"DESIGN AND EVALUATION OF FACE MOISTURIZER WITH SPECIFIC NEEDS OF SENIOR CITIZEN"

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COSMETIC TECHNOLOGY

BY

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NIRMA UNIVERSITY
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CERTIFICATE

This is to certify that the dissertation work entitled "Design and Evaluation of Face Moisturizer with Specific Needs of Senior Citizen" submitted by Mr. Chris V. Simon with Roll. No. (14MCT101) in partial fulfillment for the award of Master of Science in "Cosmetic Technology" is a bonafide research work carried out by the candidate at the Mikasa Cosmetics Limited and Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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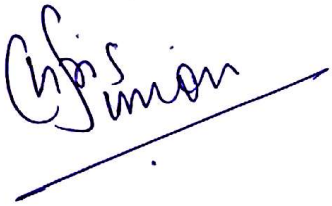
DECLARATION

I hereby declare that the dissertation entitled "Design And Evaluation Of Face Moisturizer With Specific Needs Of Senior Citizen", is based on the original work carried out by me under the guidance of Dr. Sanjeev Acharya, Associate professor, Nirma University and Mr. Vijay Zala, Assistant Manager, Mikasa Cosmetics Pvt. Ltd. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.



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I hope that I can build upon this experience and knowledge that I have gained and make a valuable contribution towards this industry in the coming future.

Chris V Simon

Abbreviations

Short name	Abbreviation
°C	Degree centigrade
Conc.	Concentration
mg	Milligram
RH	Relative humidity
IS	Indian Standard
USP	United States pharmacopeia
BP	British pharmacopeia
SC	Stratum cornieum
GAG	Glycosaminoglycans
AHA	Alphahydroxy acids
BHA	Betahydroxy acids
HA	Hyaluronic acid
HLB	Hydrophile-lipophile balance
RHLB	Required hydrophile-lipophile balance
VIT.	Vitamin
LOD	Loss on drying
UV	Ultra-violet
SCDA	Soyabean casein dextrose agar
SDA	Sougarose-dextrose agar
TBC	Total bacterial count
TYMC	Total yeast and mould count

Table of Contents

List of Figures and Tables

Abstract:

Moisturizers are among the most commonly used cosmetic product in everyday life. The characteristics of an ideal moisturizer are: S.C hydration and decrease TEWL thus increases in smoothness and softness of skin. This aids in the replenishment of lipid barrier and increases the skin's natural moisture mechanism. The natural ingredients are added to product to enhance the moisturizing activity of the cosmetic products as they are considered to be safe and popular too. The purpose of this present investigation is to design and evaluate an emulsified, low pH cosmetic composition having improved thermal stability and improved pH stability, in particular, the emulsified, low pH cosmetic composition having a pH of about 3.7 to about 4.5 and evaluate its moisturizing effects containing active Aquaxyl (Xylitylglucoside, Xylitol, Anhydroxylitol) by in-vitro skin testing methods. AQUAXYL is a derivative of two plant sugars, xylitol and glucose. Its mode of action, reinforces the antidehydration shield (Loricrin, essential lipids) together with the activation of water reserves (hyaluronic acid booster) and their movement (Aquaporins, Tight Junctions). It improves water reserves and limits water loss. The trial batches are evaluated for phyisco-chemical properties such as specific gravity, Loss on drying (LOD), Viscosity, Thermal stability, Microbial testing, Sensory analysis, In-Vitro Corneometry skin testing study. The study was conducted during the spring months (January to May). A total of five healthy male volunteers aged 55 to 65 years contributed to accomplish this study. The active cream was applied to the face (cheeks) for a duration of 1 week. The instrumental measurements were done using a corneo-meter in environmentally controlled room, with controlled temperature (18.0 to 20.6°C) and relative humidity (55 to 65%). The results recommended that the assessed outcome was very well accepted, having improved pH stablility and improved storage stability, along with improved skin hydration and skin barrier function by the product.

Keywords: Moisturizer, Aged Skin, Senior Citizen, Low pH, Aquaxyl (Xylitylglucoside, Xylitol, Anhydroxylitol), Emulsions, Corneometer

1. INTRODUCTION

1.1 INTRODUCTION TO SKIN

Skin is the largest organ of the body, with a total of 15% body's weight, reflects the persons wellbeing; both physical and emotional. Consisting of 3 layers: epidermis (outer), dermis (middle) and subcutaneous tissue. These 3 layers in combination provide various functions, chiefly among them:

- **Protection**: functions as a protective barrier, to prevent harm to internal tissues from ultraviolet (UV) light, temperature, bacteria, trauma, and toxins.
- **Barrier to infection**: acts as a physical barrier of skin; along with the presence of sebum, which has antibacterial characteristic with an acidic pH.
- **Pain receptor**: nerve endings within the skin respond to painful stimuli. They act as a protective mechanism by which an individual will move when they feel pain or discomfort, which aids in preventing pressure damage.
- **Maintains body temperature**: helps in warming the body, the blood vessels constricts and hence retains heat while dilating to further help in cooling.
- **Production of vitamin D in response to sunlight:** Vit. D help in the development of bone.
- **Production of melanin**: which is responsible for color in skin and photo-protection.
- **Communication, through touch and physical appearance**: by which an individual's state of physical wellbeing is known.

1.1.1Anatomical structure of human skin:

In an adult human, skin layers are defined into 3 main types — Hypodermis, dermis and epidermis.

The various functions of human skin can only be possible due to the unique anatomical structure of its layers. These are as follows:

- Epidermis consisting of:
  - Stratum corneum (outermost layer)
  - Viable epidermis
- Dermis

- Subcutis or subcutaneous fatty tissue

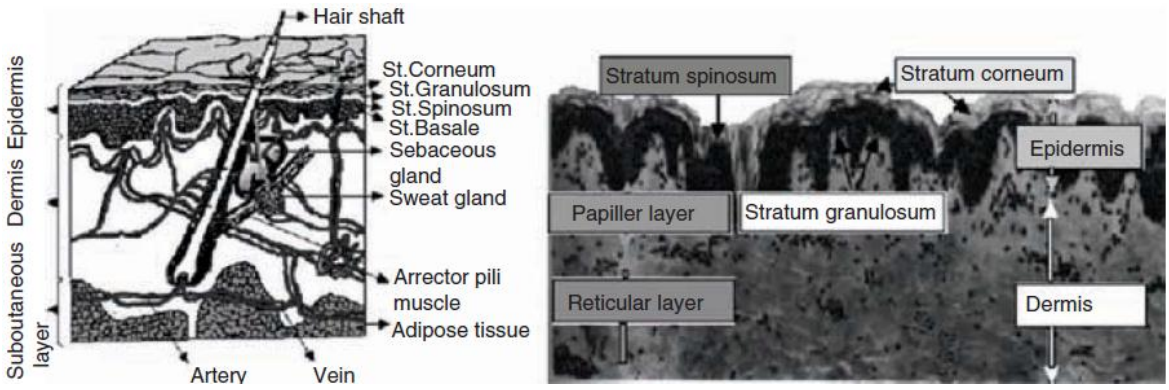

Figure 1- Structure and layers of skin [(1)]

Epidermis:

Epidermis consists of S.C (horny layer) which is in contact with the environment, as it is the outermost layer, followed by stratum granulosum (granular layer), and then stratum spinosum (prickle cell layer) and stratum basale (basal layer) [(2)]. The S.C has thickness of 10-15 cell layers, having keratinocytes (corneocytes) that are anucleated and oriented in form of bricks in the surrounding lipid and which also acts as the first barrier to any active from transdermal delivery route. The following layer, the stratum granulosum (with thickness of 1 – 3 cell) carries enzymes that can break down important cell organelles like the nuclei. By producing degrading cell organelles and keratin, keratin containing cells of this layer gradually differentiate into the corneocytes of S.C. Membrane coating granules containing precursors for intercellular lipid lamellae of the S.C are synthesized by keratinocytes. The next layer, stratum spinosum, contains two to six layers of keratinocytes that are columnar in structure that change into polygonal shapes. In this layer the tonofilaments are formed by aggregates of keratin which condenses to produce desmosomes which are cell membrane connecting structures. The stratum spinosum together with the lower stratum basale layer is known as the Malphigian layer. The stratum basale is the only layer capable of division and it also has all the typical cell organells. The keratinocytes in this layer are connected with the basement membrane (or dermo-epidermal membrane) by proteinaceous structures called hemidesmosomes and with cells of stratum spinosum layer by desmosomes. In addition to the keratinocytes, other specialized cells present in the basal layer are melanocytes, Langerhans cells and Merkel cells. Melanocytes secrete

melanosomes containing melanin (eumelanin or phaeomelanin) that protects the skin from U.V radiations and free radicals. Langerhans cells are derived from bone-marrow and as part of the immune system function as antigen presenting cells (APC) of the skin. The usual thickness of the SC is 10-25 μm, except in the palms and soles of feet, which when hydrated swells several-fold.

Dermis:

Depending on the site, the dermis thickness ranges from 3 to 5 mm. It consists of numerous connective tissues especially collagen fibrils and elastic tissues that gives support and flexibility to the dermis. Along with a network of lymphatic vessels, blood vessels, appendages and nerve endings. The three primary appendages; sebaceous glands, hair follicles and eccrine glands in the skin originate in the dermis. Hair follicles cover the entire surface of the body except palm of hands, feet and lips. Sebum secretion from sebaceous glands associated with the follicles is composed of triglycerides, free fatty acids and waxes. It plays an important role in maintenance of skin surface pH around 5 and in lubrication of skin. The eccrine glands associating with dermis secrete sweat, which is a dilute salt solution with a pH of 5, due to emotional or physical stress. Apocrine glands are present in the dermo-epidermal layer in selective regions. All appendages allow permeants to enter the lower layers of the skin without interacting the intact barrier of S.C.

Hypodermis:

The hypodermis which is also known as subcutaneous fat layer connects the layer of dermis with the underlying organs, providing insulation to the body and protection from any kind of mechanical shock.

1.2 Introduction to Moisturizers:

Research in moisturization was spearheaded by Blank in the 1950s when he demonstrated that the prime factor in dry skin is due to low moisture content in the skin.[3] These symptoms can be overcome by improving the state of hydration in S.C with humectants or occlusives and also by emollients which help in smoothing the rough surface of skin.

“Moisturization” encompasses a wide range of biophysical changes in uppermost layer of the skin, the S.C. Kligman defines the moisturizer as a topically applied substance or product that

overcomes the sign and symptoms of dry skin [4]. Moisturizers contain emollients, together with the humectants (that increase the water content and attract water to the epidermis), and the occlusives (decreasing the evaporation of water from the skin surface) are considered as the three key structural components of moisturizers. The main characteristics of an ideal moisturizer are hydration of stratum corneum and decreases transepidermal water loss (TEWL), smoothens and softens skin (acts as an emollient), helps for the restoration of the lipid barrier and enhances the skin's natural moisture retention mechanism.

Moisturizers prevent and treat dry skin, protect sensitive skin, improve skin tone and texture, and mask imperfections. Moisturizer should be cosmetically elegant and acceptable, rapid absorption providing immediate hydration and assuring long lasting effect, dermatologically hypoallergic, non-sensitizing and non-comedogenic [5]. The most valuable approach for moisturization of skin is to restore the Natural Moisturizing factor (NMF) on our skin surface. The main component of the mixture of molecules that forms a matrix on skin is lactic acid, urea, various salts and amino acids derived from degradation of the protein fillagrin in the lower region of the stratum corneum. The major constituent apart from amino acids are sodium lactate, urea, pyrollidone carboxylic acid (PCA)[6]. Naturally occurring skin lipids and sterols, as well as artificial or natural oils, humectants, emollients, lubricants, etc., may be part of the composition of commercial skin moisturizers

Moisturizers give effects primarily by:

- Reducing evaporation or increasing the integrity of skin barrier
- Increasing skin's ability to hold onto water by increasing levels of natural moisturizing factor (NMF)
- Increasing the ability of epidermis to absorb important components for circulation through aquaporin channels

1.2.1 Mechanism of action:

1.) **Occlusives:** Work by forming a thin film on surface of skin to prevent loss of moisture
2.) **Humectants:** These attract water vapour from the air to moisturize the skin
3.) **Emollients:** Improve the appearance of the skin by smoothing desquamating skin cells
4.) **Restoration of Deficient Materials:** Complex Ingredients that try to restore NMFs in skin

Occlusives cover the SC by coating it and retarding trans-epidermal water loss. Occlusives are oily substances with the ability to dissolve fats and due to its fairly effective properties are widely used.

