FORMULATION AND EVALUATION OF HYDROUS AND ANHYDROUS SKIN WHITENING PRODUCTS CONTAINING SODIUM ASCORBYL PHOSPHATE AND KOJIC ACID DIPALMITATE

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POTCHEFSTROOM
2006

Vir Ouma Ronnie

ACKNOWLEDGEMENTS

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

Above all, I thank my Heavenly Father for wonderful opportunities in life and the strength to make the best of them. Thank you, Lord, for giving me the endurance and ability to complete this study and for guiding me every step of the way.

- Dr. J.L. du Preez for being my supervisor and for guidance with the HPLC.
- Prof. A.P. Lötter, my co-supervisor, for his support, especially with the formulation of my products.
- Prof. Theo Dekker for his assistance with the release studies.
- Prof Wilna Liebenberg for her continuous interest and encouragement.
- Julia Handford for her support and for assisting with proof reading.
- Mrs. Anriëtte Pretorious for her help with the bibliography.
- Personnel at the Research Institute for Industrial Pharmacy, for the chance to gain experience and for the kindness and laughter while working in their laboratories.
- Personnel at Cenqam for their hospitality and for letting me use their equipment during the release tests.
- My fellow post-graduate students for their interest, support and friendship.
- My parents for their love, prayers and encouragement.
- My soul mate, Danie, for his continuous love, prayers, encouragement and his exceptional support and help.

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ABSTRACT

In Asia skin lightening products have grown to be the best selling skin care products, whereas in the Western hemisphere, including Europe and North America, the main demand is for the treatment of age spots and skin even toning. For African and Asian women, skin lightening is part of their culture, as lighter skin signifies increased wealth and social status. It is believed that blending vitamin C, or its derivates, with kojic acid, or its esters, could synergistically inhibit melanin synthesis. Kojic acid dipalmitate was chosen over kojic acid, as better product stability is ensured without any colour instability problems. Since vitamin C is very unstable, especially in aqueous solution, sodium ascorbyl phosphate, as a stable vitamin C derivative, was used.

In this study, kojic acid dipalmitate and sodium ascorbyl phosphate were incorporated into both hydrous - and anhydrous formulations, in order to compare the stability of these actives in the various formulations that were developed and prepared. A hydrous - and anhydrous gel, a hydrous cream and anhydrous ointment, and a hydrous - and anhydrous stick were formulated. These formulations were subjected to stability testing over a three-month period and storage at three conditions, i.e. 5°C, 25°C + 60% RH, and 40°C + 75% RH.

Various stability tests, including HPLC analysis, pH, physical examination, viscosity, relative density, spreadability, penetration, preservative efficacy and membrane release studies were done on these formulations. HPLC analysis proved the kojic acid dipalmitate to be more stable overall in anhydrous formulations. Some formulation problems were experienced with the sodium ascorbyl phosphate, which made it difficult to conclude on the stability of this active in the formulations being evaluated.

OPSOMMING

In Asië het velbleikings-produkte tot die topverkoper onder velversorgingsprodukte gegroei, terwyl in die Westerse halfrond, insluitend Europa en Noord-Amerika, die hoofaanvraag vir die behandeling van ouderdomsvlekke en veltinting is. Vir Afrika en Asiatiese vroue is velbleiking deel van hulle kultuur, aangesien 'n ligter vel toenemend gesondheid en sosiale status impliseer. Daar word geglo dat deur vitamiene C, of sy derivate, saam met kojiese suur, of sy esters, te vermeng, melaniensinteses sinergisties geïnhibeer kan word. Kojiese suur-dipalmitaat was in hierdie studie bo kojiese suur gekies, omdat dit beter produkstabiliteit waarborg, sonder enige kleurverwante onstabiliteitsprobleme. Vitamiene C is baie onstabiel, veral in waterige oplossing, daarom was natrium-askorbielfosfaat as 'n stabiele vitamien C-derivaat gebruik.

In hierdie studie was kojiese suur-dipalmitaat en natrium-askorbielfosfaat in beide hidriese - en nie-hidriese formulerings, wat in hierdie studie ontwikkel en berei is, geïnkorporeer, ten einde die stabiliteit van hierdie aktiewes in die verskeie formulerings te vergelyk. 'n Hidriese - en anhidriese gel, 'n hidriese room en anhidriese salf, en 'n hidriese - en anhidriese stiffie is geformuleer. Hierdie formulerings is oor 'n periode van drie maande aan stabiliteitstoetse blootgestel na storing by 5°C, 25°C + 60% RH, en 40°C + 75%RH.

Verskeie stabiliteitstoetse, insluitend hoë-druk vloeistofchromatografiese analises (HPLC), pH, fisiese evaluasie, viskositeit, relatiewe digtheid, spreidingsvermoë, penetrasie, preserveringseffektiwiteit en membraanvrystellingstoetse is op hierdie formulerings gedoen. HPLC analises het bewys dat kojiese suur-dipalmitaat in geheel meer stabiel in anhidriese formulerings is. Formuleringsprobleme is met die natrium-askorbielfosfaat ondervind, wat dit moeilik gemaak het om die stabiliteit van hierdie aktief in die formulerings te bepaal.

PREFACE AND OBJECTIVES

The demand for skin lightening cosmetics is immense, as many people wish to modify, or change, their skin colour, whilst others use it for depigmenting the skin in the treatment of hyperpigmentation, freckles, or lentigines.

Some skin lighteners have sensitising and cytotoxic side effects. As a result there is an increasing demand for skin lightening cosmetics that are both efficient and safe. Kojic acid, and vitamin C and its derivates, inhibit melanin production and are popular in skin lightening cosmetics, because of their low toxicity to melanocytes.

Kojic acid and vitamin C are thought to have a synergistic effect when combined in a skin lightening cosmetic. Kojic acid has a tendency to discolour those cosmetics in which it is present, due to breakdown of the kojic acid. Kojic acid dipalmitate on the other hand is a much more stable derivate, without causing colour changes. Vitamin C has long been used in skin lightening cosmetics, for controlling melanin production. Unfortunately it is unstable, especially in water, making its derivates, such as sodium ascorbyl phosphate, more attractive alternatives. Both kojic acid and vitamin C are, however, thought to be more stable in an anhydrous base.

In a previous study done by Van Rensburg (2004:153), kojic acid and sodium ascorbyl phosphate were formulated into cosmetic products and were found compatible with each other and easy to formulate. Due to the instability of kojic acid at high temperatures though, kojic acid dipalmitate was used in this study, in order to evaluate the stability of this derivate in cosmetic formulations.

Objectives of this study

The overall objective of this study was to develop stable, skin lightening cosmetics, containing both kojic acid dipalmitate and sodium ascorbyl phosphate, which are both effective and safe. The main objectives of this study therefore were:

- Formulating hydrous - and anhydrous formulations, containing both kojic acid dipalmitate and sodium ascorbyl phosphate. To formulate a hydrous - and

anhydrous gel, a hydrous cream and anhydrous ointment and a hydrous - and anhydrous stick.

- Subjecting these formulations to accelerated stability testing over a period of three months (at the onset of stability and monthly over the three-month stability period), with storage at different conditions of temperature and humidity, using a set of validated, stability indicating test methods, including:
  - Physical and chemical evaluation of the formulations.
  - Analysis of the kojic acid dipalmitate and sodium ascorbyl phosphate contents of each formulation.
  - Determination of the release of the kojic acid dipalmitate and sodium ascorbyl phosphate on the formulations (not on the sticks).
  - Preservative efficacy of these formulations.
  - Analyses of the methyl - and propyl paraben contents of each formulation.

- Finally, based on the outcomes of stability testing, comparing the stability of the actives in each of the hydrous - and anhydrous formulations, in order to determine possible stability trends and / or relationships.

- Identifying possible formulation, or other problems, and providing possible suggestions for improvement.

In order to achieve these objectives, a literature study was first done on different skin lighteners, the skin pigmentation mechanism, the stability of kojic acid and vitamin C, the stability of kojic acid dipalmitate and sodium ascorbyl phosphate, stability studies done on these actives by other authors, stability testing and different stability-indicating high-performance liquid chromatography (HPLC) methods used for the analysis of the kojic acid dipalmitate, sodium ascorbyl phosphate, and methyl - and propyl parabens. The insights gained from literature were then applied in the actual preformulation studies.

A short overview of skin lightening, the skin pigmentation mechanism, and medical and cosmetic uses of various skin lighteners are presented in chapter 1. Chapter 2 focuses on the active ingredients used in this study (kojic acid dipalmitate and sodium ascorbyl phosphate) and their synergistic effect. A discussion of the formulation of the various hydrous - and anhydrous products that were developed in this study follows in chapter 3. Chapter 4 offers a discussion of the various methods used for the stability testing undertaken in this study. The stability test outcomes of the hydrous - and anhydrous gels are presented and discussed in chapter 5, those of the hydrous cream and anhydrous ointment in chapter 6, and in chapter 7 those of the hydrous - and anhydrous sticks. Chapter 8 discusses the formulation problems that occurred during this study with recommendations for possible improvement of the formulation problems experienced and also discusses the final attempt to solve the formulation problems. The final conclusion is given in chapter 9.

CHAPTER 1

SKIN LIGHTENING

1.1 INTRODUCTION

While one half of the world's population is striving to obtain a suntanned skin, the other half strives to obtain a lighter complexion by using a skin lightener or whitener (Wiechers *et al.*, 1998:61). Throughout the centuries, substances have been sought which would lighten melanin pigmentation in the skin. Commercially available skin-lightening cosmetics have large sales in certain areas of the world, because many people wish to alter or modify their skin colour (Engasser & Maibach, 1981:143).

Especially among the Chinese population, a fair, flawless skin is the epitome of beauty, and the presence of melasma or any facial pigmentation is considered by some as "bad luck" (Lim, 1999:282).

In a study done in South Africa by Bentley-Phillips and Bayles (1975:1391), it was reported that skin lighteners are sold in abundance and used for facial improvement and the elevation of social standing. According to this study, the additional 'smoothing' effect, as the Blacks call it, is highly prized by both men and women.

Skin-lightening agents are being applied to either lighten the skin (to change or modify skin colour) or to depigment it (treatment for abnormal hyperpigmentation skin, such as melasma, freckles and actinic lentigines) (Zai & Maibach, 2001:20).

This chapter presents a brief overview on skin lightening and skin lightening agents. First the skin pigmentation mechanism is discussed, followed by the medical and cosmetic uses of skin lighteners. Finally, various skin ligtening agents are discussed, from which the most appropriate were selected for this study.

1.2 SKIN PIGMENTATION MECHANISM

The human epidermis is composed of three cell types: melanocytes, keratinocytes and Langerhans cells. Melanocytes account for between 5-10% of the cellular content and are located in the basal layer of the epidermis. The known function of melanocytes is to synthesise melanin that protect the skin from ultraviolet (UV) radiation. After production of melanin in the melanocytes, the melanin is transferred into the keratinocytes, where it becomes visible as skin colour. Only one melanocyte serves as the source of pigment for a given population of epidermal cells (Stenn, 1983:578). Melanin is the most important pigment in determining skin colour (Mitsui, 1997:22). The number and density of melanocytes in human skin is more or less the same, irrespective of the skin colour or race (Mitsui, 1997:22; Stenn, 1983:576; Zuidhoff & van Rijsbergen, 2001:53).

The first and rate-limiting step of melanin formation is mediated by the enzyme, tyrosinase. Tyrosinase catalyses the hydroxylation of tyrosine into 3,4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation of DOPA into DOPAquinone. Due to auto-oxidation and spontaneous cyclisation, DOPA produces 5,6-dihydrosyindole (DHI) melanin (Petit & Piérard, 2003:170). Other enzymes are involved in the multi-step pathway. See figure 1.1 (Petit & Piérard, 2003:171) for a simplified summary of the melanogenic pathway.

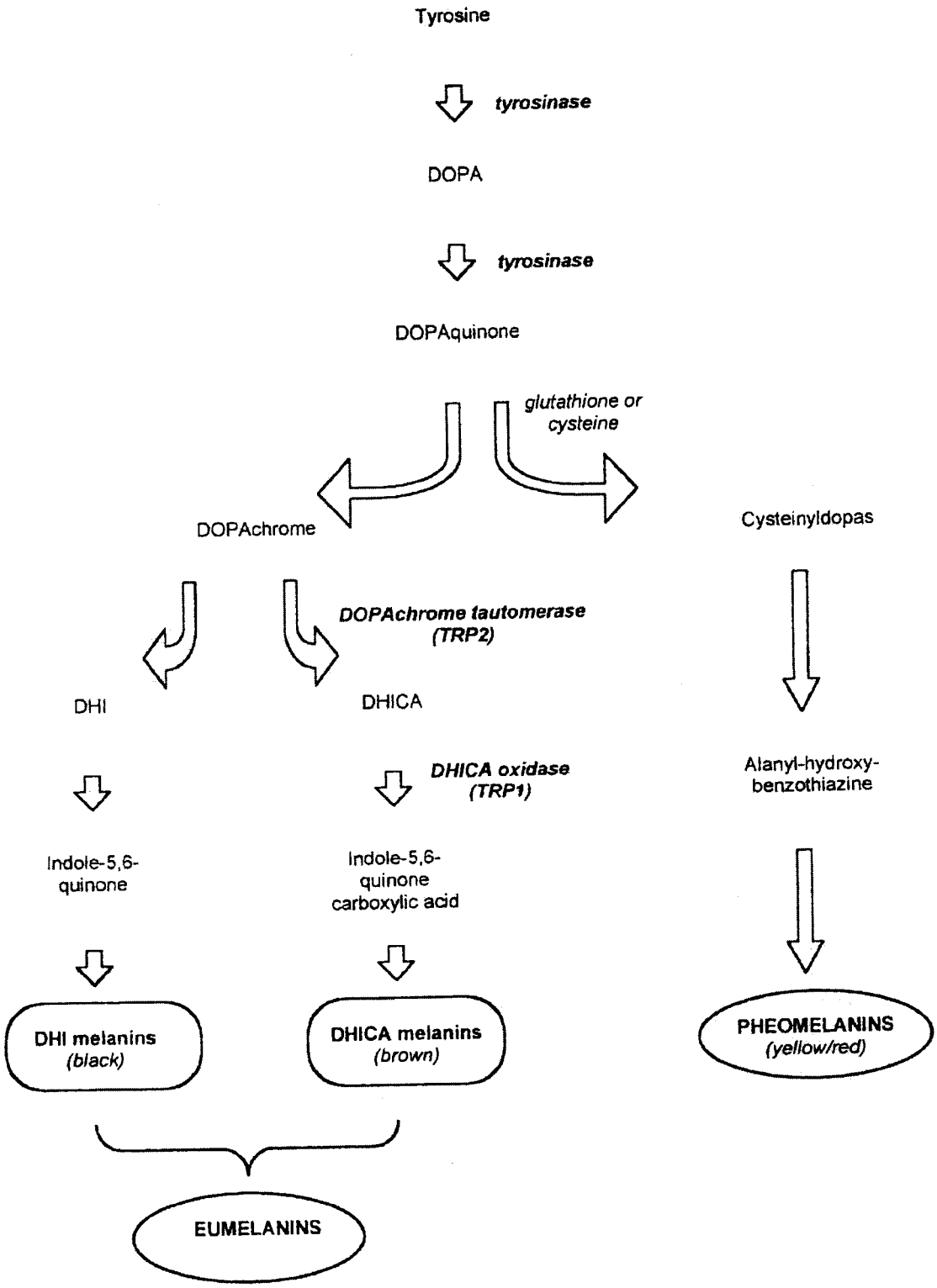

Figure 1.1 Simplified summary of the melanogenic pathway (Petit & Piérard, 2003:171).

1.3 MEDICINAL AND COSMETIC USES OF SKIN LIGHTENERS

Mainly three groups use skin whiteners, or lighteners, i.e. Asians, Africans and women over the age of forty. For African and Asian women, skin lightening is part of their culture, as a lighter skin signifies increased wealth and social status. Women over forty use skin lighteners as a cosmetic solution to treat increased skin pigmentation and uneven skin tones. Problem skin, like acne, scars, psoriasis, melasma and hyperpigmentation are also treated with skin whiteners or lighteners. Skin lightening products are expected to brighten the skin and to create a more even tone and appearance (Dayan *et al.*, 2004:96).

In recent years, the suppression of melanin synthesis has become more important in cosmetic efforts to acquire a lighter skin. In Asia, skin lightening products have grown to be the best selling skin care products, whereas in the Western hemisphere, including Europe and Northern America, the main demand is for the treatment of age spots and skin even toning (Fox, 2005:36).

Petit and Piérard (2003:169) reported that pigment disorders are multiple and occur as a result of both genetic and environmental factors. Most pigmentation disorders are the result of alterations in the density of active melanocytes, and of specific abnormalities of any part of the complex melanogenesis mechanism. Among these disorders, hypermelanosis (also known as hyperpigmentation) is a fairly common disorder and is particularly troublesome in darkly pigmented individuals (Petit & Piérard, 2003:169; Jimbow & Minamitsuji, 2001:35).

Hyperpigmentation of the face is usually due to an increased amount of melanin, either within the epidermis, or dermis, or within both. The increase in melanin content is either as a result of an increased number of functioning melanocytes, or an increased amount of melanin production without a numerical alteration of melanocytes, or both (Jimbow & Minamitsuji, 2001:35). The alteration in melanocytes is triggered by UV light, female hormones and genetic reasons, but in most cases the detailed mechanism is not clearly understood (Mitsui, 1997:148).

Melasma, one of the more common causes of facial hyperpigmentation, is a multiple clinical condition. It is characterised by slowly progressive symmetrical hypermelanosis, consisting of irregular skin coloration. Other, less frequent factors involved in the pathogenesis of melasma, are exposure to UV rays, genetic influences, cosmetics, phototoxic drugs and anticonvulsants (Haddad *et al.,* 2003:153). In addition to melasma, other causes of facial hyperpigmentation include Riehl's melanosis, photo contact dermatitis, and the sequel of inflammatory diseases, such as acne vulgaris and cutaneous lupus, and nevus of Ota (Jimbow & Minamitsuji, 2001:35).

Topical hypo- or depigmentation agents best affect those disorders where the increased melanin pigment is within the epidermis. In patients with melasma, two main groups of hypo- or depigmentation agents have been commonly used: phenolic and non-phenolic derivates. Hydroquinone is the most extensively used phenolic derivate. Phenolic thioethers are a new class of phenolic derivates, with melanocyte selective cytocidal and cytostatic effects. Non-phenolic derivates include azelaic and kojic acid (Jimbow & Minamitsuji, 2001:35).

UV protection, especially UVA radiation that causes pigmentation, is advised in addition to any skin lightening agent (Petit & Piérard, 2003:176; Zai & Maibach, 2001:22).

From the above it is thus clear that there are many causes of skin pigmentation and skin disorders, creating an increasing demand for skin lightening and depigmentation agents, whether for cosmetic or for clinical reasons. In the next section various skin lighteners are introduced, but the aim is to select an active(s) that is effective and safe to use.

1.4 SKIN LIGHTENERS

In this section, various skin lightening agents are discussed with the aim to choose safe and stable skin lightening agents for incorporation in the formulations in this study.

1.4.1 Hydroquinone

Hydroquinone is the most prescribed skin-lightening agent worldwide, despite its inconsistent effects and safety concerns (Engasser & Maibach, 1981:144; Jimbow & Minamitsuji, 2001:39).

Clinically, hydroquinone is applied topically in the treatment of melasma, freckles, senile lentigines and post-inflammatory hyperpigmentation (Engasser & Maibach, 1981:144). A decrease in pigmentation can be observed after 4 weeks of therapy, while optimal results can be seen after 6-10 weeks of therapy (Jimbow & Minamitsuji, 2001:39). The clinical efficacy depends on the hydroquinone concentration, the nature of the vehicle and the stability of the formulation. Hydroquinone is often used at 1.5-5% concentrations (Petit & Piérard, 2003:176).

There are concerns that hydroquinone may pose a health risk. This has prompted a legal debate in Korea, South Africa and elsewhere (Smith, 2001:1). When applied over long periods of time, hydroquinone has been reported to cause severe side effects. It leads to permanent de-pigmentation and causes photosensitivity of the skin (Zuidhoff & van Rijsbergen, 2001:54). Hydroquinone is a highly reactive, but a potent melanocyte cytotoxic and mutagenic compound, and for this reason it is not authorised for use in cosmetic products anymore (Petit & Piérard, 2003:176). Due to its side effects, it is prohibited in the United States (US) and in Europe (Maeyama, 2002:69).

1.4.2 Arbutin

Arbutin is an active ingredient of the crude drug, *Uvae Ursi* Folium, traditionally being used in Japan. The leaves of pear trees and certain herbs also contain arbutin (Maeda & Fukuda, 1996:765). Arbutin is the ordinary name for hydroxyquinone-ß-D-glucopyranoside. Various *in vivo* and *in vitro* tests have proven its ability to control melanin production. Studies done on cultured B16 melanoma cells, showed arbutin's ability to inhibit melanin production without influencing cellular increase, and also to lower tyrosinase activity. Arbutin's inhibition of melanin production is not based on the melanocyte cell toxicity mechanism as shown by hydroquinonemonobenzylether, but is

thought to either inhibit the activity or production of tyrosinase (Mitsui, 1997:148). A study done by Maeda and Fukuda (1996:276) showed arbutin's concentration-dependant reduction in tyrosinase activity at noncytotoxic concentrations in human melanocyte cultures. Mitsui (1997:148) reported that arbutin inhibits melanin production without being metabolised to hydroquinone.

1.4.3 Azelaic acid

Azelaic acid is a dicarboxylic acid, originally isolated from *Pityrosporum ovale*, the organism responsible for pityriasis versicolor. Azelaic acid is known to be a competitive inhibitor of tyrosinase *in vitro*, and can be used to treat melasma. It may be beneficial to use azelaic acid if prolonged treatment is anticipated, as it has fewer side effects when compared to hydroquinone (Jimbow & Minamitsuji, 2001:39).

1.4.4 Corticosteroids

In a study done by Kanwar *et al.* (1994:170), 10 patients with melasma were treated with potent, topical corticosteroid, clobetasol propionate (0.05%). In all the patients fading of pigmentation was observed after 2 weeks, with more discernible results after 4-6 weeks. Eight to ninety percent clearance of pigmentation was observed after 6-8 weeks. In some patients with cleared pigmentation, the pigmentation reappeared at the same sites after 2-3 weeks after ending treatment. The short-lived efficacy of corticosteroids may be due to their ability to suppress secretory metabolic products from the melanocytes, without causing their destruction.

1.4.5 Lactic acids and lactates

Cleopatra used to take long baths containing goats' milk, and long ago women washed their faces with wine. Although unaware of the principle, they took advantage of the beneficial properties of lactic acid and lactates. Lactic acid and its lactate salts have the ability to suppress tyrosinase formation. Good skin-whitening properties have been reported at higher concentrations (> 5% equivalent lactic acid), making use of the

independent melanogenic controlling function of the viable pigment cell (Zuidhoff & Rijsbergen, 2001:54).

1.4.6 Vitamin C and its derivates

Vitamin C, also known as ascorbic acid, has long been used in whitening cosmetics to control melanin production (Mitsui, 1997:150). According to Špiclin *et al.* (2001:271), ascorbic acid can be used to whiten the skin, because of its capability to suppress skin pigmentation and decomposition of melanin. Its effect is twofold: (i) it reduces DOPAquinone, the melanin intermediate compound in the tyrosinase reaction, which produces melanin from tyrosine, and (ii) it reduces the dark coloured reduced form (Mitsui, 1997:150). Ascorbic acid may inhibit melanin production by reducing o-quinones, so that melanin cannot be formed by the action of tyrosinase, until all vitamin C has been oxidised (Kameyama *et al.*, 1996:29). Kameyama *et al.* (1996:32) also stated that ascorbic acid probably suppresses melanin formation at various oxidative steps of melanin formation, such as 5,6-dihydroxyindole oxidation.

Mitsui (1997:150) reported that vitamin C is very safe, but very unstable, especially in an aqueous solution (Kameyama *et al.*, 1996:32). It undergoes oxidation with light exposure, and especially in aerobic conditions (copper or heavy metals in general catalyse this reaction). These reactions occur quickly in basic conditions and the compound degrades itself irreversibly in a biologically inactive form (2,3-diketo-L-gulonic acid) (Austria *et al.*, 1997:395). To overcome this problem of stability, derivates of vitamin C have been synthesised, having an action similar to ascorbic acid, but with improved chemical stability (Mitsui, 1997:150; Špiclin *et al.*, 2001:271). In the past, ascorbic acid had been administered to the skin in high doses, and had to be prepared fresh daily, because of its instability, thus increasing the cost of its use (Hernandez & Shaffer, 2000:2).

Ascorbyl esters, namely ascorbyl palmitate and phosphate, have been marketed as cosmetics for the treatment of hyperpigmentation. Ascorbyl phosphate has the commercial advantage of aqueous solubility, permitting a wide variety of cosmetic product formulations. It is also stable for at least six months and hydrolyses to L-ascorbate by phosphatases, naturally present in the skin (Colven & Pinnell, 1996:231). Currently

available ascorbyl phosphate compounds enable the formulator to incorporate the anti-oxidative activity of ascorbic acid into cosmetic formulations (Jentzsch & Streicher, 2001:55).

Magnesium-L-ascorbyl-2-phosphate (VC-PMG) is a vitamin C derivate that acts synergistically with vitamin E in several oxidation steps of melanin synthesis, it is also stable in water (Kameyama *et al.*, 1996:29). In a study done by Kameyama *et al*, (1996:32), the topical application of magnesium-L-ascorbyl-2-phosphate was effective in lightening the skin of some patients with hyperpigmentation disorders, and of some subjects with normally pigmented, healthy skin. Magnesium-L-ascorbyl-2-phosphate directly or indirectly suppresses melanin formation, catalysed by mammalian tyrosinase.

