



COMPARATIVE STUDY OF VITAMIN C CONTENT IN MARKETED COSMECEUTICALS

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Abstract

Vitamin C is known as L- ascorbic acid and is Water-Soluble Vitamin since it was observed as the factor needed for the treatment of scurvy. Vitamin C is very popular among the general population mainly due to its antioxidant properties. The human body was suggested to contain about 1.5g of vitamin C which corresponds to around 20mg/kg. In pregnant woman, an additional 5-10mg is recommended.

Sources of Vitamin C are Natural source and Synthetic source. Skin disorders include, Skin sagging caused by natural aging and oxidative stress. Wrinkle formation caused by UV exposure, smoking and medical treatments. Sunburn and hyper pigmentation caused by UV exposure.

Vitamin C , it helps to improve collagen production and immune function. It also acts as antioxidant that protects cells from damage caused by free radicals. It improves elasticity of skin. Types of cosmeceuticals are Lotions, Sunscreen, Moisturizer, Toner, and Serum. Vitamin C concentration is determined using 2,6 dichlorophenol indophenol (DCPIP) by using Iodometric titration method.

Introduction

Vitamins are essential nutrients that are required for various biochemical and physiological processes in the body. It is well known that most of the vitamins cannot be synthesized in the body and hence their supplementation in diet is essential.

Vitamins are classified on the basis of their solubility as water soluble (C and B complexes) and fat-soluble vitamins (A, D, E, K). Vitamin C or ascorbic acid (AA) was first isolated in 1923 by Hungarian biochemistry and Noble laureate Szent -Gyorgyi and synthesized by Howarth and Hirst.

Vitamin C is known as L -ascorbic acid since it was observed as the factor needed for the treatment of scurvy (in Latin scorbutus, hence, “a- scorbutus”). Since the terms “fat -soluble vitamin A” and “water-soluble” were already in use, the term vitamin C was coined.

In general, the term also includes its oxidized form, L-dehydroascorbic acid, which can easily be converted into L -ascorbic acid in the human body. In contrast to most of the vertebrates, guinea pigs, bats, passeriform birds, and primates including humans, lack L -gulono-1, 4 - lactone oxidase, and hence cannot synthesize vitamin C.

They are, therefore, fully dependent on vitamin C intake from the diet. Vitamin C is very popular among the general population mainly due to its antioxidant properties. This is reflected by the body content of vitamin C that is unusually high compared to other vitamins. The human body was suggested to contain about 1.5g of vitamin C, which corresponds to around 20mg/kg. The daily need in humans is hardly or at all assessable since it depends on many factors, including physiological status, stress, and diseases. Recommendations differ substantially from 40 to 120mg/day in various countries. This dose represents the assessment of the normal need for vitamin C in humans. In pregnant women, an additional 5-10mg is recommended (25mg in lactating mothers). The total dose of about 10mg is sufficient to prevent scurvy in the general population. (1)



Figure 1: Vitamin C Rich Foods

REVIEW OF LITERATURE

Farris Pk et al., According to Farris PK's work on topical vitamin C, cosmetic dermatology procedures involve using it topically to promote collagen synthesis, protect against UV damage, and lighten hyperpigmentation. This includes combining topical vitamin C with resurfacing procedures like microdermabrasion, laser resurfacing, or chemical peels to enhance penetration. (2)

Ahmida M.H et al., According to the method developed by Ahmida M.H., the determination of ascorbic acid {Vitamin C} is performed using a sensitive and rapid Reversed -Phase High -Performance Liquid Chromatography with UV detection {RP -HPLC-UV} method. This method is primarily used for the quantification of ascorbic acid in pharmaceutical supplements. (3) Granger M et al., Research associated with Granger M in food and nutrition includes work on

dietary vitamin C in human health, discussing its importance and bioavailability. Other studies involving M. Granger include reviews on human milk macronutrients and their impact on infant growth. (4)

Pathy K et al., Have explored the role of vitamin C in preventing diseases, its use in food preservation, and its biochemical properties. His work highlights are vitamin C's function as an antioxidant and its importance in collagen synthesis, as well as its effects on other bodily functions. (5)

OBJECTIVES OF THE STUDY

- 1) To determine the quantitative amount of vitamin C [ascorbic acid] present in a sample.
- 2) To develop simple, sensitive, and selective analytical methods for vitamin C analysis.
- 3) To apply the developed methods to real -world samples and determine vitamin C content.
- 4) To validate the developed methods according to international standards.
- 5) To compare the performance of different analytical methods [titration, iodimetry].

BIOAVAILABILITY OF VITAMIN C

Bioavailability or the effective concentration of vitamin C essentially depends on its effective absorption from intestine and renal excretion. Vitamin C consumed either with diet or dietary supplements, is absorbed by the epithelial cells of the small intestine by SVCT1 or, subsequently diffuses into the surrounding capillaries and then the Circulatory system. Circulating AA is filtered from kidney capillary bed into the Bowman's capsule through a general filtration mechanism. AA is reabsorbed through SVCT1 transporter in proximal convoluted tubule. The difference between the amount of AA filtered and reabsorbed constitutes renal excretion.

Together, intestinal absorption and renal excretion control the serum level of vitamin C and thus its bioavailability. At low concentrations, most vitamin C is absorbed in the small intestine and reabsorbed from the renal tubule. However, at high concentrations, SVCT1 is down regulated the amount of AA absorbed from the intestine and kidney. This imposes a physiological restriction on the maximal effective serum vitamin C concentration (or its bioavailability) that is attainable by oral consumption. This value has been determined to be 200 mmol/l, although "normal" physiological serum concentrations of ascorbate in healthy humans range from 60 to 100 mmol/l.

Vitamin C levels in circulating blood cells, such as platelets, are much higher than the plasma, as these cells express the SVCT2 transporter, which mediates intracellular ascorbate accumulation. Reduced bioavailability of vitamin C is Often seen in stress, alcohol intake, smoking, fever, viral illnesses, usage of antibiotics, pain killers, exposure to petroleum products or carbon monoxide, heavy metals toxicity and so on.

However, the precise mechanism behind low vitamin C level in the body is not well defined. Presumably, an increased utilization of this vitamin in these conditions and/or a decreased absorption from the gut could be a cause of decreased vitamin C level. In the event of high consumption, AA and its metabolites such as dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid are excreted via kidney in humans.

AA is generally non -toxic but at high doses (2 -6 g/day) it can cause gastrointestinal disturbances or diarrhea. The side effects are generally not serious and can be easily reversed by reducing the intake of AA. Furthermore, there is more consistent and compelling data serious health effects of vitamin C in humans. (6)

