THE FORMULATION AND EVALUATION OF UREA CONTAINING PRODUCTS

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To my wonderful parents and fiancé Johan.

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ABSTRACT

Almost every person will experience dry skin during his or her lifetime. Many people experience occasional episodes, but some have a chronic problem with xerosis that is irritating and troublesome. Moisturisers are the mainstay of treatment for dry skin, daily maintenance of normal skin, and adjunctive therapy for many skin diseases (Flynn *et al.*, 2001:387).

The objectives of this study were the formulation and evaluation of urea containing products. Seven different cosmetic products were formulated: hair gel, shampoo, facial toner, facial cleanser, day cream, foot and heel balm and a body cream. The product development program started with a preformulation study, followed by formulation of the seven skin care products, which were followed by stability testing, based on the requirements of the South African Medicines Control Council (2003:21, 22, 23) for new products. The stability of all skin care products must be matched to the expected period of usage by the consumer, as well as to the user's requirements. The formulations were tested under ICH conditions (accelerated stability studies) over a period of three months at three different storage temperatures, i.e. 5°C, 25°C/60%RH, 40°C/75%RH. Stability indicating tests that applied to these formulations, were conducted: pH, relative density, viscosity, appearance, penetration, spreadability, assay of urea, the assays of the preservatives and the release study of urea by means of membrane release.

Chapter 1 gives a literature overview of the skin and the properties and uses of urea. Chapter 2 deals with the formulation of the urea products and the chemicals that have been used. Chapter 3 describes the methods used for accelerated stability testing. Chapter 4 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 the decomposition of urea into carbon dioxide and ammonia (Beiersdorf, 2003). Higher temperature and moisture increase this decomposition and cause the pH to increase to values as high as 9 (Anon, 2003:20). However, urea compositions can be stabilised when they contain methylsulfonylmethane (MSM) (Herschler, 1981:1).

The viscosity, spreadability, penetration, relative density and appearance of the products remained more or less the same over three months, except for the day cream and the facial cleanser where phase separation occured after 1 month. The urea content decreased with time, but only dropped to below 90% in the day cream after 3 months at 40°C/75% RH. 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. The release of urea from the hair gel is about four times faster than that from the body cream and the foot and heel balm. In general, the release is influenced by the viscosity of the medium and should be faster from the gel than from the creams (Shah *et al.*, 1991:55).

In conclusion it can be said that urea was successfully formulated into the seven cosmetic products except for the facial cleanser and day cream which must be stabilised.

UITTREKSEL

Bykans elke mens sal gedurende sy of haar lewe droë vel ervaar. Baie mense ervaar periodieke episodes, maar ander het 'n chroniese probleem met xerose wat baie irriterend is. Bevogtigers is die hoofbehandeling vir droë vel, daaglikse instandhouding van 'n normale vel en bykomende terapie vir baie velsiektes (Flynn *et al.*, 2001:387).

Die doelstellings van hierdie studie was die formulering en evaluering van ureum bevattende produkte. Sewe produkte was geformuleer: haarjel, sjampoe, gesigsreiniger, verfrisser, dagroom, voet- en hakbalsem en 'n lyfroom. Die produkontwikkelingprogram het begin met 'n preformuleringstudie, gevolg deur die formulering van die sewe produkte. Daarna het stabiliteitstoetse gevolg soos vereis deur die Medisynebeheerraad van Suid-Afrika vir nuwe produkte. Die tydperk van gebruik van die velprodukte, asook die vereistes van die gebruiker, moet in ag geneem word tydens die stabiliteitstoetse. Die formulerings was onder ICH kondisies (versnelde stabiliteitstoetse) getoets oor 'n periode van drie maande by drie verskillende temperature, nl. 5°C, 25°C/60%RH, 40°C/75%RH. Stabiliteitstoetse wat op hierdie formulerings van toepassing was sluit in: pH, relatiewe digtheid, viskositeit, voorkoms, penetrasie, spreibaarheid, die analise van ureum en preserveermiddels en die vrystellingstudie van ureum d.m.v. membraanvrystelling.

Hoofstuk 1 gee 'n literatuuroorsig van die vel en die eienskappe en gebruike van urea. Hoofstuk 2 handel oor die formulering van die urea bevattende produkte en die chemikalieë wat gebruik was. Hoofstuk 3 verduidelik die metodes wat gebruik was vir die versnelde stabiliteitstoetse. Hoofstuk 4 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, dit is as gevolg van die afbraak van urea na koolstofdioksied en ammoniak (Beiersdorf, 2003). Hoër temperature en hoër vogtigheidskondisies verhoog hierdie afbraak en veroorsaak dat die pH na waardes so hoog as 9 kan toeneem (Anon, 2003:20). Ureum bevattende produkte kan gestabiliseer word wanneer metielsulfonielmetaan (MSM) bygevoeg word (Hershler, 1981:1).

Die viskositeit, spreibaarheid, penetrasie, relatiewe digtheid en voorkoms van die produkte het min of meer dieselfde gebly oor die drie maande behalwe in die geval van die gesigsreiniger en dagroom waar fase-skeiding na 1 maand plaasgevind het. Die ureum inhoud het met tyd afgeneem, maar slegs in die gaval van die dagroom tot laer as 90% gedaal. HPLC analise van die preserveermiddels het hul stabiliteit in die geformuleerde produkte bevestig. Die preserveermiddel effektiwiteits 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. Die vrystelling van ureum uit die haarjel is bykans vier keer vinniger as dié van die voet- en hakbalsem en lyfroom. 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).

In gevolgtrekking kan gesê word dat ureum suksesvol in die sewe kosmetiese produkte geformuleer was en stabiel by hoër temperature bly, behalwe die gesigsreiniger en die dagroom wat verder gestabiliseer moet word.

AIM AND OBJECTIVES

Urea is one of the most important soluble substances of the stratum corneum. In recent years this substance has become more and more important in dermatological therapy and cosmetics. Many diseases have been described that are characterised by a deficiency of urea, such as atopic dermatitis or clinical dry skin (Häntscei *et al.*,1998:155).

The aim of this study was to develop different stable urea containing topical formulations for use as cosmetic products. The stability of urea 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).

The main objectives of this study included:

- To formulate a facial toner, shampoo, hair gel, facial cream, day cream, foot and heel balm and a body cream, containing urea.

- To subject the urea containing formulations to stability indicating studies for three months under ICH conditions.

- To analyse urea by means of a stability indicating HPLC method.

- The physical and chemical evaluation of these products as required by the South African Medicines Control Council (2003:21,22,23).

- To determine the release of urea by means of membrane release studies.

- The preservative efficacy testing of these products.

CHAPTER 1

PHYSICO-CHEMICAL PROPERTIES, FUNCTION AND USES OF UREA

1.1 INTRODUCTION

Almost every person will experience dry skin during his or her lifetime. Many people experience occasional episodes, but some have a chronic problem with xerosis that is irritating and troublesome. Moisturisers are the mainstay of treatment for dry skin, daily maintenance of normal skin, and adjunctive therapy for many skin diseases (Flynn *et al.*, 2001:387).

Treatment of dry skin is aimed at restoration of the epidermal water barrier. This is accomplished with moisturising agents that are topically applied to the skin. Humectants are compounds that attract water from the dermis into the stratum corneum. Examples of humectants include urea, glycerine, propylene glycol, sodium lactate, sorbitol, honey, and pyrrolidone carboxylic acid (PCA) (Flynn *et al.*, 2001:389).

Urea is one of the most important soluble substances of the stratum corneum. In recent years this substance has become more and more important in dermatological therapy and cosmetics. Many diseases have been described that are characterised by deficiency of urea, such as atopic dermatitis or clinical dry skin. The urea content of normal skin is nearly 1%. It contributes in a significant manner to the hydration of the stratum corneum. Urea contributes approximately 3-7% to the natural moisturising factor (NMF). The NMF appears to be

responsible for the hydration status of the stratum corneum. Otherwise urea is known for its keratolytic and pruritus-easing properties, and it is a very potent humectant in moisturising creams. Its sources in the epidermis are sweat and the decomposition of arginine by arginase during the process of keratinisation (Häntschel *et al.*, 1998:155).

1.2 THE HISTORY OF UREA AND MOISTURISERS

Urea was first discovered in human urine by H.M. Rouelle in 1773. It was synthesised in 1828 by Friedrich Wohler (see Figure 1.1) and was the first organic compound to be synthesised from inorganic starting materials. It was found when Wohler attempted to synthesise ammonium cyanate, to continue a study of cyanates which he had been carrying out for several years. On treating silver cyanate with ammonium chloride solution he obtained a white crystalline material which proved identical to urea obtained from urine.

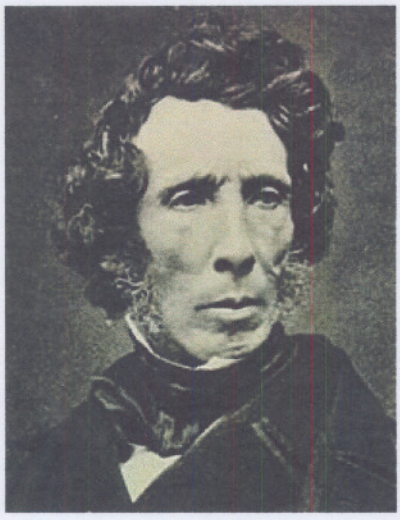

Figure 1.1 Friedrich Wohler

This discovery prompted Wohler to write triumphantly to Berzelius:-
"I must tell you that I can make urea without the use of kidneys, either man or dog. Ammonium cyanate is urea" (Fairall, 1996).

The use of moisturisers by mankind has historic roots. Ancient Egyptians frequently anointed their bodies with oils. The Bible describes applications of oils to the skin, and Ancient Greek and Roman cultures regularly applied oil-containing products. Humans have recognised the value of externally applied lipids for thousands of years, and continue to value them (Flynn *et al.*, 2001:387).

1.3 COSMECEUTICS 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 components 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).

1.3.1 The skin and its permeability

The skin is not a uniform surface. A mature human weighing 65 kg will have approximately 18000 cm^2 of skin surface area. Figure 1.2 is a diagrammatic representation of the structure of the human skin (Schaefer *et al.*, 1999:9).

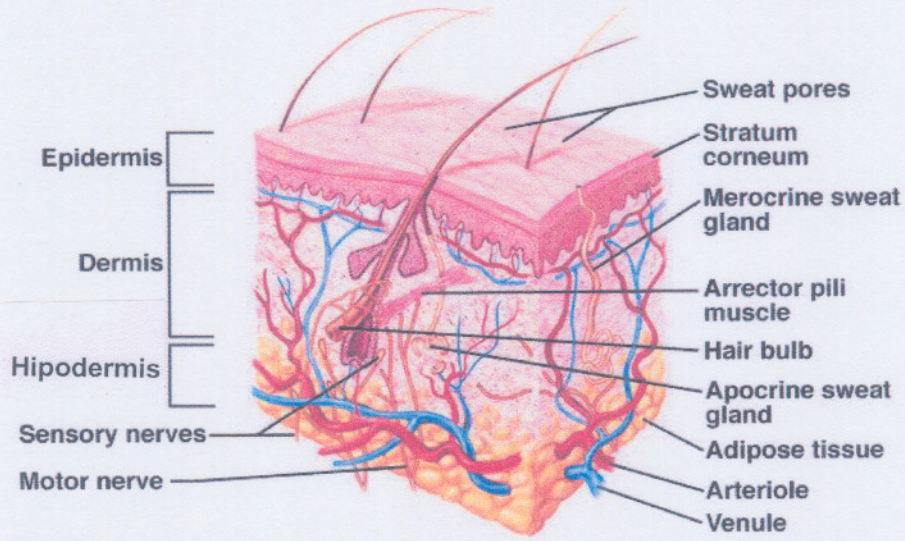

Figure 1.2 Diagrammatic representation of four compartments of the skin: stratum corneum, viable epidermis, dermis, and hipodermis

The superficial region, termed the stratum corneum, is between 10 and 20 µm thick. Underlying this region is the viable epidermis (50-100 µm), dermis (1-2 mm) and hypodermis (1-2 mm). Because of the large surface area as well as the volume of the compartments, the skin is the body's largest organ, weighing approximately 7 kg and representing more than 10% of the total body mass. Though the skin comprises a very large volume, the barrier to percutaneous absorption lies within the stratum corneum, the thinnest and smallest compartment (Schaefer *et al.*, 1999:9).

The stratum corneum consists of horny cells or corneocytes, which are flat, polyhedral, non nucleated cells approximately 40 µm long and 0,5 µm in diameter. The corneocytes are cell remnants of the terminally differentiated keratinocytes found in the viable epidermis. Their cellular organelles and cytoplasm have disappeared during the process of cornification. In turn, this is accompanied by a remodeling of the remaining protein constituents to form the corneocytes. They are composed primarily of insoluble bundled keratins surrounded by a cell envelope stabilized by cross-linked proteins and covalently bound lipid. Interconnecting the corneocytes of the stratum corneum are polar structures such as corneodesmosomes, which ascertain the cohesion of the stratum corneum (Schaefer *et al.*, 1999:10).

Intercellular lipid is generated primarily from the exocytosis of lamellar bodies during the terminal differentiation of the keratinocytes and, less importantly, from sebaceous secretion, which is predominantly deposited in the upper layers of the stratum disjunctum. The intercellular lipid is pivotal for a competent skin barrier and forms the only continuous domain in the stratum corneum. It follows a tortuous path within the stratum corneum, a structural feature that may account in part for the barrier properties of the skin (Schaefer *et al.*, 1999:10).

The stratum corneum comprises approximately 15 layers, though at sites of increased pressure (such as the soles at the feet) this number is significantly (5 - to 10-fold) increased. The upper layer, termed the *stratum disjunctum*, contains approximately 3-5 layers and is constantly undergoing desquamation. The stratum compactum (lower three layers) is thicker, more densely packed, more regular, and contains structures that more closely reflect the underlying epidermis. The lower stratum compactum has more water associated with it (30% by weight) as compared with the stratum disjunctum (15% by weight), though both are considerably less hydrated than the viable dermis (70% by weight). These differences correlate with the amino acid and lipid content of the layers. Further differences are observed for the rigidity of the cellular membranes,

perhaps reflecting the maturation process of the corneocyte cell envelope during the passage from the epidermis to the surface of the skin and the final shedding. Finally, the stratum compactum has a higher density of corneodesmosomes, suggesting that their proteolysis is required for the separation of mature corneocytes. Taken together, this indicates that the stratum corneum is not uniform, that it continuously evolves from below to the surface, and that the layers represent various stages of corneocyte and intercellular lipid maturation (Schaefer *et al.*, 1999:11).

Adsorption indicates the reversible, noncovalent interaction of compounds with structures such as the binding of drugs to keratin filaments. It is used to describe a state and not the process; it should be differentiated from the term substantivity, which refers to reversible binding. The term absorption is used to describe the process of intake of substances, as by an organism. Percutaneous absorption is thus a global term describing the passage of compounds across the skin, though it does not necessarily indicate their eventual fate. The process can be subdivided into three steps. Penetration is the entry of a substance into a particular layer or structure, such as the entrance of a compound into the stratum corneum. It is to be differentiated from the term permeation, which indicates that the compound has diffused from one layer to another distinct layer. Finally, resorption is defined as the uptake of substances through the vascular system into the central or inner compartment. Thus, we do not consider that compounds which have penetrated into the stratum corneum should be considered to be absorbed into the body (Schaefer *et al.*, 1999:22).

1.3.2 Structural basis for percutaneous absorption pathways

These routes are referred to as (1) appendiceal, (2) transcellular, and (3) intercellular (Figure 1.3). Permeability through the stratum corneum (transcorneal permeation) may be considered to occur through the intercellular

lipid domain or through the corneocytes (transcellular route). The relevance of these routes to percutaneous absorption of a compound depends upon their number per surface area and path length as well as the diffusivity and solubility of the compound in each domain. These pathways should not be treated as mutually exclusive. Hair follicles are the most important appendages in terms of surface area (Schaefer *et al.*, 1999:16).

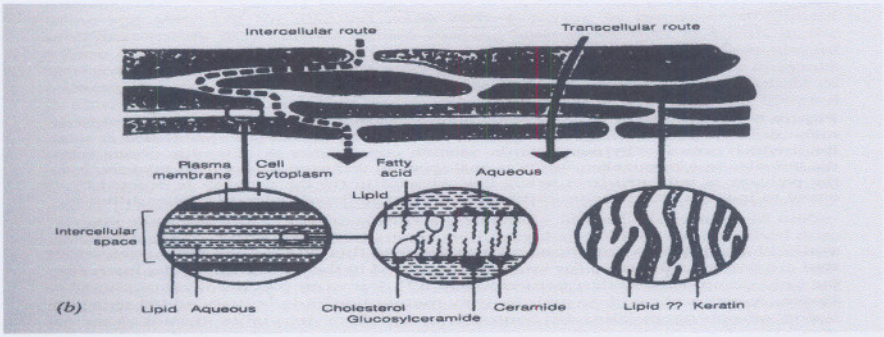

Figure 1.3 Model of penetration pathways (Schaefer *et al.*, 1999:17)

1.3.3 Transcellular vs. intercellular pathways

The rate-limiting step for permeation includes a hydrophobic barrier -i.e., the intercellular lipid. Available evidence suggests that the only continuous domain within the stratum corneum is formed by the intercellular lipid space. This suggests that the majority of compounds penetrating the stratum corneum must pass through intercellular lipid, though it does not exclude the possibility that compounds can also enter into the inner lumens of corneocytes.

Low molecular weight moisturisers like glycerol and urea are likely to undergo partition into the corneocytes and alter their water binding capacity. Thus, the

penetration of compounds into corneocytes cannot be excluded from the consideration of percutaneous absorption pathways (Schaefer *et al.*, 1999:16).

1.3.4 Skin moisturisers

In order to retain and bind water at the skin surface, hygroscopic substances are used. Examples are the active principles of NMF and particularly the sodium salt of 2-pyrrolidone-5-carboxylic acid (sodium PCA), a physiological moisturiser found in various organs, organic fluids and particularly the epidermis, including the stratum corneum.

Mixtures of sodium PCA, amino acids, urea, lactic acid, sodium lactate, and trace elements, known as "reconstituted NMFs," are also frequently used. Urea (around 2-5%) is also capable of increasing hydration of the corneum by 100%, both by an osmotic effect due to its low molecular weight and for its ability to solubilise insoluble proteins (Morganti, 1999:80).

