	1010.62
60	2092.66
120	4553.52
240	8013.27
360	10225.66

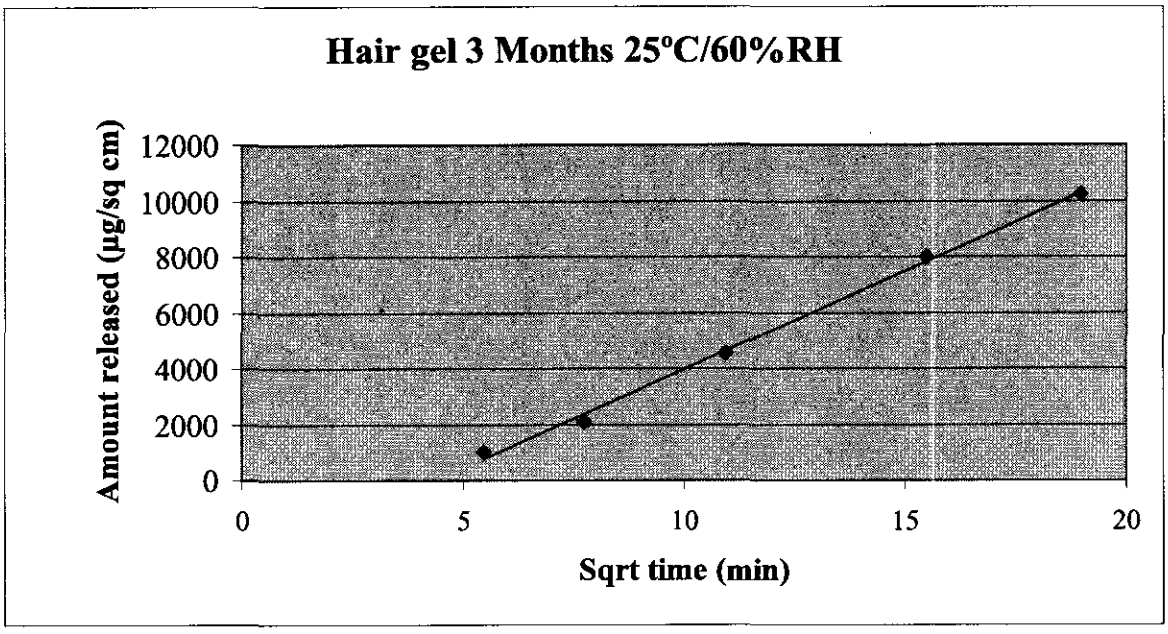


Figure 8.4 The concentration of urea released from the hair gel at 3 months 25°C/60%RH.

Table 8.10 The concentration (µg/sqcm) of urea released from the hair gel (three months) at 40°C/75%RH as a function of time.

Time (minutes)	Concentration (µg/sqcm)
30	2143.48
60	3513.60
120	5296.65
240	7326.14
360	9349.37

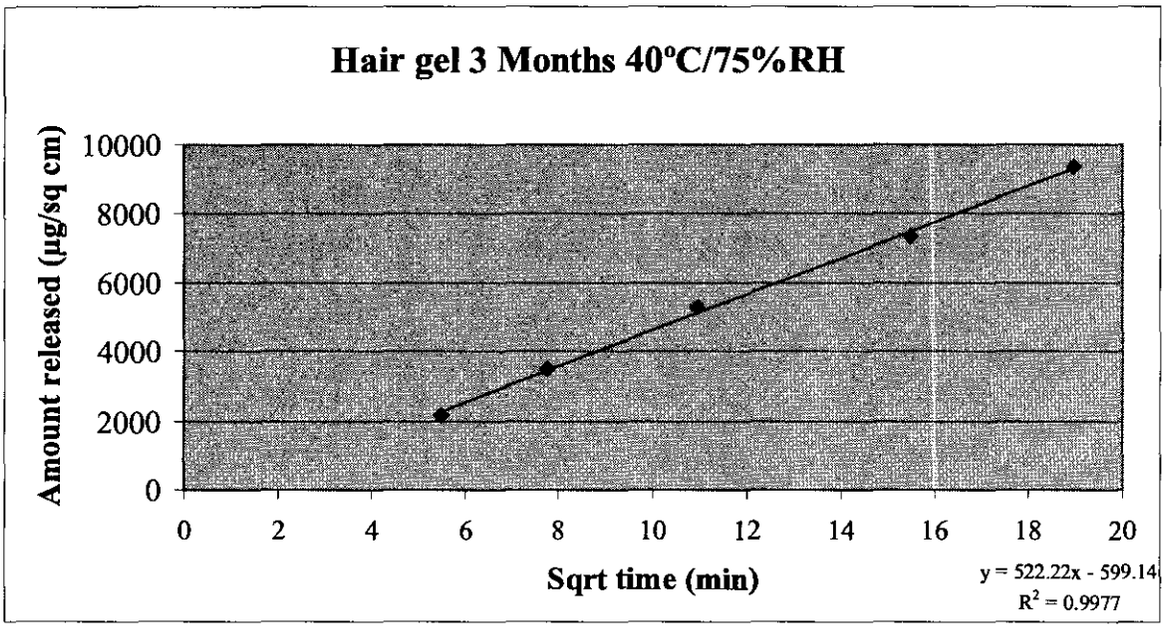


Figure 8.5 The concentration of urea released from the hair gel at 3 months 40°C/75%RH.