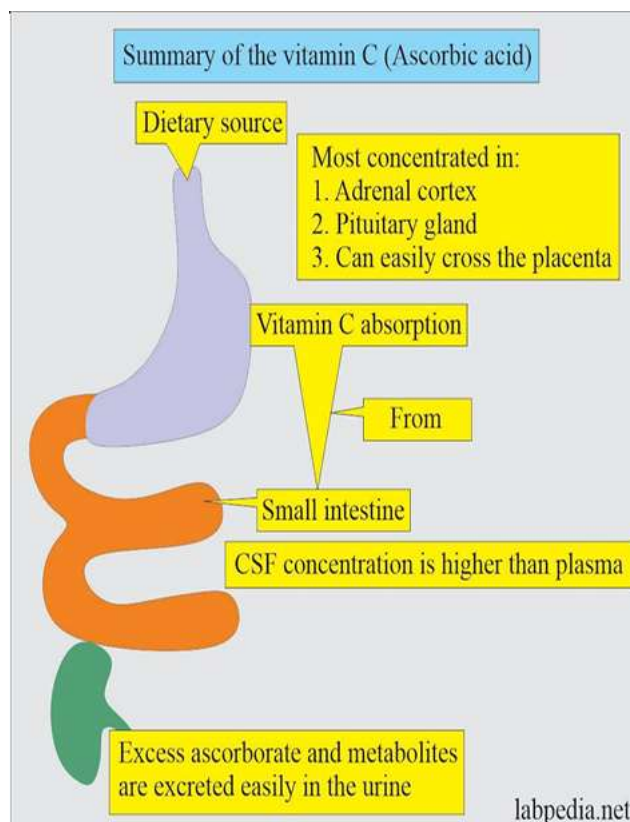


Figure 2: Bioavailability of Vitamin C

BIOCHEMICAL FUNCTIONS OF VITAMIN C

The biochemical functions of AA are largely dependent on the oxido-reduction properties of L-AA which is a co-factor for hydroxylation and activity of mono-oxygenase enzymes in the synthesis of collagen, carnitine and neurotransmitters.

AA accelerates hydroxylation reactions by maintaining the active center of metal ions in a reduced state for optimal activity of enzymes hydroxylase and oxygenase. Thus, it is crucial in the maintenance of collagen which represents about one-third of the total body protein. (7) In an experimental study AA has been shown to have involvement in synthesis and release of type IV collagen into the culture medium. Further, it has also been reported that AA 2-phosphate, a long-acting vitamin C derivative, stimulates both cell growth and the expression of mRNA for type III collagen in human osteoblast-like MG-63 cells and in normal human osteoblasts, but not the expression of type I collagen in these cells.

However, in another study Kishimoto have observed that AA induced the expression of type 1 and type 4 collagen and SVCT2 in cultured human skin fibroblasts. Collagen constitutes the principal protein of skin, bones, teeth, cartilage, tendons, blood vessels, heart valves, inter vertebral discs, cornea, eye lens. (8)

AA is essential to maintain the enzyme prolyl and lysyl hydroxylase in an active form. AA deficiency results in reduced hydroxylation of proline and lysine, thus affecting collagen synthesis. AA is also an essential co-factor for hydroxylation involved in the synthesis of muscle carnitine “ β -hydroxybutyric acid”.

Carnitine is required for the transport and transfer of long chain fatty acids into mitochondria for energy production. Further, AA is also a co-factor for the enzyme dopamine- β -hydroxylase, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine and hence essential for the synthesis of catecholamines.

In addition, AA catalyzes other enzymatic reactions involving amidation necessary for maximal activity of hormones oxytocin, vasopressin, cholecystokinin and alpha-melanotropin. It is also involved in the transformation of cholesterol to bile acids as it modulates the microsomal 7 α -hydroxylation, the rate limiting reaction of cholesterol catabolism in liver. (8)

Deficiency of AA affects this conversion and as a result cholesterol accumulates in the liver leading to hypercholesterolemia, cholesterol gall stones formation etc. (9)

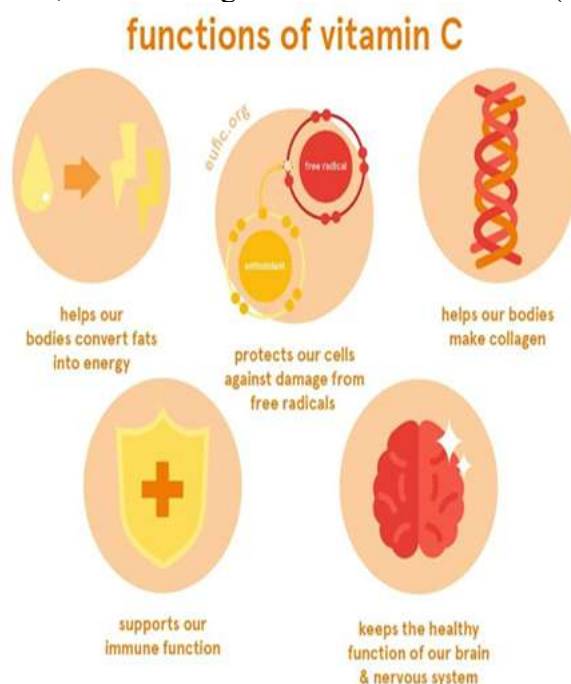


Figure 3: Functions of Vitamin C

VITAMIN C DEFICIENCY

One of the most compelling arguments for a vital role for vitamin C in skin health is the association between vitamin C deficiency and the loss of a number of important skin functions.

In particular, poor wound healing (associated with collagen function), thickening of the stratum corneum and subcutaneous bleeding (due to fragility and loss of connective tissue morphology) are extreme and rapid in onset in vitamin C deficient individuals. It is thought that similar processes occur when body stores are below optimal, although to a lesser extent. (9)

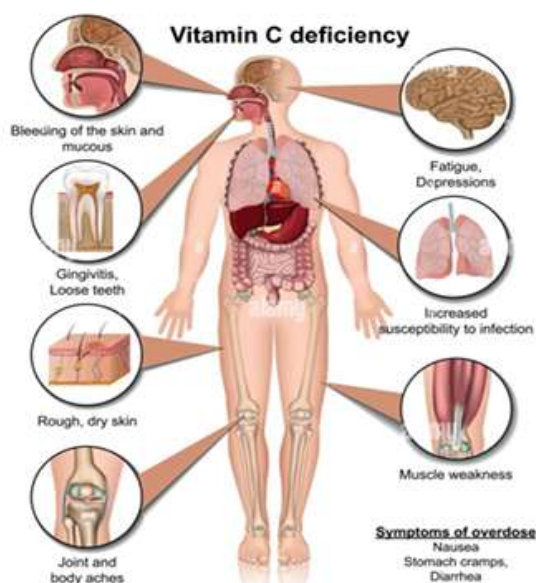


Figure 4: Vitamin C deficiency



Figure 5: Symptoms of Vitamin C deficiency

INTRODUCTION TO SKIN

Skin an Introduction

The skin is the largest organ in the body, covering its entire external surface. The skin has 3 layers—the epidermis, dermis, and hypodermis, which have different anatomical structures and functions. The skin's structure comprises an intricate network that serves as the body's initial barrier against pathogens, ultraviolet (UV) light, chemicals, and mechanical injury. This organ also regulates temperature and the amount of water released into the environment. Skin thickness varies by body region and is influenced by the thickness of the epidermal and dermal layers. Hairless skin in the palms of the hands and soles of the feet is the thickest due to the presence of the stratum lucidum, an extra layer in the epid ermis. Regions lacking this extra layer are

considered thin skin. Of these regions, the back has the thickest skin because it has a thick epidermis. The skin's barrier function makes it susceptible to various inflammatory and infectious conditions. In addition, wound healing, sensory changes, and cosmesis are significant surgical concerns. Understanding the skin's anatomy and function is crucial for managing conditions across all medical fields. (10)

Epidermis

The epidermis, the skin's outermost layer, is composed of several strata and various cell types crucial for its function.

Layers of the epidermis:

From the deepest to the most superficial, the epidermal layers are the stratum Basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The stratum Basale, also known as stratum germinativum, is separated from the dermis by the basement membrane (basal lamina) and attached to it by hemidesmosomes. The cells in this layer are cuboidal to columnar, mitotically active stem cells that constantly produce keratinocytes. This layer also contains melanocytes. The stratum spinosum, comprising 8 to 10 cell layers, is also called the prickly cell layer. This layer contains irregular, polyhedral cells with cytoplasmic processes, sometimes called spines, that extend outward and contact neighboring cells by desmosomes. Dendritic cells can be found in this layer.