1.4 PHARMACOLOGICAL ACTION

Humectants are compounds that attract water from the dermis into the stratum corneum. These agents are designed to attract water up into the outer layers of the epidermis, as opposed to trapping water found in the environment (unless the relative ambient humidity exceeds 70 to 80%). Moisturisation of the stratum corneum occurs from below, with the dermis contributing moisturisation to the skin. Examples of humectants include glycerine, propylene glycol, urea, sodium lactate, sorbitol, honey, and pyrrolidone carboxylic acid (PCA), (Flynn *et al.*, 2001:389).

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Moisturisers that contain only humectant elements will draw water into the stratum corneum but not prevent the hydrated stratum corneum from losing its increased water content. As such, they can actually increase transepidermal water loss (TEWL). The use of only humectants in skin with a defective barrier could actually contribute to a drying function of the outer layer of the skin. Thus, humectants are usually combined with occlusants (Flynn *et al.*, 2001:389). The water content of the stratum corneum should be greater than 10% for the skin to have a normal appearance and not feel rough, scaly, or dry. Ideally, the stratum corneum should have a 20 to 35% water content. Moisturisers serve to return water content to the skin with the humectants attracting water from the lower layers of the epidermis into the stratum corneum, and occlusive ingredients preventing transepidermal water loss (Flynn *et al.*, 2001:390).

Moisturisers restore epidermal lipids, which play a key role in maintaining the permeability barrier of the skin as well as increasing its plasticity, it make the skin feel smoother, a property known as emolliation. Cracks and gaps between the desquamating corneocytes are filled by moisturiser, decreasing the rough quality of the skin. Moisturisers also decrease friction on the skin, improving the lubricity (Flynn *et al.*, 2001:390).

Urea can be added to moisturisers and enhances the water-binding capacity of the stratum corneum by disrupting bonding. Urea exposes water-binding sites on corneocytes and promotes desquamation by decreasing the intercellular cementing substance between the corneocytes. Also, long-term treatment with urea has been demonstrated to decrease TEWL. A possible explanation may involve urea-induced reduction in epidermal cell proliferation which, in turn, increases the size of corneocytes. Larger corneocytes lower skin permeability, thereby lowering TEWL. It has also been shown that long-term urea application reduces the susceptibility of the skin to sodium lauryl sulfate irritation. A possible mechanism may be urea-induced alteration of the binding capacity of the stratum corneum. This protective effect (after prolonged application) has

promising clinical ramifications for the use of urea-containing moisturisers to reduce contact dermatitis from irritant stimuli (Flynn *et al.*, 2001:391).

According to Parima (2003) urea gently dissolves the intercellular matrix which results in loosening the horny layer of skin and shedding scaly skin at regular intervals, thereby softening hyperkeratotic areas. Urea also hydrates and gently dissolves the intercellular matrix of the nail plate, which can result in the softening and eventual debridement of the nail plate.

1.5 PHYSICAL AND CHEMICAL PROPERTIES

In Figure 1.4 is a model of the structure of the urea molecule. Table 1.1 summarises the physical and chemical properties of urea.

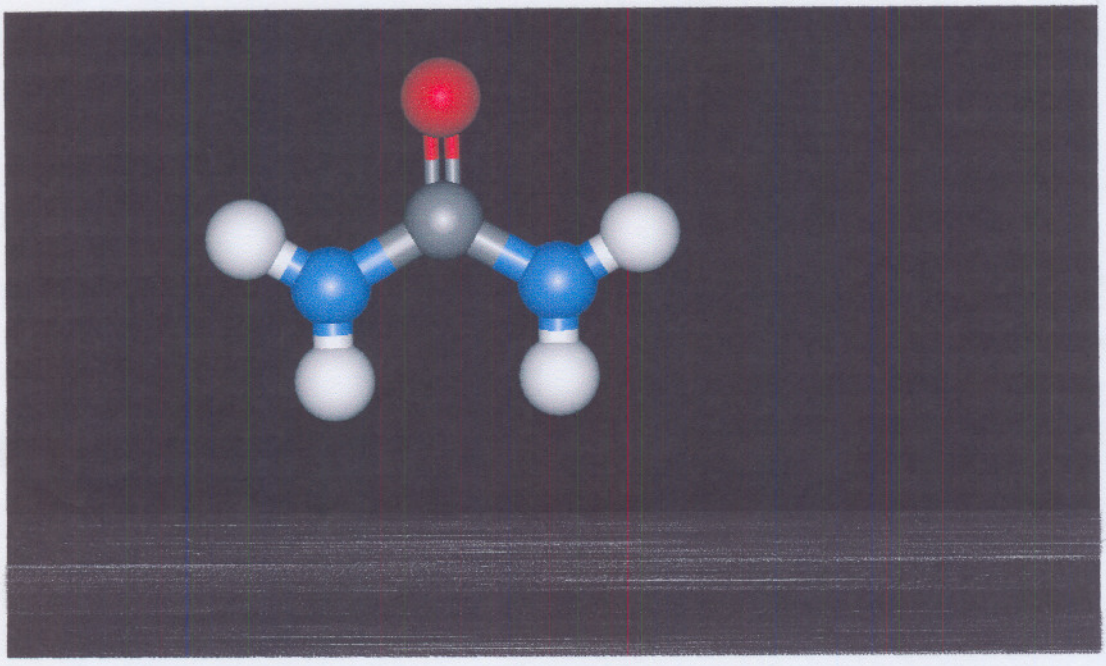

Figure 1.4 Model of the urea molecule

Table 1.1 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 ammoniacal odour on long standing (AHFS Drug Information®, 2002:3457).
pH water solution (conc.10%)	9-10
Melting point	133°C (decomposes)
Flammability (solids)	Not flammable
Explosive properties	Uncontaminated urea is not an explosion hazard. However, it may form explosive mixture subject to spontaneous detonation when contaminated with strong acid (nitric or perchloric) or nitrates.
Oxidising properties	None
Bulk density	700-800 kg/m^3
Solubility in water	1080 g/l at 20ºC

1.6 STABILITY

Upon standing, heating, or exposure to acids or alkalies, urea is hydrolysed to ammonia and carbon dioxide. Solutions of urea are unstable and cannot be sterilised by heat. Urea should be stored in well-closed containers (AHFS Drug Information®, 2002: 3457).

1.7 USES

Urea is used topically in the treatment of dry skin. At concentrations of 5-30%, urea promotes hydration of keratin and mild 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 (AHFS Drug Information®, 2002: 3457).

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

- Direct diuretic
- Wounds
- Athlete's foot
- Perfusions used in neurosurgery
- Urea infusions
- Water retention
- Urinary infection without renal lesions
- Cancer
- Hyperkeratotic conditions such as:
    - Dry skin
    - Rough skin
    - Dermatitis
    - Psoriasis
    - Xerosis
    - Ichthyosis
    - Eczema
    - Keratosis
    - Keratoderma
    - Corns
    - Calluses

- Damaged, ingrown and devitalized nails

According to Parima (2003) urea is effective for debridement and promotion of normal healing of hyperkeratolic surface lesions, particularly where healing is retarded by local infection, necrotic tissue, fibrinous or prurient debris or eschar.

Urea is a direct diuretic, meaning it can increase diuresis by boosting the function of the renal epithelia (Robert & Fils, 2000).

Wounds can be treated by spraying urea or a 2% solution of same. In Russia more concentrated solutions of urea are used to treat athlete's foot and certain related pathologies. In France urea is used in perfusions, with 500 ml flasks containing 90 g of pure urea. These perfusions are used in neurosurgery (before, during, or after) to treat brain swelling and during eye surgery (Robert & Fils, 2000).

Urea can also be taken internally: one or two grams per day are used as a treatment for water retention and related problems (such as swollen face, headaches, premenstrual water retention, enuresis). Urea can also be used to treat urinary infection in the absence of lesions. Urea possesses interesting bacteriostatic and antibacterial activities (Robert & Fils, 2000).

As far as its effect on cancerous cells, urea seems to have an antiangiogenic activity, as well as destabilising the fibrin that forms the stroma that "blankets" the tumour, and can make up 50% of the tumor mass. It seems that urea modifies the tumor's support and exposes its peripheral characteristics to the immune system (Robert & Fils, 2000).

1.8 ADVERSE EFFECTS

When used in appropriate dosage, topically applied urea preparations have a low order of toxicity. Transient stinging may occur, especially when urea preparations are applied to the face or broken or inflamed skin. Local irritation may also occur following topical application of urea (AHFS Drug Information®, 2002:3457).

1.9 PRECAUTIONS AND CONTRAINDICATIONS

Topical preparations containing urea are intended for external use only. Topical preparations should be applied with caution to the face or broken or inflamed skin. Urea should not be used near the eyes.

Some commercially available topical formulations of urea that contain sulfites may cause allergic-type reactions, including anaphylaxis and life-threatening or less severe asthmatic episodes, in certain susceptible individuals. The overall prevalence of sulfite sensitivity in the general population is unknown but probably low, such sensitivity appears to occur more frequently in asthmatic than in nonasthmatic individuals. Topical urea preparations should be discontinued if irritation or rash occurs during use (AHFS Drug Information®, 2002:3457).

1.10 ANTIMICROBIAL ACTIVITY

Although it is not yet entirely clear why urine has a germicidal and antiseptic effect, it is known that urea plays an important role here. Ammonia and salt also have a similar purifying effect. Besides killing bacteria, urine also inhibits

or destroys various viruses and fungi. Scientific research has demonstrated that both urea and ammonia have a powerful anti-viral effect. Applying urine to a fresh cut or scrape prevents infection and keeps flies away. Compresses from fresh or old urine help to combat infections and often cause them to disappear. Although urine does not entirely prevent the growth of bacteria in the urethra, it clearly has a powerful antiseptic effect when externally applied (Anon, 1999:7).

Urea is an oxidising substance which ensures that the disintegrating proteins (proteins in the area of a wound or inflammation) dissolve. If urea is present, disintegrating tissue cannot feed itself with other rotting material. It dissolves fats and other natural body secretions. Urea is even more effective when heated. Due to its strong anti-bacterial nature, urine has an inhibitive effect on the growth of tuberculosis bacille. Bacteria-inhibiting or bacteria-killing effects of urine increase with decreasing pH. Urea and ammonia, closely related, play an important role here. When brought in contact with urea, complex polymers are transformed or decomposed into monomers, which can then be endured by the body (Anon, 1999:7).

1.11 CONCLUSION

In conclusion it can be said that urea possesses all the properties that is necessary to treat hyperkeratosis, dryness, ichthyosis and as therapy in allergic (atopic) eczema. Therefore, if it is incorporated into dermatological vehicles and properly analysed and evaluated, there is a possibility that these products could be effective in the treatment of these skin problems.

CHAPTER 2

FORMULATION OF PRODUCTS CONTAINING UREA

2.1 INTRODUCTION

Urea is one of the most important end products of human protein metabolism. It has been used for many years in the treatment of chronic dry skin conditions as it is a moisturising factor (Häntschel *et al.*, 1998:155).

However, the stability of urea is somewhat of a problem in water-containing formulas that are stored for a long time. Urea can decompose into carbon dioxide and ammonia. These problems have been solved in pharmaceutical preparations by the inclusion of suitable stabilisers such as sodium lactate whereby the decomposition of urea into ammonia is minimised (Beiersdorf, 2003). The most appropriate solvents for urea are water and a water/propylene glycol (1:1 in volume) mixture (Gallardo *et al.*, 1990:845).

According to Dermadoctor.com. (2002) a concentration of 5-10% urea helps deep clean dry itchy scalp. Antibacterial agents help kill yeast and bacteria that contribute to scaly dry skin and dandruff flares.

The formulations that were developed included a hair gel, shampoo, facial toner, facial cleanser, day cream, foot and heel balm and a body cream.

2.2 FORMULATION OF A HAIR GEL

2.2.1 Purpose and function of a gel

Gels are a type of base which produce a uniform external appearance, range from transparent to semitransparent and give a moist feeling. Aqueous gels have been used in cosmetics because of their special feature of light feeling.

Aqueous gels contain a lot of moisture, they are used as a base material with water supplying, moisturising and cooling effects or as the base in cleansers for removing light makeup (Mitsui, 1997:351).

Hair growth promoters are preparations made by adding various pharmaceutical agents which are applied to the scalp to normalise its functions. By increasing the circulation in the scalp, they improve hair follicle function which in turn promotes hair growth and prevents hair loss. They also help prevent dandruff and itchiness (Mitsui, 1997:413).

2.2.1.1 Factors considered to cause hair loss

- Reduced hair follicle function due to male hormones.
- 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 that it requires for cell division from the capillaries in the dermal papilla. Therefore, 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 matrix will not

be sufficient, hence impairing cell metabolism and having an adverse effect on hair growth (Mitsui, 1997:414).

- Reduction in scalp physiological functions. 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 the dandruff is decomposed by bacteria 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. If the sebaceous glands in the upper part of the follicles secrete too much sebum, this will produce irritation to the scalp when decomposed by the bacteria on it and may give rise to seborrhoea alopecia (Mitsui, 1997:415).
- 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 (Mitsui, 1997:415).

2.2.2 Formulation

A very simple glycerine-containing treatment consists of 5-10 parts urea, 3-5 parts glycerine, and 100 parts water. This recipe is claimed to increase the volume of the hair, as well as act as a treatment for seborrhea and other scalp diseases. Effective or not for seborrhea, it should certainly provide a strong moisturising treatment. Glycerine has been claimed as an essential ingredient in a hair growth stimulant (Jungermann, 1991:374). The final formula for the hair gel is given in Table 2.1.

Table 2.1 Hair gel formula

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

2.2.3 Method

Dissolve urea and 50 ml of 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 in water. Add A to C and homogenise thoroughly. Remove the foam manually. Mix B and dissolve in 3.0 ml of water and add B to C.

2.3 FORMULATION OF A SHAMPOO

2.3.1 Purpose and function of a shampoo

A shampoo is a hair-wash cosmetic used to remove dirt from the scalp and the hair, treat dandruff and itchiness and maintain the hair in a clean and beautiful condition. In order to do this, it must have an appropriate level of cleansing power which is sufficient to remove all the dirt but will not remove too much

sebum, which is very necessary for the scalp and hair. The final formula for the shampoo is given in Table 2.2.

There is a great variety of shampoos which, in addition to their main function of cleansing, have added value in the form of conditioning, luster enhancing and styling capabilities (Mitsui, 1997:407).

2.3.1.1 Qualities characteristic to a shampoo

Shampoo must have the following qualities:

- An appropriate cleansing ability.
- Produce a lasting, rich, creamy lather.
- Protect the hair from friction damage during washing.
- The hair must have a natural luster and an appropriate softness after it has been washed, and
- They must be very safe with respect to the scalp, hair and eyes (Mitsui, 1997:407).

2.3.2 Formulation

Table 2.2 Shampoo formula

INGREDIENTS	%m/m	Activity
A. Urea	5%	Active (moisturiser)
Sodium lactate (88%)	5%	Stabiliser
Distilled water	1%	Solvent
B. Texapon N 70	14%	Surface active agent
C. Distilled water	70,5%	Solvent
Sodium chloride	4,5%	Thickening agent

2.3.3 Method

Dissolve the sodium chloride in C in a small amount of water, add insoluble B. Add the rest of the water. Then add and mix A to the BC mixture.

2.4 FORMULATION OF A FACIAL TONER

2.4.1 Purpose and function of a facial toner

A toner is the most important part of skin care. If you do not tone your skin before applying your moisturisers or treatment serums, then you are simply fooling yourself into believing that you are taking care of your skin. Applying anything on your face without toning first is useless (Westervelt, 1997:1). It refreshes, tones and moisturises the skin and prepares the skin for application of skin care (Anon, 2003:2). The final formula for the facial toner is given in Table 2.3.

Contrary to common belief, a toner is not just a skin conditioner. It is much more than that. A toner performs 4 very essential acts that no other skin care product on the market can do:

- It DEEP CLEANSES and purifies your pores, making sure that all pores are totally clear of leftover make-up, dirt, dead skin cells, toxins and other hazardous elements. It's especially important for clearing the skin of impurities found in tap water. Analyze tap water and you'll discover fluoride, chlorine and sodium which clog the pores and are very dehydrating. If this debris is not removed, you are only forcing it deeper into your pores as you apply moisturiser on top, trapping the debris that can cause bumps, blackheads, enlarged pores, improper absorption of the moisturiser, rough texture and even acne (Westervelt, 1997:1).

- It BALANCES the pH level or the natural acid mantle that protects your skin from the environment. Not too acid, not too alkaline. It can take your skin up to 30 minutes to rebalance itself after cleansing without using a toner because the sebaceous or oil glands are confused.
- HYDRATION is crucial for proper cell function. Skin can be oil dry and/or moisture dry and the moisture content is more crucial to skin health. A toner is a very nourishing form of moisture that even a moisturiser cannot provide. Also, the environment is constantly robbing your skin of hydration, especially during heat season and while travelling.
- PENETRATION of treatment and moisturisers means complete absorption of the potent nutrients, those nutrients absorbing deeper, more effectively and evenly into your skin (Westervelt, 1997:2).

2.4.2 Formulation

Table 2.3 Facial toner formula

INGREDIENTS	%m/m	Activity
A. Cremophor® RH 40	1,5%	Solubiliser
B. Propylene glycol USP	3%	Moisturiser
Ethanol 96%	15%	Preservative
Witch Hazel	2,1%	Astringent
Urea	5%	Active (moisturiser)
Sodium lactate (60%)	5%	Stabiliser
Distilled water	to 100%	Solvent

2.4.3 Method

Solubilise phase A. Mix the components of phase B to a solution and stir into phase A. Adjust the pH to 5-6

2.5 FORMULATION OF A FACIAL CLEANSER

2.5.1 Purpose and function of a facial cleanser

The most important considerations to be made regarding face cleansing cosmetics, the first step in any cosmetic routine, are:

- the object to be cleansed (skin),
- the type of dirt adhering to the skin surface,
- the type of product to be used for the cleansing, and
- the cleansing method

The purpose of face cleansers is to remove skin metabolism products, such as sebum, horny layer flakes, sebum oxidation products and sweat residues adhering to the skin; dirt and dust from the surrounding air; micro organisms; and in case of women, makeup products as well (Mitsui, 1997:323). The final formula for the facial cleanser is given in Table 2.4.

