FORMULATION AND EVALUATION OF SODIUM ASCORBYL PHOSPHATE AND KOJIC ACID CONTAINING PRODUCTS

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"Curiosity, like coffee, is an acquired need. Just a titillation at the beginning, it becomes with training a raging passion."

-Nicholas S. Thompson

To my loving parents

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ABSTRACT

The **skin**, our main defence against harmful substances such as wind, dirt, bacteria and ultraviolet radiation has also the important functions of preventing water loss, regulating temperature and receiving external stimuli. Skin **colour** varies depending on racial background, sex and the season of the year due to the exposure to sunlight. Skin colour is primarily determined by the amount of melanin produced by the melanocytes. For this reason, research for the development of **whitening products** has focused on reducing melanin production in the melanocytes, rather than bleaching of the skin.

Skin-whitening products have been widely used in the cosmetic field and clinic therapy. They either **lighten** the skin or **depigment** skin (treatment for abnormal hyperpigmentation of the skin such as freckles and melasma). Whitening agents, such as hydroquinone, **kojic acid** and **ascorbic acid** derivatives have shown efficacy in treatment of hyperpigmentation.
In this study, sodium ascorbyl phosphate and kojic acid were used as the active ingredients in skin lightening products.

Sodium ascorbyl phosphate acts as an in-vivo antioxidant, promotes collagen formation, and lightens the skin. It is a stable vitamin C derivate that protects the skin, promotes its development and improves its appearance. Kojic acid successfully fights age spots and pigmentation on face and body.

The product development program started with a literature search and a preformulation study. Existing basic formulations were used and modified to incorporate both active ingredients in a variety of skin lightening products. Stability testing followed, based on the requirements of the South African Medicine Control Council for new products.

Six skin lightening products were formulated, i.e. two **facial creams**, a **toner**, a **gel**, a **foam bath**, and a **soap**. After formulation these products were tested for their stability over a period of three months at three different storage temperatures and humidity (5°C, 25 °C + 60% RH and 40 °C + 75% RH).

UITTREKSEL

Die **vel**, ons eerste linie van beskerming teen skadelike stowwe soos wind, vuilhede, bakterië en ultraviolet strale, het ook die noodsaaklike funksies om vogverlies te voorkom, regulering van liggaamstemperatuur, en die ontvangs van eksterne stimuli. **Velkleur** wissel tussen rasse, geslag of die seisoen van die jaar afhangende van die blootstelling aan sonlig. Velkleur word hoofsaaklik bepaal deur die hoeveelheid melanien wat geproduseer word deur melanosiete, en om hierdie rede het navorsing begin fokus op die vermindering van melanien produksie in die melanosiete, eerder as bleiking van die vel.

Velbleikingsprodukte word algemeen gebruik in die kosmetiese veld en kliniese terapie. Dit word aangewend om die vel **ligter** te maak of te **depigmenteer** (behandeling van abnormale hiperpigmenteerde vel soos sproete en melasma). Bleikmiddels soos hidrokinoon, **kojiese suur** en **askorbiensuur** derivate is effektief bewys in behandeling van hiperpigmentasie.
In hierdie studie word kojiese suur en natriumaskorbielfosfaat as aktiewes aangewend in 'n reeks depigmenteringsprodukte.

Natriumaskorbielfosfaat word in-vivo aangewend as anti-oksidant, verhoger in kollageen vorming, en velverbleiker. Dit is 'n stabiele vitamien C derivaat wat die vel beskerm, die vorming daarvan stimuleer en die voorkoms verbeter. Kojiese suur beveg ouderdomsvlekke en ongewenste pigmentasie van die gesig en liggaam suksesvol.

Die produk-ontwikkelingsprogram het begin deur 'n literatuurstudie en preformuleringsstudie. Bestaande basiese formules is verander om beide aktiewe bestandele in verskeie depigmenteringsprodukte in te sluit. Stabiliteitstoetse wat gebaseer is op die vereistes van die Suid-Afrikaanse Medisynebeheerraad vir nuwe produkte, het gevolg.

Ses depigmenteringsprodukte is geformuleer, naamlik twee **gesigsrome,** 'n **velverfrisser,** 'n **gel,** 'n **badskuim en 'n seep**. Na formulering is hierdie produkte getoets vir hul stabiliteit oor 'n periode van drie maande teen drie verskillende temperature en humiditeite (5°C, 25°C + 60% RH and 40°C + 75% RH).

AIM AND OBJECTIVES

The increased demand for cosmetics, intended to lighten the skin, by people of colour, causes an increase in the number of preparations available and the need for manufacturers to increase the supply of these skin lighteners. For example, according to Lee and Kim (1995:55) anti-aging and skin whitening constitute the largest market segments of skin-care products in Korea.

For women freckles, sun stains, melasma and any form of hyper pigmentation are of serious matter, even if medical treatment is not necessary. An experienced old Japanese dermatologist in Kyoto City often told melasma patients that they do not have to treat their melasma if they intend to live past the age of 70, for it would have disappeared by then! (Due to the decrease in oestrogen). As we all know, women want immediate results, and luckily for them, there are recently researched products that have proved to be safe, efficient, and cost effective.

Kojic acid [5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one] and sodium ascorbyl phosphate [L-ascorbic acid-2-monophoshate] are two actives that are giving positive results in skin lightening and there is a great demand in the market for satisfactory skin preparations containing these ingredients.

The main objectives of this study include:

- To develop a variety of skin lightening products containing both the actives for a greater result that still are safe and effective.

- Formulation of a facial cream, gel, foam bath, astringent lotion and soap which contain both actives.

- To formulate an oil-in-water cream that contains the two actives to comply with different skin types.

- To formulate an oil-in-water cream that contains only the sodium ascorbyl phosphate.

- To study different gel formulations and choose the most complying one.

- The physical and chemical evaluation of all the products.

- To test the stability of the actives at different temperatures and humidities over a stability period of three months.

- To develop and validate a HPLC method for the simultaneous determination of kojic acid and sodium ascorbyl phosphate in cosmetic formulations.

CHAPTER 1
INTRODUCTION TO
SKIN LIGTENERS

1.1 Introduction

For many centuries, man has tried to artificially colour or bleach his skin due to many reasons like his philosophy, religion or simply the envy of another. Recorded history has many stories of how man used plant juices, fruits, especially the citrus variety where the lemon is still being used. Compounds like ammoniated mercury and hydroquinone bleaches were also common regardless the harm that was done due to massive overuse of the agents.

At present people are using harmful cortisone creams that not only lighten the skin but also damage the skin permanently and make it extremely vulnerable to sunlight. There are so many alternatives to be used where kojic acid and sodium ascorbyl phosphate are the main agents discussed here, but before we can come to that, a brief overview of the anatomy of the skin and his functions, as well as pigmentation, is necessary (McGuire, 1972:423).

1.2 The skin and its functions

The skin forms the frontier of the body, separating the external environment from the internal organs. The barrier between the external world, with its most varied conditions of temperature and humidity, and the stable internal environment of the living tissues and body fluids are the epidermis. The skin is therefore the physical protector of internal

organs and has the functions such as sensation, body temperature control, and the provision of a barrier which limits the penetration of substances into and out of the body.

The skin has two different but dependent tissues: *the cellular epidermis* and underlying connective tissues of the *dermis and hypodermis*. The hair follicles, sebaceous glands, and sweat glands are in the dermis. The dermis contains a variety of cells derived from the *mesoderm.* The fibroblasts are the most numerous of these and they are responsible for synthesising the fibrous proteins, collagen and elastin. The deepest part of the skin is the *hypodermal adipose tissue* which is composed of fat-laden cells. We are going to concentrate on the *outer layers of the skin, there where hyperpigmentation occurs and melanin is formed* (McGuire, 1972:421).

1.3 The pigmentary system

Although the dermal blood vessels and yellow hypodermal fat contribute to skin colour, the most important skin pigment is melanin, the yellow, brown or black material which is found almost exclusively in the epidermis.

The melanin pigmentary system is composed of functional units called epidermal melanin units. Each unit consists of a melanocyte that supplies melanin pigment to a group of keratinocytes. Pigmentation is determined primarily by the amount of melanin transferred to the keratinocytes (Electronic Textbook of Dermatology, 1998).

Skin pigmentation depends upon the organisation and function of epidermal melanin unit and several separate but related events:

- Melanoblast migration from the neural crest.
- Melanoblast differentiation into melanin cells.
- The rate of synthesis and melanisation of melanosomes.
- The size of melanosomes.

- Synthesis of melanin.
- The efficacy of melanosome transfer into keratin layer.
- The rate of melanosomes degradation within the keratin layer.
- The rate of synthesis and inhibition of the tyrosinase enzyme.
- Activity of tyrosinase in melanosomes (Avre skin care, 2004:1).

Skin colour varies depending on racial background, season of the year, sex and many more factors. The various human races have roughly the same number of melanocytes, cells which are responsible for forming and secreting the structured specialised brown organelles, melanosomes (packets of melanin), but dark-skinned people have more *active* cells. In black skin, there is greater production of melanosomes, higher degree of melanisation of melanosomes, and larger unaggregated melanosomes showing slower rates of degradation. In general, human skin colour appears darker near the equator where the ultraviolet light is more intense and the skin needs more protection than in the European countries. Inherited albinism has a biochemical block to melanin formation and they are extremely vulnerable to sunlight (Electronic Textbook of Dermatology, 1998).

According to Avre Skin Care (2004:1) the differences in racial skin pigmentation depend on the quality of melanin pigments produced and on the distribution and the deposition of these pigments throughout the epidermis, and also the activity of the tyrosinase (the only enzyme absolutely required for melanin production) in the melanin cells from varying skin types. Moreover, melanocytes are spider-shaped cells with long irregular arms that extend from the cell body. The arms of each melanocyte link it with surrounding skin cells. They produce pigment granules, melanosomes, which release melanin into these neighbouring cells. As the skin generates, these neighbouring cells migrate towards the skin surface and carry the pigment with them. In this way melanin is spread across the skin to give its characteristic colour. Radiation from the sun stimulates melanocytes to produce more melanin and results in skin tanning (Oceanhealth, 2003).

Skin colour is also affected to a large extent by the state of its blood circulation. The external horny layer, keratin, which is a dead protein substance, has an indigenous colour and can alter the skin appearance depending on its thickness. The importance of melanin, formed by melanocytes, is undeniable, and therefore research for the development of whitening products has focused on *reducing melanin production in the melanocytes* (Lee & Kim, 1995:51).

Three mechanisms mediate the melanocytic contribution to integumentary colour, as described in Figure 1.1:

1. Melanogenesis
    a. Synthesis of melanosomes.
    b. Catalytic oxidation of tyrosine to melanin, initiated by tyrosinase.
2. Intramelanocytic translocation of pigment granules.
3. Transfer of pigment granules from melanocytes to keratinocytes.

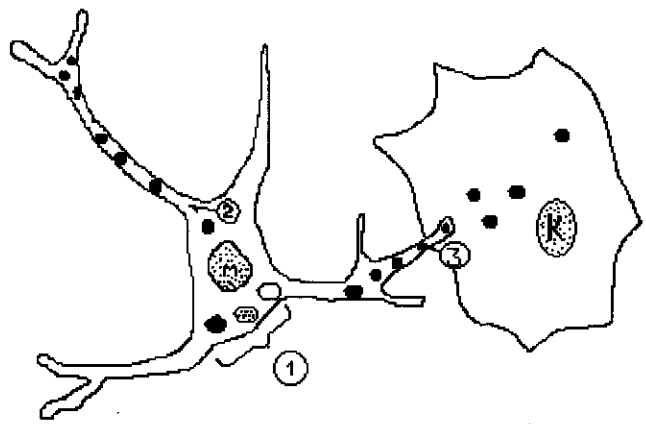

Figure 1.1 Diagram of a melanocyte (M) and keratinocyte (K) illustrating the three general mechanisms by which the melanocyte contributes to skin colour. (1) Tyrosinase is synthesised on the endoplasmic reticulum and transferred to stage 1 melanosome, which then serves as the site for melanin synthesis, that results in a fully melanised melanosome or pigment granule. (2) Intramelanocytic translocation of pigment granules.

The granules assume a perinuclear location or are dispersed through the cell, filling the dendritic processes in the darkened cell. (3) Pigment granule transfer. The dendritic tip is introduced into a keratinocyte (K) into which a packet of granules is transferred (McGuire,. 1972:422).

1.3.1 Melanin, the skin pigment

Melanin is light-absorbing and protects the skin against ultraviolet rays. Two major forms of melanin exist in humans: (1) Eumelanin, a brown to black pigment synthesised from indole-5,6-quinone and found within the ellipsoid melanosomes; and (2) phaemelanin, a yellow-red pigment found within the spherical melanosomes (Electronic Textbook of Dermatology, 1998).

According to Van Abbé *et al.* (1969:48) melanin is an oxidation product of the amino acid *tyrosine,* and it is always bound to a protein matrix. The pigment is deposited as insoluble dark brown granules in the cytoplasm of the synthesising cells, and screens the living skin cells against the adverse effects of ultraviolet irradiation in sunlight, and 'caps' of melanin granules protect the epidermal cell nuclei.

1.3.2 Tyrosinase, the enzyme behind the dark skin

Tyrosinase is a multifunctional, glycosylated, copper-containing oxidase with a molecular weight of approximately 60 to 70 kDa. It is synthesised in melanosomal ribosomes found on the rough endoplasmic reticulum. After synthesis, tyrosinase is glycosylated within Golgi, and then delivered to melanosomes via coated vesicles. Tyrosinase is a rate-limiting, essential enzyme in the biosynthesis of the skin pigment melanin. The rate limiting steps in melanogenesis are the oxidation of tyrosine and DOPA. Thus, the quantity of melanin synthesised is proportional to the amount of tyrosinase activity present in the cell (Avre skin care, 2004:1).

1.3.3 The synthesis route of melanin

The melanocytes synthesise melanin, using tyrosinase to hydroxylate tyrosine into dihydroxy phenylalanine (**DOPA**); this becomes the melanin polymer through a complex chain of oxidase reactions, see Figure 1.2. Tyrosinase also oxidizes **DOPA** into dopaquinone. Pheomelanin, a yellow or orange pigment, is synthesised via cysteinyl **DOPA**, glutathione **DOPA** and cysteinyl dopaquinone, in the presence of sulfhydryl (**-SH**) compounds like cysteine and glutathione. Eumelanin, the dark-brown pigment, is produced through the polymerisation of dopaquinone via leucodopachrome; dopachrome; 5,6-dihydroxyindole (or 5,6-dihydroxyindole-2-carboxylic acid, **DHICA**); and melanochrome. Tyrosinase plays the key role in melanin biosynthesis (Lee & Kim, 1995:51).

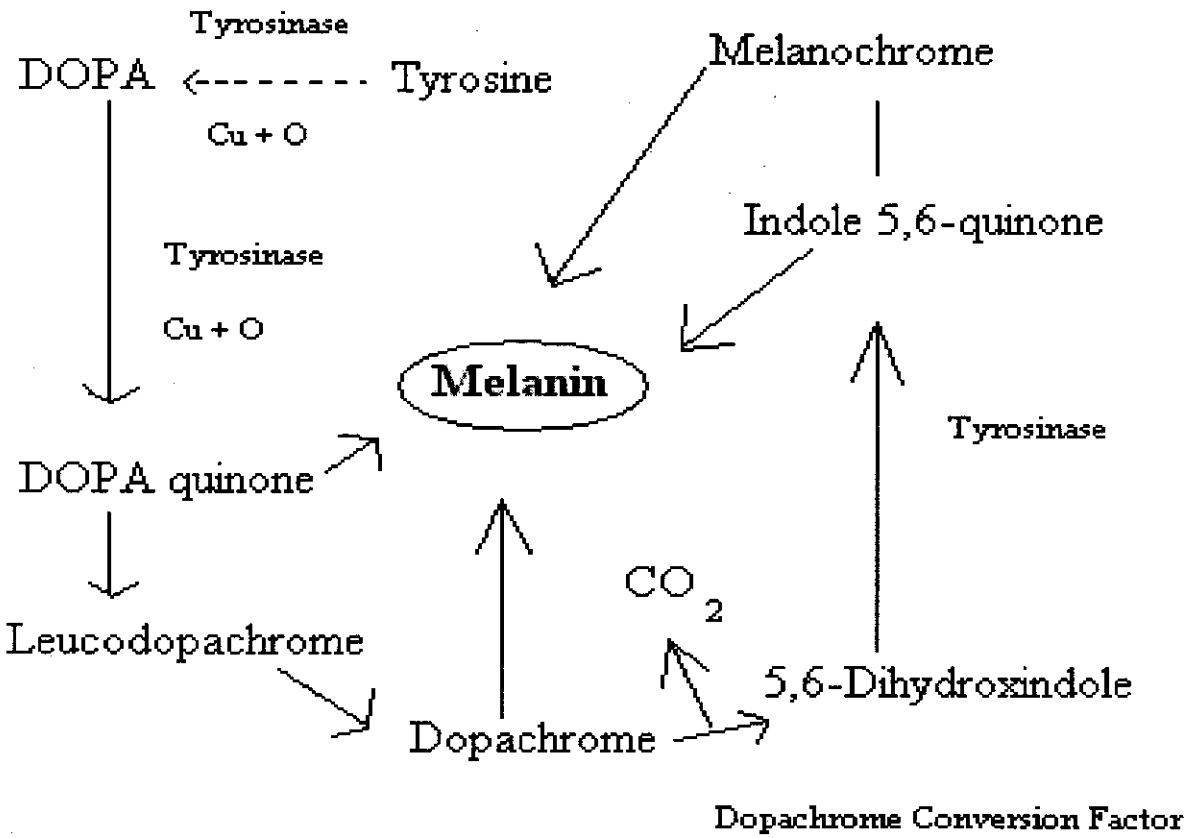

Figure 1.2. Melanin synthesis pathway (Electronic Textbook of Dermatology, 1998).

Tyrosinase is synthesised by the ribosomes of the rough endoplasmic reticulum (rER) and transported through the smooth endoplasmic reticulum (sER) to the Golgi apparatus. It is then released within membrane-bound vesicles. Meanwhile, structural melanosomal proteins are also synthesised on the rER and then incorporated into vesicles at the sER. Fusion of the two types of vesicles (tyrosinase and structural melanosomal proteins) results in the formation of a melanosome. As the melanosome matures and more melanin is deposited on its lamellar matrix, it passes into the dendrite of the melanocyte (Electronic Textbook of Dermatology, 1998).

1.4 Causes of skin hyperpigmentation

Skin hyperpigmentation can arise from various causes. Certain systemic and skin diseases can cause melanocytes to be overactive, resulting in darkening of the skin. Genetic predisposition to high activity, endocrine abnormalities, injuries, skin cancers, contraceptives, pregnancy, and agents that have an affinity for melanin such as chlorpromazine and hydroxychloroquine are also common causes for hyperpigmentation. As there are many possible causes of hyperpigmentation, these conditions can be complex and may involve various treatment options. In severe cases, surgical or laser treatment may be the only solution, but freckles, melasma, lentigines and post-inflammatory pigment may be treated effectively with topical agents such as kojic acid or sodium ascorbyl phosphate. Refer to Table 1.1 for examples of pigmentary skin disorders (Ocean Health, 2003).

Table 1.1: Pigmentary Skin Disorders of the Face, Nakayama *et al.* (2000:124).

A. Acquired

1. Melasma (chloasma)
2. Solar lentigo
3. Pigmented cosmetic dermatitis
4. Sun tanning
5. Tattoo
6. Onchronosis
7. Pigmentation due to atopic dermatitis
8. Phototoxic hyperpigmentation (Berloque dermatitis)
9. Posttraumatic hyperpigmentation
10. Others (lichen planus cum pigmentatione, pigmentsyphilis, etc.)

B. Hereditary

1. Nevus pigmentosus
2. Nevus spilus
3. Nevus of Ota
4. Ephelid

C. Skin Tumours

1. Melanoma
2. Basal cell carcinoma/epithelioma
3. Spitz nevus
4. Solar keratosis
5. Bowen's disease
6. Blue nevus
7. Others

1.5 Conclusion

To compensate for the fast growing cosmetic field and the global awareness of new hope in treatment of skin blemishes, manufacturers are spending a great deal of time and money on improvement of existing formulas and developing new products. The skin-care products dominate the Korean cosmetics market, especially skin lightening.

As was shown, melanin is primarily responsible for skin colour, thus the most effective, but safest way to treat hyperpigmentation is to focus on the melanin synthesis. Kojic acid and sodium ascorbyl phosphate work directly in on the melanin synthesis, therefore preventing hyperpigmentation before it becomes a real problem. These actives do not have the negative effect of skin thinning, for its purpose is not to destroy the melanocytes by harsh chemical methods, rather by inhibiting tyrosinase in melanocytes, thus preventing further pigmentation. Existing marks disappear after a period of time as the skin renews itself and no new pigmentation has occurred in the affected area.

CHAPTER 2
KOJIC ACID AND SODIUM ASCORBYL PHOSPHATE AS SKIN LIGHTENERS

2.1 Introduction

Although kojic acid and sodium ascorbyl phosphate are well known for their depigmentation characteristics, there are still a distinguished demand for satisfying skin care products containing them. In this chapter an overview of their history and properties are sure to give a fair motive for the formulation of the variety of products that follows.

2.2 History

After the first cure for melasma was discovered in 1977, a group started to develop a cream containing a melanogenesis inhibitor, a depigmentation agent. The Ministry of Health and Welfare of Japan rejected the 1% hydroquinone cream, because at that time, it was believed to be the cause of leukomelanoderma (skin spotting that becomes permanently white and affects the whole body, like vitiligo). Therefore, among the known chemicals that were tyrosine inhibitors, kojic acid was selected as the new depigmentation agent, because of its extremely long history of safe ingestion. In 1988 kojic acid has increasingly been used as a skin depigmenting agent in Japan. In Japanese, kojic means ferment and had been used to brew Japanese liquor (sake) made from rice (Nakayama *et al.,* 2000:140). According to Sino Lion (USA), Ltd (2003:5) kojic acid was the first well-received skin lightener after hydroquinone.

The ascorbyl acid derivatives, like ascorbyl palmitate or ascorbyl stearate, have been used for 20 years as primary depigmenting materials, in concentrations of 2 to 3%. However, because of their unstableness, salts of ascorbyl phosphate now replace other ascorbates in most products (Lee & Kim, 1995:54).

Collaborative Laboratories has formulated a product called Melarrest™A (patent pending), which consists out of kojic acid and ascorbic acid. They stated that the complex works at two levels: it inhibits the production and appearance of the pigment and stimulates migration of cells to the surface through gentle exfoliation, reducing the concentration of melanin in the epidermis.

An *in vivo* study was done on Melarrest™A by Collaborative Laboratories (Collabo, 2004), 5 female volunteers aged 25-50 with mild to moderate uneven skin tone and brown spots were selected by a dermatologist and enrolled in a 4-week clinical study. A formulation containing 5% Melarrest™A was used twice daily by each volunteer on a selected skin area. Photographs of the skin were taken before the first application and at the end of the study (Figure 2.1). These photographs were scanned and analysed using a novel digital image analysis technique to compare and determine the effectiveness of Melarrest™A in promoting even skin tone and reducing the intensity of brown spots.
In Figure 2.2 the reduction in dark pixels is shown and Figure 2.3 shows the tyrosine inhibition.

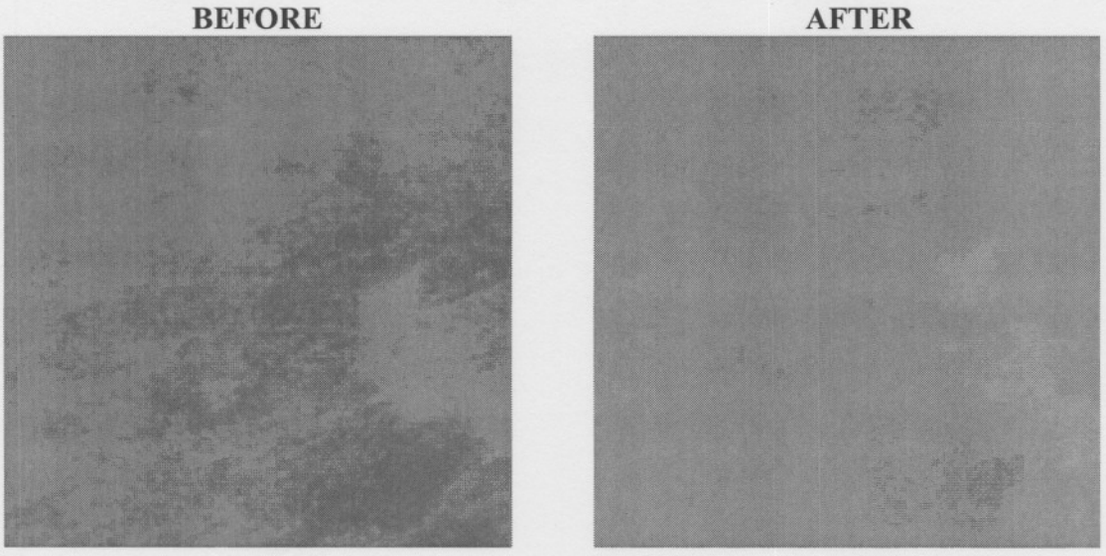

Figure 2.1 Photographs of the skin before the first application and at the end of the study (Collabo, 2004).

The post-treatment skin image contains less dark-intensity pixels and more light-intensity pixels indicating a reduction in the intensity of brown spots in skin treated with Melarrest™A. Findings of Collaborative Laboratories indicate an overall reduction of greater than 30%.

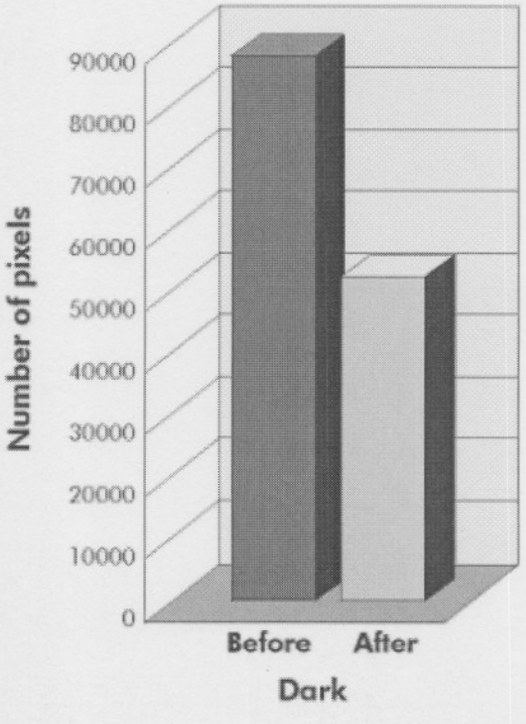

Figure 2.2 Reduction in the intensity of brown spots in skin treated with Melarrest™A (Collabo, 2004).

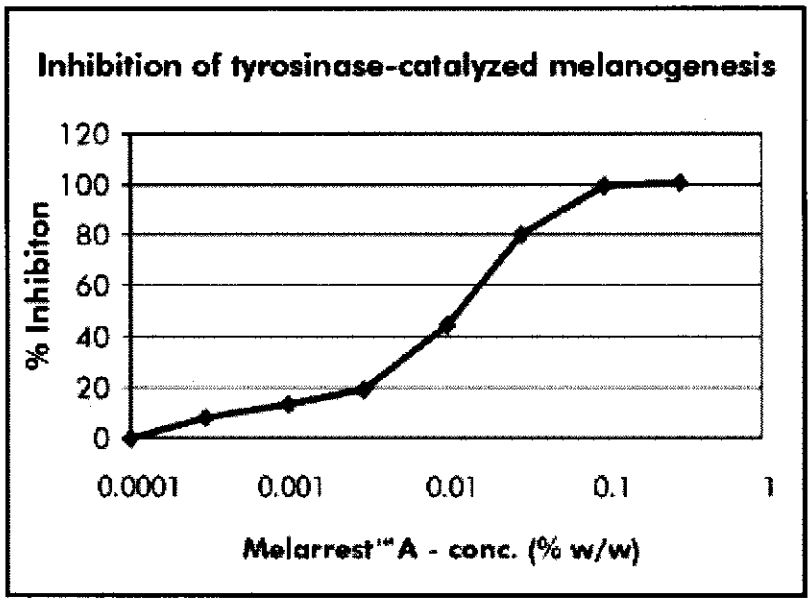

Figure 2.3 Inhibition of tyrosinase-catalyzed melanogenesis represented as concentration against % inhibition (Collabo, 2004).

In tyrosinase activity determinations, Melarrest™A shows >95% inhibition of tyrosinase activity at levels as low as 0.03% w/w. In a melanocyte cell based assay, whitening potential was also shown between 0.03% - 0.08%.

In this study kojic acid and sodium ascorbyl phosphate were also combined, but in five different preparations: a facial cream, foam bath, lotion, facial soap, and a gel. Stability tests were done on each formulation over a period of three months. The aim was not to copy Melarrest™A, but different formulas and methods were used in the preparation of the products. The compatibility of this combination of skin lightening actives was tested in different formulations and also the stability over a period of three months at three different temperatures and humidity conditions.

2.3 Pharmacological actions

Kojic acid blocks the catalytic action of the tyrosinase enzyme to prevent the conversion of tyrosine into melanin, by chelating copper ions that are indispensable for tyrosinase (Nakayama *et al.*, 2000:140).

Sodium ascorbyl phosphate blocks the auto-oxidation of DOPA and dopaquinone during the intermediate process of melanin synthesis. Sodium ascorbyl phosphate can also be used as an antioxidant, and promoter of collagen formation, thus it counter acts skin ageing (Anon., 1999:2). Because of its capability to suppress pigmentation of the skin and decomposition of melanin it can be used to whiten the skin and improve the elasticity of the skin.

A study that was done by Nakayama *et al.* (2000:130) showed that *Streptomyces fervens* produces melanin when it is cultured in a liquid medium, and the melanin synthesis can be inhibited by the presence of depigmentation agents. The important fact is that streptomyces was alive in all the culture medium, even though black eumelanin was not produced or decreased in production after kojic acid was added in various concentrations: when streptomyces was transferred to another culture medium without kojic acid, it produced melanin, turning the colour of the medium to black again. More dramatic effects of melanogenesis inhibition were also shown by Nakayama *et al.* (2000:135) where a depigmentation agent was added to the water in which black goldfish were kept. After a month or two of constant addition of kojic acid, their colour turned to yellowish brown; since they were alive and vivid, this demonstrated that only melanogenesis was inhibited, not systemic metabolism.

According to a study done by Majmudar (Anon., 1999:5) tyrosinase activity was reduced by 35% when using ascorbyl phosphate and skin lightening effect occurred in an invitro model using human epidermis. And Sakamoto demonstrated (Anon., 1999:5) that melanin production was reduced by 80% using an invitro study on melanocyte cultures.

CHAPTER 3
PHYSICO-CHEMICAL PROPERTIES OF KOJIC ACID AND SODIUM ASCORBYL PHOSPHATE

3.1 Physico-chemical characteristics

Kojic acid is also known as 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one and its molecular weight is 142.11 g/mol. The chemical formula of this substance is $C_6H_6O_4$ (Chemical Land21, 2003).

According to Chemical Land21 (2003), the chemical structure of kojic acid is given in Figure 3.1.

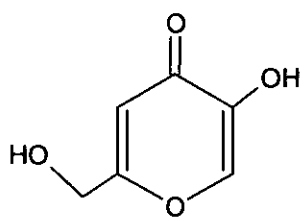

Figure 3.1 Chemical structure of kojic acid (Chemical Land21, 2003).

Sodium ascorbyl phosphate is also known as L-ascorbic acid-2-monophosphate and its molecular weight is 358.08 g/mol. The chemical formula of this substance is $C_6H_6O_9Na_3P + 2H_2O$ (Anon., 1999:2).

According to Anon. (1999:2) the chemical structure of sodium ascorbyl phosphate is given in Figure 3.2.

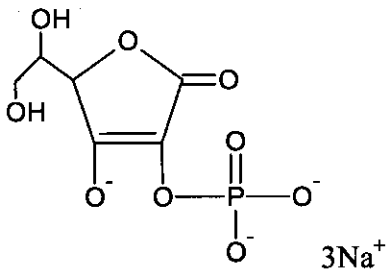

Figure 3.2 Chemical structure of sodium ascorbyl phosphate (Anon., 1999:2).

The most important physico-chemical properties of kojic acid and sodium ascorbyl phosphate are listed in Table 3.1.

Table 3.1 The physico-chemical characteristics of kojic acid (Chemical Land21, 2003) and sodium ascorbyl phosphate (Anon., 1999:2).

KOJIC ACID	**PHYSICO-CHEMICAL CHARACTERISTICS**
Chemical name	5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one
Molecular weight	142.11 g/mol
Molecular formula	$C_6H_6O_4$
Melting point	151 - 155°C
Odour	None
Appearance	White to off-white crystalline powder
pKa	7.90 - 8.03
Solubility	Freely soluble in water, ethanol, acetone, sparingly soluble in ether, ethyl acetate, chloroform, pyridine
Recommended conc.	1%

Table 3.1: The physico-chemical characteristics of kojic acid and sodium ascorbyl phosphate (continue).

SODIUM ASCORBYL PHOSPHATE	PHYSICO-CHEMICAL CHARACTERISTICS
Chemical name	L-ascorbic acid-2-monophosphate
Molecular weight	358.08 g/mol
Molecular formula	$C_6H_6O_9NaP + 2H_2O$
Melting point	Not determined
Odour	None
Appearance	White to pale beige powder
pKa	7.7
Solubility	64% in water, 13.2% in glycerol, 1.6% in propylene glycol, practically insoluble in ethanol, isopropyl myristate, cetostearyl octanol, caprylic/capric triglyceride and C12 - 15 alkyl benzoate.
Recommended conc.	3%

3.2 Stability

3.2.1 Stability of sodium ascorbyl phosphate

According to the literature and to the producer specification, ascorbyl phosphate salts are among the most stable ascorbic derivates. The stability is a result of its chemical structure. To overcome the problem of oxidation and extremely unstableness of ascorbic acid, it was chemically modified by esterification of the hydroxyl group with long-chain organic acids, resulting in a product that proved to be even more stable than ascorbyl palmitate (Austria *et al.*, 1997:796).

Moreover, vitamin C has been well-known for its inhibitory effects on melanin formation, but it has not been widely used for this function due to its instability in formulations. A number of vitamin C derivates such as ascorbyl palmitate or ascorbyl stearate, have been used for 20 years as primary depigmenting materials, however, these conventional vitamin C derivates can destabilise formulations, and hydrophilic ascorbyl phosphate salts now replace other ascorbates in most products. The addition of phosphorester groups dramatically improved the stability of ascorbic acid, because

it prevents the degradation of the compound, therefore ascorbyl *phosphate* was used. Austria, R. *et al.* (1997:795) explained that the introduction of the phosphoric group in 2 position protected the molecule from break-up of the enediol system, confirming ascorbyl phosphate as a very stable derivate of vitamin C that may be easily used in various types of cosmetic products.

Sodium ascorbyl phosphate is a stable, water soluble vitamin C derivate for at least 12 months if protected from light, metals and moisture, and stored at temperatures below 25°C in its original sealed container. It can be exposed to higher temperatures up to 80°C, but only for a short period of time. It is recommended to add sodium ascorbyl phosphate to formulations at a low temperature of 40°C (Anon., 1999:2).

Austria *et al.* (1997:797) stated that medium acid pH, rather than basic solutions, are more suitable conditions for the formulation of topical products, because this is the typical pH of the skin.

3.2.2 Stability of kojic acid

Kojic acid turns brown or yellow over time for two reasons:

1. It is not stable in light and tends to oxidize over time, which results in colour change.
2. It has a tendency to chelate with metal ions (e.g. iron) (Konsult.lv, 2003).

Kojic acid tends to be more stable in formulations with pH values between 4 and 5, but the sensitivity to heat could unfortunately not yet been solved according to Honda in an USA Patent (Honda, 1998).

The addition of phosphorester groups of sodium ascorbyl phosphate in the formulations was to improve the stability of kojic acid, as it prevents degradation of the product according to Rho (2001:1).

3.3 Toxicology

According to Zai and Maibach (2001:570) kojic acid is being used in Japan in non prescription skincare products up to a concentration of 1%. Because it is used intensively in foods (e.g., bean paste, soy, and sake) its oral safety has been studied. Zhai and Maibach (2001:570) indicated that kojic acid is a weak mutagen in bacteria, and it is nonmutagenic in eukaryotic system either in vivo or in vitro. It is also indicated that contact dermatitis may occur with sensitised patients.

According to Nicnas (2002:9) sodium ascorbyl phosphate has a low toxicity via the dermal route, since very slight erythema occurred in a study on rats. It was also stated that slight irritation of the skin occurred in a study using rabbits.

3.4 Conclusion

The efficient, but safe skin whitening characteristics of kojic acid combined with the depigmentation and anti-ageing effects of sodium ascorbyl phosphate have potential for great cosmetic products to be developed. In the following chapters the formulations and experiments done are given and explained in order to provide information regarding these chemicals and the results obtained from the tests done on these combined formulas.

CAPTER 4
FORMULATION OF TOPICAL PRODUCTS

4.1 Introduction

In topical applications, the total quantity of ingredient absorbed varies greatly based on many factors including application area size, the frequency and vigour of application, and the viscosity or thickness of the applied vehicle. These factors vary from person to person and are therefore difficult to control. In general, the larger the application areas, the more frequently will the drug be absorbed. Likewise, the thicker the applied vehicle, the more active ingredient is effectively being administered and the more the drug tends to be absorbed through the skin. Age, skin condition and application site are factors that influence drug absorption. Non-keratinised dermis is more easily penetrated by an active ingredient, and aged (thus thinner), broken or abraded skin will result in higher drug absorption as well. Finally, the solubility of the drug in the vehicle will play a role in absorption of topically applied drugs (Buckmann, 2001:151).

In the optimum topical formulation, the drug diffusion through the skin is controlled by ensuring that the drug is just soluble enough in the vehicle to encourage drug release at the desired rate. This is achieved by ensuring that the drug is entirely in solution and that minimum solvent is used. In addition, the vehicle components should increase the permeability of the stratum corneum. Lee and Kim (1995:51) advised to combine known skin lightening materials for optimal effectiveness, therefore kojic acid and sodium ascorbyl phosphate were combined. The addition of phosphorester groups of sodium ascorbyl phosphate was to improve the stability of kojic acid, as it prevents the degradation effect according to Rho (2001:1).

The hydrophilic sodium ascorbyl phosphate and kojic acid were deposited in the corresponding aqueous phases of the different formulations to ensure optimal solubility and thereby creating stable products.

4.2 Topical formulations

According to Anon.(1994:1) the characteristics for an optimum topical formulation should be:

- The concentration of the active ingredient is such that all is in solution.
- The minimum amount of solvent is used to dissolve the active ingredient, but retain a favourable partition coefficient.
- The vehicle (gel, lotion, etc.) ingredients enhance the permeability of the stratum corneum.
- The active ingredient is soluble in the vehicle, or in the case of emulsions, is soluble in the partition between the two phases.
- The rate of the diffusion of the drug within the vehicle and the rate of release of the drug within the vehicle are constant.

Several general considerations important in formulating a topical preparation are described and summarised here (Anon., 1994:2).

Considerations in formulating topical preparations

- Stability of the active ingredient
- Stability of the adjuvants
- Visual appearance
- Colour
- Odour, development of pungent odour or loss in fragrance
- Viscosity, extrudability
- Loss of water and other volatile vehicle components
- Particle size distribution of dispersed phases
- pH

- Texture, feel upon application (stiffness, grittiness, greasiness, tackiness)
- Particle contamination
- Microbial contamination/sterility (in the unopened container and under condition of use)
- Release/bioavailability (percutaneous absorption)
- Phase distribution (homogeneity/phase separation, bleeding)

4.3 Thermodynamic relationships

Flux through the skin is controlled mainly by the partition coefficient of the drug between the skin barrier and the vehicle; however, the diffusion coefficient of the drug in the skin barrier and concentration of the drug in the skin barrier also play a part in controlling flux. According to Anon. (1994:2), the partition coefficient determines the direction of the flow, or the concentration gradient. Generally, if the vehicle is a better solvent than the stratum corneum, then the drug will mainly distribute through the vehicle. Therefore, the formulator can increase the impact that the partition coefficient has on absorption by decreasing the solubility of the drug in the vehicle.

Weakly acidic drugs have a high thermodynamic activity at a low pH, while at high pH, their activity decreases. This type of active ingredient is best formulated with acidic or neutral excipients.

Weakly basic drugs have minimal activity at low pH, and are highly active at a high pH. It is best to formulate this type of active with basic or neutral excipients.

According to Van Abbé *et al.* (1969:100) skin penetration implies only that a drug passes through the epidermis and reaches the underlying dermis, and not the bloodstream. As for skin lighteners, penetration need not be further than the melanocytes in the epidermis, thus the actives need not to be absorbed in the dermis.

In Figure 4.1, a compiled figure of Knowlton and Pearce (1993:169,188) shows the location of melanocytes in the epidermis.

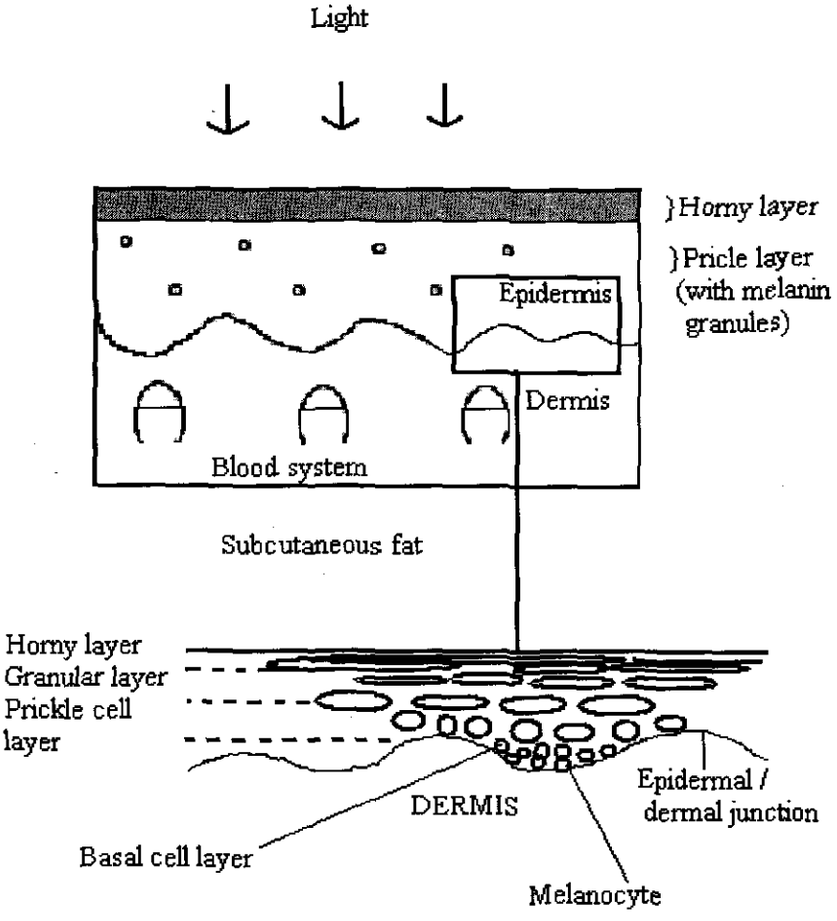

Figure 4.1 The location of the melanocytes is in the epidermis (Knowlton and Pearce, 1993:169,188).

4.4 The formulation of emulsions

Before an oil-in-water and a water-in-oil emulsion can be prepared, a brief overview of what it is and what is expected from it is needed.

4.4.1 Emulsions

An emulsion is a heterogeneous system, which has at least one immiscible, or barely miscible liquid dispersed in another liquid in the form of tiny droplets (Schueller & Romanowski, 1999:69-72; Knowlton & Pearce, 1993:90-97).

Two kinds of emulsion are described here:

1. One in which an oil is dispersed in water (o/w).
2. One in which water is dispersed in oil (w/o).

Whether an emulsion is the o/w or w/o type depends on many factors, including the concentration of each material in the system, the type of emulsifier and the processing steps used to create the emulsions.

Block (1989:336) stated that topical preparations might be more acceptable to patients as emulsions than as single-phase systems, since more components that has poor skin feel, spreadability or clothes staining ability can be placed in the internal phase of the emulsion.

According to Buchmann (2001:151) this biphasic system may be regarded in analogy to the skin or even to the skin cells which consist of lipophilic and hydrophilic components.

Emulsions, in absence of other influences, are thermodynamically unstable systems and will rapidly separate into the two phases from which they were formed. In forming an emulsion, the interfacial area between oil and water phases is increased by several orders of magnitude, the emulsion therefore possessing a much larger interfacial area than the separate phases.

4.4.2 The formulation of a whitening cream, O/W

In this kind of emulsion, oil is dispersed in water, thus the oil is in the form of very fine droplets dispersed in the continuous phase, the water. O/W emulsions spread and penetrate the skin easily with a less "oily" feel leaving a "pleasing cooling effect" on the skin after application (Knowlton & Pearce, 1993:96).

Criteria of an oil-in-water emulsion, modified from Buchmann (2001:152):

- Rub into the skin easily with a good skin feel.
- Rapid water loss should occur.
- Leaving a dry, waxy, velvety feel on the skin rather than greasy.
- Should show good skin spreadability and penetration.
- Should have an active hydration effect by the external water phase.
- Should be completely water washable.
- Should cause a cooling effect because of the evaporation of the external phase.

*All the following **target profile** skeletons were taken from Buchmann (2001:158) and adjusted to comply with the formulations that follow and aid in the functionally design of these cosmetic products.*

Target profile of an oil-in-water emulsion:

1. **Site of application:** the face
2. **Area of application:** small
3. **Target site:** the cellular epidermis
4. **Sensory properties:** a light, smooth, low viscosity cream
5. **Optical aspect:** light yellow, even coloured
6. **State of matter:** semisolid
7. **Basic type of form:** o/w emulsion
8. **Active substances:** kojic acid and sodium ascorbyl phosphate

An existing formula was taken from Anon. (1999:16) and the actives (kojic acid and sodium ascorbyl phosphate) were added with no significant change in the complexion of the emulsion.

Two facial creams were made based on the following formula, one cream contains both the actives (**cream A**), and the other cream contains only sodium ascorbyl phosphate (**cream B**).