They are one of the best tools to treat conditions such as dry skin as it decreases TEWL and also gives an emollient effect. Among the best occlusives currently found in the market are mineral oil and petrolatum. Other occlusive ingredients like propylene glycol, lanoline, dimethicone, squalene, soybean oil, grapeseed oil, lanolin, paraffin, and beeswax are commonly found. "Natural" oils like sunflower oil have been steadily gaining in popularity. Effectiveness of these agents is only optimum when they stay present upon the skin as the TEWL will return to the previous level when it is removed.

Humectants can keep the skin moist and reduce the appearance of fine lines. They are soluble in water having high water absorption properties. They attract water from their surroundings like the atmosphere (only when atmospheric humidity exceeds more than 80%) and also from the underlying epidermis. They help to hydrate the skin, and while in low-humidity conditions they may draw water from the lower layers of epidermis and dermis which will result in increasing the dry skin state. Therefore, they generally work better when combined other agents such as occlusives. Some of humectants are discussed here.

1.) **Glycerin**: It is a very effective humectant having strong hygroscopic ability which is quite similar to that of NMF.[7] This property also helps the SC to maintain high water concentration during a dry environment.

2.) **Urea**: Urea is a part of the NMF in skin. Its use can be traced back to hand creams made in the 1940s. Apart from its humectant properties, it shows a mild anti pruritic activity.[8] Several studies have demonstrated the combinatory effects of hydrocortisone, retinoic acid, and other agents with urea due to its improved penetration in skin.

3.) **Alpha Hydroxy acids (AHAs):** They are a family of naturally-found organic acids that acts as humectants; they also show exfoliating properties. Glycolic and lactic acids, obtained, respectively from sugar cane and sour milk, are widely used AHAs in moisturizing products. They are used wrinkles reduction creams or to fight the signs of aging, and to improve the overall look and feel of the skin. Alpha hydroxy acids in fruits like grapes (tartaric acid), lemons and other citrus fruits (citric acid), apples (malic acid),

almonds and apricots (mandelic acid), are the actives that may contribute to skin rejuvenation.

4.) **BHAs — Beta Hydroxy acids** are generally found in herbs and flowering plants. Salicylic acid is the most common, believed to dissolve dead skin cells to leave a smooth, even surface. While alpha hydroxy acids are water soluble, beta hydroxy acids are lipophilic — oil soluble. This means that beta hydroxy acid is more probable to penetrate into the pore which contains sebum and exfoliate the dead skin cells that are built up inside the pore. Beta hydroxy acids appear to be less irritating than alpha hydroxy acid despite their penetration properties.

5.) **Propylene Glycol**: Propylene glycol (PG) is an odorless liquid that functions as both a humectant and an occlusive.[7] It displays antimicrobial and keratolytic activity. Although PG is not a strong sensitizer itself, it alleviates the enhancing of penetrations of other allergens resulting in contact dermatitis.

Emollients fill the spaces between desquamating skin cells on the surface. They are generally grouped by their ability to spread on the skin. Emollient lipids similar to those naturally found in the skin may also increase the rate of barrier repair. By combining emollients with the different spread rates one can tailor the skin feel of a moisturizer according to specific needs. Commonly used emollient ingredients include plant oils, mineral oil, shea butter, cocoa butter, petrolatum, and fatty acids animal oils, including emu, mink, and lanolin, the latter probably the one ingredient that is most like our own skin's oil.

Restoration of deficient materials: In addition to keratin, which can bind a substantial amount of water, the stratum corneum contains a number of other hydrophilic agents called natural moisturizing factors (NMF). The NMF constitute about 20% to 30% dry weight of the stratum corneum and are found intracellularly as well as extracellularly. For example, sugars, hyaluronic acid (HA), urea, and lactate. The major contributors to the intracellular NMF are basic amino acids and their derivatives, such as pyrrolidone carboxylic and urocanic acid, comprising up to 50% weight of the total NMF. The NMF concentration varies as a function of age and skin depth In the deeper stratum corneum layers of older individuals (50–65 years), the NMF concentration is low.

Fig. 2. Composition of NMF's in skin

Components	Mole percent (%)
Amino acids	40.0
Sodium pyrrolidone carboxylic acid	12.0
Lactate	12.0
Urea	7.0
Ions (e.g., Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-})	18.5
Sugars	8.5
Ammonia, uric acid, glucosamine, creatine	1.5
Citrate and formate	0.5

1.3 Structural Changes with Age

1.3.1 Epidermis

Some clinical appearance changes in aging skin are Hair graying, sagging, skin wrinkling and thinning. Epidermal thickness has not shown any correlation with age in experimental conditions. [9] Neither has gender been implicated in thickness. The elasticity in skin decreases with age, and increases the incidence of a thinner skin caused by reduced contractile ability of the epidermis and hence lower congregation of epidermal cells. Ghadially *et al*. [10] studied the integrity of the S.C in aged vs. young subjects by tape stripping. A significantly lower number of strippings (18 ± 2) were needed to dismantle the barrier among aged individuals than for young subjects (31 ± 2 strippings). The affected barrier integrity also reduces the recovery speed in the aged skin. The normal epidermal surface patterns change to regular ridges and coarser with age leading to irregularity in skin surface.[11] The aged skin has a thin paper like nature which might seem transparent which has been implicated in the reduced thickness of epidermis. [12]

The main difference between aged skin and young skin is due to the dermal-epidermal junction between the dermis and epidermis. A series of projections (finger like) are observed between the epidermis and the dermis which increase the contact area among both layers, thereby aiding in the prevention of the epidermis from being sloughed off.[13] The downward projections are called rete pegs and the upward projections are termed as dermal papillae. They flatten with increasing

age causing decrease in the epidermal strength. Also, there are fewer keratinized and basal cells per unit area. Eventually this effect presents itself as dry skin with age.

Turnover rate of epidermal cells are found to decrease with age. The disappearance of the tetrachlorosalicylanilide, fluorescent dye, from the S.C has been shown as an indicator of cells replacement rate. [14, 15] 20 – 26 days was shown to be the replacement time in the 70+ age group and while in the age group of below 40 it was 13 – 13.5 days. The number of corneocytes that can be scrubbed off the skin in a four day period which is another indirect measure of epidermal renewal, was found to be twice higher in younger vs. aged skin. [16]

Age-related reduction in the cell turnover rate in epidermis is approximately 30% to 50% between the 3rd and 8th decade has been proved by a study of desquamation rates at selected body sites. (13)

1.3.2 Collagen Network

The synthesis of procollagen is important for skin matrix and its inhibition plays a major factor leading to the degradation of ECM components. Fibroblasts synthesizes procollagen which is regulated by the interactions between the collagen peptides and fibroblasts, while collagen present in degraded form disrupts such connections and reduces the procollagen synthesis. [17, 18]

Changes in the collagen network results from the collagen fibers' aging. Collagen molecular connections are important for enhancing tensile strength and stability to the skin, and this cross linking increases with age leading to an increase in stiffness of the skin. There are 2 main mechanisms which induces the enzyme-controlled process of maturation and development, and a glycosylation process, non-enzymatic in nature which is followed afrer tissue maturation. [19]

Proteins undergo glycosylation process, non-enzymatic in nature, known as the Maillard reaction leading to production of advanced glycosylation end products (AGE) that cause molecular damage by forming cross-links in collagen.

1.3.3 Ground Substance (Glycosaminoglycans/Proteoglycans)

The ground substance of human dermis is composed of glycoproteins, glycosaminoglycans (GAGs), and water. GAGs are polysaccharides, which are known as proteoglycans, when linked to a protein core. Hyaluronic acid (HA) and dermatan sulfate are among the most commonly found GAGs in skin. 6-sulfate and Chondroitin 4- are found in lesser quantity.

The proteoglycans and GAGs are able to bind water in the dermis up to 10,000 times of molecule size itself. [20] Due to such water attracting properties, they are said to be able to form a swelling pressure in the ECM, allowing fast diffusion of water and water soluble molecules. [21] Hence, it plays a part in the maintenance of hydration in skin and the transport of nutritional material in the ECM.

Aging leads to rapid reductions in the concentration of GAGs and HA. Fleischmajer *et al*. [22] reported a significant lowering in the concentration of GAGs from newborns to infants, with the levels being stable at middle age and then again lowering at old age. When HA was selectively stained in 30 years old subjects dermis, it showed lower amount of continuity between the elastic system and collagen. While, among subjects aged 60 years, the dermis HA precipitates were absent in surface of collagen fibers, intercellular or pericellular regions, nor between the collagen and elastic fibers. This loss of water retaining capacity and the lowering concentration of collagen bundles, might explain the dry and wrinkly appearance of aged skin. [23]

Longas *et al*.[24] found that the amount of HA and dermatan sulfate decreased respectively from 0.03% to 0.007% and 0.05% to 0.026% of weight of wet skin between 19 and 75 years of age. A chondroitin sulfate proteoglycan present in the basal lamina has been demonstrated as important in maintaining epidermal-dermal contact. After age 60, chondroitin sulfate proteoglycan concentration decreases, which may lead to the age-related changes that happen at the DEJ. [25]. Hence, a lowering amount of GAGs due to age causes changes in skin thickness and a lowering of water content.

1.3.4 Fibroblasts

They are well known as an integral part in the remodeling and production of dermal components, while being indispensable in regulation of epidermal morphogenesis.(26) Among the major cell types in the body, fibroblasts are widely used for *in vitro* tests to check the toxicology of different compounds. Among the current research findings, fibroblasts have been found to have differences depending upon the various anatomic locations(27,28) which all depends upon the local epithelial-mesenchymal interactions. Physiology of fibroblast changes from adult skin and fetal skin.(29) Fibroblasts usually have a finite replicative lifespan in-vitro, and therefore used as a model in the study of cell senescence.

1.3.5 Skin Microcirculation

Increasing age affects skin microcirculation [30], due to the significant reduction of dermal papillary loops in old skin. Li *et al* [31] using some of the currently available bio-engineering techniques for evaluation (non-invasive) of cutaneous functions to examine changes related to old age in skin microcirculation. They demonstrated that the capillary loops in dermal papillae reduces, but with age the sub papillary plexus improves in quantity. These changes lead to reduction in supply of nutrients to the epidermis, and which results in epidermis thinning and DEJ flattening. This causes the skin to look fragile and transparent.

Subsequently, as seen in spider veins, there is increase visibility in the sub papillary vascular plexus.[32]

1.4 Targets in Skin: The Surface Matters

In human skin, the first out layer is the SC, which is 10 μ m thick, having 10 – 18 cell layers of corneocytes or terminally differentiated keratinocytes.

Electron microscopy has shown that there is a distinct two-compartment arrangement of the SC where corneocytes individually are embedded in lipid matrix, to resemble a mortar and brick arrangement [9]. SC being a barrier layer in skin, is the primary target of any cosmetic treatment It is the perceiving and perceived surface in skin (dry skin presents itself here), the part where every lotion, cream, or instrument is applied. Also, prevents the penetration of actives. Irregular cohesion among corneocytes causes the appearance of dry skin, changing the reflection of light off the SC, changing both the feel and texture of skin. Effective SC hydration is extremely important factor to plasticize keratin, it being a structural protein. Thus, the properties of an excellent moisturizer are instant anti-aging effects, and increasing the strength of the lipid barrier in the extracellular spaces in SC that allows the locking in of moisture in the individual corneocytes. Humectants, water, amino acids, and lipids are part of the natural moisturizing factors (NMF) of SC. Glycerol, is among the oldest humectants currently in use, is shown to have many functions like compensating the lack of sebum [12], improving skin barrier repair [10], improving the water channels of epithelia [11], functions of aquaporins, and also influencing the rate of desquammation [13].