Sodium ascorbyl phosphate is a stable vitamin C derivative, far more stable than ascorbic acid in water. It acts on the melanin formation process to prevent hyperpigmentation and senile keratosis, and therefore has skin-lightening properties. It also protects the skin, promotes its development and improves its appearance. Sodium ascorbyl phosphate is cleaved enzymatically in the skin to release active vitamin C. Therefore it is an effective anti-oxidant, which protects cells against damage caused by free radicals. It also counteracts skin ageing in promoting collagen formation. Sodium ascorbyl phosphate is an effective water soluble anti-oxidant, which is stable in cosmetic formulation. See figure 1.2 for the stability of sodium ascorbyl phosphate in different formulations (BASF, 2005:3).

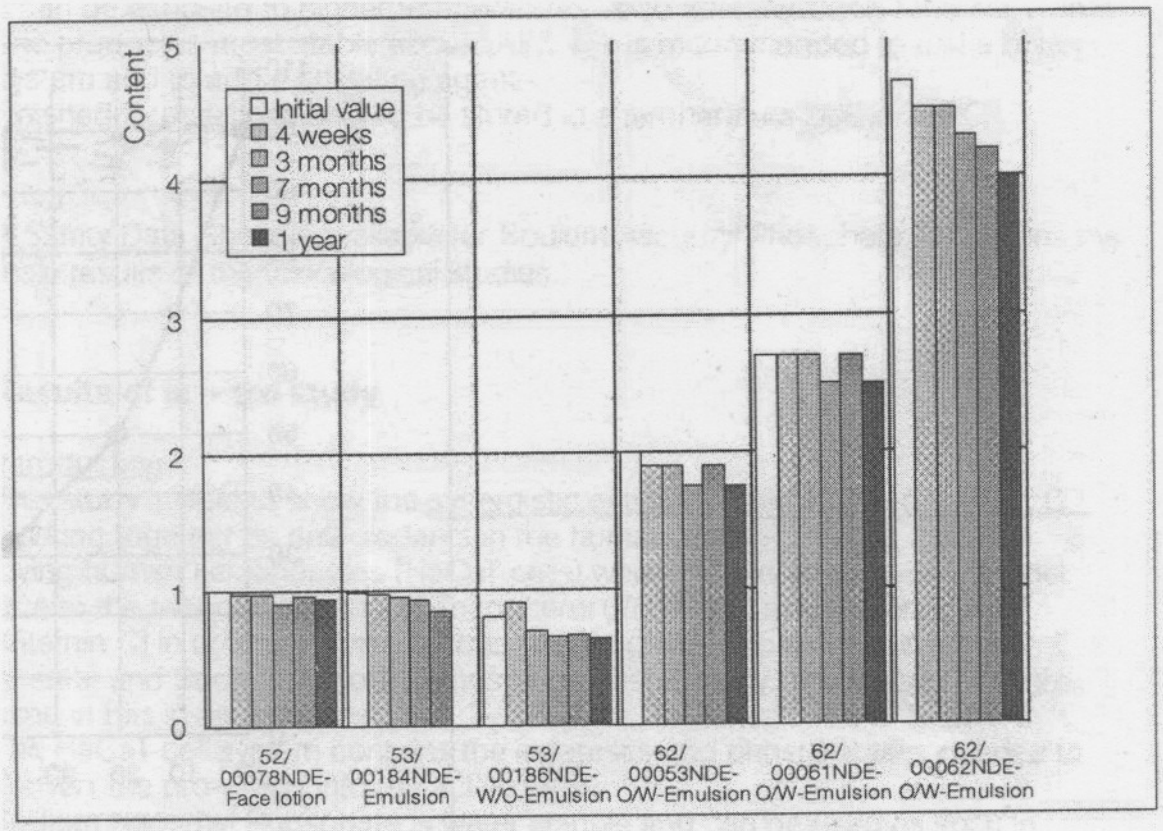

Figure 1.2 The stability of sodium ascorbyl phosphate in different formulations at 20°C, pH 6.5 (BASF, 2005:3).

Animal studies have shown significant acute photo protective and chronic photo ageing preventive effects from topical application of ascorbic acid. On the basis of circumstantial data, topical vitamin C should have a beneficial effect in the treatment of photo ageing (Colven & Pinnell, 1996:233).

- **Photo protective properties**

Vitamin C has been used in cosmetic and dermatological products, because of its many favourable effects on the skin. As an antioxidant it scavenges and destroys aggressive oxidising agents and free radicals that are involved in the process of skin ageing (Colven & Pinnell, 1996:229). The skin possesses a wide range of interlinked antioxidant, defence mechanisms to protect itself from damage by reactive oxygen species (ROS), but the capacity of these systems is limited and they can be overwhelmed by excessive exposure to ROS. Supporting the cutaneous antioxidant defence systems with exogenous antioxidants could thus prevent ROS mediated damage to the skin (Špiclin *et al.,* 2003:65).

Although not specifically tested by Colven & Pinnell (1996:231), the ascorbic acid levels of ascorbic acid-treated skin, measured after topical application, were assumed to be much higher than those obtainable with oral supplementation. In addition to this, topical ascorbate treated skin that was exposed to UV light, experienced a 66% reduction in ascorbate levels, compared to those in topically treated non-irradiated skin.

Animal studies have also shown significant acute photo protection and chronic photo ageing preventive effects from topical application of vitamin C. On the basis of circumstantial data, topical vitamin C should have a beneficial effect in the treatment of photo ageing (Colven & Pinnell, 1996:233). The combination of vitamins and sunscreens creates an ideal synergy and can therefore play an important role in the war against ageing and the maintenance of a youthful appearance (Djerassi, 1997:60).

Concentrations of 0.5-2.0% ascorbic acid are recommended by Roche Vitamins (2005:5), as an antioxidant in skin care formulations.

- **Use as a whitening agent**

As an effective reducing agent at high concentrations, ascorbic acid can momentarily retard the melanin-biosynthesis pathway, but can never eliminate it. On the contrary, the result of accumulating diphenol produces an indirect activation of this pathway when the reductant is completely depleted. This should be taken into account in the development of protective creams, which include reducing and depigmenting agents, such as ascorbic acid (Ros *et al.*, 1993:312).

Ros *et al.* (1993:309) studied the effect of ascorbic acid on the monophenolase activity of tyrosinase, using tyrosine as substrate. Various concentrations of ascorbic acid were used, with no direct effect on the enzyme. However, a shortening of the characteristic induction period of the hydroxylation reaction was observed. The evolution of this reaction is dependant on the ascorbic acid concentration. Low concentrations permit the system to reach the steady state when all ascorbic acid is consumed, whilst high concentrations do not.

Ascorbic acid may inhibit melanin production by reducing enzyme generated o-quinones (Kameyama *et al.*, 1996:29; Ros *et al.*, 1993:309), so that melanin cannot be formed by the action of tyrosinase, until all vitamin C has been oxidised (Kameyama *et al.*, 1996:29). Kameyama *et al.* (1996:32) also averred that ascorbic acid probably suppresses melanin formation at various oxidative steps of melanin formation, such as 5,6-dihydroxyindole oxidation.

- **Use in whitening cosmetics**

Ascorbic acid is difficult to stabilise in pharmaceutical formulations for use in topical delivery to the skin, for any significant period of time (Hernandez & Shaffer, 2000:2). Because of the hydrophilic character of sodium ascorbyl phosphate, it has a low ability to penetrate the skin. It is therefore important to select a suitable carrier system to deliver it to the site of action in the skin (Špiclin *et al,* 2003:66). Unfortunately, ascorbic acid, although readily soluble in water, is rapidly oxidises on exposure to air and its use in cosmetics is maximised in anhydrous systems (Djerassi, 1997:60). Hernandez & Shaffer (2000:4) stated that an anhydrous base protects ascorbic acid, or its derivates, from degradation, instability, loss of potency and loss of colour.

Concentrations of up to 5% ascorbic acid are recommended in cosmetic formulations applied for skin lightening (Roche Vitamins, 2005).

1.4.7 Kojic acid and kojic acid dipalmitate

Kojic acid, a fungal metabolic product, is increasingly being used as a skin lightening agent in skin care products marketed in Japan since 1988 (Zai & Maibach, 2001:23). It is mainly produced by microbial fermentation, using *Aspergillus oryzae* and P*enicillium*, or *Acetobacter* species. *In vivo* and *in vitro* tests have shown kojic acid's ability to inhibit melanin production (Nohynek *et al.,* 2004:93; Jimbow & Minamitsuji, 2001:41; Mitsui, 1997:149).

In a study done by Majmudar *et al.* (1998:364) kojic acid showed a dramatic decrease in tyrosinase activity when compared to other whitening agents. Compared to lactic acid,

magnesium ascorbyl phosphate and ascorbic acid, kojic acid was most effective. See figure 1.3 for results of the study done by Majmudar *et al.* (1998:365).

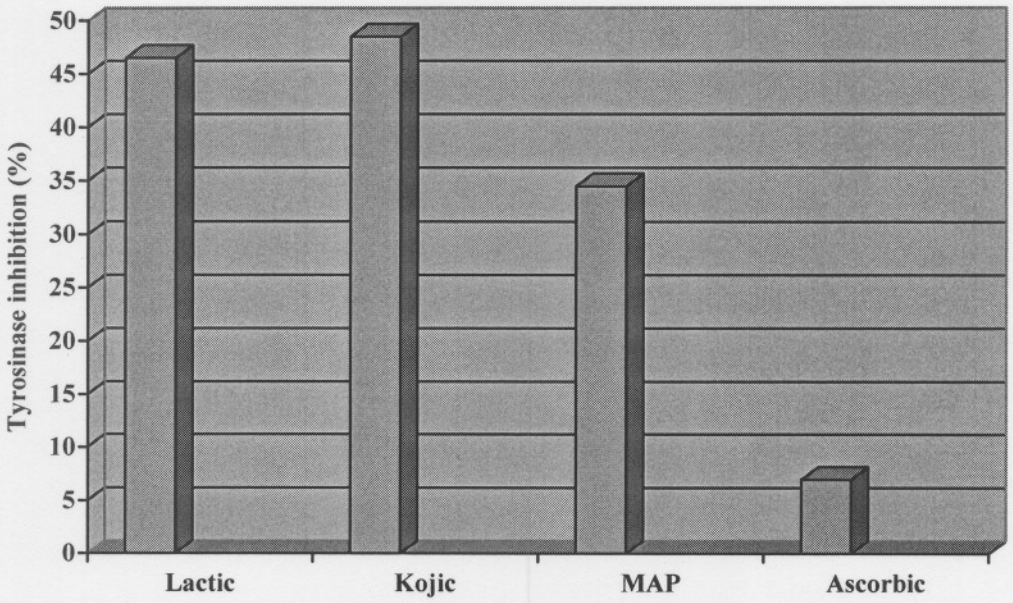

Figure 1.3 Comparison of the effect of whitening agents on tyrosinase inhibition (Majmudar *et al.*, 1998:365).

Giuseppe (1996:50) also stated that kojic acid proved to be the most useful in cosmetic applications, when compared to other skin lighteners suppressing tyrosinase directly. Figure 1.4 shows kojic acid's outstanding ability to inhibit tyrosinase activity, as reported in a study done by Simonot *et al.* (2002:53).

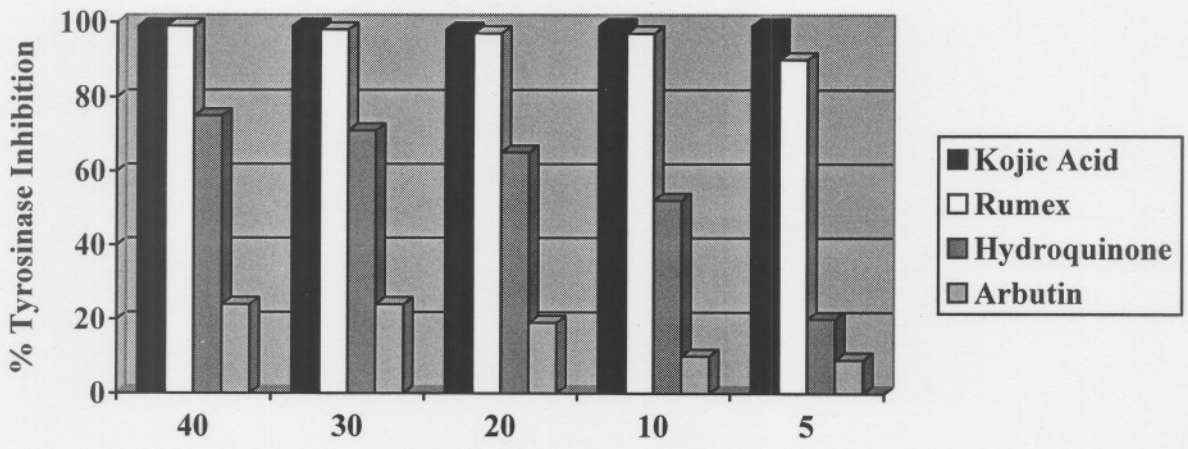

Figure 1.4 Comparison of tyrosinase inhibition of whitening agents at various concentrations (in µg/ml). Assays done in duplicate at 37°C (Simonot *et al.*, 2002:53).

Kojic acid is known to have the ability to cause those products in which it is present to discolor (Smith, 2001:1). Kojic acid can cause formulations to turn yellow to yellowish brown (Nagai & Izumi, 1983:3). This is especially evident when kojic acid is formulated in a high water content formulation (Whittemore & Neis, 1998:3). In a US patent, Igaki (1997:4) claimed that kojic acid, in an aqueous phase, or with exposure to UV light, causes discolouration or decomposition. The pH is another key degradation factor of kojic acid in a water based emulsion. At a neutral pH, kojic acid's stability will decrease rapidly (Kim, 2003:837).

Although kojic acid itself has a high ability to inhibit tyrosinase activity, converting kojic acid into an ester with an aliphatic carboxylic acid, forming kojic acid dipalmitate, further increases this ability. This increases kojic acid's stability to pH, heat and light changes. The result is an excellent storability and oil-solubility, increasing skin absorption (Nagai & Izumi, 1983:3).

Furthermore, kojic acid dipalmitate is a mild active ingredient for skin lightening products, it does not possess any cytotoxicity and it is easy to formulate. Kojic acid dipalmitate is a

natural amino acid derivate with skin whitening properties. It belongs to the class of guanidino compounds, which are ever-present in mammalian cells (Fox, 2005:36).

Kojic acid dipalmitate therefore was an obvious choice for use in this study, because it is stable under a wide range of conditions and a pH range of 4-9 (Uchem, 2004), and since it does not change colour or discolor emulsions (Smith, 2001:2).

1.5 CONCLUSION

In this chapter a brief overview on skin lightening and skin lightening agents were given. The skin lightening mechanism, as well as the medical and cosmetic uses of skin lighteners was discussed. Skin pigmentation and skin disorders were seen to have many causes, creating an increasing demand for skin lightening and depigmentation agents in recent years, whether for cosmetic or for clinical reasons.

Various skin lightening agents were then discussed in order to choose suitable skin lighteners for use in this study. Based on the knowledge gained from available literature on different skin lightening agents, sodium ascorbyl phosphate and kojic acid dipalmitate were finally chosen for incorporation in the formulations in this study, due to their ability to whiten the skin effectively and safely and because of their ease of formulation and stability.

It was concluded from the literature study that there is an increasing demand for skin lighteners for cosmetic as well as clinical use. There is a need in the market for both stable and thus effective skin lighterners. This study poses a promesing solution to the problem.

In chapter 2 sodium ascorbyl phosphate and kojic acid dipalmitate are studied and discussed in more detail.

CHAPTER 2

KOJIC ACID DIPALMITATE AND SODIUM ASCORBYL PHOSPHATE

2.1 INTRODUCTION

In chapter 1 the outcomes of a literature study on the causes of, and the increasing demand for and possible cures for skin pigmentation and skin disorders were reported. In a search for skin lightening agents for use in this study, it was seen that kojic acid dipalmitate and sodium ascorbyl phosphate are both safe and effective active ingredients for treatment, as well as potentially stable. These two actives were hence chosen for their suitability as skin lightening agents, based on their possible synergism (Hatae, 1990:1) and their low toxicity to melanocytes.

In this chapter the outcomes of a preformulation study that was performed is discussed, which comprised of an in-depth look into the properties of kojic acid dipalmitate and sodium ascorbyl phosphate.

Regarding the cytotoxic and sensitising side effects of established skin whiteners, it is evident that there is a strong need for sophisticated active ingredients with skin lightening efficacy and safety (Fox, 2005:36). Agents that inhibit melanin production, such as kojic acid, and vitamin C and its derivates, are used in whitening cosmetics, because of their low toxicity to melanocytes (Maeyama, 2002:69; Mitsui, 1997:148). The combination of the corrective and protective vitamins and UV-filters creates an ideal synergy, resulting in high performance cosmetics, which can help consumers in the war against ageing and the maintenance of a youthful appearance (Djerassi, 1997:62).

A variety of skin lightening formulations is commercially available. Two or more active compounds are more frequently used in combination therapy (Petit & Piérard, 2003:178).

In a study done by Van Rensburg (2004:153), kojic acid and sodium ascorbyl phosphate were formulated in cosmetic products. Van Rensburg (2004:154) found that kojic acid and sodium ascorbyl phosphate are compatible with each other and can easily be formulated in cosmetic products.

Due to kojic acid's known instability at high temperatures its ester, kojic acid dipalmitate, was used instead, in order to evaluate this derivates' stability in cosmetic formulations in this study.

2.2 SYNERGISM OF KOJIC ACID AND SODIUM ASCORBYL PHOSPHATE

Any synergistic effect would allow topical preparations to be formulated with a lower content in active ingredients, therefore retaining satisfactory efficacy, and having minimal sensitising potential and maximum skin safety (Ferioli *et al.*, 2001:334).

Hatae (1990:1) reported that by blending vitamin C, or its derivates, with kojic acid, or its esters, could synergistically inhibit melanin synthesis.

2.3 SODIUM ASCORBYL PHOSPHATE

2.3.1 Introduction

Vitamins have long been known for their vital role in human health, in all parts of the body, including the skin (Roche Vitamins, 2005:2). However, because of the belief that vitamins could not penetrate the skin, and because the metabolic activity of the skin was inadequately known, vitamins have not been widely used in cosmetics until recently. Now, with a better understanding of the physiology of the skin, the interest in topically applied vitamins has increased (Idson, 1993:79).

For many years, there has been an interest in cosmetics containing vitamin C (ascorbic acid), since consumers know the beneficial effects of this vitamin when ingested as citrus

fruits, or in vitamin supplements. The many beneficial properties of vitamin C in skin care have until now been under utilised, because of its instability against oxidation and the browning of products as a result of this. One way to overcome the stability problem is by temporarily blocking the active centre of the ascorbic acid molecule. This is the principle being applied by stable STAY-C® 50 (Roche Vitamins, 2005:2), which was used during this project.

2.3.2 Chemical properties and stability

Figure 2.1 shows the chemical structure of STAY-C® 50 (Roche vitamins, 2005:2).

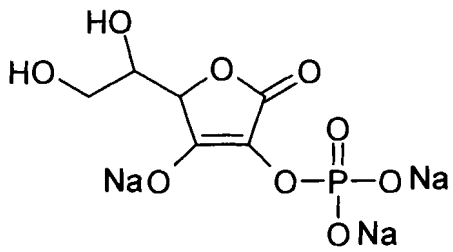

Figure 2.1 Structure of STAY-C® 50 (Roche vitamins, 2005:2).

According to Austria *et al.* (1997:797), it is reasonable to believe that the introduction of the phosphoric group in position 2 protects the enediol system of the molecule from hydrolysis. Research done by Austria *et al.* (1997:797) also revealed that the presence of phosphoric ions in a solution protect the molecule from hydrolysis, due to the ion pair effect, shifting the balance of the reaction towards the phosphorylated form.

STAY-C® 50 is the sodium salt of the monophosphate ester of ascorbic acid. It is a white powder, easily soluble in water to concentrations reaching 50%. In contrast to ascorbic acid, STAY-C® 50 is stable in aqueous solutions at a pH of 7, or higher (Roche Vitamins, 2005:2).

BASF (2005:5) describes sodium ascorbyl phosphate as a crystalline solid that is sensitive to heat, moisture, low pH values and heavy metals.

STAY-C® 50 is a provitamin. To become the biologically active ascorbic acid, the vitamin must first be freed by phosphatase (Roche Vitamins, 2005:2). Such enzymes are normal constituents of the skin (Colven & Pinnell, 1996:231).

In the production of cosmetic products, BASF (2005:5) recommends adding sodium ascorbyl phosphate to formulations at low temperature (< 40°C). It can be exposed to higher temperatures reaching 80°C, but only for a short period. Sodium ascorbyl phosphate is most stable above pH 6.5. It is recommended to use a buffer system and to add a chelating agent. Finished formulations should be stored at a temperature below 25°C.

From this discussion it is clear that the characteristics of sodium ascorbyl phosphate emphasises the stability of this vitamin C derivate.

2.4 KOJIC ACID DIPALMITATE

2.4.1 Introduction

Kojic acid dipalmitate efficiently inhibits tyrosinase activity. It is more effective than pure kojic acid. Kojic acid dipalmitate has an excellent ability to evenly tone the skin and it is used in the fight against age spots, freckles, pregnancy marks as well as general skin pigmentation disorders. When using kojic acid dipalmitate in formulations, product stability is ensured without any colour instability problems (Chemos, 2005:1).

2.4.2 Chemical properties and stability

Figure 2.2 shows the chemical structure of kojic acid dipalmitate (Uchem, 2004).

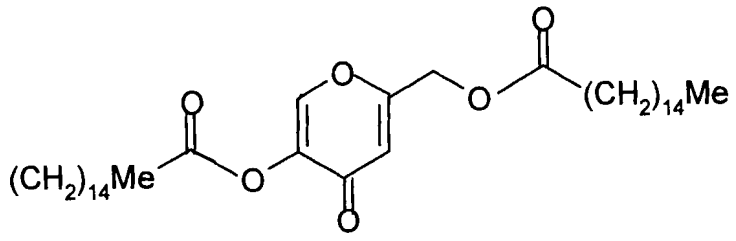

Figure 2.2 Chemical structure of kojic acid dipalmitate (Uchem, 2004).

Kojic acid dipalmitate is stable under a wide range of conditions and it does not change colour or discolour its formulations (Smith, 2001:3). Kojic acid dipalmitate offers more efficacious skin lightening effects, when compared to kojic acid. The dipalmitate derivate markedly enhances the inhibitory effects on tyrosinase activity. Kojic acid is unstable when exposed to heat and light, and tends to oxidise, resulting in a colour change (yellow or brown). The tendency of kojic acid to chelate with metal ions, such as iron, also results in a colour change. On the contrary, kojic acid dipalmitate is stable to pH, light, heat and oxidation, and it does not complex with metal ions, therefore giving colour stability (01Wholesale, 2002).

2.4.3 Optimal formulation

Kojic acid and its derivatives are known as agents which have difficulty in acquiring stability (Igaki, 1997:1). In a US patent, Igaki (1997:11) claimed that water in oil (W/O) preparations; containing kojic acid, or its derivates, tend to suffer less discoloration or decomposition in comparison to oil in water (O/W) preparations. High-performance liquid chromatography (HPLC) analysis confirmed kojic acid's lost activity, when held at room temperature for one month in an aqueous non-ionic base (Majmudar *et al.*, 1998:364). The increased stability of kojic acid in an anhydrous base is illustrated in table 2.1 (Majmudar *et al.*, 1998:366).

Table 2.1 HPLC analysis of kojic acid (Majmudar *et al.*, 1998:366)

Base	Time (weeks)	Loss (%) 23°C	Loss (%) 37°C
Aqueous	2	0	14.79
	5	87.83	86.35
Anhydrous	8	-	21.22
	12	-	19.20
	26	2.03	-

Whittemore & Neis (1998:1) reported that kojic acid dipalmitate would indefinitely keep its whiteness in an anhydrous cosmetic base, and it would maintain its skin brightening activity. This increases its commercial value over hydrous formulations.

A gradual increase in skin lightening was found over a period of 3 months with the anhydrous base (Majmudar *et al.*, 1998:366).

Clinical tests done by Majmudar *et al.*, (1998:366) revealed a gradual increase in skin lightening over a period of three months, with the anhydrous base kojic acid formulation. The non-ionic aqueous base containing kojic acid, was less effective, presumably due to a loss of kojic acid over a period of three months.

According to Nohynek *et al.* (2004:94), kojic acid has been used as a skin lightening cosmetic in Japan and other countries, in concentrations of up to 1.0-1.5% being typical.

It was therefore concluded from the literature study that incorporating kojic acid dipalmitate in an anhydrous base would further improve its stability. That is why in this study, hydrous and anhydrous products were formulated in order to investigate whether kojic acid dipalmitate would be more stable in anhydrous formulations when its stability is compared to hydrous formulations.

2.4.4 Mutagenic concerns

Since March 2003, kojic acid has practically no longer been used in Japan, when JMHW (Japan's Ministry of Health Welfare) announced that kojic acid might possibly have carcinogenic properties. Although this was not supported by any substantiating data, most cosmetic companies in Japan have stopped using kojic acid in their products (Brewster, 2004:20). Petit & Piérard (2003:177) also stated that kojic acid had been banned in Japan because of mutagenic concerns.

However, Nohynek *et al.* (2004:94) conducted a thorough investigation of the genotoxicity and general toxicity of kojic acid after topical administration, under Good Laboratory Practice (GLP) conditions. Various *in vivo* and *in vitro* tests were performed. According to these test results, Nohynek *et al.* (2004:104) concluded that the genotoxic risk for humans, using kojic acid as a skin lightener, was negligible, and in any case much less than from exposure to kojic acid in fermented food. The results obtained did suggest that consumer exposure to kojic acid from fermented foods did not pose a significant human health risk.