2.5.2 Formulation

Table 2.4 Facial cleanser formula

INGREDIENTS	% m/m	Activity
A. Cremophor® A6	1%	Emulsifying agent
Cremophor® A25	1%	Emulsifying agent
Luvitol EHO™	7%	Oil phase of emulsion
Liquid Paraffin™	8%	Oil phase of emulsion
Cetyl alcohol	1,25%	Thickening agent
GMS A/S™	2,5%	Co-emulsifying agent
B. Methylparaben	0,3%	Preservative
Propylparaben	0,2%	Preservative
Propylene Glycol	2%	Moisturiser
Sodium lactate	5%	Stabiliser
Urea	5%	Active (moisturiser)
Distilled water	to 100%	Solvent

2.5.3 Method

Heat phases A and B separately to approximately 80°C. Stir phase A into phase B and homogenise thoroughly. Cool to room temperature.

2.6 FORMULATION OF A CREAM

2.6.1 Purpose and function of a cream

A cream is a type of emulsion in which two liquids that do not mix together, like water and oil, are made into a stable dispersion. This is achieved by making the one liquid the dispersion phase that is dispersed through the other, the dispersion medium. A lipophilic active ingredient can be dissolved into a water medium when a cream is used.

The main function of a cream is to maintain the moisture balance, and to keep the skin moist and supple through the supply of water, humectants and oils (Mitsui, 1997:341). The final formula for the day cream is given in Table 2.5.

2.6.2 Day cream

2.6.2.1 Formulation

Table 2.5 Formula of a day cream

INGREDIENTS	%m/m	Activity
A. Cremophor A6®	2%	Emulsifying agent
Cremophor A25®	2%	Emulsifying agent
Luvitol EHO™	8%	Oil phase of emulsion
Cetyl alcohol	2%	Thickening agent
GMS A/S™	6%	Co-emulsifying agent
Dimethylpolysiloxane	0.2%	Glidant
B. Methyl paraben	0,3%	Preservative
Propyl paraben	0,2%	Preservative
Propylene glycol	2%	Moisturiser
Vitamin E-acetate	1%	Anti-Oxidant
Urea	5%	Active (moisturiser)
Sodium lactate 60%	5%	Stabiliser
Distilled water	to 100%	Solvent

2.6.2.2 Method.

Heat phase A and phase B separately to approx. 80°C. Stir phase A into phase B and homogenise thoroughly. Cool to room temperature.

2.6.3 Foot and heel balm

The final formula for the foot and heel balm is given in Table 2.6.

2.6.3.1 Formulation

Table 2.6 Formula of the foot and heel balm

INGREDIENTS	%m/m	Activity
A. Cremophor A6®	2%	Emulsifying agent
Cremophor A25®	2%	Emusifying 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-emusifying agent
B. Methylparaben	3%	Preservative
Propylparaben	2%	Preservative
Glycerine	5%	Moisturiser
Urea	5%	Active (moisturiser)
Distilled water	to 100%	Solvent

2.6.3.2 Method

Heat phase A and phase B separately to approximately 80°C. Stir phase A into phase B and homogenise thoroughly. Cool to room temperature.

2.6.4 Body cream

The final formula for the body cream is given in Table 2.7.

2.6.4.1 Formulation

Table 2.7 Formula of the body cream

INGREDIENTS	%m/m	Activity
A. Emulsifying ointment	30%	Oil phase of emulsion
B. Phenoxyethanol	1%	Preservative
C. Urea	10%	Active (moisturiser)
Sodium phosphate	2,5%	Buffer
Citric acid	0,5%	Buffer
Distilled water	to 100%	Solvent

A. Emulsifying ointment

INGREDIENTS	%m/m	Activity
Emulsifying wax	30%	Oil phase for
White soft paraffin	50%	ointment
Liquid paraffin	20%	preparation

(B.P, 2000:1766).

2.6.4.2 Method

Heat phase A and phase C separately to approximately 80°C. Mix phase B into phase A. Stir phase A into phase C and homogenise thoroughly. Cool to room temperature.

2.7 MATERIALS USED IN THE FORMULATIONS

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

2.7.1 Active ingredient

Table 2.8 Active ingredient used in formulations

ACTIVE INGREDIENT	SUPPLIER	BATCH NUMBER
Urea	Saarchem (UNIVAR®)	1020569

2.7.2 Solvents

A solvent must allow the optimum solubility of the solute. Table 2.9 lists all the solvents that were used in the formulations in this study.

Table 2.9 Solvents used in formulations

SOLVENT	SUPPLIER	BATCH NUMBER
Distilled water	RIIP	
Propylene glycol	ACE-Company	15719/4940
Luvitol EHO	BASF	-
Liquid Paraffin CP	ACE-Company	11141/1641
Glycerine	Saarchem (UNIVAR®)	1020733
Glycerine	Saarchem (UNIVAR®)	1018434

2.7.3 Preservatives

Table 2.10 lists all the preservatives that were used in the formulations in this study.

Table 2.10 Preservatives used in formulations

SOLVENT	SUPPLIER	BATCH NUMBER
Ethanol 96%	Labchem Ltd	E105025
Methylparaben	Galderma	JA 230076
Propylparaben	Galderma	P 13881
Phenoxyethanol	Sigma	129 H2303

2.7.4 Others

Table 2.11 lists all the materials that were used in the formulations in this study, which are neither preservatives nor solvents.

Table 2.11 Other materials used in formulations

MATERIAL	SUPPLIER	BATCH NUMBER
	Sigma	-
Dimethylpolysiloxane	Cognis LTD	-
Texapon N70	Saarchem (UNILAB®)	53047
Sodium Chloride	Saarchem (Merck Lab)	230203
Sodium Lactate 60%	BASF	-
Cremophor® RH40	-	-
Witch Hazel™	BASF	-
Cremophor A6™	BASF	-
Cremophor A25™	BASF	-
Cremophor RH 40™	-	-
Carbopol Ultrez™	BASF	-
Luvitol EHO™	Saarchem (UNILAB®)	1013865
Cetyl Alcohol	CRODA	-
GMS A/S™	Link Care	11825
Sweet oil	Riedel-de Haiën	340
Citric acid		
Sodium Phosphate dihydrate	Saarchem (UNIVAR®)	31145
Emulsi Wax™	Beige Pharm	2155
White soft paraffin™	-	-
EDTA	Saarchem (UNILAB®)	42676
Tris(hydroxymethyl) aminomethane	Saarchem (UNIVAR®)	80550
Vitamin E- acetate	BASF	-

2.8 CONCLUSION

Each of the final formulations were prepared in sufficient quantities and stored at different temperatures during stability testing.

Chapter 3 discusses the stability testing that was performed on these newly formulated products developed in this study. 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 3

STABILITY TESTING

3.1 INTRODUCTION

Stability testing may be defined as the process of evaluating a product to ensure that key attributes stay within acceptable limits. In order to make this testing meaningful, it is important to accurately establish the nature of these critical product attributes, to make sure how they change over time, and to define what degree of change is considered acceptable.

Stability data are useful as an "early warning system" that can alert the chemist to potential formulation/package-related problems. Such advance information can be helpful in many ways (Romanowski & Schueller, 2001:769).

More than other products, cosmetics are intended to be aesthetically pleasing to the consumer. For this reason consumers are likely to notice subtle changes in the odour or appearance of their favourite products. Since no product remains 100% unchanged as it ages, it is critical that the chemist anticipates the changes that may occur and make sure that they stay within limits that are acceptable.

Studying the performance of samples that are exposed to accelerated aging allows assessment of how the product will function over time. This is particularly important for cosmetic products intended to deliver "active" ingredients. If the formula is not stable, the delivery of the active ingredient may

be impaired. Properly designed stability testing can reveal such problems so that corrective action can be taken (Romanowski & Schueller, 2001:770).

Most companies have standardised test procedures for the storage of stability samples which depend on the objective of the study. Such procedures involve evaluations of samples stored at a variety of conditions and include enough samples to be statistically significant. Usually storage is done at elevated temperatures, under freeze and/or freeze thaw cycles and exposure to various types of light. Elevated temperature storage is critical, since the rate of chemical reactions roughly doubles for every 10°C increase in temperature. Storage at higher temperature allows acceleration of the aging process and certain problems are detected much sooner than they would appear at room temperature. Of course, the potential drawback is that, at high temperatures, reactions are forced to occur that would not happen at all at lower temperatures. The most common storage conditions used in the cosmetic industry are 54°C or 50°C, 45°C, 37°C or 35°C, room temperature (25°C), 4°C, freeze/thaw (Romanowski & Schueller, 2001:772).

Since many of the tests that must be conducted to evaluate product performance will affect the sample physically, multiple samples are required at each storage condition to ensure there will be enough samples left for evaluation at the end of the test period (Romanowski & Schueller, 2001:772).

3.2 STABILITY PROGRAM

Seven different cosmetic products were formulated: hair gel, shampoo, facial toner, facial cleanser, day cream, foot and heel balm and body cream. The formulations were tested under ICH conditions (ICH, 2003:4) over a period of three months at three different storage temperatures. Stability indicating tests that applied to these formulations, were conducted.

3.2.1 Storage temperatures

The physical and chemical stability of the preparations should be determined over a wide range of temperatures. All trial batches were stored at three temperatures. Controlled 5°C, 25°C and 40°C storage facilities were used during the stability period.

- 5°C ± 3°C - to determine if these formulated products would require refrigeration once on the market;
- 25°C ± 2°C /60% RH ± 5% RH- to determine the stability of these formulations at room temperature:
- 40°C ± 2°C /75% RH ± 5% RH- to accelerate stability testing (ICH, 2003:9).

3.2.2. Stability test conducted

All tests, as required by the South African Medicines Control Council (MCC, 2003:21,22,23), were conducted on the trial batches of the seven dosage forms (Tables 3.1- 3.7). All tests were done using calibrated and/or validated test apparatus, where appropriate.

Table 3.1 Stability tests conducted on the facial toner

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Relative density	✔	✔	✔	✔
Preservative assay (GC)	✔	✔	✔	✔
Preservative efficacy	✔			✔

Table 3.2 Stability tests conducted on the urea shampoo

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Relative density	✔	✔	✔	✔
Viscosity	✔	✔	✔	✔
Preservative efficacy	✔			✔

Table 3.3 Stability tests conducted on the hair gel

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Relative density	✔	✔	✔	✔
Viscosity	✔	✔	✔	✔
Preservative efficacy	✔			✔
Urea release (dissolution)	✔			✔

Table 3.4 Stability tests conducted on the foot and heel balm

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Spreadability	✔	✔	✔	✔
Preservative assay (HPLC)	✔	✔	✔	✔
Preservative efficacy	✔			✔
Penetration	✔	✔	✔	✔
Urea release (dissolution)	✔			✔

Table 3.5 Stability tests conducted on the facial cleanser

TEST	TEST INTERVALS			
	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Viscosity	✔	✔	✔	✔
Preservative assay (HPLC)	✔	✔	✔	✔
Preservative efficacy	✔			✔
Relative density	✔	✔	✔	✔

Table 3.6 Stability tests conducted on the day cream

TEST	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Viscosity	✔	✔	✔	✔
Preservative assay (HPLC)	✔	✔	✔	✔
Preservative efficacy	✔			✔
Relative density	✔	✔	✔	✔

Table 3.7 Stability tests conducted on the body cream

TEST	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Appearance	✔	✔	✔	✔
Urea assay	✔	✔	✔	✔
pH	✔	✔	✔	✔
Spreadability	✔	✔	✔	✔
Preservative assay (HPLC)	✔	✔	✔	✔
Preservative efficacy	✔			✔
Penetration	✔	✔	✔	✔
Urea release (dissolution)	✔			✔

3.3 TEST METHODS

All tests were done under Good Laboratory Practice (GLP) conditions, to ensure the accuracy of the results.

3.3.1 High performance liquid chromatography (HPLC)

HPLC analysis was used to determine the urea concentration in all the formulated products, as well as the concentration released during dissolution testing. The concentration of the preservatives in the body cream, facial cleanser, day cream and foot and heel balm was also determined with HPLC.

3.3.1.1 HPLC analysis of urea concentration

A validated HPLC method, obtained from Adcock Ingram, was used.

Chromatographic conditions:

COLUMN:	Cosmosil 5 NH_2 (Macherey-Nagel) - 4.6 x 150 mm or equivalent.
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 1 000 ml water, add 1.0 ml phosphoric acid 85% and mix. 10% **Mobile phase B:** Acetonitrile. 90%
FLOW RATE:	1.5 ml/minute
INJECTION VOLUME:	10 µl
TEMPERATURE:	15°C to 25°C
DETECTION:	UV at 200 nm
RETENTION TIME:	3.5 minutes
RUN TIME:	8.0 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) 25 standards for system suitability.

SOLVENT: Methanol

Standard preparation

Accurately weigh 125 mg urea standard into a 100 ml volumetric flask, add 5 ml water and shake until dispersed. Dilute to volume with methanol.

Sample preparation

For the 5% urea products (facial toner, shampoo, hair gel, facial cleanser, day cream, foot and heel balm): Accurately weigh 1.25 g of product into a 50 ml volumetric flask, add 25 ml methanol and shake until dispersed. Add 5.0 ml water and shake. Dilute to volume with methanol.

For the 10% urea products (body cream): Accurately weigh 1.25 g of product into a 100 ml volumetric flask, add 50 ml methanol and shake until dispersed. Add 5.0 ml water and shake. Dilute to volume with methanol.

NOTE: RETAIN SAMPLE PREPARATION FOR ANALYSIS OF PRESERVATIVE CONCENTRATION.

Procedure

Filter the standard and sample preparations through a 0.45 µm membrane filter, transfer into an autosampler vial and inject into the HPLC.

Calculation

$$\frac{\text{Sample peak area} \times \text{std mass (g)} \times \%\text{ purity} \times 100 \times 50}{\text{Standard peak area} \times 100 \times 100 \times \text{sample mass (g)}} = \text{g urea/50 g}$$

3.3.1.2 HPLC analysis of preservative concentration

The HPLC method that was used, was developed and validated at the RIIP.

3.3.1.2.1 Methylparaben & propylparaben

The HPLC parameters used were as follows.

COLUMN:	Nova-Pak C18 (Macherey-Nagel), 150 x 3.9 mm
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) 25 standards for system suitability
SOLVENT:	Mobile phase

Standard preparation

1. Weigh approximately 75 mg of methylparaben and 50 mg propylparaben accurately.
2. Transfer into a 100 ml volumetric flask and dissolve and make up to volume with solvent.
3. Dilute 10 ml of this solution to 100 ml with solvent.
4. Transfer this solution to an autosampler vial and inject into the chromatograph.

Calculation

$$\frac{\text{Sample area x mass of standard (mg) x \% potency}}{\text{Standard area x sample mass (g) x 20000}} = \% \text{ m/m}$$

3.3.1.2.2 Phenoxyethanol

The HPLC method that was used, was developed and validated at the RIIP.

The HPLC parameters used were as follows:

COLUMN:	Luna C18-2 column (Phenomenex), 150 x 4.6 mm, 5µm
MOBILE PHASE:	Acetonitrile/Water 35/65
FLOW RATE:	1.0 ml/min
INJECTION VOLUME:	10 µl
DETECTION:	UV at 220 nm
RETENTION TIME:	± 3.4 minutes
RUN TIME:	9 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) 25 standards for system suitability.
SOLVENT:	Methanol & Water 50/50

Standard preparation

1. Weigh approximately 125 mg of phenoxyethanol accurately.
2. Transfer into a 50 ml volumetric flask, and dissolve in 12.5 ml methanol. Fill the flask to volume with water.
3. Transfer 5 ml of this solution to a 50 ml volumetric flask, add 12.5 ml methanol. Fill the flask to volume with water.
4. Transfer this solution in to an autosampler vial and inject into the chromatograph.

Calculation

$$\frac{\text{Sample peak area x std mass (g) x \% purity x 100 x 50}}{\text{Standard peak area x 100 x 100 x sample mass (g)}} = \% \text{ m/m}$$

3.3.2 Gas chromatography (GC)

The GC method that was used, was developed at the RIIP. GC was used to determine the ethanol concentration of the facial toner.

The following conditions applied:

COLUMN:	Porapak Q (100 to 120 mesh)
COLUMN TEMPERATURE:	150°C

INJECTOR TEMPERATURE:	225°C
DETECTION TEMPERATURE:	275°C
INJECTION VOLUME:	2 µl

Standard preparation

720 µl of absolute ethanol and 150 µl of 1.2-propylene glycol were pipetted into a 25 ml volumetric flask. 3 ml of iso-propanol was added and diluted to volume with deionised water.

Sample preparation

5 ml of the test sample was pipetted into a 25 ml volumetric flask. 3 ml of iso-propanol was added and the sample further diluted to volume with deionised water.

Calculation

$$\frac{\text{Peak height ratio sample} \times 0.79(\text{SG}) \times 100}{\text{Peak height ratio standard}} = \% \text{ ethanol}$$

3.3.3 Urea release with enhancer cell (Dissolution testing)

The foot and heel balm, body cream and hair gel were used in the urea release studies.

In this study an USP six-spindle dissolution tester (VanKel 700) was used (Figure 3.1) for release of the active ingredient, urea. The apparatus used is calibrated using USP calibrator tablets, and checked monthly according to standard operating procedures.

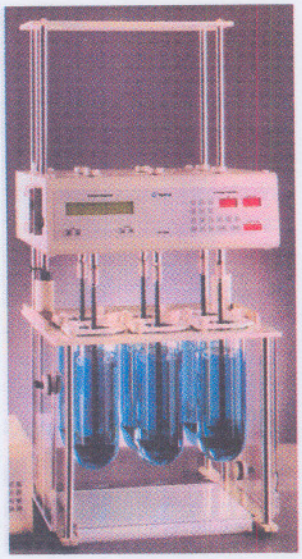

Figure 3.1 VanKel 700 dissolution tester

The enhancer cell (Figure 3.2) consisted of a cap, a washer, a membrane (cellulose-acetate membrane, 0.45 µm pore size and 30 mm diameter), a ring and the body which is the reservoir for the sample. A sample was placed in each enhancer cell. The membrane was placed on top of the sample and the O-ring was placed on top of the membrane and the cap was screwed in place.

Figure 3.2 Enhancer cell

Smaller paddles, knobs and collets customised for the enhancer cell system were used. Into six 200 ml vessels, 190 ml deionised water was transferred. To keep the dissolution medium's temperature constant at 32°C, the medium was covered with lids. The enhancer cells were dropped into the 200 ml vessels (Figure 3.3) at 20 seconds intervals. The rotation speed was set at 100 rpm. Each dissolution was run for six hours.