The o/w formulation consists of the following ingredients:

O/W WHITENING CREAM:

% m/m	Composition	Activity
A 2.0	Cremophor® A6	Emulsifier
2.0	Cremophor® A25	Emulsifier
1.0	Dimethylpolysiloxane	Solvent
5.0	Cetylstearyl alcohol	Thickening agent
B 5.0	1,2-Propylene glycol USP	Solvent
0.2	EDTA®	Complexing agent
0.2	Methyl Hydroxybenzoate	Preservative
0.02	Propyl Hydroxybenzoate	Preservative
71.46	Distilled water	Solvent
C 5.0	Cetylstearyl 2-ethylhexanoate (Luvitol EHO®)	Emollient
0.3	Carbopol® 934	Thickener
D 0.12	Sodium Hydroxide	Neutralizer
E 3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
1.0	Sodium metabisulfite	Anti-oxidant

Procedure:

Heat phases A and B separately to approximately 80 °C.

Stir phase B into phase A and homogenise thoroughly.

Mix the ingredients of phase C and stir with the homogenised mixture.

Neutralise with phase D and homogenise.

Cool to approximately 40 °C.

Add phase E and homogenise again.

Outcome and corrections:

A soft, homogeneous, light yellow cream with an even texture that applied easily. It was not too oily or too hydrous. The cream that contains only sodium ascorbyl phosphate (cream B) had the same texture, but was white of colour.

At first the preservatives were prepared separately in boiling distilled water before mixing it with the rest of phase B, but as it cooled down the parabens formed a cloudy precipitation in the water and the anti-bacterial characteristics of the preservatives could be influenced. The parabens were then dissolved in the propylene glycol instead of the water before heating, and the outcome was much better for it dissolved perfectly without any precipitation.

4.4.3 Formulation of an astringent lotion (toner)

According to Mitsui (1997:327), lotions are normally transparent liquid cosmetic products that are applied to the skin for the purpose of cleansing, and maintaining its moisture balance. Many types of lotions do exist, the one formulated is also known as a toner. Mitsui (1997:327) explains that the large amount of alcohol present in the formula causes a temporarily lowering of skin temperature when it evaporates, and this special, light feeling is described as toning. It is not only moisturising, it also

prevents the secretion of excessive sebum and sweat by drawing skin proteins together (Mitsui, 1997:333).

Criteria for an astringent lotion, modified from Mitsui (1997:329):

- It has to have an astringent action (drawing the skin together).
- It has to be aesthetically pleasing with a less oily feel.
- It has to have a cooling effect on the skin after application, due to the evaporation of the alcohol from the skin's surface.
- It has to supply moisture and humectants to the horny layer.
- It has to sustain moisturisation.
- It should have a low risk of microbial growth.
- It has to remain in a liquid at low temperatures.
- It has to inhibit sebum secretion.
- It has to feel light on the skin.

Target profile of an astringent lotion:

1. **Site of application:** the face
2. **Area of application:** small
3. **Target site:** the cellular epidermis
4. **Sensory properties:** a light spray that evaporates fast and moisturise
5. **Optical aspect:** bright, transparent, yellow, homogeneous
6. **State of matter:** solution
7. **Basic type of form:** astringent lotion/toner
8. **Active substances:** kojic acid and sodium ascorbyl phosphate

Propylene glycol serves as a humectant, that improves the feel of the skin after use, and it serves as solvent of the ingredients (Knowlton & Pearce, 1993:179). Witch hazel is added for its characteristics of refreshing the skin, removing oil and for its astringent effect (Skin care, 2004:1).

Honda, S. (1998:1) stated that the inclusion of ethanol and propylene glycol serves to improve stability of the kojic acid to heat; therefore, it was included in the formulation of the face lotion.

The following formula was taken from Anon (1999:9) and the actives (kojic acid and sodium ascorbyl phosphate) were added with no significant change in the complexion of the lotion.

The astringent lotion consists out of the following ingredients:

ASTRINGENT FACE LOTION:

% m/m	Composition	Activity
A 1.5	Cremophor RH® 40	Emulsifier
3.0	1,2 Propylene glycol USP	Solvent/humectant
15.0	Ethanol 96%	Preservative
2.0	Hammelis dist. (Witch hazel Distillate)	Astringent
B 3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
0.1	Sodium metabisulfite	Anti-oxidant
74.4	Distilled water	Solvent

Procedure:

Solubilise phase A.

Mix the components of phase B to a solution and stir into phase A.

Outcome and corrections:

A clear yellow solution that sprayed evenly from a bottle, the pH was (7.2).

Solubilising phase A led to foaming that took too long to settle. Phase A was therefore stirred by hand.

The Cremophor RH® 40 had to be dissolved in the ethanol first, before mixing it with the rest of phase A to ensure a well dissolved phase.

The sodium ascorbyl phosphate and kojic acid had to be sonicated for at least 5 minutes. It dissolved completely in the distilled water.

4.5 Formulation of a foam bath

A foam bath was formulated with the idea that an individual with the desire to achieve a lighter skin can cover the entire body at the same time, to accomplish an even and overall skin lightening effect. A luxurious foam bath with a pearly shine was formulated that the user can enjoy the product while still benefiting the even distributed skin lightening effect.

Criteria for a foam bath (Knowlton and Pearce, 1993:238):

- It should produce copious and instantaneous foam at practical product concentrations.
- The foam should be stable within fairly wide limits of temperature, in both hard and soft water, in the presence of soil and fatty impurities.
- The foam should not collapse immediately on the addition of soap, nor should it be too stable to make its removal after bathing difficult.
- It should have adequate detergency and emolliency to cleanse the body and condition the skin, providing some compensation for the loss of sebum during the bathing process.
- The product should be non-irritant to both skin and eyes, and non-sensitising to the skin.

Target profile of a foam bath:

1. **Site of application:** the body, except the face
2. **Area of application:** large
3. **Target site:** the cellular epidermis
4. **Sensory properties:** a luxurious foam bath that foams immediately when in contact with water
5. **Optical aspect:** pearly, soft creamy coloured
6. **State of matter:** milky solution
7. **Basic type of form:** solution
8. **Active substances:** kojic acid and sodium ascorbyl phosphate

The foam bath was formulated to be luxurious, using a pearlescent for sheer shine. Bath preparations should be attractive and enjoyable, for it is designed to cleanse and relax at the same time, leaving the user with a pleasant skin feel. By adding the skin lightening components, an even, all over coverage of the body is possible.

The foam bath formulation consists out of the following ingredients:

PEARLY FOAM BATH:

	% m/m	Composition	Activity
A	20.0	Texapon N® 40 / Sodium Lauryl Sulphate	Surfactant
	2.0	Euperlan® pk 771	Pearlescent
B	3.0	Sodium ascorbyl phosphate	Skin lightener
	1.0	Kojic acid	Skin lightener
	54.1	Distilled water	Solvent
C	4.0	Sodium chloride	Thickener
	9.0	Distilled water	Solvent
D	0.4	Bronidox L®	Preservative
	1.5	Sodium metabisulfite	Anti-oxidant
	5.0	Distilled water	Solvent

Procedure:

Dissolve sodium ascorbyl phosphate and kojic acid completely in the distilled water.

Dissolve the sodium chloride in the distilled water.

Mix phase B with phase A, then add phase C.

Dissolve the anti-oxidant, sodium metabisulfite in the distilled water and add to the above mixture, together with the preservative, Bronidox L®.

Outcome:

A pearly, slow flowing, light creamy coloured product that formed foam immediately after it was shaken. The product was satisfying and acceptable.

4.6 Formulation of a gel

According to Buchmann (2001:155) gels are dispersed systems, originally liquids that have a certain consistency useful and practical for topical application. Gels do not comprise two immiscible phases of opposite lyophilicity. The consistencies of the following gels are caused by different gelling agents.
It took 5 attempts in trying to achieve the optimum gel, for the acidy characteristics of the actives broke many of the gels down, resulting in a thin, watery preparation that was not fit for the purpose it was designed for.

Criteria for a gel (Kushla & Zatz, 1989:496):

- The gel should exhibit little viscosity change under the temperature variations of normal use and storage.
- The gel should not lose its characteristics due to possible microbial growth.
- The gel should not be tacky.
- The gel should be clear.
- It should contain a lot of moisture and produce a cooling effect.

Target profile of a gel:

1. **Site of application:** the face
2. **Area of application:** small
3. **Target site:** the cellular epidermis
4. **Sensory properties:** not sticky, not watery
5. **Optical aspect:** brightly yellow, transparent
6. **State of matter:** semi-solid
7. **Basic type of form:** colloidal suspension
8. **Active substances:** kojic acid and sodium ascorbyl phosphate

The following formulations were trials and therefore the activity of the compounds was not included until the final gel was formulated and accepted.

4.6.1 Formulation of gel A: HPMS Gel

Hydroxypropyl methylcellulose (HPMS) is affected by factors such as concentration and the presence of electrolytes or surfactants in the aqueous phase. In practise, hydroxypropyl methylcellulose is used to thicken and stabilise shampoos, liquid soaps, shower gels, and a wide variety of emulsion-based skin care products (Knowlton & Pearce, 1993:16). HPMS was the choice of gellant for the following formula as it has a well known ability to form a stable gel.

The following formula was the first attempt in designing a skin lightening gel that was fit for the purpose of applying it to the facial skin.

GEL A: HPMS GEL

	% m/m	Composition
A	0.1	Sodium metabisulfite
	0.1	EDTA®
	1.0	Hydroxypropyl Methylcellulose
	5.0	Glycerine
B	3.0	Sodium ascorbyl phosphate
	1.0	Kojic acid
	69.8	Distilled water
	20.0	1,2-Propylene glycol USP

Procedure:

Mix phase B with phase A and stir.

Outcome and corrections:

The gel did not form. A thin, yellow solution formed instead.

5% instead of 2% hydroxypropyl methylcellulose, (Methocel®65 HG) was then used with a little more glycerine, but it still did not comply.

Then a gel was made with the same formula as above, but without the sodium ascorbyl phosphate. No gel formed, it was still too thin and fluent.

The same procedure was followed for a gel that contained no kojic acid, and a gel formed that was still thin but much more acceptable than all the previous attempts.

The low pH value due to the characteristics of kojic acid and sodium ascorbyl phosphate was blamed for braking down the gel when it was made with HPMS; the following formulas were then tried with a much more positive outcome.

4.6.2 Formulation of gel B: Aerosil®

Aerosil® is a microcrystalline silica. Network formation results from attraction of the particles by polar forces, principally hydrogen bonding. The polarity of the distilled water results in a competition for the hydrogen bonding sites and this weakens particle-particle interactions, so that higher concentrations are required to produce a gel (Kushla & Zatz, 1989:503).

The formula for Gel B follows, containing Aerosil® as choice of gellant.

GEL B: AEROSIL GEL

% m/m	Composition
5.0	Aerosil®
3.0	Sodium ascorbyl phosphate
1.0	Kojic acid
91.0	Distilled water

Procedure:

Dissolve the sodium ascorbyl phosphate and kojic acid completely in the water.

Mix with Aerosil®.

Outcome and corrections:

A cloudy, sticky gel formed that was not too thin but still unacceptable, for a facial gel has to be smooth, transparent, and quickly absorbed without residue or stickiness.

4.6.3 Formulation of gel C: Carbopol® 934

Carbopol® resins are acrylic acid polymers that are fluffy, dry powder and according to Anon (1994:4) 100% effective. Primarily the molecule of Carbopol® resin is tightly coiled and their thickening capability limited (Figure 4.2), but after it is dispersed in water, the molecule hydrate and uncoils. Uncoiling of the Carbopol®, molecules were done in this study by neutralisation with sodium hydroxide. The neutralisation ionised the Carbopol® resin and generated negative charges along the backbone of the polymer. Repulsions of like charges then caused rapid uncoiling of the molecules (Figure 4.3).

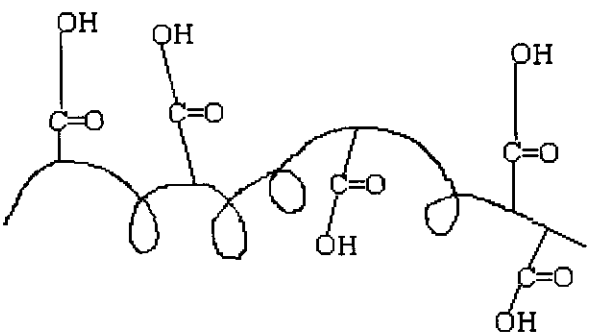

Figure 4.2 Schematic depicting molecule of Carbopol® resin in the relaxed state Anon. (1994:13).

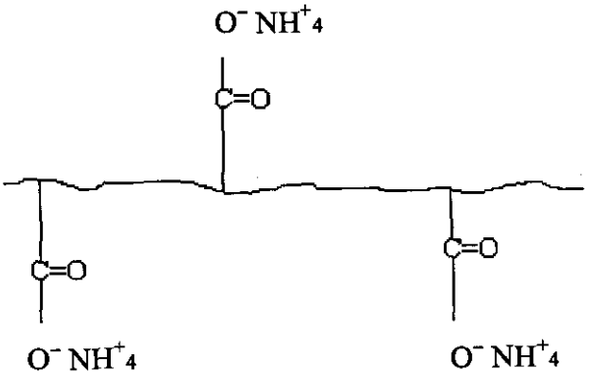

Figure 3.3. Schematic depicting molecule of Carbopol® resin in uncoiled state, Anon. (1994:13).

Carbopol® 934 was the choice of gellant in Gel C, for its efficient thickening, temperature stability, excellent shelf life, and microbial resistance.

GEL C (1): CARBOPOL® 934

% m/m		Composition
A	5.0	Luvitol EHO® Cetylstearyl 2-ethylhexanoate
	0.6	Carbopol® 934
B	3.0	Sodium ascorbyl phosphate
	1.0	Kojic acid
	85.16	Distilled water
C	0.24	Sodium hydroxide pellets
	5.0	Distilled water

Procedure:

Mix the Carbopol® 934 with the Luvitol EHO®.

Dissolve the sodium ascorbyl phosphate and kojic acid in distilled water.

Mix phase A and B.

Add the sodium hydroxide pellets last, after it was dissolved in the distilled water.

Mix well and stir with magnetic stirrer (avoid bubble formation).

Outcome and corrections:

Drops of oil appeared on the surface that contained undissolved Carbopol® 934. After stirring the mixture for a few minuets, all the Carbopol® 934 wetted thoroughly. No gel formed, the texture was more like that of thick paste.

4.6.4 Formulation of Gel C (2): Carbopol® 934

The neutralising agent, sodium hydroxide, is of great importance because it converts the acid Carbopol resins to appropriate salts. The coiled molecule of Carbopol resin is expanded to cause instantaneous thickening (Anon., 1994:4).
Carbopol® 934 was used again, this time it was not mixed with Luvitol EHO®, but with distilled water instead.

GEL C (2): CARBOPOL® 934

% m/m	Composition
1.0	Carbopol® 934
3.0	Sodium ascorbyl phosphate
1.0	Kojic acid
0.24	Sodium hydroxide pellets
94.76	Distilled water

Procedure:

Dissolve the kojic acid and sodium ascorbyl phosphate in some of the distilled water, and dissolve the sodium hydroxide pellets in approximately 5 ml distilled water.

Mix the Carbopol® 934 bit by bit into the remaining distilled water while stirring with the homogeniser.

Add the dissolved actives slowly while stirring.

Add the solution of sodium hydroxide and distilled water last.

Outcome and corrections:

A gel formed overnight but it was not acceptable. It formed clumps of gel and air bubbles were also present.

3.6.5 Formulating gel D

Anon. (1994:9) suggested that clumps of gel could be avoided by wetting the individual resin particles in ambient temperature water. Sifting the Carbopol® 934 while mixing it with the water can also be useful, as the particles can wet individually. In the case of the air bubbles, it was suggested that by reducing the mixing speed and reposition the mixer following powder dispersion, to eliminate the liquid vortex. The persistence of a vortex in the liquid dispersion during high speed mixing will incorporate and entrap air.

Carbopol® Ultrez™ 10 is an exceptionally easy-to-disperse polymer that offers a wide range of performance properties, it was chosen especially for its ability to form a clear gel. This carbomer acquire no mixing at first, after it is sprinkled on water, it needs to stand for a few minutes to be wet thoroughly, before mixing at low speed. According to Anon. (1995:2) a 500 g of dispersion at 0.5% polymer (2.5 g) will take only five minutes to completely wet without mixing.

First, the gel was made without the actives, (the placebo) and then a new gel was made that contained both the kojic acid and the sodium ascorbyl phosphate.

The following formula was used:

GEL D: CARBOPOL® ULTREZ™ 10 GEL

	% m/m	Composition
A	1.083	Carbopol Ultrez
	50.0	Distilled water
B	3.0	Sodium ascorbyl phosphate
	1.0	Kojic acid
	1.083	Tris (hydroxymethyl) amino methane
	43.83	Distilled water

Procedure:

Sprinkle Carbopol® Ultrez™ 10 slowly on the surface of the water and leave it for five minutes or until no white Carbopol particles are visible.

Homogenised at reduced speed.

Add phase B slowly to phase A and mix carefully with a spatula.

Outcome and corrections:

The placebo gel was perfect, but the gel that contained the actives was too thin. The Carbopol® Ultrez™ 10 and Tris (hydroxymethyl) amino methane were then increased to 1.17 % and the same procedure was followed as above.
A gel formed that had an even texture, not too thin, light yellow, and a pH of 6. It would have been perfect, but after a day it turned out to be too thin again.

It is known that extremely high-sheer mixers such as the homogeniser that was used can break down the polymers and this can result in a permanent viscosity loss. However, extreme care was taken not to over homogenise the gel.

4.6.6 Formulating gel E

Knowlton and Pearce (1993:20) state that Xanthan gum is a heteropolysaccharide that is soluble in cold water and is stable over a wide pH range. Zatz and Kushla (1989: 498) suggested that a suitable preservative should be used, because natural gums are subject to microbial degradation and support microbial growth.

Propyl paraben and methyl paraben were used as preservatives in the following formula of the final gel.

GEL E: XANTHAN GUM

% m/m	Composition	Activity
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
0.75	Xanthan gum	Gellant
1.0	Glycerine	Solvent for gum
93.93	Distilled water	Solvent
0.02	Propyl hydroxybenzoate	Preservative
0.2	Methyl hydroxybenzoate	Preservative
0.1	Sodium metabisulfite	Anti-oxidant

Procedure:

Dissolve the sodium ascorbyl phosphate and kojic acid in 2 parts of the distilled water.

Dissolve the parabens and sodium metabisulfite in the remaining distilled water, above 80°C.

Mix with the actives after it had cooled down.

Mix the Xantum gum and glycerine and add slowly to the mixture while stirring with homogeniser.

Outcome:

The gel that formed was bright yellow and transparent. It was not lumpy, too thin, or sticky. It remained in a gel form overnight, and had a pH of 7.5. This gel was accepted as the final gel.

4.7 Formulation of a soap

Jungermann and Lynch (1991:396) describe the basic saponification reaction. It shows the forming of soap and glycerine when fats react with an alkali.

$C_3H_5(OCOR)_3 + 3\ NaOH \rightarrow 3RCOONa + C_3H_5(OH)_3$

Fats Alkali Soap Glycerine

The neutralisation reaction between fatty acids and sodium hydroxide produced no glycerine in the finished soap.

$RCOOH + NaOH \rightarrow RCOONa + H_2O$

Fatty Acid Alkali Soap

The cleaning action of the soap is explained by Mitsui (1997:450): as an aqueous solution, it penetrates into the interface between the skin and the dirt to weaken its adhesion and make it easy to remove.

Criteria for a soap (Price, 1952):

- Must have an excellent cleansing effect.
- Must lower the surface tension of water.
- Must wet the surface to be cleaned as well as the dirt.
- Must remove oily matter by emulsification.
- Must disperse and suspend dirt particles.
- Must be harmless to the skin.
- A great loss in effectiveness in hard water should not occur.
- No irritancy to the skin.
- Fair foamability/produce a good lather in both cold and warm water.
- Must not over dry the skin.
- Must not be too hard or change shape when it becomes dry.
- Good solubility, but must not swell when put in water.
- Must have a good rinsebility.

Target profile of soap:

1. **Site of application:** the body
2. **Area of application:** large
3. **Target site:** the cellular epidermis
4. **Sensory properties:** soap that foams easy in water
5. **Optical aspect:** dark, brownish yellow coloured
6. **State of matter:** solid
7. **Basic type of form:** soap
8. **Active substances:** kojic acid and sodium ascorbyl phosphate

According to Niven (1950:8) the properties of a commercial soap depend upon the properties of the individual fatty acid salts of which it is composed. Stearic acid shows strong soap-like properties, for its saturated fatty acid is above C_{12} (the formula is $C_{18}H_{36}O_2$).

In the following formulation, sucrose was used as clarifier, while glycerine aids in the transparency of the soap. Glycerine also softens the hardness of the soap, caused by stearic acid, which was added for its rich foam forming abilities. Peanut oil was chosen for its ability to cause a lasting lather, fair cleaning, and mild effect on the skin (Thomssen and McCutcheon, 1949).

PEANUT OIL CLEAR SOAP

% m/m	Composition	Activity
A		
27.0	Peanut oil	Oil for saponification
10.0	Stearic acid	Stiffening agent
20.0	1.2-Propylene glycol	Solvent
6.0	Glycerine	Humactant
B		
8.5	Sucrose	Clarifier
4.93	Sodium hydroxide	Alkali
9.6	Distilled water	Solvent
C		
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
5.0	Distilled water	Solvent

Procedure:

Mix phase A while heating and mixing with magnetic mixer.

Dissolve the sucrose, sodium ascorbyl phosphate and kojic acid in the distilled water.

Dissolve the sodium hydroxide in 5 ml of the distilled water.

Mix phase B and phase C simultaneously into phase A.

Grease containers with Vaseline.

Pour the hot mixture into the containers and wait until it is completely cooled down

before removing the soap.

Outcome and corrections:

The soap was not clear, for foam had formed while stirring. In the second attempt the hot soap was mixed very slowly for a longer period, until it appeared to be clear and just a little bit of foam formed, but it had no influence on the appearance of the soap. The result was a clear, brownish, yellow soap that formed foam immediately in contact with water.

4.8 Conclusion

This conclusion consists out of an overview of all the formulations.

The o/w emulsion: The facial cream

The solubility of the skin lightening agents in water is very high, which is important according to Anon. (1994:3) for diffusion to the skin surface and percutaneous penetration of highly water soluble drugs are greater from vehicles having an aqueous external phase (oil-in-water emulsions).
To improve the fluidity and spreadability of the emulsion, Luvitol EHO® was added to the formula. The presence of Luvitol EHO® also improves the natural/inherently

water vapour penetrability of human skin according to Anon. (2003:1-2) and gives a soft, smooth and supple feel to the skin.

According to Block (1989:96) the inclusion of an antioxidant in an emulsion formulation may be necessary to protect, not only the active ingredients, but also formulation components (e.g., unsaturated lipids), which are oxygen-labile. The complexing/chelating agent, EDTA®, improves the stability of the product because trace amounts of metals such as copper, manganese, or iron may also initiate or catalyse oxidative reactions. Both of the creams applied easily and left the skin moisturised.

The astringent lotion:

The witch hazel is an astringent and aid in the correction of an oily skin or in removing wrinkles. It will be ideal for the face lotion to be packed in a spray bottle, due to its fluency. The user can spray the lotion directly on the face without unnecessary dissipation. The face lotion is not oily, and can be used underneath make-up, leaving the skin moisturised, while skin lightening gradually takes place.

The foam bath:

The foam bath meets the demands of its criteria: it produces instantaneous foam, it is stable at high temperatures, it does not collapse on immediate contact with a detergent, and it moisturises while it cleanse and the skin lightening actives can cover the entire body to ensure an overall skin lightening effect.
Sodium chloride was added for its thickening characteristics when reacting with the surfactant. Sodium lauryl sulphate has a very high cloud point and an exceptionally high viscosity, which makes it ideal for a creamy foam bath.

The gel:
A summary of the characteristics of the different trials is given in Table 4.1.

Table 4.1 Summary of the different gels and their characteristics.

	HPMC	Aerosil®	Carbopol®934	Carbopol®Ultrez10	Xanthan gum
Remained a gel for 24h+		✓	✓		✓
Too thin	✓			✓	
Transparent	✓			✓	✓
Sticky, tacky		✓			
Clumps of gel formed		✓	✓		
Air bubbles present			✓		

Xanthan gum is anionic and a heteropolysaccharide. It is soluble in cold water and produces a highly viscous stable solution. Xantum gum is resistant to enzymatic degradation and is stable over a wide pH range, (Knowlton and Pearce, 1993:20). Sodium ascorbyl phosphate and kojic acid are both acidic in character and this made it difficult to find a gellant that does comply and not get broken down by these actives. The wide pH range of Xanthan gum seems to be ideal in this case; therefore, Gel E was accepted as the final gel.

The soap:

The function of glycerine as a component in the soap is as follows: it enhances transparency, conditions the skin, and improves lather and appearance (Jungermann & Lynch, 1991:405). The sorbitol and glycerine retard crystal growth and the sodium hydroxide also tends to give better transparency. In the process of formulating the soap, care was taken not to stir or mix the ingredients to fast, for when foam forms before cooling down; it results in an opaque product.

Free hydroxide (OH^-) is always present in an aqueous soap solution and gives it an alkaline reaction. The pH of this soap is 10.23. Normal skin has a pH of about 5.5. Washing with soap solutions will always result in a temporary increase of the skin pH, but it will never rise beyond pH 7. Natural acidifying occurs after 5 - 10 minutes and

the original pH is re-established within 30 minutes (Jellinek, 1970: 211). Therefore, the temporary increase in pH will not be harmful to the skin.

The trial batches of these formulations were stored at three storage temperatures during the three-month stability-testing period. It was under controlled supervision at the Research Institute for Industrial Pharmacy at Potchefstroom, where the temperatures and humidities were regularly observed in their specialised storage facilities.

CAPTER 5

METHODS FOR STABILITY TESTING

5.1 Introduction

The stability profile of any product has implications for its safety, efficacy, and aesthetic properties. A cosmetic product should meet the demand of the Consumer Products (Safety) Regulations of 1989 that requires a product to be safe for its intended use, throughout its usable life, currently regarded as thirty months in the UK; moreover, the Consumer Protection act states that a product should be fit for its purpose of use (Knowlton & Pearce, 1993:435). Finally, "quality" is generally determined by the satisfaction of the user (Mitsui, 1997:5), but in the industrial situation, quality is determined at three points: (1) design, (2) manufacture and (3) sales.

The following parameters were examined:

- active content (HPLC analysis)
- membrane release
- spreadability
- viscosity
- penetration
- pH
- specific gravity
- appearance/visual assessment
- foaming ability
- preservative content of efficiency

5.2 Stability program

Six products were formulated during this study. Each of the formulations contains both kojic acid and sodium ascorbyl phosphate, except for cream B that contains only sodium ascorbyl phosphate, for the purpose of comparison.
The trial batches of these formulations were stored at three storage temperatures during the three-month stability-testing period.

5.2.1 Concentrations

Each of the six formulated products comprised of 3% m/m sodium ascorbyl phosphate and 1% m/m kojic acid, except for cream B that comprised of only 3% m/m sodium ascorbyl phosphate as active.

5.2.2 Storage temperatures

The objective of this study is to determine the stability of a product, as a function of time. All trial batches were stored at three temperatures. Controlled 5°C, 25°C + 60% RH, and 40°C + 75% RH storage facilities were used during the stability period. In Table 5.1 the storage conditions are summarised.

Table 5.1 Temperatures at which the formulations were stored.

	Cream A	Cream B	Face lotion	Gel	Foam bath	Soap
5°C	x	x		x		
25°C+60%RH	x	x	x	x	x	x
40°C+75%RH	x	x	x	x	x	x

5.3 Tests and methods

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

5.3.1 HPLC analysis

The HPLC parameters and preparations are given, as well as the formulas used in the calculations.

5.3.1.1 HPLC analysis of kojic acid and sodium ascorbyl phosphate concentrations

A high performance liquid chromatographic (HPLC) method for the simultaneous determination of kojic acid and sodium ascorbyl phosphate was developed and validated.

10% methanol in Milli-Q water was used as solvent for the creams and only Milli-Q water for the rest of the formulations.

Standard preparation of kojic acid and sodium ascorbyl phosphate:

For the preparation of the standard, 20 mg of kojic acid and 60 mg of sodium ascorbyl phosphate were accurately weighed and transferred to a 100 ml volumetric flask and filled with Milli-Q water to volume. After sonification for 10 minutes, the standard solutions were filtered and transferred into HPLC vials and analysed using the HPLC method.

Sample preparation:

Approximately 2 g of each formulation was accurately weighed and dissolved. The creams were dissolved with 10 ml methanol and filled to 100 ml with Milli-Q water. The rest of the formulations were diluted to 100 ml with Milli-Q water only. All the products were sonicated for 10 minutes and the creams were shaken for 15 minutes. The samples solutions had to be filtered through 0.45 µm filters, before it was transferred into HPLC vials and injected into the HPLC.

The following calculations were used to evaluate the results obtained and were the same for both kojic acid and sodium ascorbyl phosphate:

$$C_{std} = \frac{\text{mass weighed (mg) x potency (\%)}}{100 \times 100}$$

$$\%\ m/m = \frac{SAR \times 100 \times 100 \times C_{std}}{STR \times EqM_{sample}}$$

Where:

SAR = sample peak area

STR = standard peak area

EqM_{sample} = sample (mg) containing equivalent mass of kojic acid or sodium ascorbyl phosphate

The HPLC parameters:

Column:	Lichrospher 100-5 RP-18 ec, CC 250/4
Mobile phase:	0.185% m/v KH_2PO_4, 0.681% $C_{16}H_{36}NI$, 10% Acetonitrile in Milli-Q water. pH adjusted to 5.0 with diluted NH_4OH (1:3) or phosphoric acid.
Flow rate:	1.0 ml/minute
Injection volume:	10 µl
Detection:	UV at 255 nm
Retention time:	Kojic acid ± 2.3 minutes

	Sodium ascorbyl phosphate ± 7.8 minutes
Solvent:	Milli-Q water made up to 100 ml
Standard:	20mg : 60mg kojic acid : sodium ascorbyl phosphate
Stop time:	15 minutes
Apparatus:	Hewlett Packard 1050 HPLC, equipped with a variable wavelength UV detector, pump, injection device and integrator or recorder, or similar equipment that meets the United States Pharmacopoeia (USP) 24 standards for system suitability.

5.3.1.2 HPLC analysis of preservative concentrations

The HPLC parameters:

Column:	Lichrospher 100-5 RP-18 ec
Mobile phase:	Acetonitrile:Water (50:50)
Flow rate:	1.0 ml/minutes
Injection volume:	20 µl
Detection:	UV at 254 nm
Retension time:	Approximately 1.8 minutes and 2.6 minutes respectively for methyl and propyl hydroxybenzoate.
Solvent:	10% Methanol and Milli-Q water made up to 100 ml
Standard:	0.2% and 0.02% methyl hydroxybenzoate and propyl hydroxybenzoate.
Stop time:	5 minutes
Apparatus:	Hewlett Packard 1050 HPLC, equipped with a variable wavelength UV detector, pump, injection device and integrator or recorder, or similar equipment that meets the United States Pharmacopoeia (USP) 24 standards for system suitability.

Standard preparation of methyl and propyl hydroxybenzoate:

For the preparation of the standard 40 mg of methyl hydroxybenzoate and 4 mg of propyl hydroxybenzoate were accurately weighed and transferred to a 100 ml volumetric flask and filled with 10 ml methanol and Milli-Q water to volume. 10 ml was then withdrawn and diluted to 100 ml with Milli-Q water. After sonication for 10 minutes, the samples were filtered through 0.45 µm filters, transferred into HPLC vials, and analysed using the HPLC method.

Sample preparation:

Approximately 2 g of each formulation was accurately weighed and dissolved. The creams were dissolved with 10 ml methanol and filled to 100 ml with Milli-Q water. 10 ml was then withdrawn and diluted to 100 ml with Milli-Q water. The gel was diluted to 100 ml with Milli-Q water, and 10 ml was withdrawn and diluted again to 100 ml, using Milli-Q water as the only solvent. All the products were sonicated for 10 minutes and the creams were shaken for 15 minutes. The samples were filtered through 0.45 µm filters, before it was transferred into HPLC vials and injected into the HPLC.

The following calculations were used to evaluate the results obtained and were the same for both methyl hydroxybenzoate and propyl hydroxybenzoate:

$$\text{Concentration (m/m)} = \frac{\text{SAR x mass of standard (g)}}{\text{STR}} \times \frac{2}{\text{Sample mass (g)}}$$

Where:

SAR = sample peak area

STR = standard peak area

5.3.2 Membrane release

The scope of this study is to determine the release of the active ingredients from facial cream A, that contains kojic acid and sodium ascorbyl phosphate, and of facial cream B, that contains only sodium ascorbyl phosphate. The in vitro release from the cream preparations was measured with a release unit related to the USP dissolution apparatus, as used by Fares & Zatz (1995: 52-58) Figure 5.1; at initial and on the test samples of month three at 25°C + 60% RH and 40°C + 75% RH.
The calculations used are given in Appendix A-17.

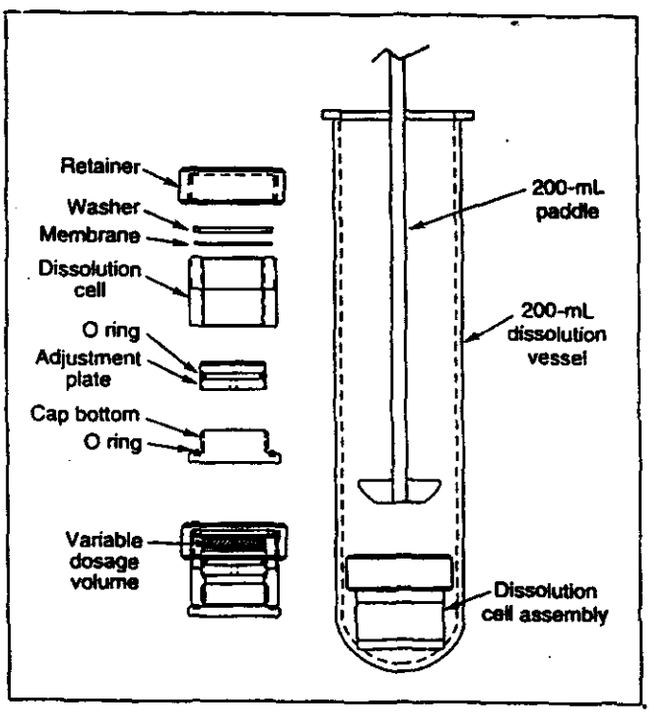

Figure 5.1 Diagrammatic representation of the release unit for the membrane release studies (Fares & Zatz, 1995:53).

HPLC conditions:

- Dissolution instrument: VanKel VK700 dissolution bath
- Dissolution apparatus: Apparatus 2 (paddle)
- Dissolution medium: Distilled water
- Dissolution volume: 190 ml
- Paddle speed: 100 rpm
- Sample volume: 500 μl
- Sample intervals: 30, 60, 120, 240, 360 minutes
- Method of analysis: HPLC

The release unit fits directly onto the VanKel VK700 six station dissolution apparatus. The release experiment was carried out six fold for each preparation.

The reservoir of the dissolution cell was filled with cream (about 3 g) and covered with the membrane (cellulose acetate; 0.45 µm pore size), taking care to exclude air bubbles between the cream and the membrane. The cell was capped and placed in the dissolution vessel containing the receptor medium (distilled water; 190 ml). The paddle speed was 100 rpm and the temperature 32°C. Samples of 500 μl were withdrawn with a micropipette at 30, 60, 120, 240 and 360 minutes.

Two standard solutions containing only kojic acid and only sodium ascorbyl phosphate were also prepared. Milli-Q water was used as solvent. 45 mg of sodium ascorbyl phosphate was weighed, transferred to a 200 ml volumetric flask, and filled to volume with Milli-Q water. Aliquots of this solution were further diluted using the Milli-Q water to yield concentrations of 22.5, 67.5, 135 and 200 µg/ml. 15 mg of

kojic acid was weighed, transferred to a 200 ml volumetric flask, and filled to volume with Milli-Q water. Aliquots of this solution were further diluted using the Milli-Q water to yield concentrations of 7.5, 22.5, 45 and 75 µg/ml. These samples were then analysed by means of HPLC methods as described in section 5.3.1.
The calculations that were used to determine the amount of active substance recovered are given in Appendix A-17.

5.3.3 Spreadability

The spreadability test was done according to the method of the RIIP (MethodSpr01) on the facial creams.
Two spreadability glass plates were used; one glass plate was clear whereas the other glass plate had a scaled 1 mm incremented grid fixed underneath. The scaled glass plate was weighed and the balance was "teared" (reading = zero). A clean syringe was filled with a sample of the cream and a rubber nozzle (6 mm in diameter) was fit securely over the syringe. A 10 mm cream sample was squeezed from the tube, onto the scaled plate, through the rubber nozzle. The sample mass was then recorded. The clear glass plate was placed on top of the sample with its sides in line with those of the scaled plate. A 100 g weight was put on top, central to the sample position and left for 60 seconds. A Vernier calliper was used to measure the diameter of the sample. The test was done in duplicate for each cream. The average mass and diameter were then calculated.

5.3.4 Viscosity

The viscosity of facial cream A and B, and the foam bath were measured, using a Brookfield Model DV-II + Viscometer, together with the Helipath stand. The samples were measured at different speed and at 25°C and time intervals of 2 minutes each.

5.3.5 Penetration

A calibrated penetrometer was used to measure the penetration of the test samples: Facial cream A and B, three times a month for three months. These three measurements were taken by allowing a twenty-four hour period to elapse in between measurements.

Without forming air bubbles, the containers were carefully and completely filled with cream sample. It was levelled and left on a flat surface for 24 hours at laboratory room temperature. A test sample was then placed at the base of the penetrometer, and its tip was repositioned to touch the surface of the sample. The penetrating object was released and held free for five seconds. The penetrating object was then clamped and the depth of penetration was measured.

5.3.6 pH

The pH meter used was calibrated with buffer solutions of pH 4 and 7. The pH was measured using the Metrohm Autotitrator 785 DMP Titrino for the facial creams. The Mettler Toledo MP 220 pH meter was used to measure the pH of the lotion, gel, 10% dilution of the foam bath in water, and a 10% dilution of the soap in water.

5.3.7 Specific gravity

This test was done on all the test samples, except the soap, over the three-month period. An empty glass polytop was weighed and the calibrated Sartorius BP211 was "teared" (reading = zero) and then filled with 9 ml water. The weight was again recorded and the water level was marked. The water was then replaced by the test sample and weighed. The following calculation was made to determine the specific gravity for the test samples:

$$\text{Specific gravity} = \frac{(\text{polytop} + \text{sample}) - (\text{empty polytop})}{(\text{polytop} + \text{water}) - (\text{empty polytop})}$$

5.3.8 Appearance / visual assessment

A visual assessment on each stored trial batch was done once a month. Changes in colour, odour, and skin feel were examined and compared.

5.3.9 Foamability

The foamability of the foam bath was measured by diluting 10 ml of the foam bath with 90 ml tap water. It was shaken for 15 seconds in a 250 ml measure cylinder. The foam formed was measured at the start and again after 30 minutes.
The foamability of the facial soap was measured by alternatively dissolving 2 g of the bar in 100 ml distilled water at 20°C and 45°C and 100 ml ordinary tap water at 20°C and 45°C. It was then shaken in 250 ml measure cylinders for 15 seconds. The foam formed was measured at the beginning and again after 30 minutes.

5.4 Conclusion

The results of the tests, described in this chapter, are given and represented graphically in Chapter 6 to Chapter 10. The outcome of each of the six preparations will be discussed separately in these chapters.

CAPTER 6

TESTS RESULTS: CREAM

6.1 Introduction

In this chapter, two different batches of the facial cream will be compared; ***cream A*** comprised of kojic acid and sodium ascorbyl phosphate whereas ***cream B*** contains only sodium ascorbyl phosphate as skin lightening active. The tests were done over a period of three months at three different temperatures and humidities. The following physical parameters of the formulated creams were investigated: pH, specific gravity, viscosity, spreadability, and appearance. Stability tests were done on all the batches, and included the membrane release at initial, and after three months at 25°C + 60% RH and 40°C + 75% RH. Microbial preservative efficacy was done by Wits Health Consortium (Pty) Ltd.

6.2.1 pH

The surface of the skin normally exhibits a slightly acidic reaction, around pH 5.5 to 6.0, strongly acid or alkaline applications act as primary irritants according to Van Abbé *et al.* (1969:96) and it is recommended to buffer within the physiological pH range of the skin. Jellinek (1970: 211) states that natural acidifying occurs after 5-10 minutes and the original pH is re-established within 30 minutes.

The pH of cream A and cream B was measured once a month for a period of three months at three different temperatures and humidities as described in Chapter 5.3.6.

6.2.1 Results

The pH of of cream A, measured over three months is given in Figure 6.1.

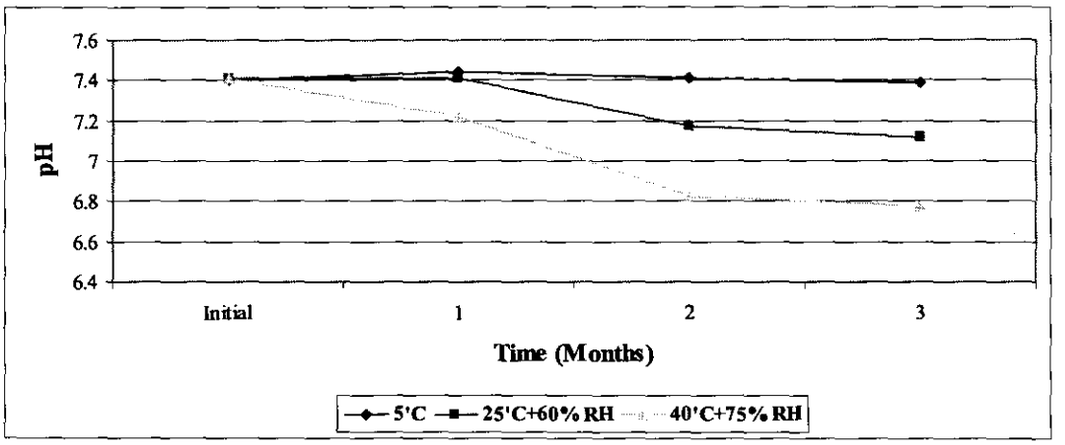

Figure 6.1 The pH of cream A over the three month stability period.

The pH of cream B, measured over three months is given in Figure 6.2.

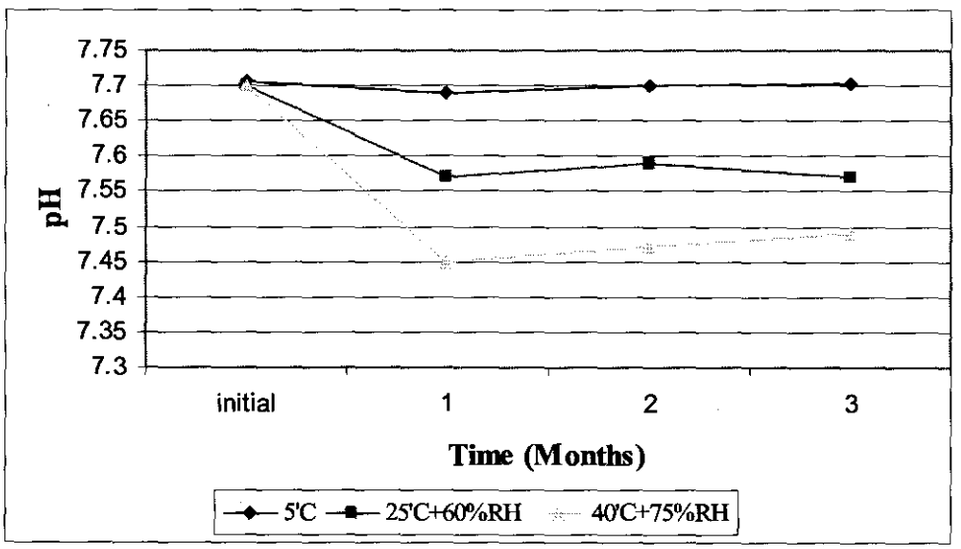

Figure 6.2 The pH of cream B over the three month stability period.

The comparison of the pH values of the batches of cream A and cream B that were stored at 40'C + 75 % RH is shown in Figure 6.3.

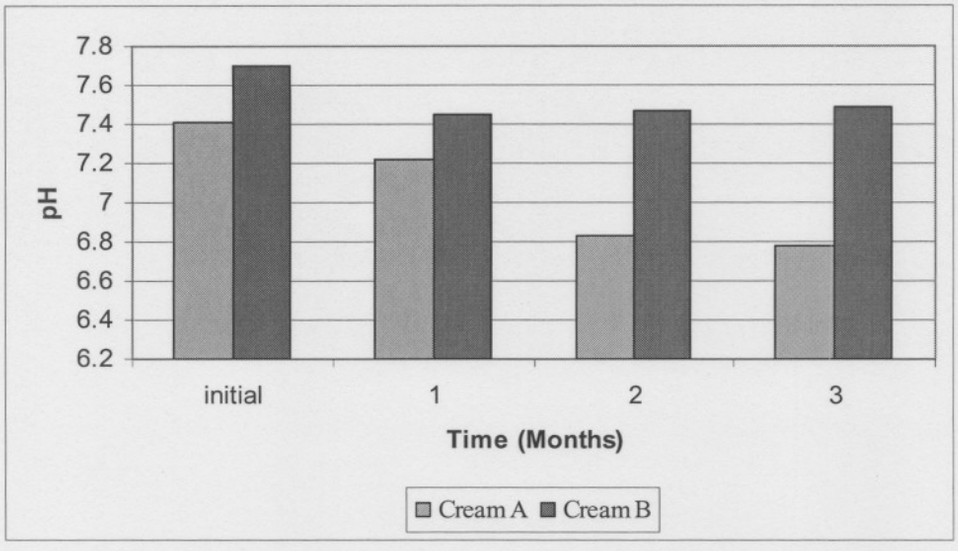

Figure 6.3 Comparison of the pH values of the batches stored at 40°C + 75% RH of cream A and cream B over the three month stability period.

6.2.2 Discussion

Figure 6.1 represents a decrease in the pH of the cream over the period of three months. Especially the sample that was stored at 40°C + 75% RH displays a large decline in pH over time. The decrease could be due to the fact that the buffer that determines the pH is part of the water phase, and the water evaporated over time. One of the characteristics of an o/w emulsion is the rapid water loss that occurs to leave behind the moisturising oil phase, see Chapter 4.4.2.