2.5 CONCLUSION

In this chapter kojic acid dipalmitate and sodium ascorbyl phosphate were discussed in detail, with the aim of emphasising their synergistic effect, effectiveness and stability, as well as their low toxicity for use in skin lightening formulations. It became clear that safe and effective skin lightening cosmetics can be formulated by using these two actives.

It was concluded from the literature study that kojic acid dipalmitate and sodium ascorbyl phosphate would be more stable in an anhydrous base. Therefore, in this study, hydrous and anhydrous products were formulated in order to compare the stability of the kojic acid dipalmitate in both kinds of formulations.

Chapter 3 is a discussion of the formulation of the cosmetic products containing kojic acid dipalmitate and sodium ascorbyl phosphate.

CHAPTER 3

FORMULATION OF COSMETICS CONTAINING KOJIC ACID DIPALMITATE AND SODIUM ASCORBYL PHOSPHATE

3.1 Introduction

According to Whittemore and Neis (1998:1), kojic acid dipalmitate will indefinitely keep its whiteness in an anhydrous cosmetic base. Furthermore, Hernandez and Shaffer (2000:4) stated that an anhydrous base protects ascorbic acid, or its derivates, from degradation, instability, loss of potency and loss of colour. Similarly Djerassi (1997:60) stated that sodium ascorbyl phosphate is rapidly oxidized on exposure to air and its use in cosmetics is maximized in anhydrous systems.

In this chapter those hydrous and anhydrous products that were formulated in this study, are discussed. The aim of this study was to formulate and identify potentially stable formulations for subsequent evaluation during a stability study, as is discussed in chapter 4. A hydrous - and anhydrous gel, a hydrous cream and an anhydrous ointment, and a hydrous - and anhydrous stick, each containing 1% kojic acid dipalmitate and 0.5% sodium ascorbyl phosphate (as Stay-C® 50), were formulated. The formulae, the procedures to prepare the formulations and a short discussion on each formulation are given. The apparent stability of these formulations was judged via visual examination over two days succeeding formulation. Only formulations which appeared potentially stable were included for testing in the subsequent stability study (see chapter 4 for discussion). The aim with the planned stability study hence was to compare the stability of the sodium ascorbyl phosphate and kojic acid dipalmitate in both a hydrous and anhydrous bases.

3.2 Formulations

Kojic acid dipalmitate and sodium ascorbyl phosphate, as Stay-C® 50, were incorporated as actives into all formulations. See Appendix D for generic names of ingredients.

3.2.1 Hydrous gel

3.2.1.1 Formula of the hydrous gel

The formula of the hydrous gel is given in table 3.1.

Table 3.1 Formula of hydrous gel

INGREDIENTS	% m/m	ACTIVITY
Hydroxypropyl methyl cellulose (HPMC)	2%	Thickener
Glycerine	10%	Moisturiser
Propylene glycol	20%	Solvent
Isopropanol	35%	Solvent
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active
Purified water	To 100%	Solvent

Procedure to prepare the formulation

- Mix isopropanol and 1,2-propylene glycol.
- Grind sodium ascorbyl phosphate with a mortar and pestle, and sift through a 0.3 μm sieve.
- Dissolve the skin lighteners in the isopropanol and propylene glycol mixture.
- Wet HPMC with the glycerine.
- Add isopropanol and propylene glycol mixture and HPMC and glycerine mixture.
- Add water to mixture.
- Stir continuously (not too fast, as air bubbles will form).
- Do not heat.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.1.2 Discussion

The hydrous gel was visually examined over two days succeeding formulation. The formulation appeared to be stable and it was incorporated in the stability study.

3.2.2 Anhydrous gel

3.2.2.1 Formula of anhydrous gel

The anhydrous gel formulation, which was not included in the stability study, is given in table 3.2.

Table 3.2 Formula of anhydrous gel (not included in the stability study)

INGREDIENTS	% m/m	ACTIVITY
Liquid paraffin	93.50%	Base
Mycrocrystalline silica	5.00%	Thickener
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active

Procedure to prepare the formulation

- Do not heat formulation.
- Pour liquid paraffin into a mixing bowl.
- Grind sodium ascorbyl phosphate with a mortar and pestle, and sift through a 0.3 μm sieve.
- Add kojic acid dipalmitate and sodium ascorbyl phosphate to liquid paraffin.
- Stir.
- Add microcrystalline silica and stir.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.2.2 Discussion

The formulation was visually examined over two days succeeding formulation, and appeared stable. After inclusion for stability testing, however, the stability test outcomes over a period of two months showed that this formulation was unstable. Only a very small amount of both actives were retrieved from the formulation during HPLC analysis. It was concluded that the formulation was too unstable for further analysis and it was withdrawn from the stability study.

An improvement on the first formulation was then attempted, in order to formulate a more stable anhydrous gel, as is discussed next. A whole new formula was formulated to address stability problems of the first gel being formulated

3.2.2.3 Formula of the improved anhydrous gel

The formula of the improved anhydrous gel is given in table 3.3.

Table 3.3 Formula of improved anhydrous gel

INGREDIENTS	%m/m	ACTIVITY
Polyethylene glycol (PEG) 4000	4.92%	Base & solvent
Polyethylene glycol (PEG) 400	93.58%	Base & solvent
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active

Procedure to prepare the formulation

- Heat PEG 4000 and PEG 400, until melted.
- Stir continuously.
- Grind sodium ascorbyl phosphate with a mortar and pestle, and sift through a 0.3 μm sieve.
- Add kojic acid dipalmitate and sodium ascorbyl phosphate.
- Stir.
- Allow cooling to room temperature.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.2.4 Discussion

This formulation was visually examined over two days succeeding formulation and appeared to be stable. It was included in the stability studies.

3.2.3 Hydrous cream and anhydrous ointment

3.2.3.1 Formula of the hydrous cream

The formula of the hydrous cream is given in table 3.4.

Table 3.4 Formula of hydrous cream

INGREDIENTS	%m/m	ACTIVITY
A. Cetyl alcohol	7%	Thickening agent
Cremophor A6®	1.5%	Emulsifying agent
Cremophor A25®	1.5%	Emulsifying agent
Liquid paraffin	12%	Oil phase of emulsion
B. Propylene glycol	8%	Solvent / Preservative
Tween 80	4%	Surface active agent
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active
Methyl paraben	0.3%	Preservative
Propyl paraben	0.2%	Preservative
C. Purified water	To 100%	Diluent

Procedure to prepare the formulation

- Mix the ingredients in A and heat the mixture.
- Dissolve the sodium ascorbyl phosphate in a little water (take some of C).
- Heat the remaining water separately to approximately 80°C.
- Add C to the obtained solution A with rigorous stirring.
- Dissolve sodium ascorbyl phosphate in a little water (take some of C).
- Mix the ingredients in B, mix with A and C.
- Continue to stir whilst cooling to room temperature.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.3.2 Discussion

The formulation was visually examined over two days succeeding formulation and appeared stable for inclusion in the stability studies.

3.2.4 Anhydrous ointment

3.2.4.1 Formula of the anhydrous ointment

The formula for the anhydrous ointment is given in table 3.5.

Table 3.5 Formula of anhydrous ointment

INGREDIENTS	%m/m	ACTIVITY
Petroleum jelly	88.5%	Base
Lanolin	10%	Base
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active

Procedure to prepare the formulation

- Heat petroleum jelly and lanolin until melted.
- Stir continuously.
- Add kojic acid dipalmitate and stir.
- Allow cooling until solidifying initiates.
- Grind sodium ascorbyl phosphate with a mortar and pestle, and sift through a 0.3 μm sieve.
- When solidifying initiates, add sodium ascorbyl phosphate gradually, while stirring with a mortar and pestle.
- Allow cooling to room temperature.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.4.2 Discussion

The formulation was first attempted by adding the sodium ascorbyl phosphate, together with the kojic acid dipalmitate, to the other melted ingredients, whilst stirring continuously. After inclusion for stability testing, however, the stability test outcomes over a period of two months revealed poor uniformity of the sodium ascorbyl phosphate in the formulation. This formulation was subsequently excluded from the stability study. However, improved uniformity of this formula was attempted by adding the sodium

ascorbyl phosphate at the end of the procedure, after grinding it in a mortar and pestle and sieving it, as well as stirring it in while solidifying takes place. The resultant formulation appeared stable from visual examination for two days, and it was incorporated in the stability studies.

3.2.5 Hydrous stick

3.2.5.1 Formula of the hydrous stick

The formula for the hydrous stick is given in table 3.6.

Table 3.6 Formula of hydrous stick

INGREDIENTS	%m/m	ACTIVITY
A. Polyethylene glycol (PEG) 4000	52.92%	Base
Polyethylene glycol (PEG) 400	33.32%	Base
Cetyl alcohol	1.76%	Thickening agent
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active
Methyl paraben	0.3%	Preservative
Propyl paraben	0.2%	Preservative
Purified water	10%	Diluent

Procedure to prepare the formulation

- Heat A until melted.
- Stir continuously.
- Dissolve the methyl paraben and propyl paraben in A.
- Add kojic acid dipalmitate and stir.
- Dissolve sodium ascorbyl phosphate in the water and add to mixture.
- Allow cooling to room temperature.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.5.2 Discussion

The hydrous stick had no formulation difficulties; it was visually examined over two days succeeding formulation, found stable and included in the stability studies.

3.2.6 Anhydrous stick

3.2.6.1 Formula of the anhydrous stick

The formula of the anhydrous stick is given in table 3.7.

Table 3.7 Formula of anhydrous stick

INGREDIENTS	%m/m	ACTIVITY
A. Petroleum jelly	79.9%	Base
Lanolin	10%	Base
Paraffin wax	8.8%	Thickening agent
Kojic acid dipalmitate	1%	Active
Sodium ascorbyl phosphate	0.5%	Active

Procedure to prepare the formula

- Heat A until melted.
- Stir continuously.
- Add kojic acid dipalmitate.
- Allow cooling until solidifying initiates.
- Grind sodium ascorbyl phosphate with a mortar and pestle, and sift through a 0.3 μm sieve.
- When solidifying initiates, add sodium ascorbyl phosphate gradually, while stirring with a mortar and pestle.
- Allow cooling to room temperature.
- Transfer the formulation into in 100 ml, white, non transparent, plastic containers, with plastic screw caps.

3.2.6.2 Discussion

As with the anhydrous ointment, the formulation was first attempted by adding the sodium ascorbyl phosphate, together with the kojic acid dipalmitate to the other melted ingredients, whilst stirring continuously. After inclusion for stability testing, however, the stability test outcomes over a period of two months, similarly to the anhydrous ointment, showed poor uniformity of the sodium ascorbyl phosphate in the formulation. This formulation was subsequently excluded from the stability study. Improved uniformity of this formula was then also attempted by adding the sodium ascorbyl phosphate at the end of the procedure, after grinding it in a mortar and pestle and sieving it, as well as stirring it in while solidifying takes place. The resultant formulation appeared stable from visual examination for two days, and it was incorporated in the stability studies.

3.3 Conclusion

In this chapter the formulae, procedures to prepare various formulations, each containing 1% kojic acid dipalmitate and 0.5% sodium ascorbyl phosphate (as Stay-C® 50), and a short discussion on each formulation were given. A hydrous - and anhydrous gel, a hydrous cream and an anhydrous ointment, and a hydrous - and anhydrous stick were formulated. The aim was to formulate and identify, by visual examination over a period of two days, potentially stable formulations for inclusion in and evaluation during subsequent stability testing. A discussion of the stability test outcomes follow in chapter 5-7.

After inclusion for stability testing, the test outcomes over a period of two months of the initially formulated anhydrous gel, showed that both actives were unstable. An improved formulation was attempted for inclusion in subsequent stability studies.

The analytical test results of the anhydrous ointment and the anhydrous stick revealed poor uniformity of the sodium ascorbyl phosphate. After 2 months of stability testing, the anhydrous ointment and anhydrous stick were excluded from the stability programme and these formulation methods were adapted in order to attempt improved sodium ascorbyl phosphate uniformity. The texture and appearance of the improved formulations were satisfactory and they were therefore acceptable for inclusion in the stability study.

Formulation difficulties with the sodium ascorbyl phosphate were identified and addressed early in this study, and after 2 months of stability testing, improved formulations of the anhydrous gel, the anhydrous ointment and the anhydrous stick were attempted. The challenge was to incorporate a hydrophilic active, as sodium ascorbyl phosphate, in a completely anhydrous formulation, as it is insoluble in the anhydrous base. This further complicated the formulation of a homogeneous, anhydrous formulation, containing sodium ascorbyl phosphate. The best known methods were applied to ensure better uniformity of the sodium ascorbyl phosphate in the formulations, namely grinding and sieving, as well as stirring it in while solidifying takes place.

Less formulation problems were experienced with the kojic acid dipalmitate, as will be seen in chapter 5-7. However, since kojic acid dipalmitate is lipophilic and insoluble in a hydrous base, it was difficult to achieve kojic acid dipalmitate uniformity in some of the hydrous formulations.

The stability test methods used are discussed in chapter 4, whilst the test outcomes of all those formulations that passed visual examination criteria, are discussed in chapter 5-7.

CHAPTER 4

METHODS FOR STABILITY TESTING

4.1 Introduction

In chapter 3 the formulation of various cosmetic skin lightening products, as well as the outcomes of their potential stability through visual examination, for inclusion in stability testing, were discussed. This chapter gives a brief introduction to stability testing, with emphasis on the stability program used for testing the products that were formulated in this study. The various stability tests and stability test methods being used are also discussed.

Cosmetic products need to comply with criteria, such as texture, performance, safety and stability. Like other products, the stability of cosmetics must be matched to the expected period of usage by the consumer, as well as to the user's requirements (Mitsui, 1997:191).

The purpose of stability testing is to provide evidence on how the quality of a drug substance, or drug product, varies with time, under the influence of a variety of environmental factors, such as temperature, humidity and light, in order to recommend appropriate storage conditions (ICH Q1A (R2), 2003:1). The stability of pharmaceutical agents in quasi drug products is established on the basis of accelerated tests of the drug, and is subjected to the same kind of strict quality regulations as ethical drug products (Mitsui, 1997:197).

4.2 Stability program

Stability testing evaluates a product's ability to maintain its original aesthetic, physical and chemical characteristics under controlled conditions designed to accelerate ageing. Such testing provides an early indication of problems that may occur in formulations. This process assists the formulator to acknowledge if the formulation is stable, meaning it will not change significantly over time (Schueller & Romanowski, 1993:47).

Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage, and that are likely to influence quality, safety, and / or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes. Validated stability-indicating analytical procedures should be applied (ICH Q1A (R2), 2003:3).

According to ICH Q1A (R2) (2003:14), accelerated stability tests are designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of formal stability studies.

Accelerated stability tests were done on kojic acid dipalmitate and sodium ascorbyl phosphate containing products. These tests were done on the formulated hydrous - and anhydrous gels, the hydrous cream, the anhydrous ointment, and the hydrous - and anhydrous sticks. The stability of the hydrous and anhydrous products is compared and discussed in Chapters 5 – 7. The accelerated stability tests were done over a three-month period at three storage temperatures.

4.2.1 Concentrations

Each of the six formulated products contained 1% kojic acid dipalmitate and 0.5% sodium ascorbyl phosphate, as discussed in chapter 3.

4.2.2 Containers

Stability studies should be conducted on the drug substance packaged in a container closure system that simulates the packaging proposed for storage and distribution (ICH Q1A (R2), 2003:2). The hydrous - and anhydrous gels, the hydrous cream and the anhydrous ointment were stored in 100 ml, white, non transparent, plastic containers, with plastic screw caps. The hydrous - and anhydrous sticks were stored in white, non transparent, plastic lip balm containers (containing a maximum of ± 6 ml), with white plastic caps.

4.2.3 Storage conditions

A drug substance should be evaluated under storage conditions that test its thermal stability and its sensitivity to moisture (ICH Q1A (R2), 2003:3). Elevated temperature storage is critical, since the rate of chemical reactions roughly double for every ten °C increase in temperature. The potential drawback is that at high temperatures you may be forcing reactions to occur that would not have happened at all at lower temperatures (Schueller & Romanowski, 1993:50).

The stability program comprised the storage of all products at temperatures and humidities of 5°C, 25°C + 60% RH, and 40°C + 75% RH, over a period of the three months. Test intervals were at onset of the stability programme (initial), at one month, two months and three months.

4.2.4 Stability tests done

All tests were done using calibrated and / or validated tests apparatus, where appropriate. The stability tests done on the six formulated products are given in table 4.1.

Table 4.1 Stability tests conducted on the formulations

TEST	FORMULATIONS					
	HYDROUS GEL	ANHYDRS GEL	HYDROUS CREAM	ANHYDRS OINTMENT	HYDROUS STICK	ANHYDRS STICK
Kojic dipalmitate assay	√	√	√	√	√	√
Sodium ascorbyl phosphate assay	√	√	√	√	√	√
Kojic acid dipalmitate release	√	√	√	√		
Sodium ascorbyl phosphate release	√	√	√	√		
pH	√		√			
Relative density	√	√	√	√		
Visual assessment	√	√	√	√	√	√
Viscosity	√	√	√	√		
Spreadability			√	√		
Penetration			√	√		
Preservative assay			√		√	
Preservative efficacy			√		√	

4.3 Stability test methods

All tests were conducted under Good Laboratory Practice (GLP) conditions, in order to ensure the accuracy of the test results being generated over the stability test period. The tests were done as discussed in the methods described below.

4.3.1 HPLC analysis

High pressure liquid chromatography (HPLC), also called high-performance liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion-exchange processes, depending on the type of stationary phase being used (USP 28, 2005:2386).

Chromatographic solvents (acetonitrile, methanol and tetrahydrofuran) used were of HPLC grade and were obtained from BDH, Poole, UK. All other chemical reagents used for sample preparation were of analytical reagent grade and obtained from E. Merck (Merck chemicals (Pty) Ltd, South Africa), or from Sigma-Aldrich (Sigma-Aldrich S.A. (Pty) Ltd). The water used was obtained from a water purification system, consisting of an Elix 5 reverse osmosis purification system, feeding a Milli-Q, academic water purification system (Millipore, Bedford, MA).

The validation of the HPLC method for the kojic acid dipalmitate is shown in Appendix A. The validated HPLC method for sodium ascorbyl phosphate, being used in this study, was validated by van Rensburg (2004:128). HPLC validation of the preservative method being used was done by the Research Institute for Industrial Pharmacy, North-West University, Potchefstroom Campus (RIIP, 2002:1).

Both kojic acid dipalmitate and sodium ascorbyl phosphate assays were done on HPLC. The HPLC parameters used are shown under each discussion of analysis below.

Kojic acid dipalmitate assays were done separately from those of sodium ascorbyl phosphate, since after various attempts it was impossible to find chromatographic conditions that were able to elute the lipophilic kojic acid dipalmitate and the hydrophilic sodium ascorbyl phosphate simultaneously. A very apolar solvent, tetrahydrofuran (THF), was used to dissolve the anhydrous bases and to release, as well as dissolve the kojic acid dipalmitate. Unfortunately THF could not dissolve the polar sodium ascorbyl phosphate. Not only was the sodium ascorbyl phosphate insoluble in the THF, the THF also caused the sodium ascorbyl phosphate to deteriorate, thus also making simultaneous analysis of the two actives impossible. The preservative assays were done on the same samples which

were prepared for the sodium ascorbyl phosphate analysis, but only afterwards and by using a different method.

4.3.1.1 HPLC analysis of the kojic acid dipalmitate

As was mentioned, THF was used as a solvent for the lipophilic kojic acid dipalmitate, as well as the anhydrous bases. An apolar solvent was chosen in order to dissolve the anhydrous bases (the anhydrous ointment and anhydrous stick had difficulty dissolving in a methanol and water solvent). Good kojic acid dipalmitate recovery was possible, as the entire active ingredient could be released from the different bases, as they all completely dissolved in the THF. Since the samples completely dissolved in the THF, satisfactory kojic acid dipalmitate recovery was possible, releasing all of the active ingredient from the different bases.

Standard preparation of kojic acid dipalmitate

100 mg of kojic acid dipalmitate was accurately weighed and transferred into a 100 ml volumetric flask. THF was added and the standard shaken by hand until well mixed, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and then filled to volume with THF. 10 ml of the standard was accurately transferred into a 50 ml volumetric flask and filled to volume with THF. Each standard was transferred into an HPLC vial for HPLC analysis.

Sample preparation

Approximately 1 g of each formulation was accurately weighed into a tared 50 ml volumetric flask, using a clean syringe for each, with a rubber tube attached to the tip. 20 ml THF was then added and the samples sonicated for approximately 20 minutes, until completely dissolved. Repetitive sonication was alternated by repetitive shaking by hand, until the base was completely dissolved. The samples were allowed to cool to room temperature and filled to volume with THF. The samples were then transferred into an HPLC vial each for HPLC analysis.

HPLC parameters:

Column:	Luna C18 (2), 150 x 4.6 mm, 5μm (Phenomenex, Torrance, CA).
Mobile phase:	Tetrahydrofuran:acetonitrile:methanol:water:acetic acid (35:30:29:5:1).
Flow rate:	1.0 ml/min.
Injection volume:	10 μl.
Detection:	UV at 250 nm.
Retention time:	Approximately 6.8 minutes.
Solvent:	Tetrahydrofuran.
Standard:	100 mg kojic acid dipalmitate.
Stop time:	10 minutes.
Apparatus:	Agilent 1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent (Agilent, Palo Alto, CA).

The standard and sample solutions, containing kojic acid dipalmitate, were injected into the HPLC. Standard and sample peak areas were measured.

4.3.1.2 HPLC analysis of the sodium ascorbyl phosphate

Difficulty was experienced with the recovery of the hydrophilic sodium ascorbyl phosphate from the anhydrous formulations. This was probability because of the anhydrous base that had difficulty dissolving in the solvents being tried. Various possible solvents, including different methanol:water concentrations were examined, but with poor sodium ascorbyl phosphate recovery from the anhydrous base. Water could not be excluded as it was the solvent for the sodium ascorbyl phosphate. THF caused the sodium ascorbyl phosphate to deteriorate. Finally, extraction with chloroform and water dissolved the anhydrous bases, resulting in better sodium ascorbyl phosphate recovery.

Standard preparation of sodium ascorbyl phosphate

50 mg of sodium ascorbyl phosphate was accurately weighed and transferred into a 100 ml volumetric flask. 20 ml of Milli-Q water was added and the standard shaken by hand until well mixed, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and then filled to volume with Milli-Q water. 10 ml of the standard was accurately transferred into a 50 ml volumetric flask and filled to volume with Milli-Q water. Each standard was transferred into an HPLC vial for HPLC analysis.

Sample preparation

Chloroform extraction was done on the anhydrous gel, the anhydrous ointment and the anhydrous stick, as it was difficult to retrieve the hydrophilic sodium ascorbyl phosphate from the anhydrous bases. 15 ml of chloroform was used to dissolve the anhydrous bases, and 15 ml of Milli-Q water was added and extracted three consecutive times. The Milli-Q water with dissolved sodium ascorbyl phosphate was then transferred into a 50 ml volumetric flask and filled to volume with Milli-Q water.

15 ml of methanol was used to dissolve the hydrous cream and the hydrous stick bases in a 50 ml volumetric flask each. 20 ml of Milli-Q water was added in order to dissolve the sodium ascorbyl phosphate, and filled to volume with Milli-Q water.

The hydrous gel was dissolved using Milli-Q water only, and filled to volume in a 50 ml volumetric flask.

HPLC parameters:

Column:	Luna C18 (2),150 x 4.6 mm, 5μm (Phenomenex, Torrance, CA).
Mobile phase:	Acetonitrile:water with 0.002 M tetrabutyl ammonium iodide (0.738 g/l), 0.005 M KH2PO4 (0.68 g/l) pH adjusted to 5.0 ± 0.05 with phosphoric acid or ammonium hydroxide 12/88.
Flow rate:	1.0 ml/min.
Injection volume:	10 μl.

Detection: UV at 255 nm.

Retention time: Approximately 3 minutes.

Solvent: Purified water.

Standard: 50 mg sodium ascorbyl phosphate.

Stop time: 8 minutes.

Apparatus: Agilent 1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent (Agilent, Palo Alto, CA).

4.3.1.3 HPLC analysis of the preservatives

After the sodium ascorbyl phosphate assay was completed, the same samples were used for the preservative assay after setting up the HPLC.

The HPLC parameters:

Column: Luna C18(2) 150 x 4.6 mm, 5 μm (Phenomenex, Torrance, CA).

Mobile phase: Methanol:water with 0.5% acetic acid 65:35.

Flow rate: 1.5 ml/min.

Injection volume: 20 μl.

Detection: UV at 255 nm.

Retention time: Approximately 2.1 and 6.8 minutes for methyl- and propyl parabens respectively.

Solvent: Purified water.

Standard: 30 mg methyl paraben and 20 mg propyl paraben.

Stop time: 10 minutes.

Apparatus: Agilent 1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent (Agilent, Palo Alto, CA).

Standard preparation of sodium ascorbyl phosphate

30 mg of methyl paraben and 20 mg of propyl paraben were accurately weighed and transferred into a 100 ml volumetric flask. Milli-Q water was added and the standard was shaken by hand until well mixed, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and then filled to volume with Milli-Q water. 10 ml of the standard was accurately transferred into a 50 ml volumetric flask and filled to volume with Milli-Q water. The standard was then transferred into an HPLC vial for HPLC analysis.

4.3.2 Membrane release

The release rate of kojic acid dipalmitate and sodium ascorbyl phosphate from the formulations were determined by means of the VanKel enhancer cell unit. As kojic acid dipalmitate is hydrophobic and sodium ascorbyl is hydrophilic, it was difficult to find a solvent that would release both actives simultaneously, and two separate release tests had to be done.

The release study was carried out with the enhancer cell unit, which was used on the VanKel 700 (Varian, North Carolina) dissolution apparatus. This apparatus used is calibrated semi-annually. Figure 4.1 illustrates the enhancer cell (Rege *et al.*, 1998:1227).