The release of urea from the hair gel is graphically given in Figure 8.6.

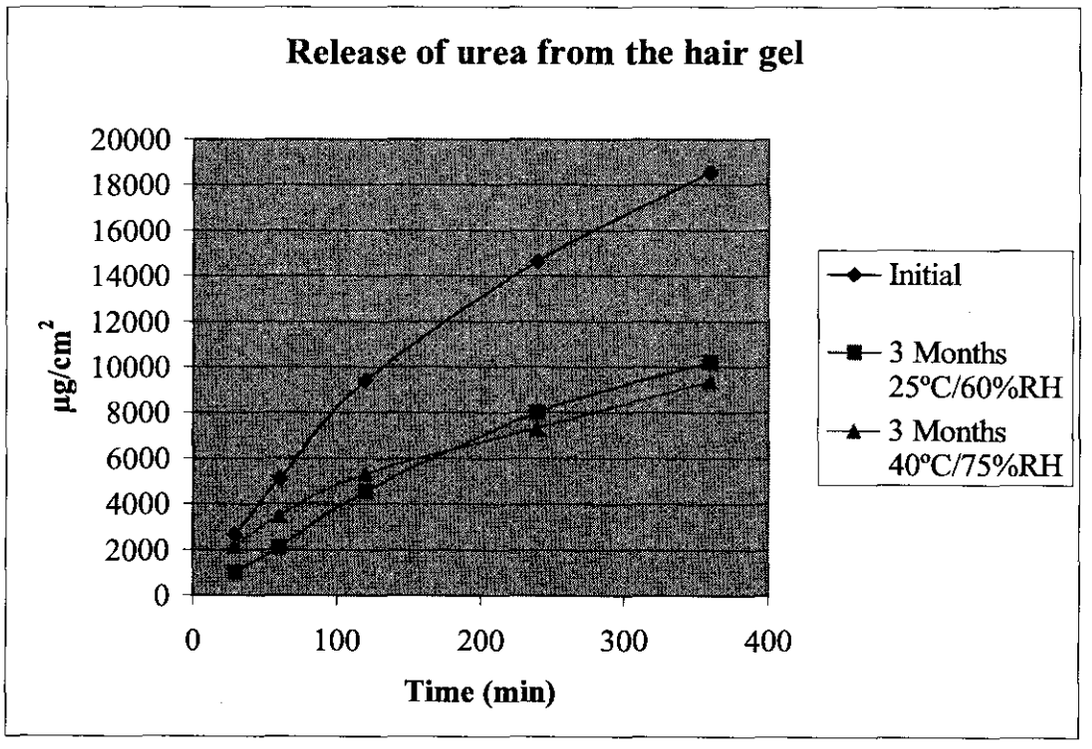


Figure 8.6 Release of urea from the hair gel over the three-month period.

8.8.2 Discussion

According to theory a plot of μg urea released per cm^2 membrane against the square root of time in minutes should produce a straight line. This is indeed the case for the hair gel. The release of urea from the hair gel decreased with time especially at the sample stored at 40°C/75%RH.

8.9 CONCLUSION

In this chapter the formulated hair gel was tested over a three-month period, using an extensive range of stability indicative tests. According to Claasen, (2003:68): the concentration of urea in the hair gel formulation decreased over time especially the sample stored at 40°C/75%RH. In this study where MSM was included in the same formulation there was no significant change in the urea concentration over time. This could be due to the stabilising effect of MSM on urea containing products (Herschler, 1981:1) by inhibiting spontaneous carbamide decomposition. A comparison of the different test results generated during the test procedures indicated that this formulated hair gel has good marketing potential.

CHAPTER 9

RESULTS AND DISCUSSION

HAIR SPRAY

9 INTRODUCTION

The following parameters of the formulated hair spray were investigated: pH, relative density, appearance, urea assay, MSM assay, and preservative efficacy. All the tests were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 4.

9.1 pH

The pH of the hair spray, measured over three months, is given in Table 9.1.

Table 9.1 pH of the hair spray measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.37		
25°C/60%RH	7.14	8.18	8.50	8.59
40°C/75%RH		8.74	9.02	9.09

9.1.1 Discussion

There was an increase in the pH with time for the samples stored at 25°C/60%RH and 40°C/75%RH. The increase in pH at 40°C/75%RH was much less than that observed for the massage cream, foot and heel balm and moisturising lotion.

9.2 RELATIVE DENSITY

The relative density of the hair spray, measured over three months, is given in Table 9.2.

Table 9.2 Relative density of the hair spray, measured over three months at different storage conditions.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		0.9608		
25°C/60%RH	0.9707	0.9702	0.9702	0.9714
40°C/75%RH		0.9702	0.9702	0.9717

9.2.1 Discussion

There were no significant changes in the relative density of the hair spray (Table 9.2). Temperature, moisture and pH didn't have any influence on the relative density.

9.3 APPEARANCE

The hair spray is a clear liquid with a peppermint flavour. Clear, white crystals formed in the spray that was stored for 1 month at 5°C. This could be due to the menthol in the formulation, which crystallises at 5°C over a period of time. There was no change in the colour, odour or texture of the other samples after the three months interval.