The stratum granulosum has 3 to 5 cell layers and contains diamond-shaped cells with keratohyalin and lamellar granules. Keratohyalin granules contain keratin precursors that aggregate, cross-link, and form bundles. The lamellar granules contain the glycolipids secreted to the cell surfaces, functioning as an adhesive to maintain cellular cohesion. The stratum lucidum comprises 2 to 3 cell layers and is present in thicker skin on the palms and soles. This thin and clear layer consists of eleidin, a transformation product of keratohyalin. The stratum corneum has 20 to 30 cell layers and occupies the uppermost epidermal layer. The stratum corneum is composed of keratin and dead keratinocytes (anucleate squamous cells) that form horny scales. This layer has the most variable thickness, especially in callused skin. Dead keratinocytes release defensins within this layer, which are part of our first line of immune defense mechanisms. (10)

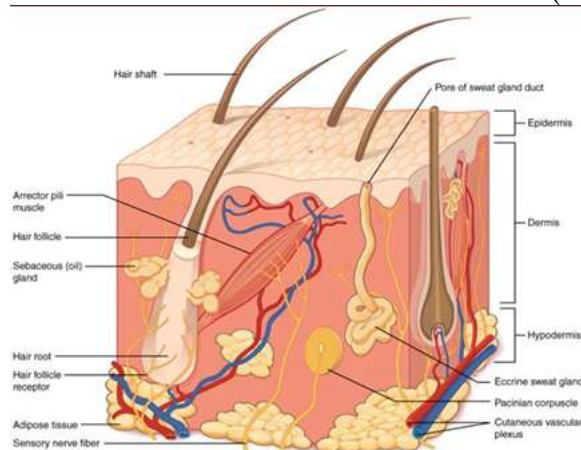


Figure 6: Structure of Skin

Cells of the epidermis

The epidermal cells include keratinocytes, melanocytes, and Langerhans and Merkel cells. Keratinocytes are the predominant cells of the epidermis, originating from the basal layer. These cells produce keratin and lipids essential for forming the epidermal water barrier. Keratinocytes also contribute to calcium regulation by enabling UVB light absorption in the skin, which is critical for vitamin D activation. Melanocytes derive from neural crest cells and primarily synthesize melanin, the main skin pigment component. These cells are found between stratum Basale cells. UVB light stimulates melanin secretion, protecting against further UV radiation exposure and acting as a built-in sunscreen. Melanin forms during the conversion of

tyrosine to dihydroxyphenylalanine by the enzyme tyrosinase. Melanin then travels from cell to cell, relying on the long processes connecting the melanocytes to the neighboring epidermal cells. Melanin granules from melanocytes transit through the lengthy processes to the cytoplasm of basal keratinocytes. This transfer occurs through cytochrome secretion, where keratinocytes phagocytose the tips of melanocyte processes. (10)

Langerhans cells are dendritic cells that act as the skin's first -line cellular immune defenders and are crucial for antigen presentation. Special stains allow visualization of these cells in the stratum spinosum. Langerhans cells are of mesenchymal origin, derived from CD34 -positive bone marrow stem cells, and are part of the mononuclear phagocytic system. These cells contain Birbeck granules and tennis racket-shaped cytoplasmic organelles. Langerhans cells express major histocompatibility complex (MHC) I and MHC II molecules, uptake antigens in the skin, and transport them to the lymph nodes. Merkel cells are oval-shaped modified epidermal cells found in the stratum Basale, directly above the basement membrane. These cells serve as mechanoreceptors for light touch and are found in the palms, soles, and oral and genital mucosa, with the highest concentration in the fingertips. Merkel cells bind to adjoining keratinocytes through desmosomes and contain intermediate keratin filaments. The cell membranes of Merkel cells interact with free nerve endings in the skin.

Dermis

The dermis is connected to the epidermis by the basement membrane. The dermis consists of 2 connective tissue layers, papillary and reticular, which merge without clear demarcation. The papillary layer is the upper dermal layer, which is thinner and composed of loose connective tissue that contacts the epidermis. The reticular layer is the deeper layer, which is thicker and less cellular. This layer consists of dense connective tissue composed of collagen fiber bundles. The dermis houses the sweat glands, hair, hair follicles, muscles, sensory neurons, and blood vessels.

Hypodermis

The hypodermis, also known as the subcutaneous fascia, is located beneath the dermis. This layer is the deepest skin layer and contains adipose lobules, sensory neurons, blood vessels, and scanty skin appendages, such as hair follicles. (10)

FUNCTIONS OF SKIN

The skin's comprehensive roles highlight its complexity and importance in maintaining overall health and well-being. These roles are discussed below.

Barrier function:

The skin has multiple protective roles, acting as a barrier against various external threats. The skin shields the body from excessive water loss or absorption, invasion by microorganisms, mechanical and chemical trauma, and UV light damage. The cell envelope establishes the epidermal water barrier, a layer of insoluble proteins on the inner surface of the plasma membrane. This barrier is formed through the cross-linking of small proline -rich proteins. Larger proteins such as cystatin, Desmoplakin, and filaggrin contribute to the barrier's robust mechanics. The lipid envelope is a hydrophobic layer attached to the outer surface of the plasma membrane. Keratinocytes in the stratum spinosum produce keratohyalin granules and bodies containing a mixture of glycosphingolipids, phospholipids, and ceramides assembled within Golgi bodies. The contents of lamellar bodies are then secreted through exocytosis into the extracellular spaces between the stratum granulosum and corneum. (11)

Immunological defense:

The skin plays a crucial role in both adaptive and innate immunity. In adaptive immunity, antigen-presenting cells initiate T-cell responses, leading to increased levels of helper T cells, such

as TH1, TH2, or TH17. In innate immunity, the skin produces various peptides with antibacterial and antifungal properties. The skin-associated lymphoid tissue is a significant component of the immune system, aiding in preventing infections, as even minor skin breaks can lead to infection. Langerhans cells are part of the adaptive immune system, presenting foreign antigens encountered in the skin to T cells. (12)

Regulation of homeostasis:

The skin plays a vital role in maintaining body temperature and water balance. This organ regulates heat exchange with the environment, particularly through the blood vessels and sweat glands. The skin manages the rate and amount of water evaporation and absorption.

Endocrine and exocrine functions:

Keratinocytes produce vitamin D by converting 7-dehydrocholesterol under UV light exposure. These cells also express the vitamin D receptor and contain enzymes that activate vitamin D, essential for the proliferation and differentiation of keratinocytes. The skin's exocrine functions include temperature control by perspiration and skin protection by sebum production. Sweat and sebaceous glands are crucial to these functions.

Sensory

functions:

The skin is equipped with nociceptors that allow for the sensation of touch, heat, cold, and pain, facilitating interaction with the environment. The skin's sensory roles are essential for an individual's movement, protection, and interaction with the environment.

Diagnostic indicator:

Skin characteristics such as pigmentation, smoothness, elasticity, and turgor provide insights into an individual's overall health status. Skin assessment is often a crucial part of a person's physical examination.