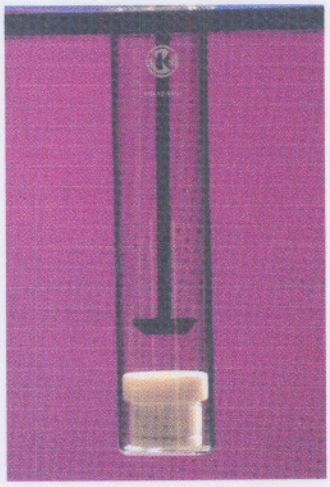

Figure 3.3 Vessel with small paddle and enhancer cell

200 µl were withdrawn with a micro-pipette at 30, 60, 120, 240 and 360 minutes and transferred to HPLC vials for HPLC analysis.

3.3.4 pH

The pH of the facial toner, hair gel and the shampoo was measured using a calibrated Mettler Toledo pH meter, equipped with a glass calomel electrode. The Mettler Toledo MP 220 pH meter was used to measure the pH of the body cream, foot and heel balm, facial cleanser and day cream. The pH meter was calibrated each time before use with buffer pH 4 and buffer pH 7.

3.3.5 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, 2000: 2071).

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 polytop was filled with the product to volume, and weighed.

Calculation

$$\frac{\text{Polytop with product - empty polytop}}{\text{Polytop with water - empty polytop}} = \text{Relative density}$$

3.3.6 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. A 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)

Figure 3.4 The small sample Adapter is shown here mounted on a Brookfield Model DV-Π+ Viscometer. With Brookfield Model TC-200 constant temperature bath (Brookfield, 1998:2)

Viscosity was determined on a Brookfield Model DV-Π+ viscometer (Figure 3.4) with Brookfield small sample adapter. The Brookfield small sample adapter (Figure 3.5) 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 3.5 Easily removable sample chamber is insulated for safe handling

The viscometer was calibrated every three months by the operator. Spindle number 25 was used. The test samples were 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.

3.3.7 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) fitted to the syringe tip. The scaled glass plate was put on a balance and tared (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.

3.3.8 Appearance

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

3.3.9 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 in 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.

3.3.10 Preservative efficacy

The microbial spoilage of cosmetics has been reported in literature for many years. Studies were conducted to evaluate the importance of the problem and investigate the primary contaminating sources, such as raw materials, personnel, water, and packaging, as well as secondary sources, such as the consumer (Devleeschouwer & Siguet, 2001:781).

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Sources of contamination can be divided into three groups:

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

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 manufacture. 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" preservative 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 25, 2002:1870).

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 25 for Category 2 products (USP 25, 2002:1871).

Table 3.8 Criteria for tested Microorganisms (Category 2)(USP 25, 2002:1871)

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 Molds	No increase from the initial calculated count at 7, 14, 28 days.

3.4 CONCLUSION

The outcomes of the test procedures, described in this chapter, are discussed and represented graphically in Chapter 4. The data that was generated during testing for each of the seven formulations will be dealt with separately and conclusions and possible relationships will be drawn from the data generated.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 FACIAL TONER

A toner refreshes, tones and moisturises the skin and prepares the skin for application of skin care (Anon, 2003:2).

The following parameters of the formulated facial toner were investigated: pH, relative density, appearance, urea assay, ethanol content and preservative efficacy. All of the tests that are discussed in 4.1 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.1.1 pH

The pH of the urea facial toner, measured over three months, is given in table 4.1.

Table 4.1 pH of the facial toner measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		5.42		
25°C/60%RH	5.43	5.58	5.75	6.10
40°C/75%RH		7.86	8.38	8.90

4.1.1.1 Discussion

- The pH of the urea toner 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 9 (Beiersdorf, 2003). This volatilisation is affected by temperature and moisture. Higher temperature and moisture increase volatilisation (Anon, 2003:20). 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).

4.1.2 Relative density

The relative density of the urea facial toner, measured over three months, is given in table 4.2.

Table 4.2 Relative density of the facial toner measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1.010		
25°C/60%RH	0.990	1.022	1.040	0.994
40°C/75%RH		1.018	1.043	1.016

4.1.2.1 Discussion

There was no significant change in the relative density of the toner (Table 4.2). Temperature, moisture and pH didn't have an influence on the relative density.

4.1.3 Appearance

The appearance, colour and odour should be evaluated, for this will determine consumer approval.

The toner is a clear transparent liquid with no odour. There were no changes in colour, odour or appearance over the three months of storage.

4.1.4 Urea assay

The concentration of urea in the toner was determined at initial, one, two and three months intervals as described in Chapter 3 (see section 3.3.1.1). An HPLC chromatogram of urea standard and facial toner sample is shown in Figure 4.1 and Figure 4.2 respectively.

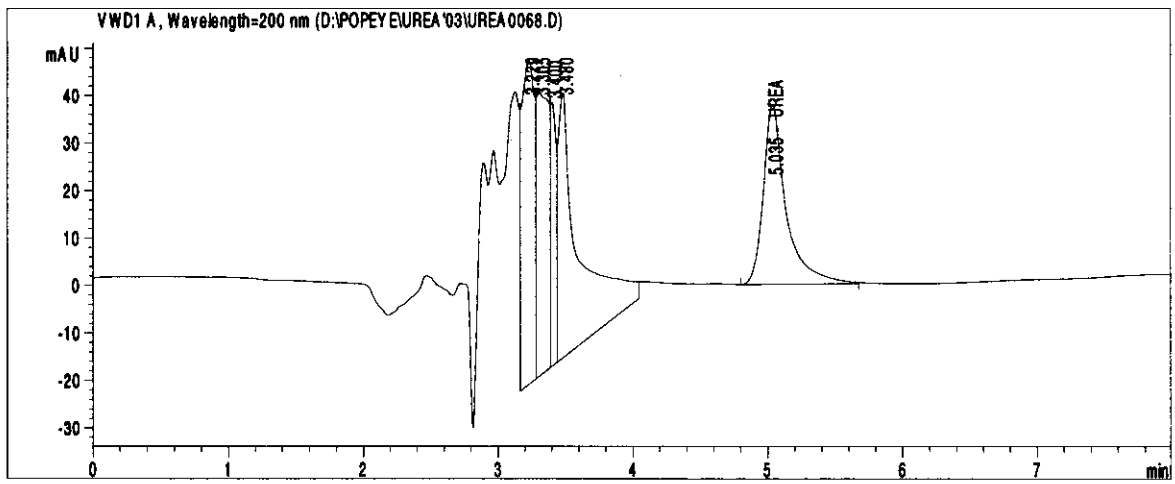

Figure 4.1 HPLC chromatogram of the urea standard

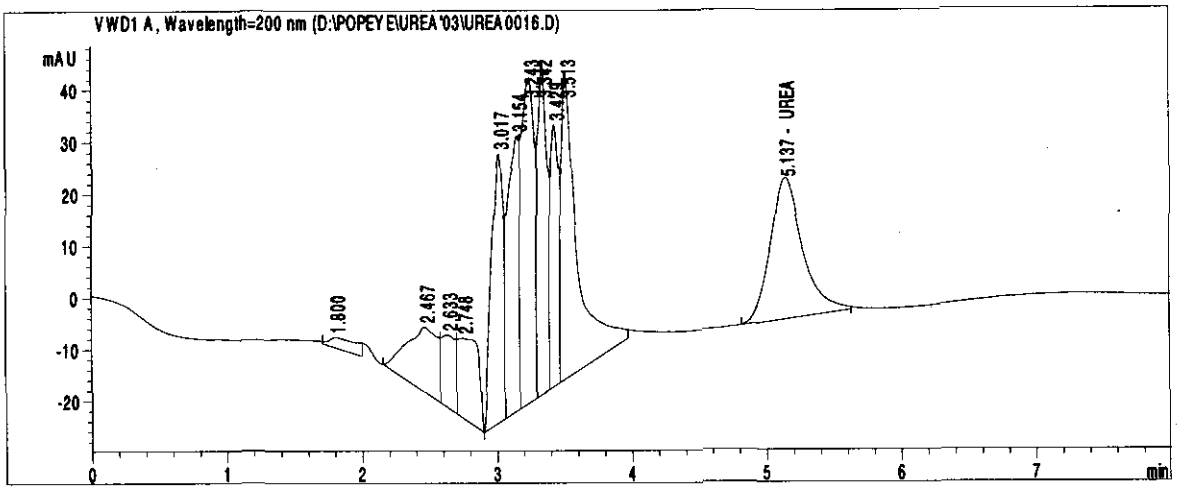

Figure 4.2 HPLC chromatogram of the urea toner sample

Table 4.3 The concentration (%) of urea in the facial toner measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.80%		
25°C/60%RH	103.70%	101.10%	97.00%	98.00%
40°C/75%RH		105.80%	97.00%	96.80%

4.1.4.1 Discussion

The concentration of urea in the formulated toner did not show any significant change. Although there was a slight decrease in urea concentration over time, it did not influence the stability.

4.1.5 Ethanol assay

Preservatives are added to cosmetics to suppress the proliferation of micro-organisms which have contaminated them and to kill them in time, thereby preventing deterioration of the product (Mitsui, 1997:201).

The ethanol concentrations of the facial toner was determined at initial and then at monthly intervals up to three months, using gas chromatography as described in Chapter 3 (see section 3.3.2). The ethanol concentration of the facial toner is given in Table 4.4.

Table 4.4 The concentration (%) of ethanol in facial toner measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.9%		
25°C/60%RH	98.5%	101.2%	102.2%	102.3%
40°C/75%RH		103.0%	103.9%	100.9%

4.1.5.2 Discussion

There was no significant change in the ethanol concentration of the toner over the three months of storage (Table 4.4). Temperature, moisture and pH didn't have an influence.

4.1.6 Preservative efficacy

The toner was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 25 Category 2 (Table 4.5 and 4.6).

Table 4.5 Preservative efficacy results of the facial toner (initial)

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	2.8	> 3.0	> 3.0	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.6 Preservative efficacy results of the facial toner (3 months), stored at 40°C/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.1.6.1 Discussion

It can be concluded that the preservative efficacy of the facial toner, complied with the requirements of the USP 25. It was therefore shown that the preservative used in the formulation was effective in protecting the facial toner against microbial contamination.

4.2 SHAMPOO

Throughout recorded history, hair has always been an important element of personal adornment. A mass of 100 000 to 150 000 flexible fibres have to be cleansed of oily deposits of sebum, sweat, entrapped desquamated scalp cells, along with residues of mousses, gels and hair sprays. All this has to be done within the span of a few minutes, leaving the individual hairs clean and free of tangles to which the structure of hair cuticles makes it particularly vulnerable.

Cleansing is clearly a dominant element of personal hygiene and, when reinforced by the aspect of attractive appearance, translated into powerful and highly marketable stimulus (Wolfram, 2001:581).

The urea shampoo was tested over a period of three months. The following parameters of the shampoo were investigated: pH, relative density, appearance, viscosity, urea assay and preservative efficacy.All of the tests were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.2.1 pH

The pH of the urea shampoo, measured over three months, is given in table 4.7.

Table 4.7 pH of the shampoo measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		4.88		
25°C/60%RH	4.84	5.07	5.39	5.82
40°C/75%RH		7.82	8.72	9.13

4.2.1.1 Discussion

The pH of the urea shampoo stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. (See 4.1.1.1)

4.2.2 Relative density

The relative density of the urea shampoo, measured over three months, is given in table 4.8.

Table 4.8 Relative density of the shampoo measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1.105		
25°C/60%RH	1.050	1.087	1.056	1.055
40°C/75%RH		1.087	1.078	1.098

4.2.2.1 Discussion

There was no significant change in the relative density of the shampoo (Table 4.8). Temperature, moisture and pH didn't have an influence on the relative density.

4.2.3 Appearance

The shampoo is a clear transparent viscous liquid with no odour. There was no changes in colour, odour or appearance over the three months of storage.

4.2.4 Viscosity

Viscosity data provides an accurate reference point in the formulation of the materials, facilitating the achievements of consistency from batch to batch and evaluates the structure achieved during storage. The viscosity of the shampoo was determined once a month for three months as described in Chapter 3 (see 3.3.6).

The average of the viscosity readings taken of the shampoo, is given in Table 4.9.

Table 4.9 The viscosity (in cP) of the shampoo measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		8734		
25°C/60%RH	9003	8158	-	7755
40°C/75%RH		9041	-	9147

4.2.4.1 Discussion

According to Table 4.9 there was a slight increase in viscosity in samples stored at 40°C/75%RH, whereas the viscosity of the samples stored at 25°C/60%RH seemed to decrease.

4.2.5 Urea assay

The concentration of urea in the shampoo was determined at initial, one, two and three monthly intervals as described in Chapter 3 (see section 3.3.1.1). (Table 4.10) An HPLC chromatogram of urea standard and shampoo is shown in Figure 4.1 and in Figure 4.3 repectively.

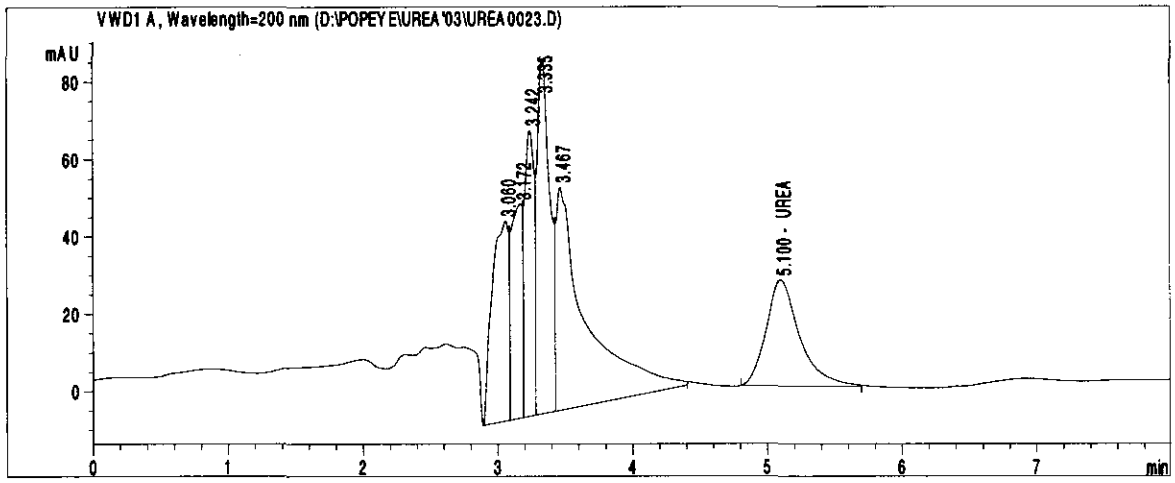

Figure 4.3 HPLC chromatogram of the urea shampoo sample

Table 4.10 The concentration (%) of urea in shampoo measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		91.90%		
25°C/60%RH	98.90%	96.00%	98.70%	98.40%
40°C/75%RH		101.40%	95.00%	93.30%

4.2.5.1 Discussion

The concentration of urea in the formulated shampoo did not show any significant change. Although there was a slight decrease in urea concentration over time, it did not influence the stability. Urea shampoo stored at 40°C/75%RH showed a decrease in concentration because urea decomposed into carbon dioxide and ammonia. (Beiersdorf, 2003). 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).

4.2.6 Preservative efficacy

The shampoo was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 25 Category 2 (Table 4.11 and Table 4.12).

Table 4.11 Preservative efficacy results of the shampoo (initial)

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	1.7	0.4	0.5	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.12 Preservative efficacy results of the shampoo (3 months), stored at 40°/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP25.

4.2.6.1 Discussion

It can be concluded that the preservative efficacy of the shampoo complied with the requirements of the USP 25. It was therefore shown that the preservative used in the formulation was effective in protecting the shampoo against microbial contamination.

4.3 HAIR GEL

The following parameters of the formulated hair gel were investigated over a period of three months: pH, relative density, appearance, viscosity, urea assay, urea release rate (dissolution) and preservative efficacy. All of the tests that are discussed in 4.3 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.3.1 pH

The pH of the urea hair gel, measured over three months, is given in table 4.13.

Table 4.13 pH of the hair gel measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		6.99		
25°C/60%RH	6.89	6.97	6.97	7.39
40°C/75%RH		7.90	8.26	8.86

4.3.1.1 Discussion

The pH of the urea hair gel stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. (See 4.1.1.1)

4.3.2 Relative density

The relative density of the urea hair gel, measured over three months, is given in table 4.14.

Table 4.14 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		1.110		
25°C/60%RH	1.051	1.146	1.157	1.083
40°C/75%RH		1.139	1.132	1.104

4.3.2.1 Discussion

There was no significant change in the relative density of the hair gel (Table 4.14). Temperature, moisture and pH didn't have an influence on the relative density.

4.3.3 Appearance

The hair gel is a clear transparent gel with no odour. No foreign particles and no stickiness were observed. There were no changes in colour, odour or appearance over the three months of storage.

4.3.4 Viscosity

Viscosity data provides an accurate reference point in the formulation of the materials, facilitating the achievements of consistency from batch to batch and evaluates the structure achieved during storage. The viscosity of the hair gel was determined once a month for three months as described in Chapter 3 (see 3.3.6).

The average of the viscosity readings taken of the hair gel, is given in Table 4.15.

Table 4.15 The viscosity (in cP) of the hair gel measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
5°C	7881	9176	-	-
25°C/60%RH		-		
40°C/75%RH		6959	-	8715

4.3.4.1 Discussion

Viscosity of urea containing products display thixotropic properties, which means that, as highly viscous gels, they liquefy without any change in the water content under the influence of mechanical stress. When the stress is removed the highly viscosity state returns (Beiersdorf, 2003:). Due to lack of sample no result at 25°C/60% RH after 3 months was obtained. No conclusion can be drawn from the results.

4.3.5 Urea assay

The concentration of urea in the hair gel was determined at initial, one, two and three monthly intervals as described in Chapter 3 (see section 3.3.1.1) (Table 4.16) An HPLC chromatogram of urea standard and hair gel is shown in Figure 4.1 and Figure 4.4 respectively.