Use of glycolic acid, which is an alpha hydroxy acid (AHA), clinically, in treatment of extremely dry skin problems, and to improve appearance of skin by desquamation acceleration,[14] lead to a new invention — functional levels of AHAs, such as maleic acids, lactic, glycolic, and tartaric

acids have excellent anti-aging effectiveness.[15,16] Apart from enhancing appearance by smoothening the skin surface, AHAs increase the dermal (GAG) contents, and improve the strength of the skin barrier [17, 18]. Salicylic acid (BHA) has been widely used to chemically exfoliate the SC, also used to treat enlarged pores, oily skin condition as well as acne.

Compounds also target SC to enhance the skin barrier, as barrier ineffectiveness leads to atopic dermatitis and sensitive skin that might trigger inflammation events [20, 21]. One leading cause of atopic dermatitis is inflammation which changes the speed of again in skin. Thus, lipid barrier in SC are important to health of skin. [22] Several strategies are used to enhance the barrier and improve the youthful appearance and skin health, including physiological lipid blends [23], glycosphingolipids [24], vegetal sterols, vitamin C (upregulates endogenous lipid production by keratinocytes), and hyaluronic acid [25], as well as enhance epidermal differentiation and proliferation. [26]

1.5 THE COSMETIC CARE OF ELDERLY SKIN

Cosmetic products for aged skin can be categorized into body care and face care. Various such products can be found in the market. Although their number is overwhelming, various common features can be mentioned.

Face Care - Skincare

Cosmetic concepts that are designed for elderly people are usually around the dry skin conditions typical for aged elderly skin. Various skincare formulas employ humectants, which imbibe excellent transient moisturizing/hydration effects. When lowering the importance of unwanted surface problems, these products have minute influence on skin dermal losses. However, evidence is mounting that certain groups of topical actives seem to change the age related signs of degenerative skin.

Body care - Skincare

For active care by lipid substitution and humidity, mainly w/o and o/w emulsions are used, which combine moisturizing action and occlusive effects. In addition to carboxylic acid salt, pyrrolidone, and urea, some other humectant agents such as hyaluronic acid and alpha-hydroxy acid which are highly efficient moisturizer are generally found. It is self-explanatory that such

formulation ingredients as propylene glycol, glycerin, and other glycols have an additive effect to their humectancy.

Nonpolar and Polar lipids are widely used in body care formulations. They act as protective lipids, emollients, and as structuring agent of the liquid crystalline bilayers between the corneocytes. These functions are usually due to fatty acids, fatty alcohols, and short- and long-chain esters, along with waxes and triglycerides. Extra beneficial effects are usually delivered by liposomes, containing sphingolipids, phospholipids and ceramides, and thereby effect the desired long-term effects, which are due to the special binding mechanisms in skin, having an anchor capacity and slow release of encapsulated and/or transported active.

2. AIM OF INVESTIGATION

Dry skin, is a common problem and its incidence and severity increase with age. It is the most common cause of skin disorders in the elderly. The cause of dry skin is not completely understood. It has a genetic component and is influenced by environmental factors, such as cold or dry climates, and the use of soaps and harsh cleansers. Age-related changes in the skin also can explain the dryness that tends to develop with age. The management of dry skin should be directed towards altering environmental factors and treating its signs and symptoms. Attention to the care of dry skin becomes more important as world population ages.

Data on the prevalence of dry skin in the elderly show a wide range of variation, from 29.5–85%.[66,67]. Although dry skin is often a cosmetic problem, it also may affect quality of life. It commonly causes symptoms such as itching, burning, stinging and a feeling of tightness. It is the most common cause of generalised pruritus in the elderly.[68]

Dry skin occurs predominantly on the extremities, but also can be seen on the sides of the torso and on the face. Features include roughness, an increase in skin markings and a scaly appearance, with other possible features of redness and cracking developing as the problem worsens.

In people older than 75 years, there is a reduced quantity of stratum corneum lipids,[69]leading to impairment of barrier function and barrier repair. Other changes in the stratum corneum in the elderly include an increase in the size of the corneocyte, greater accumulation of corneocytes and impaired desquamation due to a slower turnover of cells.[70,71] The Natural Moisturizing Factor (NMF)—found in stratum corneum cells and made up of amino acids, derivatives of amino acids and various salts—allows the stratum corneum to maintain adequate levels of water. NMF levels decline significantly with age.[72]

AQUAXYL is a derivative of two plant sugars, xylitol and glucose. Its mode of action, reinforces the antidehydration shield (Loricrin, essential lipids) together with the activation of water reserves (hyaluronic acid booster) and their movement (Aquaporins, Tight Junctions). It improves water reserves and limits water loss. Desquamation is

balanced, microrelief is smoothed. Moisturized and restructured, the skin is better equipped to combat external aggressions. Under experimental conditions Aquaxyl does not favour glycation.

With this view an attempt will be made to design and evaluate an emulsified, low pH cosmetic compositions having improved storage stability and improved pH stability, in particular, the emulsified, low pH cosmetic compositions having a pH of about 3.7 to about 4.5, with a pH drift of not more than 0.5 and evaluate its moisturizing effects containing active Aquaxyl by in-vitro skin testing methods

The aim of this study is to produce an effective and low pH stable formulation with improved phase stability, incorporating Aquaxyl and thereby improving hydration in skin. In the present study, Face moisturizing cream with Aquaxyl (0.1-3%) will be developed by using different thickening systems like Xanthum gum and Carbomer systems based in low pH conditions with fatty acid esters, PEG 150 distearate, PEG 100 glycerol stearate, hydroxypropyl starch as plasticizer. The product will be formulated by hot emulsion method. The batches will be evaluated for phyisco-chemical properties such as specific gravity, Loss on drying (LOD), Viscosity, Centrifuge test, Thermal stability, Microbial testing, Sensory analysis, and in-vitro corneometry skin testing study**.**

3. Literature Review.

3.1 Chelating Agents:

1.) Di-Sodium EDTA:

Ethylenediaminetetraacetic acid, widely abbreviated as EDTA, is an aminopolycarboxylic acid and a colourless, water-soluble solid. In personal care products, EDTA salts are used as a chelating agents. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium EDTA. [45]

2.) Sodium Gluconate:

It is the sodium salt of gluconic acid. Gluconic Acid its derivatives are naturally occurring substances used at a maximum safety concentration of 1% w/w.

3.2 Thickening Agents:

1.) A Carbomer is made up of a series of polymers mainly made from acrylic acid. They are fluffy, white powders, generally are used as gels in various products in skin care, hair care, nail care, dentrifices and make up products in industry. They aid in dispersing of insoluble solids in a liquid and preventing emulsions from breaking apart into their liquid and oil components. They are widely used to control the flow and consistency of such products.

Carbomers are large molecules prepared from monomers which are relatively small chemical compounds. Polyalkenyl polyethers and acrylic acid are the monomers used to make carbomers polymers. Although they are similar chemically, they are different in their viscosity and molecular weight. They also possess the ability to retain and absorb water, allowing the polymer to swell many times its original volume.

Carbomers are usually assigned a number like 910, 934, 940, 941 and 934P on a personal care or cosmetic product label. They indicate the specific components and molecular weight of the polymer. [46]

1. Acrypol ST-100/ Acrypol TR-2

Properties:

- Thickeners & Stabilizers

- Surfactants / Cleansing Agents
- Smoothness

Appearance:
- Liquid, Powder

Applications/ Recommended for
- Hair care (Shampoos, Conditioners & Styling) > Styling/Hair sprays
- Skin care (Facial care, Facial cleansing, Body care, Baby care) > Body care
- Sun care (Sun protection, After-sun & Self-tanning)
- Toiletries (Shower & Bath, Oral care...) > Foot care
- Hair care (Shampoos, Conditioners & Styling) > Shampoos

pH Value: Low pH functioning range of **2.5 - 3.0**

2. Carbopol Ultrez 30

INCI Name: Carbomer
Carbopol® Ultrez 30 Polymer is a cross-linked homopolymer of acrylic acid that has been designed with a unique structure to allow the thickening and stabilization of cosmetic products (47).It is polymerized in a cyclohexane and ethyl acetate cosolvent system. It helps in attaining a desired aesthetic and rheological property in a medium or low level of acid-active-containing or electrolyte conditions that are necessary for effective delivery of product to the consumer.

The attributes and effectiveness of Carbopol Ultrez 30 polymer positions itself as an ideal candidate to be used in formulation of products such as:

- Sun care
- In formulations containing electrolytes
- Facial lotions and gels
- In low pH systems (4.0 - 5.5)
- Body lotions / creams
- In high pH systems (up to pH 12)

Benefits and Features

- Rich sensory
- High clarity
- Excellent rheology and suspension performance
- At low use levels (0.1 - 0.2 wt%) when formulated in electrolyte-free systems.
- Able to thicken over a broad pH range, including low pH systems (pH 4.0 - 5.5)
- At increased use levels in systems containing increasing levels of electrolytes.
- Electrolyte tolerance
- Efficient thickening

- Easy to use

2.) Xanthan Gum:

Xanthan gum is a derived naturally with a high-molecular weight polysaccharide made by the microorganism Xanthomonas campestris by using microbial fermentation technique. Glucose units make up its main chain. The side chain is made up of trisaccharides, which contain alpha-D-mannose, having a beta-D-glucuronic acid, acetyl group, and also a pyruvate group linked to a terminal beta-D-mannose unit. These side chains total in about 60 percent of the molecule and lends various unique properties to xanthan gum.
Xanthan gum has a various versatile properties in cosmetic product applications due to it having resistance to enzymatic degradation, very stable over a wide range of pH and temperatures and also in high concentrations of salt and alcohol. It is mainly used as a thickener, and is also efficient as a stabilizer for emulsion, foams, suspensions, and solid particles in water based formulations.

- **Functionalities**

1. **Temperature and acid stability:** Xanthan gum solutions are not affected generally by changes in temperature or pH and is able to dissolve in most bases or acids.
2. **Cold water soluble:** Anionic side chains are present on the xanthan gum molecules and makes it soluble in hot as well as cold water, all the while enhancing hydration.
3. **Salt tolerance:** Even with addition of large amount of salt there is no change in Xanthan gums viscosity demonstrating its excellent compatibility in the presence of amphoteric, anionic, and non-ionic surfactants. Only a slight rise in viscosity was found when it was tested in a 250/l NaCl brine.
4. **Viscosity control:** Xanthan gums viscosity is quite stable among high temperatures and low pH values a long period of time, while under the same condition other hydrocolloids tend to lose their viscosity.
5. **Compatibility with other hydrocolloids:** It is found to have a synergistic increase in gel strength or viscosity when used with galactomannasns, particularly guar gum (higher viscosity) and locust bean gum (gel formation).
6. **Freeze/thaw stability:** It exhibits excellent freeze/thaw stability mainly because of its water binding capacity, xanthan gum solutions exhibit good freeze/thaw stability.
7. **Shear-thinning pseudoplastic rheology:** Particle suspension is possible due to the high viscosity of xanthan gum at low shear rates and this property also prevents oil droplets from coalescing. When shear is applied and the viscosity drops, the product can therefore be easily poured, scooped or squeezed out. While regaining the initial viscosity when the force is removed.

3.3 Preservatives:

1. Sodium Benzoate:

Sodium Benzoate is a common preservative in many products that market themselves as being 'natural' or 'organic.' Sodium Benzoate is a sodium salt of benzoic acid, which is naturally occurring in apples, cranberries, plums, ripe cloves, and cinnamon in low levels, and is often produced synthetically by reacting Benzoic Acid with Sodium Hydroxide (NaOH) [48].