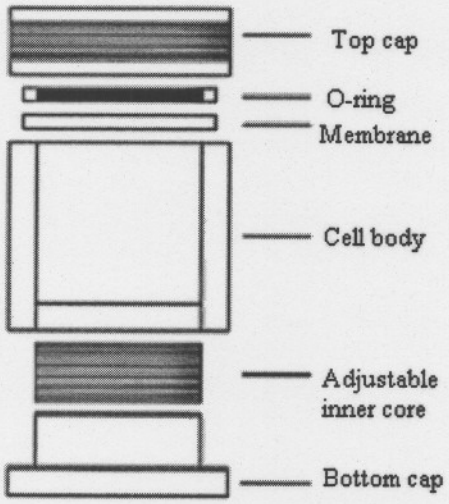

Figure 4.1 The enhancer cell (Rege *et al.*, 1998:1227).

For each release experiment, the test samples were carefully transferred into six enhancer cells (approximately 3 g of sample per enhancer cell). Care was taken to ensure that no air bubbles were present. Each enhancer cell was wiped clean, to ensure that no sample would penetrate, except through the membrane. Then each enhancer cell was covered with a cellulose acetate membrane, with a 0.45 μm pore size.

200 ml vessels were used and each filled with 190 ml of release medium. The medium was heated to 32 ± 0.5°C. When the desired temperature was reached, the enhancer cells were carefully descended into the vessels, at fixed time intervals. The paddles rotated at 100 rpm. Each release experiment was carried out for 360 minutes, with 200 μl withdrawals at 30, 60, 120, 240 and 360 minutes. Figure 4.2 illustrates the enhancer cell assembly (Rege *et al.*, 1998:1227).

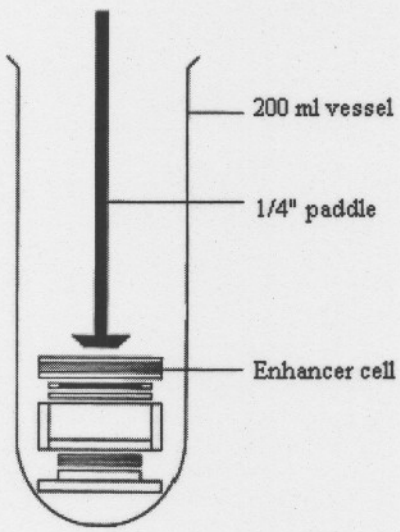

Figure 4.2 The enhancer cell assembly (Rege *et al.*, 1998:1227).

A standard sample was prepared for each release experiment. The samples and standards were each transferred into HPLC vials (vial inserts were used in the samples) HPLC analysis, according to paragraph 4.3.1.

The release medium for the kojic acid dipalmitate was 60% THF (40% being Milli-Q water). For the preparation of the standard, 100 mg of kojic acid dipalmitate was accurately weighed and transferred into a 100 ml volumetric flask. 60% of THF was added

and the standard was shaken by hand, until well mixed, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and then filled to volume with 60% of THF. 2 ml of the standard was accurately transferred into a 50 ml volumetric flask and filled to volume with 60% THF.

Purified water was used as release medium for the sodium ascorbyl phosphate. For the preparation of the standard, 50 mg of sodium ascorbyl phosphate was accurately weighed and transferred into a 100 ml volumetric flask. Milli-Q water was then added and the standard shaken by hand until mixed, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and filled to volume with Milli-Q water. 2 ml of the standard was accurately transferred into a 50 ml volumetric flask and filled to volume with Milli-Q water.

4.3.3 pH

The United States Pharmacopoeia (USP 28) (2005:2455) defines pH as the value given by a suitable, properly standardised, potentiometric instrument (pH meter), capable of reproducing pH values to ± 0.02 pH units, using an indicator electrode, sensitive to hydrogen-ion activity. As no hydrogen-ion activity is present in anhydrous formulations, only the pH of the hydrous gel and hydrous cream could be measured. A Mettler Toledo Seveneasy (Mettler Toledo, Switzerland) pH meter, which was calibrated with buffer solutions of pH 4 and 7 immediately prior to measurements, was used to measure the pH.

4.3.4 Relative density

The relative densities of the hydrous gel, the anhydrous gel, the hydrous cream and the anhydrous ointment were measured. The method used comprised a clean dry glass container which was weighed. Ten millilitres of water was accurately transferred into the container and weighed. A mark was made at the meniscus of the water. The water was then removed from the container. The container was dried with laboratory paper and was then filled with sample to the mark made at the meniscus of the water, and weighed.

The following formula was used in the calculation:

$$Density\ (g/ml) = \frac{\text{Mass of container + sample} - \text{Mass of empty container}}{\text{Mass of container + water} - \text{Mass of empty container}}$$

4.3.5 Physical assessment

A physical assessment of each stored trial batch was carried out initially and there after once a month. The physical appearance of each product was examined carefully and critically, and compared to the initial results. Any change in colour, odour, texture and skin feel was noted. According to ICH A1A (R2) (2003:14), accelerated test results are not always predictive of physical changes.

4.3.6 Viscosity

The USP 28 (2005:2510) states that viscosity is a property of fluids that are closely related to the resistance to flow. Viscosity is defined in terms of the force required to move one plane surface continuously past another under specific steady state conditions, when the space between is filled by the liquid in question. Viscosity is also defined as the sheer stress divided by the rate of shear strain (USP 28, 2005:2510).

The viscosity was determined on a Brookfield Model DV –II+ (Brookfield, United States of America) viscometer. The viscosities of the hydrous gel, the anhydrous gel, the hydrous cream and the anhydrous ointment were measured. The operator calibrates the viscometer every three months. The small sample adapter was used on the hydrous - and anhydrous gels. Different spindles were used, according to the products' assumed viscosity. The viscosity parameters are listed in table 4.2.

Table 4.2 Viscosity parameters

	Spindle	Rpm	Temperature	Time (minutes)
Hydrous gel	SC 4-25	0-60	25°C	20
Anhydrous gel	SC 4-25	0-60	25°C	20
Hydrous cream	RV 6	10	25°C	2
Anhydrous ointment	RV 7	10	25°C	2

The samples were transferred into 200 ml beakers and covered with parafilm, and left overnight to settle and to ensure that no air bubbles were present. The viscosity was measured in the morning.

4.3.7 Spreadability

The spreadabilities of the hydrous lotion and the anhydrous ointment were measured. The apparatus used consisted of two glass plates. Each sample was transferred into a clean syringe, fitted with a clean rubber tube (6 mm in diameter) to the syringe tip. The glass plate was put on the balance and tared (reading zero). Approximately 0.25 g of sample was squeezed onto the plate, and the mass accurately recorded. The glass plate was then placed on a level surface. The other glass plate was put onto the sample, after which a 100 g brass weight was added on top. It was left for 60 seconds. The longest sample diameter was measured using a Vernier caliper. Each sample was tested in duplicate.

4.3.8 Penetration

Penetration was done on the hydrous lotion and the anhydrous ointment. Penetration measures the consistency of the sample. It was done using a calibrated penetrometer. The penetration of the samples was measured three times a month, with twenty-four hour lapses between measurements, over a three-month period.

The penetrometer consists of a stand and a penetrating object, a level to check that the base is level, and a scale having 0.1 mm increments, to show the depth of penetration. All samples were transferred into a 500 ml plastic container each and the sample surfaces levelled. The samples were left for twenty-four hours, to stabilise and to ensure that no air bubbles were present. The following morning, the first measurements were taken, after

twenty-four hours the second, and after another twenty-four hours the third. After each set of measurements the sample surfaces were levelled.

4.3.9 Preservative efficacy

As in 1997, as stated by Mitsui (1997:205), there still exists no standard procedure in the regulations for the testing of the effectiveness of preservatives included in cosmetics, to prevent secondary contamination. In Japan, each cosmetic company carries out preservative efficacy tests individually on the basis of the USP.

The intention was to do preservative efficacy testing on the hydrous cream and on the hydrous stick, at onset and after 3 months. The samples were sent to a company for testing early on in the study. The samples were not returned in time, due to tremendous workload of the specific company. Only the initial results were received and are given in chapters 6 and 7.

4.4 Conclusion

A brief introduction to stability testing was given in this chapter, with the emphasis on the stability program used for testing the products being formulated in this study. The various stability tests and stability test methods were discussed in detail. The methods used were all validated.

The results of the tests being performed on the formulations, as described in this chapter, will be discussed and represented graphically in chapters 5-7. The hydrous - and anhydrous formulations are compared in each chapter. Chapter 5 compares the hydrous - and anhydrous gel, chapter 6 compares the hydrous lotion and anhydrous ointment, and chapter 7 compares the hydrous - and anhydrous sticks.

CHAPTER 5

HYDROUS - AND ANHYDROUS GELS RESULTS AND DISCUSSION

5.1 Introduction

The methods used for stability testing of the products being formulated in this study, were discussed in chapter 4. The following parameters of the formulated gels were evaluated in this chapter: kojic acid dipalmitate assay, sodium ascorbyl phosphate assay, pH, relative density, visual appearance, viscosity, kojic acid dipalmitate release rate and sodium ascorbyl phosphate release rate. Very low assay results (below) are not reported and are represented by a "-" in the tables, unless mentioned otherwise. Open spaces in the table represent time and temperature interval which was not part of the stability test period, as mentioned in chapter 4. All tests were performed under GLP conditions, as was mentioned in chapter 4.

The USP 28 (2005:2704) defines gels as semi-solid systems, consisting of either suspensions made up of small inorganic particles, or large organic molecules, interpenetrated by a liquid. Mitsui (1997:351) describes gels as a type of base, which produces a uniform external appearance, ranging from transparent to semi-transparent and giving a moist feeling. Aqueous gels have the characteristic of giving a moist and light feeling, and are often used during the summer, or by people with an oily skin. Anhydrous gels supply oil to the skin and are popular during winter and for people with a dry skin, because of their moisturising properties.

5.2 Kojic acid dipalmitate assay

The concentration of kojic acid dipalmitate in the hydrous - and anhydrous gels was determined at the onset of stability (initial), and at one -, two - and three-month intervals.

5.2.1 Results

The results of the hydrous gel are given in table 5.1, and those of the anhydrous gel in table 5.2.

Table 5.1 Kojic acid dipalmitate assay of the hydrous gel

Time	Temperature		
	5°C	25°C	40°C
Initial		103.7	
Month 1	99.2	-	-
Month 2		100.2	92.7
Month 3		90.7	77.4

Table 5.2 Kojic acid dipalmitate assay of the anhydrous gel

Time	Temperature		
	5°C	25°C	40°C
Initial		90.5	
Month 1	98.6	-	92.6
Month 2		100.6	103.6
Month 3		99.9	99.8

5.2.2 Discussion

A 5% change in assay from its initial value is considered as a significant change (Harmonised Tripartite Guideline, 2003:9).

From the stability test outcomes, the formulation of the hydrous gel appeared unstable with regards to kojic acid dipalmitate, especially at elevated temperatures. The results further suggest that the formulation was not mixed homogeneously. Assays were done numerously, but continuously gave inconsistent results. Kojic acid dipalmitate is lipophilic and thus insoluble in the hydrous gel formulation and therefore it is difficult to assure its uniformity in this formulation. More efficient mixing methods and / or homogenising of the formulation are suggested in future research and development of hydrous formulations, containing kojic acid dipalmitate. A smaller kojic acid dipalmitate particle size may also result in a more homogenous formulation. Using kojic acid, which is hydrophilic, in the hydrous formulations would also assure solubility and thus better uniformity.

The initial value of the kojic acid dipalmitate content in the anhydrous gel may suggest a formulation problem. The initial assay was also done numerously, but results remained low. If the initial result is not taken into consideration, the kojic acid dipalmitate content remained fairly stable in the anhydrous gel formulation.

In chapter 8 the formulation problems are discussed and possible solutions are provided.

5.3 Sodium ascorbyl phosphate assay

The concentration of sodium ascorbyl phosphate in the hydrous - and anhydrous gels was determined at initial and at one, two and three months.

5.3.1 Results

The results of the hydrous gel are given in table 5.3, and those of the anhydrous gel in table 5.4.

Table 5.3 Sodium ascorbyl phosphate assay of the hydrous gel

Time	Temperature		
	5°C	25°C	40°C
Initial		94.5	
Month 1	102.9	90.5	90
Month 2		95.4	94.5
Month 3		80.8	98

Table 5.4 Sodium ascorbyl phosphate assay of the anhydrous gel

Time	Temperature		
	5°C	25°C	40°C
Initial		79.9	
Month 1	80.9	73.1	-
Month 2		80.8	81.1
Month 3		110.4	91

5.3.2 Discussion

The hydrous gel appeared stable with regards to sodium ascorbyl phosphate. The contradictory results of month 1 at 5°C and of month 3 at 25°C + 60% RH, may be due to the formulation not being homogeneously mixed; a longer and more efficient mixing method is thus recommended. A smaller sodium ascorbyl phosphate particle size may result in a more homogeneous formulation.

Evaluating and concluding on the stability of the anhydrous gel was difficult, as it was severely hindered by formulation difficulties being experienced, and a few suggestions are made. The low results obtained from the anhydrous gel possibly signify a formulation problem. The conclusion was made that the hydrophilic sodium ascorbyl phosphate is insoluble in the anhydrous formulation, hence creating uniformity problems. The HPLC method may also not have been tolerable for this formulation. Various methods were examined (as was discussed in chapter 4), and the method used finally gave the best results. The assays were repeated continuously, and the average results are given in table 5.4.

In chapter 8 the formulation problems are discussed and possible solutions are provided, as well as possible reasons for the questionable increase in the sodium ascorbyl content of the anhydrous gel at 3 months at 25°C + 60% RH are given.

5.4 pH

The pH of the hydrous gel was determined initially and over the three-month period. Roche Vitamins (2005:2) states that Stay-C® 50 is stable in aqueous solutions at a pH of 7 and higher. To ensure the stability of the sodium ascorbyl phosphate in formulation, the pH must be kept at this level. Kojic acid's stability is decreased rapidly at a neutral pH, whilst it is optimally stabilised at a low pH, especially at pH 4. Kojic acid dipalmitate is stable within a pH range of 4-9 (Uchem, 2004), which greatly enhances the formulation possibilities of stable cosmetics.

5.4.1 Results

The results of the hydrous gel are given in table 5.5.

Table 5.5 The pH of the hydrous gel measured over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		9.94		
25°C + 60% RH	9.70	9.89	9.74	9.71
40°C + 75% RH		9.48	9.18	9.02

5.4.2 Discussion

The pH of the hydrous gel was above pH 7, thus ensuring the stability of the sodium ascorbyl phosphate, as recommended by Roche Vitamins (2005:2). Kojic acid dipalmitate is stable in the pH range of 4-9 (Uchem, 2004); the pH of this formulation is a fragment higher, but remains in the vicinity of pH 9 and would therefore ensure the stability of the kojic acid dipalmitate. The pH of this formulation remained at this level over the three-month period (table 5.5). The pH did show a slight decrease, when stored at high temperatures (40°C +75% RH), but it remained at ±0.5 of the initial value, showing excellent stability of the formulation. It appears that maximum stability of the hydrous gel will be ensured for a longer period, if it is stored at a temperature below 25°C.

5.5 Relative density

The relative densities of the hydrous - and anhydrous gels were determined initially, and over the three-month period.

5.5.1 Results

The results of the hydrous gel are given in table 5.6, and those of the anhydrous gel in table table 5.7.

Table 5.6 The relative density of the hydrous gel measured over three months

STORAGE CONDITION	(g/ml)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		0.951		
25°C + 60% RH	0.985	0.944	1.004	1.046
40°C + 75% RH		0.937	0.975	1.011

Table 5.7 The relative density of the anhydrous gel measured over three months

STORAGE CONDITION	(g/ml)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		1.116		
25°C + 60% RH	1.093	1.138	1.112	1.127
40°C + 75% RH		1.191	1.092	1.108

5.5.2 Discussion

There were no significant changes in the relative density of the hydrous gel (table 5.6), nor of the anhydrous gel (table 5.7). Temperature, humidity and pH did not have a significant influence on the relative density of the gels. The relative densities of the hydrous - and anhydrous gels in comparison were in the same range.

5.6 Physical assessment

The physical assessments of the hydrous - and anhydrous gels were carried out initially and over the three-month period.

5.6.1 Results

The results of the hydrous gel are given in table 5.8, and those of the anhydrous gel in table table 5.9.

Table 5.8 The physical assessment of the hydrous gel over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: Colourless	No change		
	Transparent	No change		
	Consistency: Smooth, medium thick gel. Air bubbles	No change		
	Not too oily	No change		
	Not too hydrous	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: Colourless	No change	No change	No change
	Transparent	No change	No change	No change
	Consistency: Smooth, medium thick gel. Air bubbles	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: Colourless	Very light yellow	No change	Light yellow
	Transparent	No change	No change	No change
	Consistency: Smooth, medium thick gel. Air bubbles	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change

Table 5.9 The physical assessment of the anhydrous gel over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: Colourless	No change		
	Semi-transparent	No change		
	Consistency: Soft gel	No change		
	Not too oily	No change		
	Not too hydrous	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: Colourless	No change	No change	Very light yellow
	Semi-transparent	No change	No change	No change
	Consistency: Soft gel	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: Colourless	No change	Very light yellow	Light yellow
	Semi-transparent	No change	No change	No change
	Consistency: Soft gel	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change

5.6.2 Discussion

The changes in appearance of the hydrous gel were mainly a slight change in colour, which can be attributed to the degradation of the kojic acid dipalmitate, especially when stored at high temperatures (table 5.8).

There was no significant change in the appearance of the anhydrous gel. Only a slight change of colour was observed, which can be attributed to the degradation of the kojic acid dipalmitate, especially when stored at high temperatures (table 5.9), since a slight decrease in active can result in remarkable colour change.

When compared, both the hydrous and anhydrous gels' physical appearances were relatively stable, except for changes in colour. Maximum stability of the hydrous gel appear to be ensured for a longer period, if it is stored at a temperature below 25°C.

5.7 Viscosity

The viscosities of the hydrous - and anhydrous gels were determined initially and over the three-month period.

5.7.1 Results

The results of the hydrous gel are given in table 5.10, and those of the anhydrous gel in table 5.11.

Table 5.10 The viscosity of the hydrous gel measured over three months

STORAGE CONDITION	VISCOSITY, cP			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		6571		
25°C + 60% RH	7168	11947	10667	6997
40°C + 75% RH		8363	12715	9130.5

Table 5.11 The viscosity of the anhydrous gel measured over three months

STORAGE CONDITION	VISCOSITY, cP			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		5462		
25°C + 60% RH	5717	5120	4722	3670
40°C + 75% RH		4352	4267	2134

5.7.2 Discussion

The viscosity values fluctuated marginally. The skin feel and spreadability stayed the same, thus can be concluded that the fluctuation was insignificant.

5.8 Kojic acid dipalmitate release

The release rates of the kojic acid dipalmitate of the hydrous - and anhydrous gels were determined by dissolution, at initial and after three months.

5.8.1 Results

The results of the hydrous gel are given in figure 5.1 and those of the anhydrous gel in table figure 5.2. See appendix B for the test results used in figure 5.1 and 5 .2.

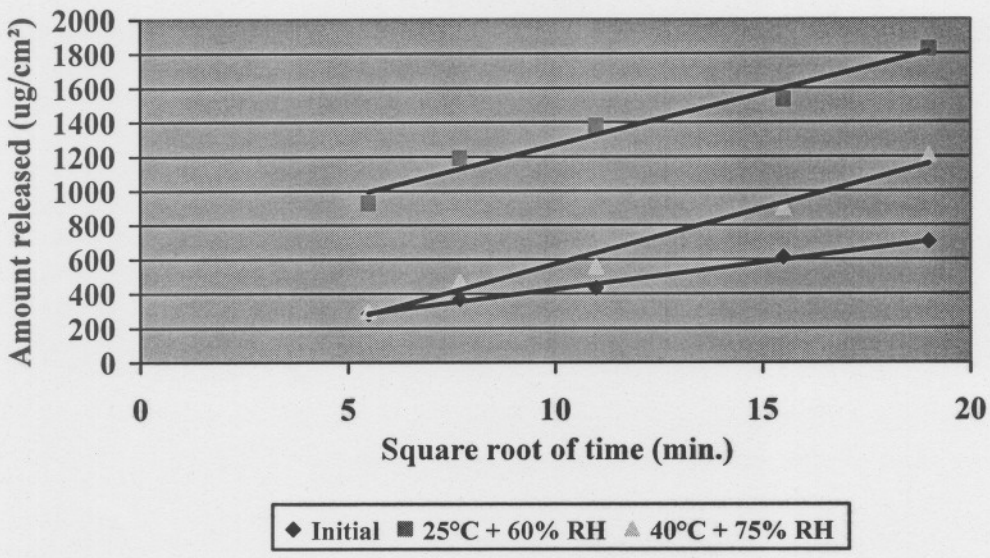

Figure 5.1 Release rate of the kojic acid dipalmitate from the hydrous gel at initial, at 3 months (25°C + 60% RH) and at 3 months (40°C + 60% RH).

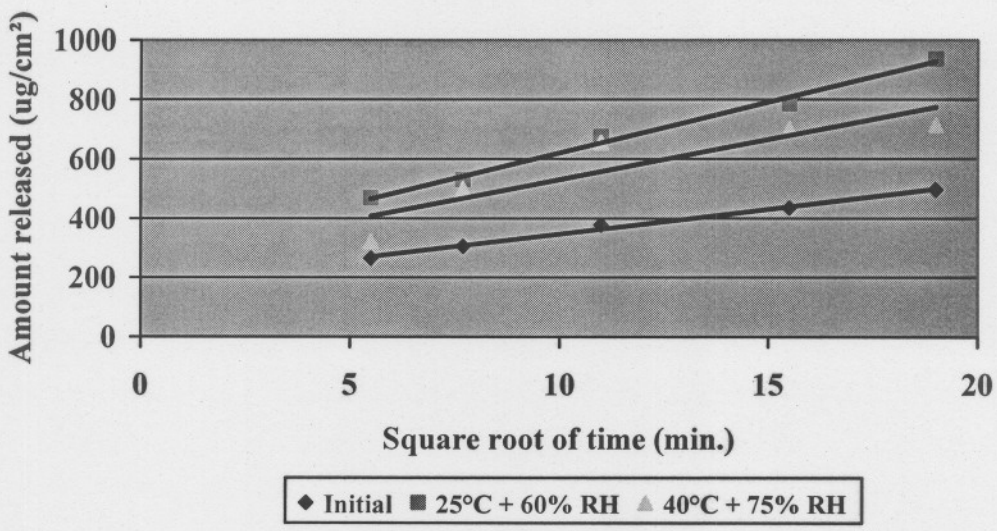

Figure 5.2 Release rate of the kojic acid dipalmitate from the anhydrous gel at initial, at 3 months (25°C + 60% RH) and at 3 months (40°C + 75% RH).

5.8.2 Discussion

The theory of release rate is that the amount released per unit area against square root of time should produce a straight line. The hydrous gel complies with this theory. The initial value of the kojic acid dipalmitate release rate is lower than the kojic acid dipalmitate release rate after 3 months. This may be due to an air bubble present under the membrane surface. Care was taken to remove as many air bubbles as possible, inevitably very small air bubbles still remained. As time passed during this 6 hour test, these small air bubbles moved to the surface, accumulated and formed a big air bubble which considerably decreased the membrane surface.

The anhydrous gel also complies with theory and produced a straight line. The initial release rate is slightly lower that that after 3 months. This might be due to experimental error or the presence of air bubbles.

Kojic acid dipalmitate was released to a great extend from both formulations. There is a relationship between release rate and efficacy of the product. Since the kojic acid dipalmitate was sufficiently released from the hydrous - and anhydrous gels, the active should be available to act on the skin.

5.9 Sodium ascorbyl phosphate release rate

The release rates of the sodium ascorbyl phosphate of the hydrous - and anhydrous gels were determined by dissolution, at initial and after three months.

5.9.1 Results

The results of the hydrous gel are given in figure 5.3 and those of the anhydrous gel in table figure 5.4. See appendix B for the test results used in figure 5.3 and 5.4.

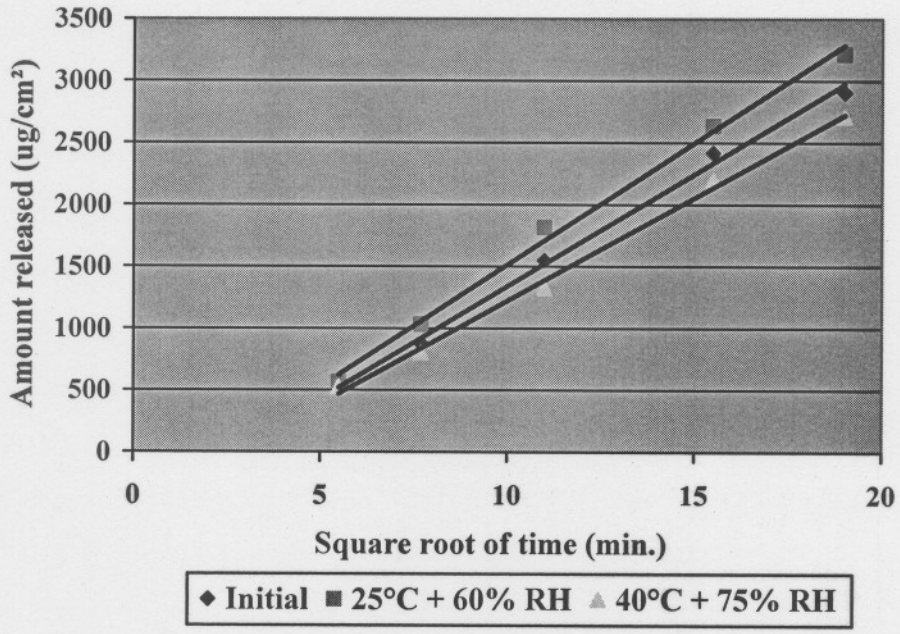

Figure 5.3 Release rate of the sodium ascorbyl phosphate from the hydrous gel initial, at 3 months (25°C + 60% RH) and at 3 months (40°C + 75% RH).