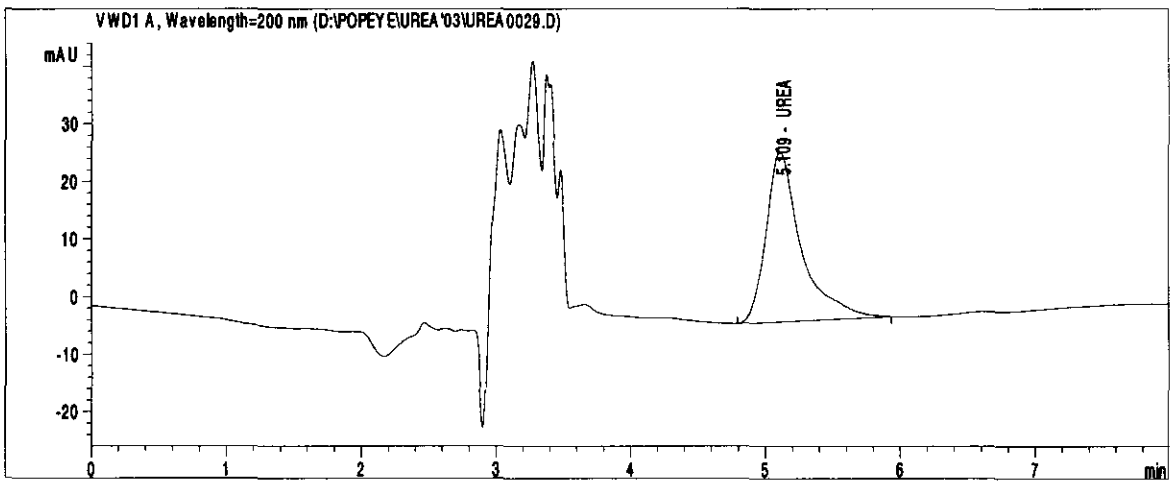

Figure 4.4 HPLC chromatogram of the urea hair gel sample

Table 4.16 The concentration (%) of urea of hair gel measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		105.00%		
25°C/60%RH	103.90%	103.40%	99.30%	99.30%
40°C/75%RH		98.30%	98.30%	97.60%

4.3.5.1 Discussion

The concentration of urea in the formulated hair gel did not show any significant change. Although there was a slight decrease in concentration, it did not influence the stability.

4.3.6 Urea release

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved. The use of potent medicaments, capable of penetrating the natural defensive barrier, demands a serious examination.

4.3.6.1 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 released from the hair gel was determined at initial and after three months storage at 25°C/60%RH and 40°C/75%RH.

The release experiment was done six fold and the average release was calculated for each analysis point.

The concentration of urea that was released from the hair gel (initial) as function of time, is given in Table 4.17 and Figure 4.5.

Table 4.17 The concentration (µg/sqcm) of urea released from the hair gel (initial) as a function of time

Time (minutes)	Concentration (µg/sqcm)
30	1598
60	3769
120	5476
240	7463
360	10258

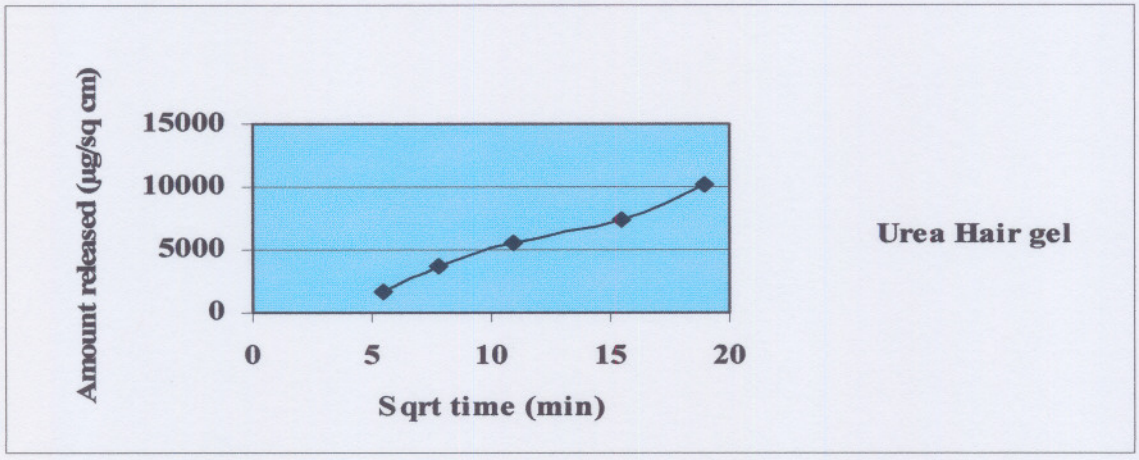

Figure 4.5 The concentration of urea released from the hair gel at initial.

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 4.18 and Figure 4.6 and Table 4.19 and Figure 4.7 respectively

Table 4.18 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	5405
60	8799
120	13812
240	21852
360	28493

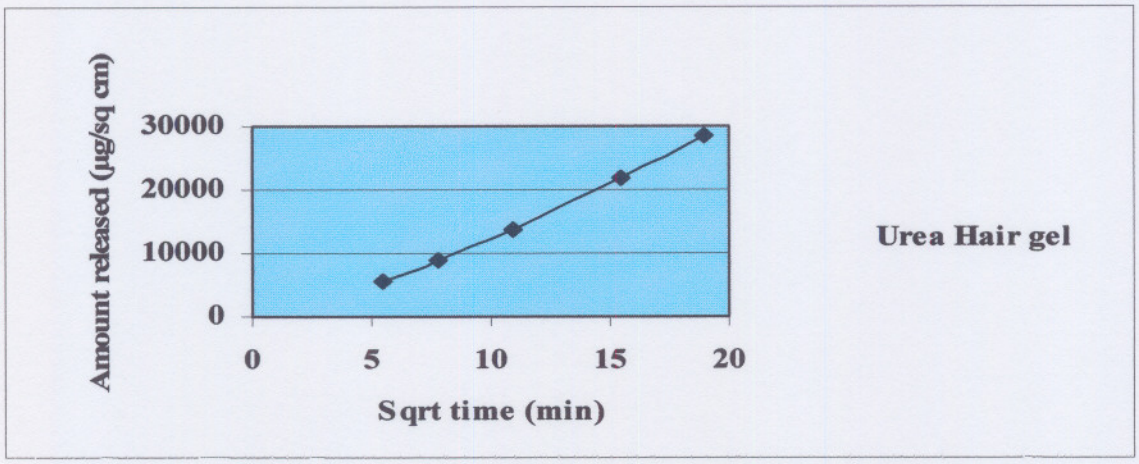

Figure 4.6 The concentration of urea released from the hair gel (three months) at 25°C/60%RH

Table 4.19 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	1433
60	2866
120	5732
240	11464
360	17196

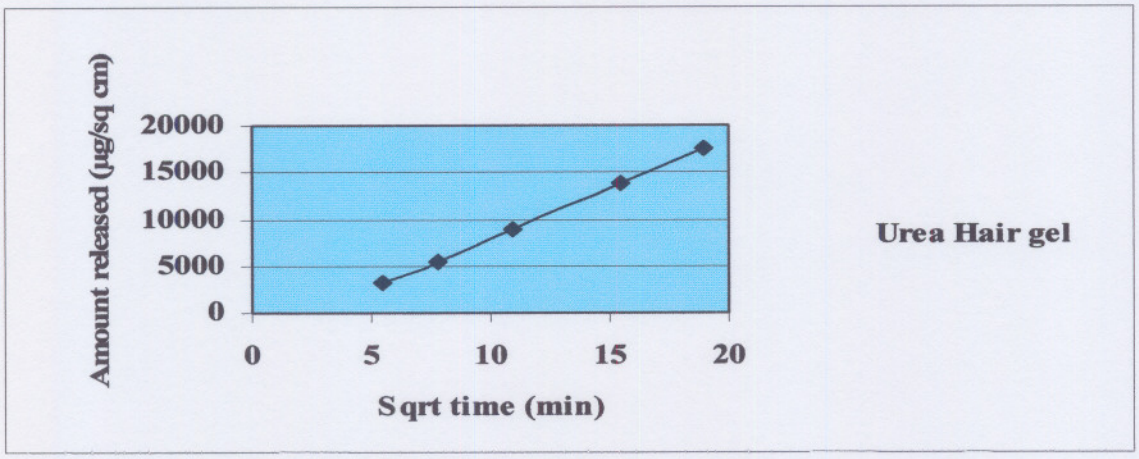

Figure 4.7 The concentration of urea released from the hair gel (three months) at 40°C/75%RH

4.3.6.2 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 (Figure 4.5- 4.7) for the urea hair gel. One can speculate that it is possible, that because of the degradation of urea to ammonia, the pH became alkaline, and urea became less soluble in the base. Due to the better solubility, of urea in the aqueous dissolution medium, the release rate increased with time.

4.3.7 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 25 Category 2 (Table 4.20 and Table 4.21).

Table 4.20 Preservative efficacy results of the hair gel (initials)

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	5.0×10^5	1.4	1.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	1.6	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	1.7	1.5	0.5	No increase from
C. albicans	4.9×10^5	2.7	> 3.0	> 3.0	initial inoculum

Comments: Sample fails the requirements of USP 25.

Table 4.21 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	Day 14	Day 28	
E. coli	5.5×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	6.5×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	6.0×10^6	2.3	> 3.0	> 3.0	increase at 28 days
A. niger	3.0×10^5	2.0	> 3.0	> 3.0	No increase from
C. albicans	5.5×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.3.7.1 Discussion

It can be concluded that the preservative efficacy of the hair gel, after 3 months at 40°C/75% RH, complied with the requirements of the USP 25. The failure in the preservative efficacy at initial could be due to a contaminated container or experimental error.

4.4 FACIAL CLEANSER

Skin cleansers are products that clean and refresh the skin by removing soil or dirty materials to help keep the skin's physiological condition normal (Kaneko & Sakamoto, 2001:499).

The following parameters of the formulated facial cleanser were investigated: pH, relative density, appearance, viscosity, urea assay, preservative content and preservative efficacy.All of the tests that are discussed in 4.4 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.4.1 pH

The pH of the urea facial cleanser, measured over three months, is given in table 4.22.

Table 4.22 pH of the facial cleanser taken over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		5.79		
25°C/60%RH	5.76	6.12	6.51	7.10
40°C/75%RH		8.12	8.15	8.22

4.4.1.1 Discussion

The pH of the urea facial cleanser stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. (See 4.1.1.1)

4.4.2 Relative density

The relative density of the urea facial cleanser, measured over three months, is given in table 4.23.

Table 4.23 Relative density of the facial cleanser measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1.053		
25°C/60%RH	0.986	0.989	1.024	0.973
40°C/75%RH		0.991	1.043	0.999

4.4.2.1 Discussion

There was no significant change in the relative density of the facial cleanser (Table 4.33). Temperature, moisture and pH didn't have an influence on the relative density.

4.4.3 Appearance

The facial cleanser is a white cream with no odour. It spreads smoothly and no grittiness was observed. After one month of storage at 25°C/60%RH and 40°C/75%RH, the cream separated into two layers. A small amount of water was found at the bottom of the containers, a sign of phase separation. It may have been a result of the differences in relative density between the two phases in the cream (Knowlton & Pearce, 1993: 98). There was no change in the odour or colour of the cleanser after three months.

4.4.4 Viscosity

Viscosity data provides an accurate reference point in the formulation of the materials, facilitating the achievements of consistency from batch to batch and evaluates the structure achieved during storage. The viscosity of the facial cleanser was determined once a month for three months as described in Chapter 3 (see 3.3.6).

The average of the viscosity readings taken of the facial cleanser, is given in Table 4.24.

Table 4.24 The viscosity (in cP) of the facial cleanser measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		4876		
25°C/60%RH	5920	2553	3215	2467
40°C/75%RH		2601	2121	2342

4.4.4.1 Discussion

The viscosity of the facial cleanser decreased from one month (Table 4.24). The decrease in viscosity can be explained as a result of the separation of the cleanser into two layers because of the differences in relative density between the two phases in the cleanser. A large decrease in viscosity is a sign of instability.

4.4.5 Urea assay

The concentration of urea in the facial cleanser was determined at initial, one, two and three months intervals as described in Chapter 3 (see section 3.3.1.1) (Table 4.25). An HPLC chromatogram of urea standard and facial cleanser sample is shown in Figure 4.1 and Figure 4.8 respectively.

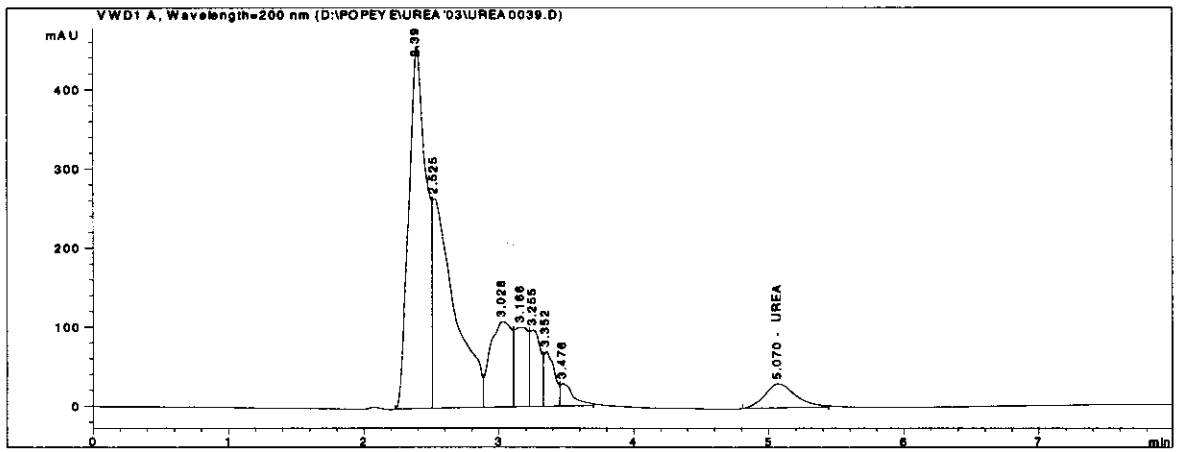

Figure 4.8 HPLC chromatogram of the urea facial cleanser sample.

Table 4.25 The concentration (%) of urea of facial cleanser measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		101.80%		
25°C/60%RH	103.70%	101.10%	97.00%	98.00%
40°C/75%RH		105.80%	97.00%	96.80%

4.4.5.1 Discussion

The concentration of urea in the formulated facial cleanser did not show any significant change. Although there was slight decrease in concentration, it did not influence the stability.

4.4.6 Methyl- and propylparaben assay

Preservatives are added to cosmetics to suppress the proliferation of micro-organisms 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 facial cleanser were determined at initial and then monthly for 3 months, using HPLC chromatography as described in Chapter 3 (see section 3.3.1.2.1). An HPLC chromatogram of methyl- and propylparaben standard is shown in Figure 4.9 and of the facial cleanser sample in Figure 4.10.

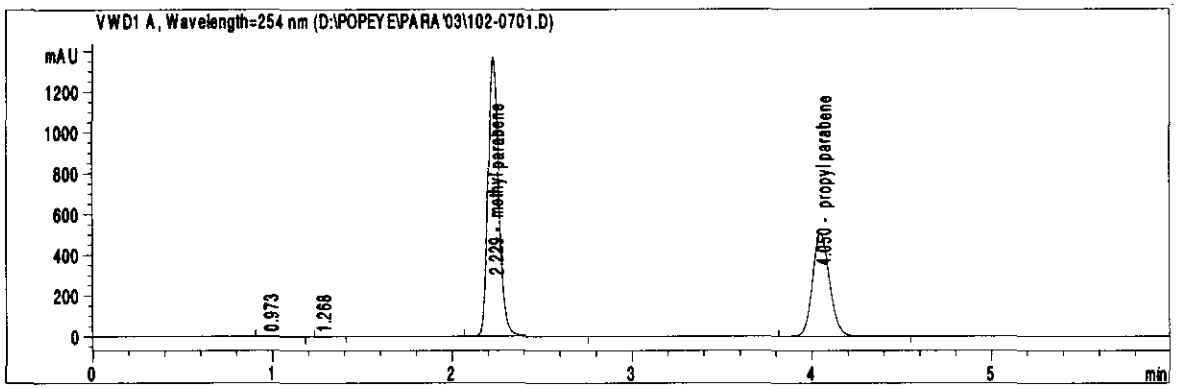

Figure 4.9 HPLC chromatogram of the methyl- and propylparaben standard

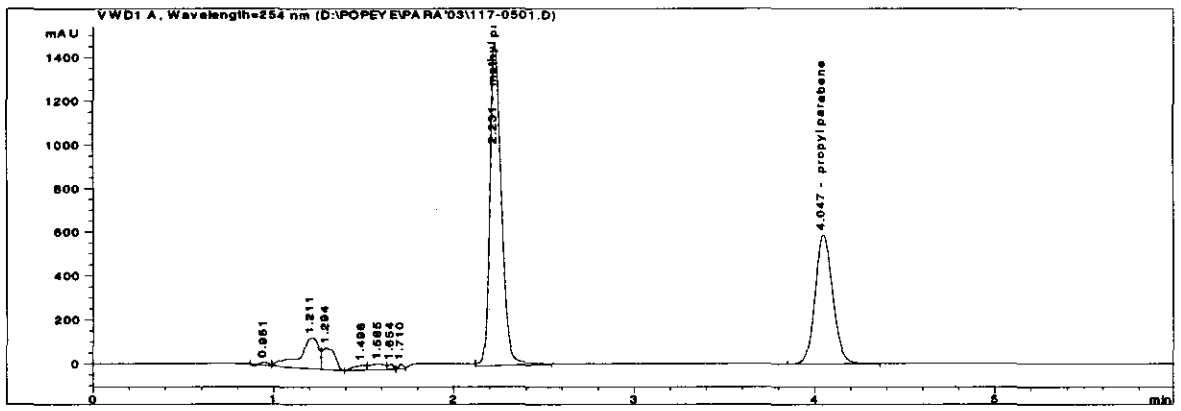

Figure 4.10 HPLC chromatogram of the facial cleanser sample

The results follow in Tables 4.26 and 4.27.

Table 4.26 The concentration (%) of propylparaben in the urea facial cleanser measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		102.00%		
25°C/60%RH	109.20%	99.30%	104.90%	109.00%
40°C/75%RH		101.90%	107.70%	104.90%

Table 4.27 The concentration (%) of methylparaben in facial cleanser measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		102.00%		
25°C/60%RH	110.20%	100.40%	108.00%	110.80%
40°C/75%RH		98.80%	105.900%	100.80%

4.4.6.1 Discussion

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

4.4.7 Preservative efficacy

The facial cleanser was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 25 Category 2 (Table 4.28 and Table 4.29).