Cell division, desquamation, and shedding in the skin:

Cell division occurs in the stratum Basale. Basal cells (young keratinocytes) begin the synthesis of keratinous tonofilaments, which are grouped into bundles called Tono fibrils. Older keratinocytes are then pushed into the stratum spinosum after mitosis. Skin cells begin to produce keratohyalin granules with intermediate-associated proteins, filaggrin, and trichohyalin in the upper part of the spinous layer. This process helps aggregate keratin filaments and convert granular cells into cornified cells, known as keratinization. Cells also produce lamellar bodies during this stage. Keratinocytes continue to move into the stratum granulosum afterward, where they become flattened and diamond-shaped. The cells accumulate keratohyalin granules mixed between Tono fibrils. Keratinocytes then continue to the stratum corneum, flattening and losing organelles and nuclei. The keratohyalin granules turn Tono fibrils into a homogenous keratin matrix. Cornified cells reach the surface and are desquamated when desmosomes disintegrate. The proteinase activity of kallikrein-related serine peptidase is triggered by lowered pH near the surface. The processes of skin shedding and desquamation vary slightly by body region. Hairless skin comprises more layers, with the addition of the stratum lucidum. Thus, keratinocytes in body regions with hairless skin go through more layers before reaching the surface. (13)



Figure 7: Vital Functions of Skin

CLINICAL BENEFITS OF VITAMIN C ON SKIN

The skin is a multi - functional organ, the largest in the body, and its appearance generally reflects the health and efficacy of its underlying structures. It has many functions, but its fundamental role is to provide a protective interface between the external environment and an individual's tissues, providing shielding from mechanical and chemical threats, pathogens, ultraviolet radiation and even dehydration. Being in constant contact with the external environment, the skin is subject to more insults than most organs, and where the first visible signs of aging occur. The skin is composed of two main layers with quite different underlying structures- the outer most epidermis and the deeper dermis.

The epidermis fulfils most of the barrier functions of the skin and is predominantly made up of cells, mostly Keratinocytes. The keratinocytes are arranged in layers throughout the epidermis; as these cells divide and proliferate away from the basal layer, which is closest to the dermis, they begin to differentiate.

This process is called Keratinization, and involves the production of specialized structural proteins, secretion of lipids, and the formation of a cellular envelope of cross -linked proteins. During differentiation, virtually all of the sub cellular organelles disappear, including the nucleus.

The cytoplasm is also removed, although there is evidence that some enzymes remain. In contrast, the dermal skin layer provides strength and elasticity, and includes the vascular, lymphatic and neuronal systems. It is relatively acellular and is primarily made up of complex extracellular matrix proteins, being particularly rich in collagen fibers, which make up to 75% of the dermis dry weight. (5)

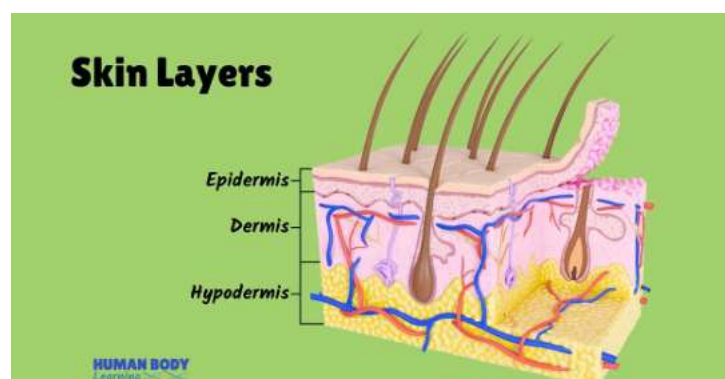


Figure 8: Layers of Skin

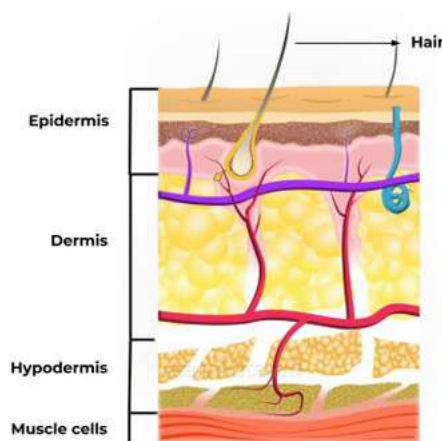


Figure 9: Structure of Skin

Type of skin damage	Causes	Skin structure affected	Evidence of protection by Vitamin C
Sunburn	Acute and excessive UV exposure	Cell death of all skin cells, with associated inflammation	Improving skin vitamin C and Vitamin E levels can improve resistance to UV exposure
Photo aging, oxidant-induced damage	Chronic UV over exposure, cigarette smoking	Damaged collagen and elastin matrix, thinning of the epidermal layer	Decreased signs of aging with higher fruit and vegetable intake
Hyper pigmentation	Chronic UV exposure and environmental stresses	Excessive pigment formation and propagation of melanocytes in the epidermis	Nutrition studies showing improved skin color with higher fruit and vegetable intake
Wrinkle formation	Natural aging, oxidative stress, UV exposure, smoking, medical treatments	Dermal layer changes, deterioration of collagen and elastic fibers	Lessening of wrinkle depth following vitamin C supplementation
Skin sagging	Natural aging, oxidative stress damage, extreme weight loss	Loss of elastin and collagen fibers, thinning of skin layers, loss of muscle tone	Improved skin tightness in individuals with higher fruit and vegetable intake
Surface roughness	Chemical and UV exposure, physical abrasion, allergy and inflammation	Stratum corneum, loss of skin moisture barrier function	Vitamin C enhances production of barrier lipids in cell culture
Dry skin	Medications, illness, extreme temperature, low humidity and wind exposure	Stratum corneum, loss of skin barrier lipids and natural moisturizing factor	Vitamin C enhances production of barrier lipids in cell culture
Excessive scar formation, generation of keloids	Infective wound healing	Fibroblast function, collagen and elastin formation	Supplementation improves wound healing, enhances collagen formation by fibroblasts in vitro

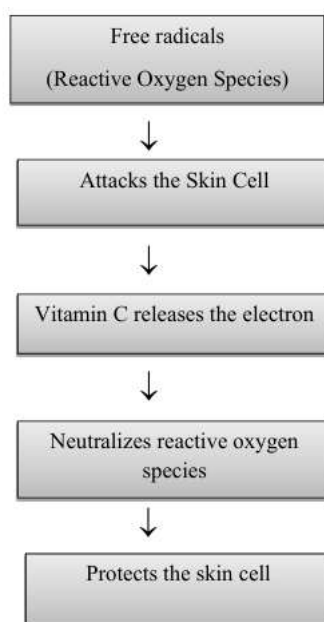
Table 1: Types of Skin Damage



MECHANISM OF ACTION

1) As potent Antioxidant:

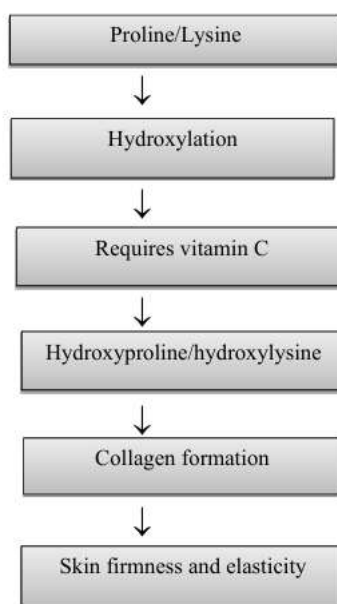
Environmental factors, such as solar radiation, pollution, and smoking can accelerate damage to the skin through the generation of so-called “oxidative stress”. Vitamin C is one of the most potent antioxidants in the skin. it neutralizes the oxidative stress by a process of electron transfer and/or donation. (9)