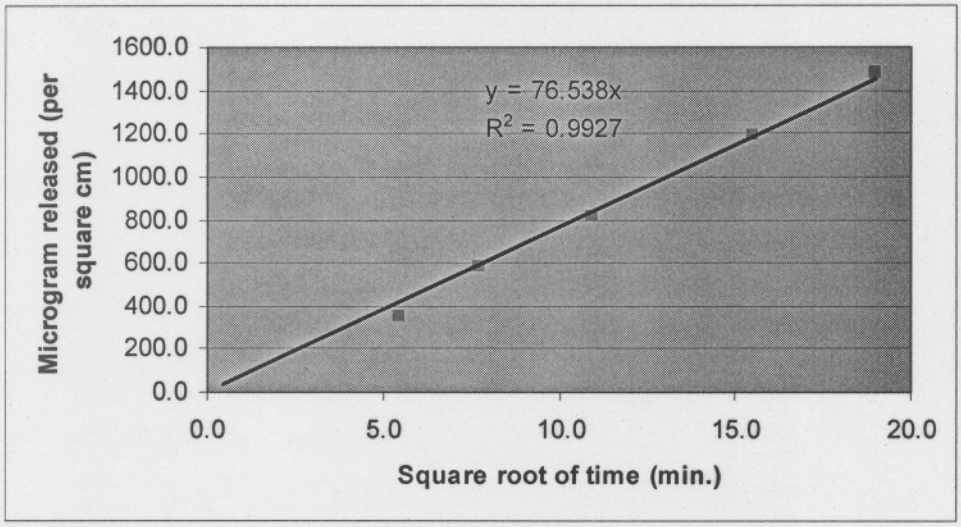

Figure 5.4 Release rate of the sodium ascorbyl phosphate from the anhydrous gel at initial.

5.9.2 Discussion

The hydrous gel complies with the straight line theory (paragraph 5.8.2 above). There was no significant change in release rate of the sodium ascorbyl phosphate from the hydrous gel over the three months of testing, except for a slight decrease in the rate at high temperatures. It can be concluded that since the active is released from the hydrous gel formulation, it will be free to act on the skin.

The anhydrous gel also complies with the straight line theory. The sodium ascorbyl phosphate content of the anhydrous gel was determined at 3 months (25°C + 60% RH and 40°C + 75% RH). Due to a problem with the HPLC instrument, the data could not be generated and was hence not included.

5.10 Conclusion

In this chapter the test results of the hydrous - and anhydrous gels were presented and discussed.

The hydrous gel formulation was found unstable with regards to kojic acid dipalmitate, and the results also suggested that the formulation was not homogeneous. This was probably due to the lipophilic kojic acid dipalmitate being insoluble in a hydrous formulation. The kojic acid dipalmitate content of the anhydrous gel remained fairly stable, except for the initial values.

The hydrous gel appeared to be stable with regards to sodium ascorbyl phosphate. Evaluating and concluding on the stability of the anhydrous gel was difficult, as it was severely hindered by formulation difficulties being experienced. Some contradictory results found in the assays might point to a formulation problem. The conclusion was made that as sodium ascorbyl phosphate is hydrophilic it is insoluble in the anhydrous formulation, creating uniformity problems. A smaller particle size of sodium ascorbyl phosphate is inevitable. Longer and more efficient mixing methods may also address the formulation problems experienced.

The pH of the hydrous gel was found stable and favourable with regards to sodium ascorbyl phosphate, as well as kojic acid dipalmitate. The viscosity of both formulations remained stable throughout the stability test period. Relative density also did not significantly change over the three-month test period. Physical examinations showed a slight change in colour in both the hydrous - and anhydrous gel formulations. This may have been caused by a slight decrease in kojic acid dipalmitate, or of the ester (kojic acid dipalmitate) being converted to kojic acid, which is known to cause cosmetic products to turn yellowish (chapter 1). A slight decrease in sodium ascorbyl phosphate could also be responsible for the slight change in colour. Excellent release rate results of the kojic acid dipalmitate and sodium ascorbyl phosphate were found from both the formulations. The actives will thus be available to act on the skin.

Except for the hydrous gel formulation, which does not comply with regards to stability of the kojic acid dipalmitate, the hydrous gel proved to be a stable formulation. The anhydrous formulation appeared to be stable with regards to kojic acid dipalmitate. However, no definite conclusion to the sodium ascorbyl phosphate stability in the formulations could be made, due to formulation difficulties. Some contradictory results may suggest a formulation problem; which could be addressed by using a smaller particle

size of sodium ascorbyl phosphate and by using longer and more efficient mixing methods. Still it would remain difficult to incorporate a hydrophilic active in an anhydrous formulation.

In chapter 8 the formulation problems are discussed and possible solutions are provided, as well as possible reasons for the questionable increase in the sodium ascorbyl content of the anhydrous gel at 3 months at 25°C + 60% RH are given.

CHAPTER 6

HYDROUS LOTION AND ANHYDROUS OINTMENT RESULTS AND DISCUSSION

6.1 Introduction

The stability test results of the hydrous - and anhydrous gels were discussed in the previous chapter. The parameters of the formulated gels that are evaluated and discussed in this chapter include the kojic acid dipalmitate assay, sodium ascorbyl assay, preservative assay (only on the hydrous cream), pH, relative density, visual appearance, viscosity, preservative efficacy (only on the hydrous cream), penetration, spreadability, kojic acid dipalmitate release rate and sodium ascorbyl phosphate release rate. Very low assay results (below) are not reported and are represented by a "-" in the test result tables, unless mentioned otherwise. Open spaces in the table represent time and temperature intervals which were not part of the stability test period, as mentioned in chapter 4. All tests were performed under GLP conditions.

A cream is a type of emulsion in which two liquids that do not mix together, such as water and oil, are made into a stable dispersion, by making one the dispersion phase and dispersing it through the other which acts as the dispersion medium. The main functions of creams are 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 USP 28 (2005:2705) defines an ointment as semi-solid preparations intended for external application to the skin. The anhydrous ointment that was formulated in this study consisted of an absorption base, in accordance with the USP 28 (2005:2705), because of its consistence of hydrophilic petrolatum and lanolin.

6.2 Kojic acid dipalmitate assay

The concentration of kojic acid dipalmitate in the hydrous cream and anhydrous ointment was determined at the onset of stability (initial), and at one -, two - and three-month intervals.

6.2.1 Results

The results of the hydrous cream are given in table 6.1, and those of the anhydrous ointment in table 6.2.

Table 6.1 Kojic acid dipalmitate assay of the hydrous cream

Time	Temperature		
	5°C	25°C	40°C
Initial		99.1	
Month 1	101.7	97.2	98.4
Month 2		89.8	94.1
Month 3		90.8	94.5

Table 6.2 Kojic acid dipalmitate assay of the anhydrous ointment

Time	Temperature		
	5°C	25°C	40°C
Initial		100.9	
Month 1	99.0	100.7	100.0
Month 2		99.0	91.3
Month 3		98.9	94.5

6.2.2 Discussion

A 5% change in assay from its initial value is considered as a significant change (ICH Harmonised Tripartite Guideline, 2003:9). The hydrous cream showed a decrease of more than 5% from initial value after two months already. The increase in the percentage of kojic acid dipalmitate at 40°C + 75% RH, after two and three months, may have been as a result of water evaporating from the formulation, and thus an increase in the percentage of

the active due to a decrease in the formulation base. The hydrous cream thus appeared unstable with regards to kojic acid dipalmitate.

High temperatures (40°C + 75% RH) resulted in a more than 5% decrease in active in the anhydrous ointment. At storage conditions of 25°C + 60% RH and 5°C, the anhydrous ointment remained stable during the three-month study. Storing the anhydrous ointment at temperatures below 25°C would ensure its maximum stability. The anhydrous ointment appeared to be significantly more stable, when compared to the hydrous cream.

The anhydrous ointment shows excellent stability at 25°C + 60% RH. Elevated temperature storage is critical, since the rate of chemical reactions roughly double for every ten °C increase in temperature. The potential drawback is that at high temperatures you may be forcing reactions to occur that would not have happened at all at lower temperatures (Schueller & Romanowski, 1993:50). This might be an explanation for the low values at 40°C + 75% RH.

6.3 Sodium ascorbyl phosphate assay

The concentration of sodium ascorbyl phosphate in the hydrous cream and anhydrous ointment was determined at initial and at one, two and three months.

6.3.1 Results

The results of the hydrous cream are given in table 6.3, and those of the anhydrous ointment in table 6.4.

Table 6.3 Sodium ascorbyl phosphate assay of the hydrous cream

Time	Temperature		
	5°C	25°C	40°C
Initial		94.6	
Month 1	98.3	101.6	93.9
Month 2		96.3	71.4
Month 3		95.4	110.7

Table 6.4 Sodium ascorbyl phosphate assay of the anhydrous ointment

Time	Temperature		
	5°C	25°C	40°C
Initial		103.4	
Month 1	108.3	100.7	102.0
Month 2		104.4	113.0
Month 3		97.4	92.6

6.3.2 Discussion

It is difficult to conclude if the hydrous cream formulation was stable with regards to sodium ascorbyl phosphate. A substantial decrease in active was found at 2 months (40°C + 75% RH), and the contradictory increase in active at 3 months (40°C + 75% RH) may suggest formulation problems. The hydrous cream's uniformity may be improved by using sodium ascorbyl phosphate having a smaller particle size.

The anhydrous formulation showed good stability, but at 3 months the active showed a decrease. A formulation problem may explain some of the contradictory results. It appeared as if the anhydrous ointment was unstable with regards to sodium ascorbyl phosphate. The sodium ascorbyl phosphate is hydrophilic and thus insoluble in the anhydrous ointment and complicates a uniform formulation.

It is difficult to conclude whether these formulations are stable or not, due to formulation problems. Some contradictory results confirm this. Chapter 8 offers possible explanations for the formulation problems encountered and suggestions for improvements.

6.4 Preservatives assays

The assays of the methyl - and propyl parabens in the hydrous cream were determined at initial and at one, two and three months.

6.4.1 Results

The results of the assays of the preservatives in the hydrous cream are given in tables 6.5 and 6.6.

Table 6.5 Methyl paraben assay of the hydrous cream

Time	Temperature		
	5°C	25°C	40°C
Initial		103.0	
Month 1	95.1	96.0	102.7
Month 2		72.1	70.4
Month 3		89.4	69.9

Table 6.6 Propyl paraben assay of the hydrous cream

Time	Temperature		
	5°C	25°C	40°C
Initial		104.9	
Month 1	102.7	105.4	97.4
Month 2		102.7	97.1
Month 3		106.6	100.9

6.4.2 Discussion

The methyl paraben assay outcomes decreased with time. It is concluded that this preservative does not comply with the formulation. It is suggested that another preservative be used in future preparation of the hydrous cream formulation. Some contradictory results also may indicate a formulation problem. See chapter 8 for possible suggestions for improvement.

The propyl paraben remained more stable than the methyl paraben. The propyl paraben showed a decrease among the higher storage conditions. Other preservatives in future formulation of the hydrous cream should be considered.

6.5 pH

The pH of the hydrous cream was determined at onset and monthly over the three month period. Roche Vitamins (2005:2) states that Stay-C® 50 is stable in aqueous solutions at a pH of 7 and higher. To ensure the stability of the sodium ascorbyl phosphate in the formulated product, the pH must be kept at this level. Kojic acid's stability is decreased rapidly at a neutral pH, and is optimally stabilised at a low pH, especially at pH 4. Kojic acid dipalmitate is stable within a pH range of 4-9 (Uchem, 2004), which greatly enhances the formulation possibilities of stable cosmetics.

6.5.1 Results

The results of the hydrous cream are given in table 6.7.

Table 6.7 The pH of the hydrous cream measured over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		7.95		
25°C + 60% RH	8.01	7.85	7.79	7.69
40°C + 75% RH		7.70	7.47	7.30

6.5.2 Discussion

The pH of the hydrous cream was above pH 7, thus ensuring the stability of the sodium ascorbyl phosphate, as recommended by Roche Vitamins (2005:2). Kojic acid dipalmitate is stable in the pH range of 4-9 (Uchem, 2004), the pH of this formulation would therefore ensure the stability of the kojic acid dipalmitate. The formulation's pH was maintained at this level. The pH remained stable during the three-month stability period (table 6.7). The pH did show a slight decrease, when stored at high temperatures (40°C + 75% RH), but remained at a range of ± 0.5 of the initial value, showing excellent stability of the formulation. Maximum stability of the hydrous cream should thus be ensured for a longer period, if it is stored at a temperature below 25°C.

6.6 Relative density

The relative density of the hydrous cream and anhydrous ointment was determined at initial and monthly over the three-month period.

6.6.1 Results

The results of the hydrous cream are given in table 6.8, and those of the anhydrous ointment in table 6.9.

Table 6.8 The relative density of the hydrous cream measured over three months

STORAGE CONDITION	(g/ml)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		0.930		
25°C + 60% RH	1.053	0.983	1.011	1.001
40°C + 75% RH		0.943	1.009	1.022

Table 6.9 The relative density of the anhydrous ointment measured over three months

STORAGE CONDITION	(g/ml)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		0.808		
25°C + 60% RH	0.863	0.871	0.817	0.844
40°C + 75% RH		0.874	0.796	0.698

6.6.2 Discussion

There were no significant changes in the relative density of the hydrous cream (table 6.8). The relative density of the anhydrous ointment remained fairly stable, except for a small decline at high temperatures (table 6.9). Temperature, humidity and pH thus did not have a significant influence on the relative density.

The relative density of the hydrous cream was slightly higher when compared to the anhydrous ointment.

6.7 Physical assessment

The physical appearance assessment of the hydrous cream and anhydrous ointment, was carried out at initial and monthly over the three-month period.

6.7.1 Results

The results of the hydrous cream are given in table 6.10, and those of the anhydrous ointment in table 6.11.

Table 6.10 The visual assessment of the hydrous cream over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: White	No change		
	Smooth, fluent	No change		
	Air bubbles	No change		
	Not too oily	No change		
	Not too hydrous	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: White	No change	Cream	Cream
	Smooth, fluent	No change	No change	No change
	Air bubbles	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: White	Cream	Very light yellow	Light yellow
	Smooth, fluent	No change	No change	No change
	Air bubbles	No change	No change	No change
	Not too oily	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Applies easily	No change	No change	No change

Table 6.11 The visual assessment of the anhydrous ointment over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: Light yellow	No change		
	Smooth	No change		
	Very thick, non-fluent	No change		
	No air bubbles	No change		
	Oily	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: Light yellow	No change	No change	No change
	Smooth	No change	No change	No change
	Very thick, non-fluent	No change	No change	No change
	No air bubbles	No change	No change	No change
	Oily	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: Light yellow	No change	Slightly darker, light yellow	Slightly darker, light yellow
	Smooth	No change	No change	No change
	Very thick, non-fluent	No change	No change	No change
	No air bubbles	No change	No change	No change
	Oily	No change	No change	No change
	Applies easily	No change	No change	No change

6.7.2 Discussion

The changes in appearance of the hydrous cream and anhydrous ointment mainly comprised a slight change in colour, which could be attributed to some degradation of the kojic acid dipalmitate with time, especially when stored at high temperatures (tables 6.10 and 6.11), since even a slight decrease in the active can result in a remarkable colour change. For the anhydrous ointment, the change in colour was minuscule. In comparison, based on physical examination, the anhydrous ointment appeared more stable than the hydrous cream.

6.8 Viscosity

The viscosity of the hydrous cream and anhydrous ointment was determined at initial and monthly over the three-month period.

6.8.1 Results

The results of the hydrous cream are given in table 6.12, and those of the anhydrous ointment in table 6.13.

Table 6.12 The viscosity of the hydrous cream measured over three months

STORAGE CONDITION	VISCOSITY, cP			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		9100		
25°C + 60% RH	18900	7200	14300	46400
40°C + 75% RH		7600	10800	45600

Table 6.13 The viscosity of the anhydrous ointment measured over three months

STORAGE CONDITION	VISCOSITY, cP			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		34800		
25°C + 60% RH	43200	55200	28400	50000
40°C + 75% RH		19600	48400	72800

6.8.2 Discussion

The initial decrease in viscosity of the hydrous cream at one month may due to the cream still settling before going into the final resting stage, or due to experimental error. The increase in viscosity after three months may be due to the water evaporating during the continued exposal of the cream to high temperatures. Maximum stability of the hydrous cream may be ensured for a longer period, if it is stored at a temperature below 25°C.

The anhydrous ointment showed no significant change in viscosity over the three-month period. It remained fairly stable throughout the stability period. The anhydrous ointment

appeared more stable when compared to the viscosity results of the hydrous cream. The viscosity of the anhydrous ointment remained fairly stable throughout the stability period.

6.9 Preservative efficacy

The preservative efficacy of the hydrous cream was determined at onset of the stability programme only.

6.9.1 Results

The results of the hydrous cream are given in table 6.14.

Table 6.14 Preservative efficacy results of the hydrous lotion (initial)

Test organism	Initial I inoculum	Log unit reduction Day 7	Day 14	Day 28	Specified limits for category 2 products
E. coli	3.1×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days
P. aeruginosa	2.0×10^5	>3.0	>3.0	>3.0	
S. aureus	3.6×10^5	>3.0	>3.0	>3.0	
A. niger	1.0×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
C. albicans	1.9×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with requirements of USP 28 (2005:2705).

6.9.2 Discussion

The initial test results showed good preservative efficacy of the hydrous cream. The company who tested it concluded that the samples complied with the requirements of USP 28 (2005:2705).

6.10 Penetration

The penetration of the hydrous cream and anhydrous ointment was determined at initial and monthly over the three-month period.

6.10.1 Results

The results of the hydrous cream are given in table 6.15, and those of the anhydrous ointment in table 6.16.

Table 6.15 The penetration of the hydrous cream measured over three months

STORAGE CONDITION	(mm)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		40.90		
25°C + 60% RH	42.37	39.84	32.93	30.99
40°C + 75% RH		39.07	34.58	34.91

Table 6.16 The penetration of the anhydrous ointment measured over three months

STORAGE CONDITION	(mm)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		26.83		
25°C + 60% RH	29.05	26.56	26.51	26.15
40°C + 75% RH		24.78	24.40	23.63

6.10.2 Discussion

The penetration of the hydrous cream decreased a little with time. This correlated with the increase in viscosity (table 6.12). The decrease in penetration may have been due to water evaporating from the formulation when exposed to high temperatures.

There was no significant change in the penetration of the anhydrous ointment. The anhydrous ointment appeared to keep its physical properties better than the hydrous cream. This correlates with the viscosity results (tables 6.12 and 6.13).

6.11 Spreadability

The spreadability of the hydrous cream and anhydrous ointment was determined at initial and monthly over the three-month period.

6.11.1 Results

The results of the hydrous cream are given in table 6.17, and those of the anhydrous ointment in table 6.18.

Table 6.17 The spreadability of the hydrous cream measured over three months

STORAGE CONDITION	(mm)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		58.11		
25°C + 60% RH	55.08	56.48	51.14	55.00
40°C + 75% RH		52.56	49.42	60.60

Table 6.18 The spreadability of the anhydrous ointment measured over three months

STORAGE CONDITION	(mm)			
	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C		24.49		
25°C + 60% RH	26.70	25.05	23.84	24.88
40°C + 75% RH		25.69	22.66	24.80

6.11.2 Discussion

There were no significant changes in the spreadability of the hydrous cream (table 6.17), or of the anhydrous ointment (table 6.18). Temperature, humidity and pH appeared not to have had a significant influence on the relative density.

The spreadibility of the anhydrous ointment was significantly lower than that of the hydrous cream, when compared. This is consistent with the higher viscosity results of the anhydrous ointment (table 6.13), when compared to the hydrous cream.

6.12 Kojic acid dipalmitate release

The release rate of the kojic acid dipalmitate of the hydrous cream and anhydrous ointment was determined by performing release tests at initial and at three months.

6.12.1Results

The results of the hydrous cream are given in figures 6.1 and 6.2, and those of the anhydrous ointment in figures 6.3 and 6.4. See appendix B for the test results used in figures 6.1 and 6.2.

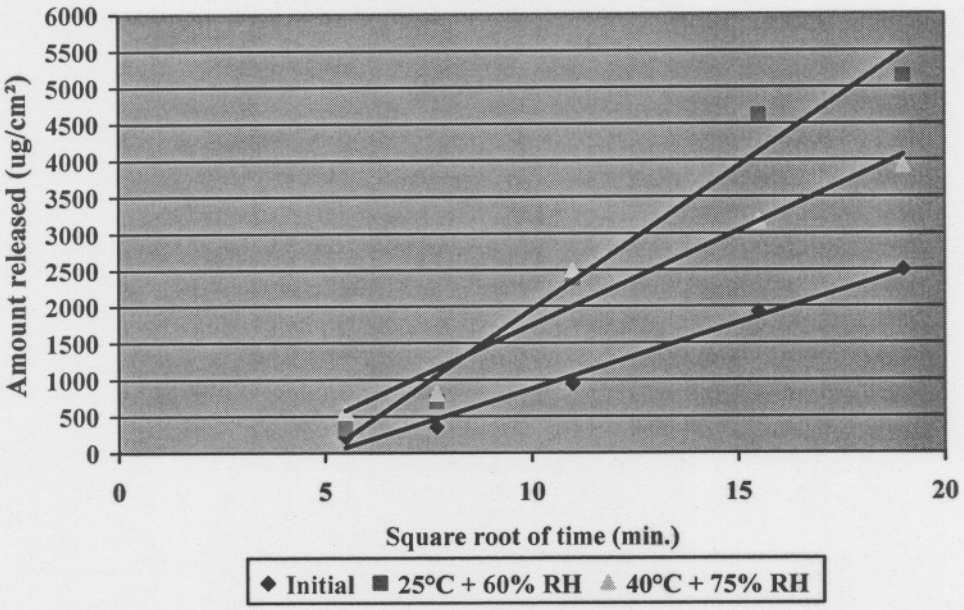

Figure 6.1 Release rate of the kojic acid dipalmitate from the hydrous cream at initial, at 3 months (25°C + 60% RH) and at 3 months (40°C + 60% RH)

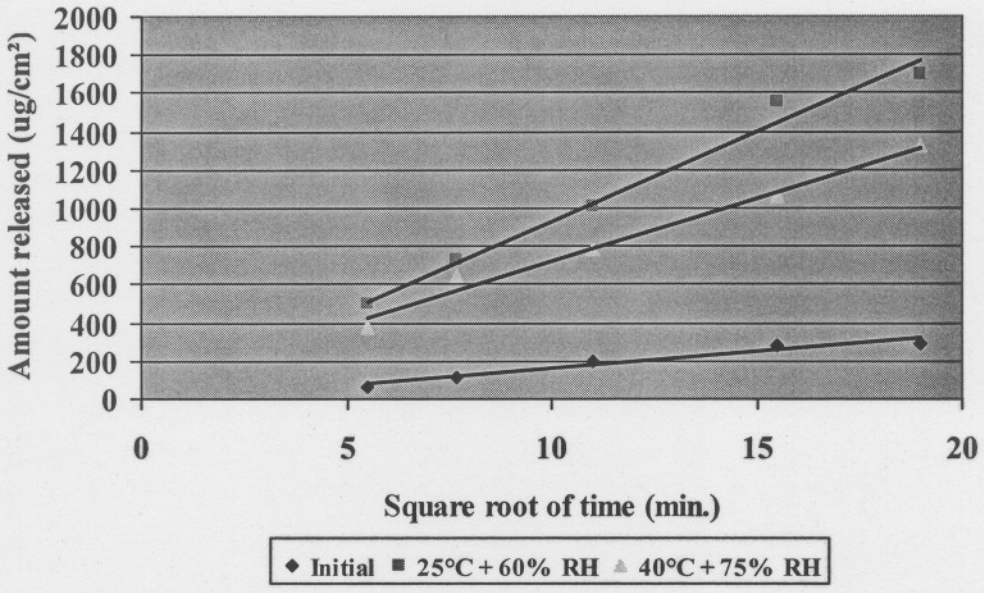

Figure 6.2 Release rate of the kojic acid dipalmitate from the anhydrous ointment at initial, at 3 months (25°C + 60% RH) and at 3 months (40°C + 60% RH)

6.12.2Discussion

The theory of release rate is that microgram per square cm released, against square root of time, should produce a straight line. The hydrous cream complies with this theory. The release rate of kojic acid dipalmitate at initial is much lower than after 3 months. At the end of the test, when the enhancer cell containers were washed, the hydrous cream was completely watery, fluent and transparent, and it had a yellowish colour. This was only the case after 3 months, for both 25°C + 60% RH and 40°C + 75% RH, whilst the hydrous cream was an emulsion at initial . The reason for the increased release rate after 3 months may be due to the fluid-like consistency of the cream, which made release of the active much easier.

The anhydrous ointment complies with the release rate theory and produced a straight line. The initial value of the kojic acid dipalmitate release was significantly lower than after 3 months. There was no logical explanation for this. Release rate tests are extremely time consuming, but it would be advisable to do the tests in duplicate in future studies.

The hydrous cream showed a higher release of kojic acid dipalmitate than the anhydrous ointment. Since the hydrous cream was a complete fluid after the three months of testing, it was difficult to compare the two formulations, as in practical application of the hydrous cream on the skin, the hydrous cream would be an emulsion. The hydrophobic nature of kojic acid dipalmitate explains the slower release from the anhydrous ointment formulation.