Table 4.28 Preservative efficacy results of the facial cleanser (initials)

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.29 Preservative efficacy results of the facial cleanser (3 months), stored at 40°C/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.4.7.1 Discussion

It can be concluded that the preservative efficacy of the facial cleanser complied with the requirements of the USP 25. It was therefore shown that the preservatives used in the formulation were effective in protecting the facial cleanser against microbial contamination.

4.5 DAY CREAM

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

The following parameters of the formulated day cream were investigated: pH, relative density, appearance, viscosity, urea assay, preservative content and preservative efficacy. All of the tests that are discussed in 4.5 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.5.1 pH

The pH of the urea day cream, measured over three months, is given in Table 4.30.

Table 4.30 pH of the day cream measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		5.33		
25°C/60%RH	5.31	5.46	5.59	5.90
40°C/75%RH		7.62	7.89	8.07

4.5.1.1 Discussion

The pH of the urea day cream stored at 40°C/75%RH was higher than the pH of the samples stored at 25°C/60%RH. (See 4.1.1.1).

4.5.2 Relative density

The relative density of the urea day cream, measured over three months, is given in Table 4.31.

Table 4.31 Relative density of the day cream measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1.112		
25°C/60%RH	0.884	1.049	1.001	1.013
40°C/75%RH		1.075	1.027	0.997

4.5.2.1 Discussion

There was no significant change in the relative density of the day cream (Table 4.31). Temperature, moisture and pH didn't have an influence on the relative density.

4.5.3 Appearance

The day cream is a white cream with no odour. It spreads smoothly and no grittiness was observed. After one month of storage at 25°C/60%RH and 40°C/75%RH, the cream separated into two layers. A small amount of water was found at the bottom of the containers, a sign of phase separation. It may have been a result of the differences in relative density between the two phases in the cream (Knowlton & Pearce, 1993: 98). There was no change in the odour or colour of the day cream after the three months.

4.5.4 Viscosity

Viscosity data provides an accurate reference point in the formulation of the materials, facilitating the achievements of consistency from batch to batch and evaluates the structure achieved during storage. The viscosity of the day cream was determined once a month for three months as described in Chapter 3 (see 3.3.6).

The average of the viscosity readings taken of the day cream, is given in Table 4.32.

Table 4.32 The viscosity (in cP) of the day cream measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		1517		
25°C/60%RH	2668	1363	1219	1555
40°C/75%RH		2102	1603	1613

4.5.4.1 Discussion

The viscosity of the day cream decreased from one month (Table 4.32). The decrease in viscosity can be explained as a result of the separation of the day cream into two layers because of the differences in relative density between the two phases. A large decrease in viscosity is a sign of instability.

4.5.5 Urea assay

The concentration of urea in the day cream was tested at initial, one, two and three monthly intervals as described in Chapter 3 (see section 3.3.1.1) (Table

4.33). An HPLC chromatogram of urea standard and day cream sample is shown in Figure 4.1 and Figure 4.11 respectively.

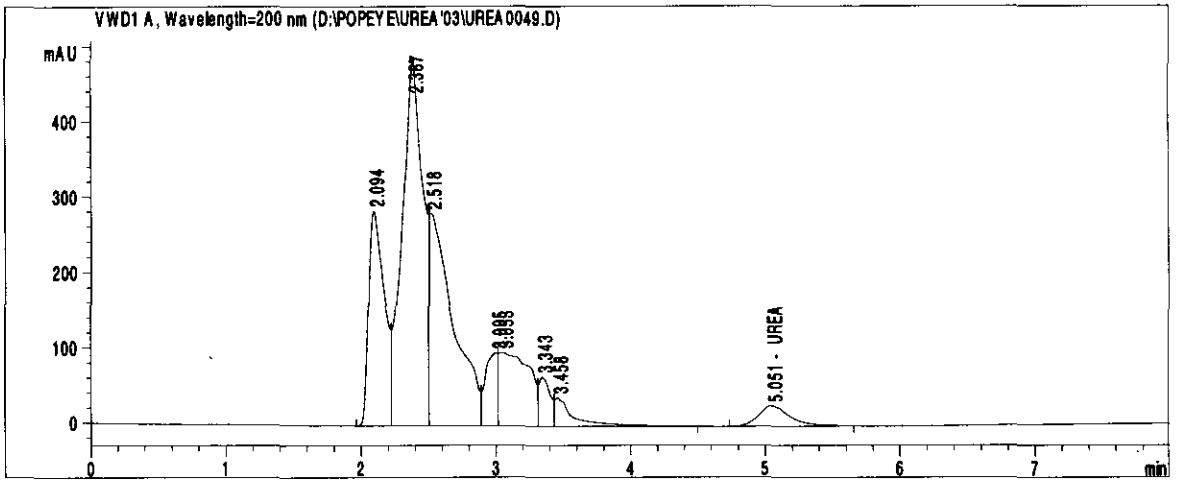

Figure 4.11 HPLC chromatogram of the urea day cream sample

Table 4.33 The concentration (%) of urea of day cream measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		107.00%		
25°C/60%RH	104.70%	102.80%	102.00%	92.30%
40°C/75%RH		98.50%	95.50%	89.30%

4.5.5.1 Discussion

The concentration of urea in the formulated day cream decreased with time, especially in the sample that was stored at 40°C/75%RH, where the urea concentration dropped to below 90% after 3 months storage. A possible explanation could be the decomposition of urea into carbon dioxide and ammonia and the separation of the day cream into two layers, indicating instability of the formulation.

4.5.6 Methyl- and propylparaben assay

Preservatives are added to cosmetics to suppress the proliferation of micro-organisms 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 day cream were determined at the initial and then monthly for 3 months, using HPLC chromatography as described in Chapter 3 (see section 3.3.1.2.1). A high performance liquid chromatogram of methyl- and propylparaben standard is shown in Figure 4.9 and of the day cream sample in Figure 4.12.

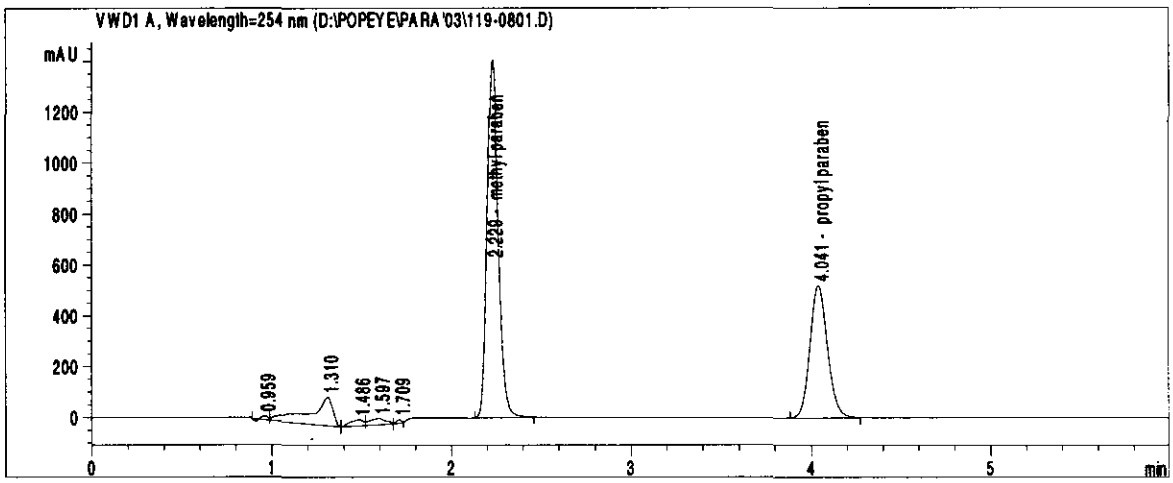

Figure 4.12 HPLC chromatogram of the day cream sample.

The results follow in Tables 4.34 and 4.35.

Table 4.34 The concentration (%) of propylparaben of day cream measured over three months.

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		102.30%		
25°C/60%RH	104.90%	102.50%	103.40%	105.00%
40°C/75%RH		101.40%	106.00%	106.00%

Table 4.35 The concentration (%) of methylparaben of day cream measured over three months.

STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
5°C		102.00%		
25°C/60%RH	110.20%	100.40%	108.00%	110.80%
40°C/75%RH		98.80%	105.90%	100.80%

4.5.6.2 Discussion

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

4.5.7 Preservative efficacy

The day cream was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 25 Category 2 (Table 4.36 and Table 4.37).

Table 4.36 Preservative efficacy results of the day cream (initial)

Test organism	Initial inoculum	Log Unit Reduction Day 7	Day 14	Day 28	Specified limits for Category 2 products
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	2.6	> 3.0	> 3.0	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.37 Preservative efficacy results of the day cream (3 months), stored at 40°C/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.5.7.1 Discussion

It can be concluded that the preservative efficacy of the day cream complied with the requirements of the USP 25. It was therefore shown that the preservatives used in the formulation were effective in protecting the day cream against microbial contamination.

4.6 FOOT AND HEEL BALM

The following parameters of the formulated foot and heel balm were investigated: pH, appearance, spreadability, penetration, urea assay, preservative content, urea release rate (dissolution) and preservative efficacy.All of the tests 4.6 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.6.1 pH

The pH of the urea foot and heel balm, measured over three months, is given in table 4.38.

Table 4.38 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		5.33		
25°C/60%RH	5.31	5.46	5.59	5.90
40°C/75%RH		7.62	7.89	8.07

4.6.1.1 Discussion

The pH of the urea heel balm 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 9 (Beiersdorf, 2003). This volatilisation is affected by temperature and moisture. Higher temperature and moisture increase volatilisation (Anon, 2003:20). 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).

4.6.2 Appearance

The foot and heel balm is a thick, white rich cream with no odour. It spreads smoothly, and felt rich in texture with no grittiness. There was no change in the odour or colour of the foot and heel balm after the three months.

4.6.3 Spreadability

Spreadability test results show how easily the foot and heel balm is applied to the affected areas. The spreadability of the balm was determined once a month for three months as described in Chapter 3 (see section 3.3.7).

The spreadability results of the foot and heel balm is given in Table 4.39.

Table 4.39 The spreadability (mm) of the foot and heel balm over a three month period

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		35.44		
25°C/60%RH	32.52	31.18	31.60	32.37
40°C/75%RH		30.53	32.56	33.08

4.6.3.1 Discussion

As can be seen in Table 4.39, there was no significant change in spreadability over the three months test intervals.

4.6.4 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 3 (see section 3.3.9).

The penetration results of the foot and heel balm is given in Table 4.40.

Table 4.40 The penetration (mm) of the foot and heel balm over a three month period

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		18.26		
25°C/60%RH	17.47	17.09	15.24	14.25
40°C/75%RH		17.42	14.53	13.11

4.6.4.1 Discussion

Table 4.40 illustrates a slight decrease in penetration for the second and third month samples. It may be that the heel balm was still settling before it went into the final resting stage. This is not troublesome because a balm is supposed to be very thick.

4.6.5 Urea assay

The concentration of urea in the heel balm was tested at initial, one, two and three monthly intervals as described in Chapter 3 (see section 3.3.1.1) (Table 4.41). An HPLC chromatogram of urea standard and foot and heel balm sample is shown in Figure 4.1 and Figure 4.13 respectively.

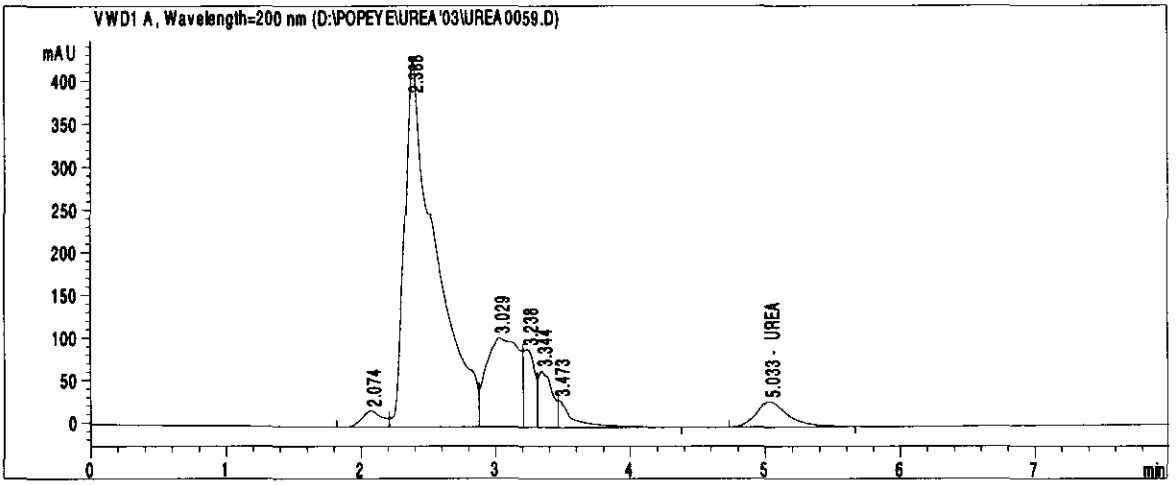

Figure 4.13 HPLC chromatogram of the urea foot and heel balm sample.

Table 4.41 The concentration (%) of urea of foot and heel balm measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		103.00%		
25°C/60%RH	110.30%	100.20%	99.10%	95.40%
40°C/75%RH		106.80%	95.10%	94.90%

4.6.5.1 Discussion

Urea foot and heel balm stored at 25°C/60%RH and 40°C/75%RH showed a decrease in concentration. A possible explanation could be the decomposition of urea into carbon dioxide and ammonia.

4.6.6 Methyl- and propylparaben assay

Preservatives are added to cosmetics to suppress the proliferation of micro-organisms 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 three months, using HPLC chromatography as described in Chapter 3 (see section 3.3.1.2.1). An HPLC chromatogram of methyl- and propylparaben standard is shown in Figure 4.9 and of the foot and heel balm sample in Figure 4.14.

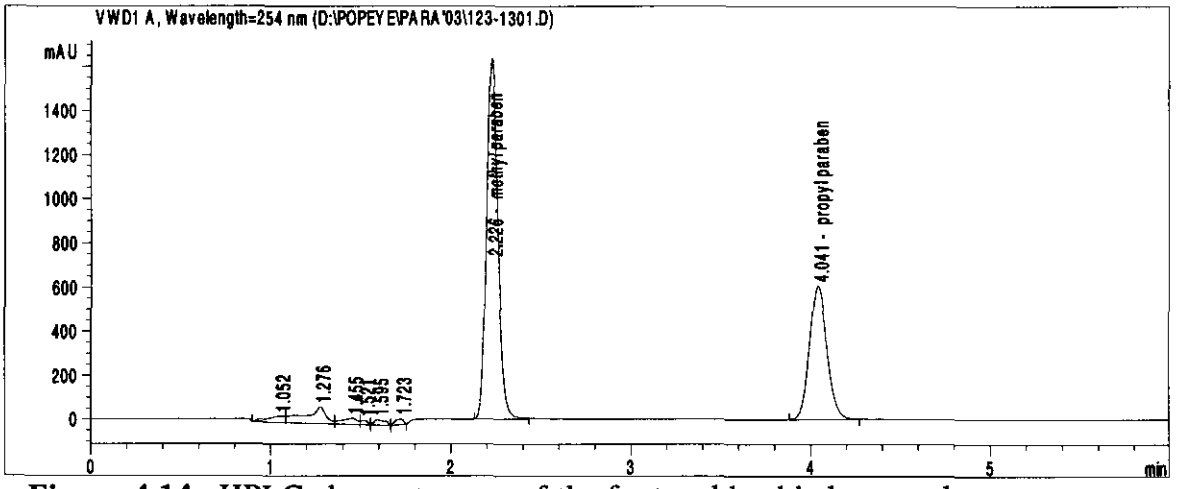

Figure 4.14 HPLC chromatogram of the foot and heel balm sample.

The results follow in Tables 4.42 and 4.43.

Table 4.42 The concentration (%) of propylparaben of foot and heel balm measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		105.50%		
25°C/60%RH	110.60%	107.50%	103.30%	110.60%
40°C/75%RH		102.30%	107.00%	110.60%

Table 4.43 The concentration (%) of methylparaben of foot and heel balm measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		108.50%		
25°C/60%RH	110.50%	100.40%	106.40%	110.10%
40°C/75%RH		98.60%	106.40%	107.20%

4.6.6.1 Discussion

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

4.6.7 Urea release

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved. The use of potent medicaments, capable of penetrating the natural defensive barrier, demands a serious examination.

4.6.7.1 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 heel balm was determined initial and after three months storage at 25°C/60%RH and 40°C/75%RH.

The release experiment was done six fold and the average release was calculated for each analysis point.

The concentration of urea that was released from the foot and heel balm (initial) as function of time, is given in Table 4.44 and Figure 4.15.

Table 4.44 The concentration (µg/sqcm) of urea released from the foot and heel balm (initials) as a function of time

Time (minutes)	Concentration (µg/sqcm)
30	790
60	1463
120	2476
240	3757
360	5035

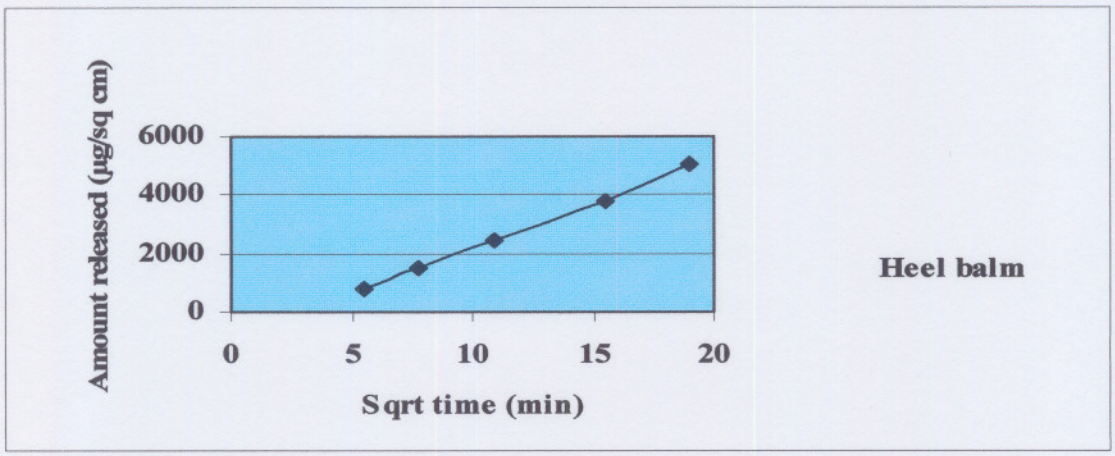

Figure 4.15 The concentration of urea released from the foot and heel balm at initial

The concentration of urea that was released from the heel balm after three months at 25°C/60%RH and 40°C/75%RH as function of time is given in Table 4.45 and Figure 4.16 and Table 4.46 and Figure 4.17 respectively.