FDA limits the use of sodium benzoate to 2.5% in rinse off products and 0.5% in leave on products by the European Union Cosmetic Directive.[50] Despite being a synthetic chemical used in 'natural' and 'organic' products, it serves its purpose as a preservative without exposing consumers to negative impacts under ideal conditions. It is not known as an allergen or sensitizer, nor is it itself toxic in the concentrations used in food and cosmetics.

2. Potassium Sorbate:

Potassium Sorbate is the potassium salt (neutralization reaction of an acid and a base) of sorbic acid. Sorbic acid is naturally occurring in the rowan berry, however, it is primarily produced synthetically. Potassium sorbate has been regarded as a safe replacement for parabens in cosmetics and personal care products. Use of sorbic acids and its salts (potassium sorbate) are limited to 0.6% by the EU. Potassium sorbate is considered generally safe by the FDA, and has been found to be relatively non-toxic [49, 50]. A study conducted in 1990 found potassium sorbate to show "a very low level of mammalian toxic," even at up to 10% of the diet, devoid of carcinogenic activity, and non-mutagenic in in vitro and in vivo tests [51]. Potassium sorbate is also considered a nonirritant to the eyes and only slightly irritating to the skin. Potassium sorbate is considered one of the safest synthetic preservatives used in cosmetics[52]. By and large, potassium sorbate is regarded as generally safe, is not a carcinogen and has relatively low toxicity and mutagenic activity. As of 2006, the Cosmetic Ingredient Review lists potassium sorbate as safe for use in cosmetics and did not find it to be mutagenic within accepted guidelines (0.00003% – 0.7%) [53]

3. Phenoxyethanol:

Phenoxyethanol is a glycol ether made from alkyl ethers of ethylene glycol, though it can be derived from natural sources. Also known as Ethylene glycol monophenyl ether, it is a bactericide (kills bacteria) that is used in cosmetics, pharmaceuticals, and vaccines.

Phenoxyethanol ($C_8H_{10}O_2$) has increased in usage over that past 5 years because it is a much safer alternative to formaldehyde releasing preservatives and does not have the same negative connotation as parabens [54]. However, phenoxyethanol is not without its own concerns. It use is limited to 1% in Japan and the EU, and it is not suggested for use in baby products as per a warning issued by the FDA.

The Cosmetic Ingredient Review declared that phenoxyethanol is "practically non-toxic" to humans when administered orally or through the skin [55, 56]

3.4 Actives:

1.) Potassium Lactate+ Aqua+ L-Lactic Acid (Natural)

Alpha Hydroxy Acids (AHAs) are versatile ingredients, with effects ranging from hydration to anti-aging. Their actions can be tuned by adjusting the concentration and pH of the formulation L-Lactic Acid is natural and can be considered 'the body's own AHA', since this form is exclusively produced by the metabolic conversion of glucose or glycogen in the body. Compared to other AHAs, L-Lactic Acid has the best therapeutic index (skin renewal vs. potential irritation).

The amount of lactic acid and lactate present depends on the pH of the formulation. To illustrate this pH effect, in Figures 1 and 2, the cell renewal properties and irritation potential of glycolic acid are compared to those of L-Lactic Acid1. The increase in cell renewal is highest at 25% at pH 3, where the effect can be attributed to the direct effect of lactic acid on cell renewal.

At pH 7 virtually all of the lactic acid is present as lactates. The renewal effect of the cell can be due to the moisture level of the skin effected by the lactates. The NMF of the skin being a major constituent of the skin, plays a major role in the maintenance of proper skin hydration, which is required for the optimal functioning of the natural cell renewal process.

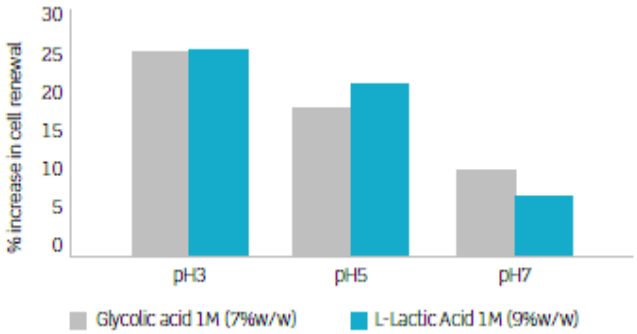

Fig. 3. Cell renewal rates at different pH levels

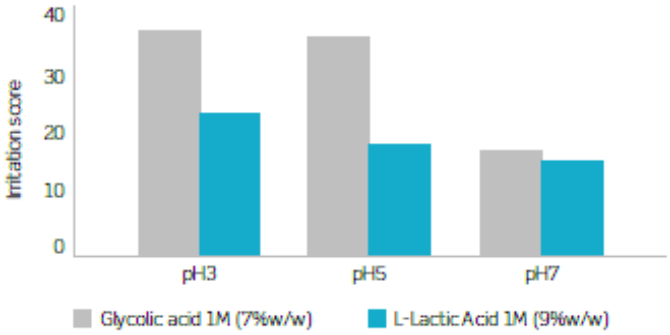

Fig.4. Irritation scores at different pH

Properties

INCI: Potassium Lactate (and) Aqua (and) Lactic Acid
Preservative- Free
Aspect: Clear, colorless, aqueous liquid
Recommended use level: 5-15%
Recommended pH: 4.0-5.0
COSMOS and ECOCERT approved. [57]

Absorption of lactic acid

In vitro study human skin o/w emulsion, 5% conc

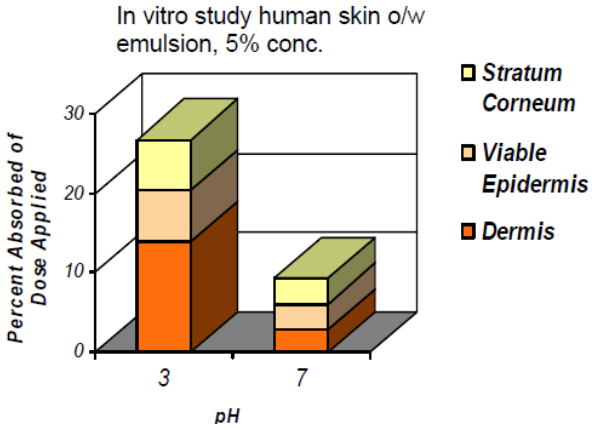

Fig. 5 Absorption of lactic acid in skin

1. **Sodium PCA (Pyrrolidone Carbonic Acid)**

Sodium PCA is the sodium salt of pyroglutamic acid (also known as PCA). PCA is a naturally occurring component of human skin and a part of the "natural moisturizing factors" (NMF) that maintain a healthy epidermis. Sodium PCA is very hygroscopic, attracting moisture from the air.

It imparts a moist feeling to hair and skin. Sodium PCA applied to the skin is absorbed to a limited extent. It is noncomedogenic, nonirritating to the eye and skin even at concentrations up to 50%, and does not contribute to phototoxicity or sensitization. It is rapidly biodegradable. Soluble in water and ethanol and insoluble in oils, it is used for its powerful humectant properties in many skin and hair care products including gels, creams, lotions, shampoos, conditioners, lipsticks and foundations. [57]

Recommended Usage Rate: 0.2 4%

Appearance: Clear, syrupy liquid

Preservative: None, product as supplied is unpreserved

Solubility: Soluble in water

INCI: Sodium PCA

2. **Aquaxyl (Xylitylglucoside, Xylitol, Anhydroxylitol)**

AQUAXYL is a derivative of two plant sugars, xylitol and glucose. Its mode of action, reinforces the antidehydration shield (Loricrin, essential lipids) together with the activation of water reserves (hyaluronic acid booster) and their movement (Aquaporins, Tight Junctions).[73]

It is based on the sugar chemistry which is dedicated to continuous improvement of hydration. Glucose and xylitol association which are humectant and hygroscopic molecules are able to trap free water which lead to Xylitylglucoside synthesis obtained from vegetable and natural origins.

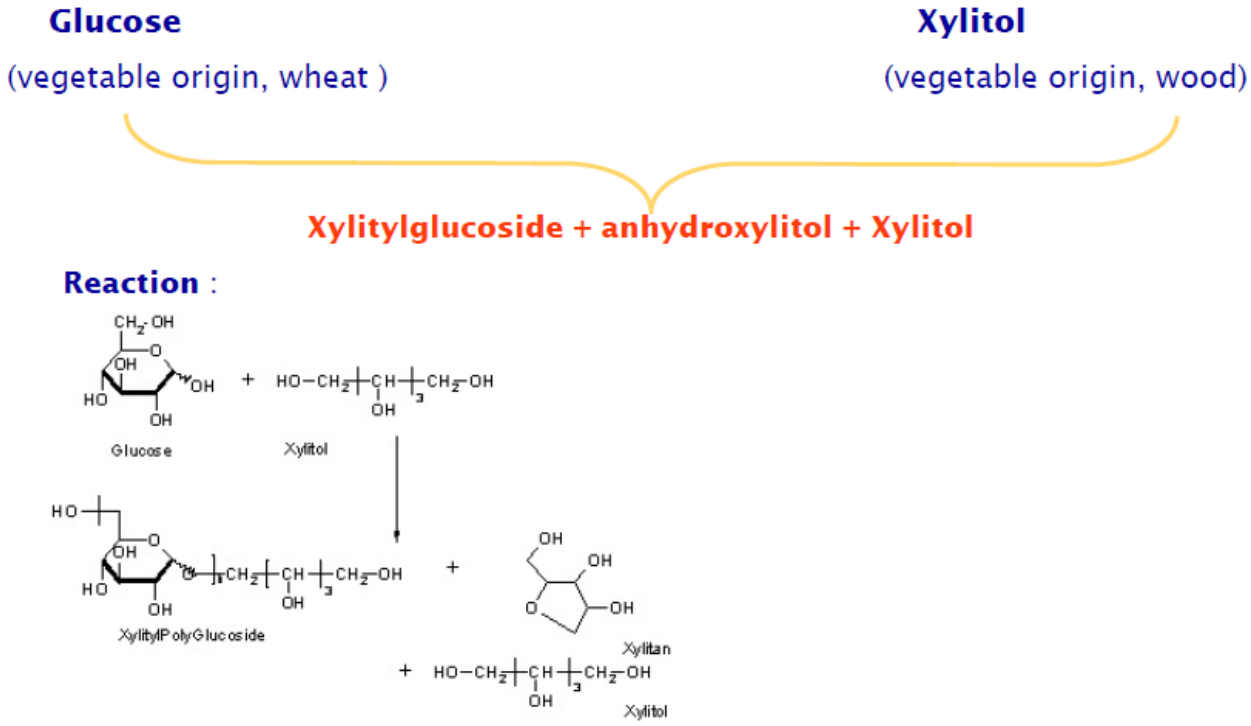

Fig. 6 Synthesis of Aquaxyl.

Aquaxyl- Hydra Concept: Harmonization of Skin Hydrous Flow:

A hydrous flow balance is essential for an optimum hydration state. Aquaxyl balance this ater reserves and water loss scale by increasing the GAG's synthesis and epidermal water content and also increasing barrier/ceramide function along with reduction in TEWL. Aquaxyl significantly improves epidermis hydration from the first application. This effect is confirmed after 15 days and one month of application with immediate to long term relief. It significantly stimulates ceramide synthesis and thereby improving the barrier function. Aquaxyl significantly stimulates the glycosaminoglycans content making the trapped water available within the skin. It also decreases significantly skin water loss versus placebo after one month of treatment resulting in stronger and more resistant skin.