9.4 UREA ASSAY

The concentration of urea in the hair spray was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 9.3 and graphically in Figure 9.1.

Table 9.3 The concentration (%) of urea in the hair spray measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		98.60%		
25°C/60%RH	100.07%	99.56%	99.88%	99.47%
40°C/75%RH		98.84%	96.64%	96.89%

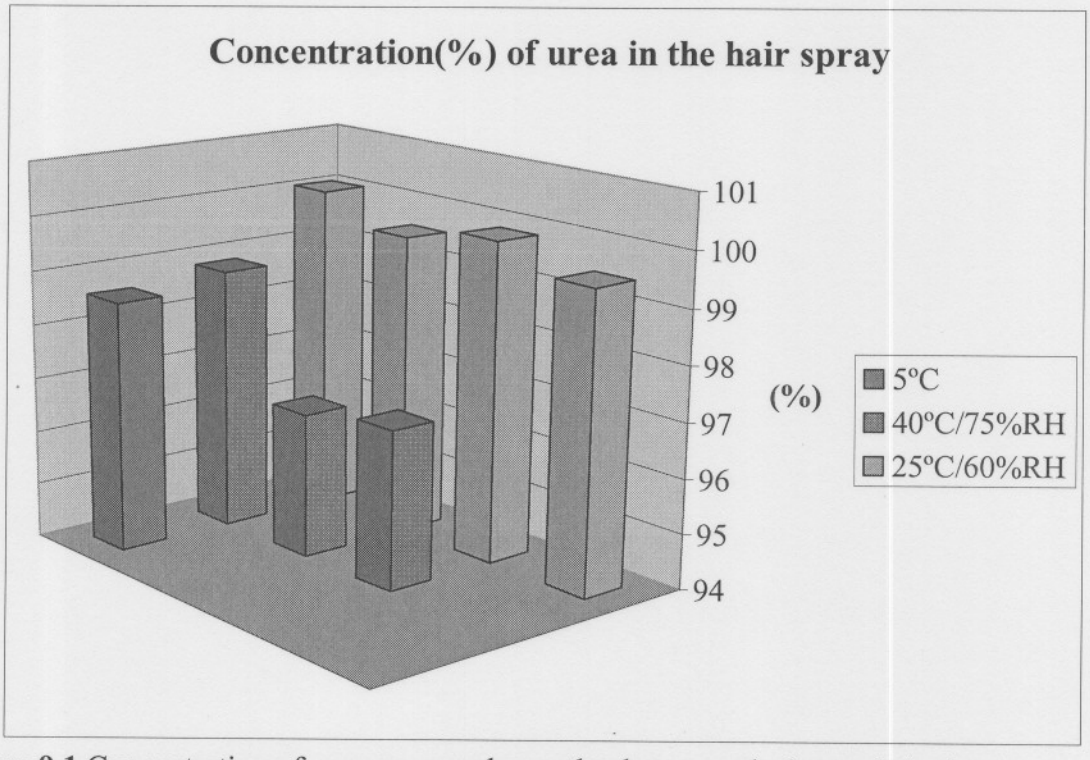


Figure 9.1 Concentration of urea measured over the three months intervals in the hair spray.

9.4.1 Discussion

The concentration of urea in the formulated hair spray decreased with time, especially in the sample that was stored at 40°C/75%RH. This decrease, however, was not significant.

9.5 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the hair spray was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 9.4 and graphically in Figure 9.2.

Table 9.4 The concentration (%) of MSM in the hair spray measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.84%		
25°C/60%RH	106.19%	103.76%	103.89%	103.33%
40°C/75%RH		101.45%	100.71%	99.53%