Another option to consider is the explanation of Wade and Weller (1994:310,411) that the parabens which were used as preservatives, are known to decrease the pH of weakly buffered solutions.

The stability of the product depends very much on the pH value of the formulation. According to Anon. (1999:4) the best stability for sodium ascorbyl phosphate is obtained at pH values above 6.5, but still medium acid pH (see section 3.2.1). All the

batches of cream A and cream B remained above pH 6.5 and below 7.75. Sodium ascorbyl phosphate is a crystalline solid that is sensitive to heat and moisture, finished products should therefore be stored at temperatures below 25°C (Anon., 1999:4). According to a Honda (1998), the optimum pH level for kojic acid to be stable, would be between 4 and 5. This could be the reason why the kojic acid assays showed a remarkable decline in cream A, see section 6.8.

As shown in Figure 6.3, the decrease of pH in the 40°C + 75% RH samples of cream A were higher than the samples of cream B. Kojic acid has a significant sensitivity to higher temperatures. This problem of influence by heat has not so far been solved, according to Honda (1998).

6.3 Specific gravity

The specific gravity of the creams was determined once a month for three months, as was described in section 5.3.7.

6.3.1 Results

The relative density results of the facial cream are given in Table 6.1.

Table 6.1 The specific gravity (g/cm^3) of cream A and cream B over the three month stability period.

Storage condition	Initial	Month 1	Month 2	Month 3
Cream A				
5°C	0.933	0.931	0.933	0.933
25°C + 60% RH	0.932	0.974	0.939	0.954
40°C + 75% RH	0.934	0.954	0.939	0.944
Cream B				
5°C	0.947	0.934	0.962	0.935
25°C + 60% RH	0.939	0.958	0.928	0.955
40°C + 75% RH	0.942	0.931	0.974	0.932

6.3.2 Discussion

There was no significant change in the specific gravity of cream A or cream B (Table 6.1). Temperature and humidity did not have an influence on the specific gravity.

6.4 Viscosity

The higher the viscosity the better the droplets of the dispersed phase are held in place. Schueller and Ramanowski (1999:148) state that viscosity measurements help to determine whether a product is of proper consistency and it indicates the product's stability. Moreover, viscosity measurement is an indicator of the thickness and flow properties and also the changing of a product over time.
The viscosities of cream A and cream B are shown Table 6.2.

6.4.1 Results

Table 6.2 The viscosities of cream A over a period of three months at three different temperatures and humidities.

Viscosity values (cP)	Initial	Month 1	Month 2	Month 3
5°C	59435	44647	47091	40103
25°C+60%RH	53975	45073	48241	43113
40°C+75%RH	54388	43894	48268	43150

Table 6.3 The viscosities of cream B over a period of three months at three different temperatures and humidities.

Viscosity values (cP)	Initial	Month 1	Month 2	Month 3
5°C	71880	64793	74549	82465
25°C+60%RH	76477	71707	74721	81505
40°C+75%RH	74279	75428	74726	82479

6.4.2 Discussion

The viscosity of cream A had a slight decrease over time, and cream B showed a small but significant increase in viscosity. The difference in viscosity between the two creams may be because of the presence of kojic acid in cream A or it might just be that cream B was thicker after formulation than cream A. This correlates with the penetration results that follow.

6.5 Penetration

If separation has occurred, it can be determined with penetration measurements. There is a close resemblance with the viscosity measurements. The penetration of the cream was determined once a month for three months as described in section 5.3.5.

6.5.1 Results

The penetration of the three batches of cream A and cream B are given in Table 6.4 and Table 6.5 respectively.

Table 6.4 Penetration results (mm) of cream A over the three month stability period.

Cream A Initial				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	30.3	31.1	31.3	30.9
25°C+60%RH	30.2	31.2	33.2	31.6
40°C+75%RH	30.1	31.5	30.7	30.8
Month 1				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	31.2	30.4	31.1	30.9
25°C+60%RH	32.0	31.0	32.3	31.8
40°C+75%RH	31.3	31.4	32.9	31.9
Month 2				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	32.6	32.1	32.9	32.5
25°C+60%RH	32.1	33.9	32.4	32.8
40°C+75%RH	32.7	33.6	33.0	33.1
Month 3				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	33.9	32.1	32.0	32.7
25°C+60%RH	33.3	33.1	32.9	33.1
40°C+75%RH	33.8	34.2	34.0	34.0

Table 6.5 Penetration results (mm) of cream B over the three month stability period.

Cream B				
Initial				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	33.3	31.7	30.1	31.7
25°C+60%RH	30.2	29.9	30.2	30.1
40°C+75%RH	33.2	30.1	31.3	31.5
Month 1				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	30.1	30.2	30.4	30.2
25°C+60%RH	32.8	29.6	30.3	30.9
40°C+75%RH	28.6	31.3	31.1	30.3
Month 2				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	31.1	31.3	32.3	31.6
25°C+60%RH	32.2	33.1	30.1	31.8
40°C+75%RH	32.1	31.2	33.5	32.3
Month 3				
Temperature	**Day 1**	**Day 2**	**Day 3**	**Average**
5°C	31.2	31.1	30.4	30.9
25°C+60%RH	32.2	32.6	33.5	32.8
40°C+75%RH	33.8	32.3	30.1	32.1

6.5.2 Discussion

As can be seen in Table 6.4, there was a slight increase in penetration for cream A, and Table 6.5 reveals that there was no significant change in the penetration results for cream B over time. The slight increase in penetration for cream A can be due to the decrease in the viscosity (see section 6.4). Cream B showed good stability and the conclusion can be made that no separation had occurred.

6.6 Spreadability

Spreadability measurements determine with what ease a cream can be applied to the skin. The spreadability of cream A and cream B was determined once a month over a three month period, as described in section 5.3.3.

6.6.1 Results

The results of cream A and cream B are given in Table 6.6.

Table 6.6 The spreadability (mm) of the three bathes of cream A and cream B over a period of three months.

Cream A	Initial	Month 1	Month 2	Month 3
5°C	40.01	40.65	40.29	40.74
25°C+60%RH	41.54	40.05	41.81	40.20
40°C+75%RH	40.17	40.67	41.03	42.31
Cream B				
5°C	38.68	38.68	38.68	38.68
25°C+60%RH	38.37	38.37	38.37	38.37
40°C+75%RH	39.46	39.46	39.46	39.46

6.6.2 Discussion

Table 6.6 shows no significant change in spreadability over a three month period at three different temperatures and humidities. The lower spreadability of cream B corresponds with the higher viscosity results compared to cream A.

6.7 Visual assessment

The visual assessment of cream A and cream B were done once a month for three months as described in section 5.3.8.

6.7.1 Results

The appearance of cream A and cream B is given in Table 6.7.

Table 6.7 The visual assessment of cream A over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	Colour: light yellow	Darker yellow	Darker yellow	Darker yellow
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	No change
	Homogeneous	No change	No change	No change
	Thin but not fluent	No change	No change	No change
	Air bubbles present	No change	No change	No change
25°C + 60%RH	Colour: light yellow	Darker yellow	Darker yellow	Darker yellow
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	No change
	Homogeneous	No change	No change	No change
	Thin but not fluent	No change	No change	No change
	Air bubbles present	No change	No change	No change
40°C + 75%RH	Colour: light yellow	Brown	Golden brown	Black/brown
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	Hydrous
	Homogeneous	Not uniform	Not uniform	Not uniform
	Thin but not fluent	No change	No change	Fluent
	Air bubbles present	No change	No change	No change

Table 6.8 The visual assessment of cream B over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	Colour: white	No change	No change	No change
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	No change
	Homogeneous	No change	No change	No change
	Thin but not fluent	No change	No change	No change
	Air bubbles present	No change	No change	No change
25°C + 60%RH	Colour: white	No change	No change	No change
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	No change
	Homogeneous	No change	No change	No change
	Thin but not fluent	No change	No change	No change
	Air bubbles present	No change	No change	No change
40°C + 75%RH	Colour: white	Off-white	Off-white	Off-white
	Soft feel	No change	No change	No change
	Applies easily	No change	No change	No change
	Not too oily/hydrous	No change	No change	No change
	Homogeneous	No change	No change	No change
	Thin but not fluent	No change	No change	No change
	Air bubbles present	No change	No change	No change

6.7.2 Discussion

The changes in appearance of cream A and cream B are mainly in colour; especially cream A, which can be ascribed to the degradation of the kojic acid with time. Even a small breaking down in active can result in a remarkable colour change. The samples that were stored at 40°C + 75% RH became fluent, changed to a brown colour and were not uniform anymore, therefore the creams should be stored below 25°C + 60%

RH in an amber container. Cream B had a slight change in colour, but did not become fluent like cream A. The thinning of cream A confirms the results of viscosity testing that decreased over time.

6.8 Assays of cream A and cream B

HPLC assays were done on cream A and cream B at 5°C, 25°C + 60% RH and 40°C + 75% RH over a three month stability period. Figure 6.4 represents a chromatogram of a standard solution containing the actives.

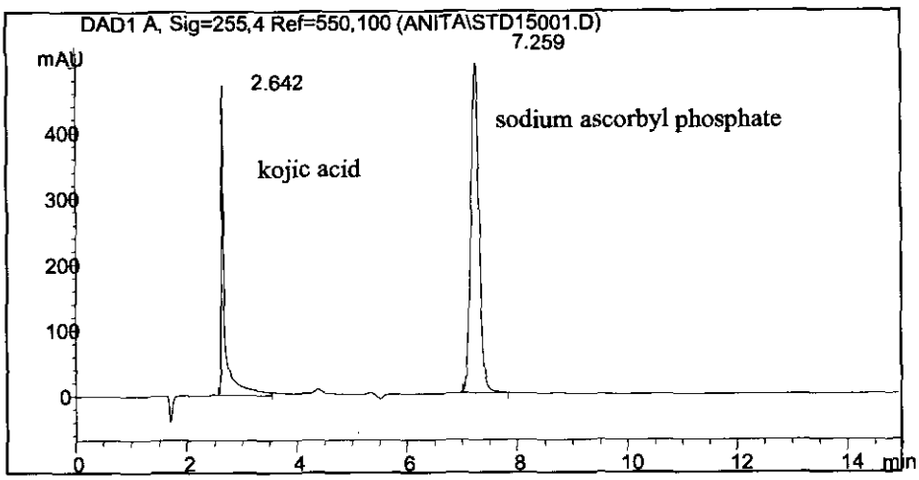

Figure 6.4 Chromatogram of a standard solution of kojic acid and sodium ascorbyl phosphate.

6.8.1 Results of cream A and cream B

A chromatogram of cream A and cream B which was stored at 25°C + 60 % RH for three months is represented in Figure 6.5 and Figure 6.6 respectively.

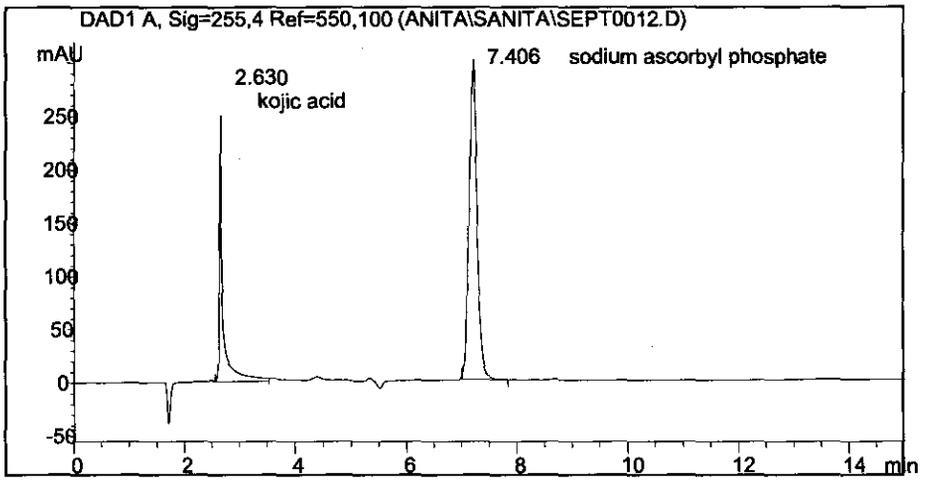

Figure 6.5 Chromatogram of a sample of cream A which was stored at 25°C + 60 % RH for three month stability period.

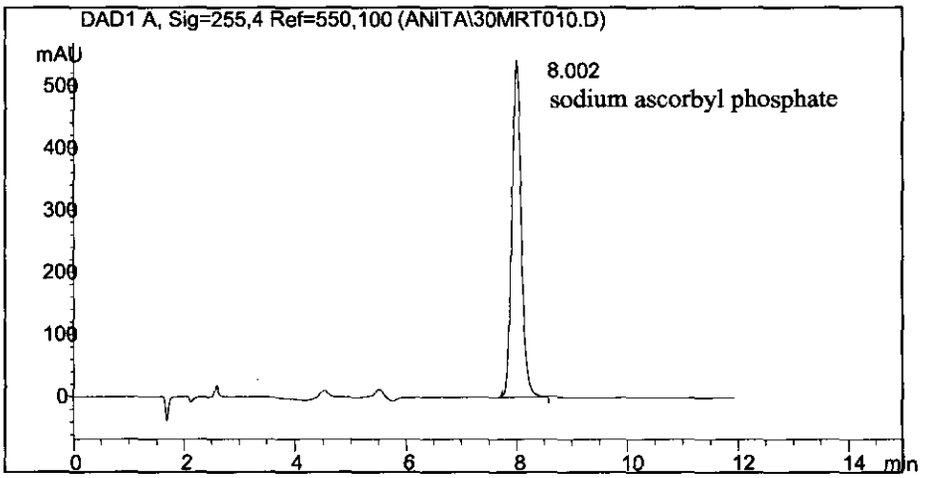

Figure 6.6 Chromatogram of a sample of cream B which was stored at 25°C + 60 % RH for three month stability period.

The following graphs represent the assay test results of the kojic acid and sodium ascorbyl phosphate in cream A over a three month period (See Appendix B for the assay results).

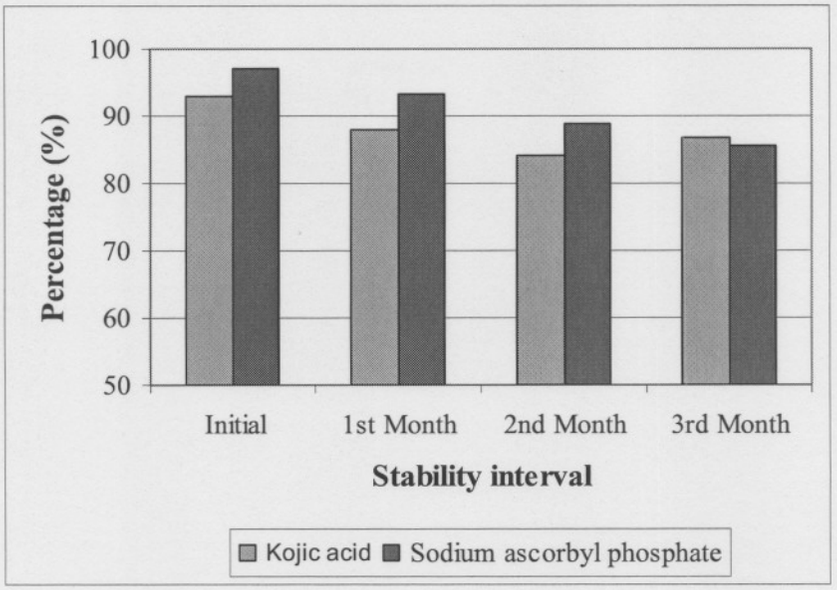

Figure 6.7 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in cream A after three months of storage at 5°C.

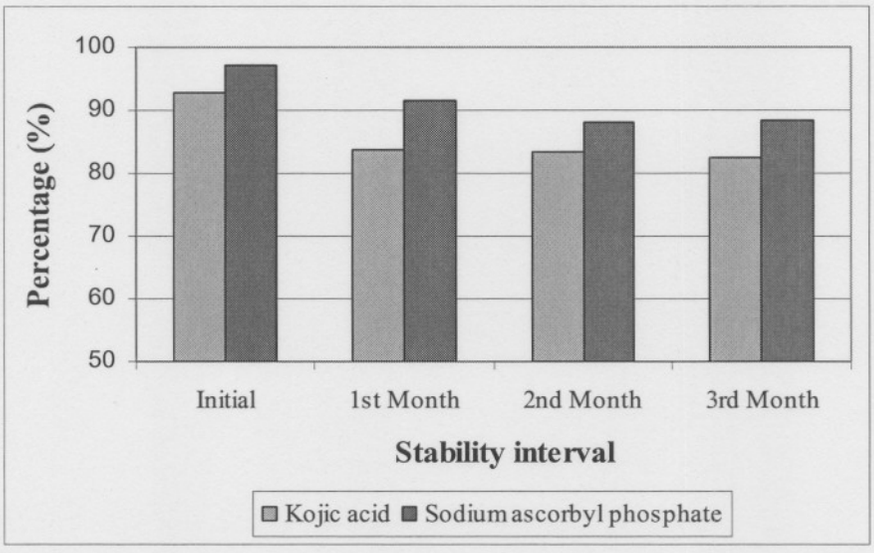

Figure 6.8 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in cream A after three months of storage at 25°C + 60% RH.

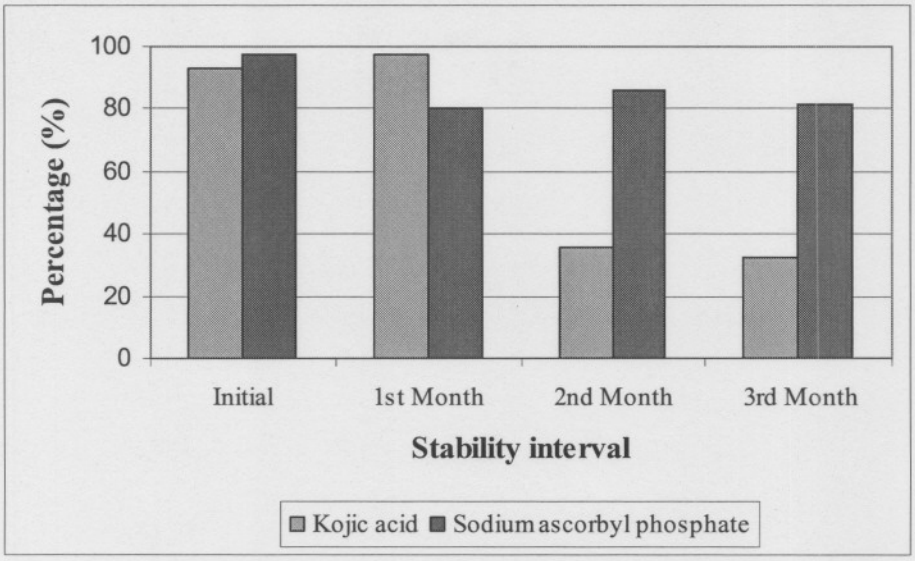

Figure 6.9 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in cream A after three months of storage at 40°C + 75%RH.

The following graph represents the assay test results of the sodium ascorbyl phosphate in cream B over a three month period (See Appendix B for the HPLC results).

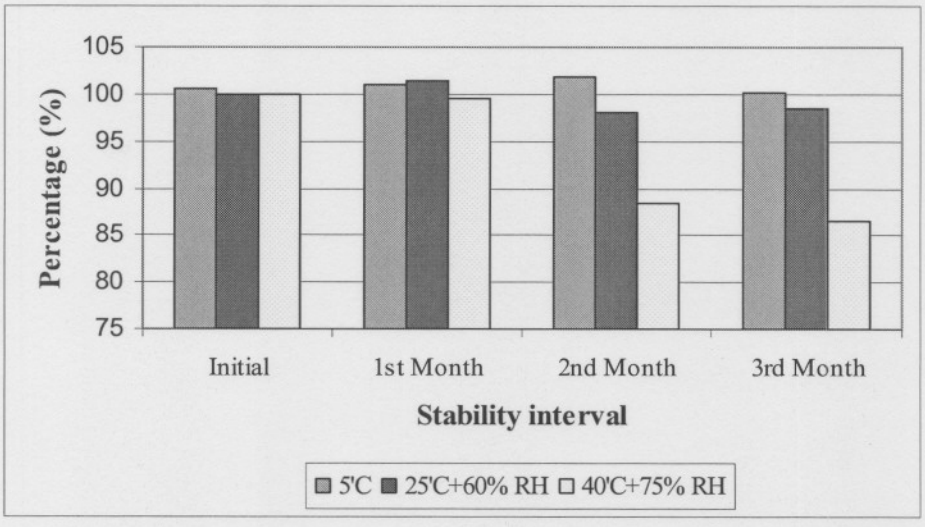

Figure 6.10 HPLC assay results showing the percentage sodium ascorbyl phosphate present in cream B after three months stability period.

The following graph represents a comparison between sodium ascorbyl phosphate concentrations of cream A and cream B which was stored at 25˚C + 60% RH over the three month stability period.

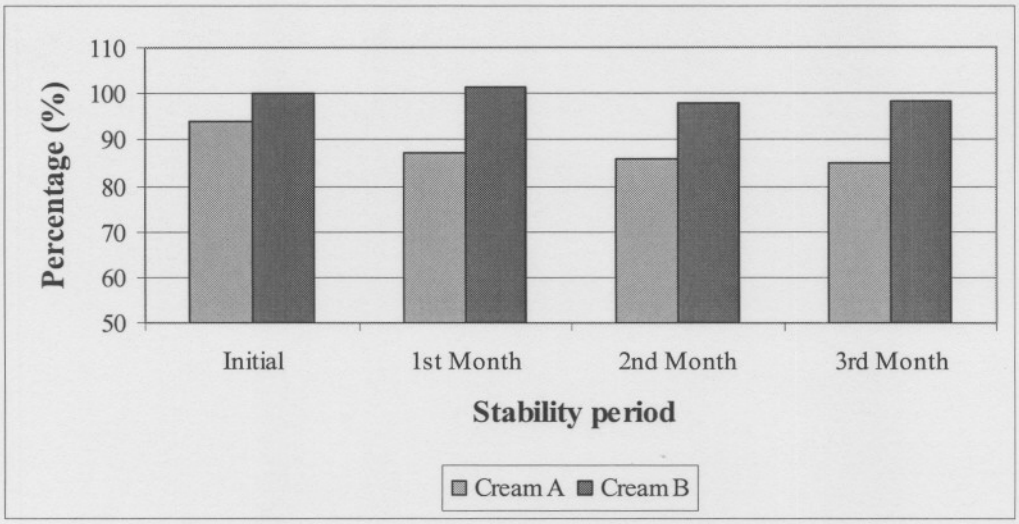

Figure 6.11 HPLC assay results of a comparison of the sodium ascorbyl phosphate concentration between cream A and cream B that was stored at 25˚C + 60% RH over the three month stability period.

6.8.2 Discussion

The samples of cream A that was stored at 5˚C showed a decline of ± 6% for kojic acid and ± 12% for sodium ascorbyl phosphate (Figure 6.7). At 25˚C + 60% RH the samples showed a slightly sharper decline for kojic acid (± 10%), but sodium ascorbyl phosphate showed a decline similar to the samples that was stored at 5˚C. It can be clearly seen how sensitive kojic acid is to heat, at 40˚C + 75% RH the percentage dropped with 60.6% after three months whilst sodium ascorbyl phosphate showed a decline of only 15.6%. Kojic acid tends to oxidise over time (Konsult.lv:2003), which explains the major colour change that was observed in the visual assessment in section 6.7. The samples of cream B that was stored at 5˚C showed an insignificant decline of less than 1%. The percentage sodium ascorbyl phosphate present in the batches that was stored at 25˚C + 60% RH also showed a very small decline of 1.6%. The heat sensitivity of sodium ascorbyl phosphate can be

seen in the results obtained from the samples that were stored at 40°C + 75% RH, the percentage dropped with 13.5%. The samples of cream A and cream B that were stored at 25°C + 60% RH for three months, were compared in Figure 6.11, it is clear that the sodium ascorbyl phosphate was more stable when formulated as the only active.

6.9 Kojic acid and sodium ascorbyl phosphate membrane release

Membrane release tests are used to determine if the active ingredients have been released from the formulation, as well as to determine the release rate.

6.9.1 Concentrations of Kojic acid and sodium ascorbyl phosphate released from cream A and cream B.

These tests were done to prove that the actives were released from cream A and cream B. The concentrations of kojic acid and sodium ascorbyl phosphate released from cream A, and the sodium ascorbyl phosphate released from cream B, were determined at initial and after three months storage at 25°C + 60% RH and 40°C + 75% RH for 3 months as described in section 5.3.2. The results of the initial batches of cream A and cream B will be given prior to the comparison of the release rates at different months of stability testing in section 6.10.

6.9.1.1 Results

A high performance liquid chromatogram of the simultaneous release of kojic acid and sodium ascorbyl phosphate, obtained during the release rate test, is shown in Figure 6.12.

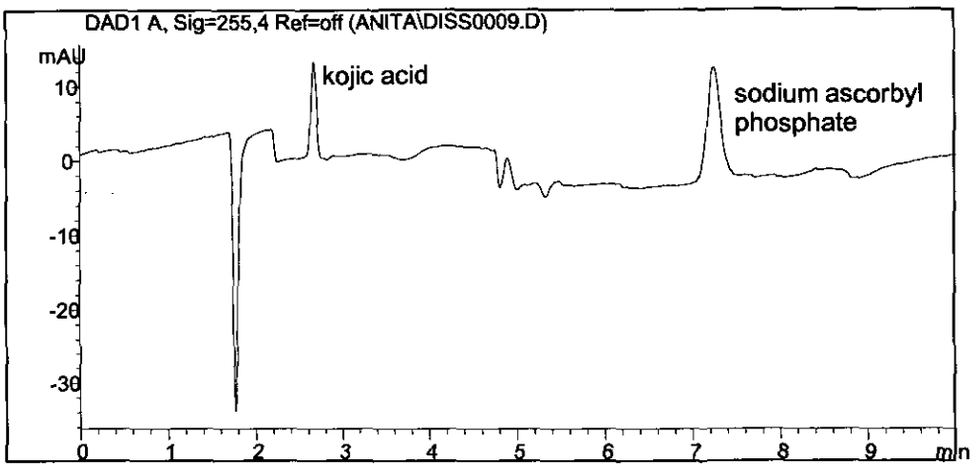

Figure 6.12 HPLC chromatogram of the simultaneous release of kojic acid and sodium ascorbyl phosphate, obtained during the release rate test of a sample that was stored at 25°C + 60% RH for 3 months.

The amount of kojic acid released, as function of time, from cream A (B/N: Initial (A)), is given in Figure 6.13.

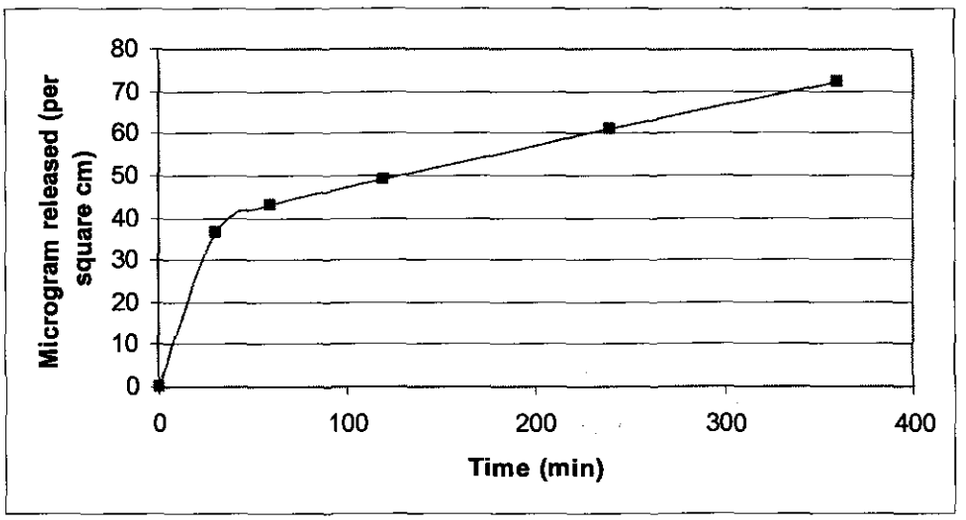

Figure 6.13 The concentration of kojic acid released over six hours, from the initial batch of cream A.

The release rate of kojic acid, as function of square root of time, from the initial batch of cream A is given in Figure 6.14.

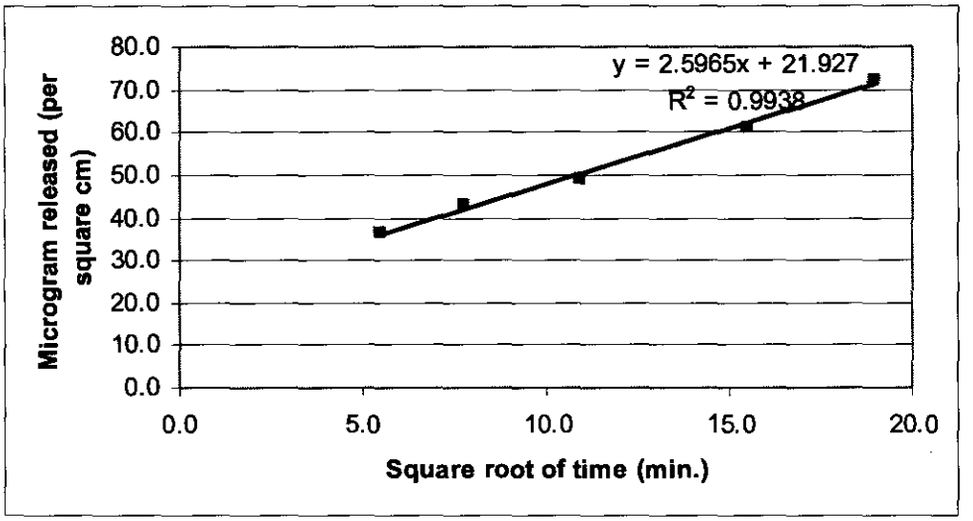

Figure 6.14. Release rate of the initial batch of kojic acid from cream A.

The amount of sodium ascorbyl phosphate released, as function of time, from cream A (B/N: Initial (A)), is given in Figure 6.15.

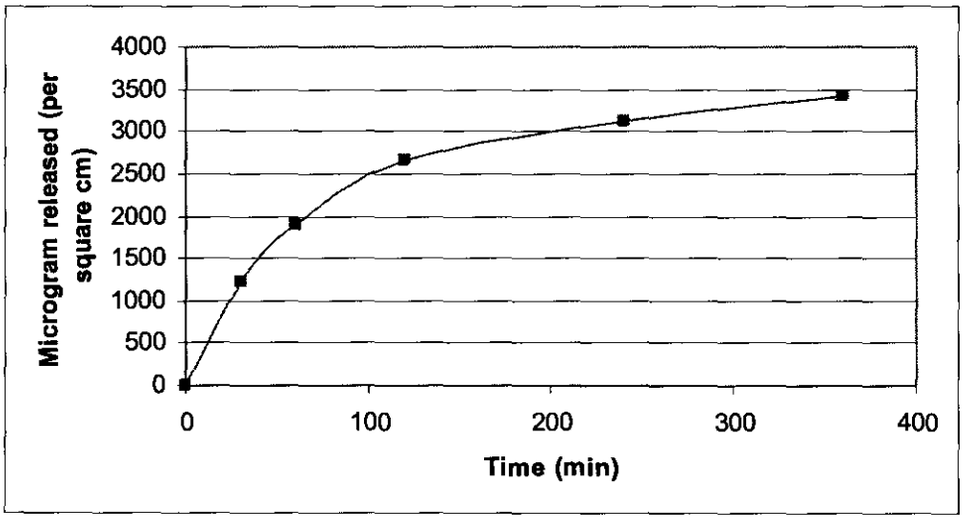

Figure 6.15. The concentration of the sodium ascorbyl phosphate released over six hours from the initial batch of cream A.

The release rate of sodium ascorbyl phosphate, as function of square root of time, from the initial batch of cream A is given in Figure 6.16.

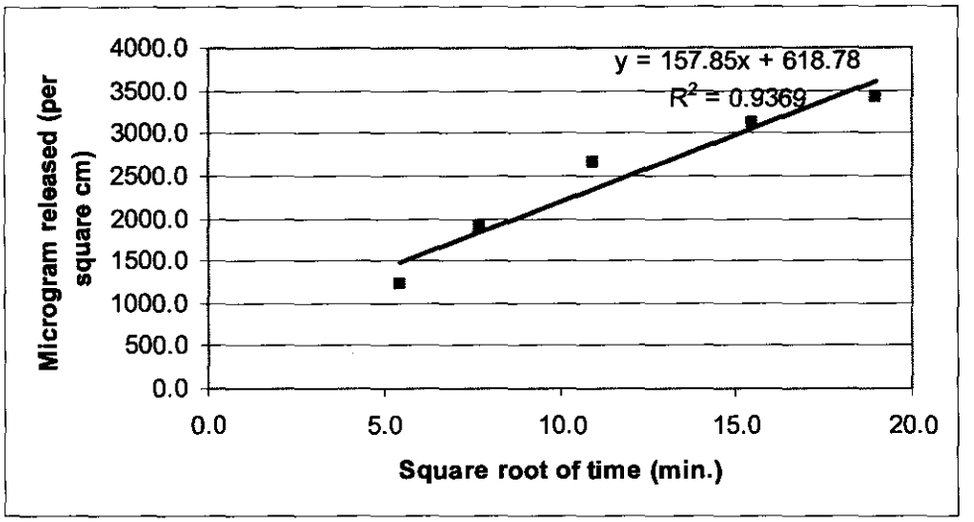

Figure 6.16 Release rate of the initial batch of sodium ascorbyl phosphate from cream A.

The concentration of sodium ascorbyl phosphate release, as function of time, from cream B (B/N: Initial (B)), is given in Figure 6.17.

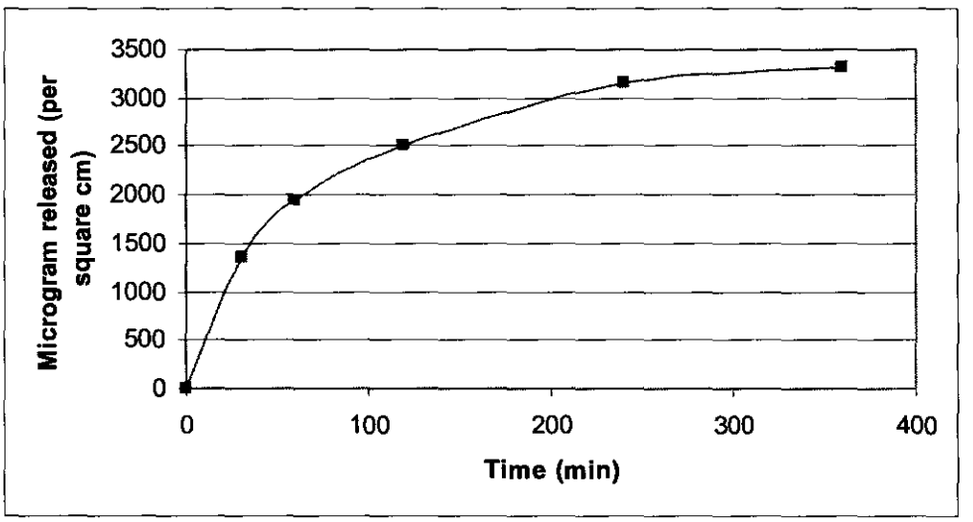

Figure 6.17 The concentration of the sodium ascorbyl phosphate released over six hours from the initial batch of cream B.

The release rate of sodium ascorbyl phosphate, as function of square root of time, from the initial batch of cream B is given in Figure 6.18.

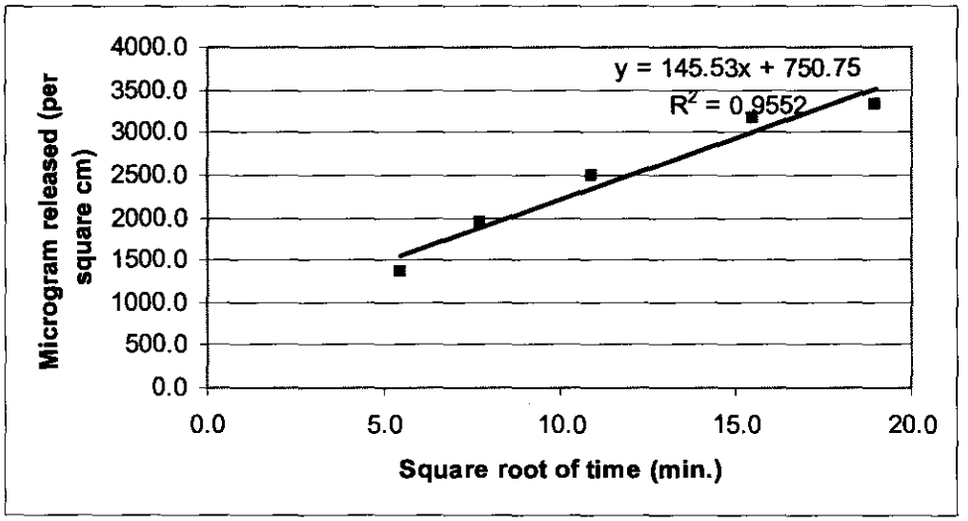

Figure 6.18 Release rate of the initial batch of sodium ascorbyl phosphate from cream B.

6.9.1.2 Discussion

The chromatogram obtained during the HPLC analysis of the membrane release is displayed in Figure 6.12. The kojic acid peak is visible at approximately 2 minutes and the sodium ascorbyl phosphate is visible at approximately 7 minutes. The peaks are very small because of the very low concentrations that were released.

The initials of cream A and cream B showed effective release for kojic acid and sodium ascorbyl phosphate, with acceptable regression values as displayed on the graphs.

A comparison of the release rates at different months of stability testing of cream A and cream B will now be given.

6.10 Comparison between the two creams.

The release rate from samples of cream A and cream B over the three months of storage can prove changes in physical and chemical parameters of the creams.

6.10.1 Results

Figures 6.19 – 6.20 represent the comparison between the release rates of kojic acid at the different months and temperatures of stability testing. Figures 6.21 – 6.22 and Figures 6.23 – 6.24 represent the comparison between the release rates of sodium ascorbyl phosphate at the different months of stability testing of cream A and cream B respectively. Figures 6.25 and 6.26 represent the comparison between the samples of kojic acid in cream A at 25°C + 60% RH and 40°C + 75% RH respectively over the three month stability period. The same values were used as in Figures 6.19 and 6.20, but were added to the text for the sake of completion and to show the decline in release rate clearer.

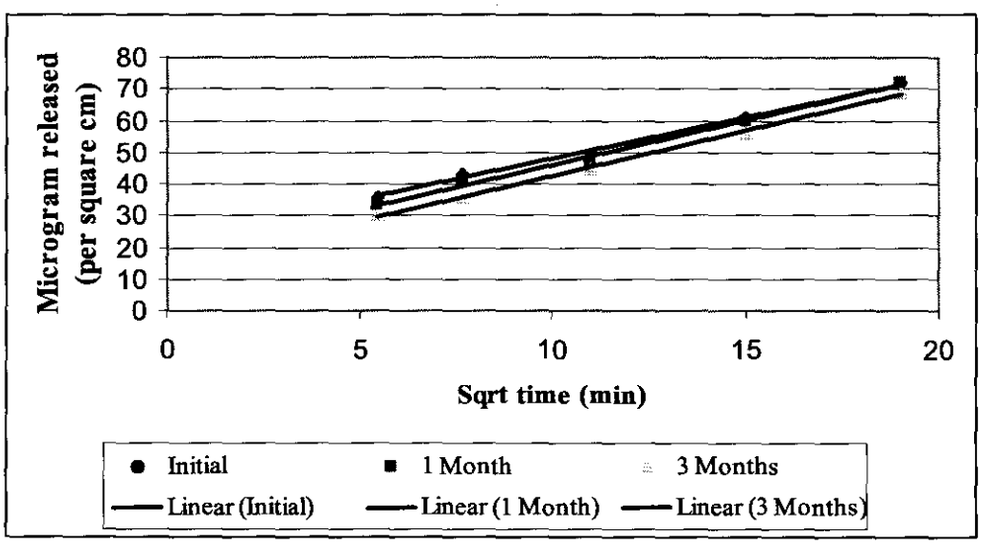

Figure 6.19 Release rate of the kojic acid from the samples of cream A that was stored at 25°C + 60% RH over a period of three months.

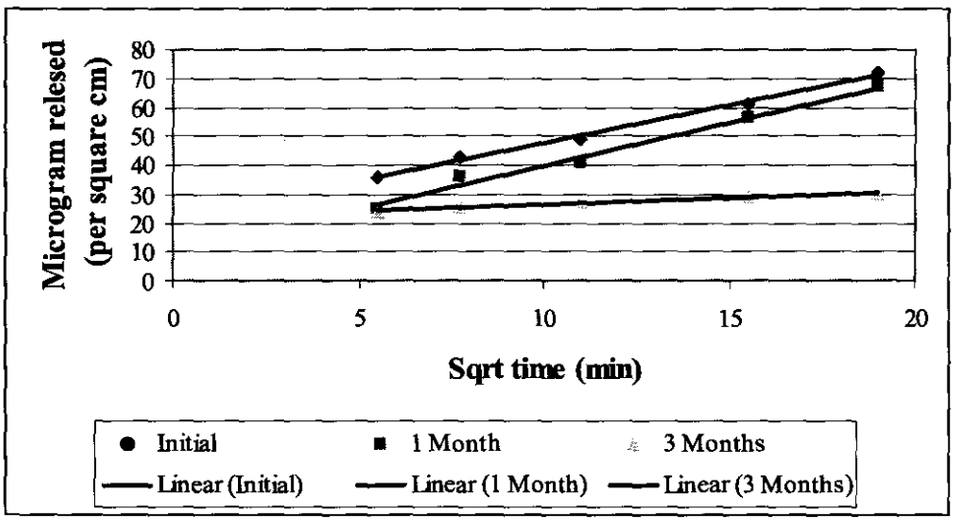

Figure 6.20 Release rate of the kojic acid from the samples of cream A that was stored at 40°C + 75% RH over a period of three months.

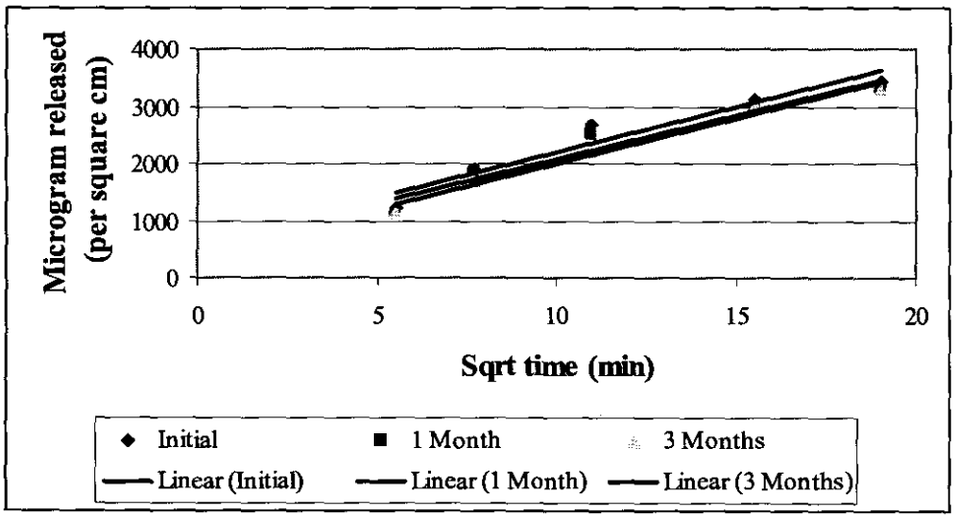

Figure 6.21 Release rate of the sodium ascorbyl phosphate from the samples of cream A that was stored at 25°C + 60% RH over a period of three months.

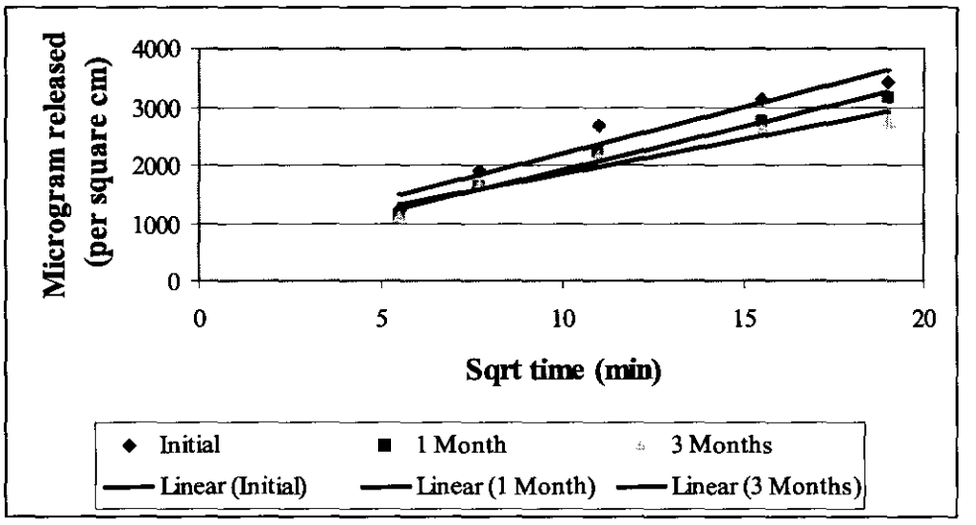

Figure 6.22 Release rate of the sodium ascorbyl phosphate from the samples of cream A that was stored at 40°C + 75% RH over a period of three months.