6.13 Sodium ascorbyl phosphate release rate

The release rate of the sodium ascorbyl phosphate of the hydrous cream was determined by performing release tests at initial and at three months. Testing at the onset of the stability programme showed the release of very little sodium ascorbyl phosphate, and at an inconsistent rate. The reason for this was that the anhydrous base is insoluble in the release medium (MilliQ water). The water could not be replaced with another release medium, as it is the suitable solvent for the sodium ascorbyl phosphate. It is presumed that if the anhydrous ointment is applied to the skin, the sodium ascorbyl phosphate would be released from the formulation. No definite conclusion can, however, be made and would require clinical testing to confirm.

6.13.1 Results

The results of the hydrous cream are given in figure 6.3. See appendix B for the test results used in figure 6.3.

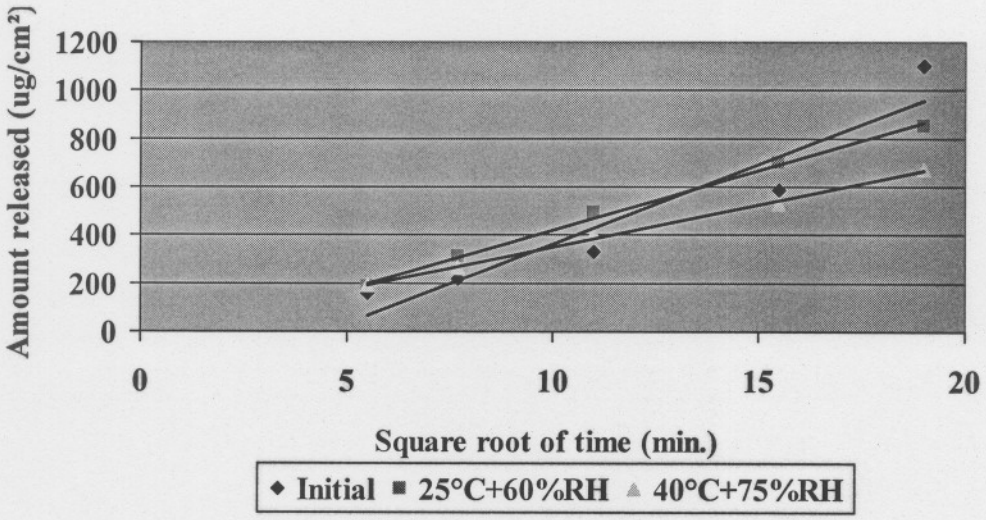

Figure 6.3 Release rate of the sodium ascorbyl phosphate from the hydrous cream at initial, at 3 months (25°C + 60% RH) and cream at 3 months (40°C + 60% RH)

6.13.2 Discussion

The hydrous cream complied with the straight line theory. There was a slight decrease in release rate of the sodium ascorbyl phosphate, but the active was released at an excellent rate. The active will thus be free to act on the skin.

6.14 Conclusion

In this chapter the results of the stability tests being done on the hydrous cream and the anhydrous ointment were presented and discussed. The hydrous cream appeared unstable with regards to kojic acid dipalmitate. The anhydrous ointment appeared to be stable with regards to kojic acid dipalmitate when stored at temperatures below 25°C. It was difficult to conclude whether the formulations were stable with regards to sodium ascorbyl phosphate, as some formulation difficulties may have negatively influenced the test outcomes. The hydrous cream appeared stable, whereas the anhydrous ointment appeared unstable. The hydrous cream's uniformity may be improved by using sodium ascorbyl phosphate having a smaller particle size. Both preservatives appeared unstable in both formulations, other preservatives is suggested for future formulation. The pH, relative density, penetration and spreadability remained fairly stable during the three-month stability test period. Physical examination showed a slight change in colour of both

formulations, but in comparison the anhydrous ointment appeared more stable than the hydrous cream.

The viscosity of the hydrous cream initially decreased, possibly due to the cream still settling, whilst after 3 months the results showed an increase in test results, probably due to water evaporating from the formulation over time. The anhydrous ointment's viscosity remained fairly stable. The release medium of the kojic acid dipalmitate completely dissolved the hydrous cream after three months, making it difficult to compare with the initial results. The anhydrous ointment showed good release of the kojic acid dipalmitate. Sodium ascorbyl phosphate release was done only on the hydrous cream, showing a slightly decreasing, but stable release rate of the active.

It was difficult to conclude whether these formulations were stable with regards to both actives, and some contradictory results may be solved by addressing formulation difficulties. Chapter 8 offers possible explanations for the formulation problems encountered and suggestions for improvements, as well as possible reasons for the questionable results are given.

CHAPTER 7

HYDROUS AND ANHYDROUS STICK RESULTS AND DISCUSSION

7.1 Introduction

The previous chapter presented the stability test results of the hydrous cream and the anhydrous ointment. In this chapter the parameters of the stick formulations that are evaluated and discussed include the kojic acid dipalmitate assay, sodium ascorbyl assay, preservative assay (only on the hydrous stick), visual appearance, viscosity and preservative efficacy (only on the hydrous stick). Very low assay results were not included in the study and are represented by a "-" in the tables, unless mentioned other. Open spaces in the table represent time and temperature intervals which were not part of the stability test period, as mentioned in chapter 4. All tests were performed were under GLP conditions.

The sticks were formulated in order to apply the skin lightener on a specific area on the skin, such as on freckles, or age spots on the face or hands.

7.2 Kojic acid dipalmitate assay

The concentration of kojic acid dipalmitate in the hydrous - and anhydrous sticks was determined at initial and at one, two and three months.

7.2.1 Results

The results of the hydrous stick are given in table 7.1 and those of the anhydrous stick in table 7.2.

Table 7.1 Kojic acid dipalmitate assay of the hydrous stick

Time	Temperature		
	5°C	25°C	40°C
Initial		99.5	
Month 1	97.9	101.9	95.2
Month 2		99.2	95.6
Month 3		94.8	96.7

Table 7.2 Kojic acid dipalmitate assay of the anhydrous stick

Time	Temperature		
	5°C	25°C	40°C
Initial		99.5	
Month 1	97.8	99.7	99.5
Month 2		94.8	91.0
Month 3		94.9	97.0

7.2.2 Discussion

The hydrous stick formulation showed excellent kojic acid dipalmitate stability. A slight decrease in active during the three-month stability test period was found, but not more than 5%, which proves the formulation's stability.

Overall, the anhydrous stick also showed excellent stability over the three-month stability period, even at high temperatures. After two months, however, the 40°C + 75% RH sample showed, a substantial decrease in active, probably indicative of a formulation problem. A longer and more efficient mixing method, as well as the use of kojic acid dipalmitate having a smaller particle size, to improve product uniformity, are recommended. A definite conclusion with regards the stability of the anhydrous stick was difficult, because of the negative result obtained at 2 months (40°C + 75% RH), as a result of formulation difficulties experienced. It appeared as if the anhydrous stick formulation was stable. The hydrous stick proved to remain stable over the three months of testing.

7.3 Sodium ascorbyl phosphate assay

The concentration of sodium ascorbyl phosphate in the hydrous - and anhydrous sticks was determined at initial and at one, two and three months.

7.3.1 Results

The results of the hydrous stick are given in table 7.3 and those of the anhydrous stick in table 7.4.

Table 7.3 Sodium ascorbyl phosphate assay of the hydrous stick

Time	Temperature		
	5°C	25°C	40°C
Initial		90.6	
Month 1	93.3	-	-
Month 2		101.2	107.8
Month 3		89.1	91.7

Table 7.4 Sodium ascorbyl phosphate assay of the anhydrous stick

Time	Temperature		
	5°C	25°C	40°C
Initial		103.1	
Month 1	112.4	102.6	-
Month 2		86.1	112.1
Month 3		99.5	98.6

7.3.2 Discussion

Due to obvious formulation problems it was difficult to conclude whether the hydrous stick was stable or not. Different methods were attempted to find the best possible sodium ascorbyl phosphate recovery. Due to the extent of repeated testing, no more hydrous sticks were left for testing at 1 month (25°C + 60% RH and 40°C + 75% RH). The formulation could be improved by using sodium ascorbyl phosphate having a smaller particle size.

The anhydrous stick appeared to remain fairly stable, except for the slight decrease in active at 2 months (25°C + 60% RH). Two contradictory results representing increases in active may also signify the now familiar formulation problems concerning the sodium ascorbyl phosphate. Chapter 8 offers possible explanations for the formulation problems encountered and suggestions for improvements.

It was difficult to conclude on the stability of the sodium ascorbyl phosphate in the anhydrous stick formulation. The sodium ascorbyl phosphate is hydrophilic and thus insoluble in the anhydrous base, it is therefore difficult to assure uniformity. The conclusion was made that the formulation was not successful with regards to sodium ascorbyl phosphate. It is recommended that the lipophilic sodium ascorbyl palmitate should be investigated for possible future formulations.

7.4 Preservative assay

7.4.1 Results

The results of the hydrous stick are given in table 7.5 and those of the anhydrous stick in table 7.6.

Table 7.5 Methyl paraben assay of the hydrous stick

	Temperature		
Time	5°C	25°C	40°C
Initial		97.7	
Month 1	98.3	97.8	-
Month 2		49.9	38.2
Month 3		45.4	47.1

Table 7.6 Propyl paraben assay of the hydrous stick

	Temperature		
Time	5°C	25°C	40°C
Initial		105.3	
Month 1	106.3	112.0	115.0
Month 2		104.8	101.2
Month 3		102.7	99.4

7.4.2 Discussion

The methyl paraben was without doubt instable in this formulation, as its assay results decreased significantly over time. It is concluded that this preservative is unsuitable for

use in this formulation. The propyl paraben remained more stable than the methyl paraben. The propyl paraben seemed stable at first, but at 3 months (40°C + 75% RH) its assay results decreased with more than 5%. The use of alternative preservatives are recommended for use in future preparation of the hydrous stick. Some contradictory results may also be indicative of a formulation problem. See chapter 8 for possible suggestions for improvement.

7.5 Physical assessment

The physical appearance assessment of the hydrous - and anhydrous stick was carried out at initial and monthly over the three-month period.

7.5.1 Results

The results of the hydrous stick are given in table 7.7, and those of the anhydrous stick in table 7.8.

Table 7.7 The visual assessment of the hydrous stick over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: Pearly white	No change		
	Smooth, thick	No change		
	No air bubbles	No change		
	Not too hydrous	No change		
	Not too oily	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: Pearly white	No change	No change	No change
	Smooth, thick	No change	No change	No change
	No air bubbles	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Not too oily	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: Pearly white	No change	No change	No change
	Smooth, thick	No change	No change	No change
	No air bubbles	No change	No change	No change
	Not too hydrous	No change	No change	No change
	Not too oily	No change	No change	No change
	Applies easily	No change	No change	No change

Table 7.8 The visual assessment of the anhydrous stick over three months

STORAGE CONDITION	INITIAL	MONTH 1	MONTH 2	MONTH 3
5°C	Colour: Light yellow	No change		
	Smooth	No change		
	Thick	No change		
	No air bubbles	No change		
	Oily	No change		
	Applies easily	No change		
25°C + 60% RH	Colour: Light yellow	No change	No change	No change
	Smooth	No change	No change	No change
	Thick	No change	No change	No change
	No air bubbles	No change	No change	No change
	Oily	No change	No change	No change
	Applies easily	No change	No change	No change
40°C + 75% RH	Colour: Light yellow	No change	Slightly darker, light yellow	Slightly darker, light yellow
	Smooth	No change	No change	No change
	Thick	No change	No change	No change
	No air bubbles	No change	No change	No change
	Oily	No change	No change	No change
	Applies easily	No change	No change	No change

7.5.2 Discussion

The hydrous stick showed no significant change in visual appearance over the three-month period (table 7.7). The change in colour of the anhydrous stick was insignificant. The hydrous stick hence maintained its visual appearance satisfactory, possibly indicative of its stability. However, most assay results to this point, except those of kojic acid dipalmitate, do not confirm this apparent stability of the formulation.

7.6 Preservative efficacy

The preservative efficacy of the hydrous stick was determined at onset of the stability programme.

7.6.1 Results

The results of the hydrous stick are given in table 7.8.

Table 7.9 Preservative efficacy results of the hydrous stick (initial)

Test organism	Initial I inoculum	Log unit reduction			Specified limits for category 2 products
		Day 7	Day 14	Day 28	
E. coli	3.1×10^5	>3.0	>3.0	>3.0	Not less than 2.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days
P. aeruginosa	2.0×10^5	>3.0	>3.0	>3.0	
S. aureus	3.6×10^5	>3.0	>3.0	>3.0	
A. niger	1.0×10^5	>3.0	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
C. albicans	1.9×10^5	>3.0	>3.0	>3.0	

Comments: Sample complies with requirements of USP 28 (2005:2705).

7.6.2 Discussion

Initial test results showed good preservative efficacy of the hydrous stick. The company who tested it concluded that it complied with requirements of USP 28 (2005:2705).

7.7 Conclusion

In this chapter the stability test results of the hydrous - and anhydrous sticks were presented and discussed. The hydrous stick showed excellent stability with regards to kojic acid dipalmitate. It was difficult to make a definite conclusion on the stability of the anhydrous stick, because of one negative result obtained at 2 months (40°C + 75% RH,) resulting from formulation difficulties. From physical examination, it appeared as if the anhydrous stick formulation was stable. Formulation problems made it difficult to conclude whether the hydrous stick was stable with regards to sodium ascorbyl phosphate. The anhydrous stick appeared to be stable with regards to this active, except for one contradictory result. The formulation could be improved by using sodium ascorbyl phosphate having a smaller particle size.

Both preservatives proved to be unstable in the hydrous stick formulation, although the propyl paraben showed considerably better stability than the methyl paraben. Alternative preservatives are recommended in future formulation attempts.

Both formulations showed good physical stability, with the hydrous stick showing no colour change and the anhydrous stick only an almost insignificant change in colour.

Chapter 8 offers possible explanations for the formulation problems encountered and suggestions for improvements, as well as possible reasons for the questionable results are given.

CHAPTER 8
APPROACH TO SOLVE FORMULATION PROBLEMS

8.1 Introduction

This chapter discusses the formulation problems experienced throughout this study. Possible suggestions to solve the formulation problems are suggested. A final approach to solve the formulation problems were attempted by formulating a batch of anhydrous gel and anhydrous ointment, containing kojic acid dipalmitate and ascorbyl *palmitate* (lipophilic), is also discussed.

8.2 Discussion on formulation problems

It is clear from the assay results of the sodium ascorbyl phosphate represented in chapters 5-7, that problems occurred during formulation and / or that the validated analytical method, adapted from Van Rensburg (2004:128), was unsuitable for the assay.

The method used to add the sodium ascorbyl phosphate into the formulations was the one suggested by BASF (2005:24), according to which the product should be cooled to 40°C followed by the mixing of the powder into the formulation. During the previous study by Van Rensburg the sodium ascorbyl phosphate used was obtained from BASF and during stability trials the analytical method was used successfully. During this study, Stay-C® 50 from Roche was used.

It was clear during this study that the Stay-C® 50 raw material used, mainly consisted of larger, thus heavy particles, causing it to descend to the base of the container when in suspension (in aqueous and non-aqueous liquids), before solidification took place. It is thus assumed that when transferred into the packaging for stability testing, due to this lack of uniformity, the container(s) that were filled first would have had a lower concentration of sodium ascorbyl phosphate, compared to the container(s) filled last. This possibly

caused the variation in the test results being generated over the three-month stability testing, since there was no control over which container was tested when.

It is difficult to comment on the stability of the actives in the products formulated. The visual and other physical results showed no significant changes. The release studies done on both actives showed satisfactory release rate, which also add to the argument that the actives did not break down in the formulations. Due to the poor state of the assay results as a result of possible formulation problems, a definite conclusion on the stability of the kojic acid dipalmitate and sodium ascorbyl phosphate in the present formulations cannot be reached. It was however clear from the physical results that there was no significant breakdown of the ascorbic acid part of the sodium ascorbyl phosphate.

Possible recommendations for addressing the problem being identified are the following:

- It is suggested that for those formulations containing water, since sodium ascorbyl phosphate is soluble in water, approximately 10% of the water should be used to dissolve the sodium ascorbyl phosphate first, before adding it into the emulsion at 40°C, whilst continuously stirring. This should homogenise the product before it becomes too viscous.

- Both the anhydrous and hydrous formulations should be improved with grinding, or milling, of the sodium ascorbyl phosphate, into a very fine powder, and with subsequent sieving before adding to the formulations. These smaller and uniform particles would enhance the homogeneity of the active throughout the formulation, together with mixing it well, until solidifying of the base.

- Another problem arises from the fact that it is difficult to incorporate a hydrophilic active, such as sodium ascorbyl phosphate, into an anhydrous formulation, as it is insoluble in the anhydrous base. The same applies to incorporating a lipophilic active, such as kojic acid dipalmitate, into a hydrous formulation, as it is insoluble in the hydrous base. Hydrophilic actives (kojic acid and sodium ascorbyl phosphate) for the hydrous formulations and lipophilic actives (kojic acid dipalmitate and

ascorbyl palmitate) for the anhydrous formulation is suggested to ensure better solubility and thus better uniformity.

Unsuitable and varying particle size may complicate product uniformity. This may be resolved by incorporating actives having a suitable and uniform particle size, hence homogenising the formulations.

8.3 Final attempt to solve formulation problems

In an approach to solve the formulation problems a final attempt was initiated. The suggestion to formulate hydrous formulations containing hydrophilic actives and anhydrous formulations containing lipophilic actives was investigated. In a study done by van Rensburg in 2004, two hydrous creams and a hydrous gel containing between 71-93% water was formulated. When stored at temperatures below 25°C the products remained fairly stable. Therefore another batch of hydrous products was not formulated again. A batch of anhydrous products containing kojic acid dipalmitate and ascorbyl palmitate was formulated and assayed numerously.

The anhydrous gel and anhydrous ointment formulation discussed in chapter 3 was formulated. The only difference being that the hydrophilic sodium ascorbyl phosphate was replaced with the lipophilic ascorbyl palmitate. The actives were milled with a mortar and pestle to ensure better uniformity. These formulations will be referred to as anhydrous gel B and anhydrous ointment B.

During the formulation of the anhydrous gel B and anhydrous ointment B, the following observations were made:

- With preparation of both the anhydrous gel B and anhydrous ointment B the actives were added by trituration with increasing amount of molten base.
- Anhydrous ointment B presented with small white lumps, possibly indicating on the lipophilic actives agglomerating to each other. The ointment was allowed to settle for 24 hours. Small amounts of anhydrous ointment B was transferred to a

mortar and pestle mixing (rubbing) out the lumps by adding small amounts of ointment at a time. All visible lumps were rubbed out.

- Anhydrous gel B appeared to be stable, but after 24 hours, the actives were visible in a silverish mass at the surface of the formulation, indicating the separation of anhydrous gel B. Small transparent lumps were visible on close inspection. Care was taken to homogenise the actives throughout the anhydrous gel B by pouring small amounts of gel on a glass plate and rubbing out the lumps with a spatula. All visible lumps were rubbed out.

The samples of anhydrous gel B and anhydrous ointment B were done on the validated method used for the kojic acid dipalmitate (refer to appendix A).

HPLC parameters:

Column:	Luna C18 (2), 150 x 4.6 mm, 5µm (Phenomenex, Torrance, CA).
Mobile phase:	Tetrahydrofuran:methanol:water:acetic acid (60:20:20:5).
Flow rate:	1.0 ml/min.
Injection volume:	10 µl.
Detection:	UV at 245 nm.
Retention time:	Approximately 3 minutes for the ascorbyl palmitate and approximately 17 minutes for the kojic acid dipalmitate.
Solvent:	Tetrahydrofuran.
Standard:	100 mg kojic acid dipalmitate and 50 mg ascorbyl palmitate.
Stop time:	25 minutes.
Apparatus:	Agilent 1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent (Agilent, Palo Alto, CA).

The results of the assay experiments are given in tables 8.1 – 8.4.

Table 8.1 Assay results of ascorbyl palmitate content

	Anhydrous gel B		
	Sample1	Sample2	Sample3
Initial1	115.3	104.6	98.3
Initial2	104	95.3	87

Table 8.2 Assay results of ascorbyl palmitate content

	Anhydrous ointment B		
	Sample1	Sample2	Sample3
Initial1	189.1	176.2	178.1
Initial2	130.1	108.6	189.8

Table 8.3 Assay results of kojic acid dipalmitate content

	Anhydrous gel B		
	Sample1	Sample2	Sample3
Initial1	104.2	101.4	97.4
Initial2	114.1	108.6	110.8

Table 8.4 Assay results of kojic acid dipalmitate content

	Anhydrous ointment B		
	Sample1	Sample2	Sample3
Initial1	113.6	100.9	111.5
Initial2	118.5	110.3	114.7

Despite numerous assays done on the anhydrous gel B and anhydrous ointment B, it was without any success. The products were mixed well before sample extraction was done. The HPLC samples were clear and transparent, indicating that both the base and actives dissolved in the solvent. Possibly because of the large molecular size of the dipalmitate

and palmitate, it appeared as if the anhydrous gel B and anhydrous ointment B kept on agglomerating and separating on microscopic level. This was not visually observable but can be concluded from the assay results.

8.4 Conclusion

This chapter discussed the formulation problems and the final attempt to solve the problems experienced. Possible suggestions were given on solving the formulation problems. These suggestions were applied in the formulation of anhydrous products containing the lipophilic actives kojic acid dipalmitate and ascorbyl palmitate. As illustrated through the assay results of anhydrous gel B and anhydrous ointment B, this attempt did not have the expected positive outcome.

CHAPTER 9

FINAL CONCLUSION

This chapter is a conclusion on the results of the present study. The anhydrous formulations appeared to be more stable with regards to kojic acid dipalmitate, except for the hydrous stick, which also showed excellent stability. The formulations showed no significant decrease in kojic acid dipalmitate, nor a significant change in colour, thus confirming the stability of this active in the anhydrous gel, the anhydrous ointment, and the hydrous - and anhydrous sticks.

According to the assay results, the methylparaben and propylparaben were unstable in these formulations therefore; alternative preservatives are suggested for possible future formulations of the hydrous cream and hydrous stick.

Several formulation problems were experienced throughout this study. Various attempts were initiated to solve the problems, and products were formulated numerously in order to attempt to get better uniformity. A new anhydrous gel, a modified method for formulating the anhydrous ointment and anhydrous stick was attempted after 2 months of stability testing which revealed the inconsistency of the products. The result was better uniformity, but still not satisfactory homogeniety. The use of hydrophilic actives in hydrous formulations and lipophilic actives in anhydrous formulations were investigated. Van Rensburg (2004) formulated hydrous products containing kojic acid and sodium ascorbyl phosphate (both hydrophilic). These formulations remained stable when stored at temperatures below 25°C. An anhydrous gel B and anhydrous ointment B containing kojic acid dipalmitate and ascorbyl palmitate (both lipophilic) was formulated as a final attempt to solve the problems experienced. Various assay results revealed that this attempt was also not successful.

It is difficult to comment on the stability of the actives in the formulated products due to the varying assay results and the numerous unsuccessful attempts to solve this. The visual and physical tests' results supports the argument that breakdown of the actives did not take place. The sample preparation was not a problem, as the samples were completely clear,

indicating that both the actives and bases dissolved in the solvent, which adds to the argument that the anhydrous gel B and anhydrous ointment B separated on microscopical level, though this was not visible with the eye. A definite conclusion on the stability of the actives can inevitably not be made. The sodium ascorbyl phosphate and ascorbyl palmitate were so heavily hindered by formulation problems that it is impossible to comment on its stability. The kojic acid dipalmitate appeared to stay stable in the formulations, with regard to the visual and physical tests' results. Though no definite conclusion on the stability of the kojic acid dipalmitate can be made from this study, it appears, as claimed by Chemos (2005:1), that kojic acid dipalmitate is more stable than kojic acid and has less product instability problems, especially in anhydrous formulations (Whittemore & Neis, 1998:1; Majmudar *et al.*, 1998:366).

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APPENDIX

APPENDIX A: Validation of HPLC method of kojic acid dipalmitate

APPENDIX B: Membrane release data sheets

APPENDIX C: Publication

APPENDIX D: Generic and trade names

APPENDIX A

SUMMARY

Test	Result
Specificity	Complies
Range	20.5-287.0 µg/ml
Linearity	Kojic acid dipalmitate $R^2 = 0.9999$
Accuracy	Kojic acid dipalmitate 101.0%
Precision	Kojic acid dipalmitate RSD 0.97%

1. ORIGIN OF METHOD

The method was developed and validated by Marike Ganz in cooperation with Dr Jan du Preez at the Analytical Technology Laboratory, Research Focus Area 9.2, School of Pharmacy, North West University.

2. CHROMATOGRAPHIC CONDITIONS

Analytical instrument: Agilent 1100 series HPLC equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software or equivalent (Agilent, Palo Alto, CA).

Column: Luna C18 (2),150x4.6 mm, 5µm (Phenomenex, Torrance, CA)

Mobile phase: Tetrahydrofuran/acetonitrile/methanol/water/acetic acid. 35/30/29/5/1

Flow rate: 1.0 ml/min.

Injection volume: 10 µl.

Detection: UV at 250 nm.

Retention time: Approximately 6.6 minutes.

Solvent: Mobile phase.

3. SAMPLE PREPARATION

Weigh approximately 1 g of each formulation accurately into a tared 50 ml volumetric flask, using a syringe with a tube attached to the tip. THF was then added, and the samples were sonicated for approximately 20 minutes, until completely dissolved. The samples were sonicated and shaken repeatedly by hand during this period, until the base was completely dissolved. The samples were allowed to cool to room temperature, filled to volume and then transferred to a HPLC vial and injected into the chromatograph.