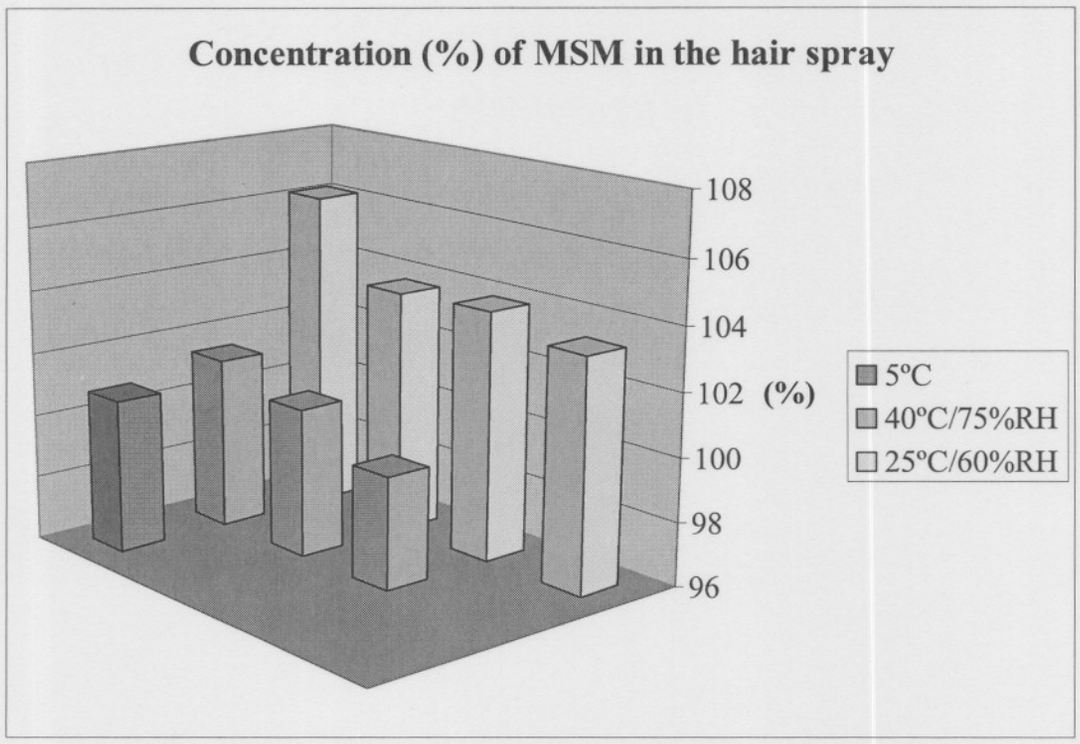


Figure 9.2 Concentration of MSM measured over the three months intervals in the hair spray.

9.5.1 Discussion

The concentration of MSM in the formulated hair spray did not show any significant change. The high initial value could be due to experimental error.

9.6 PRESERVATIVE EFFICACY

The hair spray was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 9.5 and Table 9.6).

Table 9.5 Preservative efficacy results of the hair spray (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	$\mathbf{5.5 \times 10^5}$	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 9.6 Preservative efficacy results of the hair spray (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

9.6.1 Discussion

The preservative efficacy of the hair spray complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the spray against microbial contamination.

9.7 CONCLUSION

In this chapter the formulated hair spray was tested over a three-month period, using an extensive range of stability indicative tests. A comparison of the different test results generated during the test procedures indicated that this formulated hair spray has good marketing potential.

CHAPTER 10

RESULTS AND DISCUSSION

LIP ICE

10 INTRODUCTION

The following parameters of the formulated lip ice were investigated: appearance, urea assay, MSM assay, and preservative efficacy. All the tests were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 4.

10.1 APPEARANCE

The visual inspection of the lip ice is a method used to determined consumer approval. The lip ice was soft, slightly oily and crumbles easily; it applies easily to the lips and gives a wet sensation to the lips. There was no change in the colour or texture of the lip ice after the three months interval.

10.2 UREA ASSAY

The concentration of urea in the lip ice was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 10.1 and graphically in Figure 10.1.

Table 10.1 The concentration (%) of urea in the lip ice measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		93.06%		
25°C/60%RH	92.94%	85.75%	81.85%	82.89%
40°C/75%RH		86.88%	82.33%	85.94%

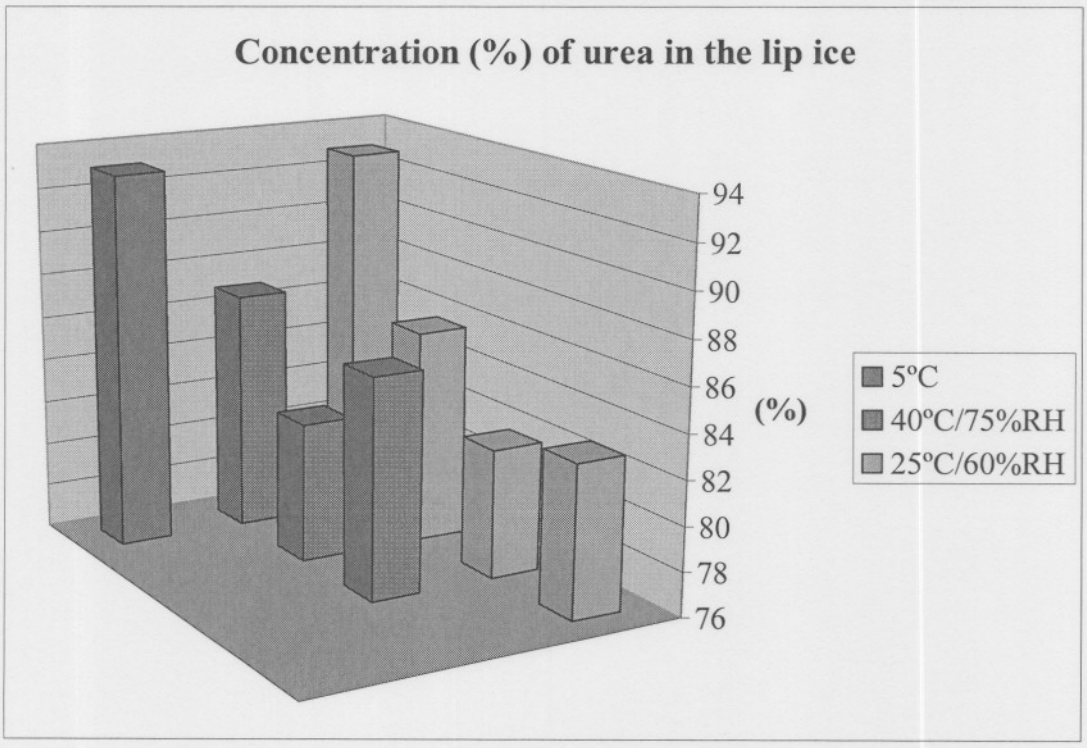


Figure 10.1 Concentration of urea measured over the three months intervals in the lip ice.