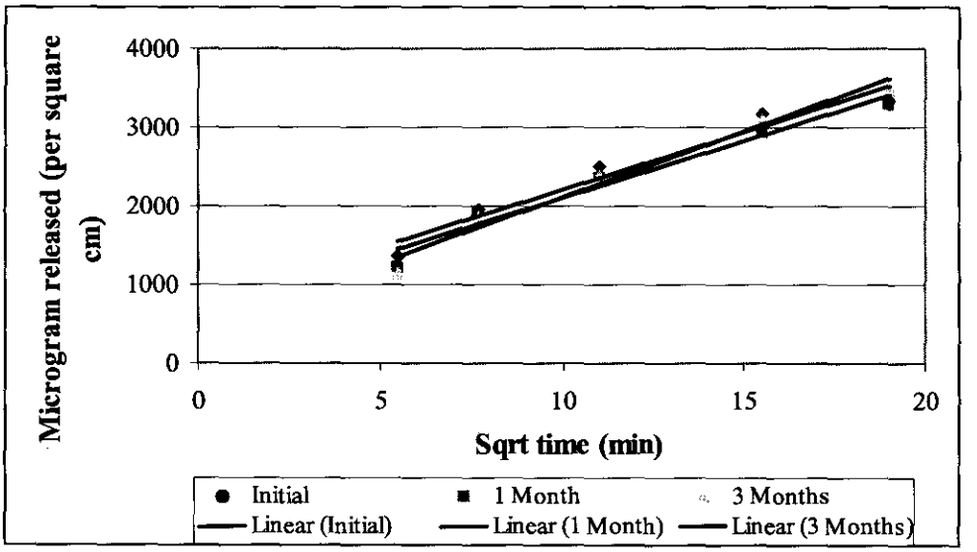

Figure 6.23 Release rate of the sodium ascorbyl phosphate from the samples of cream B that was stored at 25°C + 60% RH over a period of three months.

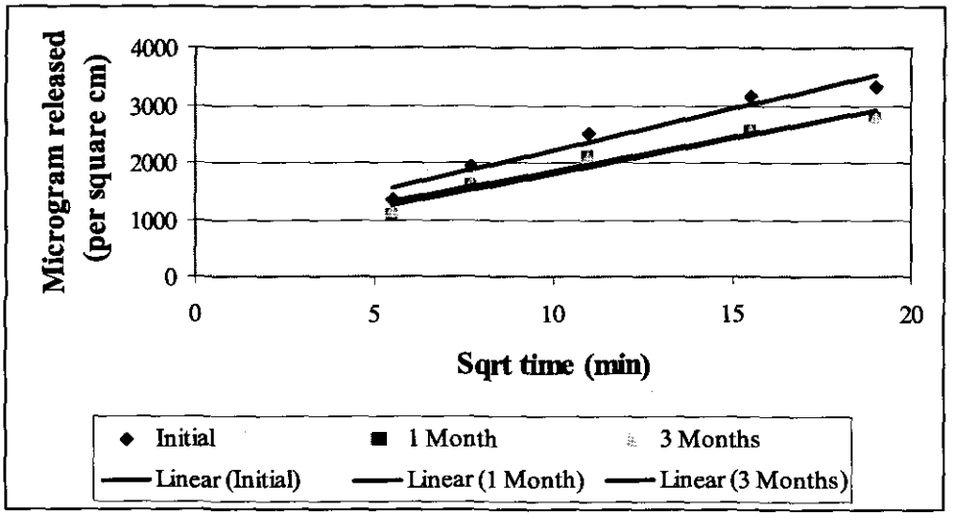

Figure 6.24 Release rate of the sodium ascorbyl phosphate from the samples of cream B that was stored at 40°C + 75% RH over a period of three months.

The sodium ascorbyl phosphate in cream A and in cream B showed no significant increase or decrease in the release rate over time, therefore the stability is accepted to be good and is confirmed by the HPLC assay results, see section 6.8.

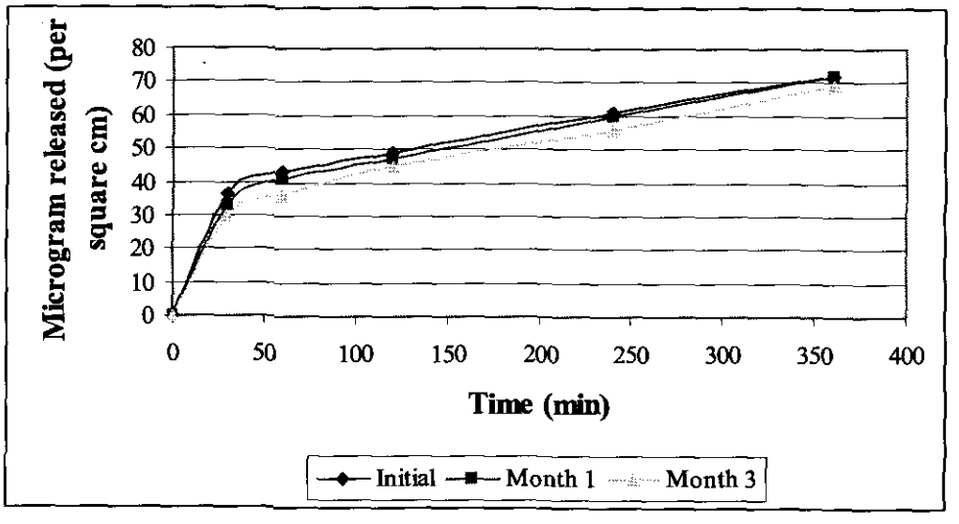

Figure 6.25 Comparison between the release rates of kojic acid from the samples of cream A that was stored at 25°C + 60% RH over a period of three months.

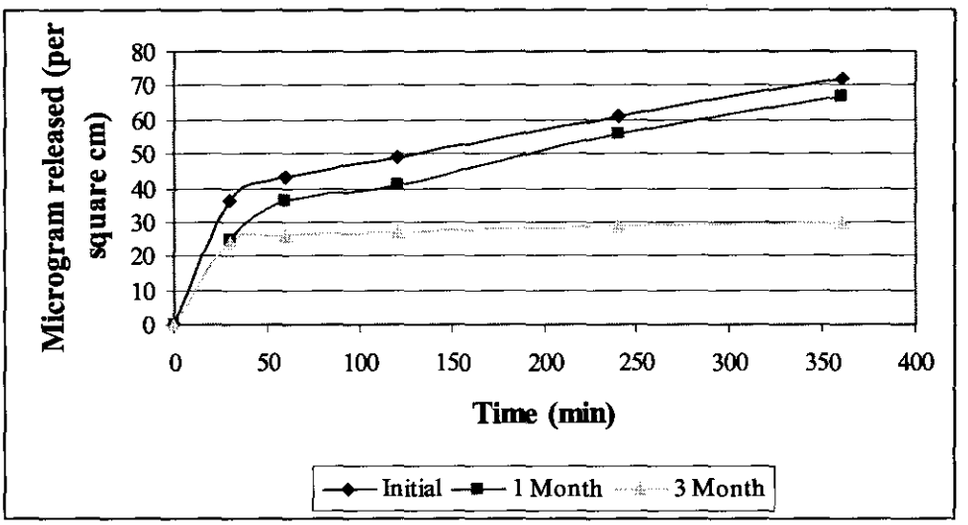

Figure 6.26 Comparison between the release rates of kojic acid from the samples of cream A that was stored at 40°C + 60% RH over a period of three months.

6.10.2 Discussion

Figures 6.19 – 6.26 represent all the samples of cream A and cream B that was tested for their stability. The rate of the concentration released of kojic acid and sodium ascorbyl phosphate that was released at each test interval was compared for every batch. The release rate of kojic acid decreased significantly after storage for 3 months at 40°C + 75% RH. This correlate well with the HPLC assay results, see Figure 6.9, which can be due to the instability of kojic acid at high temperatures.

Figures 6.25 and 6.26 show the decline that occurred in the release rate of kojic acid clearly. The graphics of sodium ascorbyl phosphate release against time in minutes were not included, for the graphics of the square root of time were sufficient enough to see that no significant increase or decrease had occurred.

6.11 Preservative testing

Preservation is an anti-microbial process, used to control the prevention of microbial spoilage of cosmetics and toiletry products. Moreover, preservation should enable a

formulation to cope adequately with all micro-organisms likely to enter the product, during repeated use (Knowlton & Pearce 1993:449).

The hydroxybenzoate concentrations of the creams were determined once a month at three different temperatures and humidities, using HPLC chromatography as was described in section 5.3.1.2.

6.11.1 Results of preservative testing in cream A

The methyl hydroxybenzoate concentrations in cream A over the three month period at the three different temperatures and humidities are depicted in Figure 6.27.

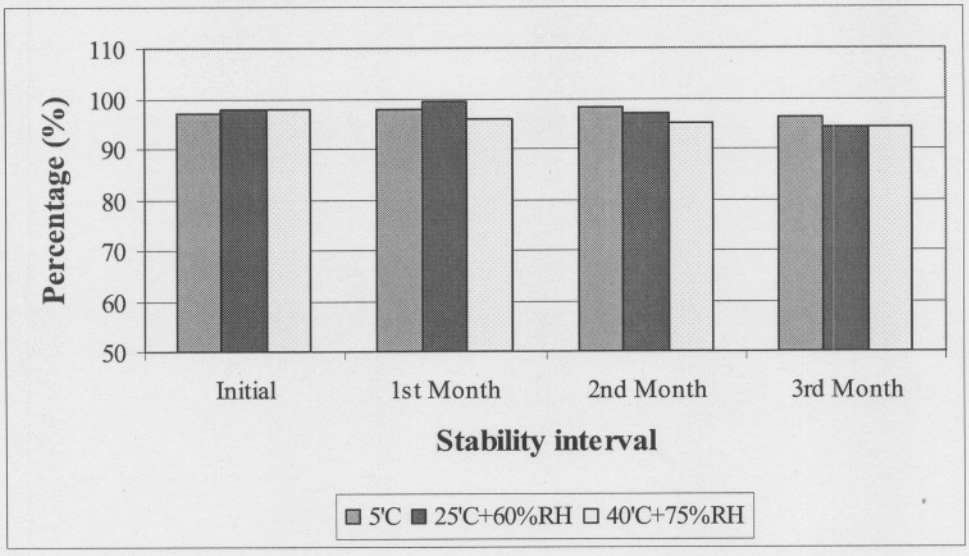

Figure 6.27 Methyl hydroxybenzoate concentrations in cream A over the three month stability period.

The propyl hydroxybenzoate concentrations in cream A over the three month stability period at the three different temperatures and humidities are given in Figure 6.28.

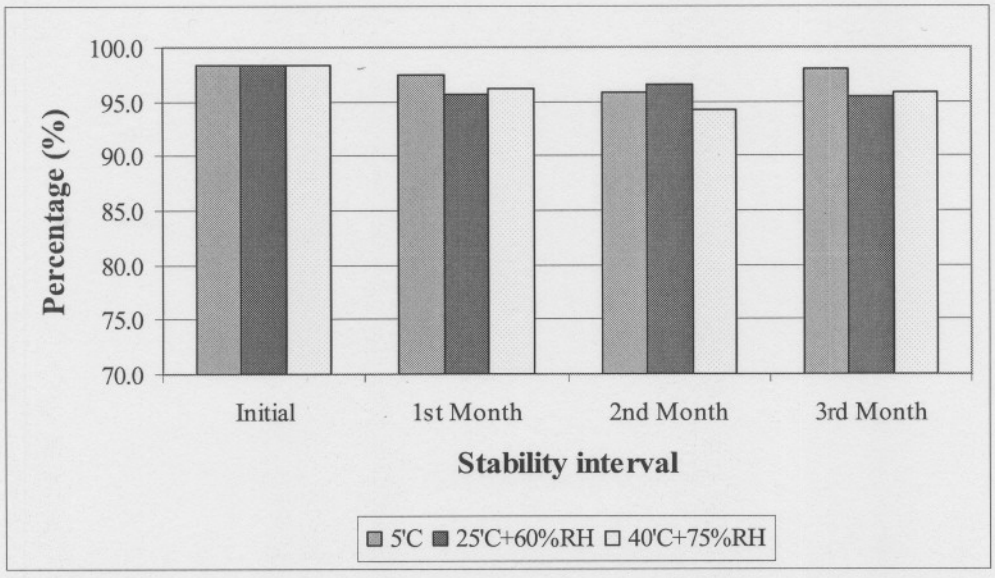

Figure 6.28 Propyl hydroxybenzoate concentrations in cream A over the three month stability period.

6.11.2 Results of preservative testing in cream B

The methyl hydroxybenzoate concentrations in cream B over the three month period at the three different temperatures and humidities are depicted in Figure 6.29.

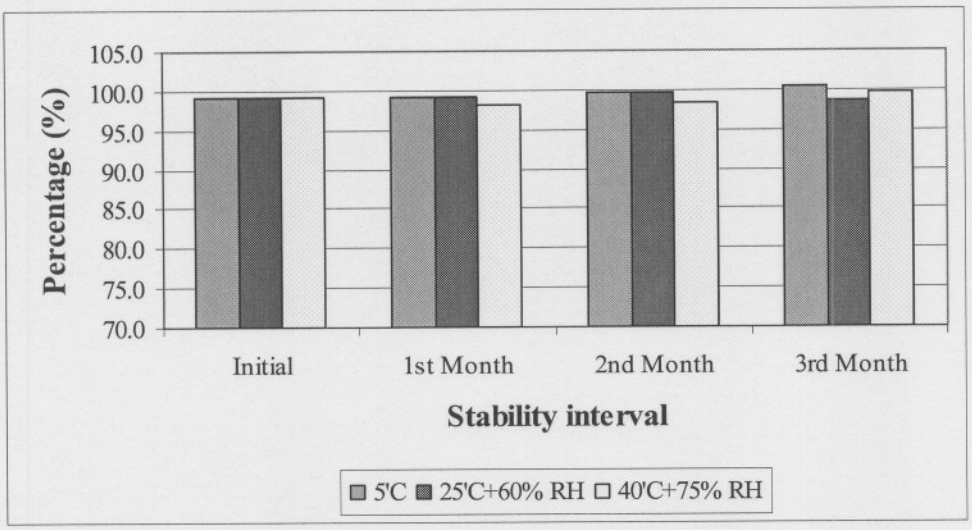

Figure 6.29 Methyl hydroxybenzoate concentrations in cream B over the three month stability period.

The propyl hydroxybenzoate concentrations in cream B over the three month period at the three different temperatures and humidities are depicted in Figure 6.30.

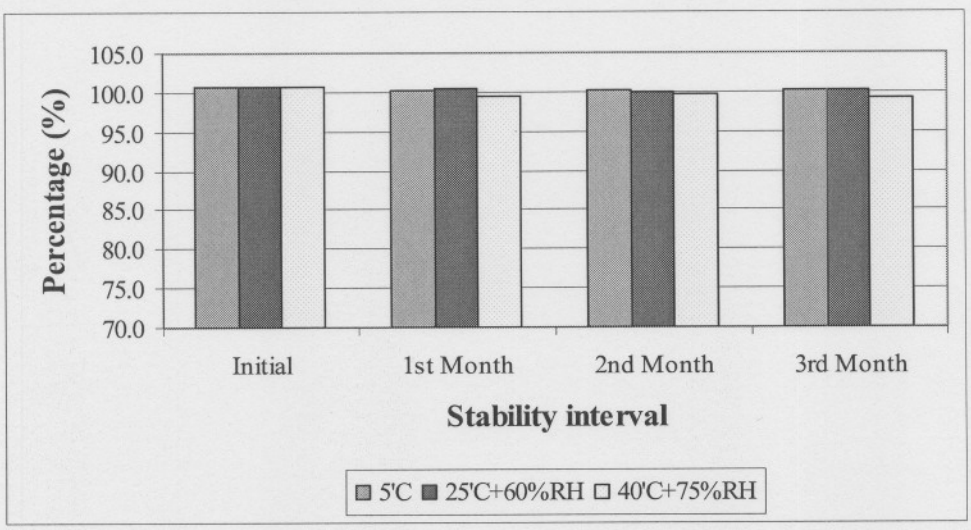

Figure 6.30 Propyl hydroxybenzoate concentrations in cream B over the three month stability period.

6.11.3 Discussion

The percentage of the concentrations of the preservatives in cream A and cream B, methyl and propyl hydroxybenzoate, are shown in Figures 6.28, 6.29, 6.30 and 6.31 respectively. There was a decrease in the concentrations of both the preservatives over the period of three months. The required concentrations for methyl and propyl hydroxybenzoate are 0.02% – 0.3% and 0.01% - 0.6% respectively (Wade and Weller, 1994:310,411). The decrease in concentration is not significant, since the variation could be due to experimental variation. All the values were above 90%. The preservative efficacy tests that were done by Wits Health Consortium (Pty) Ltd. on samples of cream A and cream B stated that the samples complied with the requirements of USP 25 and USP 26 and the specified microbial limits. There was no significant microbiological growth that interfered with the stability of the formulations.

6.12 Conclusion

In this chapter, cream A and cream B were tested over a three month period, using different stability indicative test methods. Cream A contains kojic acid and sodium ascorbyl phosphate whereas cream B only contains sodium ascorbyl phosphate as active.

A decrease in the pH of the cream A and cream B over the period of three months, especially the batch that was stored at 40°C + 75% RH, should not influence the effectiveness of the sodium ascorbyl phosphate, for its stability is best at pH values above 6.5. The kojic acid showed a decrease in stability that can be correlated to the pH decrease over time, for kojic acid is most stable in formulations with pH values between four and five.

There was no significant change in the specific gravity of cream A or cream B, see Table 6.1. Temperature and humidity did not have an influence on the specific gravity.

The viscosity results showed a decrease for cream A, whereas cream B had a small increase. This correlates well with the penetration results, since the penetration for cream A showed a slight increase, thus the cream became thinner with time. The spreadability revealed no significant change over time for both the creams, but the measurements showed that cream B was definitely thicker than cream A.

The sensitivity of kojic acid to heat can clearly be seen from the results obtained during HPLC assay testing. The kojic concentration present in cream A that was stored at 40°C + 75% RH over a three month stability period, decreased significantly compared to the much more stable sodium ascorbyl phosphate.

In the membrane release graphics it became clear that the decrease in concentration of kojic acid in cream A, which were stored at 40°C + 75% RH over three months, had a large influence on the concentration of active released from the cream.

Preservative efficacy testing was done by Wits Health Consortium (Pty) Ltd.
All the samples complied with the requirements of USP 25 and USP 26 and the specified microbial limits.

CAPTER 7
TEST RESULTS: FACIAL TONER

7.1 Introduction

Toners are used after the face has been cleansed using face cleansing cosmetics mainly to moisturise the skin and supply it with humectants. Mitsui (1997:328) describes a toner to have an astringent action as well as supplying moisture and humectants to the horny layer of the skin, it also gives a light feeling to the skin and prevents makeup from spoiling.

In this chapter the tests done on the toner are described and results are discussed. The tests were done over a period of three months at three different temperatures and humiditIES. The following physical parameters were investigated: pH, specific gravity, visual assessment, and the assays from HPLC chromatography. Microbial preservative efficacy was done by Wits Health Consortium (Pty) Ltd.

7.2 pH of the toner

The pH of the toner was measured once a month for a period of three months at three different temperatures and humidity as described in section 5.3.6.

7.2.1 Results

The pH values of the toner measured over the three month stability period is given in Figure 7.1.

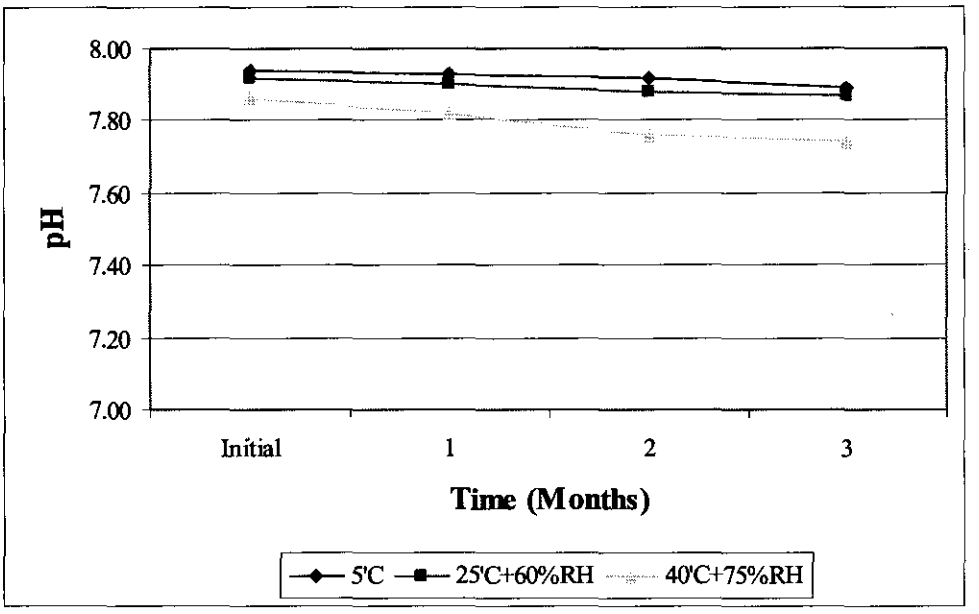

Figure 7.1 The pH of the toner over the three month stability period.

7.2.2 Discussion

Figure 7.1 represents a small decrease in the pH of the toner over the period of three months. The batch that was stored at 40°C + 75% RH displays a larger decline in pH over time. For a broader discussion on the stability of kojic acid and sodium ascorbyl phosphate, refer to section 3.2.1. The pH of the toner tends to be between 7 and 8, and since the optimum pH for sodium ascorbyl phosphate is 6.5 and for kojic acid is between the values of 4 and 5, the effectiveness of the toner could be influenced.

7.3 Specific gravity

The specific gravity of the toner was determined once a month for three months, as was described in section 5.3.7.

7.3.1 Results

The relative density results of three samples of the toner are given in Table 7.1.

Table 7.1 The specific gravity (g/cm^3) of the three batches of the toner over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	1.04	1.03	1.04	1.03
25°C+60%RH	1.04	1.00	1.00	1.00
40°C+75%RH	1.04	1.06	0.97	1.01

7.3.2 Discussion

There was no significant change in the specific gravity of the toner (Table 7.1). Temperature and humidity did not have an influence on the specific gravity.

7.4 Microbial preservative efficacy

Preservative efficacy testing was done by Wits Health Consortium (Pty) Ltd. All the samples complied with the requirements of USP 25 and USP 26 and the specified microbial limits.

7.5 Visual assessment

The appearance measurements of the toner were done once a month for three months as described in section 5.3.8.

7.5.1 Results

The visual assessment of the toner is given in Table 7.2.

Table 7.2 The visual assessment of the toner over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	**Colour: bright yellow**	No change	No change	**Darker yellow**
	Easy sprayable	No change	No change	No change
	Applies easily	No change	No change	No change
	Homogeneous	No change	No change	No change
	Transparent	No change	No change	No change
	Completely fluent	No change	No change	No change
25°C + 60%RH	**Colour: light yellow**	**Darker yellow**	**Brown/orange**	**Brown**
	Easy sprayable	No change	No change	No change
	Applies easily	No change	No change	No change
	Homogeneous	No change	No change	No change
	Transparent	No change	No change	No change
	Completely fluent	No change	No change	No change
40°C + 75%RH	**Colour: light yellow**	**Brown**	**Dark brown**	**Black/brown**
	Easy sprayable	No change	No change	No change
	Applies easily	No change	No change	No change
	Homogeneous	No change	No change	No change
	Transparent	**Intransparent**	**Intransparent**	**Intransparent**
	Completely fluent	No change	No change	No change

7.5.2 Discussion

The visual change that occurred was primarily in the *colour* of the toner. It changed from light yellow and transparent to shades of brown and intransparent, especially the samles that were subjected to 40°C + 75% RH. The colour change also increased with time over the stability period of three months.

7.6 Assays

The concentrations of kojic acid and sodium ascorbyl phosphate in the toner over a period of three months and at 5°C, 25°C + 60% RH and 40°C + 75% RH were determined using HPLC chromatography as was described in section 5.3.1.

7.6.1 Results

Figure 7.2 represents a chromatogram of the toner obtained during the assays of a sample stored at 25°C + 60% RH for three months.

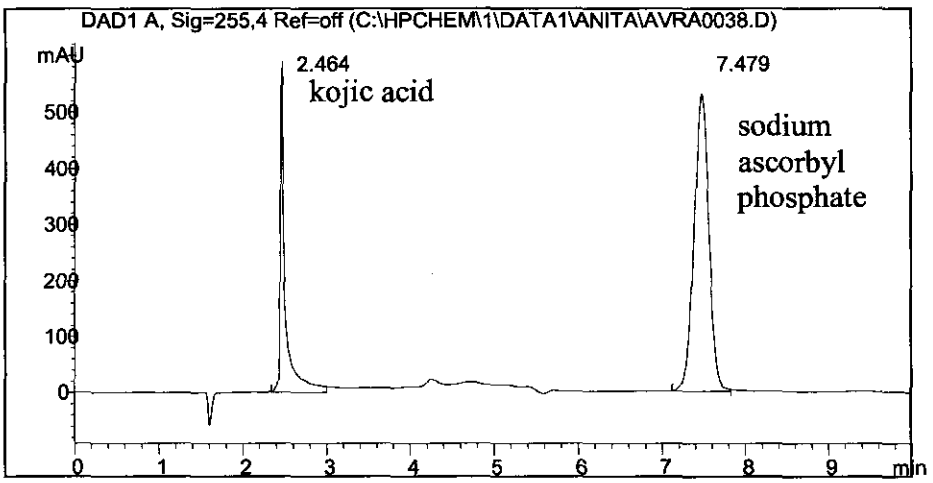

Figure 7.2 A chromatogram of the toner obtained during the assays of a sample stored at 25°C + 60% RH for three months.

Figures 7.3 – 7.5 represent the assay test results of the kojic acid and sodium ascorbyl phosphate in the toner over a three month period (See Appendix B for HPLC results).

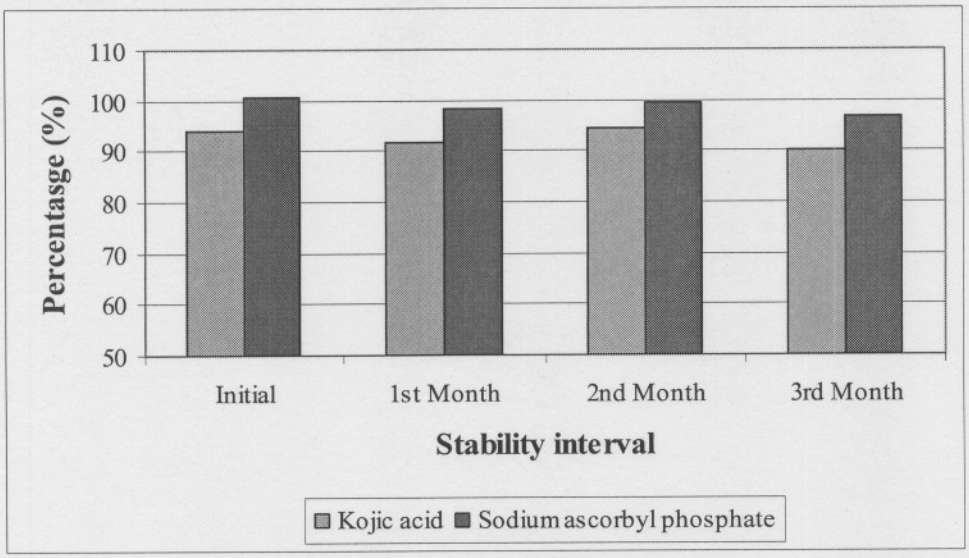

Figure 7.3 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the toner after three months of storage at 5°C.

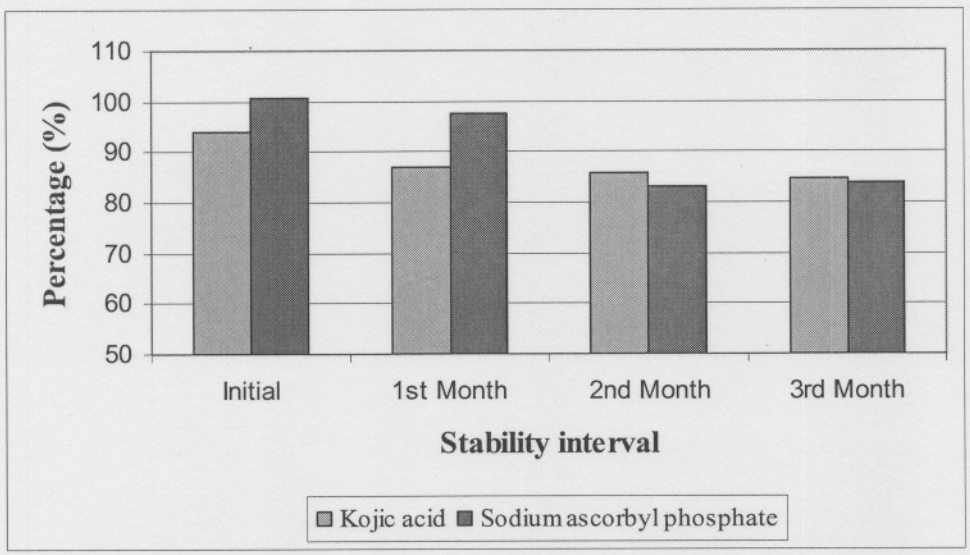

Figure 7.4 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the toner after three months of storage at 25°C + 60% RH.

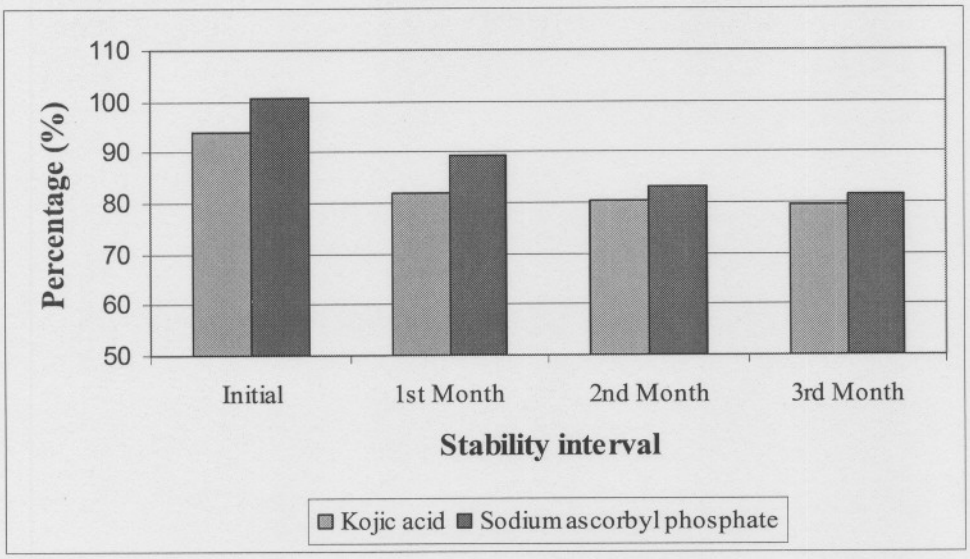

Figure 7.5 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the toner after three months of storage at 40°C + 75% RH.

7.6.2 Discussion

The samples that were stored at 5°C showed a decline of 3.8% for kojic acid and 4% for sodium ascorbyl phosphate (Figure 7.3). At 25°C + 60% RH the samples showed a slight sharper decline for kojic acid of 9.5%, and sodium ascorbyl phosphate showed a decline of 16.8% (refer to Appendix B). The concentration of kojic acid present in the toner was remarkably higher in comparing to the other formulations, which were also stored at 40°C + 75% RH. This is because of the inclusion of propylene glycol and ethanol in the formulation, according to Honda (1998) inclusion of these ingredients serves to markedly improve stability of the kojic acid to heat. The percentage dropped with only 14.2%, in comparison with 60.6% in cream A (section 6.8.2), whilst sodium ascorbyl phosphate showed a decline of 19.3%.

Kojic acid tends to oxidise over time (Konsult.lv:2003), which explains the major colour change that was observed in the visual assessment in section 7.5. The addition of phosphorester groups of sodium ascorbyl phosphate was to improve the stability of kojic acid, as it prevents the degradation effect according to Rho (2001:1).

7.7 Conclusion

In this chapter, the three batches of the toner were subject to different stability indicative test methods. The pH measurements showed a small decline over a period of three months. The optimum pH value of the toner would be about 5.5 (pH of the skin), but it average about 7.90. This may influence the effectiveness of the toner because kojic acid is most stable at pH values between 4 and 5, while sodium ascorbyl phosphate is stable above 6.5.

The specific gravity did not reveal any significant changes. A large colour change occurred over time and with increased temperature it showed a break down in the actives, and it is confirmed with the stability tests done on the HPLC, see section 7.6.

Preservative efficacy testing that was done by Wits Health Consortium (Pty) Ltd. Results showed that all the samples complied with the requirements of USP 26 and the specified microbial limits.

CAPTER 8
TEST RESULTS: FOAM BATH

8.1 Introduction

In this chapter the tests done on the foam bath are described and results are discussed. The tests were done over a period of three months at three different temperatures and humidities. The following physical parameters were investigated: pH, specific gravity, visual assessment, viscosity, foamability and the assays of kojic acid and sodium ascorbyl phosphate. Microbial preservative efficacy was done by Wits Health Consortium (Pty) Ltd.

8.2 pH of the foam bath

The pH of the foam bath was measured once a month for a period of three months at 5°C, 25°C + 60% RH and 40°C + 75% RH as described in section 5.3.6.

8.2.1 Results

The pH values of the foam bath over the three months stability period are given in Figure 8.1.

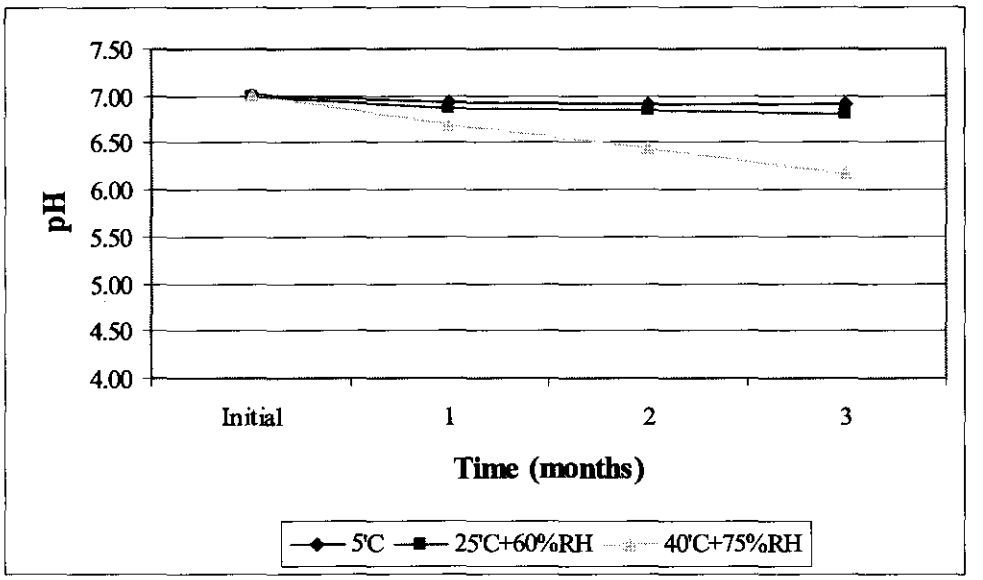

Figure 8.1 The pH of the foam bath over the three month stability period.

8.2.2 Discussion

Figure 8.1 indicates a small decrease in the pH of the foam bath over the period of three months. The sample that was stored at 40°C + 75% RH displays a larger decline in pH over time. The pH of the foam bath tends to be between 6 and 7 and since the optimum pH for sodium ascorbyl phosphate is 6.5, the effectiveness of sodium ascorbyl phosphate should not be influenced, but kojic acid might become unstable (see section 3.2 for explanation of stability).

8.3 Specific gravity

The specific gravity of the foam bath was determined once a month for three months, as was described in section 5.3.7.

8.3.1 Results

The specific gravity results of the foam bath are given in Table 8.1.

Table 8.1 The specific gravity (g/cm^3) of the foam bath over the three month stability period.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	1.040	1.085	1.083	1.076
25°C+60% RH	1.039	1.033	1.048	1.032
40°C+75% RH	1.041	1.047	1.078	1.059

8.3.2 Discussion

There was no significant change in the specific gravity of the foam bath (Table 8.1). Temperature and humidity did not have an influence on the specific gravity.

8.4 Microbial preservative efficacy

Preservative efficacy testing was done by Wits Health Consortium (Pty) Ltd. All the samples complied with the requirements of the USP 26 and the specified microbial limits.

8.5 Visual assessment

The appearance measurements of the foam bath were done once a month for three months as described in section 5.3.8.

8.5.1 Results

The visual assessment of the foam bath is given in Table 8.2.

Table 8.2 The visual assessment of the foam bath over the three month stability period at three different temperatures and humidities.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	Colour: pearly cream	No change	No change	No change
	Colour of foam: white	No change	No change	No change
	Slow flowing	No change	No change	No change
	Forms foam easily	No change	No change	No change
	Homogeneous	No change	No change	No change
25°C + 60% RH	Colour: pearly cream	yellow	Gold	Gold
	Colour of foam: yellow	yellow	yellow	yellow
	Slow flowing	No change	No change	No change
	Forms foam easily	No change	No change	No change
	Homogeneous	No change	No change	No change
40°C + 75% RH	Colour: pearly cream	Brown	Golden brown	Dark brown
	Colour of foam: yellow	yellow	yellow	yellow
	Slow flowing	No change	Thicker	Thicker
	Forms foam easily	No change	No change	No change
	Homogeneous	Separated	Separated	Separated

8.5.2 Discussion

The colour of the foam bath changed from pearly cream-coloured to dark brown when exposed to 40°C + 75% RH for three months. The foam colour became yellow. The flow characteristics also changed to a thicker, slower flowing formulation that corresponds with the viscosity results (see section 8.6) which also increased over the three month stability period. The consistency became less homogeneous with time.

8.6 Viscosity

The viscosity of the foam bath was determined once a month for three months as described in section 5.3.4.

8.6.1 Results

The viscosity of the foam bath is given in Table 8.3.

Table 8.3 The viscosity (in cP) of the foam bath, measured over a three month stability period at 2 minute intervals.

Viscosity values (cP)	Initial	Month 1	Month 2	Month 3
5°C	302.6	302.0	301.7	301.5
25°C+60%RH	325.9	369.6	324.3	358.5
40°C+75%RH	327.2	419.9	616.2	736.5

8.6.2 Discussion

As can be seen in Table 8.3, the overall trend was that the viscosity of the batches remained stable over the test period, except at 40°C + 75% RH. The increase in the viscosity after three months at 40°C + 75% RH was probably due to the instability and sensitivity of the ingredients in the foam bath to increased temperatures.

8.7 Foamability

The foamability of the foam bath was determined once a month for three months on the samples that was stored at three different temperatures and humidities, as described in section 5.3.9.

8.7.1 Results

The foamability results of the foam bath are given in Figure 8.2.

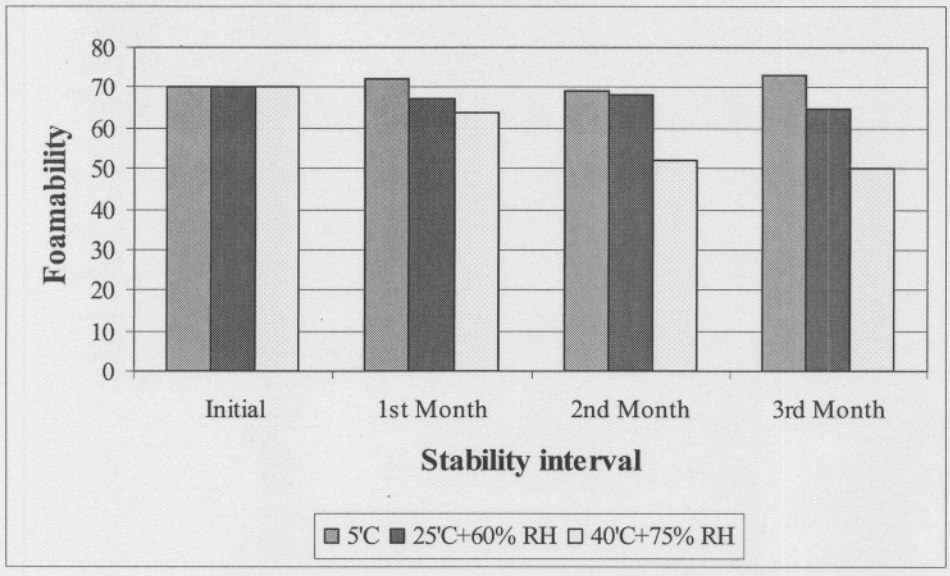

Figure 8.2 The foamability results (in cm^3) of the foam bath over the three month stability period.

8.7.2 Discussion

As can be seen in Figure 8.2, the foamability decreased slightly in the samples stored at 5˚C and 25˚C + 60% RH. The batches stored at 40˚C + 75% RH showed a more significant decrease. The foam became yellow after one month. The foam of the 40˚C + 75% RH samples had smaller bubbles and were denser than at initial. All the samples had approximately the same foam forming ability but the speed at which it decreased was much faster for the batches stored at higher temperatures (refer to Appendix C for values and calculations).

8.8 Assays

The concentrations of kojic acid and sodium ascorbyl phosphate in the foam bath over a period of three months and at 5˚C, 25˚C + 60% RH and 40˚C + 75% RH were determined using HPLC chromatography as was described in section 5.3.1.
Figure 8.3 represents a chromatogram of a sample of the foam bath from the sample which was stored at 25˚C + 60 % RH for three months.

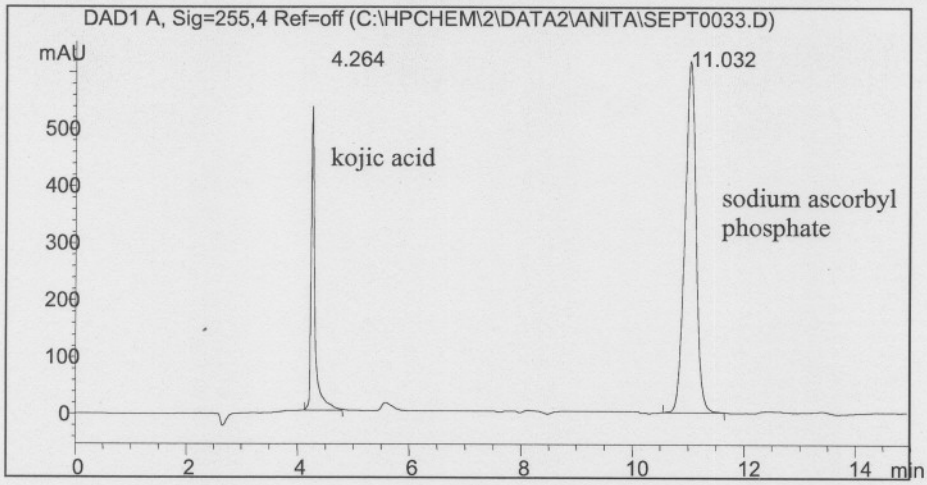

Figure 8.3 Chromatogram of a sample of the foam bath from the sample which was stored at 25°C + 60% RH over the three month stability period.

8.8.1 Results

The following graphs represent the assay test results of the kojic acid and sodium ascorbyl phosphate in the foam bath over a three month stability period (Refer to Appendix B for the HPLC results).

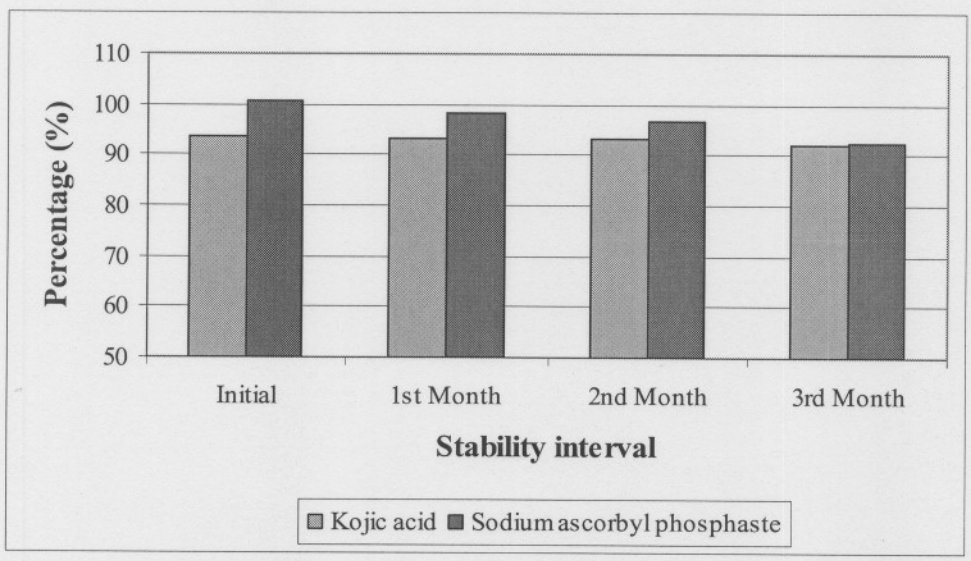

Figure 8.4 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the foam bath after three months of storage at 5°C.

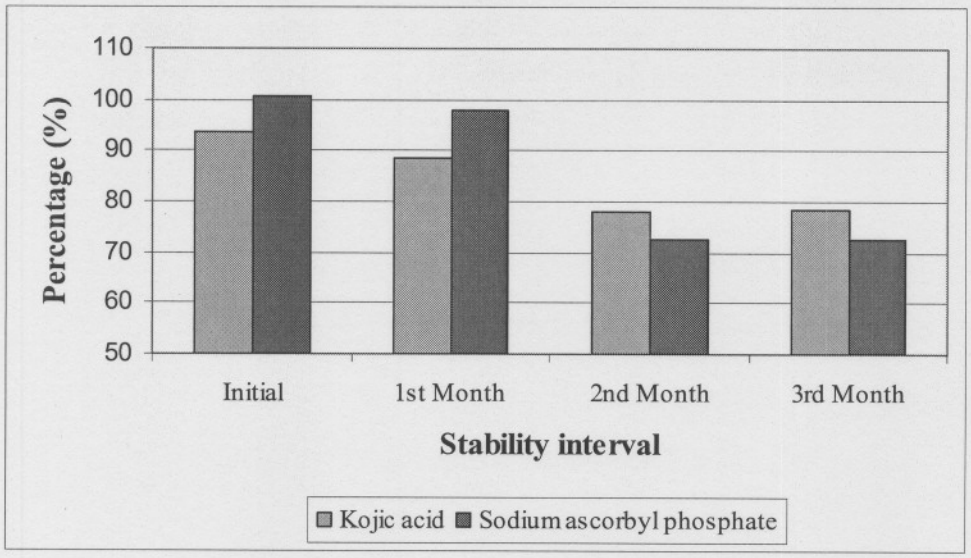

Figure 8.5 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the foam bath after three months of storage at 25°C + 60% RH.