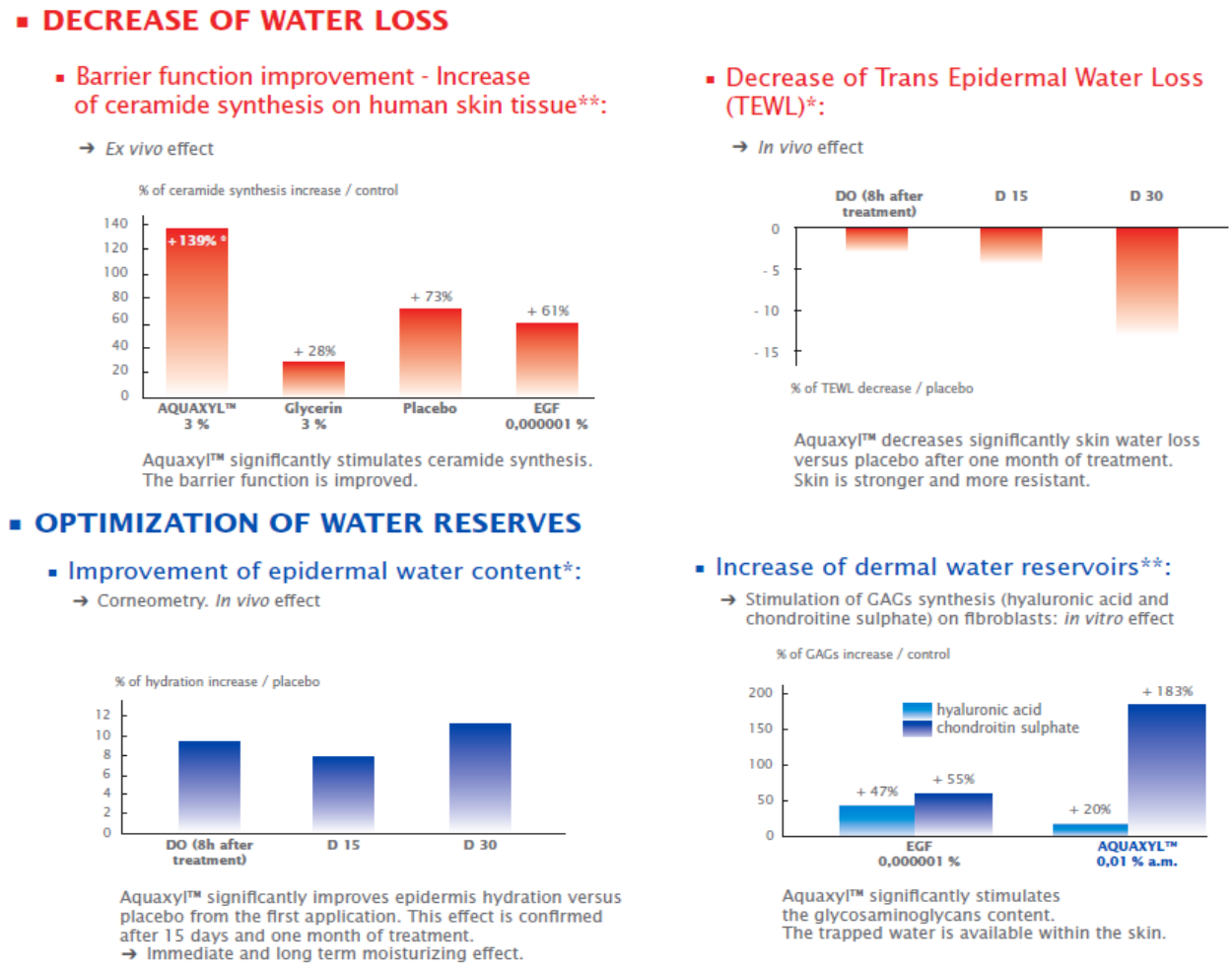

Fig. 7 Aquaxyl properties; Hydra Concept

3.4.1 Aquaxyl- In Vitro Evaluation on Glycation:

As Aquaxyl is composed of sugar derivatives (xylitylglucosides + anhydroxylitol + xylitol), it is important to make sure that it does not favour glycation.[43,44] Glycation is the reaction which results in a covalent bond between a target protein and a sugar, in particular glucose. In the body this is a slow, spontaneous, non-enzymatic reaction which takes place in the extracellular environment. This phenomenon alters the proteins and their functions. Glycation increases with age, and provides a partial explanation for the cross-linking of collagens. An invitro model used to assess the effect of Aquaxyl, based on the measurement of the formation of derivatives of glycation between lysine (a fairly reactive amino acid) and glucose-6-phosphate tested the formation of products derived from lysine after incubation for 14 days by measurement of the fluorescence emitted by certain of these derivatives, known as advanced glycation end-products (AGE), validated

by using two reference inhibitors, aminoguanidine (AG) and rutin, proved that Aquaxyl does not favour glycation.

3.5 Silicones:

1. **Dimethicone:**

Dimethicone (also known as polydimethylsiloxane or PDMS) is technically called a silicone-based polymer. More simply, it's a silicone oil with certain properties that make it extremely popular in today's personal care properties

It provides a smooth application. For skin care products, it fills in uneven texture and fine lines, which helps create a smooth and flawless look in products like primers, foundations, and lotions. It also provides a protective cover on skin, which is supposed to help keep moisture in, leaving skin hydrated for longer.

Dimethicone is an emulsifier and a moisturizer that makes it a highly effective additive to skin and hair care products. The chemical is also used as an emollient to make the surface of the skin more flexible and help the skin retain moisture

2. **Cyclopentasiloxane**

Cyclopentasiloxane is one of the most common ingredients used in personal care products today. Synthetically manufactured, it is a silicone derivative that carries a variety of skin and hair applications, such as hair spray and sunscreen lotion, among others. Cyclopentasiloxane is finding more and more popularity in hair care products, and those where a microscopic protective layer may benefit the skin

One of the biggest benefits of cyclopentasiloxane is its lower cost. For example, cyclopentasiloxane may be used as a cheap alternative to organic compounds like vegetable glycerin, and its concentrations in skin care items can be more easily varied because it is synthetically manufactured. This chemical also has low viscosity (meaning that it's not very thick), and does not leave significant residue behind on the epidermis giving the skin a smoother, healthier feel. Cyclopentasiloxane also doesn't cool during evaporation, which is helpful in personal care products because it helps decrease possible discomfort.

Cyclopentasiloxane is also considered an emollient that soothes and softens the skin by keeping moisture locked in. It may also correct dryness by preventing water loss and prevents scaling by holding more water on the skin's surface. This chemical is also an excellent lubricant that sufficiently stretches water layers on the surface of the skin.

The silicone often acts as a waterproofing agent, lubricates the skin, and offers a temporary shine. Cyclopentasiloxane may also have skin healing properties, because of its ability to create a protective layer.

Cyclopentasiloxane is used in concentrations ranging from as low as 0.1% to levels well into the double-digits. At the lower end, the chemical is found in hand and body moisturizers, as it provides enhanced feeling of soft skin. Irritation is kept to the minimum, while the good feel effect without cooling does find favor among regular users. At the higher end, it carries active antiperspirant compounds to the skin and hair and also provides a hair cuticle coat. It's compatibility with most personal care ingredients such as mineral oil, ethanol, and fatty acid esters make it a common ingredient in eye liners, drugstore eye cream, foundation creams, lip liners, and eye shadow.

3.6 Natural Oils:

1. **Almond Oil**

The use of almond oil for skin treatments is the high level of fatty acids contained in the final oil mixture. Fatty acids serve as a natural emollient for the skin, and can help the skin lock in moisture by forming a protective barrier. Emollients are especially effective for conditions like acne, eczema, psoriasis, or rosacea.

Another reason why almond oil is beneficial is because of its ability to plump and firm the skin. Almond oil works as a natural face exfoliator to help to remove dead skin cells and stimulate the growth of healthier skin. When used topically, the formulated almond oil for skin products or treatments is absorbed by the fatty cells of the skin. The moisture in the oil is then utilized by the tissues to repair damaged cells and give skin a more youthful appearance.

3.7 Emollients:

1. **Shea butter:**

It is known to have be hydrating to the skin and may help to build collagen. Shea butter is fantastic at retaining your skin's moisture. In a test where participants' arms were placed in water containing ethanol, researchers found that shea butter was able to help the skin totally recover within two hours. After three to four hours, it improved skin barrier [58].

In another study, a cream with 5% shea and a placebo cream were applied to volunteer's forearms. The moisturizing effects peaked at one hour but continued for eight hours [59].

Shea butter also may stimulate collagen production. In two studies shea butter was proven to help regenerate thinning skin, lessen wrinkles from sun damage, improve complexion, and promote healing [59]. In a study with rats, collagen was further shown to boost collagen production (British Journal of Dermatology). [60]

2. **Kokum butter:**

Kokum fruit, which is from the Garcina Indica Tree is popular as the ripe fruit whose pulp is used to add sourness as a substitute for tamarind in many recipes. The kokum kernels are rich in an essential fatty acids and are used to extract a rich butter that is known as kokum butter. It has a mild to non-existent fragrance and looks a lot like shea or cocoa butter. Kokum Butter has excellent emollient properties and very high oxidative stability. These properties help kokum butter make its way into many formulations like lip balms, lotions and soaps.

Produced from the seeds of the Kokum tree's (Garcinia Indica) fruit, Kokum Butter is refined resulting in a white butter with a mild to nonexistent odor. Kokum Butter has a smooth dense texture suitable for cosmetic, confectionary and toiletry applications. Kokum Butter is highly resistant to oxidation and often used as a Cocoa Butter substitute.

Kokum Butter is used extensively in dry skin therapies, hair conditioners, and inflamed skin treatments and various other skin care and beauty products. Kokum Butter is non-comedogenic and it does not clog pores. It helps cells oxygenation and makes the nutrients more readily available to the hair and thereby **promotes hair growth**.

The antioxidants in the butter helps in the regeneration of cells and regular use helps **prevent wrinkles**. It gets **quickly absorbed by the skin** and does not leave any greasy feeling, making it ideal for day time use too.

3. **CCTG (Caprylic/Capric Triglyceride):**

CCTG is primarily mixed esters of caprylic (C8) & (C10) acids. It is a fully saturated medium-chain triglyceride with unique application as a pharmaceutical excipient & cosmetic emollient. It is water resistant with high stability, spreadability and is oxidatively stable. It acts as excellent plasticizer & emollient for skin creams & lotions. Also, aids in the dispersion of colorants thus finding applications in color cosmetics like lipsticks, foundations & other color cosmetics. It is used as solvent/delivery vehicle for active compounds and as masking agent in perfumes.

3.8 Emulsifiers:

The Steareth ingredients are prepared by reacting ethylene oxide with stearyl alcohol where the numerical value in the name corresponds to the average number of units of ethylene oxide. For example, Steareth-5 is prepared using an average of 5 units of ethylene oxide reacted with stearyl alcohol.

1. Brij S2-SO(TH)-(Steareth-2),
2. Brij S21-SO-(TH)(Steareth-21)

The concentration of the emulsifiers was selected by calculating the Required HLB Value of the system. All trials were worked around ~ 8.3 HLB Value.

3.9 Structuring Agent:

1. Structure XL (Hydroxypropyl Starch Phosphate)

Structural XL is a starch-based rheology modifier that provides excellent stabilization in emulsion products. This starch, delivered as a powder, is a new concept to simply create and process elegant and stable personal care emulsions. The ease of use and immediate dispersability in cold water make it ideal for use in continuous manufacturing processes.

STRUCTURE XL starch can aid in emulsion stabilization, aesthetics enhancement and viscosity-build. An emulsion containing STRUCTURE XL starch will have outstanding stability over a broad temperature range (-30°C up to 50°C). It also brings body to the formulation and a conditioning after feel. STRUCTURE XL starch is readily cold water dispersible so that no pre-mixes are needed. STRUCTURE XL starch can be added to the oil phase or to adjust batch properties also at the end of a production.

3.10 Anti-Oxidant:

1. **Vitamin E (Alpha Tocopherol)**

Tocopheryl acetate is a form of vitamin E, a natural skin-conditioning agent and antioxidant. It is the ester of acetic acid and tocopherol and is often used as an alternative to pure tocopherol (or undiluted vitamin E) because it is considered more stable and less acidic.