10.2.1 Discussion

The concentration of urea in the formulated lip ice decreased with time. The low concentration of urea in the lip ice could be due to poor solubility of the urea crystals in the waxes of the lip ice formulation.

10.3 METHYLSULFONYLMETHANE (MSM) ASSAY

The concentration of MSM in the lip ice was tested at initial, one, two and three monthly intervals as described in Chapter 4 and given in Table 10.2 and graphically in Figure 10.2.

Table 10.2 The concentration (%) of MSM in the lip ice measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		77.92%		
25°C/60%RH	33.37%	112.61%	30.71%	66.03%
40°C/75%RH		60.80%	63.12%	42.98%

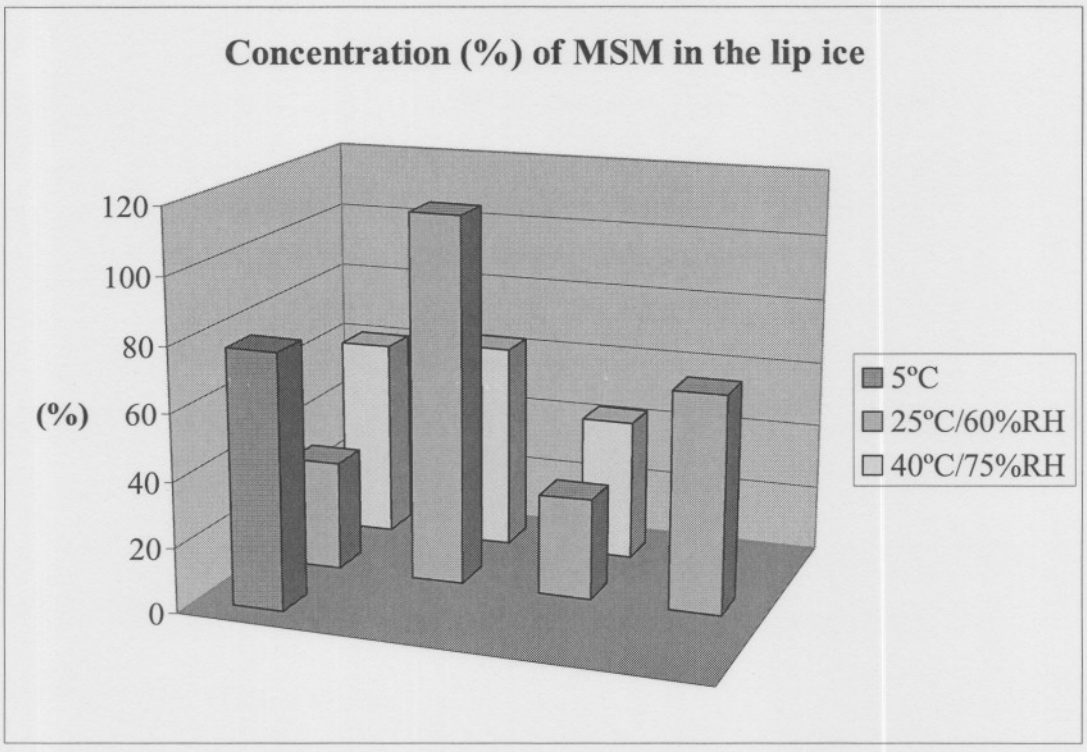


Figure 10.2 Concentration of MSM measured over the three months intervals in the lip ice.

10.3.1 Discussion

The low concentrations of MSM in the lip ice are due to poor solubility in the waxes in the lip ice formulation. The MSM crystals did not dissolve uniformly into the waxes, leading to a huge variation in the results.

10.4 PRESERVATIVE EFFICACY

The lip ice was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 27 Category 2 (2004) (Table 10.3 and Table 10.4).

Table 10.3 Preservative efficacy results of the lip ice (initial).

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

Table 10.4 Preservative efficacy results of the lip ice (3 months), stored at 40°C/75%RH.

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day14	Day 28	
E. coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial count at 14 days, And no increase from the 14 days count at 28 days.
P.aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
S.aureus	5.5×10^5	>3.0	>3.0	>3.0	
A. niger	3.8×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days.
C.albicans	5.0×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with the requirements of USP 27.

10.4.1 Discussion

The preservative efficacy of the lip ice complied with the requirements of the USP 27. It was therefore shown that the preservatives used in the formulation were effective in protecting the lip ice against microbial contamination.

10.5 CONCLUSION

In this chapter the formulated lip ice was tested over a three-month period, using an extensive range of stability indicative tests. A comparison of the different test results generated during the test procedures indicated that this formulated lip ice does not have good marketing potential. Results showed a big variation in the concentrations of the actives, because of poor solubility of the actives in the waxes of the lip ice formulation. This formulation could be improved by using a substance, in which the actives (good water soluble) are more soluble in, such as polyethylene glycol, which is a more water-soluble substance than the waxes.

CHAPTER 11

FINAL CONCLUSION

The formulated urea and MSM containing products were tested ove3r a three-month period, using an extensive range of stability indicative test methods. The three storage conditions utilised were 5°C, 25°C/60%RH and 40°C/75%RH.