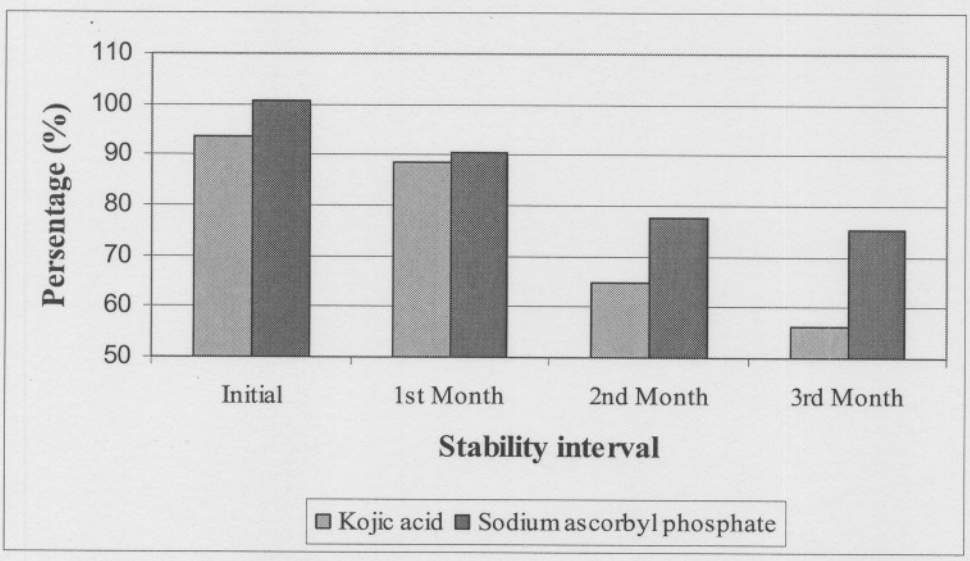

Figure 8.6 HPLC assay results showing the percentage kojic acid and sodium ascorbyl phosphate present in the foam bath after three months of storage at 40°C + 75% RH.

8.8.2 Discussion

The assays of the foam bath revealed a small decline in kojic acid in the batches that was stored at 5°C over the three month stability period, whereas the batches stored at 25°C + 60% RH showed a decline of 15.1%. The batches stored at 40°C + 75% RH had a major decline of 37.4%. This drop in concentration percentage can be due to the instability of kojic acid to heat.

The sodium ascorbyl phosphate in the batches that was stored at 5°C showed a small decrease in concentration, but the batches stored at 25°C + 60% RH had a decrease of 27.9% and the batches that was stored at 40°C + 75% RH had a drop in concentration percentage of 25.1%. This can be due to the sensitivity of sodium ascorbyl phosphate to heat.

8.9 Conclusion

The pH results revealed that a decrease occurred over time, which might have an influence on kojic acid's stability, since it requires a lower pH value (see section 3.2). The assays confirmed a remarkable decrease in the concentration of kojic acid over time, and sodium ascorbyl phosphate also decreased significantly. The specific gravity stayed uniform, while the viscosity increased in the third month, especially the samples stored at 40°C + 75% RH. This was confirmed by the visual assessment where it was clear that the foam bath became more viscous with time. The foam became yellow. The colour changed due to kojic acid's sensitivity to high temperatures, and revealed the characteristic brown change. The foamability showed a decrease with time, but the samples stored at 40°C + 75% RH foamed very well when shaken, the foam just decreased faster compared to the batches that were stored at lower temperatures for a shorter period.

CAPTER 9
TEST RESULTS: GEL

9.1 Introduction

In this chapter the tests done on the gel are described and results are discussed. The tests were done over a period of three months at three different temperatures and humidity. The following physical parameters were investigated: pH, specific gravity, visual assessment, and the assays from HPLC chromatography. Microbial preservative efficacy was done by Wits Health Consortium (Pty) Ltd.

9.2 pH of the gel

The pH of the gel was measured once a month for a period of three months for samples stored at 5°C, 25°C + 60% RH and 40°C + 75% RH as described in section 5.3.6.

9.2.1 Results

The pH values of the gel over the three month stability period are given in Figure 9.1.

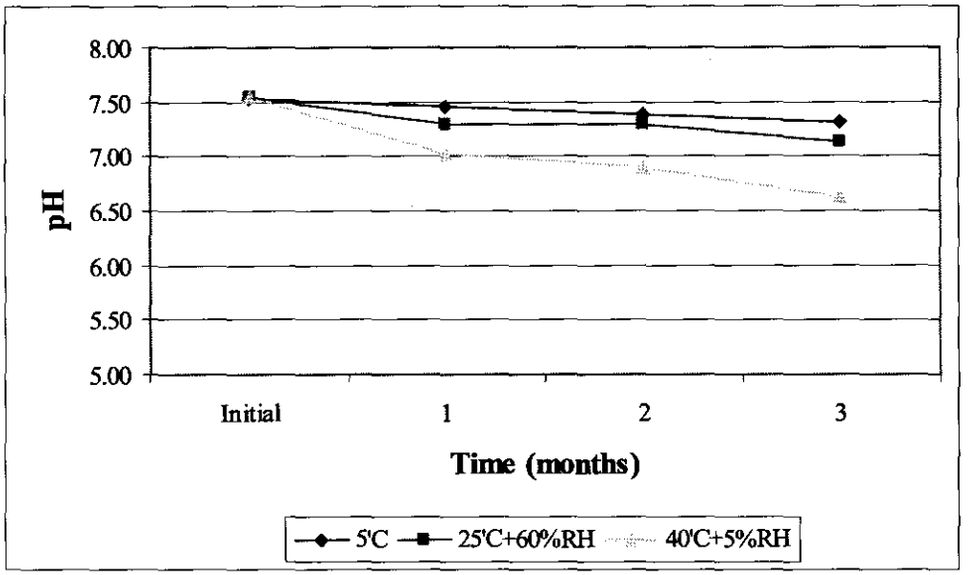

Figure 9.1 The pH of the gel over the three month stability period.

9.2.2 Discussion

Figure 9.1 represents a small decrease in the pH of the gel over the period of three months. The sample that was stored at 40°C + 75% RH displays a larger decline in pH over time (refer to section 3.2 for a broader discussion on the stability of kojic acid and sodium ascorbyl phosphate). The pH of the gel tends to be between 6.6 and 7.5 and since the optimum pH for sodium ascorbyl phosphate is 6.5 and for kojic acid is between 4 and 5, the effectiveness of the gel could be influenced.

9.3 Specific gravity

The specific gravity of the gel was determined once a month for three months, as was described in section 5.3.7.

9.3.1 Results

The specific gravity results of the gel are given in Table 9.1.

Table 9.1 The specific gravity (g/cm^3) of the three batches of the gel over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	1.054	0.993	1.023	1.027
25°C+60%RH	1.052	0.972	1.056	1.034
40°C+75%RH	1.051	1.001	1.075	1.071

9.3.2 Discussion

There was no significant change in the specific gravity of the gel (Table 9.1). Temperature and humidity did not have an influence on the specific gravity.

9.4 Visual assessment

The appearance measurements of the gel were done once a month for three months as described in section 5.3.8.

9.4.1 Results

The visual assessment of the gel is given in Table 9.2.

Table 9.2 The visual assessment of the gel over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	**Colour: light yellow**	No change	No change	No change
	Transparent	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
	Fluent and cool	**More fluent**	No change	No change
25°C + 60%RH	**Colour: light yellow**	**Colour: orange**	**Colour: brown**	**Colour: black**
	Transparent	No change	No change	**Intransparent**
	Air bubbles	No change	No change	**No air bubbles**
	Not too oily	No change	No change	No change
	Not too hydrous	No change	**Too hydrous**	**Too hydrous**
	Applies easily	No change	**Too thin**	**Too thin**
	Fluent and cool	**More fluent**	**Completely fluent**	**Completely fluent**
40°C + 75%RH	**Colour: light yellow**	**Colour: brown**	**Colour: black**	**Colour: black**
	Transparent	No change	**Intransparent**	**Intransparent**
	Air bubbles	**No air bubbles**	**No air bubbles**	**No air bubbles**
	Not too oily	No change	No change	No change
	Not too hydrous	**Too hydrous**	**Too hydrous**	**Too hydrous**
	Applies easily	**Too thin**	**Too thin**	**Too thin**
	Fluent and cool	**Completely fluent**	**Completely fluent**	**Completely fluent**

9.4.2 Discussion

The appearance changed from a light yellow and transparent gel, to an intransparent and black fluid, over the 3 month stability period. Although Xanthan gum is known for its wide pH stability range, the decrease in pH (see section 9.2) may be responsible for the breakage of the gum. The increased temperature result in a faster breakage of the gel as can be seen of the sample that was stored at 40°C + 75% RH compared to

the samples at 5°C and 25°C + 60% RH. The colour change is due to the breaking down of the actives, in particular the kojic acid that is known for its colour changing when the formulation becomes unstable.

9.5 Assays

The concentrations of kojic acid and sodium ascorbyl phosphate in the gel over a period of three months and at 5°C, 25°C + 60% RH and 40°C + 75% RH were determined using HPLC chromatography as was described in section 5.3.1. Figure 9.2 represents a chromatogram of a sample from the sample stored at 25°C + 60% RH for three months.

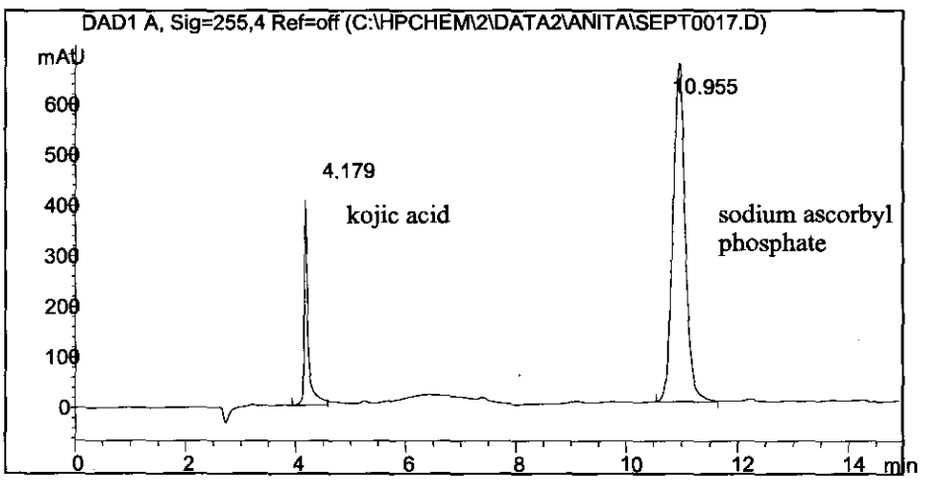

Figure 9.2 A chromatogram of a sample gel from the sample stored at 25°C + 60% RH for three months.

9.5.1 Results

The percentage kojic acid present in the samples over the three month stability period is shown in Figure 9.3 and the percentage sodium ascorbyl phosphate is shown in Figure 9.4.

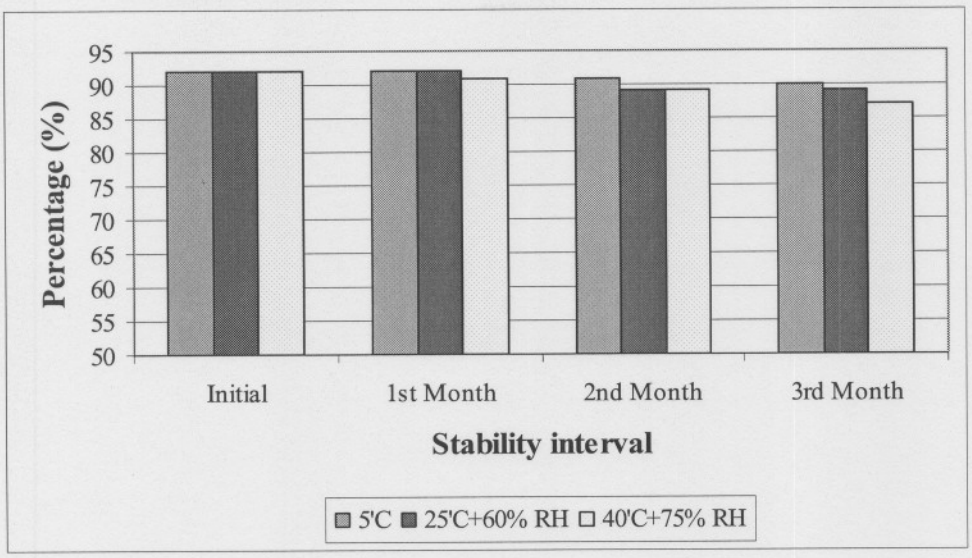

Figure 9.3 Kojic acid concentrations in the gel over the three month stability period.

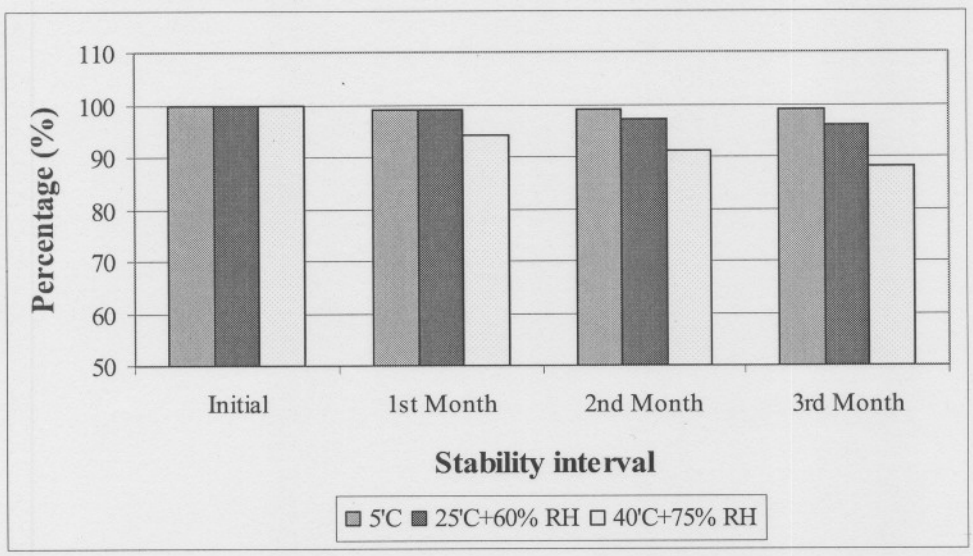

Figure 9.4 Sodium ascorbyl phosphate concentrations in the gel over the three month stability period.

9.5.2 Discussion

The decrease in kojic acid in the samples stored at 5°C for three months was only 2%, while the samples stored at 25°C + 60% RH and 40°C + 60% RH, showed a decrease of 3% and 5% respectively. This proves that a small decrease in concentration can

result in a major colour change (section 9.4). The concentration of sodium ascorbyl phosphate decreased with only 1% in the samples stored at 5°C, the samples stored at 25°C + 60% RH had a decrease of 4%. A decrease of 12% occurred in the samples stored at 40°C + 75% RH.

9.6 Preservative testing

The hydroxybenzoate concentrations of the gel were determined once a month for a period of three months at 5°C, 25°C + 60% RH and 40°C + 75% RH, using HPLC chromatography, as was described in section 5.3.1.2.

9.6.1 Results

The methyl hydroxybenzoate concentrations of the gel at 5°C, 25°C + 60% RH and 40°C + 75% RH for the three month stability period are given in Figure 9.5.

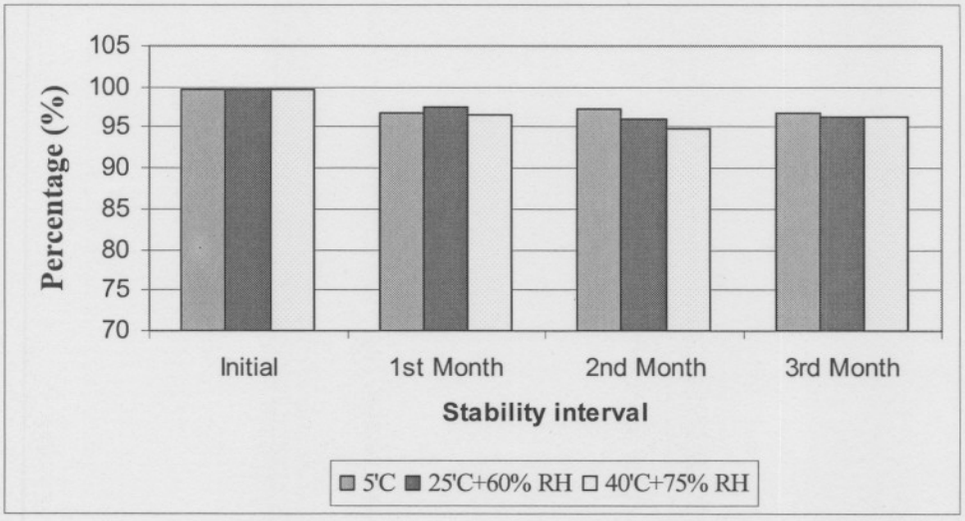

Figure 9.5 Methyl hydroxybenzoate concentrations in the gel over the three month stability period.

The propyl hydroxybenzoate concentrations of the gel at 5°C, 25°C + 60% RH and 40°C + 75% RH for the three month stability period are given in Figure 9.6.

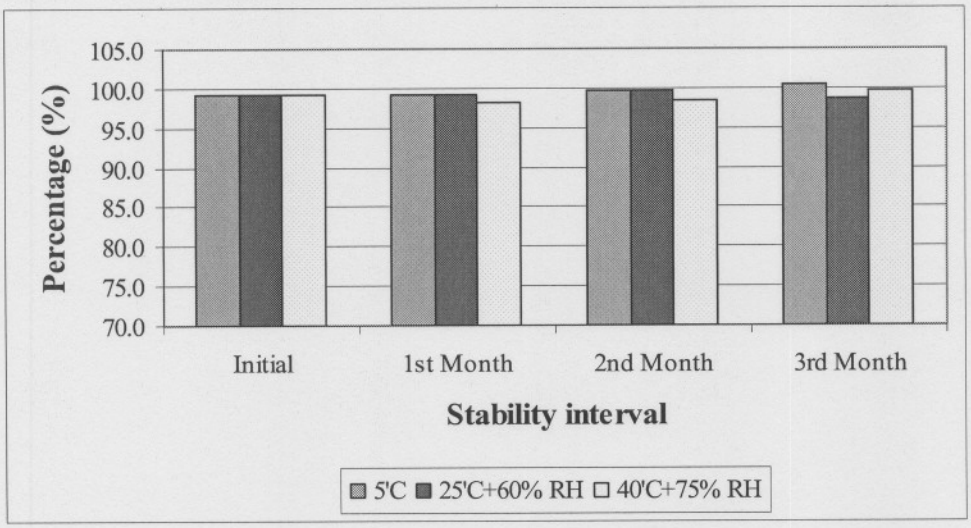

Figure 9.6 Propyl hydroxybenzoate concentrations in the gel over the three month stability period.

9.6.2 Discussion

The percentage of the concentrations of the preservatives, methyl and propyl hydroxybenzoate, is shown in Figures 9.5 and 9.6 respectively. There was a decrease in the concentrations of both the preservatives over the period of three months. The decrease in concentration may be due to experimental variation. The preservative efficacy tests that were done by Wits Health Consortium (Pty) Ltd. showed that the samples complied with the requirements of USP 26 and the specified microbial limits. There was no significant microbial growth.

9.7 Conclusion

After many trials (see section 4.6) a gel was finalised consisting of Xanthan gum, it is a thin, but not fluent, transparent gel with a light yellow colour and no odour. The pH value varies between 6.6 and 7.5. The stability of sodium ascorbyl phosphate is optimal at a pH of 6.5, and kojic acid is most stable at pH values between 4 and 5, this may have an influence on the effectiveness of the whitening properties of the gel.

The specific gravity measurements showed no significant change (see Table 9.1). Temperature and humidity did not have an influence on the specific gravity.

The visual assessment indicated a colour change and breaking of the gel. The yellow, transparent gel changed to brown/black and intransparent over the three month period at the different temperatures and humidity. The breakage of Xanthan gum usually occurs if unsuitable preservatives were used, because natural gums are subject to microbial degradation and it supports microbial growth (Kushla & Zatz, 1989: 498), but as the Wits Health Consortium (Pty) Ltd. stated that all the batches of the gel complied with the requirements of USP 26 and the specified microbial limits, it would be very unlikely to be the cause. The HPLC analysis revealed that there was a decrease in preservative concentration over the three month stability period that was between 100 $\pm$ 10%, which Wade and Weller stated to be the maximum percentage breakdown allowable (Wade and Weller, 1994:310, 411).

Sodium ascorbyl phosphate and kojic acid are both sensitive to heat and light, it is therefore recommended to store the gel at temperatures below 25°C + 60% RH and away from light, in a tube of approximately 15 ml to prevent the preparation from ageing.

CAPTER 10
TEST RESULTS: SOAP

Dirt has been defined as matter in the wrong place (Price, 1952).

10.1 Introduction

Soap has an extremely long history and was already being used for washing the body before the time of Christ. According to Mitsui (1997:447) the production of soap started in the 8th century in the Italian port town of Savona and this is the origin of the French word savon, the English word soap and the German word seifen. It was later made in Venice and other places and then a soap industry was started in Marseilles.

Soap bars were developed that would clean the skin while medically preventing hyper-pigmentation and which have cosmetic advances such as anti-ageing. It was meant to be used together with a range of depigmentation products for more complete skin care.

In this chapter the tests done on the soap are described and results are discussed. The samples of soap consist each out of one bar (50 g) and the tests were done over a period of three months at three different temperatures and humidities. The following physical parameters were investigated: pH, visual assessment, foamability and the assays from HPLC chromatography.

10.2 pH of the soap

Research in the field of detergency has shown that there is an optimum pH value at which they work best. In Price (1952) the best alkalinity for soap is stated to be in the neighbourhood of pH 10.5.

The pH of the soap was measured once a month for a period of three months for samples stored at 5°C, 25°C + 60% RH and 40°C + 75% RH as described in section 5.3.6.

10.2.1 Results

The pH values of the soap over three months are given in Figure 10.1.

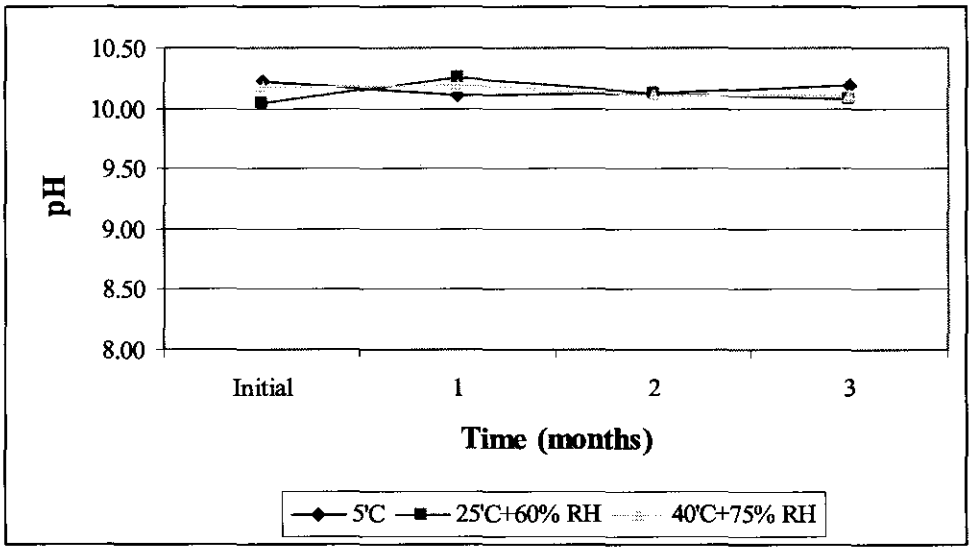

Figure 10.1 The pH of the soap over the three month stability period.

10.2.2 Discussion

The pH values did not differ significantly between the samples at different temperatures and humidities during the three month stability period.

10.3 Visual assessment

The appearance measurements of the soap were done once a month for three months as described in section 5.3.8.

10.3.1 Results

The visual assessment of the three batches of the soap is given in Table 10.1.

Table 10.1 The visual assessment of the soap over a period of three months.

Storage condition	Initial	Month 1	Month 2	Month 3
5°C	**Colour: orange**	No change	No change	No change
	Semi-transparent	No change	No change	No change
	Foams easily	No change	No change	No change
	No cracking	No change	No change	No change
	Odourless	No change	No change	No change
	Uniform and solid	No change	No change	No change
25°C + 60%RH	**Colour: orange**	No change	**Colour: brown**	**Colour: brown**
	Semi-transparent	No change	**Intransparent**	**Intransparent**
	Foams easily	No change	No change	No change
	No cracking	No change	No change	No change
	Odourless	No change	No change	No change
	Uniform and solid	No change	No change	No change
40°C + 75%RH	**Colour: orange**	**Colour: brown**	**Colour: dark brown**	**Colour: black**
	Semi-transparent	**Intransparent**	**Intransparent**	**Intransparent**
	Foams easily	No change	No change	No change
	No cracking	No change	No change	No change
	Odourless	No change	No change	No change
	Uniform and solid	**Sticky**	**Hard**	**Hard**

10.3.2 Discussion

The soap was formed in bars of about 50 g each, it is solid, transparent, and forms foam easily with water contact. The colour changed from orange to brown in the sample stored at 25°C + 60% RH after two months, and to black in the sample stored at 40°C + 75% RH for three months. According to Thomssen and McCutcheon (1949:222) a transparent soap can be made opaque by melting it, and then cooling it slowly, therefore the change in transparency may have occurred during the exposure to higher temperatures.

The colour change to brown or black can also be due to the heat sensitivity of both kojic acid and sodium ascorbyl phosphate; this is confirmed in the HPLC assays (see section 10.5). Kojic acid tends to change colour (yellow to brown) when exposed to heat (Sino Lion USA, Ltd. 2003). The soap became sticky and very hard after three months at 40°C + 75% RH. The stickiness can be explained by the reaction of the sucrose to the heat (Thomssen & McCutcheon, 1949:225).

10.4 Foamability

Foam may be considered as a gas-in-liquid system in which bubbles of gas are dispersed more or less stable in a continuous liquid phase (Niven, 1950:82).
Foam, according to Price (1952:66), is a better criterion of cleaning power than the lowering of surface tension as it has an important role in the removal of dirt. Foam also acts as indicator of the life of the detergent during cleaning.

The foamability of the soap was determined once a month for three months on the samples stored at three different temperatures and humidities, as described in section 5.3.9.

10.4.1 Results

In Harris (1967) foam formation is described as a measurable function, common to good soaps which in turn are good detergents.

The foamability results of the soap are given in Figure 10.2.

It represents the three different storage temperatures at which the batches were stored that was dissolved in different types of water under two different temperatures. This was done to compare the influence of hard water and temperature on the foamability.

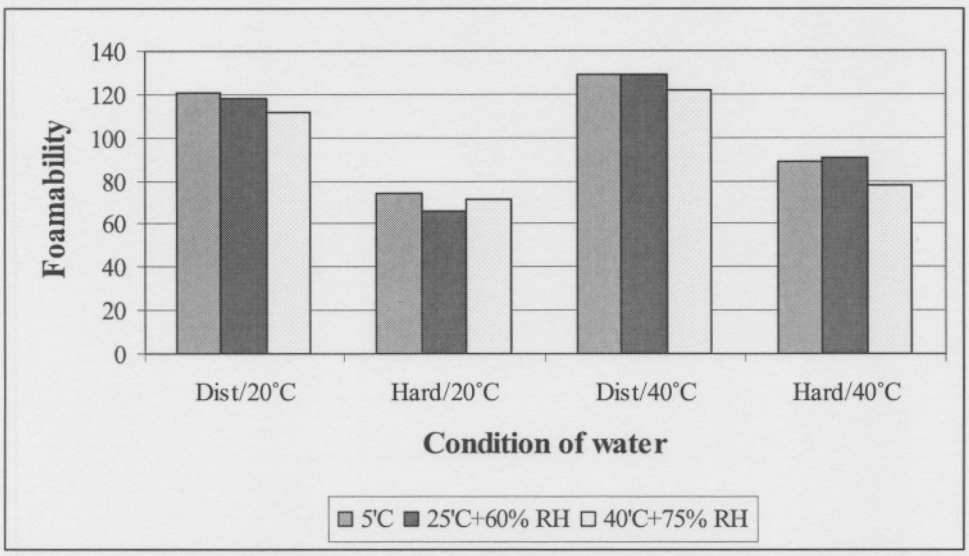

Figure 10.2 The foamability results (in cm^3) of the soap over a three month stability period.

10.4.2 Discussion

No data are generally available to show a direct correlation between foam and detergency (Harris, 1967). But as was stated earlier, foam acts as indicator of the life of a detergent and it does have an importance in cleaning.

The warmer water had a favourable effect on the soap, and it correlates with the fact explained by Price (1952:67) that detergents containing chains of eighteen carbon atoms are most efficient at higher temperatures (stearic acid contains 18 carbon atoms). The soap contains no builders (also referred to as blockers) that could impart water softening qualities, thus the tap water that contains calcium and magnesium resulted in poor foamability. The water in Potchefstroom is known to have high calcium content, since the older houses experience great difficulty with closed water pipes and taps. But the water is not considered very hard, and it is confirmed with the results showing a relative small decrease in foamability.

From the foamability results (See appendix C) it became clear that the soap had not foam significantly less with increased temperatures or storage time, but what happened was that the decrease in volume and density was faster within the thirty minute test period from the batches that was stored at 40°C + 75% RH.
From Figure 10.2 it can be seen that the changes in foamability occurred when the samples of soap were dissolved in different types of water and temperatures. The soap that was dissolved in warmer water gave higher foamability results in both distilled and hard water.

Since it is known that the actives are sensitive to high temperatures, it will be advisable not to use water with temperatures above that of normal tap water, for the foamability was not influenced too much by using water at low temperatures, and this will secure the life of the actives.

10.5 Assays

The concentrations of kojic acid and sodium ascorbyl phosphate in the soap over a period of three months and at 5°C, 25°C + 60% RH and 40°C + 75% RH were determined using HPLC chromatography as was described in section 5.3.1.

A chromatogram of a sample of the sample that was stored at 25°C + 60% RH for three months is represented in Figure 10.3.

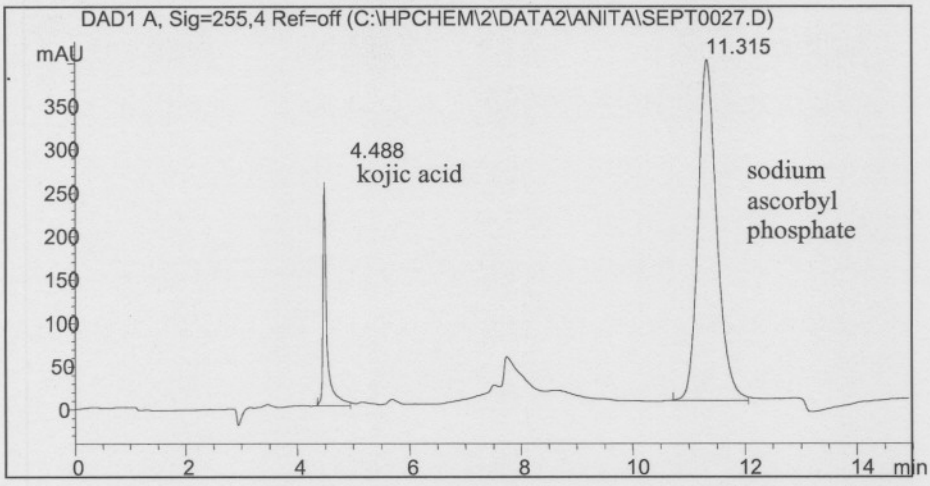

Figure 10.3 A chromatogram of a soap sample that was stored at 25˚C + 60% RH for three months.

10.5.1 Results

The concentration kojic acid present in the samples over the three month stability period is shown in Figure 10.4 and the concentration sodium ascorbyl phosphate is shown in Figure 10.5. Tests were also done to see if the concentration of the actives would be influenced when the soap was dissolved in water that was at 40˚C prior to HPLC injection, the results was then compared with the original assay results of the samples stored at 25˚C + 60% RH for 1 month (Figure 10.6).

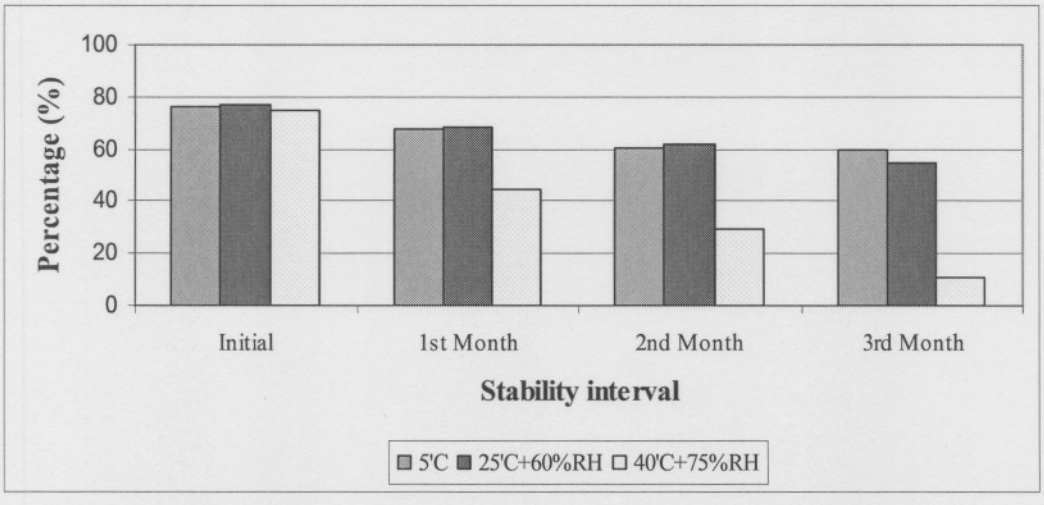

Figure 10.4 Kojic acid concentrations in the soap over the three month stability period.

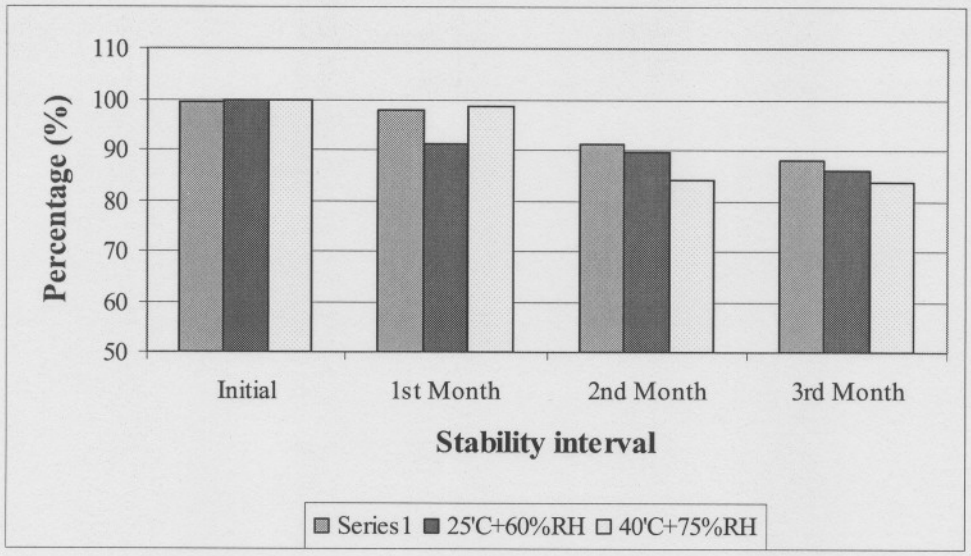

Figure 10.5 Sodium ascorbyl phosphate concentrations in the soap over the three month stability period.

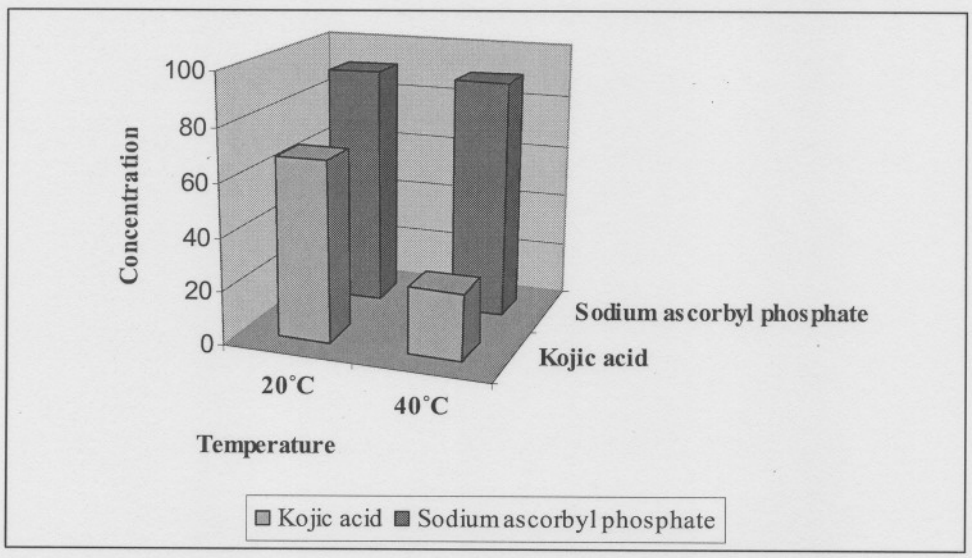

Figure 10.6 Comparison of the influence of water temperature on the concentration (%) of kojic acid and sodium ascorbyl phosphate of the samples stored at 25°C + 60% RH for one month.

10.5.2 Discussion

The concentrations of kojic acid and sodium ascorbyl phosphate are shown in Figures 10.4 and 10.5 respectively. There is a sizeable breakdown in kojic acid concentrations

in the sample that was stored at 40°C + 75% RH (see Figure 10.4). Sodium ascorbyl phosphate concentrations showed a smaller decrease within the sample that was stored at 40°C + 75% RH, but a significant breakdown had occurred, for the percentage concentration dropped with 15.8%. This maybe due to the warm distilled water that was used to dissolve the samples before analyses were done, but the considerable decrease in the batch that was stored at 40°C + 75% RH supports the known characteristic of significant sensitivity that kojic acid has for high temperatures.

The batches stored at 25°C + 60% RH for one month were dissolved in warm water (40°C) to see what the influence would be on the actives (if one should use the soap in warm water). The results were compared to the results obtain in 10.5.1. The effect on kojic acid was more significant, since sodium ascorbyl phosphate decreased with only 1% in the warm water, and kojic acid decreased with 43%.

10.6 Conclusion

The pH results remained between 10.05 and 10.23, which is according to Price (1952) the ideal pH range for soap.

The appearance tests revealed that the soap bars did not undergo much visual change in the first month of stability storage. The colour change occurred after the second month of exposure to temperatures above 25°C + 60% RH; this was due to the heat sensitivity of kojic acid that causes colour changes in formulations (see stability of kojic acid, section 3.2). After three months of stability storage, the soap bars had become sticky and hard. Since it is formulated in a small bar (50 g) and is meant to be used daily, the colour change will not be relevant, for if it is used daily it will only last about a month.

The foamability results did not differ significantly within the same storage temperatures or samples, but the influence of warm and hard water could be seen clearly. The warmer water had a favourable effect on the soap, but the hard water caused lower foamability results.

The HPLC assays confirmed the colour change in the soap to be due to the instability of kojic acid to high temperatures. Sodium ascorbyl phosphate also had a sizable decrease in concentration, but was more stable than kojic acid. The comparison test showed how sensitive kojic acid is to heat when the soap was dissolved in warm water prior to HPLC analysis. The sodium ascorbyl phosphate concentration did not decrease, and according to Anon. (1999:5) sodium ascorbyl phosphate may be exposed to 40°C, and even to higher temperatures, but only for a short time. This was confirmed with the stability results that showed a decrease in sodium ascorbyl phosphate concentration when it was stored at 40°C + 75% RH for three months.

CAPTER 11
VALIDATION OF A METHOD FOR THE SIMULTANEOUS DETERMINATION OF KOJIC ACID AND SODIUM ASCORBYL PHOSPHATE

11.1 Introduction

Sottafotorri *et al.* (1998:213) stated that in the near future, cosmetic industries were going to have to comply with the sixth amendment to the 76/768/EEC Council Directive, which introduced the availability of both a fuller dossier and labelling of cosmetic ingredients. Moreover, the cosmetic formulations are complex mixtures of different chemical compounds with different chemical properties. Therefore, new analytical methods, which can detect and quantify such compounds in commercial products, have to be found.

This chapter describes the method that was developed and validated as part of this study.

11.2 Chromatographic conditions

Analytical instrument: Hewlett Packard 1050 HPLC, equipped with a variable wavelength UV detector, pump, injection device and integrator or recorder, or similar equipment that meets the United States Pharmacopoeia (USP) 24 standards for system suitability.

Column:	Lichrospher 100-5 RP-18 ec
Mobile phase:	0.185% KH_2PO_4, 0.681% BAI, 10% Acetonitrile in Milli-Q water. pH adjusted to 5.0 with diluted NH_4OH (1:3).
Flow rate:	1.0 ml/minutes
Injection volume:	10 µl
Detection:	UV at 255 nm
Retention time:	Kojic acid ± 2.3 minutes
	Sodium ascorbyl phosphate ± 7.8 minutes
Solvent:	Milli-Q water made up to 100 ml
Standard:	20:60 kojic acid:sodium ascorbyl phosphate
Stop time:	15 minutes

11.3 Sample preparation

Approximately 2 g of each product was accurately weighed and dissolved. The creams were dissolved with 10 ml methanol and filled to 100 ml with Milli-Q water. The rest of the formulations were diluted to 100 ml with Milli-Q water only. All the products were sonicated for 10 minutes and the creams were shaken for 15 minutes. The samples had to be filtered through 0.45 µm filters, before it was transferred into HPLC vials and injected into the HPLC.

11.4 Standard preparation

Approximately 0.20 g kojic acid and 0.60 g sodium ascorbyl phosphate were weighed accurately and dissolved in 70 ml Milli-Q water in a 100 ml volumetric flask. It was then sonicated on an ultrasonic bath for 10 minutes. The flask was left to cool down and then filled to volume with Milli-Q water. No filtering was necessary, and the samples were transferred into a HPLC autosampler vial and injected into the HPLC.

11.5 Calculations

The preparations contained 3% (m/m) sodium ascorbyl phosphate and 1% kojic acid. Thus it contains 30 mg/g sodium ascorbyl phosphate and 10 mg/g kojic acid.

11.6 System suitability parameters

The following parameters were used:

- Analyse six replicate injections of a standard solution.
- Calculate the relative standard deviation of the peak areas obtained for the active ingredients.
- Calculate the number of theoretical plates, using the 5-sigma method.

The system would be suitable to perform the analysis if the following criteria were met:

- RSD not more than 1.0%,
- Number of plates more than 3500 per column for both actives,
- USP tailing factor not more than 1.5.

11.7 Validation test procedure and acceptance criteria

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose (UNITED STATES, 1995:1).

11.7.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present (UNITED STATES, 1995:2).

Method

1. Prepare a sample from a placebo product that does not contain the active being tested.
2. Diluted a standard solution 1:1 with:
   - Water
   - 0.1 M hydrochloric acid
   - 0.1 M sodium hydroxide
   - 10% hydrogen peroxide.
3. Store these solutions overnight in closed test tubes at 40°C to allow degradation.
4. Inject the samples into the chromatograph with a stop time of 30 minutes.
5. 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.

11.7.2 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample (UNITAD STATES, 1995:6).

Method

Preparation of standards:

1. Prepare a standard solution as described under standard preparation.
2. Inject variable volumes into the chromatograph to obtain standards from 60-120% of the expected sample concentration.

Accepted criteria

Linear regression analysis should yield a regression coefficient (r^2) of ≥ 0.99.

11.7.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (UNITED STATES, 1995:4).

Method

1. Measure in triplicate 80% (1.2 g), 100% (2.0 g) and 120% (2.4 g) of the placebo into 100 ml volumetric flasks.
2. Spike these samples with concentrations of 80%, 100% and 120% of the expected sample concentrations.
3. Inject into the chromatograph in duplicate.

Accepted criteria

Recovery must be between 98% and 102%.

11.7.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility (UNITED STATES, 1995:4).

11.7.4.1 Intra-day precision (repeatability)

Method

1. Measure approximately 1.2 g, 2.0 g and 2.4 g (in triplicate) of sample cream each into a 100 ml volumetric flask, add 10 ml MeOH and fill to volume with Milli-Q water.
2. Inject into the chromatograph in duplicate.

11.7.4.2 Inter-day precision

Method

1. 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 day three, a different analyst should perform the analysis.

Accepted criteria

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

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

11.7.5 Ruggedness

11.7.5.1 Stability of sample solutions

Method

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 re-analyse over a period of 24 hours 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 case of degradation, special precautions should be followed to compensate for the loss.

11.7.5.2 System repeatability

Method

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

Acceptance criteria

The peak area and retention times should have an RSD of $\leq 2\%$.

11.7.5.3 Robustness

Method

1. Make deliberate changes to the chromatographic conditions to determine the method tolerance towards changes.
2. Change the flow, wavelength, and injection volume, 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.

11.7.6 System suitability (system and method performance characteristics)

Method

1. Calculate the chromatographic performance characteristics of the separation, such as 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 K' and the RSD must be less than 2%.

11.8 Summary of validation results

The validation of the method for the simultaneous determination of kojic acid and sodium ascorbyl phosphate in skin preparations yielded the following results, as summarised in Table 11.1.

Table 11.1 Summary of validation results

Test	**Result**
Specificity	Complies
Range	Kojic acid: 20 - 240 µg/ml
	Sodium ascorbyl phosphate: 60 - 720 µg/ml
Linearity	Kojic acid: R square = 0.998
	Sodium ascorbyl phosphate: R square = 0.998
Accuracy	Kojic acid: 99.2%
	Sodium ascorbyl phosphate: 99.0%
Precision	Kojic acid: RSD < 1%
	Sodium ascorbyl phosphate: RSD < 1%
Ruggedness	Complies
Robustness	Complies

11.9 Validation results

11.9.1 Specificity

The results of the samples prepared from the placebos are shown in Figure 11.1-11.5. Figures 11.6 – 11.9 are chromatograms of samples that have been stressed for 24 hours as described under section 11.7.1. Figure 11.10 represents a chromatogram of a standard solution. Figures 11.11 and 11.12 represent the peak purity tests for both kojic acid and sodium ascorbyl phosphate.