Table 4.45 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	1209
60	1795
120	2760
240	3792
360	4610

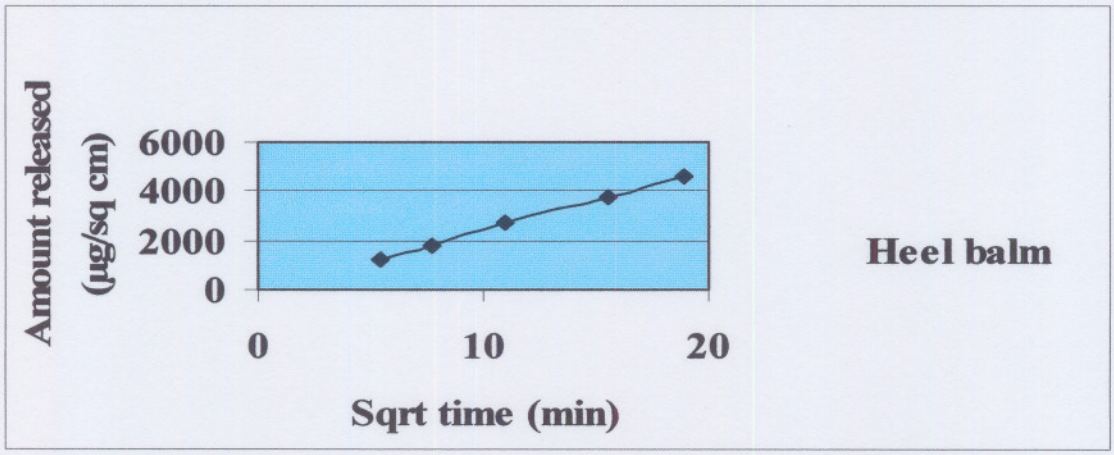

Figure 4.16 The concentration of urea released from the foot and heel balm (three months) at 25°C/60%RH

Table 4.46 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	1384
60	1903
120	2644
240	4003
360	4733

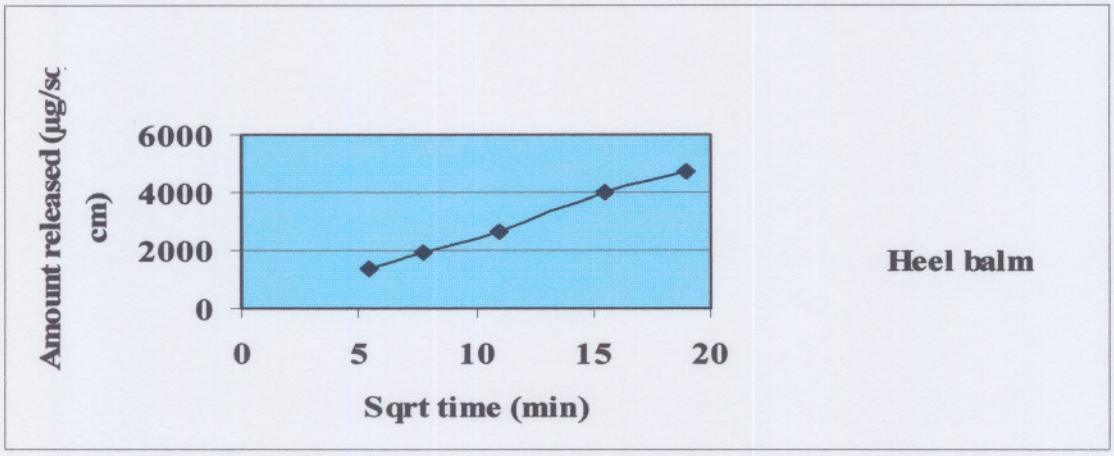

Figure 4.17 The concentration of urea released from the foot and heel balm (three months) at 40°C/75%RH

4.6.7.1.1 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 (see Figure 4.15 - 4.17) for the urea foot and heel balm.

4.6.8 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 25 Category 2 (Table 4.47 and Table 4.48).

Table 4.47 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	Day 14	Day 28	
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.48 Preservative efficacy results of the foot andheel balm (3 months), stored at 40°C/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.6.8.1 Discussion

It can be concluded that the preservative efficacy of the foot and heel balm complied with the requirements of the USP 25. It was therefore shown that the preservatives used in the formulation were effective in protecting the foot and heel balm against microbial contamination.

4.7 UREA BODY CREAM

The following parameters of the formulated body cream were investigated: pH, appearance, spreadability, penetration, urea assay, preservative content, urea release rate (dissolution) and preservative efficacy. All of the tests that are discussed in 4.7 were performed according to the requirements of the MCC under GLP conditions, as were discussed in Chapter 3.

4.7.1 pH

The pH of the urea body cream, measured over three months, is given in table 4.49.

Table 4.49 pH of the urea body cream measured over three months at different storage conditions

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		5.96		
25°C/60%RH	6.22	6.09	6.50	6.84
40°C/75%RH		7.21	7.96	7.85

4.7.1.1 Discussion

The pH of the urea cream stored at 40°C/75%RH was higher than the pH of the products stored at 25°C/60%RH. (See 4.1.1.1).

4.7.2 Appearance

The urea cream is a thick, white-yellow rich cream with no odour. It spreads smoothly, and felt rich in texture with no grittiness. There was no change in the odour or colour of the urea body cream after the three months.

4.7.3 Spreadability

Spreadability test results show how easily the urea body cream is applied to the skin. The spreadability of the cream was determined once a month for three months as described in Chapter 3 (see section 3.3.7).

The spreadability results of the urea body cream is given in Table 4.50.

Table 4.50 The spreadability (mm) of the urea body cream over a three month period

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		34.92		
25°C/60%RH	45.03	37.17	39.82	40.15
40°C/75%RH		38.18	36.13	35.46

4.7.3.1 Discussion

As can be seen in Table 4.50, there was no significant change in spreadability over the three months test intervals.

4.7.4 Penetration

Penetration is a useful way to determine if phase separation has occurred. The penetration of the urea body cream was determined once a month for three months as described in Chapter 3 (see section 3.3.9).

The penetration results of the urea body cream is given in Table 4.51.

Table 4.51 The penetration in (mm) of the urea cream over a three month period

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		21.10		
25°C/60%RH	21.42	16.35	20.30	20.12
40°C/75%RH		17.20	18.35	14.61

4.7.4.1 Discussion

As can be seen in Table 4.51, there was no significant change in penetration over the three months test intervals, except for the three month sample at the storage temperature of 40°C/75%RH, but it could be due to experimental error.

4.7.5 Urea assay

The concentration of urea in the urea cream was tested at initial, one, two and three monthly intervals as described in Chapter 3 (see section 3.3.1.1) (Table 4.52). An HPLC chromatogram of urea standard and urea body cream sample is shown in Figure 4.1 and Figure 4.18 respectively.

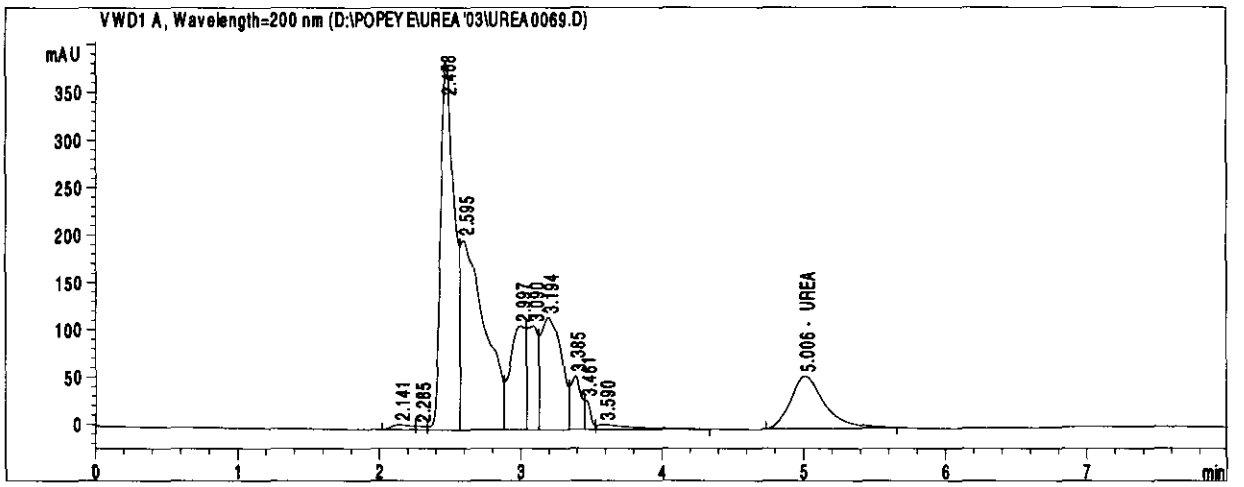

Figure 4.18 HPLC chromatogram of the urea body cream sample

Table 4.52 The concentration (%) of urea in urea body cream measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		100.40%		
25°C/60%RH	105.70%	99.80%	98.70%	94.90%
40°C/75%RH		101.80%	94.10%	93.30%

4.7.5.1 Discussion

Urea body cream stored at 25°C/60%RH and 40°C/75%RH showed a decrease in concentration with time because urea decomposed into carbon dioxide and ammonia. This can cause the pH to increase to values as high as 9 (Beiersdorf, 2003). 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). Although there was a decrease in concentration, it did not influence the stability.

4.7.6 Phenoxyethanol assay

Preservatives are added to cosmetics to suppress the proliferation of micro-organisms which have contaminated them and to kill them in time, thereby preventing deterioration of the product (Mitsui, 1997: 201). The phenoxyethanol concentration in the urea body cream was determined at initial and then monthly for three months, using HPLC chromatography as described in Chapter 3 (see section 3.3.1.2.2). An HPLC chromatogram of phenoxyethanol standard is shown in Figure 4.19 and of the urea body cream sample in Figure 4.20.

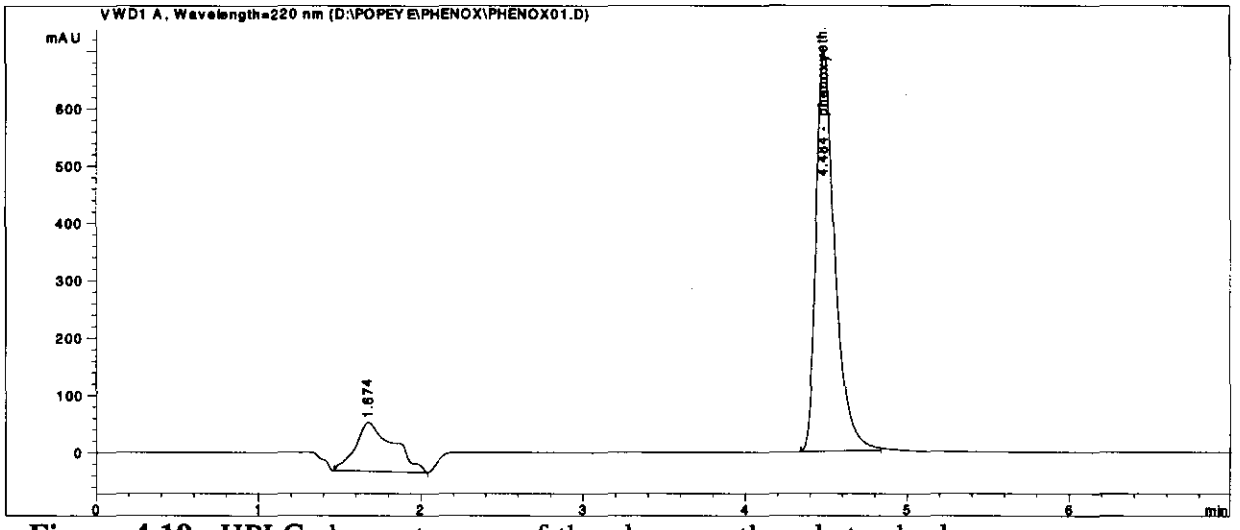

Figure 4.19 HPLC chromatogram of the phenoxyethanol standard

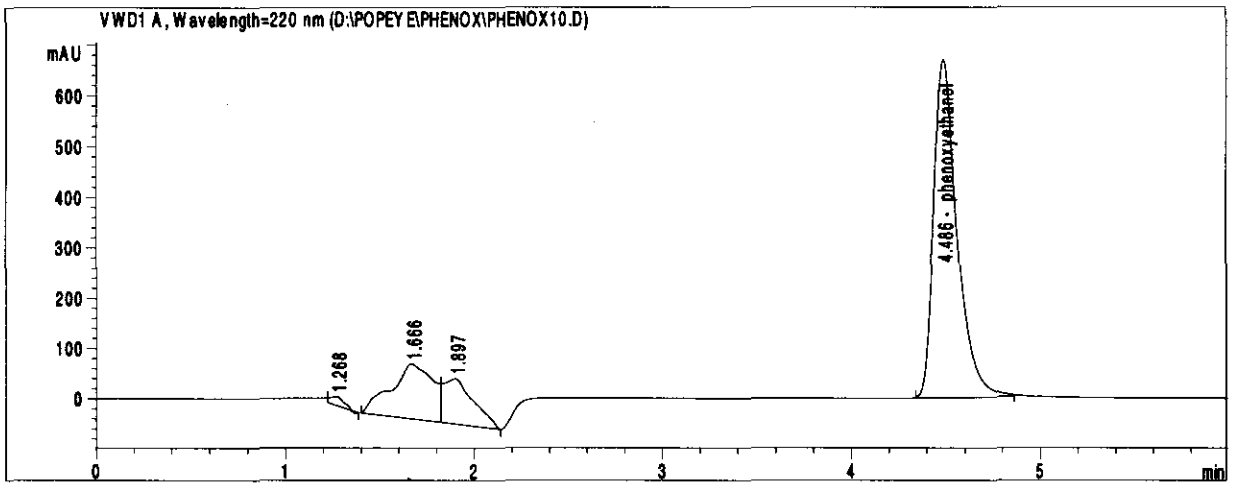

Figure 4.20 HPLC chromatogram of the urea body cream sample.

The results follow in Table 4.53

Table 4.53 The concentration (%) of phenoxyethanol in urea body cream measured over three months

STORAGE TEMPERATURE	INITIAL	1 MONTH	2 MONTHS	3 MONTHS
5°C		102.20%		
25°C/60%RH	102.30%	101.40%	101.10%	102.60%
40°C/75%RH		99.50%	101.00%	101.70%

4.7.6.1 Discussion

The concentration of phenoxyethanol in the formulated urea body cream remained stable over the three month storage period at all temperatures.

4.7.7 Urea release

The release of active medicaments into the skin tissue is a prerequisite for pharmacological activity to be achieved. The use of potent medicaments, capable of penetrating the natural defensive barrier, demands a serious examination.

4.7.7.1 Concentration of the urea released from the urea body cream

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

The concentration of urea that was released from the urea body cream (initial) as function of time, is given in Table 4.54 and Figure 4.21.

Table 4.54 The concentration (µg/sqcm) of urea released from the urea body cream (initial) as a function of time

Time (minutes)	Concentration (µg/sqcm)
30	1772
60	3167
120	5437
240	7952
360	10406

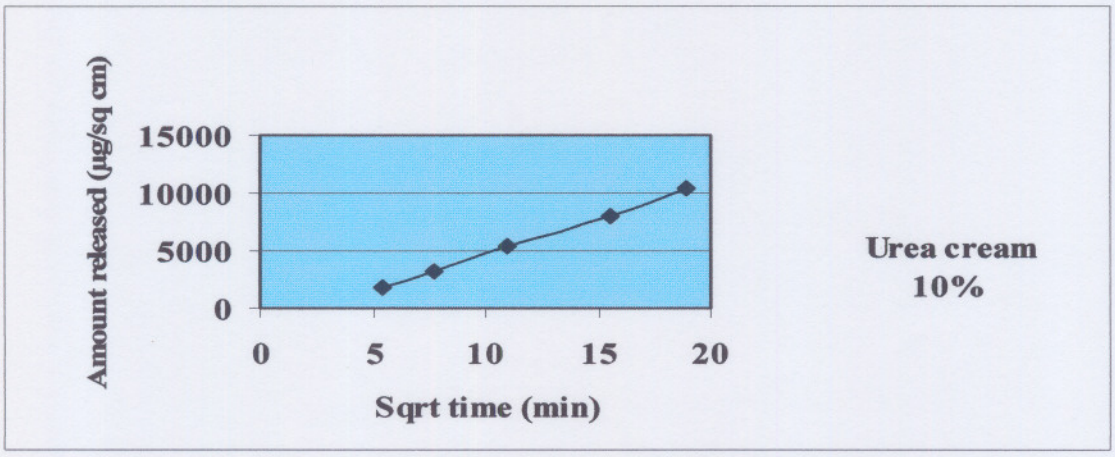

Figure 4.21 The concentration of urea released from the urea body cream at initial

The concentration of urea that was released from the urea body cream at three months at 25°C/60%RH and 40°C/75%RH as function of time is given in Table 4.55 and Figure 4.22 and Table 4.56 and Figure 4.23 respectively. In Figure 4.24 the rate at which urea is released from the hair gel, foot and heel balm and urea body cream, after 3 months at 25°C/60% RH, is compared.