The following is a summary of the most significant test results generated, and the possible conclusion that can be drawn from that.

- The pH of the urea and MSM containing samples stored at 40°C/75%RH were higher than the pH of the samples stored at 25°C/60%RH. Urea decomposes into carbon dioxide and ammonia (Beiersdorf, 2003:1). This can cause the pH to increase to values as high as 11. This volatilisation is affected by temperature and moisture. Higher temperature and moisture increase volatilisation (Anon, 2003:42). Urea compositions can be stabilised when they contain methylsulfonylmethane (MSM), such compositions soften skin strengthen nails and provide other benefits when applied topically (Herschler, 1981:1). By comparing the pH results of the foot and heel balm and hair gel, obtained by Claasen, (2003:63) and the pH results optained in this study, it could be concluded that there was no improvement in the pH by including MSM to the same formulation. Results showed that urea still decomposed into carbon dioxide and ammonia, although MSM was included in the formulation. A small decompostion can lead to a huge increase in the pH.

- Viscosity of urea and MSM containing products display thixotropic properties, which means that, they liquefy without any change in the water content under the influence of mechanical stress. When the stress is removed the highly viscous state returns (Beiersdorf, 2003:1).

- The appearance of the products remained the same over three months, except the hair spray stored for 1 month at 5°C, where the menthol in the formulation formed crystals, and the foot and heel balm that become yellow after 3 months due to the high oil content.

- The spreadability of the products remained the same over three months.

- The penetration and relative density of the products remained more or less the same over the three months.

- According to Claasen, (2003), the HPLC analysis of the hair gel and the foot and heel balm showed a decrease in urea concentration with time. Results optained in this study showed that there was no significant change in the urea concentration with time. This could be due to the stabilising effect of MSM on urea containing products by inhibiting spontaneous carbamide decomposition or it could be an experimental variation. The urea concentration in the lip ice showed a big variation with low concentrations, due to poor solubility of urea in the waxes of the formulation. Using polyethylene glycol instead of the waxes might improve the formulation.

- HPLC analysis of the preservatives namely methyl- and propylparaben confirm their stability in the formulated products.

- GC analysis showed in slight decrease in the MSM concentration over time, which was not significant. The MSM concentration in the lip ice showed a big variation

with very low concentrations, due to the poor solubility of MSM in the waxes of the formulation.

- Preservative efficacy results proved that the products were sufficiently protected from microbial growth by the added methyl- and propylparaben, and the other preservatives used in the formulations.

- The release study indicated that urea is released at a steady rate from all four preparations tested. The most important conclusion is that urea is released from the four preparations, i.e. the urea active compound is, in all four products, available for bio-absorption. The release of MSM was not tested, because MSM was included into the formulation due to its stabilising effect on urea and not as a active ingrdient.

In conclusion, it can be said that urea and MSM were successfully formulated into the six formulations. The pH results optained in this study showed that MSM had no effect on the decomposition of urea into carbon dioxide and ammonia, because the pH increased over time. Results obtained by Claasen, (2003) showed the same without MSM in the formulation. However, the urea concentration in the tested products remained more stable compared to the results obtained by Claassen (2003). Altough including, the MSM helped to inhibit spontaneous decomposition of urea, and gave higher urea concentrations, it could not be for sure that MSM had a stabilising effect on urea and better stabilisers for urea could be investigated in the futher studies.

REFERENCES

ACD/ChemSketch – Freeware Version. Advanced Chemistry Development Inc. Toranto, Ontario. Version 5.12 / 10 November 2003.

ANON, 2003. UREA: its role in cosmetics. Skin care forum, 21. [Web:]

http://www.scf-online.com/English/issue 21/urea-21-e.htm [Date of access: 21 January 2004].

BEIERSDORF. 2003. Eucerin®.[Web:]

[http://www.eucerin.co.uk/product - info/galenics.htm1.] [Date of access: 21 January 2004]. 4p.

BRONAUGH, R.L. & COLLIER, S.W. 1993. In vitro methods for measuring skin permeation. (*In* Zats, J.L., *ed.* Skin permeation, fundamentals and application. Wheaton, Ill.: Allured Publishing Corporation. p. 93 - 111).

BRITISH PHARMACOPOEIA. 2003. London: HMSO. 2488p.

BROOKFIELD. 1998. Brookfield viscometer/rheometers. Massachusetts: Brookfield engineering laboratories, Inc. p. 2-22.

BUYUKTIMKIN, N., BUYUKTIMKIN, S. & RYTTING, J.H. 1997. Chemical means of transdermal drug permeation enhancement. (*In* Gosh, T.K., Pfister, W.R. & Yum, S.I., *eds.* Transdermal and topical drug delivery systems. Buffalo Grove, I11.: Interpharm. p.357-475).

CARSTENSEN, J.T. 1990. Drug stability: principles and practices. Vol 43. New York: Marcel Dekker Inc. p.2 - 22.

CHIEN, Y.W. 1987. Advances in transdermal systemic medication. (*In* Chien, Y.W., *ed.* Transdermal controlled systemic medications. New York, N.Y. : Marcel Dekker. p. 1-22).

CLAASEN, C.I. 2003. The formulation and evaluation of urea containing products. Potchefstroom: PU vir CHO. Dissertation – M.Sc. p. 63.