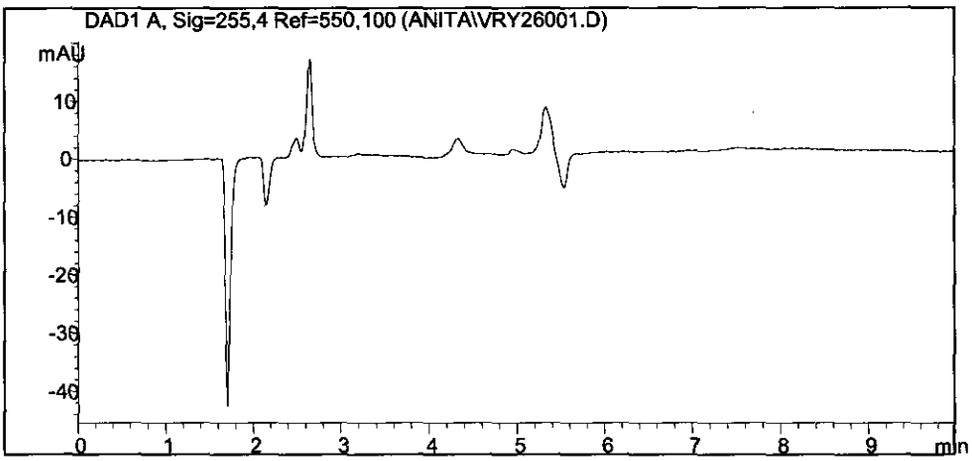

Figure 11.1 Chromatogram of the cream placebo.

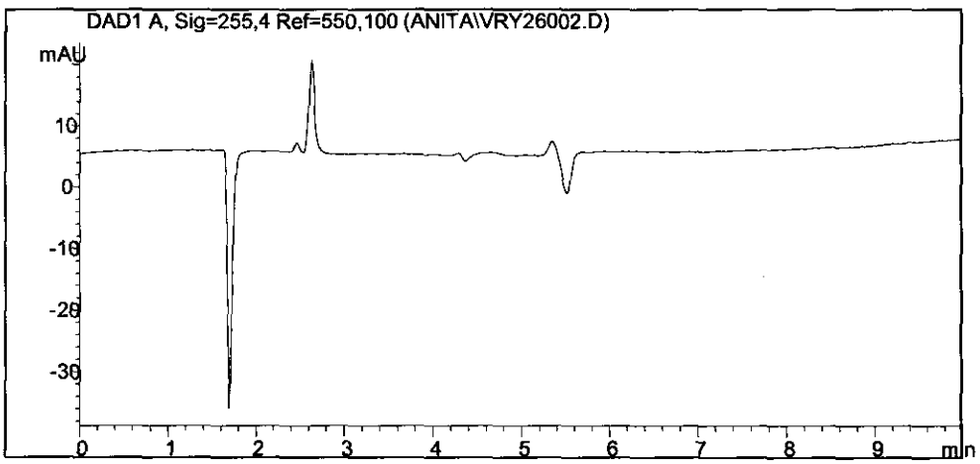

Figure 11.2 Chromatogram of the toner placebo.

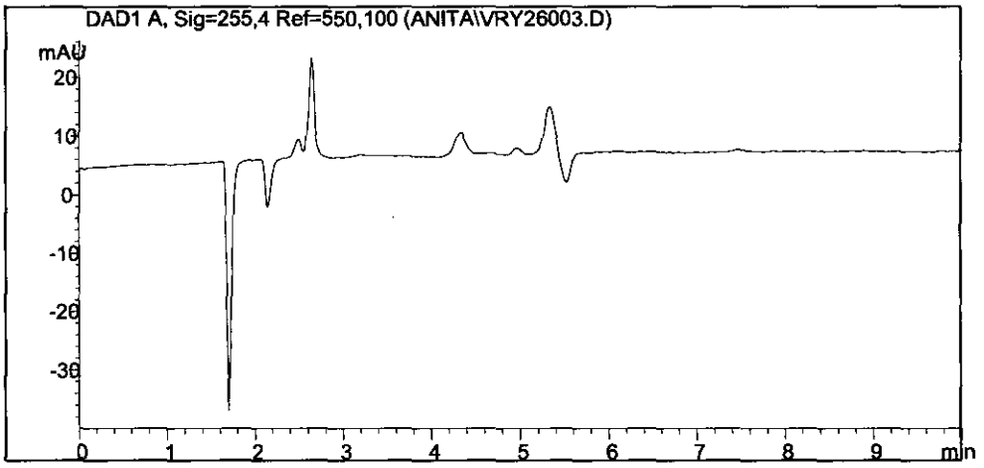

Figure 11.3 Chromatogram of the gel placebo.

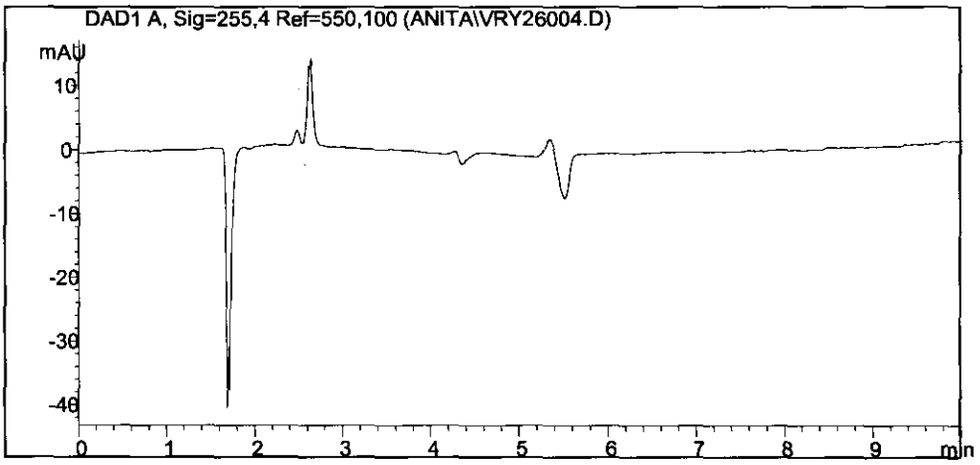

Figure 11.4 Chromatogram of foam bath placebo

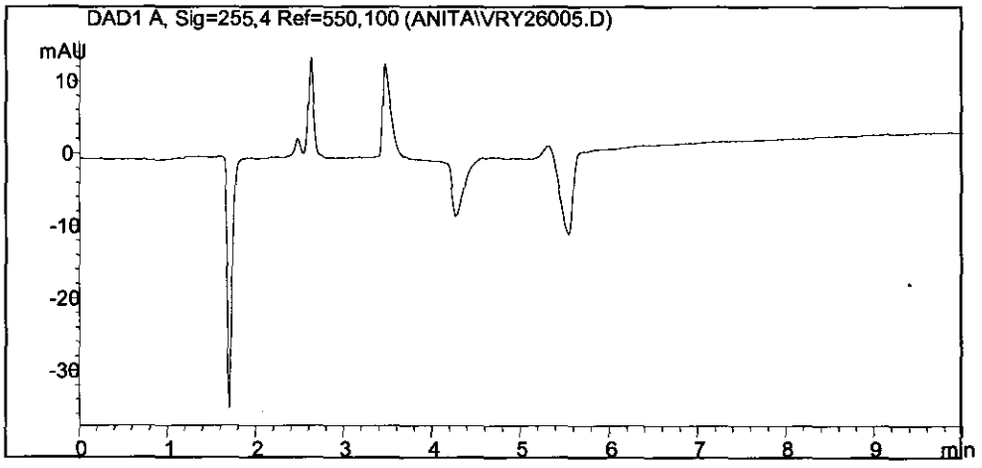

Figure 11.5 Chromatogram of the soap placebo.

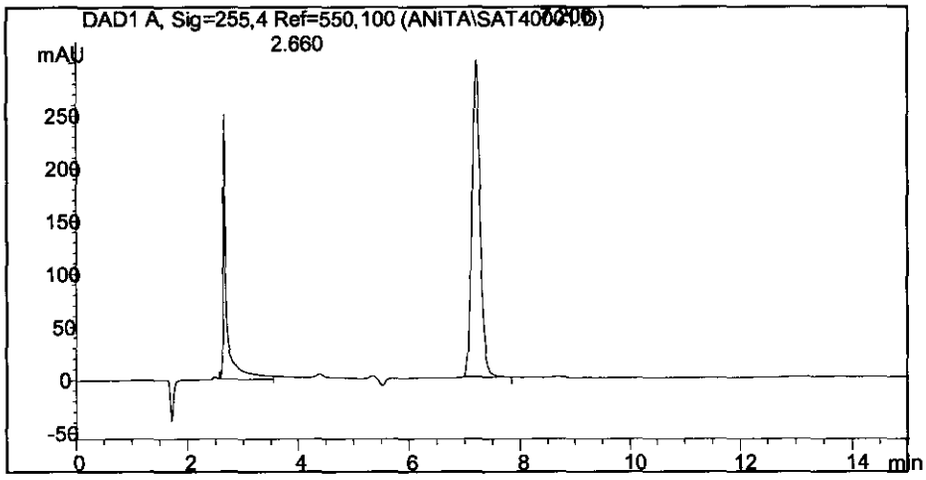

Figure 11.6 Chromatogram of the sample stressed in water at 40°C for 24 hours.

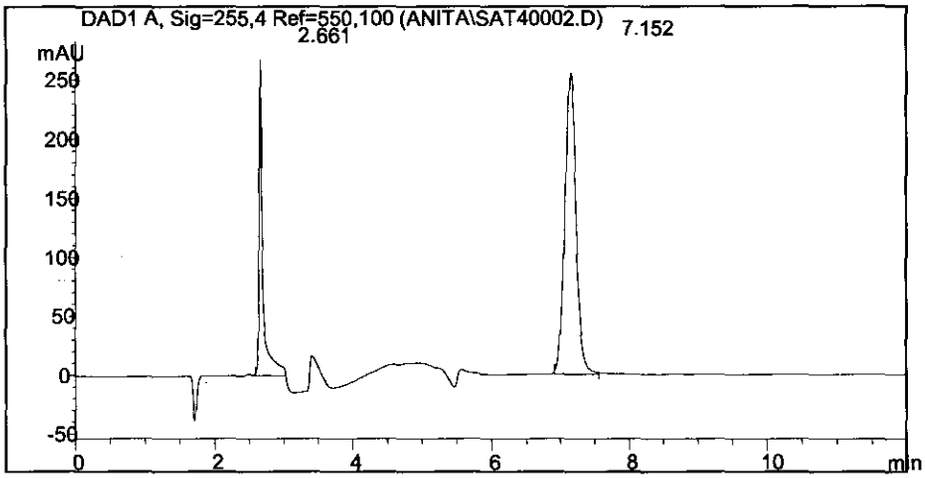

Figure 11.7 Chromatogram of the sample stressed in 0.1 M hydrochloric acid at 40°C for 24 hours.

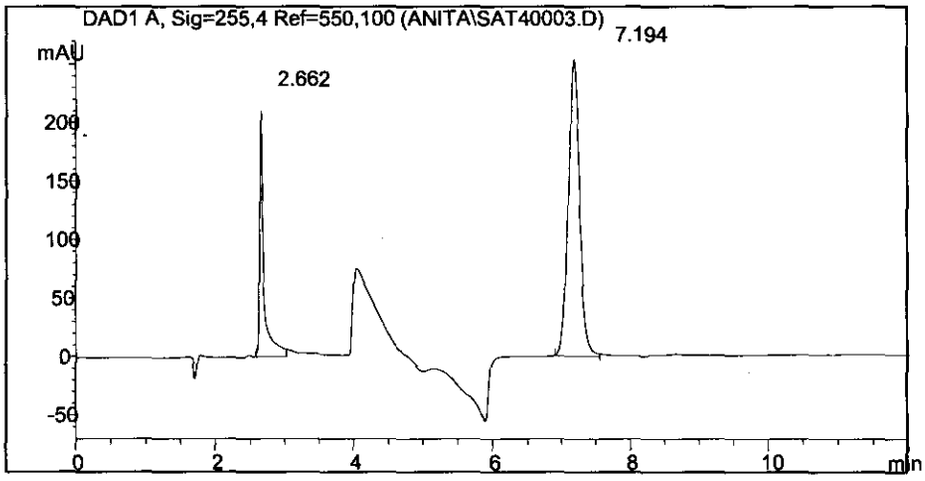

Figure 11.8 Chromatogram of the sample stressed in 0.1 M sodium hydroxide at 40°C for 24 hours.

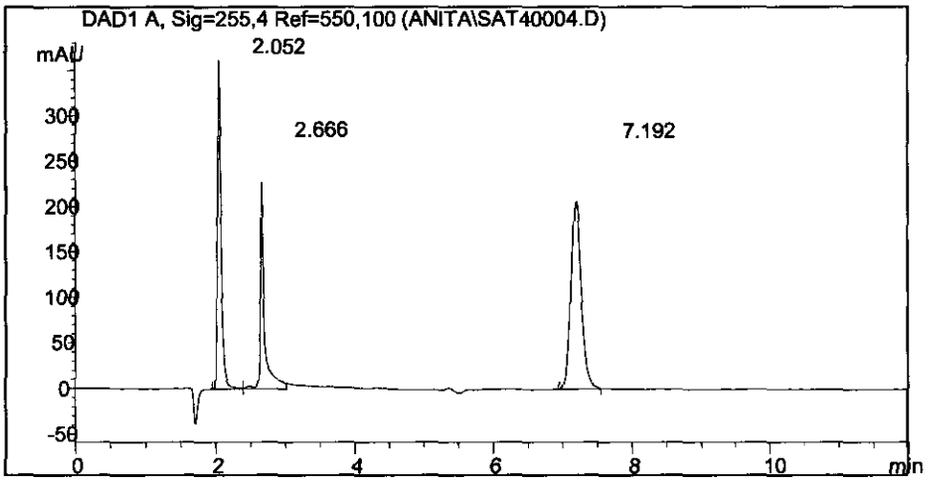

Figure 11.9 Chromatogram of the sample stressed in 10% hydrogen peroxide at 40°C for 24 hours.

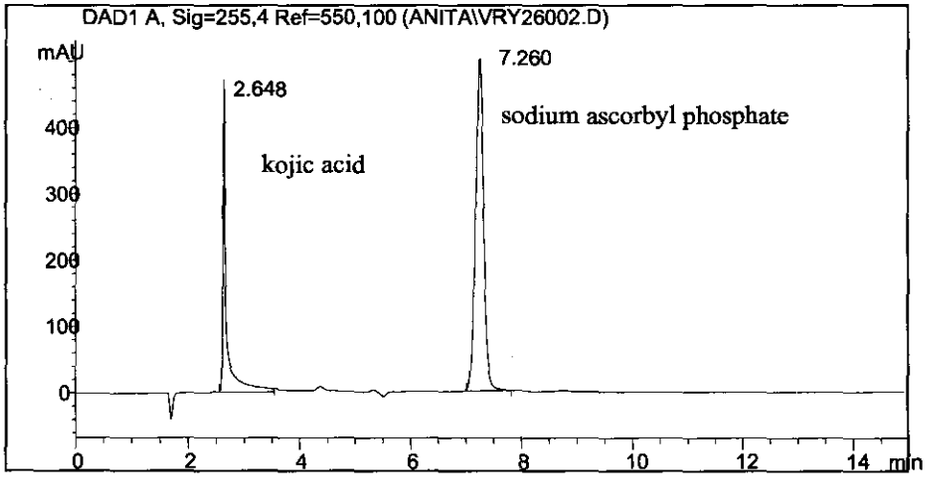

Figure 11.10 Chromatogram of a standard solution.

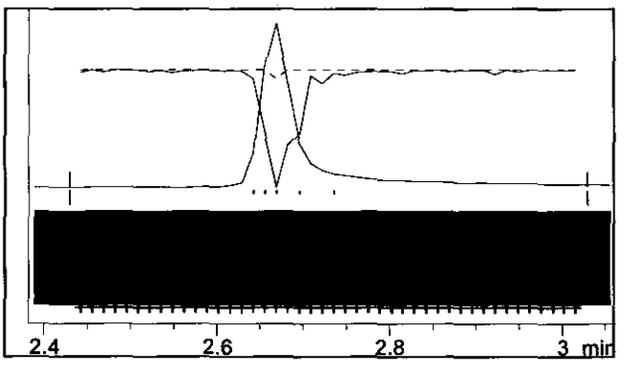

Figure 11.11 Peak purity test of kojic acid.

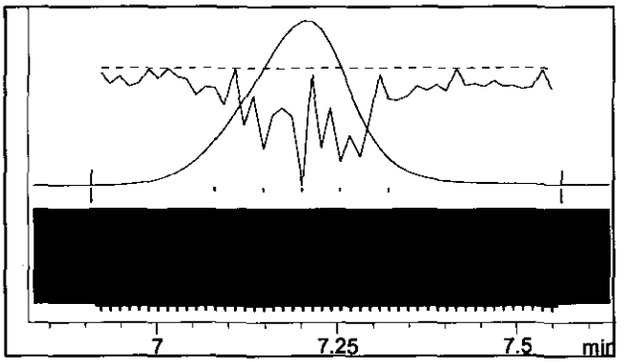

Figure 11.12 *Peak purity test of sodium ascorbyl phosphate.*

Conclusion

None of the ingredients in the placebo interfered with the analyte peaks. Peak purity testing of the peaks, after forced degradation in water, showed that the peaks remained pure, thus proving that the method was stability-indicating.

11.9.2 Linearity and range of kojic acid and sodium ascorbyl phosphate

Tables 11.2 – 11.5 summarise the linearity results of kojic acid and sodium ascorbyl phosphate, respectively. The linear regression curves for kojic acid and sodium ascorbyl phosphate are shown in Figure 11.13 and Figure 11.14 respectively.

Table 11.2 Peak area and concentration found for kojic acid.

Injection volume (µl)	Conc. (µg/ml)	Area 1	Area 2	Mean
10	20	18	18	18
10	60	321	356	338
10	100	740	710	725
10	118	867	902	884
10	140	1077	1083	1080
10	160	1332	1347	1340
10	180	1490	1501	1495
10	200	1710	1700	1705
11	220	1840	1879	1859
12	240	2100	2100	2100

Table 11.3 Regression parameters for kojic acid.

Regression parameters			
R squared	0.998	**Lower 95%**	**Upper 95%**
Intercept	-214.3	-264.0	-164.6
Slope	9.52	9.21	9.84

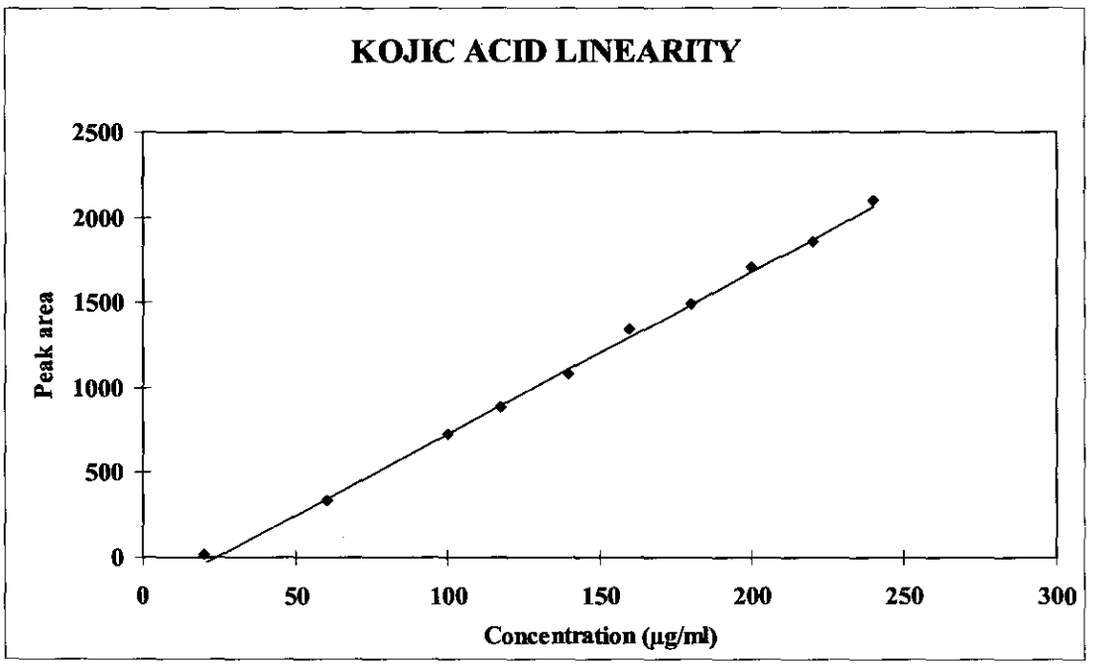

Figure 11.13 Linear regression curve for kojic acid.

Table 11.4 Peak area and concentration found for sodium ascorbyl phosphate.

Injection volume (µl)	Conc. (µg/ml)	Area 1	Area 2	Mean
10	60	45	52	48
10	180	1026	1099	1063
10	300	2023	2068	2045
10	353	2557	2588	2573
10	420	3222	3247	3235
10	480	3993	3612	3803
10	541	4321	4413	4367
10	600	4966	5003	4985
11	660	5613	5544	5578
12	720	6117	6230	6173

Table 11.5 Regression parameters of sodium ascorbyl phosphate.

Regression parameters			
R squared	0.998	**Lower 95%**	**Upper 95%**
Intercept	-646.1	-788.1	-504.0
Slope	9.35	9.05	9.65

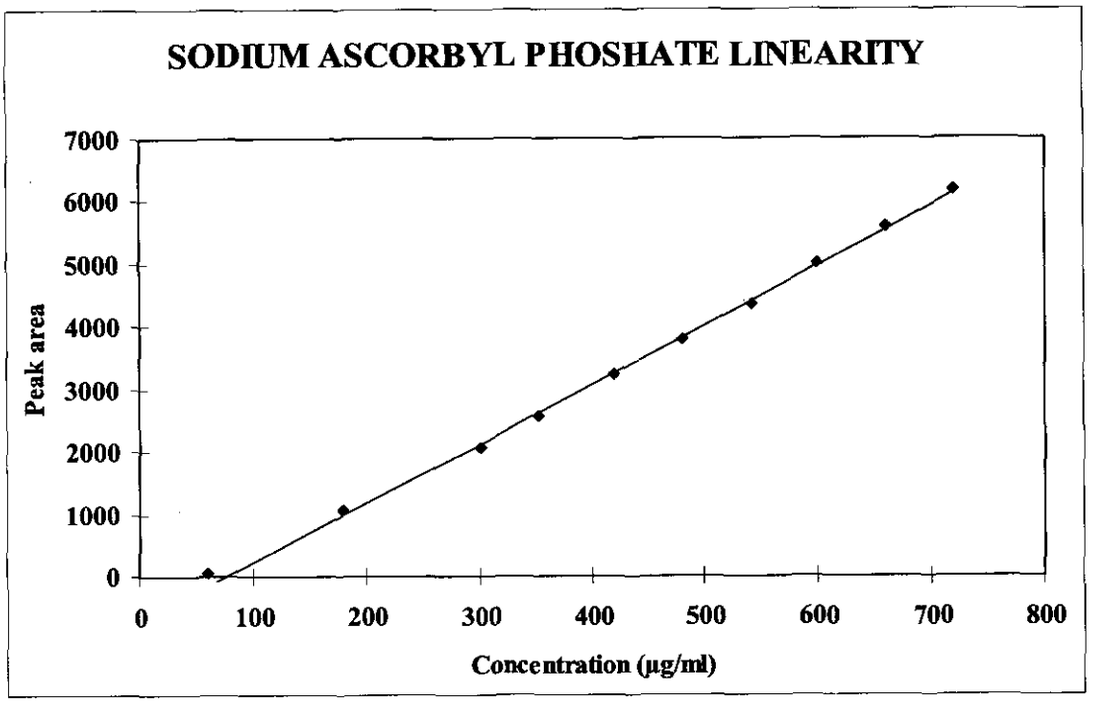

Figure 11.14 Linear regression curve for sodium ascorbyl phosphate.

Conclusion

This method is linear over the concentration range of 20 – 240 µg/ml for kojic acid, and 60 – 720 µg/ml for sodium ascorbyl phosphate. The method met the acceptance criteria and was suitable for single point calibration.

11.9.3 Accuracy

Table 11.6 and table 11.7 represent the percentage kojic acid recovered and confidence intervals respectively. Table 11.8 and table 11.9 represent the percentage sodium ascorbyl phosphate recovered and confidence intervals respectively.

Table 11.6 Percentage kojic acid recovered.

Conc. spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovered (%)
160.00	1802	1811	1807	156.6	98
160.00	1803	1799	1801	156.1	98
160.00	1798	1799	1799	155.9	97
200.00	2301	2296	2299	199.3	100
200.00	2293	2299	2296	199.0	100
200.00	2305	2300	2303	199.6	100
240.00	2780	2784	2782	241.2	100
240.00	2789	2781	2785	241.4	101
240.00	2779	2772	2776	240.6	100

Table 11.7 Confidence intervals for kojic acid.

Statistical analysis	
Mean	99.2
SD	1.2
% RSD	1.2
95% confidence intervals	
Lower limit	98.3
Upper limit	100.2
Estimated median	99.6
Confidence Level (95.0%)	1.0

Table 11.8 Percentage sodium ascorbyl phosphate recovered.

Conc. spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovered (%)
480	4499	4502	4501	473	99
480	4506	4512	4509	474	99
480	4497	4513	4505	474	99
600	5689	5672	5681	597	100
600	5699	5702	5701	600	100
600	5692	5703	5698	599	100
720	6720	6740	6730	708	98
720	6733	6723	6728	708	98
720	6741	6756	6749	710	99

Table 11.9 Confidence intervals for sodium ascorbyl phosphate.

Statistical analysis	
Mean	99.0
SD	0.6
% RSD	0.6
95% confidence intervals	
Lower limit	98.5
Upper limit	99.5
Estimated median	98.7
Confidence Level (95.0%)	0.5

Conclusion

Over the concentration range of 160 – 240 µg/ml for kojic acid, and 480 - 720 µg/ml for sodium ascorbyl phosphate, the method yielded and accuracy of 99.2% and 99.0%, respectively.

11.9.4 Precision

Tables 11.10 – Table 11.13.1 represent the results obtained in the intra-day and inter-day of kojic acid and sodium ascorbyl phosphate, respectively.

11.9.4.1 Intra-day precision

Table 11.10 Intra-day precision results for kojic acid.

Conc. found (µg/ml)	Recovered (%)
196.3	98.2
198.6	99.3
196.8	98.4
196.4	98.2
197.8	98.9
199.1	99.6
198.4	99.2
198.9	99.4
199.8	99.9
Mean	99.01
SD	0.59
RSD %	0.59

Table 11.11 Intra-day precision results for sodium ascorbyl phosphate.

Conc. found (µg/ml)	Recovered (%)
598.50	99.7
597.39	99.6
596.82	99.5
591.50	98.6
588.26	98.0
592.63	98.8
591.11	98.5
595.90	99.3
602.52	100.4
Mean	99.16
SD	0.70
RSD %	0.70

11.9.4.2 Inter-day precision

Table 11.12 Inter-day precision results for kojic acid.

	Day 1	Day 2	Day 3
	98.2	98.9	99.1
	98.9	99.7	98.4
	99.6	100.8	98.9
Mean	98.90	99.83	98.77
SD	0.55	0.79	0.30
RSD %	0.55	0.79	0.31

Table 11.12.1 ANOVA: Single factor for kojic acid.

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	296.7	98.90	0.446
Day 2	3	299.5	99.83	0.936
Day 3	3	296.3	98.77	0.139

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**
Between days	1.98	2.0	0.991	1.956	0.222
Within days	3.04	6.0	0.507	--	--
Total	5.02	8.0	--	--	--

SS = Sum of squares

DF = Degrees of freedom

MS = Mean of squares

F = F ratio

Table 11.13 Inter-day precision results for sodium ascorbyl phosphate.

	Day 1	Day 2	Day 3
	98.6	98.4	100.0
	98.0	98.3	99.2
	98.8	99.9	100.0
Mean	98.47	98.86	99.74
SD	0.31	0.75	0.40
RSD %	0.31	0.76	0.40

Table 11.13.1 ANOVA: Single factor for sodium ascorbyl phosphate.

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	295.40	98.47	0.142
Day 2	3	296.59	98.86	0.850
Day 3	3	299.22	99.74	0.243

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**
Between days	2.55	2.0	1.274	3.096	0.119
Within days	2.47	6.0	0.412	--	--
Total	5.02	8.0	--	--	--

Conclusion

Precision was satisfactory with RSD values less than 4%. There were no significant differences between "within day" and "between day" variances of kojic acid and sodium ascorbyl phosphate respectively, in fact the between day variances were less than the within day variances for kojic acid, which indicate that the method should perform well even when executed by another analyst on different equipment.

11.9.5 Ruggedness

11.9.5.1 Stability of sample solutions

Table 11.14 Stability results of kojic acid.

Time (hours)	Peak Area	% Remaining
0	2328	100
1	2327	100
2	2327	100
3	2326	100
4	2325	100
5	2324	100
6	2324	100
7	2323	100
8	2320	100
9	2311	99
10	2308	99
11	2303	99
12	2300	99
13	2292	98
14	2290	98
15	2284	98
16	2281	98
17	2279	98
18	2279	98
19	2268	97
20	2254	97
21	2250	97
22	2245	96
23	2245	96
24	2244	96
	Mean	98
	SD	0.07
	RSD %	0.07

Table 11.15 Stability results of sodium ascorbyl phosphate.

Time (hours)	Peak Area	% Remaining
0	6324	100
1	6323	100
2	6322	100
3	6320	100
4	6318	100
5	6318	100
6	6317	100
7	6313	100
8	6311	100
9	6310	100
10	6309	100
11	6307	100
12	6307	100
13	6306	100
14	6306	100
15	6304	100
16	6302	100
17	6300	100
18	6298	100
19	6298	100
20	6295	100
21	6294	100
22	6291	99
23	6290	99
24	6288	99
	Mean	100
	SD	0.06
	RSD %	0.06

Conclusion

A 3.6% breakdown of kojic acid was observed over the 24-hour period but no significant breakdown for sodium ascorbyl phosphate was observed.

11.9.5.2 System repeatability

Table 11.16 System repeatability for kojic acid and sodium ascorbyl phosphate.

	Kojic acid		Sodium ascorbyl phosphate	
	Area	Retention time	Area	Retention time
	2303	2.68	6301	7.23
	2313	2.66	6298	7.23
	2309	2.65	6333	7.23
	2333	2.68	6301	7.23
	2308	2.66	6208	7.21
	2323	2.68	6311	7.20
Mean	2315	2.67	6293	7.22
SD	10.2	0.01	39.5	0.01
RSD %	0.44	0.44	0.63	0.18

Conclusion

System performance was acceptable with RSD values of 0.44% and 0.63% for peak area and RSD values of 0.44 % and 0.18 % for retention times of kojic acid and sodium ascorbyl phosphate, respectively.

11.9.5.3 Robustness

Deliberate changes were made to the chromatographic conditions to determine the robustness of the method.

The following changes were made:

Mobile phase: The acetonitrile concentration was varied from 10% to 30%

Injection volume: 8 – 12 µl

Flow rate: 0.8 – 1.2 ml/min

Detection wavelength: 250 – 280 nm, recommended with reference wavelength of 500 nm and band width of 100.

Column: Luna C18(2), 150 x 4.6mm, 5 µm column, 100 A pores, 21.5 % carbon load, end capped, Phenomenex, Torrance, CA was used.

Conclusion

The method was not affected by any changes made to the analytical conditions and proved to be robust. The change in retention times with the Luna C18 was insignificant.

11.10 Chromatographic performance parameters

Reference: USP 24, 2000. p.1923

Retention time (minutes)

kojic acid: 2.374

sodium ascorbyl phosphate: 7.876

Number of theoretical plates (N)column

kojic acid: 47919

sodium ascorbyl phosphate: 79423

USP tailing factor (T)

kojic acid: 1.171

sodium ascorbyl phosphate: 1.158

Capacity factor (K')

kojic acid: 3.112

sodium ascorbyl phosphate: 1.643

Resolution between peaks (Rr)

kojic acid and sodium ascorbyl phosphate: 30.948

CONCLUSION

After all the tests were done on all the formulations, it was clear that kojic acid and sodium ascorbyl phosphate are compatible as skin whitening agents in a variety of cosmetic products. These actives did show a decrease in concentration when exposed to temperatures above 25°C + 60% RH, but the tests results revealed that the formulations remained stable when stored below 25°C. The batches of cream A and cream B that were stored at 25°C + 60% RH for three months, were compared in Figure 6.11, it became clear that the sodium ascorbyl phosphate was more stable when formulated as the only active.

The cream and the toner kept its soft skin feel, fast absorbing and moisturising capabilities, but the visual assessment confirmed that the products should not be exposed to temperatures above 25°C for too long. Xanthan gum was chosen as gellant after many trials, since it has a wide pH range and is resistant to enzymatic degradation. The gel became more fluent with time, but if it could be presented in a small tube (10 ml), it will be ideal for applying to the face as it is quick absorbing and non-sticking. The foam bath and the soap had extraordinary foamability. The soap showed a decrease in foamability when exposed to hard water, but was still accepted to have good cleaning and skin whitening value.

It would be recommended that all the formulations should be presented in small dosages to reduce the possibility of becoming instable. Kojic acid caused a colour change in the formulations due to oxidation and its sensitivity to heat, and for this reason the products should be kept below 25°C. HPLC analysis showed that even a small decrease in concentration caused a significant change in colour; therefore the containers should be containing no more than a month's supply of product.

The validation method for HPLC analysis performed well and complied with all the acceptance criteria for the simultaneous determination of kojic acid and sodium ascorbyl

phosphate in the preparations, for stability testing, quality control and batch release purposes. This method could thus be regarded as being stability-indicating.

Kojic acid and sodium ascorbyl phosphate should really be considered as the futures skin whitening wonder, for it is compatible with each other, easily formulated into cosmetic products, and showed no major stability problems when stored at low temperatures. The research showed that the toxicology for both the actives was neglectable when formulated in topical products.

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APPENDIX

APPENDIX A Membrane release data sheets

APPENDIX B Assays

APPENDIX C Stability tests data sheets

APPENDIX D Conference contribution

APPENDIX E Publication

APPEDIX A

Membrane release

Cream A

- **Kojic acid** **A1 – A5**
- **Sodium ascorbyl phosphate** **A6 - A11**

Cream B

- **Sodium ascorbyl phosphate** **A12 - A16**

Calculations **A17**

Membrane release of kojic acid

Product:	Cream A
Batch no.:	Initial (A)
Stability period:	Initial
Container:	Plastic jar
API:	Kojic acid
Strength:	1 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP-18 ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 7.5 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
3.022	662	68	79	91	115	133	0.77	0.89	1.03	1.30	1.50
3.014	660	69	81	92	114	135	0.78	0.91	1.04	1.29	1.52
3.012	671	69	81	92	116	136	0.78	0.91	1.04	1.31	1.53
3.251	672	65	78	90	111	133	0.73	0.88	1.02	1.25	1.50
3.114	662	67	80	89	110	134	0.76	0.90	1.00	1.24	1.51
3.179	660	68	79	91	115	134	0.77	0.89	1.03	1.30	1.51
Ave	665					**Ave %**	0.8	0.9	1.0	1.3	1.5
%RSD	0.15					**%RSD**	0.00	0.00	0.00	0.00	0.37

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	662	68	79	91	115	133	37	43	49	62	72
3.0918	660	69	81	92	114	135	37	44	50	61	73
3.1242	671	69	81	92	116	136	37	44	50	63	73
3.1276	672	65	78	90	111	133	35	42	49	60	72
3.2364	662	67	80	89	110	134	36	43	48	59	72
3.2997	660	68	79	91	115	134	37	43	49	62	72
Ave	665					**Ave %**	36	43	49	61	72
%RSD	0.15					**%RSD**	0.00	0.00	0.00	0.00	0.37

Membrane release of kojic acid

Product: Cream A
Batch no.: (A25)1
Stability period: 25°C + 60% RH
Container: Plastic jar
API: Kojic acid
Strength: 1 % m/m

Analytical: HPLC: Hewlett Packard 1050
Wavelength: 255 nm

Column: Lichrospher 100-5 RP-18 ec

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 500 µl
Dilution: None
Apparatus: VanKel
Temperature: 32 °C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 7.5 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
3.021	660	60	76	87	110	130	0.68	0.86	0.98	1.24	1.47
3.055	661	68	76	87	113	136	0.77	0.86	0.98	1.28	1.54
3.048	662	56	76	89	112	133	0.63	0.86	1.01	1.26	1.50
3.118	668	56	75	89	110	139	0.63	0.85	1.01	1.24	1.57
3.112	669	62	78	87	109	132	0.70	0.88	0.98	1.23	1.49
3.192	665	70	79	86	116	134	0.79	0.89	0.97	1.31	1.51
Ave	664					**Ave %**	0.7	0.9	1.0	1.3	1.5
%RSD	0.38					**%RSD**	8.06	1.96	0.57	2.69	1.49

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	660	60	76	87	110	130	32	41	47	59	70
3.0918	661	68	76	87	113	136	37	41	47	61	73
3.1242	662	56	76	89	112	133	30	41	48	60	72
3.1276	668	56	75	89	110	139	30	40	48	59	75
3.2364	669	62	78	87	109	132	33	42	47	59	71
3.2997	665	70	79	86	116	134	38	43	46	63	72
Ave	664					**Ave %**	33	41	47	60	72
%RSD	0.38					**%RSD**	8.06	1.96	0.57	2.69	1.49

Membrane release of kojic acid

Product: Cream A
Batch no.: (A25)3
Stability period: 25°C + 60 % RH / 3 months
Container: Plastic jar
API: Kojic acid
Strength: 1 % m/m

Analytical: HPLC: Hewlett Packard 1050
Wavelength: 255 nm

Column: Lichrospher 100-5 RP 18 ec

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 500 µl
Dilution: None
Apparatus: VanKel
Temperature: 32 °C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 7.5 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
3.012	666	54	69	84	104	129	0.61	0.78	0.95	1.17	1.46
3.188	664	56	69	82	104	128	0.63	0.78	0.93	1.17	1.45
3.201	662	55	64	82	105	127	0.62	0.72	0.93	1.19	1.43
3.192	668	57	68	81	106	128	0.64	0.77	0.91	1.20	1.45
3.144	663	56	66	84	104	124	0.63	0.75	0.95	1.17	1.40
3.208	661	54	67	86	102	127	0.61	0.76	0.97	1.15	1.43
Ave	664					**Ave %**	0.6	0.8	0.9	1.2	1.4
%RSD	0.38					**%RSD**	0.00	1.49	1.20	0.96	0.79

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	666	54	69	84	104	129	29	37	45	56	70
3.0918	664	56	69	82	104	128	30	37	44	56	69
3.1242	662	55	64	82	105	127	30	35	44	57	69
3.1276	668	57	68	81	106	128	31	37	44	57	69
3.2364	663	56	66	84	104	124	30	36	45	56	67
3.2997	661	54	67	86	102	127	29	36	46	55	69
Ave	664					**Ave %**	30	36	45	56	69
%RSD	0.38					**%RSD**	0.00	1.49	1.20	0.96	0.79

Membrane release of kojic acid

Product:	Cream A
Batch no.:	(A40)1
Stability period:	40°C + 75% RH / 1 month
Container:	Plastic jar
API:	Kojic acid
Strength:	1 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 7.5 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
3.0121	662	49	69	77	100	122	0.55	0.78	0.87	1.13	1.38
3.0555	663	48	64	79	101	122	0.54	0.72	0.89	1.14	1.38
3.0652	668	47	69	78	109	123	0.53	0.78	0.88	1.23	1.39
3.2141	665	47	68	79	106	125	0.53	0.77	0.89	1.20	1.41
3.124	664	42	62	76	108	129	0.47	0.70	0.86	1.22	1.46
3.2169	661	40	63	72	103	121	0.45	0.71	0.81	1.16	1.37
Ave	664					**Ave %**	0.5	0.7	0.9	1.2	1.4
%RSD	0.08					**%RSD**	9.89	4.56	3.25	1.44	0.40

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	662	49	69	77	100	122	26	37	42	54	66
3.0918	663	48	64	79	101	122	26	35	43	55	66
3.1242	668	47	69	78	109	123	25	37	42	59	66
3.1276	665	47	68	79	106	125	25	37	43	57	67
3.2364	664	42	62	76	108	129	23	33	41	58	70
3.2997	661	40	63	72	103	121	22	34	39	56	65
Ave	664					**Ave %**	25	36	41	56	67
%RSD	0.08					**%RSD**	9.89	4.56	3.25	1.44	0.40

Membrane release of kojic acid

Product:	Cream A
Batch no.:	(A40)3
Stability period:	40°C + 75% RH / 3 months
Container:	Plastic jar
API:	Kojic acid
Strength:	1 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water	
Volume:	190 ml	
Amount withdraw:	500 µl	
Dilution:	None	
Apparatus:	VanKel	
Temperature:	32 °C	
Agitation:	Paddle	
Speed:	100 rpm	
Membrane diameter:	2.25 cm	
Membrane surface area:	3.978	cm*cm

Concentration of standard: 7.5 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
3.026	666	45	49	49	53	58	0.51	0.55	0.55	0.60	0.66
3.044	664	44	48	50	52	57	0.50	0.54	0.56	0.59	0.64
3.162	662	42	48	51	53	54	0.47	0.54	0.58	0.60	0.61
3.118	668	46	49	52	53	53	0.52	0.55	0.59	0.60	0.60
3.112	663	42	47	50	56	56	0.47	0.53	0.56	0.63	0.63
3.048	661	43	49	49	52	51	0.49	0.55	0.55	0.59	0.58
Ave	664					**Ave %**	0.5	0.5	0.6	0.6	0.6
%RSD	0.38					**%RSD**	2.29	0.00	0.00	0.94	6.38

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	666	45	49	49	53	58	24	26	26	29	31
3.0918	664	44	48	50	52	57	24	26	27	28	31
3.1242	662	42	48	51	53	54	23	26	28	29	29
3.1276	668	46	49	52	53	53	25	26	28	29	29
3.2364	663	42	47	50	56	56	23	25	27	30	30
3.2997	661	43	49	49	52	51	23	26	26	28	28
Ave	664					**Ave %**	24	26	27	29	30
%RSD	0.38					**%RSD**	2.29	0.00	0.00	0.94	6.38

Membrane release of sodium ascorbyl phosphate

Product:	Cream A
Batch no.:	Initial (A)
Stability period:	Initial
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 225 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
3.0024	2105	240	375	520	610	670	25.6	40.0	55.5	65.1	71.5
3.0118	2114	242	377	523	611	675	25.8	40.2	55.8	65.2	72.0
3.0144	2101	235	379	524	609	677	25.1	40.4	55.9	65.0	72.2
3.0476	2111	239	369	527	612	672	25.5	39.4	56.2	65.3	71.7
3.1247	2121	246	372	520	613	671	26.3	39.7	55.5	65.4	71.6
3.0997	2099	242	370	519	616	673	25.8	39.5	55.4	65.7	71.8
Ave	2109					**Ave %**	25.7	39.9	55.7	65.3	71.8
%RSD	0.14					**%RSD**	0.42	0.67	0.10	0.49	0.22

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	240	375	520	610	670	1223	1911	2650	3109	3415
3.0918	2114	242	377	523	611	675	1233	1921	2666	3114	3440
3.1242	2101	235	379	524	609	677	1198	1932	2671	3104	3451
3.1276	2111	239	369	527	612	672	1218	1881	2686	3119	3425
3.2364	2121	246	372	520	613	671	1254	1896	2650	3124	3420
3.2997	2099	242	370	519	616	673	1233	1886	2645	3140	3430
Ave	2109					**Ave %**	1227	1905	2661	3118	3430
%RSD	0.14					**%RSD**	0.42	0.67	0.10	0.49	0.22

Membrane release of sodium ascorbyl phosphate

Product:	Cream A
Batch no.:	(A25)1
Stability period:	25°C+60%RH / 1 Month
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 225 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
3.0121	2111	220	365	491	581	650	25.2	41.9	56.3	66.6	74.5
3.0881	2106	221	366	489	588	649	25.3	42.0	56.1	67.4	74.4
3.1652	2110	223	359	490	573	651	25.6	41.2	56.2	65.7	74.6
3.2147	2111	219	362	485	589	654	25.1	41.5	55.6	67.5	74.9
3.2164	1219	226	367	488	579	650	25.9	42.1	56.0	66.4	74.6
3.1999	2115	227	359	492	581	652	26.0	41.2	56.4	66.6	74.8
Ave	1962					**Ave %**	25.5	41.6	56.1	66.7	74.6
%RSD	0.10					**%RSD**	1.57	0.83	0.10	0.00	0.16

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	2111	220	365	491	581	650	1205	1999	2689	3182	3560
3.0918	2106	221	366	489	588	649	1210	2005	2678	3221	3556
3.1242	2110	223	359	490	573	651	1221	1966	2684	3139	3564
3.1276	2111	219	362	485	589	654	1200	1983	2657	3226	3579
3.2364	1219	226	367	488	579	650	1238	2010	2673	3171	3561
3.2997	2115	227	359	492	581	652	1243	1966	2695	3182	3572
Ave	1962					**Ave %**	1220	1988	2679	3187	3565
%RSD	0.10					**%RSD**	1.57	0.83	0.10	0.00	0.16

Membrane release of sodium ascorbyl phosphate

Product:	Cream A
Batch no.:	(A25)3
Stability period:	25°C+60%RH / 3 Months
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 225 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
3.2964	2009	215	311	420	560	610	24.1	34.9	47.1	62.8	68.4
3.0228	2001	216	312	419	561	612	24.2	35.0	47.0	62.9	68.6
3.1372	2010	216	315	421	566	610	24.2	35.3	47.2	63.5	68.4
3.1376	2011	214	314	425	567	611	24.0	35.2	47.7	63.6	68.5
3.2334	2004	210	316	418	562	609	23.5	35.4	46.9	63.0	68.3
3.1927	2004	211	316	419	569	613	23.7	35.4	47.0	63.8	68.7
Ave	2007					**Ave %**	24.0	35.2	47.1	63.3	68.5
%RSD	0.12					**%RSD**	0.94	0.80	0.12	0.80	0.25