2) Antiaging effect:

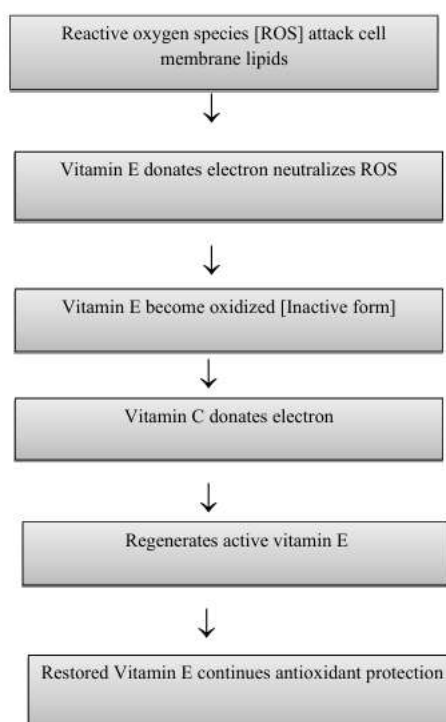
Vitamin C is essential for collagen biosynthesis and is thought to have an anti-aging effect. More importantly, it serves as a cofactor for prolyl and lysyl hydroxylase, key enzymes that cross-link and stabilize collagen fibers. Vitamin C also directly activates the transcription factors involved in collagen synthesis and stabilizes procollagen messenger Rna (mRna) that regulates type i and iii collagen synthesis. In addition,

vitamin C increases the gene expression of collagen and synthesis of the tissue inhibitor of mmP-1, which decreases collagen degradation. (9)



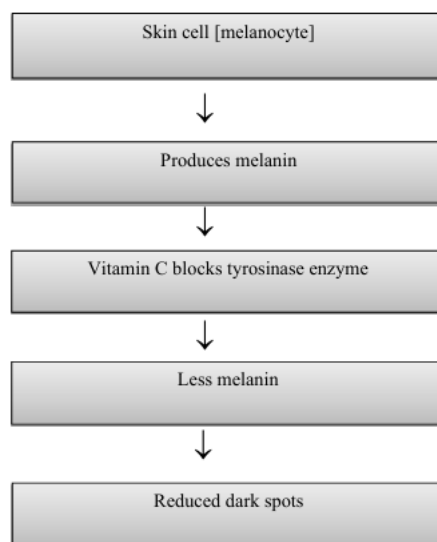
3) Replenisher of Vitamin E:

Aside from its antiaging and photo protective effects, vitamin C is also known to be the primary Replenisher of Vitamin E. Vitamin E is a lipophilic antioxidant and has a primary role in protecting cell membranes against oxidative stress and maintaining the collagen network in the skin. Similar to Vitamin C, its levels are rapidly depleted after UV exposure. Several clinical studies have proved the synergistic antioxidant effect of Vitamins C and E in photo protection. (9)



4) Anti pigmentary effect:

Vitamin C also plays a role as an anti-pigmentation agent. It interacts with copper ions at tyrosinase active sites and inhibits the action of the enzyme tyrosinase —the main enzyme responsible for the conversion of tyrosine into melanin —thereby decreasing melanin formation. A clinical study examining the effect of a topical formulation containing 25-percent Vitamin C and a chemical penetration enhancer reported a significant decrease in pigmentation caused by melasma after 16 weeks. Although Vitamin C has been shown to suppress melanin production, its clinical effects may not be as effective as other topical products containing hydroquinone. (9)



VITAMIN C CONTENT IN SKIN

Normal skin contains high concentrations of vitamin C, with levels comparable to other body tissues and well above plasma concentrations, suggesting active accumulation from the circulation. Most of the vitamin C in the skin appears to be in intracellular compartments, with concentrations likely to be in the millimolar range. It is transported into cells from the blood vessels present in the dermal layer. Skin vitamin C levels have not often been reported and there is considerable variation in the published levels, with a 10-fold range across a number of independent studies. Levels are similar to that found in numerous other body organs. The variation in reported levels most likely reflects the difficulty in handling skin tissue, which is very resilient to degradation and solubilisation, but may also be due to the location of the skin sample and the age of the donor. (9)

Tissue	Vitamin C content (mg/100g net weight)
Adrenal glands	30-40
Pituitary glands	40-50
Liver	10-16
Spleen	10-15
Lungs	7
Kidneys	5-15
Heart muscle	5-15
Skeletal muscle	3-4
Brain	13-15
Skin epidermis	6-64

Table 2: Vitamin C content of skin and a comparison to with oher tissues

TOPICAL APPLICATIONS OF VITAMIN C

When plasma levels are low, some vitamin C can be delivered to the epidermal layer by topical application, although the efficacy of this is dependent on the formulation of the cream or serum used on the skin.

Vitamin C, as a water-soluble and charged molecule, is repelled by the physical barrier of the terminally differentiated epidermal cells.

It is only when pH levels are below 4 and vitamin C is present as ascorbic acid then some penetration occurs, but whether this result in increased levels in the metabolically compromised stratum corneum is unknown.

A great deal of effort has been put into the development of ascorbic acid derivatives for the purpose of topical application.

Such derivatives need to ensure stabilization of the molecule from oxidation and also overcome the significant challenge of skin penetration. (6)

METHODS USED FOR DETERMINATION OF VITAMIN C

Spectrophotometric Method:

UV Spectrophotometry is mostly used to determine ascorbic acid as it can absorb UV rays and is a simple method. This method can be used for vitamin C tablets, fresh or packaged fruit juices, solid fruits and vegetables (Desai and Desai, 2019). To determine the content of total vitamin C, a well - established method is the 2, 4-dinitrophenyl hydrazine (2, 4-DNPH) method. There is a coupling reaction between 2,4 DNPH and ascorbic acid. This ascorbic acid content in different fruits and vegetables can be deter mined by using this method. In this method the total amount of vitamin C includes both Ascorbic acid and Dehydroascorbic acid. In this method, bromine water in the presence of acetic acid oxidizes the ascorbic acid into dehydroascorbic. Then a known amount of 2, 4 DNPH is added which gives a coupling reaction. Solutions are kept for 3 hours. After 3 hours 85% H₂SO₄ is added which gives a colored solution. The absorbance of these solutions is measured using UV spectrophotometer and the ascorbic acid content is then determined.

Iodine Titration Method:

Vitamin C (C₆H₈O₆) can also be determined by iodometric titration. There are several oxidizing agents such as potassium iodate, potassium permanganate and potassium dichromate which have higher reduction potential values than iodine. Higher the reduction potential value, stronger is the oxidizing agent. Thus, potassium iodate,

potassium permanganate and potassium dichromate are stronger oxidizing agents than iodine (I₂).
 Oxidizing agent + KI -----> I₂ + reduced form of oxidizing agent
 In this titration, potassium iodate is added to a solution of ascorbic acid which contains a strong acid like sulphuric acid and potassium iodide (KI). The potassium iodate on reacting with KI, liberates I₂. The iodine produced reacts with ascorbic acid leading to the formation of dehydroascorbic acid (C₆H₆O₆) and iodide ions (I⁻).

$$\text{KIO}_3 + 5\text{KI} + 6\text{H}^+ \longrightarrow 3\text{I}_2 + 6\text{K}^+ + 3\text{H}_2\text{O}$$

$$\text{C}_6\text{H}_6\text{O}_6 + \text{I}_2 \longrightarrow \text{C}_6\text{H}_6\text{O}_6 + 2\text{I}^- + 2\text{H}^+$$

The potassium iodide is always added in excess to ensure completion of reaction and to dissolve the iodine. After complete utilization of ascorbic acid, the excess I₂ remaining in the solution can be titrated with sodium thiosulphate solution using starch as an indicator. Starch forms an intense blue colored complex with I₂ and at the end point of the titration the blue color disappears.

$$\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \longrightarrow \text{Na}_2\text{S}_4\text{O}_6$$

 By the amount of sodium thiosulphate used in the titration the remaining I₂ after reaction with ascorbic acid can be calculated. Thus, iodometry is an indirect titration for estimating the concentration of ascorbic acid.