CHIMCO. 1997. Urea. Material Safety data Sheets.

[http://www.chimco.bg/eng1/ureatecheng.htm.] [Date of access: 26 April 2004].

DEVLEESCHOUWER, M. & SIQUET, F. 2001. Stability Control: Microbial Test. (*In* Barel, A., Paya, M & Maibach, H., *eds.* Handbook of Cosmetic Science and Technology. New York: Marcel Dekker. 886p).

EPSTEIN, H. & SIMON, A. 2001. Emulsion-based skin care products: Formulated and measuring their moisturising benefits. (*In* Barel, A., Paya, M & Maibach, H., *eds* Handbook of Cosmetic Science and Technology. New York: Marcel Dekker. 886p).

FLYNN, G.L. & WEINER, N.D. 1993. Topical and transdermal delivery-provinces of realism. (*In* Gurny, R. & Teubner, A., *eds.* Dermal and transdermal drug delivery. new insights and perspectives : Second International Symposium of the International Association for Pharmaceutical Technology (APV) Frankfurt, 11-13 November 1991 Stuttgart : Wissenschaftliche Verlagsgesellschaft).

FOLDVARI, M. 2000. Non-invasive administration of drugs through the skin: challenges in delivery system design. *Pharmaceutical science and technology today*, 3:417-425.

FRIEDLER, H.P. 1977. Harnstoff: Eigenschaften-Wirkurg-Verwendung. Berufs.Dermatosen, 25(2). [Web:] http://search.epnet.com [Date of access: 21January 2004].

HERSCHLER, R. 1981. Preparation containing methylsulfonylmethane and methods of use and purification. United States Patent 4,477,469: p.1-18.

HORVATH, K., NOKER, P.E., SOMFAI-RELLE, S., GLAVITS, R., FINANCSEK, I. & SCHAUSS, A.G. 2004. Toxicity of methylsulfonylmethane in rats. [Web:] [http://www.elsevier.com/locate/foodchemtox] [Date of access: 3 February 2004]. 4p.

HUNTING, A.L.L .1983. Decorative cosmetics. *A formulary of cosmetic preparations*,Vol. 1. 115p.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH): Q1A(R2). 2000. Stability testing of new drug substances and products. p. 4.

IT WORKS MARKETING. 2003. Applicator ingredients. [Web:] http:www.iwr.com/itworks/applicators-ingredients.html [Date of access: 3 January 2004].

JONES,W.E. 1987. MSM reviewed. *Journal of equine veterinary science*, 7(2).[Web:] [http://www.redwing.org/HTMLarts/msmreva.htm] [Date of access: 21 January 2004].

LAWRENCE, R.M. 2002. Methylsulfonylmethane (MSM): a double blind study of its use in degenerative arthritis. [Web:] [http://www.redwings.org/HTMLarts/msmdjdstudy.htm] [Date of access: 21 January 2004].

LOREN, K. 2000. MSM face & body cream. Calm Cream. [Web:]

http://www.bulkmsm.com/retailmsm/msmcream/index.html [Date of access: 2 December 2003].

LUND, W., *ed.* 1994. The pharmaceutical codex. 12th ed. London :The Pharmaceutical Press. 1117p.

MAGDASSI, S. & TOUITOU, E. *eds.* 1999. Cosmetics and Delivery Systems. (*In* Magdassi S. & Touitou E., Novel Cosmetic Delivery Systems. Vol. 19. New York: Marcel Dekker. 357p).

McEUOY, G.K., *ed.* 2002. AHFS: drug information 2002. Bethesda, Md.: American Society of Hospital Pharmacists. 3756p.

MEDICINES CONTROL COUNCIL. 2003. Stability Studies. Addendum 5. Republic of South Africa. p. 21-23.

MITSUI, T. 1997. New cosmetic science. Amsterdam : Elsevier. 499p.

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 13:318-325.

PARIMA Inc. 40% Urea. [Web:]

[http://www.bradpharm.com/product%20insert/IL129Carmo140.pdf.] [Date of access: 11 February 2003].2p.

RICHMOND, V.L. 1986. Incorporation of methylsulfonylmethane sulfur into guinea pig serum proteins. *Life science,* 39: 263-268.

RIEGER, M.M. 1993. Factors affecting sorption of topical applied substances. (*In* Zats, J.L., *ed.* Skin permeation, fundamentals and application. Wheaton, Ill. : Allured Publishing Corporation. p. 33-72).

RIVIERE, J.E. 1993. Biological factors in absorption and permeation. (*In* Zats, J.L., *ed.* Skin permeation, fundamentals and application. Wheaton, Ill. : Allured Publishing Corporation. p. 113-125).

ROBERT & FILS. 2000. Therapeutic Uses of Urea. [Web:]

[http://www.robertetfils.com/en/info-sante/is200207uree.htm1] [Date of access: 11 February 2004].

ROYAL BODY CARE. 2002. MSM with nanoclusters. [Web:]

[http://www.rbcglobenet.com/Research.htm#msm.] [Date of access: 24 February 2004].

ROY, S.D. 1997. Preformulation aspects of transdermal drug delivery systems. (*In* Ghosh, T.K., Pfitser, W.R. & Yum, S.I., *eds.* Transdermal and topical drug delivery systems. Buffalo Grove, Ill. : Interpharm Press. p. 139-166).