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	2009	215	311	420	560	610	1152	1666	2249	2999	3267
3.0918	2001	216	312	419	561	612	1157	1671	2244	3005	3278
3.1242	2010	216	315	421	566	610	1157	1687	2255	3031	3267
3.1276	2011	214	314	425	567	611	1146	1682	2276	3037	3272
3.2364	2004	210	316	418	562	609	1125	1692	2239	3010	3262
3.2997	2004	211	316	419	569	613	1130	1692	2244	3048	3283
Ave	2007					**Ave %**	1144	1682	2251	3022	3272
%RSD	0.12					**%RSD**	0.94	0.80	0.12	0.80	0.25

Membrane release of sodium ascorbyl phosphate

Product:	Cream A
Batch no.:	(A40)1
Stability period:	40°C+75%RH / 1 Month
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water	
Volume:	190 ml	
Amount withdraw:	500 µl	
Dilution:	None	
Apparatus:	VanKel	
Temperature:	32 °C	
Agitation:	Paddle	
Speed:	100 rpm	
Membrane diameter:	2.25 cm	
Membrane surface area:	3.978	cm*cm

Concentration of standard: 225 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
3.1444	2105	200	303	418	512	550	21.3	32.3	44.6	54.6	58.7
3.1472	2114	201	304	416	511	542	21.4	32.4	44.4	54.5	57.8
3.1331	2101	200	306	417	511	610	21.3	32.7	44.5	54.5	65.1
3.2421	2111	203	309	420	510	611	21.7	33.0	44.8	54.4	65.2
3.2217	2121	206	310	418	512	609	22.0	33.1	44.6	54.6	65.0
3.2921	2099	207	302	419	516	613	22.1	32.2	44.7	55.1	65.4
Ave	2109					**Ave %**	21.6	32.6	44.6	54.6	62.9
%RSD	0.14					**%RSD**	1.73	0.16	0.12	0.39	5.35

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	200	303	418	512	550	1019	1544	2130	2610	2803
3.0918	2114	201	304	416	511	542	1024	1549	2120	2604	2762
3.1242	2101	200	306	417	511	610	1019	1560	2125	2604	3109
3.1276	2111	203	309	420	510	611	1035	1575	2141	2599	3114
3.2364	2121	206	310	418	512	609	1050	1580	2130	2610	3104
3.2997	2099	207	302	419	516	613	1055	1539	2136	2630	3124
Ave	2109					**Ave %**	1034	1558	2130	2610	3003
%RSD	0.14					**%RSD**	1.73	0.16	0.12	0.39	5.35

Membrane release of sodium ascorbyl phosphate

Product:	Cream A
Batch no.:	(A40)3
Stability period:	40°C+75%RH / 3 Months
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 225 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
3.121	2010	212	312	402	499	512	23.7	34.9	45.0	55.8	57.3
3.1146	2009	213	316	401	489	513	23.8	35.4	44.9	54.7	57.4
3.2136	2011	214	314	401	486	512	24.0	35.1	44.9	54.4	57.3
3.2189	2011	211	312	402	495	519	23.6	34.9	45.0	55.4	58.1
3.1998	2008	209	314	406	493	521	23.4	35.1	45.4	55.2	58.3
3.2198	2013	210	312	405	497	519	23.5	34.9	45.3	55.6	58.1
Ave	2010					**Ave %**	23.7	35.1	45.1	55.2	57.8
%RSD	0.07					**%RSD**	0.47	0.00	0.37	0.20	0.68

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	2010	212	312	402	499	521	1133	1668	2149	2667	2737
3.0918	2009	213	316	401	489	522	1139	1689	2144	2614	2742
3.1242	2011	214	314	401	486	523	1144	1679	2144	2598	2737
3.1276	2011	211	312	402	495	529	1128	1668	2149	2646	2774
3.2364	2008	209	314	406	493	527	1117	1679	2170	2635	2785
3.2997	2013	210	312	405	497	526	1123	1668	2165	2657	2774
Ave	2010					**Ave %**	1131	1675	2153	2636	2758
%RSD	0.07					**%RSD**	0.47	0.00	0.37	0.20	0.68

Membrane release of sodium ascorbyl phosphate

Product:	Cream B
Batch no.:	Initial (B)
Stability period:	Initial
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water
Volume:	190 ml
Amount withdraw:	500 µl
Dilution:	None
Apparatus:	VanKel
Temperature:	32 °C
Agitation:	Paddle
Speed:	100 rpm
Membrane diameter:	2.25 cm
Membrane surface area:	3.978 cm*cm

Concentration of standard: 225 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
3.1121	2010	249	365	465	589	620	27.9	40.8	52.0	65.9	69.4
3.1142	2009	255	366	471	599	621	28.5	40.9	52.7	67.0	69.5
3.2125	2010	259	361	470	589	623	29.0	40.4	52.6	65.9	69.7
3.0217	2011	257	362	469	587	629	28.8	40.5	52.5	65.7	70.4
3.1651	2013	256	367	473	592	624	28.6	41.1	52.9	66.2	69.8
3.2198	2014	256	361	464	594	620	28.6	40.4	51.9	66.5	69.4
Ave	2011					**Ave %**	28.6	40.7	52.4	66.2	69.7
%RSD	0.10					**%RSD**	1.37	0.55	0.11	0.42	0.00

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	2010	249	365	465	589	620	1331	1950	2485	3147	3313
3.0918	2009	255	366	471	599	621	1363	1956	2517	3201	3318
3.1242	2010	259	361	470	589	623	1384	1929	2511	3147	3329
3.1276	2011	257	362	469	587	629	1373	1934	2506	3137	3361
3.2364	2013	256	367	473	592	624	1368	1961	2527	3163	3334
3.2997	2014	256	361	464	594	620	1368	1929	2479	3174	3313
Ave	2011					**Ave %**	1364	1943	2504	3162	3328
%RSD	0.10					**%RSD**	1.37	0.55	0.11	0.42	0.00

Membrane release of sodium ascorbyl phosphate

Product: Cream B
Batch no.: (B25)1
Stability period: 25°C+60%RH / 1 Month
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 3 % m/m

Analytical: HPLC: Hewlett Packard 1050
Wavelength: 255 nm

Column: Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium: Distilled water
Volume: 190 ml
Amount withdraw: 500 µl
Dilution: None
Apparatus: VanKel
Temperature: 32 °C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 225 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
3.0121	2120	232	381	465	579	650	24.6	40.4	49.3	61.3	68.9
3.1125	2122	239	387	469	579	649	25.3	41.0	49.7	61.3	68.8
3.1487	2129	234	389	471	578	651	24.8	41.2	49.9	61.2	68.9
3.1467	2128	241	382	469	571	654	25.5	40.5	49.7	60.5	69.2
3.2567	2127	246	381	463	579	650	26.1	40.4	49.0	61.3	68.9
3.2094	2119	237	380	472	581	652	25.1	40.3	50.0	61.5	69.1
Ave	2124					**Ave %**	25.2	40.6	49.6	61.2	68.9
%RSD	0.02					**%RSD**	1.05	0.13	0.75	0.17	0.16

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	2120	232	381	465	579	650	1174	1928	2353	2929	3288
3.0918	2122	239	387	469	579	649	1209	1958	2373	2929	3284
3.1242	2129	234	389	471	578	651	1184	1968	2383	2924	3292
3.1276	2128	241	382	469	571	654	1219	1933	2373	2889	3306
3.2364	2127	246	381	463	579	650	1245	1928	2342	2929	3289
3.2997	2119	237	380	472	581	652	1199	1922	2388	2939	3299
Ave	2124					**Ave %**	1205	1939	2369	2923	3293
%RSD	0.02					**%RSD**	1.05	0.13	0.75	0.17	0.16

Membrane release of sodium ascorbyl phosphate

Product:	Cream B
Batch no.:	(B25)3
Stability period:	25°C+60%RH / 3 Months
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water	
Volume:	190 ml	
Amount withdraw:	500 µl	
Dilution:	None	
Apparatus:	VanKel	
Temperature:	32 °C	
Agitation:	Paddle	
Speed:	100 rpm	
Membrane diameter:	2.25 cm	
Membrane surface area:	3.978	cm*cm

Concentration of standard: 225 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
3.1141	1999	212	341	442	579	648	23.8	38.3	49.7	65.0	72.8
3.2594	1999	216	348	446	579	649	24.3	39.1	50.1	65.0	72.9
3.2467	2001	217	342	448	578	650	24.4	38.4	50.3	64.9	73.0
3.1998	2005	218	341	449	571	651	24.5	38.3	50.4	64.1	73.1
3.0487	2004	210	349	441	579	650	23.6	39.2	49.5	65.0	73.0
3.1021	2009	211	348	439	581	652	23.7	39.1	49.3	65.3	73.3
Ave	2003					**Ave %**	24.0	38.7	49.9	64.9	73.0
%RSD	0.25					**%RSD**	0.23	1.01	0.34	0.17	0.32

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	1999	212	341	442	579	648	1138	1830	2372	3107	3477
3.0918	1999	216	348	446	579	649	1159	1867	2393	3107	3482
3.1242	2001	217	342	448	578	650	1164	1835	2404	3101	3488
3.1276	2005	218	341	449	571	651	1170	1830	2409	3064	3493
3.2364	2004	210	349	441	579	650	1127	1873	2366	3107	3488
3.2997	2009	211	348	439	581	652	1132	1867	2356	3117	3499
Ave	2003					**Ave %**	1148	1850	2383	3100	3488
%RSD	0.25					**%RSD**	0.23	1.01	0.34	0.17	0.32

Membrane release of sodium ascorbyl phosphate

Product:	Cream B
Batch no.:	(B40)1
Stability period:	40°C+75%RH / 1 Month
Container:	Plastic jar
API:	Sodium ascorbyl phosphate
Strength:	3 % m/m
Analytical:	HPLC: Hewlett Packard 1050
Wavelength:	255 nm
Column:	Lichrospher 100-5 RP 18ec

Membrane release conditions:

Medium:	Distilled water	
Volume:	190 ml	
Amount withdraw:	500 µl	
Dilution:	None	
Apparatus:	VanKel	
Temperature:	32 °C	
Agitation:	Paddle	
Speed:	100 rpm	
Membrane diameter:	2.25 cm	
Membrane surface area:	3.978	cm*cm

Concentration of standard: 225 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
3.1241	1997	209	299	399	472	512	23.6	33.7	45.0	53.2	57.7
3.0642	1995	204	298	387	479	513	23.0	33.6	43.6	54.0	57.8
3.2115	2004	205	297	385	469	512	23.1	33.5	43.4	52.9	57.7
3.2645	1990	200	298	386	471	519	22.5	33.6	43.5	53.1	58.5
3.2181	1992	201	301	382	468	521	22.7	33.9	43.1	52.7	58.7
3.1108	2000	201	302	388	476	519	22.7	34.0	43.7	53.6	58.5
Ave	1996					**Ave %**	22.9	33.7	43.7	53.3	58.2
%RSD	0.08					**%RSD**	1.97	0.50	1.42	0.42	0.68

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	1997	209	299	399	472	541	1125	1610	2148	2541	2756
3.0918	1995	204	298	387	479	537	1098	1604	2083	2579	2762
3.1242	2004	205	297	385	469	549	1104	1599	2073	2525	2756
3.1276	1990	200	298	386	471	544	1077	1604	2078	2535	2794
3.2364	1992	201	301	382	468	539	1082	1620	2056	2519	2805
3.2997	2000	201	302	388	476	538	1082	1626	2089	2562	2794
Ave	1996					**Ave %**	1095	1610	2088	2544	2778
%RSD	0.08					**%RSD**	1.97	0.50	1.42	0.42	0.68

Membrane release of sodium ascorbyl phosphate

Product: Cream B
Batch no.: (B40)3
Stability period: 40°C+75%RH / 3 Months
Container: Plastic jar
API: Sodium ascorbyl phosphate
Strength: 3 % m/m

Analytical: HPLC: Hewlett Packard 1050
Wavelength: 255 nm

Column: Lichrospher 100-5 RP 18ec

Membrane release conditions:
Medium: Distilled water
Volume: 190 ml
Amount withdraw: 500 µl
Dilution: None
Apparatus: VanKel
Temperature: 32 °C
Agitation: Paddle
Speed: 100 rpm
Membrane diameter: 2.25 cm
Membrane surface area: 3.978 cm*cm

Concentration of standard: 225 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
3.0958	2113	212	309	399	472	521	22.5	32.9	42.4	50.2	55.4
3.0597	2112	213	300	398	479	519	22.7	31.9	42.3	50.9	55.2
3.1545	2114	218	301	394	469	527	23.2	32.0	41.9	49.9	56.1
3.2648	2115	219	301	396	471	528	23.3	32.0	42.1	50.1	56.2
3.1414	2120	217	308	399	468	526	23.1	32.8	42.4	49.8	55.9
3.2264	2119	220	306	382	476	523	23.4	32.5	40.6	50.6	55.6
Ave	2116					**Ave %**	23.0	32.4	42.0	50.3	55.7
%RSD	0.14					**%RSD**	1.85	0.49	2.15	0.42	0.19

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	2113	212	309	399	472	541	1077	1570	2027	2398	2647
3.0918	2112	213	300	398	479	537	1082	1524	2022	2433	2636
3.1242	2114	218	301	394	469	549	1107	1529	2001	2382	2677
3.1276	2115	219	301	396	471	544	1113	1529	2012	2393	2682
3.2364	2120	217	308	399	468	539	1102	1565	2027	2377	2672
3.2997	2119	220	306	382	476	538	1118	1554	1941	2418	2657
Ave	2116					**Ave %**	1100	1545	2005	2400	2662
%RSD	0.14					**%RSD**	1.85	0.49	2.15	0.42	0.19

Calculations

$$\text{Mass released } (\mu g/ml) \text{ in time (min.)} = \frac{\text{Standard conc.}(\mu g/ml) \times \text{Peak Area}}{\text{Average area of system suitability}}$$

$$\text{Release rate } (\mu g/cm^2) \text{ in time (min.)} = \frac{\text{Mass released} \times \text{Volume (190 ml)}}{\text{Membrane surface area (3.9777)}}$$

Membrane surface area = πr^2

Where membrane radius is = 1.125cm

APPENDIX B

Assays of kojic acid

Assays of sodium ascorbyl phosphate

Assays of Methyl hydroxybenzoate

Assays of Propyl hydroxybenzoate

Assays of kojic acid

Product: Cream A
Strength: 1% m/m
Wavelength: 255nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2332	2330	2341	2361
Ave STD B	2351	2320	2339	2352
Mass of STD A	200mg	200mg	200mg	200mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.328	2893	2894	2894	213.2	0.916	92
	2.106	2433	2436	2435	198.3	0.941	94
Month 1: 5°C	2.099	2234	2230	2232	182.3	0.868	87
	2.057	2199	2194	2197	183.2	0.890	89
Month 1: 25°C+60%RH	2.133	2240	2234	2237	179.8	0.843	84
	2.017	1977	1974	1976	168.0	0.833	83
Month 1: 40°C+75%RH	2.136	2103	2100	2102	168.8	0.790	79
	2.186	2212	2218	2215	173.8	0.795	79
Month 2: 5°C	2.097	2166	2140	2153	176.1	0.840	84
	2.100	2159	2174	2167	176.9	0.842	84
Month 2: 25°C+60%RH	2.038	1986	2102	2044	172.0	0.844	84
	2.143	2198	2223	2211	176.9	0.825	83
Month 2: 40°C+75%RH	2.091	1107	783	945	77.5	0.371	37
	2.090	907	806	857	70.3	0.336	34
Month 3: 5°C	2.096	2210	2197	2204	180.3	0.860	86
	2.077	2189	2210	2200	181.7	0.875	87
Month 3: 25°C+60%RH	2.052	2024	2018	2021	168.9	0.823	82
	2.102	2135	2112	2124	173.3	0.824	82
Month 3: 40°C+75%RH	2.018	691	939	815	69.3	0.343	34
	2.037	749	714	732	61.6	0.302	30

Assays of kojic acid

Product: Face Lotion
Strength: 1% m/m
Wavelength: 255nm

Container: 200ml plastic bottle
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2332	2241	2220	2236
Ave STD B	2300	2239	2224	2239
Mass of STD A	200mg	200mg	200mg	200mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.010	2174	2216	2195	187.4	0.933	93
	2.007	2292	2147	2220	189.7	0.945	94
Month 1: 5°C	2.053	2078	2212	2145	187.3	0.913	91
	2.050	2100	2210	2155	188.5	0.920	92
Month 1: 25°C+60%RH	2.045	1998	2032	2015	176.7	0.864	86
	2.018	1994	1989	1992	177.0	0.877	88
Month 1: 40°C+75%RH	2.091	1997	2013	2005	171.9	0.822	82
	2.089	1995	1991	1993	171.0	0.819	82
Month 2: 5°C	2.011	2110	2094	2102	187.7	0.933	93
	2.004	2110	2156	2133	191.1	0.953	95
Month 2: 25°C+60%RH	2.194	2603	2313	2458	190.7	0.869	87
	2.044	2141	2004	2073	172.6	0.844	84
Month 2: 40°C+75%RH	2.055	2023	1978	2001	165.7	0.807	81
	2.050	1999	1951	1975	164.0	0.800	80
Month 3: 5°C	2.014	2021	2053	2037	181.6	0.902	90
	2.077	2154	2169	2162	186.8	0.900	90
Month 3: 25°C+60%RH	2.049	2099	2115	2107	175.0	0.854	85
	2.030	2087	1756.8	1922	170.9	0.842	84
Month 3: 40°C+75%RH	2.012	1679	1916	1798	161.2	0.801	80
	2.012	1726	1832	1779	159.5	0.793	79

Assays of kojic acid

Product: Foam bath
Strength: 1% m/m
Wavelength: 255nm

Container: 200ml plastic bottle
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2306	2442	2312	2330
Ave STD B	2321	2423	2329	2334
Mass of STD A	200mg	200mg	200mg	200mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.024	2231	2239	2235	189.5	0.936	94
	2.024	2241	2237	2239	189.8	0.937	94
Month 1: 5°C	2.041	2100	2243	2172	190.8	0.935	93
	2.004	2141	2024	2083	186.3	0.930	93
Month 1: 25°C+60%RH	2.128	2234	2247	2241	188.8	0.887	89
	2.100	2034	2324	2179	186.0	0.886	89
Month 1: 40°C+75%RH	2.031	2304	2198	2056	181.5	0.894	89
	2.028	1987	2043	2015	178.1	0.878	88
Month 2: 5°C	2.016	2221	2219	2220	197.4	0.979	98
	2.102	2399	2386	2393	204.1	0.971	97
Month 2: 25°C+60%RH	2.003	1815	1815	1815	162.5	0.811	81
	2.026	1857	1941	1899	168.1	0.830	83
Month 2: 40°C+75%RH	2.080	1556	1430	1493	128.7	0.619	62
	2.032	1641	1434	1538	135.6	0.667	67
Month 3: 5°C	2.069	2265	2278	2272	196.8	0.951	95
	2.033	2241	2230	2236	197.2	0.970	97
Month 3: 25°C+60%RH	2.027	1780	1791	1786	157.9	0.779	78
	2.017	1779	1782	1781	158.2	0.784	78
Month 3: 40°C+75%RH	2.024	1439	1325	1382	122.4	0.605	60
	2.020	1418	1225	1322	117.3	0.581	58

Assays of kojic acid

Product: Gel
Strength: 1% m/m
Wavelength: 255nm

Container: 100ml plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2331	2361	2333	2329
Ave STD B	2339	2358	2328	2331
Mass of STD A	200mg	200mg	200mg	200mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.001	2310	2316	2313	180.3	0.901	90
	2.003	2410	2415	2413	187.8	0.938	94
Month 1: 5°C	2.13399	2355	2352	2354	197.7	0.927	93
	2.10081	2257	2259	2258	192.7	0.917	92
Month 1: 25°C+60%RH	2.03398	2111	2110	2111	186.0	0.915	91
	2.01407	2113	2099	2106	187.5	0.931	93
Month 1: 40°C+75%RH	2.02972	2112	2105	2109	186.3	0.918	92
	2.04486	2107	2110	2109	184.9	0.904	90
Month 2: 5°C	2.0421	2140	2137	2139	187.8	0.919	92
	2.0641	2140	2144	2142	186.1	0.901	90
Month 2: 25°C+60%RH	2.02562	2022	2010	2016	178.4	0.892	89
	2.03411	2011	2023	2017	177.8	0.889	89
Month 2: 40°C+75%RH	2.09101	2141	2132	2137	183.2	0.916	92
	2.09014	2047	2011	2029	174.0	0.870	87
Month 3: 5°C	2.0119	2042	2051	2047	182.4	0.906	91
	2.0212	2056	2049	2053	182.1	0.901	90
Month 3: 25°C+60%RH	2.06689	2047	1843	1945	168.7	0.816	82
	2.0049	2213	2080	2147	192.0	0.957	96
Month 3: 40°C+75%RH	2.0149	2017	2012	2015	179.3	0.890	89
	2.06239	2020	2031	2026	176.1	0.854	85

Assays of kojic acid

Product: Soap
Strength: 1% m/m
Wavelength: 255nm

Container: foil cover
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2555	2545	2560	2549
Ave STD B	2565	2550	2554	2541
Mass of STD A	200mg	200mg	200mg	200mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.022	1985	1977	1981	152.8	0.756	76
	2.012	1988	1982	1985	153.9	0.765	76
Month 1: 5°C	2.036	1801	1813	1807	138.4	0.680	68
	2.004	1747	1750	1749	136.1	0.679	68
Month 1: 25°C+60%RH	2.001	1782	1761	1772	138.1	0.690	69
	2.021	1777	1769	1773	136.8	0.677	68
Month 1: 40°C+75%RH	2.114	1277	1284	1281	94.5	0.447	45
	2.113	1263	1271	1267	93.5	0.443	44
Month 2: 5°C	2.023	1603	1609	1606	123.8	0.612	61
	2.036	1599	1582	1591	121.8	0.598	60
Month 2: 25°C+60%RH	2.080	1733	1726	1730	129.7	0.623	62
	2.117	1766	1779	1773	130.6	0.617	62
Month 2: 40°C+75%RH	2.023	798	800	799	61.6	0.304	30
	2.110	833	816	825	60.9	0.289	29
Month 3: 5°C	2.113	1722	1712	1717	126.7	0.600	60
	2.056	1611	1604	1608	121.9	0.593	59
Month 3: 25°C+60%RH	2.054	1475	1469	1472	111.8	0.544	54
	2.062	1540	1455	1498	113.3	0.549	55
Month 3: 40°C+75%RH	2.053	302	309	306	23.2	0.113	11
	2.091	304	300	302	22.5	0.108	11

Assays of sodium ascorbyl phosphate

Product: Cream A
Strength: 1% m/m
Wavelength: 255nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	6684	6672	6659	6713
Ave STD B	6663	6660	6668	6652
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.064	7029	6997	7013	610.0	2.956	99
	2.098	7033	7065	7049	603.2	2.875	96
Month 1: 5°C	2.100	6259	6267	6263	535.5	2.550	85
	2.057	6234	6248	6241	544.7	2.648	88
Month 1: 25°C+60%RH	2.096	6657	6667	6662	570.6	2.722	91
	2.017	6232	6220	6226	554.1	2.746	92
Month 1: 40°C+75%RH	2.136	6199	6201	6200	521.1	2.439	81
	2.187	6362	6359	6361	522.2	2.388	80
Month 2: 5°C	2.033	6202	6211	6207	548.0	2.695	90
	2.071	6292	6298	6295	545.7	2.635	88
Month 2: 25°C+60%RH	2.039	6103	6111	6107	537.8	2.638	88
	2.144	6809	6707	6758	566.0	2.641	88
Month 2: 40°C+75%RH	1.991	5789	5723	5756	519.1	2.607	87
	2.097	6305	6182	6244	534.6	2.549	85
Month 3: 5°C	2.045	6197	6210	6204	544.7	2.664	89
	2.119	6177	6172	6175	523.2	2.469	82
Month 3: 25°C+60%RH	2.052	5711	5672	5692	497.9	2.426	81
	1.981	5298	5310	5304	480.8	2.427	81
Month 3: 40°C+75%RH	2.019	5113	5096	5105	454.0	2.249	75
	2.038	5124	5172	5148	453.6	2.226	74

Assays of sodium ascorbyl phosphate

Product: Cream B
Strength: 3% m/m
Wavelength: 255nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	5687	5662	5772	5674
Ave STD B	5722	5660	5690	5669
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.111	6373	6379	6376	637.4	3.02	101
	2.113	6373	6377	6375	636.7	3.01	100
Month 1: 5°C	2.041	5990	5996	5993	619.6	3.04	101
	2.042	5999	5986	5993	619.3	3.03	101
Month 1: 25°C+60%RH	2.111	6497	6455	6476	647.4	3.07	102
	2.114	6399	6410	6405	639.3	3.02	101
Month 1: 40°C+75%RH	2.190	7176	7083	7130	687.0	3.14	105
	2.191	6439	6440	6440	620.2	2.83	94
Month 2: 5°C	2.044	6077	6035	6056	625.2	3.06	102
	2.041	6033	6015	6024	622.8	3.05	102
Month 2: 25°C+60%RH	2.150	6439	6448	6444	632.4	2.94	98
	2.161	6511	6512	6512	635.9	2.94	98
Month 2: 40°C+75%RH	2.180	6005	6028	6017	582.4	2.67	89
	2.192	6001	6011	6006	578.2	2.64	88
Month 3: 5°C	2.110	6321	6329	6325	632.6	3.00	100
	2.131	6480	6488	6484	642.1	3.01	100
Month 3: 25°C+60%RH	2.165	6535	6543	6539	637.4	2.94	98
	2.148	6472	6481	6477	636.3	2.96	99
Month 3: 40°C+75%RH	2.036	5069	5077	5073	525.8	2.58	86
	2.014	5011	5014	5013	525.2	2.61	87

Assays of sodium ascorbyl phosphate

Product: Face lotion
Strength: 3% m/m
Wavelength: 255nm

Container: 200ml Plastic bottle
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	6680	6651	6689	6680
Ave STD B	6677	6642	6692	6675
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.010	6852	6847	6850	612.0	3.045	102
	2.007	6742	6733	6738	602.6	3.002	100
Month 1: 5°C	2.053	6923	6918	6921	605.3	2.948	98
	2.050	6903	6917	6910	605.2	2.953	98
Month 2: 5°C	2.004	6724	6733	6729	602.8	3.008	100
	2.047	6923	6941	6932	608.0	2.970	99
Month 3: 5°C	2.066	6976	6985	6981	606.6	2.936	98
	2.091	6999	6991	6995	600.6	2.872	96
Month 1: 25°C+60%RH	2.015	6631	6563	6597	587.9	2.918	97
	2.013	6639	6610	6625	590.8	2.935	98
Month 2: 25°C+60%RH	2.196	6788	6779	6784	554.7	2.526	84
	2.055	5810	5824	5817	508.2	2.473	82
Month 3: 25°C+60%RH	2.049	5874	5886	5880	515.1	2.513	84
	2.030	5793	5787	5790	512.2	2.524	84
Month 1: 40°C+75%RH	2.091	6527	6540	6534	560.9	2.682	89
	2.089	6511	6549	6530	561.1	2.685	90
Month 2: 40°C+75%RH	2.196	6627	6639	6633	542.3	2.470	82
	2.194	5772	5780	5776	550.3	2.508	84
Month 3: 40°C+75%RH	2.012	5097	5016	5057	493.5	2.452	82
	2.012	5027	5032	5030	490.8	2.439	81

Assays of sodium ascorbyl phosphate

Product: Gel
Strength: 3% m/m
Wavelength: 255nm

Container: 100ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	6680	6685	6672	6710
Ave STD B	6686	6689	6679	6702
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.001	6680	6682	6681	599.6	2.997	100
	2.003	6729	6716	6723	602.6	3.008	100
Month 1: 5°C	2.034	6881	6889	6885	607.7	2.988	100
	2.008	6691	6690	6691	598.2	2.979	99
Month 1: 25°C+60%RH	2.034	6888	6884	6886	607.8	2.988	100
	2.014	6779	6773	6776	604.0	2.999	100
Month 1: 40°C+75%RH	2.030	6512	6499	6506	575.4	2.835	95
	2.045	6539	6529	6534	573.7	2.805	94
Month 2: 5°C	2.008	6701	6712	6707	599.6	2.986	100
	2.034	6821	6823	6822	602.1	2.960	99
Month 2: 25°C+60%RH	2.026	6690	6698	6694	593.3	2.929	98
	2.034	6687	6681	6684	590.0	2.900	97
Month 2: 40°C+75%RH	2.091	6700	6699	6700	575.2	2.751	92
	2.090	6612	6619	6616	568.3	2.719	91
Month 3: 5°C	2.014	6784	6780	6782	604.6	3.002	100
	2.017	6759	6766	6763	602.0	2.985	100
Month 3: 25°C+60%RH	2.067	6851	6862	6857	595.6	2.882	96
	2.005	6440	6449	6445	577.1	2.878	96
Month 3: 40°C+75%RH	2.015	6111	6113	6112	544.6	2.703	90
	2.062	6102	6107	6105	531.4	2.577	86

Assays of sodium ascorbyl phosphate

Product: Foam bath
Strength: 3% m/m
Wavelength: 255nm

Container: 200ml Plastic bottle
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	6683	6680	6672	6692
Ave STD B	6720	6688	6679	6684
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.024	6890	6881	6886	610.9	3.019	101
	2.024	6879	6872	6876	609.8	3.013	100
Month 1: 5°C	2.051	6890	6984	6937	607.1	2.959	99
	2.005	6610	6617	6614	592.1	2.953	98
Month 1: 25°C+60%RH	2.031	6744	6744	6744	596.2	2.936	98
	2.029	6798	6739	6769	599.0	2.952	98
Month 1: 40°C+75%RH	2.031	6267	6211	6239	551.5	2.716	91
	2.028	6214	6209	6212	549.8	2.711	90
Month 2: 5°C	2.015	6674	6683	6679	595.1	2.953	98
	2.062	6747	6751	6749	587.6	2.850	95
Month 2: 25°C+60%RH	2.128	5454	5411	5433	458.4	2.154	72
	2.100	5379	5433	5406	462.1	2.200	73
Month 2: 40°C+75%RH	2.124	5018	5011	5015	493.6	2.324	77
	2.120	5021	5019	5020	495.1	2.335	78
Month 3: 5°C	2.013	6301	6310	6306	562.4	2.794	93
	2.073	6614	6622	6618	573.2	2.765	92
Month 3: 25°C+60%RH	2.128	5454	5411	5433	458.4	2.154	72
	2.100	5379	5433	5406	462.1	2.200	73
Month 3: 40°C+75%RH	2.080	5116	5104	5110	482.4	2.319	77
	2.134	5111	5116	5114	470.6	2.205	74

Assays of sodium ascorbyl phosphate

Product: Soap
Strength: 3% m/m
Wavelength: 255nm

Container: foil cover
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	6709	6710	6711	6709
Ave STD B	6709	6705	6713	6703
Mass of STD A	600mg	600mg	600mg	600mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.011	6750	6744	6747	600.1	2.984	99
	2.014	6766	6769	6768	601.0	2.984	99
Month 1: 5°C	2.036	6721	6718	6720	590.3	2.899	97
	2.011	6710	6708	6709	596.7	2.967	99
Month 1: 25°C+60%RH	2.095	6657	6667	6662	568.8	2.715	90
	2.078	6614	6629	6622	569.9	2.743	91
Month 1: 40°C+75%RH	2.055	6312	6317	6315	549.6	2.674	89
	2.054	6362	6359	6361	553.9	2.697	90
Month 2: 5°C	2.036	6333	6330	6332	556.2	2.732	91
	2.031	6292	6298	6295	554.4	2.730	91
Month 2: 25°C+60%RH	2.016	6101	6107	6104	541.5	2.686	90
	2.017	6118	6114	6116	542.3	2.689	90
Month 2: 40°C+75%RH	2.031	6051	6047	6049	532.7	2.623	87
	2.111	6044	6032	6038	511.6	2.423	81
Month 3: 5°C	2.116	6612	6628	6620	559.6	2.644	88
	2.114	6613	6617	6615	559.7	2.647	88
Month 3: 25°C+60%RH	2.038	6011	6017	6014	527.8	2.590	86
	2.047	6023	6024	6024	526.3	2.571	86
Month 3: 40°C+75%RH	2.062	6000	6003	6002	520.6	2.525	84
	2.041	5822	5823	5823	510.2	2.500	83

Assays of Methyl Hydroxybenzoate

Product: Cream A
Strength: 0.2% m/m
Wavelength: 254nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	2774	2779	2771	2774
Ave STD B	2822	2770	2773	2776
Mass of STD A	40mg	40µg	40mg	40mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.058	2779	2810	2794.5	39.1	0.195	98
	2.062	2779	2772	2775.5	38.7	0.194	97
Month 1: 5°C	2.061	2816	2810	2813	39.3	0.196	98
	2.057	2799	2798	2798.5	39.1	0.196	98
Month 1: 25°C+60%RH	2.094	2879	2872	2875.5	39.5	0.198	99
	2.124	2956	2951	2953.5	40.0	0.200	100
Month 1: 40°C+75%RH	2.048	2717	2723	2720	38.2	0.191	96
	2.088	2783	2779	2781	38.3	0.192	96
Month 2: 5°C	2.043	2786	2789	2787.5	39.2	0.196	98
	2.033	2777	2780	2778.5	39.3	0.197	98
Month 2: 25°C+60%RH	2.039	2717	2716	2716.5	38.3	0.192	96
	2.013	2742	2748	2745	39.2	0.196	98
Month 2: 40°C+75%RH	1.991	2699	2692	2695.5	39.0	0.195	97
	2.097	2706	2710	2708	37.2	0.186	93
Month 3: 5°C	2.045	2769	2760	2764.5	38.9	0.195	97
	2.054	2722	2721	2721.5	38.1	0.191	95
Month 3: 25°C+60%RH	2.052	2696	2685	2690.5	37.7	0.189	94
	2.023	2659	2651	2655	37.8	0.189	94
Month 3: 40°C+75%RH	2.019	2651	2653	2652	37.8	0.189	94
	2.014	2647	2639	2643	37.8	0.189	94

Assays of Methyl Hydroxybenzoate

Product: Cream B
Strength: 0.2% m/m
Wavelength: 254nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	1802	1800	1798	1799
Ave STD B	1799	1806	1801	1797
Mass of STD A	40mg	40mg	40mg	40mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.022	1827	1821	1824	39.6	0.198	99
	2.021	1822	1826	1824	39.7	0.198	99
Month 1: 5°C	2.180	1966	1969	1968	39.7	0.198	99
	2.191	1972	1976	1974	39.6	0.198	99
Month 1: 25°C+60%RH	1.999	1802	1807	1805	39.7	0.198	99
	2.001	1802	1804	1803	39.6	0.198	99
Month 1: 40°C+75%RH	2.000	1789	1781	1785	39.2	0.196	98
	2.000	1788	1786	1787	39.3	0.196	98
Month 2: 5°C	2.014	1821	1823	1822	39.8	0.199	99
	2.016	1834	1839	1837	40.0	0.200	100
Month 2: 25°C+60%RH	2.010	1824	1829	1827	39.9	0.200	100
	2.010	1826	1826	1826	39.9	0.200	100
Month 2: 40°C+75%RH	2.070	1859	1865	1862	39.5	0.198	99
	2.060	1842	1841	1842	39.3	0.196	98
Month 3: 5°C	2.002	1831	1836	1834	40.3	0.201	101
	2.008	1833	1839	1836	40.2	0.201	100
Month 3: 25°C+60%RH	2.050	1839	1841	1840	39.4	0.197	99
	2.049	1847	1846	1847	39.6	0.198	99
Month 3: 40°C+75%RH	2.050	1860	1869	1865	40.0	0.200	100
	2.050	1855	1854	1855	39.8	0.199	99

Assays of Methyl Hydroxybenzoate

Product: Gel
Strength: 0.2% m/m
Wavelength: 254nm

Container: 1 Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	1800	1803	1799	1798
Ave STD B	1792	1811	1802	1799
Mass of STD A	40mg	40mg	40mg	40mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.011	1833	1830	1832	40.0	0.200	100
	2.003	1843	1839	1841	40.4	0.202	101
Month 1: 5°C	2.004	1842	1840	1841	40.4	0.202	101
	2.009	1836	1832	1834	40.1	0.201	100
Month 1: 25°C+60%RH	2.010	1802	1807	1805	39.5	0.197	99
	2.011	1802	1804	1803	39.4	0.197	99
Month 1: 40°C+75%RH	2.014	1809	1799	1804	39.4	0.197	98
	2.016	1801	1806	1804	39.3	0.197	98
Month 2: 5°C	2.050	1855	1859	1857	39.8	0.199	100
	2.051	1844	1847	1846	39.5	0.198	99
Month 2: 25°C+60%RH	2.010	1803	1802	1803	39.4	0.197	99
	2.010	1826	1826	1826	39.9	0.200	100
Month 2: 40°C+75%RH	2.074	1859	1865	1862	39.5	0.197	99
	2.085	1842	1841	1842	38.8	0.194	97
Month 3: 5°C	2.091	1859	1852	1856	39.0	0.195	98
	2.093	1863	1860	1862	39.1	0.195	98
Month 3: 25°C+60%RH	2.014	1811	1810	1811	39.5	0.198	99
	2.017	1803	1800	1802	39.3	0.196	98
Month 3: 40°C+75%RH	2.049	1829	1822	1826	39.2	0.196	98
	2.050	1833	1826	1830	39.2	0.196	98

Assays of Propyl Hydroxybenzoate

Product: Cream A
Strength: 0.02% m/m
Wavelength: 254nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	247	240	239	255
Ave STD B	243	244	240	264
Mass of STD A	4mg	4mg	4mg	4mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.020	229	231	230	4.0	0.020	99
	2.053	230	228	229	3.9	0.019	97
Month 1: 5°C	2.061	231	230	230.5	3.9	0.020	98
	2.057	228	230	229	3.9	0.019	97
Month 1: 25°C+60%RH	2.094	229	231	230	3.8	0.019	96
	2.124	231	234	232.5	3.8	0.019	96
Month 1: 40°C+75%RH	2.048	229	228	228.5	3.9	0.019	97
	2.088	225	230	227.5	3.8	0.019	95
Month 2: 5°C	2.098	231	232	231.5	3.9	0.019	96
	2.135	233	234	233.5	3.8	0.019	95
Month 2: 25°C+60%RH	2.015	224	229	226.5	3.9	0.020	98
	2.062	227	223	225	3.8	0.019	95
Month 2: 40°C+75%RH	2.043	226	228	227	3.9	0.019	97
	2.169	226	229	227.5	3.7	0.018	92
Month 3: 5°C	2.026	231	228	229.5	4.0	0.020	99
	2.034	229	225	227	3.9	0.019	97
Month 3: 25°C+60%RH	2.091	226	229	227.5	3.8	0.019	95
	2.090	229	230	229.5	3.8	0.019	96
Month 3: 40°C+75%RH	2.067	225	222	223.5	3.8	0.019	94
	2.005	225	223	224	3.9	0.019	97

Assays of Propyl Hydroxybenzoate

Product: Cream B
Strength: 0.02% m/m
Wavelength: 254nm

Container: 200ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	165	166	168	165
Ave STD B	166	167	165	164
Mass of STD A	4mg	4mg	4mg	4mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.022	166	168	167	4	0.02	100
	2.021	168	169	169	4	0.02	101
Month 1: 5°C	2.180	182	179	181	4	0.02	100
	2.191	183	179	181	4	0.02	100
Month 1: 25°C+60%RH	1.999	169	162	166	4	0.02	100
	2.001	166	166	166	4	0.02	101
Month 1: 40°C+75%RH	2.000	165	162	164	4	0.02	99
	2.000	165	164	165	4	0.02	100
Month 2: 5°C	2.014	167	166	167	4	0.02	100
	2.016	169	164	167	4	0.02	100
Month 2: 25°C+60%RH	2.010	167	165	166	4	0.02	100
	2.010	167	164	166	4	0.02	100
Month 2: 40°C+75%RH	2.070	170	169	170	4	0.02	99
	2.060	171	170	171	4	0.02	100
Month 3: 5°C	2.002	166	167	167	4	0.02	101
	2.008	165	165	165	4	0.02	100
Month 3: 25°C+60%RH	2.050	167	170	169	4	0.02	100
	2.049	171	170	171	4	0.02	101
Month 3: 40°C+75%RH	2.050	169	165	167	4	0.02	99
	2.050	167	171	169	4	0.02	100

Assays of Propyl Hydroxybenzoate

Product: Gel
Strength: 0.02% m/m
Wavelength: 254nm

Container: 100ml Plastic jar with screw cap
Instrument: HPLC: Hewlett Packard 1050
Column: Lichrospher 100-5 RP-18 ec

	Initial	1st Month	2nd Month	3rd Month
Ave STD A	246	247	251	246
Ave STD B	244	249	249	243
Mass of STD A	4mg	4mg	4mg	4mg

Stability interval	Sample mass	Area 1	Area 2	Area average	µg/ml	g/100 g	%
Initial	2.020	230	231	231	4.0	0.020	100
	2.053	230	230	230	3.9	0.020	98
Month 1: 5°C	2.098	233	237	235	3.9	0.020	98
	2.135	238	239	239	3.9	0.019	97
Month 1: 25°C+60%RH	2.102	239	233	236	3.9	0.020	98
	2.144	241	236	239	3.9	0.019	97
Month 1: 40°C+75%RH	2.043	230	228	229	3.9	0.020	98
	2.169	239	241	240	3.9	0.019	97
Month 2: 5°C	2.015	227	224	226	3.9	0.020	98
	2.005	226	219	223	3.9	0.019	97
Month 2: 25°C+60%RH	2.026	224	229	227	3.9	0.020	98
	2.034	227	223	225	3.9	0.019	97
Month 2: 40°C+75%RH	2.091	231	228	230	3.8	0.019	96
	2.090	226	229	228	3.8	0.019	95
Month 3: 5°C	2.098	231	228	230	3.8	0.019	95
	2.053	229	225	227	3.9	0.019	96
Month 3: 25°C+60%RH	2.067	226	229	228	3.8	0.019	96
	2.005	211	224	218	3.8	0.019	95
Month 3: 40°C+75%RH	2.015	225	222	224	3.9	0.019	97
	2.062	225	223	224	3.8	0.019	95

Calculations

Kojic acid:

$$\text{Concentration } (\mu g/ml) = \frac{\text{Average Area x Standard concentration}}{\text{Average Standard Area}} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Mass } (g/100g) = \frac{\text{Concentration } (\mu g/ml)}{200} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Percentage } (\%) = \text{Mass } (g/100g) \times 100$$

Sodium ascorbyl phosphate:

$$\text{Concentration } (\mu g/ml) = \frac{\text{Average Area x Standard concentration}}{\text{Average Standard Area}} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Mass } (g/100g) = \frac{\text{Concentration } (\mu g/ml)}{200} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Percentage } (\%) = \frac{\text{Mass } (g/100g)}{3} \times 100$$

Methyl hydroxybenzoate:

$$\text{Concentration } (\mu g/ml) = \frac{\text{Average Area x Standard concentration}}{\text{Average Standard Area}} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Mass } (g/100g) = \frac{\text{Concentration } (\mu g/ml) \times 0.2}{\text{Standard concentration } (\mu g/ml)}$$

$$\text{Percentage } (\%) = \frac{\text{Mass } (g/100g)}{0.2} \times 100$$

Propyl hydroxybenzoate:

$$\text{Concentration } (\mu g/ml) = \frac{\text{Average Area x Standard concentration}}{\text{Average Standard Area}} \times \frac{2}{\text{Mass of sample (g)}}$$

$$\text{Mass } (g/100g) = \frac{\text{Concentration } (\mu g/ml) \times 0.02}{\text{Standard concentration } (\mu g/ml)}$$

$$\text{Percentage } (\%) = \frac{\text{Mass } (g/100g)}{0.02} \times 100$$

APPENDIX C

Table C1: Ph results of the formulations over the three month stability period

Table C1				
Product	**Initial**	**1 Month**	**2 Months**	**3 Months**
Cream A				
5°C	7.40	7.44	7.41	7.39
25°C+60%RH	7.41	7.41	7.17	7.12
40°C+75%RH	7.41	7.22	6.83	6.78
Ceam B				
5°C	7.70	7.51	7.50	7.70
25°C+60%RH	7.71	7.57	7.56	7.57
40°C+75%RH	7.73	7.45	7.62	7.49
Face lotion				
5°C	7.91	7.93	7.92	7.89
25°C+60%RH	7.80	7.90	7.88	7.87
40°C+75%RH	7.86	7.82	7.76	7.74
Foam bath				
5°C	7.01	6.94	6.91	6.91
25°C+60%RH	7.00	6.87	6.84	6.80
40°C+75%RH	7.02	6.70	6.45	6.17
Gel				
5°C	7.53	7.45	7.39	7.32
25°C+60%RH	7.54	7.30	7.29	7.13
40°C+75%RH	7.54	7.02	6.89	6.63
Soap				
5°C	10.23	10.11	10.12	10.19
25°C+60%RH	10.05	10.25	10.13	10.07
40°C+75%RH	10.17	10.19	10.10	10.10

Table C2: Specific gravity results of the formulations over the three month stability period

Description	Initial	1 Month	2 months	3 months
Cream A				
5°C	0.933	0.931	0.933	0.933
25°C + 60% RH	0.932	0.974	0.939	0.954
40°C + 75% RH	0.934	0.954	0.939	0.944
Cream B				
5°C	0.947	0.934	0.962	0.935
25°C + 60% RH	0.939	0.958	0.928	0.955
40°C + 75% RH	0.942	0.931	0.974	0.932
Lotion				
5°C	1.049	1.036	1.041	1.034
25°C+60%RH	1.042	1.006	1.003	1.002
40°C+75%RH	1.047	1.061	0.975	1.014
Foam bath				
5°C	1.040	1.085	1.083	1.076
25°C+60%RH	1.039	1.033	1.048	1.032
40°C+75%RH	1.041	1.047	1.078	1.059

Table C3: Spreadability results of cream A and cream B

Cream A	Initial	1 Month	2 Months	3 Months
5°C	40.01	40.65	40.29	40.74
25°C+60%RH	41.54	40.05	41.81	40.2
40°C+75%RH	40.17	40.67	41.03	42.31
Cream B				
5°C	38.68	38.68	38.68	38.68
25°C+60%RH	38.37	38.37	38.37	38.37
40°C+75%RH	39.46	39.46	39.46	39.46