4. STANDARD SOLUTION

For the preparation of the standard, 100 mg of kojic acid dipalmitate was accurately weighed and transferred to a 100 ml volumetric flask. THF was added and then the standard was shaken by hand, after which it was sonicated for five minutes, until completely dissolved. After sonification, the standard was allowed to cool to room temperature and then filled to volume with THF. 10 ml of the standard was accurately transferred to a 50 ml volumetric flask and filled to volume with THF. The standard was then transferred into a HPLC vial and injected into the chromatograph.

5 CALCULATIONS

$$\frac{\text{Sample area X mass of standard (g) X potency of standard}}{\text{Standard area X Sample mass X 100}} = \text{\% of label claim}$$

6. SYSTEM SUITABILITY PARAMETERS

- Make duplicate injections of a standard solution.

- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates for the kojic acid dipalmitate peaks.

The system is suitable to perform the analysis if the following criteria are met:

- RSD of 2 injections not more than 2 %.
- The column must have more than 3000 theoretical plates for kojic acid dipalmitate.

7. VALIDATION TEST PROCEDURE AND ACCEPTANCE CRITERIA

7.1. Specificity

Method

1. Prepare a sample from a placebo product that does not contain the substances being tested with this method.
2. Dilute a standard solution 1:1 with water, 0.1M hydrochloric acid, 0.1M sodium hydroxide and 10% hydrogen peroxide respectively.
3. Store these solutions overnight in closed test tubes at 40 °C to degrade.
4. Inject the samples into the chromatograph and examine the chromatograms to determine whether any additional peaks were formed.

ACCEPTANCE CRITERIA:

1. The placebo should not contain any peaks that will interfere with the determination of the actives.
2. Extra peaks formed in the stressed samples should be discernible from those of the active components.

7.2. Linearity

Preparation of standards.

1. Prepare a standard solution as described under standard preparation.
2. Inject variable volumes into the chromatograph to obtain standards from 10-150 % of the expected sample concentration.

ACCEPTANCE CRITERIA:

Linear regression analysis should yield a regression coefficient (R squared) of ≥ 0.99.

7.3. Accuracy

1. Measure 3 times 0.8g, 3 x 1.0g and 3 x 1.2g mg of placebo into 100 ml volumetric flasks.
2. Spike with known amounts of active at concentrations of approximately 80, 100 and 120% of the expected sample concentration.
3. Inject into the chromatograph in duplicate.

ACCEPTANCE CRITERIA:

Recovery must be between 98-102%.

7.4. Precision

7.4.1. Intra-day precision (repeatability)

1. Measure approximately 3 x 0.8g, 3 x 1.0g and 3 x 1.2g of cream or lotion into 100 ml volumetric flasks and fill to volume with solvent.
2. Inject into the chromatograph in duplicate.

7.4.2. Inter-day precision

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

ACCEPTANCE CRITERIA:

Repeatability must be better than 2%, (n = 9).

Inter- day precision must be better than 5% (n = 9).

7.5. Ruggedness

7.5.1. Stability of sample solutions

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

ACCEPTANCE CRITERIA:

Sample solutions should not be used for a period longer than it takes to degrade by 2%, and in the case of degradation special precautions should be followed to compensate for the loss. The sample stability proved acceptable stability for the medium used during the analysis.

7.5.2 System repeatability

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

ACCEPTANCE CRITERIA:

The peak area and retention times should have an RSD of 2% or less.

7.6. Robustness

1. Make deliberate changes to the chromatographic conditions to determine the method's tolerance towards changes.
2. Change the flow rate, wavelength, injection volume, acetonitrile concentration and use a similar column from a different manufacturer.

ACCEPTANCE CRITERIA:

The method should be able to tolerate a 5 % variance in the chromatographic conditions.

7.7. System and method performance characteristics (system suitability)

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

ACCEPTANCE CRITERIA:

The USP tailing factor must be less than 1.5 and the RSD must be less than 2%.

8. VALIDATION RESULTS

8.1. SPECIFICITY:

A sample prepared from the placebo anhydrous gel is shown in Figure 1. Figure 2 is a chromatogram of a standard solution. Figure 3 shows a sample chromatograph of the anhydrous gel. Stress tests could not be performed as kojic acid dipalmitate immediately precipitates in contact with a watery solvent.

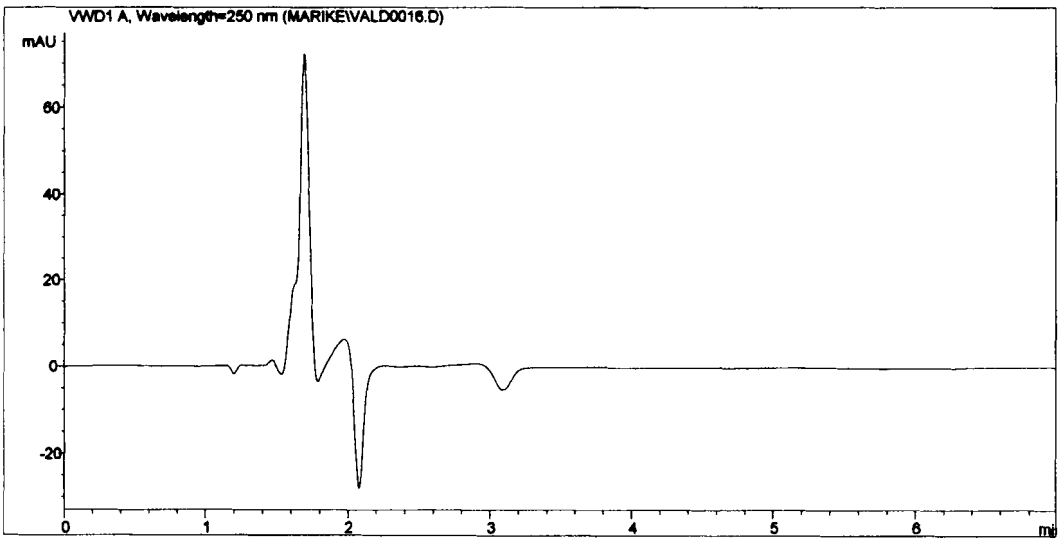

Figure 1. Placebo of the anhydrous gel.

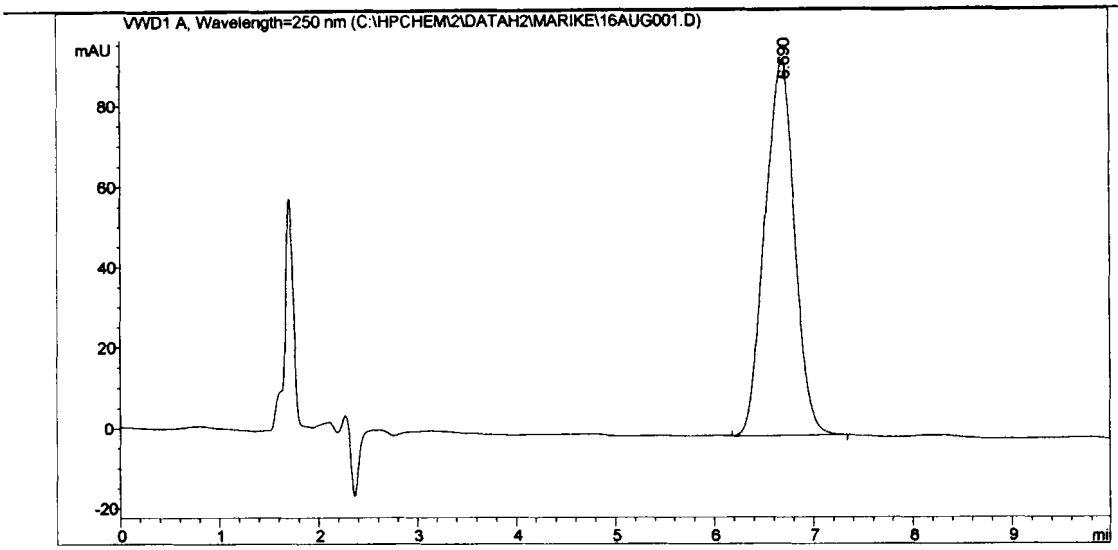

Figure 2. Chromatogram of a standard solution.

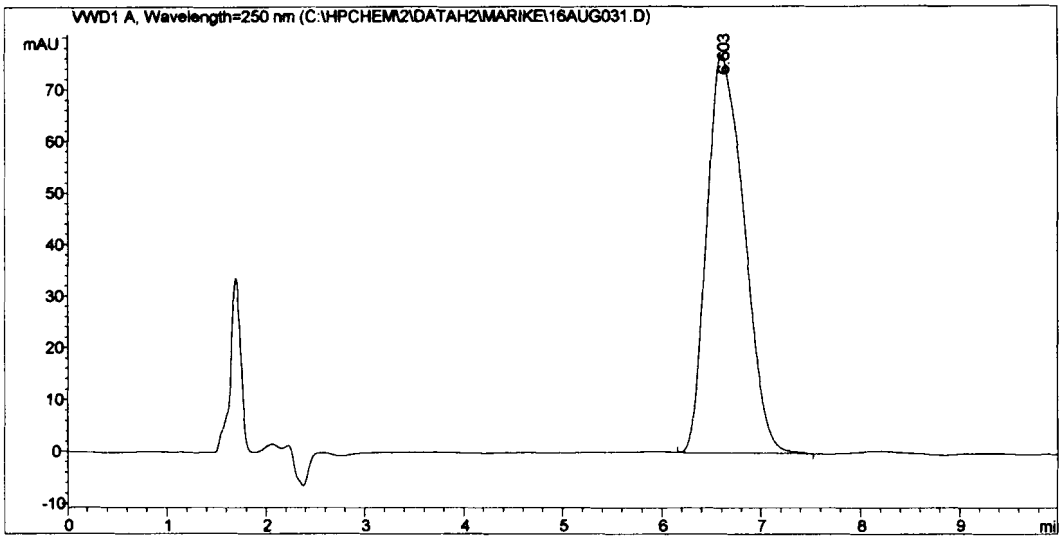

Figure 3. Sample of the anhydrous gel 25°C + 65RH 1 month.

Conclusion:

None of the ingredients in the placebo interfered with the analyte peaks. Extra peaks formed during forced degradation did not interfere with the remainder of the analyte peaks, proving that the method is stability-indicating.

9. Kojic acid dipalmitate:

9.1. LINEARITY AND RANGE:

Results:

µg/ml	Area 1	Area 2	Mean
20.5	203	207	205.0
41.0	403	408	405.5
82.0	828	817	822.5
123.0	1214	1226	1220.0
164.0	1648	1647	1647.5
205.0	2053	2046	2049.5
246.0	2458	2466	2462.0
287.0	2867	2863	2865.0

Regression statistics:

R Squared	0.99996	Lower 95%	Upper 95%
Intercept	-1.32203	-10.91531	8.27124
Slope	20.00246	9.945352	10.05711

Conclusion:

The method is linear over the concentration range 20.5-287.0 µg/ml. The method is suitable for single point calibration.

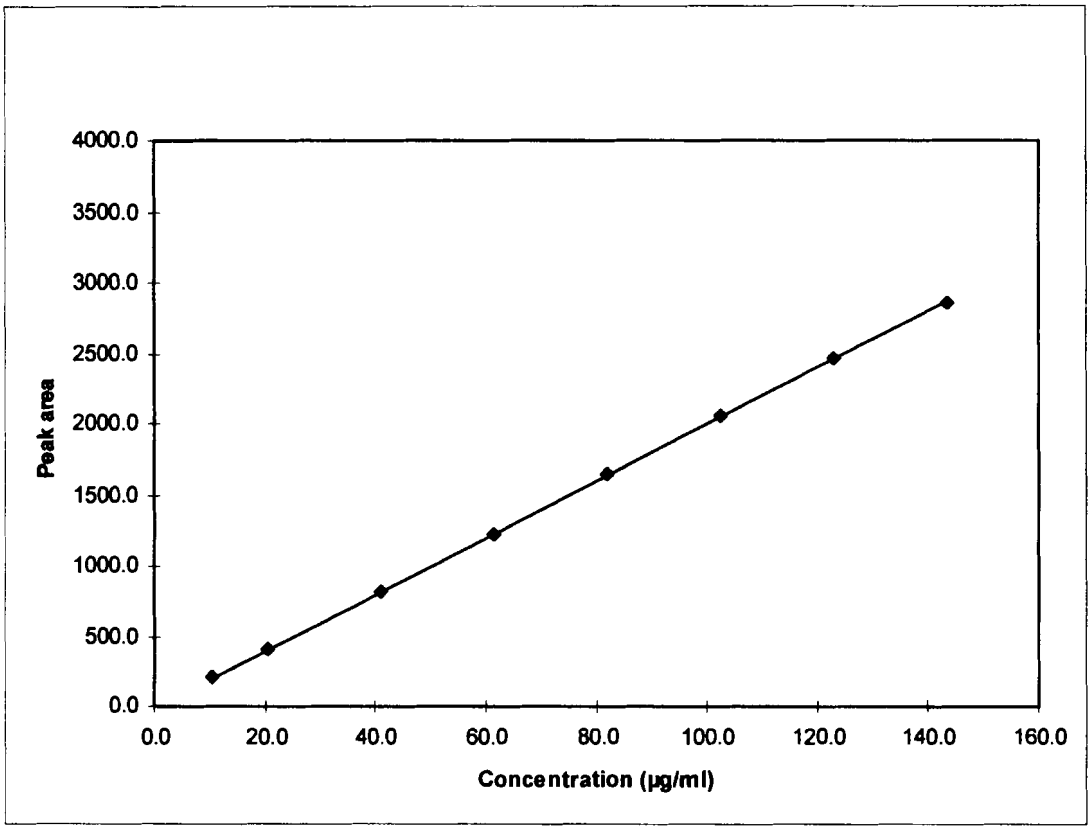

Figure 91. Linear regression graph for kojic acid dipalmitate.

9.2. ACCURACY:

Conc. spiked				Recovery	
µg/ml	Area 1	Area 2	Mean	µg/ml	%
167.5	1705.1	1705.4	1705.3	170.6	101.9
167.5	1684.2	1701.2	1692.7	169.4	101.1
167.5	1723.2	1715.8	1719.5	172.1	102.7
209.4	2121.2	2095.8	2108.5	211.0	100.7
209.4	2095.8	2098.3	2097.1	209.8	100.2
209.4	2104.3	2107.6	2105.9	210.7	100.6
251.3	2524.1	2525.7	2524.9	252.6	100.5
251.3	2526.2	2520.5	2523.3	252.4	100.5

251.3	2522.1	2531.7	2526.9	252.8	100.6

Statistical analysis	
Mean	101.0
SD	0.8
% RSD	0.8
95% confidence intervals	
Lower limit	100.4
Upper limit	101.6
Estimated median	100.6
Confidence Level	0.6

Conclusion:

Over the range of 83.8-125.6 % of the sample concentration, the method yielded a mean recovery of 101.0 %.

9.3. PRECISION:

9.3.1. Intermediate (intra-day) precision:

Mass (g)	Area		Mean	Conc. µg/mg	%
0.8310	1520.4	1566.5	1543.5	97.4	97.4
0.8171	1545.2	1544.6	1544.9	99.2	99.2
0.8243	1549.3	1569.6	1559.5	99.2	99.2
1.1286	2109.2	2094.1	2101.7	97.7	97.7
1.1347	2143.5	2116.1	2129.8	98.5	98.5
1.0979	2066.8	2023.2	2045.0	97.7	97.7
1.2500	2289.4	2313.3	2301.4	96.6	96.6
1.2159	2220.8	2269.8	2245.3	96.9	96.9
1.2960	2418.7	2476.8	2447.8	99.1	99.1
			Mean		98.03
			SD		0.95
			RSD %		0.97

Conclusion:

Precision was satisfactory with a RSD of 0.97 %.

9.3.2. Inter-day precision:

	DAY 1 %	DAY 2 %	DAY 3 %	Inter day
	97.7	98.9	99.3	
	98.5	98.5	98.7	
	97.7	97.7	99.4	
Mean	97.95	98.00	99.15	98.37
SD	0.36	0.24	0.31	0.56
RSD %	0.37	0.24	0.31	0.56

ANOVA single factor statistics:

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	293.856	97.952	0.194
Day 2	3	293.989	97.996	0.085
Day 3	3	297.456	99.152	0.143

ANOVA					
Source of	SS	df	MS	F	P-value
Inter day	2.777	2.000	1.388	9.873	0.013
Intra day	0.844	6.000	0.141		
Total	3.760	8			

SS = sum of squares

df = degrees of freedom

MS = mean squares

F = F ratio

Conclusion:

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

9.4. RUGGEDNESS:

9.4.1. STABILITY OF SAMPLE SOLUTIONS:

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

Results:

Time (hours)	Peak Area	% Remaining
0	1995.3	100.0
1	1967.9	98.6
2	1974.2	98.9
3	1977.2	99.1
4	1990.1	99.7
5	2000.5	100.3
6	2006.1	100.5
7	2012.3	100.9
8	2012.4	100.9
9	2014.2	100.9
10	2003.0	100.4
11	2007.1	100.6
12	1996.7	100.1
Mean	1996.7	100.07
SD	15.98	0.75
RSD %	0.80	0.80

Conclusion:

The methyl paraben is stable over a period of 12 hours.

9.4.2. SYSTEM REPEATABILITY:

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

Results.

	Area	Retention time (minutes)
	2030	6.619
	2023	6.620
	2027	6.620
	2021	6.621
	2088	6.622
	2005	6.622
Mean	2032	6.620
SD	28.43	0.001
RSD %	1.40	0.02

Conclusion:

System performance proved well with RSD values of 1.40 % for peak area and 0.02 % for retention time respectively.

14. SYSTEM SUITABILITY PARAMETERS.

- Inject a standard solution in duplicate.
- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates for the paraben peaks.
- Use the 5-sigma method to calculate the parameters.

The system is suitable to perform the analysis if the following criteria are met:

- RSD of 2 injections not more than 2 %.
- The column must have more than 3 000 theoretical plates for all the analytes.

15. CONCLUSION:

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

APPENDIX B

Membrane release

Kojic acid dipalmitate

Sodium ascorbyl phosphate

Membrane release of hydrous gel

Product: Hydrous gel
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: Distilled water
Volume: 190 ml
Amount withdrawn: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 40.844µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	57.067	7	8	10	14	14	5.1	5.6	7.0	9.8	9.8
3.0918	57.5	10	9	10	13	14	7.0	6.3	7.0	9.1	9.8
3.1242	60.893	9	10	13	16	17	6.3	7.0	9.1	11.2	11.9
3.1276		10	14	17	37	43	7.0	9.8	11.9	25.8	30.0
3.2364		7	15	15	17	18	4.9	10.5	10.5	11.9	12.3
3.2997		8	10	13	13	21	5.6	7.0	9.1	9.1	14.7
Ave	58					**Ave %**	6.0	7.7	9.1	12.8	14.7
%RSD	0.00					**%RSD**	4.09	9.09	11.54	2.73	16.59

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	7	8	10	14	14	243	267	334	467	467
3.0918	2114	10	9	10	13	14	334	300	334	434	467
3.1242	2101	9	10	13	16	17	300	334	434	534	567
3.1276	2111	10	14	17	37	43	334	467	567	1234	1434
3.2364	2121	7	15	15	17	18	233	500	500	567	585
3.2997	2099	8	10	13	13	21	267	334	434	434	700
Ave	2109					**Ave %**	285	367	434	612	704
%RSD	0.14					**%RSD**	4.09	9.09	11.54	2.73	16.59

Membrane release of anhydrous gel

Product:	Anhydrous gel
Batch no.:	
Stability period:	Initial
Container:	Plastic jar
API:	Kojic acid dipalmitate
Strength:	1% m/m
Analytical:	HPLC: Agilent 1100
Wavelength:	250 nm
Column:	Luna C18 (2), 5µm

Membrane release conditions:

Medium:	60:40 THF:Water
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32°C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978cm*cm

Concentration of standard: 40.748 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	101.8	14	16	20	22	24	5.5	6.2	7.7	8.5	9.2
3.0918	107.72	14	15	24	25	27	5.4	5.8	9.2	9.6	10.4
3.1242	107.06	16	13	16	22	26	6.2	5.0	6.2	8.5	10.0
3.1276	106.52	11	18	18	23	25	4.2	6.9	6.9	8.9	9.6
3.2364		18	20	23	26	32	6.9	7.7	8.9	10.0	12.3
3.2997		13	17	22	24	28	5.0	6.5	8.5	9.2	10.8
Ave	106					**Ave %**	5.5	6.4	7.9	9.1	10.4
%RSD	0.00					**%RSD**	4.45	3.03	4.88	4.23	7.41

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	14	16	20	22	24	263	294	368	405	442
3.0918	2114	14	15	24	25	27	258	276	442	460	497
3.1242	2101	16	13	16	22	26	294	239	294	405	478
3.1276	2111	11	18	18	23	25	202	331	331	423	460
3.2364	2121	18	20	23	26	32	331	368	423	478	589
3.2997	2099	13	17	22	24	28	239	313	405	442	515
Ave	2109					**Ave %**	265	304	377	435	497
%RSD	0.14					**%RSD**	4.45	3.03	4.88	4.23	7.41

Membrane release of Hydrous cream

Product: Hydrous cream
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 250 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 40µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	106.52	11	22	72	146	150	4.2	8.2	27.1	55.2	56.7
3.0918	101.8	13	23	100	121	142	5.0	8.7	38.0	45.8	53.7
3.1242	107.72	13	19	32	99	140	4.8	7.0	12.1	37.4	52.9
3.1276	107.06	12	17	45	104	156	4.5	6.4	17.0	39.3	59.0
3.2364		10	17	33	81	152	3.8	6.4	12.5	30.6	57.5
3.2997		13	21	32	87	92	4.9	7.9	12.1	32.9	34.8
Ave	106					**Ave %**	4.5	7.4	19.8	40.2	52.4
%RSD	0.00					**%RSD**	7.58	1.78	37.84	27.74	20.91

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	11	22	72	146	150	202	392	1293	2637	2709
3.0918	2114	13	23	100	121	142	239	415	1813	2186	2565
3.1242	2101	13	19	32	99	140	228	334	578	1788	2529
3.1276	2111	12	17	45	104	156	217	307	813	1878	2818
3.2364	2121	10	17	33	81	152	181	307	596	1463	2745
3.2997	2099	13	21	32	87	92	235	379	578	1571	1662
Ave	2109					**Ave %**	217	356	945	1921	2505
%RSD	0.14					**%RSD**	7.58	1.78	37.84	27.74	20.91

Membrane release of cream A

Product: Anhydrous ointment
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 250 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: THF
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 40.844 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	57.067	0	4	12	14	15	0.0	2.6	8.2	9.8	10.4
3.0918	57.5	2	2	6	8	8	1.4	1.4	4.2	5.9	5.2
3.1242	60.893	0	6	7	8	6	0.0	3.8	4.7	5.6	4.2
3.1276		4	4	5	5	6	2.8	2.8	3.5	3.5	4.2
3.2364		2	3	3	5	7	1.4	1.9	2.2	3.8	4.9
3.2997		3	4	4	10	11	1.7	2.5	2.4	6.8	7.7
Ave	58					**Ave %**	1.2	2.5	4.2	5.9	6.1
%RSD	0.00					**%RSD**	71.19	1.82	68.46	25.50	22.27

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	0	4	12	14	15	0	123	394	467	497
3.0918	2114	2	2	6	8	8	67	67	202	280	250
3.1242	2101	0	6	7	5	6	0	183	227	267	200
3.1276	2111	4	4	5	5	6	133	133	167	167	200
3.2364	2121	2	3	3	5	7	68	90	107	183	233
3.2997	2099	3	4	4	10	11	83	119	117	324	367
Ave	2109					**Ave %**	59	119	202	281	291
%RSD	0.14					**%RSD**	71.19	1.82	68.46	25.50	22.27

Membrane release of hydrous gel

Product: Hydrous gel
Batch no.:
Stability period: 25°C+60%RH / 3 Months
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 250 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium:	60% THF
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32°C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 60.804 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	44.056	15	21	22	23	30	18.4	25.8	27.0	28.2	36.8
3.0918	50.94	13	17	20	26	21	16.0	20.9	24.6	31.9	25.8
3.1242	53.604	19	21	29	30	34	23.3	25.8	35.6	36.8	41.7
3.1276		12	17	18	20	27	14.7	20.9	22.1	24.6	33.1
3.2364		16	21	23	26	33	19.6	25.8	28.2	31.9	40.5
3.2997		20	25	29	32	42	24.6	30.7	35.6	39.3	51.6
Ave	50					**Ave %**	19.4	25.0	28.8	32.1	38.3
%RSD	0.00					**%RSD**	15.79	9.84	14.89	17.20	19.25

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	15	21	22	23	30	879	1231	1290	1349	1759
3.0918	2114	13	17	20	26	21	762	997	1173	1524	1231
3.1242	2101	19	21	29	30	34	1114	1231	1700	1759	1993
3.1276	2111	12	17	18	20	27	704	997	1055	1173	1583
3.2364	2121	16	21	23	26	33	938	1231	1349	1524	1935
3.2997	2099	20	25	29	32	42	1173	1466	1700	1876	2462
Ave	2109					**Ave %**	928	1192	1378	1534	1827
%RSD	0.14					**%RSD**	15.79	9.84	14.89	17.20	19.25

Membrane release of Hydrous cream

Product:	Hydrous cream
Batch no.:	
Stability period:	25°C+60%RH / 3 Months
Container:	Plastic jar
API:	Kojic acid dipalmitate
Strength:	1% m/m
Analytical:	HPLC: Agilent 1100
Wavelength:	250 nm
Column:	Luna C18 (2), 5µm

Membrane release conditions:

Medium:	60% THF
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32°C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 40.5 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	60.1	10	30	116	163	187	6.7	19.8	77.7	109.2	125.3
3.0918	58.83	8	17	49	156	190	5.4	11.4	32.8	104.5	127.3
3.1242	61.075	7	12	29	120	210	4.7	8.0	19.4	80.4	140.7
3.1276	61.73	10	24	69	198	178	6.7	16.1	46.2	132.7	119.3
3.2364		8	19	55	169	60	5.4	12.7	36.9	113.3	40.2
3.2997		19	30	128	59	140	12.7	20.1	85.8	39.5	93.8
Ave	60					**Ave %**	6.9	14.7	49.8	96.6	107.8
%RSD	0.00					**%RSD**	43.55	0.91	8.07	36.07	14.61

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	10	30	116	163	187	320	947	3713	5217	5986
3.0918	2114	8	17	49	156	190	256	544	1568	4993	6082
3.1242	2101	7	12	29	120	210	224	384	928	3841	6722
3.1276	2111	10	24	69	198	178	320	768	2209	6338	5697
3.2364	2121	8	19	55	169	60	256	608	1760	5409	1921
3.2997	2099	19	30	128	59	140	608	960	4097	1888	4481
Ave	2109					**Ave %**	331	702	2379	4615	5148
%RSD	0.14					**%RSD**	43.55	0.91	8.07	36.07	14.61

Membrane release of Anhydrous ointment

Product: Anhydrous ointment
Batch no.:
Stability period: 25°C+60%RH / 3 Months
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 250 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: 60% THF
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 61.086 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	104.55	20	21	29	56	59	11.6	12.2	16.9	32.5	34.3
3.0918	105.89	16	27	34	53	60	9.3	15.7	19.8	30.8	34.9
3.1242	104.5	16	26	35	56	64	9.3	15.1	20.3	32.5	37.2
3.1276	105.45	17	28	42	66	78	9.9	16.3	24.4	38.4	45.3
3.2364		22	30	38	60	46	12.8	17.4	22.1	34.9	26.7
3.2997		16	25	40	45	61	9.3	14.5	23.2	26.2	35.5
Ave	105					Ave %	10.4	15.2	21.1	32.5	35.6
%RSD	0.00					%RSD	11.21	7.64	15.14	9.82	1.63

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	20	21	29	56	59	555	583	805	1555	1638
3.0918	2114	16	27	34	53	60	444	750	944	1471	1666
3.1242	2101	16	26	35	56	64	444	722	972	1555	1777
3.1276	2111	17	28	42	66	78	472	777	1166	1832	2165
3.2364	2121	22	30	38	60	46	611	833	1055	1666	1277
3.2997	2099	16	25	40	45	61	444	694	1110	1249	1693
Ave	2109					Ave %	495	726	1009	1555	1703
%RSD	0.14					%RSD	11.21	7.64	15.14	9.82	1.63

Membrane release of hydrous gel

Product:	Hydrous gel
Batch no.:	
Stability period:	40°C+60%RH / 3 Months
Container:	Plastic jar
API:	Kojic acid dipalmitate
Strength:	1% m/m
Analytical:	HPLC: Agilent 1100
Wavelength:	250 nm
Column:	Luna C18 (2), 5µm

Membrane release conditions:

Medium:	60% THF
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32°C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 60.804 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	76.86	8	21	22	23	16	5.9	16.6	17.4	18.2	12.7
3.0918	76.529	7	10	12	15	24	5.5	7.9	9.3	11.9	19.0
3.1242	76.679	9	11	12	14	25	7.1	8.7	9.5	11.1	19.8
3.1276	77.536	8	10	12	14	20	6.3	7.6	9.5	11.1	15.8
3.2364		8	11	18	20	21	6.3	8.7	14.2	15.8	16.6
3.2997		8	12	13	57	86	6.3	9.5	10.3	45.1	68.0
Ave	77					**Ave %**	6.3	9.8	11.7	18.8	25.3
%RSD	0.00					**%RSD**	3.16	36.16	30.44	71.33	109.38

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	8	21	22	23	16	283	793	831	869	604
3.0918	2114	7	10	12	15	24	264	378	442	566	906
3.1242	2101	9	11	12	14	25	340	415	453	529	944
3.1276	2111	8	10	12	14	20	302	365	453	529	755
3.2364	2121	8	11	18	20	21	302	415	680	755	793
3.2997	2099	8	12	13	57	86	302	453	491	2153	3248
Ave	2109					**Ave %**	299	470	558	900	1208
%RSD	0.14					**%RSD**	3.16	36.16	30.44	71.33	109.38

Membrane release of Hydrous cream

Product: Hydrous cream
Batch no.:
Stability period: 40°C+60%RH / 3 Months
Container: Plastic jar
API: Kojic acid dipalmitate
Strength: 1% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: 60% THF
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 60.75 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	105.16	18	23	92	106	136	10.0	13.0	52.0	59.9	76.9
3.0918	107.67	18	40	92	190	203	10.2	22.6	52.0	107.4	114.7
3.1242	106.93	23	26	50	81	100	13.0	14.7	28.3	45.8	56.5
3.1276	110.14	20	38	143	89	99	11.3	21.5	80.8	50.3	56.0
3.2364		20	26	82	129	148	11.3	14.7	46.4	72.9	83.7
3.2997		25	28	97	108	184	14.1	15.8	54.8	61.0	104.0
Ave	107					**Ave %**	11.7	17.1	52.4	66.2	82.0
%RSD	0.00					**%RSD**	17.70	8.29	2.70	0.85	16.55

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	18	23	92	106	136	478	621	2484	2862	3672
3.0918	2114	18	40	92	190	203	486	1080	2484	5130	5481
3.1242	2101	23	26	50	81	100	621	702	1350	2187	2700
3.1276	2111	20	38	143	89	99	540	1026	3861	2403	2673
3.2364	2121	20	26	82	129	148	540	702	2214	3483	3996
3.2997	2099	25	28	97	108	184	675	756	2619	2916	4968
Ave	2109					**Ave %**	557	814	2502	3163	3915
%RSD	0.14					**%RSD**	17.70	8.29	2.70	0.85	16.55

Anhydrous ointment

Product:	Anhydrous ointment
Batch no.:	
Stability period:	40°C+60%RH / 3 Months
Container:	Plastic jar
API:	Kojic acid dipalmitate
Strength:	1% m/m
Analytical:	HPLC: Agilent 1100
Wavelength:	250 nm
Column:	Luna C18 (2), 5µm

Membrane release conditions:

Medium:	60% THF
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32°C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 61.086 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	120.85	19	29	33	33	50	10.3	15.7	17.9	17.9	27.1
3.0918	102.92	19	21	29	41	50	10.3	11.4	15.7	22.2	27.1
3.1242	115.02	12	27	29	45	49	6.5	14.6	15.7	24.4	26.5
3.1276	112.3	9	24	27	42	51	4.9	13.0	14.6	22.8	27.6
3.2364		18	28	32	41	53	9.8	15.2	17.3	22.2	28.7
3.2997		11	22	32	44	55	6.0	11.9	17.3	23.8	29.8
Ave	113					**Ave %**	7.9	13.6	16.4	22.2	27.8
%RSD	0.00					**%RSD**	27.27	13.91	1.65	13.41	4.87

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	19	29	33	33	50	492	750	854	854	1294
3.0918	2114	19	21	29	41	50	492	543	750	1061	1294
3.1242	2101	12	27	29	45	49	310	699	750	1164	1268
3.1276	2111	9	24	27	42	51	233	621	699	1087	1319
3.2364	2121	18	28	32	41	53	466	724	828	1061	1371
3.2997	2099	11	22	32	44	55	285	569	828	1138	1423
Ave	2109					**Ave %**	379	651	785	1061	1328
%RSD	0.14					**%RSD**	27.27	13.91	1.65	13.41	4.87

Membrane release of cream A

Product: Hydrous gel
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.50%

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 20.74 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1269	387.09	217	346	599	923	1100	11.7	18.7	32.4	49.9	59.5
3.5602	386.64	205	356	618	971	1194	11.1	19.3	33.4	52.5	64.6
3.3459	372.79	205	351	626	962	1114	11.1	19.0	33.9	52.0	60.3
3.3457	387.09	186	322	557	900	1104	10.0	17.4	30.1	48.7	59.7
3.3793		200	353	640	934	1121	10.8	19.1	34.6	50.5	60.6
3.3139		183	310	556	928	1112	9.9	16.8	30.1	50.2	60.2
Ave	383					**Ave %**	10.8	18.4	32.4	50.7	60.8
%RSD	0.00					**%RSD**	8.48	5.30	3.59	0.27	0.53

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	217	346	599	923	1100	560	894	1548	2385	2842
3.0918	2114	205	356	618	971	1194	530	921	1597	2509	3085
3.1242	2101	205	351	626	962	1114	530	907	1617	2486	2878
3.1276	2111	186	322	557	900	1104	480	832	1439	2325	2852
3.2364	2121	200	353	640	934	1121	517	912	1654	2413	2896
3.2997	2099	183	310	556	928	1112	473	801	1437	2398	2873
Ave	2109					**Ave %**	515	878	1548	2419	2905
%RSD	0.14					**%RSD**	8.48	5.30	3.59	0.27	0.53

Membrane release of cream A

Product: Anhydrous gel
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 104.16 µg/ml

Sample mass(g)	Area STD	Area samples at time (min)					Mass released (µg/ml) in time (min.)				
		30	60	120	240	360	30	60	120	240	360
3.1124	1698.7	120	192	270	396	496	7.4	11.7	16.5	24.2	30.3
3.0918	1710.6	112	194	271	398	487	6.9	11.9	16.6	24.3	29.8
3.1242	1699.2	117	196	274	412	510	7.2	12.0	16.7	25.2	31.2
3.1276	1708.4	110	188	274	391	481	6.7	11.5	16.7	23.9	29.4
3.2364		130	207	297	432	549	7.9	12.7	18.2	26.4	33.6
3.2997		133	211	297	426	523	8.1	12.9	18.1	26.0	32.0
Ave	1704					**Ave %**	7.4	12.1	17.1	25.0	31.0
%RSD	0.00					**%RSD**	5.29	4.73	4.80	3.67	2.66

Sample mass(g)	Area STD	Area samples at time (min)					Release rate (µg/square cm) in time (min)				
		30	60	120	240	360	30	60	120	240	360
3.1124	2105	120	192	270	396	496	351	561	788	1156	1448
3.0918	2114	112	194	271	398	487	328	566	791	1162	1422
3.1242	2101	117	196	274	412	510	343	573	800	1203	1489
3.1276	2111	110	188	274	391	481	321	550	800	1141	1404
3.2364	2121	130	207	297	432	549	379	604	867	1261	1603
3.2997	2099	133	211	297	426	523	388	616	866	1244	1527
Ave	2109					**Ave %**	352	578	818	1194	1482
%RSD	0.14					**%RSD**	5.29	4.73	4.80	3.67	2.66

Membrane release of hydrous cream

Product: Hydrous cream
Batch no.:
Stability period: Initial
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 101.64 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	1740.6	52	80	117	174	407	3.0	4.7	6.8	10.1	23.7
3.0918	1741.8	55	79	113	189	417	3.2	4.6	6.6	11.0	24.3
3.1242	1742.8	58	73	124	249	405	3.4	4.2	7.2	14.5	23.6
3.1276	1766.6	56	80	126	212	395	3.3	4.7	7.3	12.3	23.0
3.2364	1745.3	59	82	132	226	380	3.4	4.8	7.7	13.1	22.1
3.2997		59	82	104	225	374	3.4	4.8	6.0	13.1	21.8
Ave	1747					**Ave %**	3.3	4.6	6.9	12.4	23.0
%RSD	0.00					**%RSD**	6.29	1.26	5.36	12.00	4.11

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	52	80	117	174	407	144	222	324	483	1130
3.0918	2114	55	79	113	189	417	151	219	314	525	1159
3.1242	2101	58	73	124	249	405	161	203	344	692	1125
3.1276	2111	56	80	126	212	395	156	222	350	589	1097
3.2364	2121	59	82	132	226	380	164	228	367	628	1056
3.2997	2099	59	82	104	225	374	164	228	289	625	1039
Ave	2109					**Ave %**	157	220	331	590	1101
%RSD	0.14					**%RSD**	6.29	1.26	5.36	12.00	4.11

Membrane release of cream A

Product: Hydrous gel
Batch no.:
Stability period: 25°C+60%RH / 3 Months
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 20.3µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	361.39	217	351	694	968	1090	12.4	20.1	39.7	55.3	62.3
3.0918	365.32	207	393	681	973	1119	11.8	22.5	38.9	55.6	64.0
3.1242	343.52	196	390	660	948	1287	11.2	22.3	37.7	54.2	73.5
3.1276	350.4	204	384	649	897	1082	11.7	21.9	37.1	51.3	61.8
3.2364		219	376	663	1007	1238	12.5	21.5	37.9	57.6	70.8
3.2997		210	380	646	998	1256	12.0	21.7	36.9	57.0	71.8
Ave	355					**Ave %**	11.9	21.7	38.0	55.2	67.4
%RSD	0.00					**%RSD**	1.82	3.85	3.63	1.57	7.05

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	217	351	694	968	1090	593	958	1895	2642	2975
3.0918	2114	207	393	681	973	1119	565	1072	1858	2656	3055
3.1242	2101	196	390	660	948	1287	535	1065	1802	2588	3513
3.1276	2111	204	384	649	897	1082	558	1048	1772	2449	2953
3.2364	2121	219	376	663	1007	1238	598	1026	1809	2750	3380
3.2997	2099	210	380	646	998	1256	572	1037	1763	2725	3429
Ave	2109					**Ave %**	570	1034	1816	2635	3217
%RSD	0.14					**%RSD**	1.82	3.85	3.63	1.57	7.05

Membrane release of Hydrous cream

Product: Hydrous cream
Batch no.:
Stability period: 25°C+60%RH / 3 Months

Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm

Column: Luna C18 (2), 5µm

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
200 µl
Amount withdraw:
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 20.316 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	357.99	69	101	169	241	292	4.0	5.8	9.7	13.8	16.7
3.0918	353.43	64	107	173	233	298	3.7	6.1	9.9	13.3	17.1
3.1242	351.44	61	105	176	264	320	3.5	6.0	10.1	15.1	18.3
3.1276	350.68	70	117	180	276	339	4.0	6.7	10.3	15.8	19.4
3.2364	360.48	72	121	189	244	297	4.1	6.9	10.8	14.0	17.0
3.2997		69	129	194	280	325	4.0	7.4	11.1	16.0	18.6
Ave	355					**Ave %**	3.9	6.5	10.3	14.7	17.8
%RSD	0.00					**%RSD**	0.34	12.44	6.94	7.60	5.36

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	69	101	169	241	292	190	276	462	659	797
3.0918	2114	64	107	173	233	298	175	293	473	637	815
3.1242	2101	61	105	176	264	320	168	287	481	723	874
3.1276	2111	70	117	180	276	339	190	320	492	755	927
3.2364	2121	72	121	189	244	297	197	331	517	667	811
3.2997	2099	69	129	194	280	325	189	353	531	766	889
Ave	2109					**Ave %**	185	310	493	701	852
%RSD	0.14					**%RSD**	0.34	12.44	6.94	7.60	5.36

Membrane release of hydrous gel

Product: Hydrous gel
Batch no.: (25)1
Stability period: 40°C+75%RH / 3 Months
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 200 µl
Dilution: None
Apparatus: VanKel 700
Temperature: 32°C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 20.68 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	368.37	170	263	415	662	923	9.6	14.9	23.5	37.5	52.3
3.0918	368.2	202	309	549	920	1070	11.4	17.5	31.1	52.1	60.6
3.1242	360.9	192	278	495	835	984	10.9	15.8	28.0	47.3	55.8
3.1276	364.36	202	306	503	790	993	11.4	17.3	28.5	44.7	56.2
3.2364	362.62	193	308	478	806	982	10.9	17.5	27.1	45.7	55.6
3.2997	365.46	200	311	496	863	1056	11.3	17.6	28.1	48.9	59.8
Ave	365					**Ave %**	10.9	16.8	27.7	46.0	56.7
%RSD	0.40					**%RSD**	7.87	8.05	8.28	12.37	6.62

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	170	263	415	662	923	459	713	1123	1792	2498
3.0918	2114	202	309	549	920	1070	547	836	1486	2490	2896
3.1242	2101	192	278	495	835	984	520	752	1340	2260	2663
3.1276	2111	202	306	503	790	993	547	828	1360	2137	2686
3.2364	2121	193	308	478	806	982	521	834	1294	2181	2658
3.2997	2099	200	311	496	863	1056	541	842	1342	2335	2856
Ave	2109					**Ave %**	522	801	1324	2199	2709
%RSD	0.14					**%RSD**	7.87	8.05	8.28	12.37	6.62

Membrane release of Hydrous cream

Product: Hydrous cream
Batch no.:
Stability period: 40°C+60%RH / 3 Months
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 0.5% m/m

Analytical: HPLC: Agilent 1100
Wavelength: 255 nm
Column: Luna C18 (2), 5µm

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	200 µl
Dilution:	None
Apparatus:	VanKel 700
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 20.188 µg/ml

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Mass released (µg/ml) in time (min.) 30	60	120	240	360
3.1124	354.28	66	97	136	194	239	3.8	5.6	7.8	11.2	13.8
3.0918	347.75	87	99	153	237	239	5.0	5.7	8.8	13.7	13.8
3.1242	346.42	68	101	147	180	244	3.9	5.8	8.5	10.4	14.1
3.1276	349.76	64	92	136	183	225	3.7	5.3	7.9	10.6	13.0
3.2364	347.36	77	92	146	207	269	4.4	5.3	8.4	12.0	15.5
3.2997	351.77	57	88	151	152	243	3.3	5.1	8.7	8.8	14.0
Ave	350					**Ave %**	4.0	5.5	8.4	11.1	14.0
%RSD	0.36					**%RSD**	6.38	4.75	5.35	10.93	0.82

Sample mass(g)	Area STD	Area samples at time (min) 30	60	120	240	360	Release rate (µg/square cm) in time (min) 30	60	120	240	360
3.1124	2105	66	97	136	194	239	182	268	374	535	659
3.0918	2114	87	99	153	237	239	239	273	422	654	659
3.1242	2101	68	101	147	180	244	188	279	405	497	673
3.1276	2111	64	92	136	183	225	177	254	375	505	621
3.2364	2121	77	92	146	207	269	212	254	403	571	742
3.2997	2099	57	88	151	152	243	157	243	417	419	670
Ave	2109					**Ave %**	192	262	399	530	671
%RSD	0.14					**%RSD**	6.38	4.75	5.35	10.93	0.82

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Release studies of skin lighteners containing kojic acid dipalmitate

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Kojic acid is known to have the ability to cause products, in which it is present, to discolour (Smith, 2001:1). Converting kojic acid into the ester, increases its stability to pH, heat and light. Kojic acid dipalmitate is a mild active ingredient for skin lightening products (Fox, 2005:36). When using kojic acid dipalmitate in formulations, product stability is ensured without any colour instability problems (Chemos, 2005:1). In a study by Majmudar *et al.* (1998:366), the increased stability of kojic acid in an anhydrous base, was proven. Whittemore & Neis (1998:1) also reported that kojic acid dipalmitate would indefinitely keep its whiteness in an anhydrous cosmetic base and would maintain its skin brightening activity. This increases its commercial value over hydrous formulations.

Release studies were carried out with the enhancer cell unit, which was used on the VanKel 700 dissolution apparatus. The release studies were done on hydrous and anhydrous formulations, containing kojic acid dipalmitate, in order to compare the stability of these formulations. The formulations were subjected to a stability test period of three months, and storage at 25°C + 60% RH and 40°C + 75% RH.

The release rates of a hydrous - and anhydrous gel, and a hydrous lotion and anhydrous ointment were tested. The tests were done on each formulation at onset of stability (initial) and after 3 months for storage conditions of 25°C + 60% RH and 40°C + 75% RH.

Figures 1 and 2 show the release rates of the hydrous gel and of the anhydrous gel at initial and after 3 months at 25°C + 60% RH and 40°C + 75% RH.

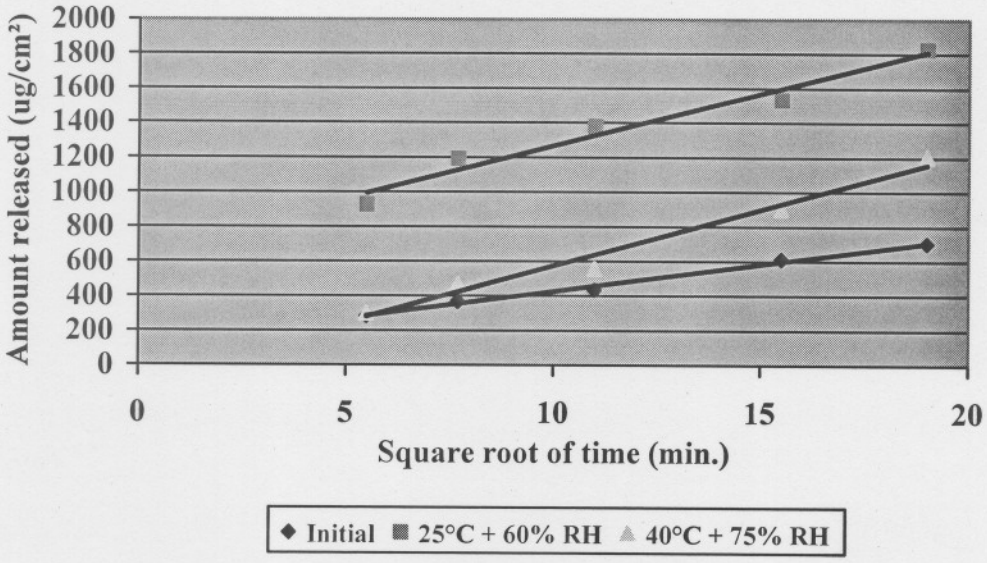

Figure 1 Release rate of kojic acid dipalmitate from the hydrous gel.

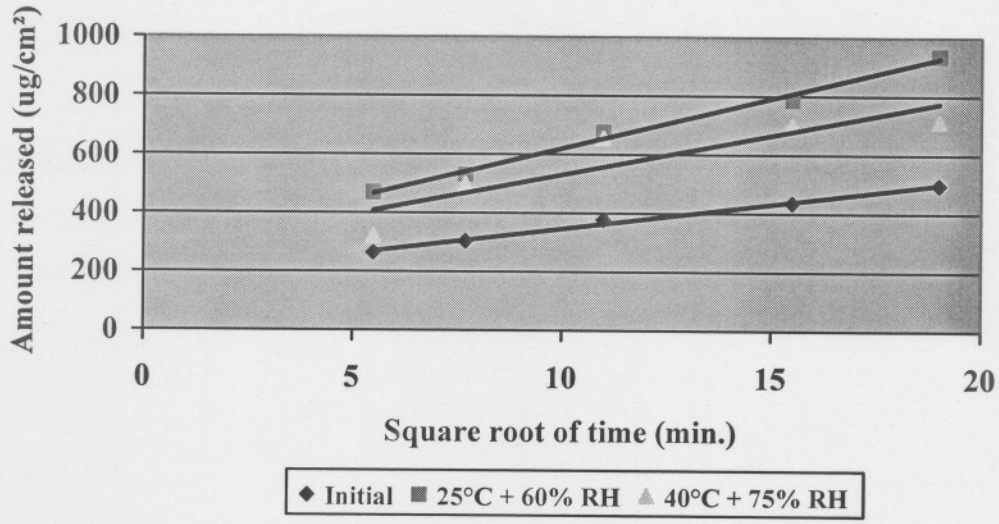

Figure 2 Release rate of kojic acid dipalmitate from the anhydrous gel.

It was found that kojic acid dipalmitate was significantly released in both the hydrous and anhydrous formulations, including those formulations being subjected to high temperatures (40°C + 75% RH). The release of kojic acid dipalmitate increased with an increase in storage temperature, which is questionable.

Experimental

For each release test, the test samples were carefully transferred into six enhancer cells (approximately 3 g of sample per enhancer cell). Care was taken to ensure that no air bubbles were present. Each enhancer cell was wiped clean, to ensure that no sample would

penetrate, except through the membrane. Then the enhancer cells were each covered with a cellulose acetate membrane, with a 0.45 μm pore size. 200 ml vessels were used filled with 190 ml of release medium (60% tetrahydrofuran). The medium was heated to 32 ± 0.5°C. When the required temperature was reached, the enhancer cells were carefully descended into the vessels, at fixed time intervals. The paddles rotated at 100 rpm. Each release test was carried out for 360 minutes, with 200 μl withdrawals done at 30, 60, 120, 240 and 360 minutes.

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APPENDIX D
GENERIC AND TRADE NAMES

TRADE NAME	GENERIC NAME	MANUFACTURER/ SUPPLIER
Aerosil	Colloidal silicon dioxide	FMC, United States of America
Ascorbyl palmitate	Ascorbyl palmitate	Sigma-Aldrich Laborchemikallen
Cethyl alcohol	Cethyl alcohol	Merck chemicals, South Africa
Chremophor-A6	Ceteareth-6 & stearyl alcohol	BASF, Germany
Chremophor-A25	Ceteareth-25	BASF, Germany
Glycerin	Glycerin	Merck chemicals, South Africa
Hydroxypropylmethylcellulose	HPMC (65 HG)	Fluka, South Africa
Kojic acid dipalmitate	Kojic acid dipalmitate	Spec-Chem, South Africa
Liquid paraffin	Liquid paraffin	Merck chemicals, South Africa
Methyl-4-hydroxybenzoate	Methyl-4-hydroxybenzoate	Sigma-Aldrich Laborchemikallen
Polyethylene glycol	PEG 400	Merck chemicals, South Africa
Polyethylene glycol	PEG 4000	Merck chemicals, South Africa
Polysorbate	Tween 80	Atlas Chem
Propan-2-ol	Propan-2-ol	Merck chemicals, South Africa
Propylene glycol	Propylene glycol	Merck chemicals, South Africa
Propylparahydroxy benzoate	Propylparahydroxy benzoate	Warren Chem Specialities, South Africa
Sodium ascorbyl phosphate	Stay-C	Roche vitamins