It is a fat-soluble vitamin that can be isolated from vegetable oils. Vitamin E protects cell membranes from damage by oxygen free radicals. It can prevent premature aging of the skin induced by UV irradiation and lipid peroxidation. Used in 0.5-2.5% concentration for skin effects, 0.1-1.0% for stabilizing oils in products. [61]

Tocopherols and tocotrienols can rapidly scavenge lipid peroxyl free radicals by acting as chain breaking antioxidants, thus preventing them from reacting with other lipids. This process is key to limiting the propagation of lipid peroxidation in membranes. It should be noted that tocopherol has the potential to act as a pro-oxidant rather than an anti-oxidant when co-antioxidants like vitamin C are not available to neutralize the tocopherol radical and when oxidative stress is mild.[62]

3.11 Skin pH in the elderly

The acidic skin surface pH (pHSS) plays a crucial role in the maintenance of a vital stratum corneum (SC) and an intact epidermal barrier. However, ageing affects the pH of the skin, both pHSS and the pH of the stratum corneum (pHSC).

Three molecular mechanisms responsible for the acidification of skin appear to be disturbed with increasing age: a decreased NHE1 expression, a reduced breakdown of phospholipids into acidifying FFAs, and a reduced breakdown of filaggrin into natural moisturising factors.[33,34] In addition, sebum and sweat production are decreased in the elderly, thereby further decreasing the buffer capacity of the skin.[35,36] The age-related pHSS increase alters several functions in the skin of the elderly, and therefore may have negative effects. An increased pHSS impairs SC function, which may cause disturbed lipid processing, putatively via influencing the two key lipid-processing enzymes β-glucocerebrosidase and acidic sphingomyelinase, which both exhibit highest activity at low pH. Therefore, their activity will be reduced with increasing age.[37].

Furthermore, the microbial defense mechanisms of the SC are impaired due to an increased skin pH. As mentioned above, physiologic pH-values ensure normal microbial

colonization of the skin, whereas aged skin exhibits an increased susceptibility to pathogens facilitating bacterial infections.[38,39]Thus, an age-dependent increase of pHSS very likely promotes the colonization of the skin surface with an altered microbial flora, contributing to the development of a characteristic body odour.[40,41]

Ditre et al.,[42] among others, showed an increased skin thickness caused by increased synthesis of collagen and possibly elastic fibres after treatment with a pH 3.5 lotion in a placebo-controlled trial. Therefore, based on our findings, it is recommended that skin care products for continuous application (e.g. daily) should be formulated taking into account age-related changes of the skin, in particular increased pH and reduced hydration. Skin surface pH ranges between 4 and 6 and this acidity of the skin surface is important for physiological skin functions. A reduction in acidity of SC, usually seen aged skin, may negatively affect normal functioning of the skin, such as antimicrobial capacity, barrier permeability, and cohesion and integrity of the SC. Either leave on or rinse off Skin care products for this segment should ideally be formulated with a lower pH, e.g. of 4, which helps in normalizing the age-related elevation of skin pH and thereby help to maintain proper skin functioning and a allowing healthy skin.[74]

3.12 Specific Gravity- Pycnometer Method

This method determines the ratio of the weight of a unit volume of the sample at a specified temperature to the weight of a unit volume of water at the same temperature. Usually the reference substance is water which always has a density of 1 gram per milliliter or 1 gram per cubic centimeter.

The scope of this method is applicable to all oils and liquid fats and solvents. Specific gravity bottles with well-fitting ground glass joints are usually used.

3.13 Loss on drying (LOD):

Test method to determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample. It determines the amount of volatile matter which is driven off under the conditions specified.

A weighed sample of the test material is dried under the conditions specified for the product. The usual conditions are a) 105°C at atmospheric pressure or b) 80°C under

vacuum equivalent to 24 inches of mercury. The results can be reported as 1) loss on drying or 2) percent volatiles

Note: Care must be taken to avoid moisture pick-up from the atmosphere. The most accurate measurements can be expected from samples removed the first time the sample container is opened. Because of the hygroscopic nature of Carbopol polymers, moisture pick-up each time the sample container is opened will influence the loss on drying

3.14 Viscosity

This procedure determines the viscosity of a fluid by the use of a Brookfield Viscometer. Viscosity is the measure of fluid friction which can be considered as the internal friction resulting when a layer of fluid is made to move in relationship to another layer. It can also be termed as a measure of the ratio of shearing stress to rate of shear.

Viscosity is a principal parameter when any flow measurements of fluids, such as liquids, semi-solids, gases and even solids are made. Brookfield deals with liquids and semi-solids. Viscosity measurements are made in conjunction with product quality and efficiency.

3.15 Thermal Stability

This test is an experiment in which samples are subjected to different environmental conditions for a set period of time. These conditions vary in temperature and light levels and are meant to simulate what will happen to the product during its life cycle.[(75)]

At select intervals samples are evaluated for various physical, chemical and performance characteristics to see how they have changed. If the changes are minimal according to the specified standards, then the formula is said to have "passed" stability testing. This reinforces the confidence that when the formula is shipped to stores and ultimately customers, it will still be as good as when it was first manufactured.

The underlying assumption in stability testing is that increasing storage temperature speeds up any aging reactions that will occur. A handy rule of thumb is that a sample stored at $45^{o}c$ for 8 weeks is equivalent to one that is stored at room temperature for one year. This is not an exact predictor, but is good enough for the purposes of cosmetic products.

3.16 Microbial Testing

This method involves enumeration of colonies on a non-selective agar medium (Plate count). The possible inhibition of microbial growth by the sample shall be neutralized to allow detection of viable microorganisms. Sample creams are subjected to standard conditions and microbial colonies are determined in accordance with Total bacterial count (TBC) and Total Yeast/Mould Count.

3.17 pH Testing

A pH Meter is a scientific instrument that measures the hydrogen-ion concentration (or pH) in a solution, indicating its acidity or alkalinity.[76] The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode. It usually has a glass electrode plus a calomel reference electrode, or a combination electrode.

3.18 Skin Testing Meter- Corneometer (CM 825)

The Measuring Principle. The measurement is based on capacitance measurement of a dielectric medium.(77) The Corneometer® CM 825 measures the change in the dielectric constant due to skin surface hydration changing the capacitance of a precision capacitor.

3.19 Centrifuge Test (Emulsion Stability)

Instability of an emulsion is detected because of creaming, i.e. the appearance of an oil phase at the surface of the system or by the sedimentation of components having a higher density.(78) Therefore use of centrifugation methods are employed in order to speed up the potential destabilization process. The Remi High Speed Centrifuge works by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density.

4.0 EXPERIMENTAL WORK

Materials and Equipment Used:

4.1 Materials Used:

Table 8 - List of materials used

Function	Ingredients
Solvent	Water (Distilled)
Chelating Agent	Sodium Gluconate, Disodium EDTA
Thickening Agent	Acrypol ST-100 Acrypol ET-1 Acrypol TR-2 Xanthan Gum Dehydroxy Xanthan Gum Carbopol Ultrez 30 PEG 150 Distearate
Preservative	Sodium Benzoate, Potassium Sorbate,
Actives	Potassium Lactate+ Aqua+ L-Lactic Acid (Natural), Sodium PCA (Pyrrolidone Carbonic Acid), Aquaxyl (Xylitylglucoside, Xylitol, Anhydroxylitol)
Silicones	Dimethicone Cyclopentasiloxane
Natural Oils	Almond Oil
Emollients	Shea butter, Kokum butter, CCTG (Caprylic/Capric Triglyceride)

Emulsifier	Brij S2-SO(TH)-(Steareth-2), Brij S21-SO-(TH)(Steareth-21)
Structuring Agent	Structure XL(Hydroxypropyl Starch Phosphate)
Anti-Oxidant	Vitamin E (Alpha Tocopherol)
Perfume	Fuity Note Base

4.2 List of Equipment Used

Table-9 List of equipment used with company name

EQUIPMENTS	COMPANY NAME
Hot plate	Pathak Electrical Works - Mumbai
Mechanical Stirrer	Remi Elektrotechnik Ltd - Vasai
Viscometer LVT (Analog) (Helipath Stand)	Brookfield Engineering Laboratories, Inc
Centrifuge	Remi Elektrotechnik Ltd - Vasai
Skin Meter	Courage Khazana - Germany
Hot air oven	Pathak Electrical Works – Mumbai Tempo Instruments & Equipments Pvt. Ltd.
Humidity chamber	Remi Elektrotechnik Ltd – Vasai (REMI CHM – 6 PLUS)
Digital Balance	TX3202L Shimadzu & Metlor Toledo AB204-S
Laminar Air Flow-UV chamber	Klenzaids
Colony Counter	Lapiz, Medica Instruments Mfg. Co.

Incubater/ BOD Incubator	Meta- lab Scientific Industries - Mumbai
Autoclave	Meta- lab Scientific Industries - Mumbai
Mechanical Homogenizer	Remi Elektrotechnik Ltd – Vasai
pH meter	DP 505/Toshcon industry – Ajmer Digital instruments corporation

4.3 FORMULATION OF PRODUCT

4.3.1 Composition of Final Face Moisturizer Batch

Table 10. Composition of final batch

Phase	Ingredient	Quantity (%)	Function
A	Water	70-85	Solvent
A	Sodium Gluconate	0.05-0.1	Chelating Agent
A	Xanthan Gum/Carbopol Ultrez 30	0.1-2	Thickening Agent
A	Potassium Sorbate	0.1-1	Preservative
A	Sodium Benzoate	0.1-1	Preservative
B	Potassium Lactate+ Aqua+ L-Lactic Acid (Natural)	0.1-5	Active
B	Sodium PCA (Pyrrolidone Carbonic Acid)	0.4-4	Active
B	Aquaxyl (Xylitylglucoside, Xylitol, Anhydroxylitol)	0.1-6	Active
C	Dimethicone	0.1-5	Occlusive
C	Cetyl Alcohol	0.1-6	Emulsifier
C	Almond Oil	0.1-10	Occlusive
C	CCTG (Caprylic/Capric Triglyceride)	0.1-5	Emollient
C	Brij S2-SO(TH)-(Steareth-2)	1-5	Emulsifier
C	Brij S21-SO-(TH)(Steareth-21)	1-3	Emulsifier

C	Shea Butter (Refined)	1-4	Emollient
C	Kokum Butter (Refined)	1-4	Emollient
D	Structure XL(Hydroxypropyl Starch Phosphate)	0.5-3	Structuring Agent
E	Vitamin E (Alpha Tocopherol)	0.05-1	Vitamin
E	Perfume	0.3-1	Fragrance

4.3.2 Method of Preparation

1. Sprinkle the Xanthan Gum into a gentle vortex and mix at 1200 rpm until uniform. Add EDTA and incorporate slowly the preservative system at 1500 rpm. Start to heat at 75 °C and reduce mixing at 500 rpm while heating.
2. Prepare PART C and start to heat at 80 °C.
3. Add PART C to PART A under high speed mixing 3000 rpm (Emulsification). Stop heating.
4. Homogenize for 5min at 3000rpm.
5. Sprinkle PART D to the batch. Mix at 2000 rpm until homogeneous. Cool down under low mixing at 1000 rpm.
6. Add PART B below 45 °C and wait until homogeneous. Mix until RT at 1000 rpm.
7. Under mixing (1000 rpm), add PARTH E one after the other to base. Wait until uniform between each addition.

Note: All trials were worked around ~ 8.3 HLB Value. Formulations were prepared by hot emulsion method.