Table C4: The viscosity of cream A at 5°C, Initial

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	235400
3	1.0	24.9	2	100200
4	2.5	24.9	2	48470
5	5.0	24.9	2	27590
6	10.0	25.0	2	17100
7	20.0	25.0	2	10440
8	50.0	25.0	2	5675
9	100.0	25.0	2	3677
10	50.0	25.0	2	5939
11	20.0	25.0	2	11550
12	10.0	25.0	2	18600
13	5.0	24.9	2	27950
14	2.5	25.0	2	39110
15	1.0	25.0	2	64190
16	0.5	24.9	2	275640
17	0.0	25.0	2	0.0

Table C5: The viscosity of cream A at 25°C+60%RH, Initial

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	179100
3	1.0	24.9	2	100210
4	2.5	24.9	2	48460
5	5.0	24.9	2	27620
6	10.0	25.0	2	170090
7	20.0	25.0	2	10435
8	50.0	24.9	2	5685
9	100.0	24.9	2	3677
10	50.0	24.9	2	5960
11	20.0	25.0	2	11550
12	10.0	25.0	2	18677
13	5.0	24.9	2	27760
14	2.5	25.0	2	39210
15	1.0	25.0	2	64100
16	0.5	24.9	2	97085
17	0.0	24.0	2	0.0

Table C6: The viscosity of cream A at 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	223000
3	1.0	25.0	2	100100
4	2.5	25.0	2	48550
5	5.0	24.9	2	27445
6	10.0	25.0	2	17020
7	20.0	24.9	2	10645
8	50.0	25.0	2	5670
9	100.0	24.9	2	3465
10	50.0	25.0	2	5980
11	20.0	25.0	2	11400
12	10.0	25.0	2	18433
13	5.0	24.9	2	27400
14	2.5	25.0	2	40215
15	1.0	24.9	2	64100
16	0.5	24.9	2	212400
17	0.0	25.0	2	0.0

Table C7: The viscosity of cream A: 1 Month, 5°C

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	166200
3	1.0	25.0	2	88750
4	2.5	25.0	2	44600
5	5.0	25.0	2	22550
6	10.0	24.9	2	16670
7	20.0	24.9	2	11590
8	50.0	24.9	2	6260
9	100.0	25.0	2	4440
10	50.0	25.0	2	6280
11	20.0	25.0	2	12140
12	10.0	25.0	2	22240
13	5.0	25.0	2	29850
14	2.5	25.0	2	45600
15	1.0	25.0	2	77430
16	0.5	25.0	2	115100
17	0.0	24.9	2	0.0

Table C8: The viscosity of cream A: 1 Month, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	171800
3	1.0	25.0	2	94180
4	2.5	25.0	2	45830
5	5.0	25.0	2	28310
6	10.0	24.9	2	17340
7	20.0	24.9	2	10920
8	50.0	24.9	2	5927
9	100.0	25.0	2	3749
10	50.0	25.0	2	6263
11	20.0	25.0	2	12150
12	10.0	25.0	2	19500
13	5.0	25.0	2	28790
14	2.5	25.0	2	44150
15	1.0	25.0	2	75580
16	0.5	25.0	2	111600
17	0.0	25.0	2	0.0

Table C9: The viscosity of cream A: 1 Month, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	133300
3	1.0	25.0	2	77980
4	2.5	24.9	2	53030
5	5.0	25.0	2	32870
6	10.0	25.0	2	21360
7	20.0	25.0	2	13820
8	50.0	24.9	2	7546
9	100.0	25.0	2	4619
10	50.0	25.0	2	7618
11	20.0	25.0	2	11200
12	10.0	24.9	2	21780
13	5.0	24.9	2	29850
14	2.5	25.0	2	52550
15	1.0	25.0	2	78580
16	0.5	25.0	2	112300.0
17	0.0	25.0	2	0.0

Table C10: The viscosity of cream A: 2 Months, 5°C

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	144500
3	1.0	25.0	2	67650
4	2.5	24.9	2	52100
5	5.0	25.0	2	38580
6	10.0	25.0	2	26500
7	20.0	25.0	2	14450
8	50.0	24.9	2	6820
9	100.0	25.0	2	4430
10	50.0	25.0	2	7618
11	20.0	25.0	2	13200
12	10.0	24.9	2	22240
13	5.0	24.9	2	33750
14	2.5	25.0	2	51580
15	1.0	25.0	2	76740
16	0.5	25.0	2	146200.0
17	0.0	25.0	2	0.0

Table C11: The viscosity of cream A: 2 Months, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	153600
3	1.0	25.0	2	94810
4	2.5	25.0	2	48710
5	5.0	25.0	2	32030
6	10.0	25.0	2	21540
7	20.0	25.0	2	13170
8	50.0	25.0	2	6875
9	100.0	25.0	2	4277
10	50.0	25.0	2	6935
11	20.0	25.0	2	13350
12	10.0	25.0	2	20940
13	5.0	25.0	2	31190
14	2.5	25.0	2	42810
15	1.0	25.0	2	91780
16	0.5	25.0	2	141600
17	0.0	25.0	2	0.0

Table C12: The viscosity of cream A: 2 Months, 40˚C+75%RH

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	150200
3	1.0	25.0	2	88720
4	2.5	25.0	2	45630
5	5.0	24.9	2	33120
6	10.0	25.0	2	24210
7	20.0	24.9	2	16850
8	50.0	25.0	2	6720
9	100.0	24.9	2	5580
10	50.0	25.0	2	7280
11	20.0	25.0	2	10200
12	10.0	25.0	2	19860
13	5.0	24.9	2	30320
14	2.5	25.0	2	44870
15	1.0	24.9	2	99680
16	0.5	24.9	2	140780
17	0.0	25.0	2	0.0

Table C13: The viscosity of cream A: 3 Months, 5˚C

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	133200
3	1.0	25.0	2	55820
4	2.5	25.0	2	49880
5	5.0	25.0	2	37630
6	10.0	25.0	2	28850
7	20.0	25.0	2	11420
8	50.0	25.0	2	5830
9	100.0	25.0	2	3890
10	50.0	25.0	2	5210
11	20.0	25.0	2	11200
12	10.0	25.0	2	22240
13	5.0	25.0	2	31370
14	2.5	25.0	2	48750
15	1.0	25.0	2	52540
16	0.5	25.0	2	103720.0
17	0.0	25.0	2	0.0

Table C14: The viscosity of cream A: 3 Months, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	130100
3	1.0	25.0	2	66850
4	2.5	25.0	2	38650
5	5.0	25.0	2	48540
6	10.0	25.0	2	29850
7	20.0	25.0	2	22150
8	50.0	25.0	2	10100
9	100.0	25.0	2	5210
10	50.0	25.0	2	9890
11	20.0	25.0	2	12100
12	10.0	25.0	2	15520
13	5.0	25.0	2	29870
14	2.5	25.0	2	38500
15	1.0	25.0	2	59560
16	0.5	25.0	2	129800
17	0.0	25.0	2	0.0

Table C15: The viscosity of cream A: 3 Months, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	127520
3	1.0	24.9	2	77850
4	2.5	25.0	2	48750
5	5.0	25.0	2	30680
6	10.0	24.9	2	19200
7	20.0	25.0	2	10800
8	50.0	25.0	2	7280
9	100.0	25.0	2	5180
10	50.0	25.0	2	7280
11	20.0	25.0	2	11200
12	10.0	25.0	2	18750
13	5.0	24.0	2	31330
14	2.5	24.9	2	48250
15	1.0	24.9	2	77640
16	0.5	25.0	2	125540
17	0.0	25.0	2	0.0

Table C16: The viscosity of cream B at 5˚C, Initial

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	249500
3	1.0	24.9	2	151200
4	2.5	24.9	2	75100
5	5.0	24.9	2	47750
6	10.0	25.0	2	32100
7	20.0	25.0	2	22560
8	50.0	25.0	2	10550
9	100.0	25.0	2	10422
10	50.0	25.0	2	10530
11	20.0	25.0	2	19710
12	10.0	25.0	2	31810
13	5.0	24.9	2	43070
14	2.5	25.0	2	71500
15	1.0	25.0	2	115200
16	0.5	24.9	2	187200
17	0.0	25.0	2	0.0

Table C17: The viscosity of cream B at 25˚C+60%RH, Initial

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	234200
3	1.0	24.9	2	150000
4	2.5	24.9	2	88540
5	5.0	24.9	2	55650
6	10.0	25.0	2	42100
7	20.0	25.0	2	19800
8	50.0	24.9	2	11210
9	100.0	24.9	2	9850
10	50.0	24.9	2	10530
11	20.0	25.0	2	18750
12	10.0	25.0	2	32660
13	5.0	24.9	2	49860
14	2.5	25.0	2	80450
15	1.0	25.0	2	133400
16	0.5	24.9	2	210150
17	0.0	24.0	2	0.0

Table C18: The viscosity of cream B at 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	221100
3	1.0	25.0	2	144570
4	2.5	25.0	2	82880
5	5.0	24.9	2	50840
6	10.0	25.0	2	30200
7	20.0	24.9	2	14500
8	50.0	25.0	2	11330
9	100.0	24.9	2	8870
10	50.0	25.0	2	10120
11	20.0	25.0	2	16870
12	10.0	25.0	2	31680
13	5.0	24.9	2	51850
14	2.5	25.0	2	88450
15	1.0	24.9	2	140780
16	0.5	24.9	2	210150
17	0.0	25.0	2	0.0

Table C19: The viscosity of cream B: 1 Month, 5°C

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	250700
3	1.0	25.0	2	168000
4	2.5	25.0	2	75580
5	5.0	25.0	2	50990
6	10.0	25.0	2	34310
7	20.0	25.0	2	22530
8	50.0	24.9	2	11610
9	100.0	24.9	2	5880
10	50.0	25.0	2	11120
11	20.0	25.0	2	19530
12	10.0	25.0	2	28070
13	5.0	25.0	2	40910
14	2.5	24.9	2	63350
15	1.0	24.9	2	16710
16	0.5	25.0	2	172600
17	0.0	25.0	2	0.0

Table C20: The viscosity of cream B: 1 Month, 25˚C+60%RH

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	244200
3	1.0	25.0	2	15250
4	2.5	25.0	2	77450
5	5.0	25.0	2	44980
6	10.0	24.9	2	32240
7	20.0	25.0	2	22910
8	50.0	25.0	2	111420
9	100.0	25.0	2	4242
10	50.0	25.0	2	15660
11	20.0	24.9	2	20230
12	10.0	24.9	2	31160
13	5.0	25.0	2	44550
14	2.5	25.0	2	68870
15	1.0	25.0	2	119640
16	0.5	25.0	2	222800
17	0.0	25.0	2	0.0

Table C21: The viscosity of cream B: 1 Month, 40˚C+75%RH

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	256700
3	1.0	25.0	2	153000
4	2.5	25.0	2	87820
5	5.0	25.0	2	50510
6	10.0	25.0	2	35270
7	20.0	24.9	2	23810
8	50.0	25.0	2	11670
9	100.0	24.9	2	3432
10	50.0	25.0	2	16550
11	20.0	25.0	2	21660
12	10.0	25.0	2	32510
13	5.0	25.0	2	47030
14	2.5	25.0	2	71260
15	1.0	25.0	2	120600
16	0.5	25.0	2	199600
17	0.0	25.0	2	0.0

Table C22: The viscosity of cream B: 2 Months, 5˚C

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	242100
3	1.0	25.0	2	156000
4	2.5	25.0	2	76520
5	5.0	25.0	2	55820
6	10.0	25.0	2	36340
7	20.0	25.0	2	22640
8	50.0	25.0	2	11880
9	100.0	24.9	2	6200
10	50.0	25.0	2	14120
11	20.0	24.9	2	19260
12	10.0	25.0	2	27780
13	5.0	25.0	2	41960
14	2.5	24.9	2	62210
15	1.0	24.9	2	155600
16	0.5	25.0	2	189800
17	0.0	25.0	2	0.0

Table C23: The viscosity of cream B: 2 Months, 25˚C+60%RH

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	254300
3	1.0	25.0	2	158400
4	2.5	25.0	2	80620
5	5.0	24.9	2	50030
6	10.0	25.0	2	34130
7	20.0	24.9	2	22590
8	50.0	25.0	2	11130
9	100.0	24.9	2	6230
10	50.0	25.0	2	10270
11	20.0	25.0	2	19620
12	10.0	25.0	2	30710
13	5.0	25.0	2	46790
14	2.5	25.0	2	77200
15	1.0	25.0	2	113200
16	0.5	25.0	2	205600
17	0.0	25.0	2	0.0

Table C24: The viscosity of cream B: 2 Months, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	238700
3	1.0	25.0	2	140400
4	2.5	25	2	82540
5	5.0	25.0	2	50630
6	10.0	25.0	2	35570
7	20.0	25.0	2	24170
8	50.0	25.0	2	11100
9	100.0	25.0	2	6250
10	50.0	24.9	2	10900
11	20.0	25.0	2	22230
12	10.0	25.0	2	31450
13	5.0	25.0	2	44430
14	2.5	25.0	2	76520
15	1.0	24.9	2	133400
16	0.5	24.9	2	212600
17	0.0	25.0	2	0.0

Table C25: The viscosity of cream B: 3 Months, 5°C

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	233100
3	1.0	24.9	2	177880
4	2.5	25.0	2	84260
5	5.0	25.0	2	55950
6	10.0	24.9	2	47440
7	20.0	24.9	2	24420
8	50.0	24.9	2	11150
9	100.0	24.9	2	7200
10	50.0	25.0	2	10100
11	20.0	25.0	2	22410
12	10.0	25.0	2	30120
13	5.0	25.0	2	44850
14	2.5	25.0	2	89850
15	1.0	24.9	2	177850
16	0.5	24.9	2	220400
17	0.0	25.0	2	0.0

Table C26: The viscosity of cream B: 3 Months, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	233020
3	1.0	25.0	2	175700
4	2.5	25.0	2	99560
5	5.0	25.0	2	58400
6	10.0	25.0	2	42100
7	20.0	25.0	2	22190
8	50.0	25.0	2	11050
9	100.0	25.0	2	6850
10	50.0	25.0	2	10300
11	20.0	25.0	2	19800
12	10.0	25.0	2	37850
13	5.0	25.0	2	42170
14	2.5	25.0	2	88740
15	1.0	25.0	2	164800
16	0.5	25.0	2	210040
17	0.0	25.0	2	0.0

Table C27: The viscosity of cream B: 3 Months, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	244700
3	1.0	24.9	2	172200
4	2.5	24.9	2	93580
5	5.0	24.9	2	60590
6	10.0	24.9	2	37550
7	20.0	24.9	2	25460
8	50.0	25.0	2	11050
9	100.0	25.0	2	6680
10	50.0	25.0	2	10200
11	20.0	25.0	2	23580
12	10.0	24.9	2	31240
13	5.0	25.0	2	55080
14	2.5	24.9	2	88370
15	1.0	25.0	2	166800
16	0.5	24.9	2	210100
17	0.0	25.0	2	0.0

Table C28: The viscosity of the foam bath at 5°C, Initial

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	240.9
3	1.0	24.9	2	344.9
4	2.5	24.9	2	335.9
5	5.0	24.9	2	315.9
6	10.0	24.9	2	293.9
7	20.0	24.9	2	290.9
8	50.0	24.9	2	289.1
9	100.0	25.0	2	281.9
10	50.0	25.0	2	299.9
11	20.0	25.0	2	304.9
12	10.0	25.0	2	291.9
13	5.0	24.9	2	315.9
14	2.5	24.9	2	332.9
15	1.0	24.9	2	359.9
16	0.5	24.9	2	239.9
17	0.0	25.0	2	0.0

Table C29: The viscosity of the foam bath at 25°C+60%RH, Initial

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	25.0	2	415.9
3	1.0	24.9	2	387.9
4	2.5	24.9	2	330.9
5	5.0	24.9	2	311.9
6	10.0	25.0	2	301.9
7	20.0	25.0	2	292.9
8	50.0	24.9	2	284.4
9	100.0	24.9	2	276.5
10	50.0	24.9	2	287.3
11	20.0	25.0	2	293.4
12	10.0	25.0	2	299.9
13	5.0	24.9	2	305.9
14	2.5	25.0	2	323.9
15	1.0	25.0	2	359.9
16	0.5	24.9	2	415.9
17	0.0	24.0	2	0.0

Table C30: The viscosity of the foam bath at 40˚C+75%RH, Initial

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	419.9
3	1.0	25.0	2	389.9
4	2.5	25.0	2	335.9
5	5.0	25.0	2	311.9
6	10.0	25.0	2	305.9
7	20.0	24.9	2	293.9
8	50.0	25.0	2	283.3
9	100.0	24.9	2	276.5
10	50.0	25.1	2	287.3
11	20.0	25.1	2	293.4
12	10.0	25.1	2	299.9
13	5.0	25.1	2	305.9
14	2.5	25.1	2	323.9
15	1.0	25.0	2	359.9
16	0.5	25	2	419.9
17	0.0	25.0	2	0.0

Table C31: The viscosity of the foam bath: 1 Month, 5˚C

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.1	2	0.0
2	0.5	25.0	2	239.9
3	1.0	24.9	2	359.9
4	2.5	24.9	2	337.9
5	5.0	25.0	2	311.9
6	10.0	24.9	2	299.9
7	20.0	25.0	2	293.9
8	50.0	25.0	2	289.1
9	100.0	25.0	2	291.9
10	50.0	24.9	2	299.9
11	20.0	24.9	2	305.9
12	10.0	24.9	2	299.9
13	5.0	24.9	2	311.9
14	2.5	24.9	2	287.9
15	1.0	24.9	2	359.9
16	0.5	24.9	2	239.9
17	0.0	24.9	2	0.0

Table C32: The viscosity of the foam bath: 1 Month, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	359.9
3	1.0	25.0	2	389.9
4	2.5	25.1	2	395.9
5	5.0	25.0	2	377.9
6	10.0	25.0	2	368.9
7	20.0	25.0	2	368.9
8	50.0	25.0	2	359.9
9	100.0	25.0	2	342.5
10	50.0	25.0	2	354.5
11	20.0	24.9	2	362.9
12	10.0	24.9	2	359.9
13	5.0	24.9	2	369.9
14	2.5	24.9	2	383.9
15	1.0	25.0	2	389.9
16	0.5	24.9	2	359.9
17	0.0	24.9	2	0.0

Table C33: The viscosity of the foam bath: 1 Month, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	24.9	2	0.0
2	0.5	24.9	2	479.9
3	1.0	24.9	2	439.9
4	2.5	24.9	2	359.9
5	5.0	24.9	2	445.9
6	10.0	24.9	2	439.9
7	20.0	25.0	2	413.9
8	50.0	25.0	2	395.9
9	100.0	25.0	2	370.7
10	50.0	25.0	2	388.7
11	20.0	25.0	2	401.9
12	10.0	25.0	2	419.9
13	5.0	24.9	2	431.9
14	2.5	24.9	2	383.9
15	1.0	24.9	2	445.7
16	0.5	25.0	2	479.9
17	0.0	24.9	2	0.0

Table C34: The viscosity of the foam bath: 2 Months, 5˚C

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.1	2	0.0
2	0.5	25.0	2	249.9
3	1.0	25.0	2	355.9
4	2.5	25.0	2	334.9
5	5.0	25.1	2	310.9
6	10.0	25.1	2	290.9
7	20.0	25.0	2	287.9
8	50.0	25.0	2	289.1
9	100.0	25.0	2	290.9
10	50.0	25.0	2	301.9
11	20.0	25.0	2	303.9
12	10.0	24.9	2	299.9
13	5.0	24.9	2	311.9
14	2.5	25.0	2	298.9
15	1.0	25.0	2	369.9
16	0.5	25.0	2	228.9
17	0.0	25.0	2	0.0

Table C35: The viscosity of the foam bath: 2 Months, 25˚C+60%RH

No.	Speed (rpm)	Temperature (˚C)	Time (minutes)	Viscosity (cP)
1	0.0	25.1	2	0.0
2	0.5	25.1	2	299.9
3	1.0	25.1	2	329.9
4	2.5	24.9	2	335.9
5	5.0	25.1	2	323.9
6	10.0	25.1	2	320.9
7	20.0	25.1	2	319.4
8	50.0	25.1	2	318.9
9	100.0	25.1	2	299.9
10	50.0	25.1	2	311.9
11	20.0	25.1	2	317.9
12	10.0	25.1	2	323.9
13	5.0	25.1	2	323.9
14	2.5	25.1	2	347.9
15	1.0	25.0	2	389.9
16	0.5	25.0	2	299.9
17	0.0	25.0	2	0.0

Table 36: The viscosity of the foam bath: 2 Months, 40°C+75%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.1	2	0.0
2	0.5	25.1	2	599.9
3	1.0	25.1	2	629.9
4	2.5	25.1	2	623.9
5	5.0	25.0	2	617.9
6	10.0	25.1	2	608.9
7	20.0	25.1	2	607.4
8	50.0	25.1	2	579.4
9	100.0	25.1	2	572.2
10	50.0	25.1	2	575.3
11	20.0	25.0	2	594.5
12	10.0	25.0	2	608.9
13	5.0	25.0	2	611.9
14	2.5	25.0	2	632.9
15	1.0	25.0	2	659.9
16	0.5	25.0	2	719.8
17	0.0	25.1	2	0.0

Table C37: The viscosity of the foam bath: 3 Months, 5°C

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	25.0	2	242.9
3	1.0	25.0	2	354.0
4	2.5	25.0	2	339.5
5	5.0	24.9	2	310.9
6	10.0	24.9	2	297.9
7	20.0	24.9	2	286.9
8	50.0	24.9	2	289.1
9	100.0	25.0	2	294.4
10	50.0	25.0	2	288.9
11	20.0	25.0	2	292.9
12	10.0	25.0	2	299.9
13	5.0	25.0	2	312.9
14	2.5	25.0	2	313.9
15	1.0	25.0	2	359.9
16	0.5	25.0	2	238.9
17	0.0	25.0	2	0.0

Table C38: The viscosity of the foam bath: 3 Months, 25°C+60%RH

No.	Speed (rpm)	Temperature (°C)	Time (minutes)	Viscosity (cP)
1	0.0	25.0	2	0.0
2	0.5	24.9	2	299.9
3	1.0	24.9	2	389.9
4	2.5	24.9	2	371.9
5	5.0	24.9	2	353.9
6	10.0	24.9	2	359.9
7	20.0	24.9	2	355.9
8	50.0	25.0	2	359.4
9	100.0	25.0	2	329.0
10	50.0	24.9	2	339.9
11	20.0	24.9	2	343.4
12	10.0	24.9	2	338.9
13	5.0	24.9	2	353.4
14	2.5	24.9	2	371.9
15	1.0	24.9	2	389.9
16	0.5	24.9	2	419.9
17	0.0	24.9	2	0.0

Table C39: The viscosity of the foam bath: 3 Months, 40°C+75%RH

No.	**Speed (rpm)**	**Temperature (°C)**	**Time (minutes)**	**Viscosity (cP)**
1	0.0	25.0	2	0.0
2	0.5	25.0	2	839.8
3	1.0	25.0	2	809.8
4	2.5	25.0	2	755.8
5	5.0	25.0	2	755.8
6	10.0	25.0	2	749.8
7	20.0	25.0	2	727.3
8	50.0	25.0	2	684.9
9	100.0	25.1	2	699.9
10	50.0	25.1	2	685.5
11	20.0	25.1	2	688.4
12	10.0	25.1	2	698.9
13	5.0	25.1	2	695.8
14	2.5	25.0	2	695.8
15	1.0	25.0	2	719.8
16	0.5	25	2	839.8
17	0.0	25.0	2	0.0

Table C40: Penetration results of cream A and cream B

Cream A		Initial		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	30.3	31.1	31.3	30.9
25°C+60%RH	30.2	31.2	33.2	31.6
40°C+75%RH	30.1	31.5	30.7	30.8
		Month 1		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	31.2	30.4	31.1	30.9
25°C+60%RH	32.0	31.0	32.3	31.8
40°C+75%RH	31.3	31.4	32.9	31.9
		Month 2		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	32.6	32.1	32.9	32.5
25°C+60%RH	32.1	33.9	32.4	32.8
40°C+75%RH	32.7	33.6	33.0	33.1
		Month 3		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	33.9	32.1	32.0	32.7
25°C+60%RH	33.3	33.1	32.9	33.1
40°C+75%RH	33.8	34.2	34.0	34.0
Cream B		Initial		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	33.3	31.7	30.1	31.7
25°C+60%RH	30.2	29.9	30.2	30.1
40°C+75%RH	33.2	30.1	31.3	31.5
		Month 1		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	30.1	30.2	30.4	30.2
25°C+60%RH	32.8	29.6	30.3	30.9
40°C+75%RH	28.6	31.3	31.1	30.3
		Month 2		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	31.1	31.3	32.3	31.6
25°C+60%RH	32.2	33.1	30.1	31.8
40°C+75%RH	32.1	31.2	33.5	32.3
		Month 3		
Temperature	Day 1	Day 2	Day 3	Day Average
5°C	31.2	31.1	30.4	30.9
25°C+60%RH	32.2	32.6	33.5	32.8
40°C+75%RH	33.8	32.3	30.1	32.1

Table C41: Foamability of the soap at 20°C in distilled water

Stability interval	Height of foam (cm3)	Decreased foam after 30min. (cm3)	Foamability
Initial	130	10	120
Month 1: 5°C	135	18	117
Month 2: 5°C	138	16	122
Month 3: 5°C	140	16	124
Month 1:25°C+60%RH	145	18	127
Month 2: 25°C+60%RH	136	18	118
Month 3: 25°C+60%RH	125	16	109
Month 1: 40°C+75%RH	135	26	109
Month 2: 40°C+75%RH	130	16	114
Month 3: 40°C+75%RH	130	18	112

Table C42: Foamability of the soap at 40°C in distilled water

Stability interval	Height of foam (cm3)	Decreased foam after 30 min.(cm3)	Foamability
Initial	146	10	136
Month 1: 5°C	148	15	133
Month 2: 5°C	150	12	138
Month 3: 5°C	150	14	136
Month 1: 25°C+60%RH	142	16	126
Month 2: 25°C+60%RH	146	10	136
Month 3: 25°C+60%RH	140	15	125
Month 1: 40°C+75%RH	140	15	125
Month 2: 40°C+75%RH	138	18	120
Month 3: 40°C+75%RH	140	18	122

Table C43: Foamability of the soap at 20°C in hard water

Stability interval	Height of foam (cm3)	Decreased foam after 30 min.(cm3)	Foamability
Initial	100	20	80
Month 1: 5°C	100	24	76
Month 2: 5°C	90	22	68
Month 3: 5°C	105	26	79
Month 1: 25°C+60%RH	95	30	65
Month 2: 25°C+60%RH	98	32	66
Month 3: 25°C+60%RH	95	28	67
Month 1: 40°C+75%RH	98	34	64
Month 2: 40°C+75%RH	105	30	75
Month 3: 40°C+75%RH	96	34	62

Table C44: Foamability of the soap at 40°C in hard water

Stability interval	Height of foam (cm3)	Decreased foam after 30 min.(cm3)	Foamability
Initial	110	20	90
Month 1: 5°C	115	26	89
Month 2: 5°C	115	24	91
Month 3: 5°C	110	24	86
Month 1: 25°C+60%RH	112	20	92
Month 2: 25°C+60%RH	118	22	96
Month 3: 25°C+60%RH	110	26	84
Month 1: 40°C+75%RH	116	24	92
Month 2: 40°C+75%RH	100	30	70
Month 3: 40°C+75%RH	100	28	72

Table C45: Foamability of the foam bath

Stability interval	Height of foam (cm3)	Decreased foam after 30 min.(cm3)	Foamability
Initial	110	40	70
Month 1: 5°C	120	48	72
Month 2: 5°C	115	46	69
Month 3: 5°C	128	55	73
Month 1: 25°C+60%RH	115	48	67
Month 2: 25°C+60%RH	120	52	68
Month 3: 25°C+60%RH	115	50	65
Month 1: 40°C+75%RH	120	56	64
Month 2: 40°C+75%RH	110	58	52
Month 3: 40°C+75%RH	110	60	50

APPENDIX D

CONFERENCE CONTRIBUTION

VAN RENSBURG, A., LöTTER, A.P., DU PREEZ, J.L. & SWANEPOEL, E. 2004. Formulation of kojic acid and sodium ascorbyl phosphate containing products. Poster to be presented at the 25th Annual Congress of the Academy of Pharmaceutical Sciences, September, 12-15, Grahamstown, South-Africa.

Pages D1-D8 represents the poster.

INTRODUCTION

The **skin**, our main defence against harmful substances such as wind, dirt, bacteria and ultraviolet radiation has also the important functions of preventing water loss, regulating temperature and receiving external stimuli. Skin **colour** varies depending on racial background, sex and the season of the year due to the exposure to sunlight. Skin colour is primarily determined by the amount of melanin produced by the melanocytes. For this reason, research for the development of **whitening products** has focused on reducing melanin production in the melanocytes, rather than bleaching of the skin.

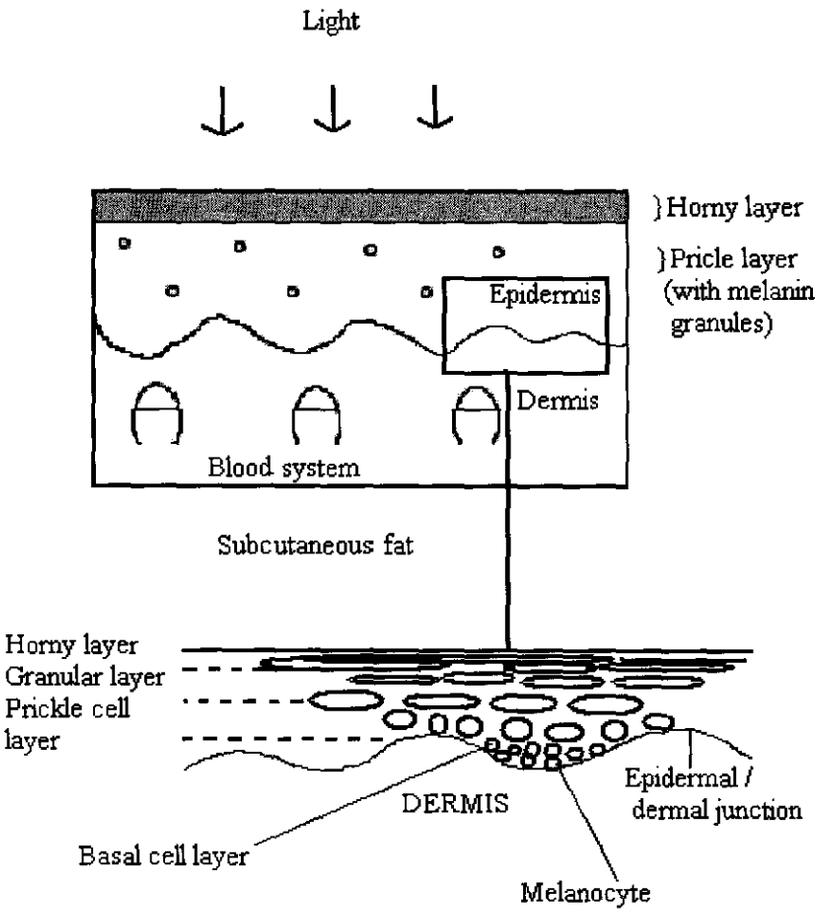
Skin-whitening products have been widely used in the cosmetic field and clinic therapy. They either **lighten** the skin or **depigment** skin (treatment for abnormal hyperpigmentation skin such as freckles and melasma.) Whitening agents, such as hydroquinone, **kojic acid** and **ascorbic acid** derivatives have shown efficacy in treatment of hyperpigmentation.

In this study, sodium ascorbyl phosphate and kojic acid were used as the active ingredients in skin lightening products.

Sodium ascorbyl phosphate acts as an in-vivo antioxidant, promotes collagen formation, and lightens the skin. It is a stable vitamin C derivate that protects the skin, promotes its development and improves its appearance. Kojic acid successfully fights age spots and pigmentation on face and body.

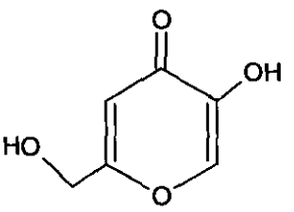
The product development program started with a literature search and preformulation study. Existing basic formulations were used and modified to incorporate both active ingredients in a variety of skin lightening

products. Stability testing followed, based on the requirements of the South African Medicine Control Council for new products.

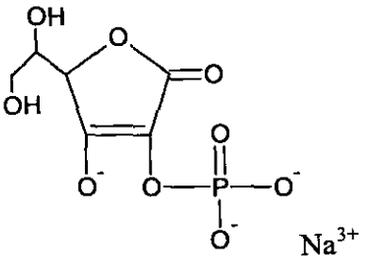


The location of the melanocytes is in the epidermis

CHEMICAL STRUCTURES



Kojic acid



Sodium ascorbyl phosphate

Chemical names:

Kojic acid: 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one

Sodium ascorbyl phosphate: L-ascorbic acid-2-monophosphate

Pharmacological actions:

Kojic acid blocks the catalytic action of the tyrosinase enzyme to prevent the conversion of tyrosine into melanin, by chelating copper ions that are indispensable for tyrosinase.

Sodium ascorbyl phosphate blocks the auto-oxidation of DOPA and dopaquinone during the intermediate process of melanin synthesis.

FORMULAS

O/W WHITENING CREAM:

% m/m	Composition	Activity
2.0	Cremophor® A6	Emulsifier
2.0	Cremophor® A25	Emulsifier
1.0	Dimethylpolysiloxane	Solvent
5.0	Cetylstearyl alcohol	Thickening agent
5.0	1,2-Propylene glycol USP	Solvent
0.2	EDTA®	Complexing agent
0.2	Methyl Hydroxybenzoate	Preservative
0.02	Propyl Hydroxybenzoate	Preservative
71.46	Distilled water	Solvent
5.0	Cetylstearyl 2-ethylhexanoate (Luvitol EHO®)	Emollient
0.3	Carbopol® 934	Thickener
0.12	Sodium Hydroxide	Neutralizer
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
1.0	Sodium metabisulfite	Anti-oxidant

W/O FACE LOTION:

% m/m	Composition	Activity
1.5	Cremophor RH® 40	Emulsifier
3.0	1,2 Propylene glycol USP	Solvent
15.0	Ethanol 96%	Preservative
2.0	Hammelis dist. (Witch hazel Distillate)	Astringent
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
0.1	Sodium metabisulfite	Anti-oxidant
74.4	Distilled water	Solvent

PEARLY FOAM BATH:

% m/m	Composition	Activity
20.0	Texapon N® 40/ Sodium Lauryl Sulphate	Surfactant
2.0	Euperlan® pk 771	Pearlescent
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
54.1	Distilled water	Solvent
4.0	Sodium chloride	Thickener
9.0	Distilled water	Solvent
0.4	Bronidox L®	Preservative
1.5	Sodium metabisulfite	Anti-oxidant
5.0	Distilled water	Solvent

GEL:

% m/m	Composition	Activity
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
0.75	Xantum gum	Gellant
1.0	Glycerine	Solvent for gum
93.93	Distilled water	Solvent
0.02	Propyl hydroxybenzoate	Preservative
0.2	Methyl hydroxybenzoate	Preservative
0.1	Sodium metabisulfite	Anti-oxidant

PEANUT OIL CLEAR SOAP		
% m/m	**Composition**	**Activity**
27.0	Peanut oil	Oil for saponification
10.0	Stearic acid	Stiffening agent
20.0	1.2-Propylene glycol	Solvent
6.0	Glycerine	Humactant
8.5	Sucrose	Clarifier
4.93	Sodium hydroxide	Alkali
9.6	Distilled water	Solvent
3.0	Sodium ascorbyl phosphate	Skin lightener
1.0	Kojic acid	Skin lightener
5.0	Distilled water	Solvent

RESULTS AND DISCUSSION

All formulations were stored over a stability period of three months in ovens of 5°C , 25°C + 60% RH and 40°C + 75% RH.
The tests done on the formulations included the following: membrane release, HPLC concentration analysis, pH valuations, specific gravity, and spreadability of the cream, viscosity, foamability, and visual assessment.

The stability tests revealed that the formulations that were stored below 25°C were stable and all in good condition. HPLC analysis revealed that Kojic acid and sodium ascorbyl phosphate showed a decrease in concentration in all the formulations, especially the batches stored at 40°C + 75% RH over a three month stability period. Kojic acid caused a colour change in the formulations due to oxidation and its sensitivity to heat. If all the products

could be stored below 25°C in small containers, there will be no stability problems.

The cream and the lotion kept its soft skin feel, fast absorbing and moisturising capabilities. The gel became more fluent, but if it could be presented in a tube, it will be ideal for applying to the face as it is quick absorbing and non-sticking. The foam bath and the soap had extraordinary foamability. The soap showed a decrease in foamability when exposed to hard water, but was still accepted to have good cleaning and skin whitening value.

PUBLICATIONS

Development and validation of a stability indicating HPLC assay method for the simultaneous determination of kojic acid and sodium ascorbyl phosphate in cosmetic formulations.

Van Rensburg, A., du Preez, J.L. & Lötter, A.P.

Research Institute for Industrial Pharmacy, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

Abstract

An HPLC method for the simultaneous determination of kojic acid and sodium ascorbyl phosphate in cosmetic formulations was developed and validated. A Lichrospher 100-5 RP-18 ec column and a mobile phase of 0.185% KH_2PO_4, 0.681% $C_{16}H_{36}NI$, and 10% Acetonitrile in Milli-Q water were used. The injection volume was 10 µl, with UV detection at 255 nm at ambient temperature. The retention time for kojic acid was approximately 2.6 minutes and for sodium ascorbyl phosphate approximately 7.3 minutes. The method was linear over the range of 20 – 240 µg/ml for kojic acid, and 60 – 720 µg/ml for sodium ascorbyl phosphate, with an accuracy of 99.2% and 99.0% for kojic acid and sodium ascorbyl phosphate respectively, and precision of 0.59% and 0.70% for kojic acid and sodium ascorbyl phosphate respectively. Forced degradation and peak purity analysis were used to prove that the method is stability indicating.

Keywords: Method validation, HPLC, kojic acid, sodium ascorbyl phosphate

Introduction

Kojic acid (5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) and sodium ascorbyl phosphate ($C_6H_6O_9Na_3P + 2H_2O$) have shown efficacy in treatment of hyperpigmentation through preventing melanin production.

Sottafotorri *et al.* (1998:213) stated that in the near future, cosmetic industries were going to have to comply with the sixth amendment to the 76/768/EEC Council Directive, which introduced the availability of both a fuller dossier and labelling of cosmetic ingredients. Moreover, the cosmetic formulations are complex mixtures of different chemical compounds with different chemical properties. Therefore, new analytical methods, which can detect and quantify such compounds in commercial products, have to be found. The HPLC method for the simultaneous assay of kojic acid and sodium ascorbyl phosphate in cosmetic formulations were not yet been developed up till now.

Experimental

Chemicals and reagents

All the chemicals used were of HPLC or analytical reagent grade. Water was purified in-house by means of an Elix 10 and a Milli-Q purification system (Millipore, Bedford, Ma).

Materials and equipment for HPLC

A Hewlett Packard 1050 series HPLC (HP, Palo Alto, CA) equipped with a HP1050 quanternary gradient pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev.A.06.02 data acquisition and analysis software was used, with a Lichrospher 100-5 RP-18 ec column and a mobile phase of 0.185% KH_2PO_4, 0.681% BAI, and 10% Acetonitrile in Milli-Q water at a flow rate of 1 ml/minute. The injection volume was 10 µl, with UV detection at 255 nm at ambient temperature.

The retention time for kojic acid was approximately 2.6 minutes and for sodium ascorbyl phosphate approximately 7.3 minutes.

Sample preparation

Approximately 2 g of each product was accurately weighed and dissolved. The creams were dissolved with 10 ml methanol and filled to 100 ml with Milli-Q water. The rest of the formulations were diluted to 100 ml with Milli-Q water only. All the products were sonicated for 10 minutes and the creams were shaken for 15 minutes. The samples had to be filtered through 0.45 μm filters, before it was transferred into HPLC vials and injected into the HPLC.

Standard preparation

Approximately 0.20 g kojic acid and 0.60 g sodium ascorbyl phosphate were weighed accurately and dissolved in 70 ml Milli-Q water in a 100 ml volumetric flask. It was then sonicated on an ultrasonic bath for 10 minutes. The flask was left to cool down and then filled to volume with Milli-Q water. No filtering was necessary, and the samples were transferred into a HPLC autosampler vial and injected into the HPLC.

Validation

The method was validated according to the 1995 ICH-Q2a guidelines. Linearity and range were established by preparing a standard containing both kojic acid and sodium ascorbyl phosphate over the range of 180 – 240 μg/ml for kojic acid and 480 - 720 μg/ml for sodium ascorbyl phosphate respectively. Accuracy was determined by weighing the appropriate amount of cream placebo into 100 ml volumetric flasks and then spiking it by adding standard solution to obtain 3 samples each of 80%, 100%, and 120% of the expected sample concentrations. Precision was tested by analysing nine samples of the same homogenous suspension on day one (three at 80%, three at 100% and three at 120% of the expected sample concentration), and analysing three samples again on two more days, once by a different analyst on another instrument. Sample stability was assessed by leaving a prepared sample in the autosampler at

ambient temperature and injecting it hourly over a 24 hour period. Repeatability was determined by injecting the sample solution six times. Specificity was tested by forced degradation. A standard solution containing kojic acid, 1 M sodium hydroxide and 5% hydroxide peroxide, and stored at 40°C overnight to degrade and then analysed. Robustness was tested by making deliberate changes to the operation conditions and noting the effects.

Results and discussion

The validation results are given in Table 1.

Table 1: Validation results

Test	**Result**
Specificity	Complies
Range	Kojic acid: 180 - 240 µg/ml
	Sodium ascorbyl phosphate: 480 - 720 µg/ml
Linearity	Kojic acid: R square = 0.998
	Sodium ascorbyl phosphate: R square = 0.998
Accuracy	Kojic acid: 99.2%
	Sodium ascorbyl phosphate: 99.0%
Precision	Kojic acid: RSD < 1%
	Sodium ascorbyl phosphate: RSD < 1%
Ruggedness	Complies
Robustness	Complies

Samples analysed after forced degradation had extra peaks, due to breakdown of the samples, but none of the degradation products interfered with the kojic acid and sodium ascorbyl phosphate peaks. Diode array peak purity analysis showed that the peaks of kojic acid and sodium ascorbyl phosphate remained pure. No interference was found from the sample that was prepared from a cream placebo (Figure 2). Small changes in the flow rate, detection wavelength, mobile phase composition and injection volume did not affect the separation. A Luna C18(2), 150 x 4.6 mm, 5 µm column, was found equally suitable to perform the analysis, proving that the method is robust.

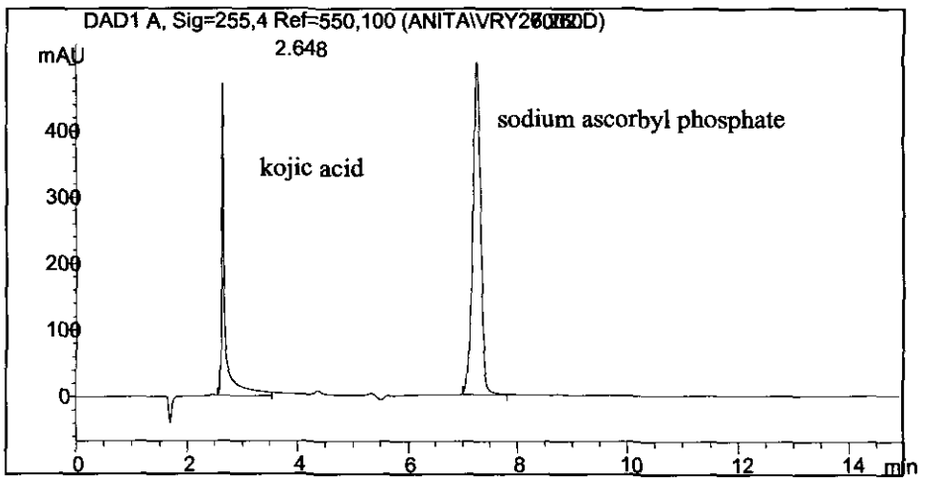


Figure 1: Chromatogram of a standard solution.

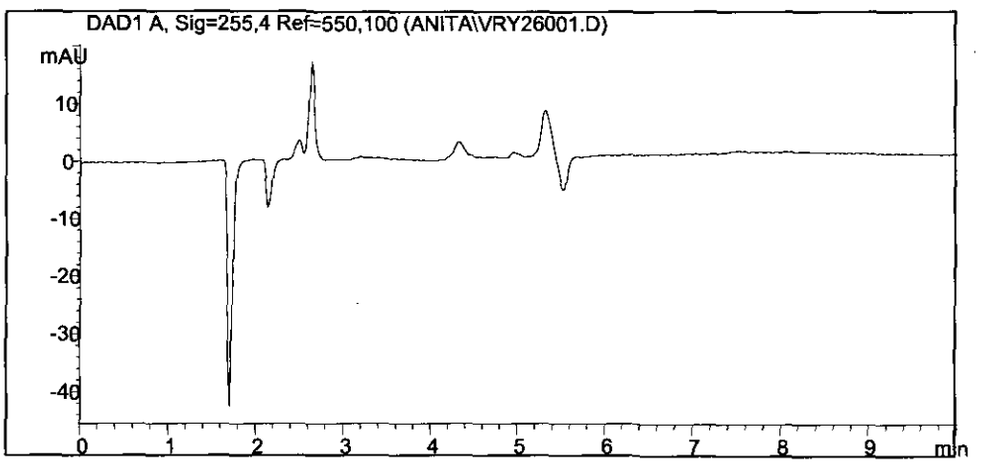


Figure 2: Chromatogram of cream placebo.

Conclusion

The method was used over a period of twelve weeks to analyse stability samples placed at accelerated environmental conditions, and it proved to be reliable and easy to execute.

References

SOTTAFOTORRI, E., ANZALDI, M., BALBI, A. & TONELLO, G. 1998. Simultaneous HPLC determination of multiple components in a commercial cosmetic cream. *Journal of pharmaceutical and biomedical analysis,* 18:213-217, Feb.

UNITED STATES. Food and Drug Administration. 1995. Guideline for industry. Text on validation of analytical procedures. (ICH-Q2A) [Web:] http://www.fda.gov/cder/guidance/ich.q2a.pdf [Date of access: 23 June 2004].