Table 4.55 The concentration (µg/sqcm) of urea released from the the urea body cream (three months) at 25°C/60%RH as a function of time

Time (minutes)	Concentration (µg/sqcm)
30	2428
60	3359
120	4678
240	6589
360	8008

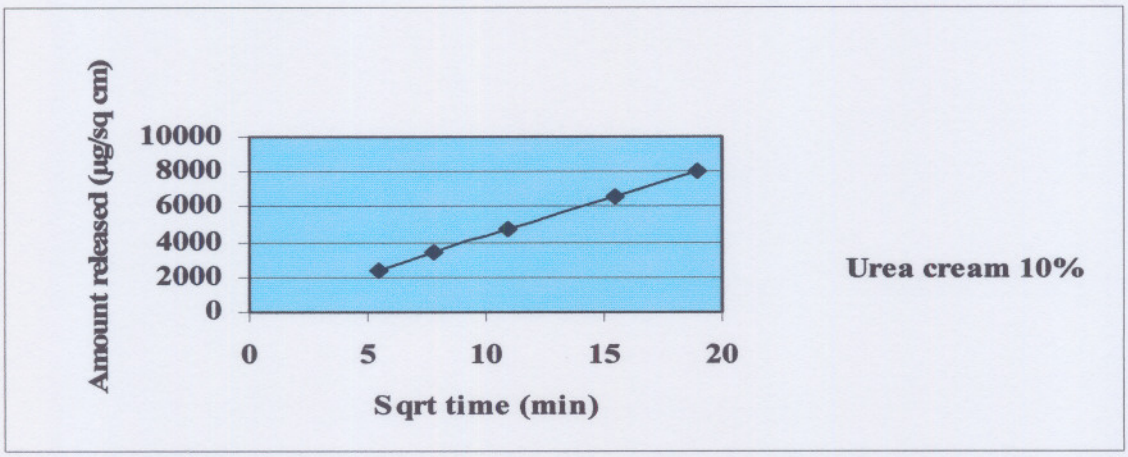

Figure 4.22 The concentration of urea released from the urea body cream (three months) at 25°C/60%RH

Table 4.56 The concentration (µg/sqcm) of urea released from the urea body cream (three months) at 40°C/75%RH as a function of time

Time (minutes)	Concentration (µg/sqcm)
30	2580
60	3557
120	4924
240	6801
360	8918

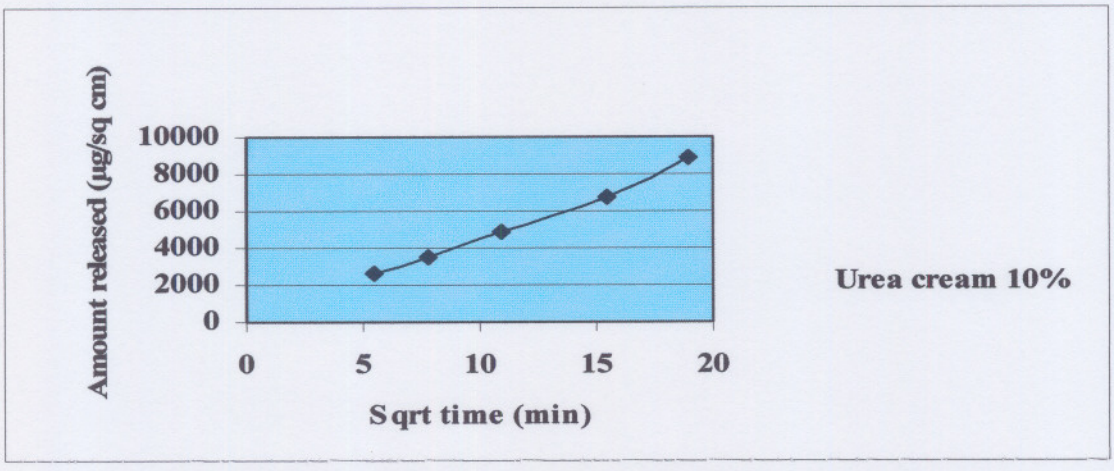

Figure 4.23 The concentration of urea released from the urea body cream (three months) at 40°C/75%RH

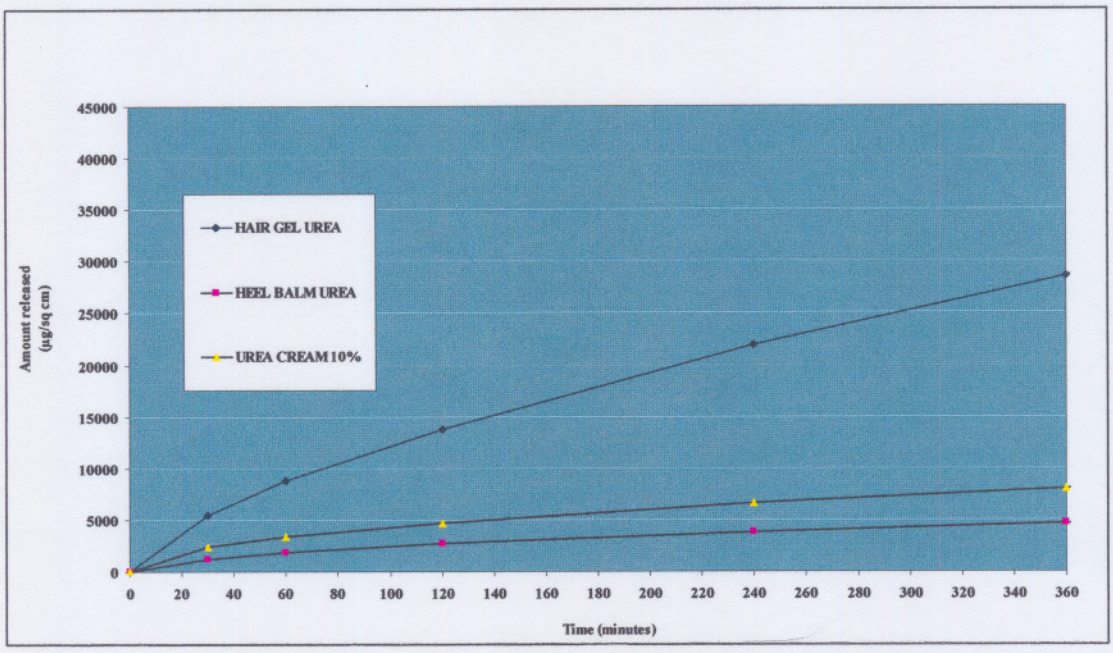

Figure 4.24 The concentration of urea released from the hair gel, foot and heel balm and body cream after 3 months at 25°C/60%RH

4.7.7.1.1 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 (see Figure 4.21- 4.23) for the urea body cream. From Figure 4.24 it is clear that urea is released from the hair gel at a much faster rate than from the body cream and foot and heel balm. The release rate is influenced by the viscosity of the medium and could be faster from the gel than from the creams (Shah *et al.*, 1991:55).

4.7.8 Preservative efficacy

The toner was sent to the University of the Witwatersrand and the test was carried out according to guidelines given by the USP 25 Category 2 (Table 4.57 and Table 4.58).

Table 4.57 Preservative efficacy results of the body cream (initial)

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	5.0×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	7.0×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	1.4×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A. niger	2.2×10^5	> 3.0	> 3.0	> 3.0	No increase from
C. albicans	4.9×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

Table 4.58 Preservative efficacy results of the body cream (3 months), stored at 40°C/75% RH

Test organism	Initial inoculum	Log Unit Reduction			Specified limits for Category 2 products
		Day 7	Day 14	Day 28	
E. coli	1.8×10^5	> 3.0	> 3.0	> 3.0	>2.0 log reduction
P. aeruginosa	3.2×10^5	> 3.0	> 3.0	> 3.0	at 14 days & no
S. aureus	7.5×10^6	> 3.0	> 3.0	> 3.0	increase at 28 days
A .niger	2.1×10^5	> 3.0	> 3.0	> 3.0	No increase from
C .albicans	4.2×10^5	> 3.0	> 3.0	> 3.0	initial inoculum

Comments: Sample complies with the requirements of USP 25.

4.7.8.1 Discussion

It can be concluded that the preservative efficacy of the urea body cream complied with the requirements of the USP 25. It was therefore shown that the preservative used in the formulation was effective in protecting the body cream against microbial contamination.

4.8 CONCLUSION

The formulated urea products were tested over 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 conclusions that can be drawn from that.

- The pH of the urea containing samples 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 (Beiersdorf, 2003). This can cause the pH to increase to values as high as 9. 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).
- Viscosity of urea 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).
- 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 three months.
- The appearance of the products remained the same over three months, except the facial cleanser and the day cream. After one month of storage at 25°C/60%RH and 40°C/75%RH, they separated into two layers. A small amount of water was found at the bottom of the containers, a sign of separation. It may have been a result of the difference in relative density between the two phases (Knowlton & Pearce, 1993:98).
- HPLC analysis showed a decrease in urea concentration with time , but only in the case of the day cream did it drop to below 90% after storage at 40°C /75%RH for three months. Stabilising the formulation with methylsulfonylmethane (MSM) could solve the problem.
- HPLC analysis of the preservatives namely methyl- and propylparaben and phenoxyethanol confirmed their stability in the formulated products.
- 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 all three preparations tested. The most important conclusion is that urea is released from the three preparations, i.e. the urea active compound is, in all three products, available for bio-absorption.
- The release of urea from the gel is about four times faster than that from the two creams. In general, the release rate is influenced by the viscosity of the medium and should be faster from the gel than from the creams (Shah *et al.*, 1991:55).

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APPENDIX A

N. Claasen., J.L. du Preez., A.P. Lötter & Erna Swanepoel. 2003. Formulation and stability of urea containing products. Poster presented at the 2003 International Conference on Pharmaceautical and Pharmacological Science, September 2003, Durban, South Africa.

PURPOSE

This study focussed on a literature study to develop a suitable urea containing formulation. Different formulations were developed for use in various cosmetic products. The formulations were tested under ICH conditions (accelerated stability studies). Stability indicating methods were used to test the active ingredient and preservatives in the products. The release of urea from the formulations was tested by means of membrane release studies.

BACKGROUND

Urea is one of the most important soluble substances of the stratum corneum. In recent years this substance has become more and more important in dermatological therapy and cosmetics. Many diseases have been described that are characterised by a deficiency of urea, such as atopic dermatitis or clinical dry skin (1). Moisturisers are the mainstay of treatment for dry skin. Treatment of dry skin is aimed at restoration of the epidermal water barrier. This is accomplished with moisturising agents that are topically applied to the skin. Moisturisers that contain only humectant elements will draw water into the stratum corneum but not prevent the hydrated stratum corneum from losing its increased water content. Urea enhances the water-binding capacity of the stratum corneum by disrupting hydrogen bonding. Urea exposes water-binding sites on corneocytes and promotes desquamation by decreasing the intercellular cementing substance between the corneocytes.

Also, long-term treatment with urea has been demonstrated to decrease transepidermal water loss (TEWL). A possible explanation may involve urea-induced reduction in epidermal cell proliferation which, in turn, increases the size of corneocytes. Larger corneocytes lower skin permeability, thereby lowering TEWL (4). The content of urea for normal skin is nearly 1%. It

contributes in a significant manner to the hydration of the stratum corneum. Besides amino acids, lactate, and other substances, urea contributes approximately 3-7% to the natural moisturising factor (NMF). Otherwise urea is known for its keratolytic and pruritus-easing properties, and it is a very potent humectant in moisturising creams. Its sources in the epidermis are sweat and the decomposition of arginine by arginase during the process of keratinisation (1).
However, the stability of urea is somewhat of a problem in water-containing formulas that are stored for a long time. Urea can decompose into carbon dioxide and ammonia. These problems have been solved in pharmaceutical preparations by the inclusion of suitable stabilisers such as sodium lactate. The decomposition of urea into ammonia is minimised (2).
5-10% urea deep cleans dry itchy scalp. Antibacterial agents help kill yeast and bacteria that contribute to scaly dry skin and dandruff flares (3).

MATERIALS AND METHODS

Seven different cosmetic products were formulated: hair gel, shampoo, facial toner, facial cleanser, day cream, foot and heel balm, body cream.
The formulations were tested under ICH conditions (accelerated stability studies) over a period of three months at three different storage temperatures, i.e. 5°C, 25°C/60%RH, 40°C/75%RH. Stability indicating tests that applied to these formulations, were conducted.

- Assay of phenoxyethanol (Preservative)
- Assay of urea (Active)
- Assay of methyl & propyl paraben (preservative)
- pH
- Viscosity
- Spreadability
- Relative density

➢ Penetration

RESULTS

Table 1: The concentration (%) of phenoxyethanol assay results over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Urea cream	5°C	102.30%	102.20%		
	25°C/60%RH		101.40%	101.10%	102.60%
	40°C/75%RH		99.50%	101.00%	101.70%

Table 2: The concentration (%) of urea assay results over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Toner	5°C	103.70%	101.80%		
	25°C/60%RH		101.10%	97.00%	98.00%
	40°C/75%RH		105.80%	97.00%	96.80%
Shampoo	5°C	98.90%	91.90%		
	25°C/60%RH		96.00%	98.70%	98.40%
	40°C/75%RH		101.40%	95.00%	93.30%
Hair gel	5°C	103.90%	105.00%		
	25°C/60%RH		103.40%	99.30%	99.30%
	40°C/75%RH		98.80%	98.30%	97.60%
Heel balm	5°C	110.30%	103.00%		
	25°C/60%RH		100.20%	99.10%	95.40%
	40°C/75%RH		106.80%	95.10%	94.90%
Facial cleanser	5°C	100.40%	102.30%		
	25°C/60%RH		103.08%	98.90%	97.80%
	40° /75%RH		104.00%	96.60%	91.50%
Day cream	5°C	104.70%	107.00%		
	25°C/60%RH		102.80%	102.00%	92.30%
	40°C/75%RH		98.50%	95.50%	89.30%
Urea cream	5°C	105.70%	100.40%		
	25°C/60%RH		99.80%	98.70%	94.90%
	40°C/75%RH		101.80%	94.10%	93.30%

Table 3: The concentration (%) of propyl paraben assay results over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Heel balm	5°C	110.60%	105.50%	103.30%	110.60%
	25°C/60%RH		107.50%	107.00%	110.60%
	40°C/75%RH		102.30%		
Facial cleanser	5°C	109.20%	102.00%	104.90%	109.00%
	25°C/60%RH		99.30%	107.70%	104.90%
	40°C/75%RH		101.90%		
Day cream	5°C	104.90%	102.30%	103.40%	105.00%
	25°C/60%RH		102.50%	106.00%	106.00%
	40°C/75%RH		101.40%		

Table 4: The concentration (%) of methyl paraben assay results over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Heel balm	5°C	110.50%	108.50%	106.40%	110.10%
	25°C/60%RH		100.40%	106.40%	107.20%
	40°C/75%RH		98.60%		
Facial cleanser	5°C	110.20%	102.00%	108.00%	110.80%
	25°C/60%RH		100.40%	105.90%	100.80%
	40°C/75%RH		98.80%		
Day cream	5°C	106.30%	100.60%	105.30%	106.10%
	25°C/60%RH		102.20%	104.60%	102.00%
	40°C/75%RH		97.30%		

Table 5: The pH of the urea containing products measured over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Toner	**5°C**	5.43	5.42	5.75	6.1
	25°C/60%RH		5.58		
	40°C/75%RH		7.86	8.38	8.9
Shampoo	**5°C**	4.84	4.88	5.39	5.82
	25°C/60%RH		5.07		
	40°C/75%RH		7.82	8.72	9.13
Hair gel	**5°C**	6.89	6.99	6.97	7.39
	25°C/60%RH		6.97		
	40°C/75%RH		7.9	8.26	8.86
Heel balm	**5°C**	5.34	4.97	5.64	6.09
	25°C/60%RH		5.06		
	40°C/75%RH		7.17	7.68	7.95
Facial cleanser	**5°C**	5.76	5.79	6.51	7.1
	25°C/60%RH		6.12		
	40°C/75%RH		8.12	8.15	8.22
Day cream	**5°C**	5.31	5.33	5.59	5.9
	25°C/60%RH		5.46		
	40°C/75%RH		7.62	7.89	8.07
Urea cream	**5°C**	6.22	5.96	6.5	6.84
	25°C/60%RH		6.09		
	40°C/75%RH		7.21	7.96	7.85

Table 6: The viscosity in (cP) of the urea containing products measured over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Shampoo	5°C	9003	8734	-	7755
	25°C/60%RH		8158		
	40°C/75%RH		9041	-	9147
Hair gel	5°C	7881	9176	-	-
	25°C/60%RH		-		
	40°C/75%RH		6959	-	8715
Facial cleanser	5°C	5920	4876	3215	2467
	25°C/60%RH		2553		
	40°C/75%RH		2601	2121	2342
Day cream	5°C	2668	1517	1219	1555
	25°C/60%RH		1363		
	40°C/75%RH		2102	1603	1613

Table 7: The spreadability in (mm) of the urea containing products measured over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Heel balm	5°C	32.52	35.44	31.6	32.37
	25°C/60%RH		31.18		
	40°C/75%RH		30.53	32.56	33.08
Urea cream	5°C	45.03	34.92	39.82	40.15
	25°C/60%RH		37.17		
	40°C/75%RH		38.18	36.13	35.46

Table 8: The relative density in (g/ml) of the urea containing products measured over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Toner	5°C	0.99	1.01	1.04	0.994
	25°C/60%RH		1.022		
	40°C/75%RH		1.018	1.043	1.016
Shampoo	5°C	1.05	1.105	1.056	1.055
	25°C/60%RH		1.087		
	40°C/75%RH		1.087	1.078	1.098
Hair gel	5°C	1.051	1.11	1.157	1.083
	25°C/60%RH		1.146		
	40°C/75%RH		1.139	1.132	1.104
Facial cleanser	5°C	0.986	1.053	1.024	0.973
	25°C/60%RH		0.989		
	40°C/75%RH		0.991	1.043	0.999
Day cream	5°C	0.884	1.112	1.001	1.013
	25°C/60%RH		1.049		
	40°C/75%RH		1.075	1.027	0.997

Table 9: The penetration in (mm) of the urea containing products measured over three months.

PRODUCT	STORAGE TEMPERATURE	INITIALS	1 MONTH	2 MONTHS	3 MONTHS
Heel balm	5°C	17.47	18.26	15.24	14.25
	25°C		17.09		
	40°C		17.42	14.53	13.11
Urea cream	5°C	21.42	21.1	20.3	20.12
	25°C		16.35		
	40°C		17.2	18.35	14.61

CONCLUSIONS

- A method for liquid chromatographic separation was used for determining the amount of phenoxyethanol present in the formulation. This product showed good stability.

- A method for liquid chromatographic separation was used for determining the amount of urea present in the formulations. These products showed good stability.

- A method for liquid chromatographic separation was used for determining the amount of methyl and propyl paraben present in the formulations. These products showed good stability.

- The pH of the urea containing products stored at 40°C/75%RH was higher than the pH of the products stored at 25°C/60%RH. Urea can decompose into carbon dioxide and ammonia. This can cause the pH to increase as high as 9 (2). This volatilisation is affected by temperature and moisture. Higher temperature and moisture increase volatilisation (5).

- Viscosity of urea containing products display thixotropic properties, which means that, as highly viscous gels, they liquefy without any change in the water content under the influence of mechanical stress. When the stress is removed the high viscosity state returns (2).

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

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

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