4.3 3 Table 11- Composition of all trials:

Phase	Ingredient	Quantity (%)	1	2	3	4	5	6	7	8	9	10	11	12	Function
A	Water	70-85	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Solvent
A	Di-Sodium EDTA	0.05-0.1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Chelating Agent
A	Acrypol ST-100		N	N	N	N	0.5-1.5	N	N	N	N	N	N	N	Thickening Agent
A	Acrypol TR-2		0.5-1.5	N	N	N	N	N	N	N	N	N	N	N	Thickening Agent
A	Xanthan Gum	0.5-1.5	N	N	0.5-1.5	0.5-1.5	N	0.5-1.5	0.5-1.5	N	N	0.5-1.5	0.5-1.5	N	Thickening Agent
A	Dehydroxy Xanthan Gum		N	N	N	N	N	N	N	0.5-1.5	0.5-1.5	N	N	N	Thickening Agent
A	Carbopol Ultrez 30	0.5-1.5	N	N	N	N	N	N	N	N	N	N	N	0.5-1.5	Thickening Agent
A	PEG 150 Distearate		N	0.5-1.5	N	N	N	N	N	N	N	N	N	N	Thickening Agent
A	Potassium Sorbate	<1	N	N	N	N	N	N	N	N	N	N	N	N	Preservative
A	Sodium Benzoate	0.1-1	N	N	N	N	N	N	N	N	N	N	N	N	Preservative
A	Phenoxyethanol	0.1-1	0.1-1	0.1-1	N	0.1-1	0.1-1	N	0.1-1	0.1-1	N	N	N	N	Preservative
B	Potassium Lactate	1-5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Active
B	Sod. PCA	2-5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Active
B	Aquaxyl	2-5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Active
C	Dimethicone	1-5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Occlusive
C	Cetyl Alcohol	0.5-3	N	N	N	N	N	N	N	N	N	0.5-3	0.5-3	0.5-3	Emulsifier
C	Almond Oil	5-10	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Occlusive
C	CCTG	2-6	-	-	-	-	-	-	Y	Y	Y	Y	Y	Y	Emollient
C	Brij S2-SO(TH)-(Steareth-2)	1.0-5.0	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Emulsifier
C	Brij S21-SO-(TH)(Steareth-21)	1.0-5.0	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Emulsifier
C	Shea Butter	2.0-4.0	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Emollient
C	Kokum Butter	2.0-4.0	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Emollient
D	Structure XL(Hydroxypropyl starch Phosphate)	0.5-2	-	-	-	-	-	-	-	-	Y	Y	Y	Y	Structuring Agent
E	Vitamin E (Alpha tocopherol)	0.2-1.0	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Vitamin
E	Perfume	0.3-1.0	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Fragrance

4.4 EVALUATION OF FACE MOISTURIZER:

Evaluation of product as per Bureau of Indian Standards: The emulsions with different concentrations of ingredients were evaluated according to Bureau of Indian standard [63].

(A)Physicochemical evaluation

(B)In vitro evaluation

4.4.1 (A) Physicochemical Evaluation:

1.) Specific Gravity: Specific gravity bottles with well-fitting ground glass joints, 25 ml. capacity were used. Testing is done at 28o c temperature.

Procedure: 1) The Sample was cooled to approximately 1-3°C below specified temperature and the bottle was filled until it overflowed, holding the bottle on its side in such a manner as to prevent the entrapment of air bubbles. 2) It is then warmed to the specified temperature using the warmth of the hand. Overflow is wiped off, bottle is capped; cleaned, dried and weighed. 3) Calculation of specific gravity is done.

2.) Loss on drying (LOD): Care was taken to avoid moisture pick-up from the atmosphere. Because of the hygroscopic nature of Carbopol polymers, moisture is picked-up each time the sample container is opened and will influence the loss on drying. Hot air oven was controlled at 105 ± 2°C. Balance capable of ±0.0001 g accuracy is used and Heat safe petridish are used. The weighings are all done with the cover in place. Heating was carried out by placing the petridish in the oven with its cover beside it. At the end of the specified heating period, the petridish was removed from the oven, and allowed to reach room temperature. At time of weighing, the cover is placed tightly on the petridish. Since the actual weight of sample is recorded and used in the calculation, a slight deviation from 1.000 gram is acceptable.[64,65]

Procedure: 1. Prepare the petridish and cover by heating 30 minutes in the 105°C oven. Allow to cool.

2. Tare balance and accurately weigh both petridish and cover and record weight.

3. Remove the cover and add 2-3 (±0.1) g of sample to the bottle.

4. Replace cover and reweigh immediately. Record weight to the nearest 0.1mg. Obtain the weight of the sample by difference.

5. Heat the sample at 105°C for 2 hour

6. At the end of the drying time, remove the petirdish and its cover from the oven (remembering to place cover over petridish but in an angled position so that petriplate is not sealed). Allow the material to reach room temperature and then replace cover and weigh.

3.) Viscosity:

The Brookfield Viscometer measures viscosity by measuring the force required to rotate a spindle in a fluid. Brookfield Viscometer LVT (Analog) model is used, wherein the viscosity is obtained by multiplying the reading by a constant related to the particular rotational speed used. Spindle LV 4/64 @ 0.3 RPM and calculated using the viscosity chart.

Procedure:

1. Check to confirm that the viscometer has been calibrated. If not, calibrate using method Lab-III-13.

2. The sample container and quantity should be approximately the same as for the Calibration Standard. Equilibrating the temperature of the sample to the temperature designated (28 °C) in the specification (±1°C).

3. Confirming that the viscometer is level using the bubble level on the back of the instrument.

4. Immerse the spindle (LV 4/64) designated in the product specification into the sample to the groove on the spindle shaft. Do not allow air bubbles to be formed. Attach the spindle to the viscometer. The spindle should not touch the bottom or sides of the container and should be centered. Reconfirm that the viscometer is level

5. Set the speed (@ 0.3 RPM) as designated in the product specification, start the viscometer and read at constant reading.

6. Use the conversion chart to convert the dial readings to centipoises.

7. When done, turn motor and power off. Clean spindle and place in spindle holder.

4) Thermal Stability:

Each trial was subjected to thermal stability testing in different conditions such as 40°C/75% RH, 25°C RT, and 55°C using Humidity chambers and Hot air ovens.

Procedure: With the help of spatula, insert the cream into glass bottle and tap it to settle to the bottom. Fill up to two-third capacity of bottle and insert plug and tighten the cap. Keep the filled bottle erect inside the incubator at 45 ± °C for 48 h. The sample shall be taken to have passed the test, if on removal from the incubator shows no oil separation or any other phase separation.

(5)Microbial testing: Microbial testing was done in accordance to IS 14648. The Final Trial batch 11 and 12 were subjected to microbial testing after 1 month at RT. Testing was done for Total bacterial count (TBC) and Total Yeast/Mould Count (TYMC)

Procedure: (1) Soybean Casein Digest Agar (SCDA)/ Sabourand Dextrose Agar (SDA) culture media was prepared by dissolving the components or the dehydrated complete medium in water by mixing while heating. Sterilize in the autoclave at 121°C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7.3 ± 0.2 when measured at room temperature.

(2) Modified Letheen Broth (1:9 dilution) components was dissolved successively in boiling water until their complete dissolution. Mix gently to avoid foam. Dispense the medium into suitable containers. Sterilize in the autoclave at 121°C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7.2 ± 0.2 when measured at room temperature.

(3) Product sample 1g is transferred to 9ml diluent carefully under HEPA Filter.

(4) The prepared samples are incubated @30°-35°C for 24-48 hours for microbial enumeration (Total Bacterial Count) and also incubated @20°-25°C for 3-5 days for microbial enumeration (Total Yeast/Mould Count)

(6) pH testing: Oil-in-Water Emulsion Cream was weighed accurately. 5+0.01 g of the cream in a 100 ml beaker was taken. Determination of the pH of the emulsion at 27°C was done using the pH meter.

Procedure: (1) Sample is allowed to stand for 30 mins to stabilize temperature

(2) Electrode of pH meter is immersed into sample and beaker turned slightly to obtain good contact with sample

(3) Electrode is immersed for minimum 30 secs to allow meter reading to stabilize.
(4) pH value is read and recorded to nearest tenth of a whole number
(5) Electrode is rinsed with Distilled water, and dabbed lightly with tissues to remove any film formed on it.

(7) Sensory Analysis: Prepared samples of selected stable emulsion trials 11 and 12 were given to a panel of 5 members with experience of sensory evaluation. The panel was asked to fill up the evaluation form based on product attributes on a scale of 5 points, in proper sequence according to protocol. Proper test conditions were maintained throughout the procedure. At the start of the procedure, the marked application site was cleaned with an alcohol swab and one drop of cream was used to rub the area in a circular manner.

- **Parameters** selected according to the claims needed to be substantiated for the product.
    - Fragrance
    - Product Consistency
    - Spreadability (At time of application)
    - Stickiness (At time of application)
    - Oiliness (At time of application)
    - Softness (After Absorption, softness on touch)
    - Moisturization (After 1min of Application)
    - Overall Likeability

Evaluation of Senior Citizen Product

Name of product: Face Moisturizer

Parameters		Score
1. Fragrance	1 2 3 4 5 (Dislike … Like)	
2. Product Consistency	1 2 3 4 5 (Thin … Thick)	
3. Spreadability (At time of application)	1 2 3 4 5 (Difficult to spread … Easily spread)	
4. Stickiness (At time of application)	1 2 3 4 5 (Sticky … Non-Sticky)	
5. Oiliness (At time of application)	1 2 3 4 5 (Oily … Non-Oily)	
6. Softness (After Absorption, softness on touch)	1 2 3 4 5 (Not-soft … Very-Soft)	
7. Moisturization (After 1min of Application)	1 2 3 4 5 (Dislike … Like)	
8. Overall Likeability	1 2 3 4 5 (Dislike … Like)	
9. Remarks *(if any)*:		

Date of Evaluation:

Sample Code/Batch No:

Name & Signature of the Volunteer:

Fig 12. Sensory Analysis Evaluation Form to be filled up by the panel.

(8) Centrifuge Test: Remi Centrifuge was used for the test at 3000 RPM for 30 mins.

Procedure: The selected emulsions are heated upto 50^{o}c temperature was taken to be filled into 2 glass containers with cylindrical shapes (inner diameter 2.8 cm) and rotated at a constant rotation speed of 3000 rpm. The emulsions were treated in this way for 30 min. At the end of the process they are individually checked for creaming.

4.4.2 (B) In vitro evaluation:

Invitro skin hydration/moisture content study was done using Courage Khazana skin testing meter with CORNEOMETER ® CM825 probe.

Objective: The objective of this study was to evaluate the *in vivo* effects of two skin care products (Face Moisturising Cream and Body Butter) in healthy dry skin type male subjects. The evaluation was performed using:

Corneometry (measurement of the moisturizing effect of the superficial layers of the skin)

Population: 5 healthy male subjects, aged between 55 and 65 years old, having skin of all skin types (dry, combination, oily, normal), were selected for this study.

Study design

1. Single blind study
2. Non comparative study
3. Subjects served as their own reference.

Study schedule and duration

The study began on May 19-20, 2016 and ended on July 1-2, 2016.

Evaluations and measurements were carried out according to schedule presented thereafter:

DATE	T0	T+ 2 HOURS	T+4 HOURS	T+6 HOURS	T+8 HOURS	T+ 2 WEEKS
	19-20/5/16					1-2/6/16
*Skin moisture measurement by corneometry	Yes	Yes	Yes	Yes	Yes	Yes

Fig. 13 Corneometry Schedule

Cutaneous acceptability, Tolerance

No unwanted or adverse event has been reported by the subjects during the study time course.

Therefore we can conclude that the tested products of Face Moisturizer cream for senior citizen**,** applied 4 times daily on face during the study period were perfectly well tolerated by 100% the subjects of this test.