High Performance Liquid Chromatography (HPLC) Method:

The High-performance liquid chromatography (HPLC) technique deserves an increasing interest mainly due to its rapidity, selectivity, specificity properties and simple sample preparation procedure. The sample preparation and sample extraction are important steps to minimize degradation of L-AA. Metaphosphoric acid (MPA) is the most common reagent used as it can function both as an extractant and stabilizer. It inhibits ascorbate oxidation, metal catalysis, and precipitates proteins. MPA can be used with other organic acids and modifiers like acetic acid, methanol etc. and stabilizers like ethylenediamine acetic acid (EDTA) and monosodium glutamate (MSG). L-AA has higher stability in MPA than oxalic acid and trichloroacetic acid. The analysis of liquid samples is done by directly injecting the sample in HPLC after dilution with initial mobile phase. The solid samples are first homogenized with the extractant, followed by centrifugation or filtration and then injected in HPLC after dilution with mobile phase. The reversed phase - HPLC is the most common approach used because of the non-volatile and hydrophilic nature of vitamin C. Other methods like ion-exclusion, ion-exchange, ion-pair and hydrophilic liquid chromatography have been used. Ultra-High performance liquid chromatography (UHPLC) has also been used in analysis of vitamin C due to shorter time of analysis and lower solvent consumption when compared with other approaches. L-AA shows a strong absorption in the UV region (245-270 nm) and this makes UV absorbance the most popular detection technique. Other detection techniques like mass spectrometry, fluorescence and electrochemical detection can also be used. The HPLC method is a procedure used extensively in research and in the food industry due to its high accuracy and the ability to measure both AA and the oxidized form of vitamin C, dehydroascorbic acid (DHA).

Enzymatic method:

The enzyme ascorbate oxidase catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. The dehydroascorbic acid formed as a result is reacted with o-phenylenediamine to produce a 2,2-anhydroquinoxaline. The absorption of 2,2-anhydroquinoxaline with respect to a specially prepared blank is observed at 358 nm using a UV spectrophotometer. The increase in the absorbance of the quinoxaline is an indicator of the amount of ascorbic acid present and that has reacted with oxygen in the presence of ascorbate oxidase.

DCPIP method:

The DCPIP method for vitamin C estimation is based on a redox titration Principle - it uses 2,6-dichlorophenolindophenol [DCPIP], a colored dye, which gets reduced by ascorbic acid to a colorless form.

PRINCIPLE:

Ascorbic acid [vitamin C] is a strong reducing agent. DCPIP in its oxidized form is blue in alkaline medium and pink in acidic medium. In the assay, the medium is kept acidic [usually with metaphosphoric acid or acetic acid] so DCPIP appears pink.

When ascorbic acid is present, it reduces the pink DCPIP to its colorless leuco form. The endpoint is the first persistent pink color after all vitamin C has been oxidized -indicating excess DCPIP is present.

Ascorbic acid +DCPIP (pink) it gives dehydroascorbic acid +DCPIP (colorless).

MATERIALS AND METHODS

Materials:

Chemicals and Reagents

Indicator solution was prepared by dissolving 100mg of reagent in 50ml of distilled water along with 125mg sodium carbonate (Na_2CO_3), and the volume was made up to 100ml with distilled water.

- 1% Oxalic acid solution (freshly prepared).
- Distilled water
- Samples
- Liquid sample
- Cream sample
- Glassware and apparatus
- Burette
- Pipettes (1ml)
- Conical flasks (100ml)
- Volumetric flask
- Measuring cylinders

Method:

Preparation of Sample Solutions

- For the liquid sample, 1ml of sample was pipetted out and the volume was made up to 5ml with distilled water.
- For the cream sample, 0.5g of the sample was accurately weighed and dissolved in 15ml distilled water.

Titration Procedure

- The prepared sample solution was transferred into a 100ml conical flask.
- To this, 1ml of 1% oxalic acid solution was added.
- Subsequently, 5ml of acetone was added to the mixture.
- A burette was filled with the indicator solution.
- The sample solution in the conical flask was titrated against the indicator solution.
- The titration was continued until the endpoint was reached, which was observed as a distinct color change from blue to pink in acidic medium.

Observation:

Endpoint: Blue Pink (acidic medium)

**Formula:**

Vitamin C (mg) = Volume of DCPIP used x Concentration of DCPIP x Equivalent factor
 Sample weight
 Equivalent factor = 0.176mg

RESULTS:

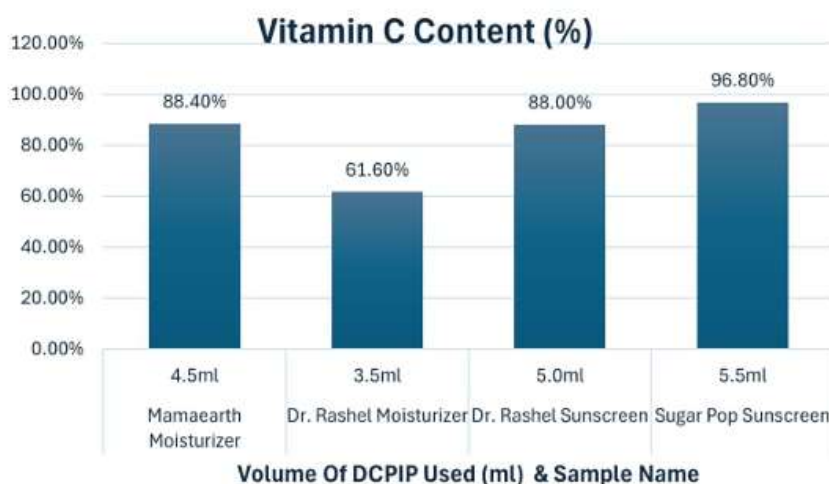
The vitamin C content of different cosmetic and skincare formulations (moisturizers, sunscreens, serums, and toners) was estimated by titration with DCPIP reagent.

The Mama earth moisturizer exhibited the highest vitamin C content (88.4%), followed closely by DR. R sunscreen (88%).

In contrast, the Dr. Rashel moisturizer showed comparatively lower vitamin C content (61.6%), suggesting a weaker formulation or degradation of the vitamin during storage. Sugar Pop sunscreen had the highest vitamin C content overall (96.8%), indicating strong antioxidant fortification.