SCHUELLER, R. & ROMANOWSKI, P. 1999. Beginning cosmetic chemistry. Carol Illinois : Allured. 177p.

SHAH, V., HANUS, J., NOORIZADEH, C. & SKELLY, J. 1991. In vitro release of hydrocortisone from topical preparations and automated procedure. Pharmaceutical Research, 8: p. 55 – 59.

SHAW, N.O. 1998. Topical delivery of Ivermectin: Vehicle design and formulation. Potchefstroom: PU for CHO. Thesis-M.Sc. 161p.

SUPER VITAMIN OUTLET. COM. 2002. MSM, methylsulfonylmethane. [Web:]

[http://www.supervitaminoutlet.com/Info_Pages/details/msm_details.htm] [Date of access: 20 August 2003].

UNITED STATES PHARMACOPOEIAL CONVENSION. 2004. USP 27: NF 20. United states pharmacopeial convention, Rockville, Md, USA.

VITA-FLEX NUTRITION. 2001. Nutritional significance and medical application of MSM: a biological active sulfur compound. [Web:]

http://www.redwing.org/HTMLarts/nusigmsma.htm [Date of access: 21 January 2004].

WALTERS, K.A. 1989. Penetration enhancers and their use in transdermal therapeutic systems. (*In* Hadgraft, J. & Guy, R.H., *eds*. Transdermal drug delivery: developmental issues and research initiatives. New York, N.Y. : Dekker. p. 197-233).

WOLFRAM, L., 2001. Hair Cosmetics. (*In* Barel, A., Paye, M. & Maibach, H., *eds.* Handbook of Cosmetic Science and Technology. New York: Marcel Dekker. 886p).

ZATS, J.L. 1993. Scratching the surface: rationale and approaches to skin permeation. (*In* Zats, J.L., *ed.* Skin permeation fundamentals and application. Wheaton, Ill.: Allured. p. 11-31).

APPENDIX A

J.M Botha, E. Swanepoel & A.P. Lötter. 2004. The stabilising effect of methylsulfonylmethane (MSM) on urea containing formulations. Poster presented at the 25th Silver Jubilee Annual Congress of the Academy of Pharmaceutical Sciences, 12 – 15 September, Rodes University, Grahamstown, South Africa.

The stabilizing effect of methylsulfonylmethane (MSM) on urea containing formulations.

J.M. Botha[1], A.P. Lötter[1], E. Swanepoel[1]
[1] Research Institute for Industrial Pharmacy, School of Pharmacy, North-West University, Potchefstroom Campus, Potchefstroom, 2520, South Africa.

Abstract

suitable Urea and MSM (methylsulfonylmethane) containing formulations for use in moisturizing creams and lotions, creams, gels, ointments and the like. The formulations were subjected to stability

when such compositions contain carbamide (urea). Also, carb

ure of methylsulfonylmethane (MSM)

Figure 2 Chem

Methylsulfonylmethane (MSM) (Figure 1) is not a vitamin, nor a drug, but rather a popular dietary supplement used in a variety of conditions including pain, inflammation, allergies, arthritis, exercise recovery, infections and the maintenance of normal keratin levels in hair, skin and nails (Horvath et al., 2002: 1459).

As one of the major soluble substances of the stratum corneum, urea (Figure 2) has a growing importance in dermatological therapy and cosmetics. Diseases such as atopic dermatitis or clinical dry skin are marked by a deficiency of urea (Anon., 1999 : 1).

Materials and methods

(containing 5% urea and MSM), moisturizing lotion (containing 5% urea and MSM), hair gel (containing 5% ura and MSM), and lip ice (containing 2.5% urea and MSM). The formulations were tested unde
density, viscosity, penetration, spreadability and appearance.
MSM content was determined using a GC method, while HPLC was used to determine the urea, methyl- and propyl paraben content.

Results

Figures 3 and 4 represent examples of a GC and HPLC chromatogram of MSM and urea respectively.

Figure 3 GC chromatogram of MSM in the foot and heel balm formulation.

Figure 4 HPLC chromatogram of urea in the foot and heel balm formulation.

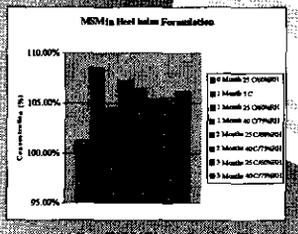


Figure 5 The concentration (%) MSM in the heel balm formulation.

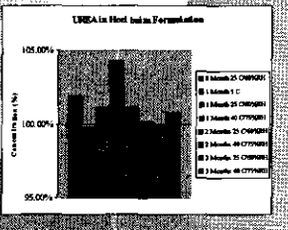


Figure 6 The concentration (%) urea in the heel balm formulation.

Conclusion

stability (Figure 5). Lower concentrations were obtained for the lip ice due to the poor solubility in the vessel. HPLC analysis of urea (Figure 6), methyl- and propyl paraben content showed that the actives
There was no significant change in the penetration, relative density, pH, spreadability, viscosity and appearances of the products over the three-month period.

YUNIBESITI YA BOKONE-BOPHIRIMA
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT

Potchefstroom Campus