Sl. No	Sample Name	Volume of DCPIP used(ml)	Vitamin C content (%)
1	Mama earth Moisturizer	4.5ml	88.4%
2	Dr. Rashel Moisturizer	3.5ml	61.6%
3	Dr. Rashel Sunscreen	5.0ml	88.0%
4	Sugar Pop Sunscreen	5.5ml	96.8%

Table 3: Vitamic C content in moisturizers and sunscreen

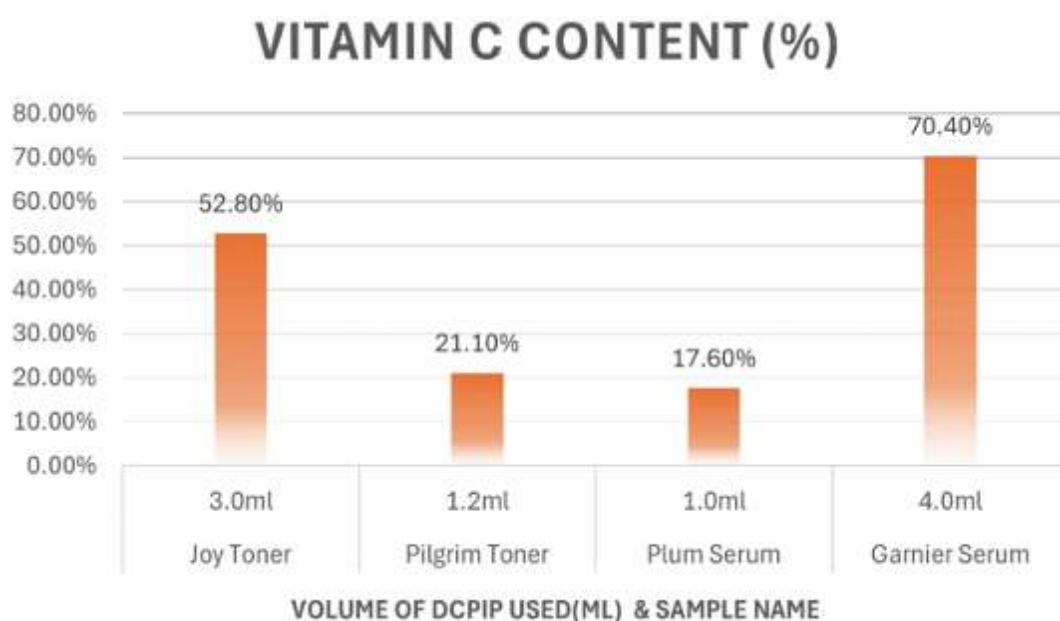


Among the toners, Joy toner showed moderate vitamin C content (52.8%), whereas Pilgrim toner showed very low content (21.1%).

Serums, which are usually marketed as concentrated antioxidant formulations, showed highly variable results. Garnier serum contained a significant amount (70.4%), while Plum Serum had the lowest vitamin C content (17.6%).

Sl. No	Sample Name	Volume of DCPIP used(ml)	Vitamin C content (%)
1	Joy Toner	3.0ml	52.8%
2	Pilgrim Toner	1.2ml	21.1%
3	Plum Serum	1.0ml	17.6%
4	Garnier Serum	4.0ml	70.4%

Table 4: Vitamin C content in Serums and Toners



Sl. No.	Sample	Volume of DCPIP used	Vit C (mg)	Vit C (%)
1	Joy Toner	5 ml	0.528	52.8%
2	Pilgrim Toner	1.2 ml	0.21	21.1%
3	Plum Serum	1ml	0.176	17.65
4	Garnier Serum	0.1ml	0.01	0.4%
5	Mama earth moisturizer	0.799ml	0.799	88.1%
6	Dr. Rashel moisturizer	0.616ml	0.616	61.6%
7	Dr. Rashel Sunscreen	0.88ml	0.888	96.8%
8	Sugar pop sunscreen	0.968ml	0.968	96.8%

Table 5: Determination of Vitamin C content in Topical Products

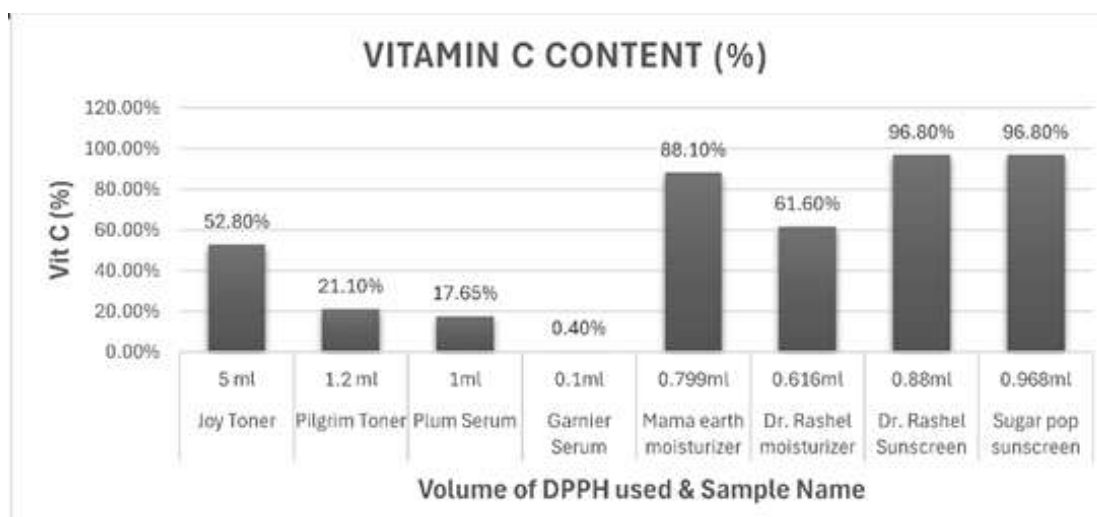
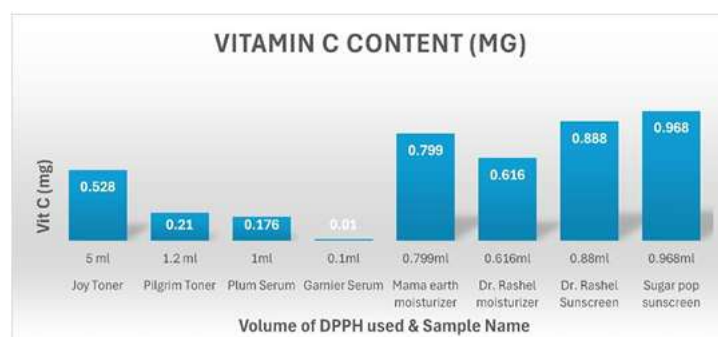


Table 6: Physical Evaluation

Formulation (Brand)	Appearance/Color	Odor	pH ($\pm$ SD)	Consistency/ Homogeneity
Mama earth Moisturizer	White, smooth cream	Pleasant	5.8 ± 0.1	Uniform, no grittiness
Dr. Rashel Moisturizer	Off- white cream	Mild fragrance	5.6 ± 0.2	Smooth, uniform
Dr. Rashel Sunscreen	White lotion	Mild	5.9 ± 0.1	Homogenous
Sugar Pop Sunscreen	White, slightly viscous	Pleasant	6.1 ± 0.1	Uniform
Joy Toner	Transparent liquid	Floral	5.4 ± 0.1	Clear, no precipitation
Pilgrim Toner	Clear solution	Mild	5.2 ± 0.2	Clear, uniform
Plum Serum	Transparent viscous liquid	Mild citrus	5.7 ± 0.1	Clear, uniform
Garnier Serum	Transparent gel- like	Citrus	5.5 ± 0.1	Uniform, clear

Formulation (Brand)	Spread ability (sec)*	Washability	Viscosity(cPs)**	Stability (7 days)
Mama earth Moisturizer	12	Easily washable	28,500	Stable
Dr. Rashel Moisturizer	15	Easily washable	26,200	Stable
Dr. Rashel Sunscreen	10	Easily washable	18,400	Stable
Sugar Pop Sunscreen	11	Washable	19,200	Stable
Joy Toner	-	Washable	650	Stable
Pilgrim Toner	-	Washable	700	Slight haziness
Plum Serum	-	Washable	9,800	Stable
Garnier Serum	-	Washable	10,200	Stable

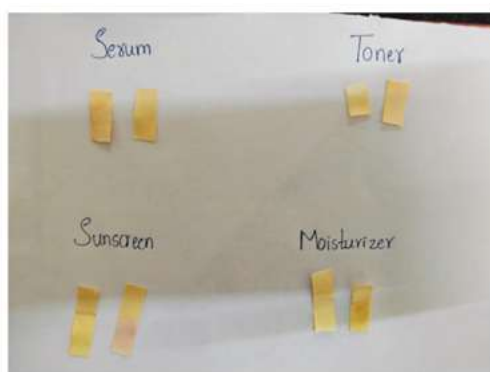


Figure 10: Ph Determination

DISCUSSION

The present study aimed to determine the vitamin C content in various cosmetic formulation such as moisturizers, sunscreens, serums and toners. The results obtained highlight the variability in vitamin C concentration among different products, which can be attributed to differences in formulation type, source of vitamin C, and storage stability. Vitamin C is a water -soluble antioxidant widely used in cosmetics for its skin brightening, anti -aging, and photoprotective properties. However, it is highly unstable and prone to oxidation when exposed to light, air, or high temperatures, leading to a gradual loss of activity.

The determination method employed - typically titrimetric analysis using DCPIP

method- proved reliable for quantifying ascorbic acid content. The colorimetric endpoint observed was distinct, allowing for accurate calculation of concentration. However, in products containing other antioxidants or colored ingredients, slight interferences may have occurred, leading to marginal deviations in results. Therefore, sample preparation and filtration are critical to ensure accuracy

CONCLUSION

From the analysis, it may be concluded that not all marketed vitamin C containing products deliver equal concentrations of the active ingredient. Sunscreens and certain moisturizers appear to retain higher vitamin C content, whereas some serums and toners show degradation or low incorporation. This highlights the importance of formulation and stability studies to ensure efficacy.

SUMMARY

The present study aimed to determine the Vitamin C content in selected cosmetic formulations using the 2,6 -dichlorophenol indophenol (DCPIP) redox titration method. Vitamin C (ascorbic acid) is an essential water -soluble vitamin recognized for its antioxidant, anti -aging, and skin -brightening effects. It plays a crucial role in collagen synthesis, protecting the skin from oxidative stress, and reducing hyperpigmentation, which makes it a key ingredient in cosmeceutical products. Various marketed formulations —including moisturizers, sunscreens, serums, and toners — were tested for their Vitamin C concentration. The results revealed significant variation across products. Sunscreens (e.g., Sugar Pop sunscreen 96.8%) and certain moistur izers (e.g., Mama Earth 88.4%) retained high levels of Vitamin C, suggesting effective formulation strategies and stability. In contrast, some serums and toners, despite being advertised as concentrated antioxidant products, contained relatively lower amou nts (e.g., Plum serum 17.6%, Pilgrim toner 21.1%). These differences highlight that Vitamin C stability largely depends on product type, formulation components, packaging, and storage conditions. The findings underscore the importance of assessing marketed products to confirm label claims and ensure their effectiveness.

In conclusion, the study demonstrated that the DCPIP method is a simple and reliable tool for comparing Vitamin C content in cosmetic formulations. It provides meaningful insights into the necessity of routine quality evaluation of cosmeceuticals to guaran tee consumer benefits and therapeutic effectiveness storage of vitamin C based skin care products.

FUTURE SCOPE

1. Advanced Analytical Validation – Employing highly sensitive and selective techniques such as High -Performance Liquid Chromatography (HPLC) and Ultra -High-Performance Liquid Chromatography (UHPLC) for confirmatory quantification of Vitamin C and its derivatives.
2. Stability Studies – Conducting systematic stability assessments of Vitamin C in cosmetic formulations under various storage conditions (temperature, light, humidity) to address its susceptibility to oxidation
3. Comparative Studies – Expanding the investigation to compare herbal/natural formulations with synthetic counterparts to evaluate variations in stability, bioavailability, and overall efficacy.
4. Nano formulation Approaches – Exploring novel delivery systems such as Vitamin C-loaded nano emulsions, liposomes, and nanoparticles for improved skin penetration, controlled release, and prolonged activity.
5. In -vitro and In -vivo Evaluations – Performing biological studies to assess antioxidant potential, collagen biosynthesis, and dermal penetration, thereby linking analytical quantification with therapeutic effectiveness.

6. Regulatory and Consumer Awareness – Establishing standardized quality parameters for Vitamin C-containing cosmeceuticals to ensure product authenticity and to promote consumer confidence in marketed claims.

REFERENCES

1. Dosedel M, Jirkovsk y E, Macakova K, Krcmova LK, Javorska L, Pourova J, Mercolini, On Behalf of The Oeonom. Vitamin C-Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. *Nutrients*. 2021 Feb 13;13(2):615.
2. Farris Pk. Cosmetical vitamins: vitamin C. In: Draelos ZD, Dover JS, Alam M, eds. *Cosmeceuticals. Procedures in Cosmetic Dermatology*. 2 nd ed. New York: Saunders Elsevier; 2009:51-56
3. Ahmida, M.H. 2009. Determination of ascorbic acid in vitamin C (Tablets) by high performance liquid chromatography. *Asian J Chem*.21:6463-6467.
4. Granger, M.; Eck, P. Dietary vitamin C in human health. *Adv. Food Nutr. Res*. 2018, 83, 281-310
5. Pathy, K.2018. Process for preparation of vitamin C and method for determination of vitamin C in tablets. *SF J Chem Res*. 2(1):2
6. Bartlett MK, Jones CM, Ryan AE. Vitamin C and wound healing: II. Ascorbic acid content and tensile strength of healing wounds in human beings. *N Engl J med* 1942; 226:474-81.
7. <https://www.slideshare.net/slideshow/estimation-of-vitamin-c-by-iodine-titration-pptx/258478514>(date:07.03.2025 time: 15.00) <https://search.app/cF7e1Z3kmz5E7AoDA> (date:07.03.2025 time: 15.00) <https://www.slideshare.net/slideshare/ipqc-fpqc-tests-for-creams/250677265> (date:07.03.2025 time: 15.00)
8. Tortara GJ, Derrickson B. *Principles of anatomy and physiology*, 14th ed. Danvers, MA: Wiley:2014
9. Manela-Azulay M, Bagatin E. Cosmeceuticals vitamins. *Clin Dermatol*. 2009; 27:469-474
10. Colvin RM, Pinnell SR. Topical vitamin C in aging. *Clin Dermatol* 1996; 14:227-234.
11. Silva, C. R., Simoni, J. A., Collins, C. H. and Volpe, P. L. 1999. Ascorbic acid as a standard for iodometric titrations. An analytical experiment for general chemistry. *Journal of chemical education*. 76(10): 1421-1422.
12. Swati Raman, Anjali sehrawat, Rupa Upadhyay, Swati singh, 2023: Different methods used for determination of vitamin C:58-60
13. Manela-Azulay M, Bagatin E. Cosmeceuticals vitamins. *Clin Dermatol*. 2009; 27:469- 474