5. Result and Discussion:

5.1.1 Specific Gravity

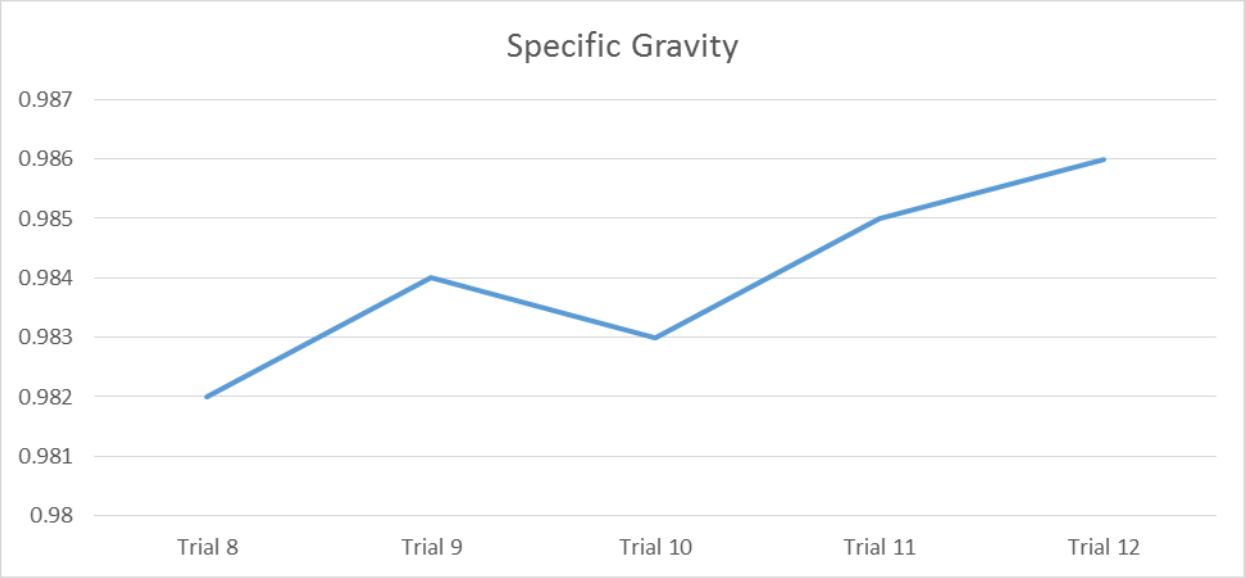

Fig. 14 Chart of Specific Gravity; Physicochemical test

5.1.2 Loss on drying

Results:

Batch No.	% Loss on drying
8	70.14
9	71.69
10	73-76
11	73-76
12	73-76

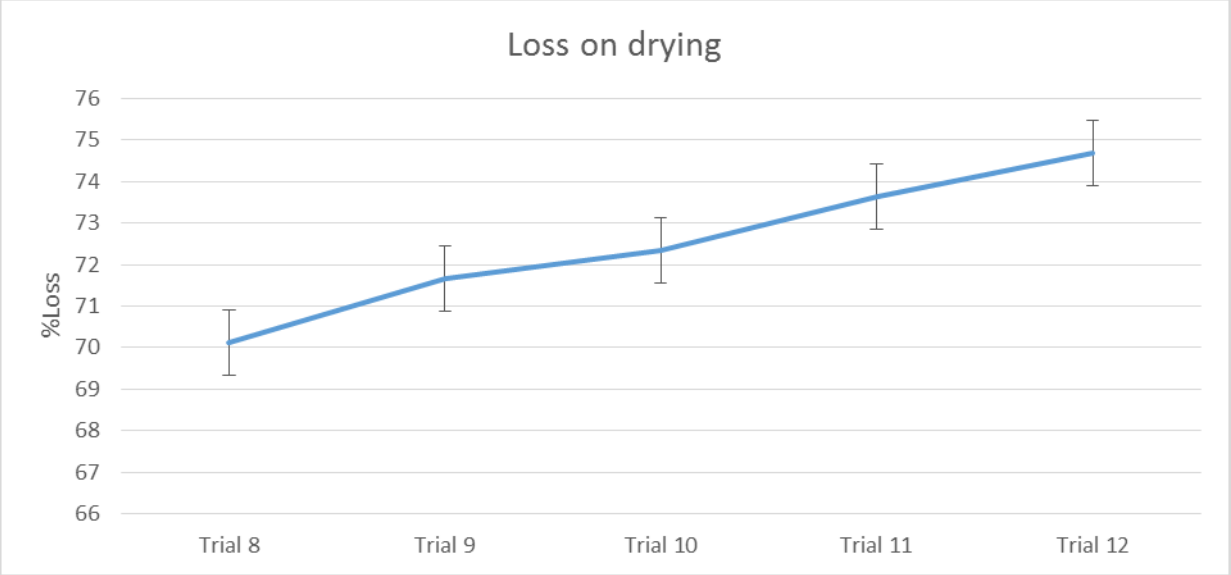

Fig. 15 Chart of Loss on drying; Physicochemical test

5.1.3 Viscosity Test

Results:

Batch No.	Viscosity (cP)
8	290k
9	275k
10	155k- 250k
11	165-250k
12	170-250k

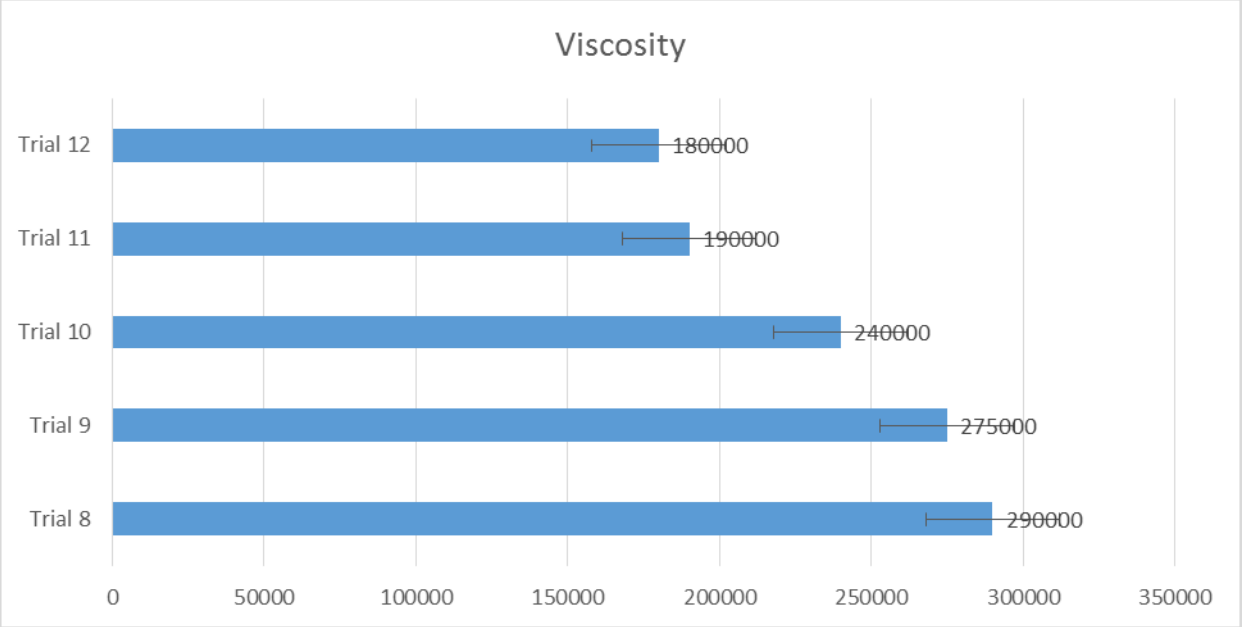

Fig.16 Chart of viscosity test; Physicochemical Test

5.1.4 Thermal Stability.

5.1.4.1 Freeze Thaw Cycle Test:

Batch No.		1	2	3	4	5	6	7	8	9	10	11	12
	Parameters												
After 7 days	Pass/Fail	Failed	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass	Pass
Alternating - 10°C/+ 40°C													
	Appearance	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade
	Water Separation	Separation	Separation	Separation	Separation	Separation	Separation	Separation	No separation	No separation	No separation	No separation	No separation

5.1.4.2 Constant 25^{o} C Temperature Stability Test:

Batch No.		1	2	3	4	5	6	7	8	9	10	11	12
	Parameters												
After 14 days	Pass/Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass	Pass

Constant 25°C													
	Fragrance stability	80%	80%	50%	80%	50%	100%	100%	100%	100%	100%	100%	100%
	Appearance	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade
	Phase Separation	Separation	Separation	Separation	Separation	Separation	Separation	Separation	No separation	No separation	No separation	No separation	No separation

5.1.4.3 Constant 40º C Temperature Test:

Batch No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Parameters												
After 14 days	Pass/Fail	Failed	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass	Pass
Constant 40°C													
	Fragrance stability	50%	50%	50%	70%	70%	70%	70%	100%	80%	80%	80%	80%
	Appearance	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	Dull White emulsion	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade	White Shiny Emulsion No color fade
	Phase Separation	Separation	Separation	Separation	Separation	Separation	Separation	Separation	No separation	No separation	No separation	No separation	No separation

5.1.4.4 Constant 55º C Temperature Stability Test:

Batch No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Parameters												
After 14 days	Pass/Fail	Failed	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass	Pass
Constant 55°C													
	Appearance	Dull Colored emulsion	Dull Colored emulsion	Dull Colored emulsion	Dull colored emulsion	Dull colored emulsion	Dull colored emulsion	Dull colored emulsion	Peach colored Shiny Emulsion	Peach colored Shiny Emulsion	Peach Shiny Emulsion No color	Peach colored Shiny Emulsion No color	Peach colored Shiny Emulsion No color

									No color fade	No color fade	fade	fade	fade
	Phase Separation	Separation	Separation	Separation	Separation	Separation	Separation	Separation	No separation	No separation	No separation	No separation	No separation

5.1.4.5 Sunlight Stability Test:

Batch No.		**8**	**9**	**10**	**11**	**12**
	Parameters					
After 7 days	Pass/Fail	Pass	Pass	Pass	Pass	Pass
Sunlight						
	Appearance	Peach Shiny Emulsion Minor color fade after 6 days	Peach Shiny Emulsion Minor color fade after 6 days	Peach Shiny Emulsion No color fade	Peach Shiny Emulsion No color fade	Peach Shiny Emulsion No color fade

5.1.5 Microbial testing

5.1.5.1 Result of Batch 11

TBC	TFC
1000	100
<10	<10

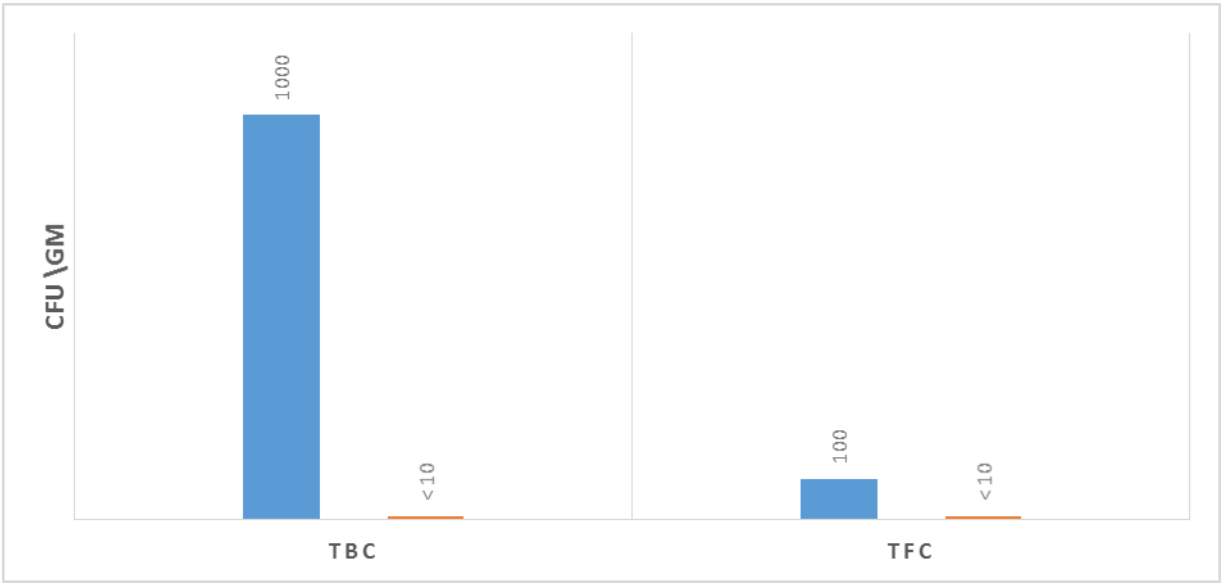

Fig. 22 Graph of Microbial study of Batch 11

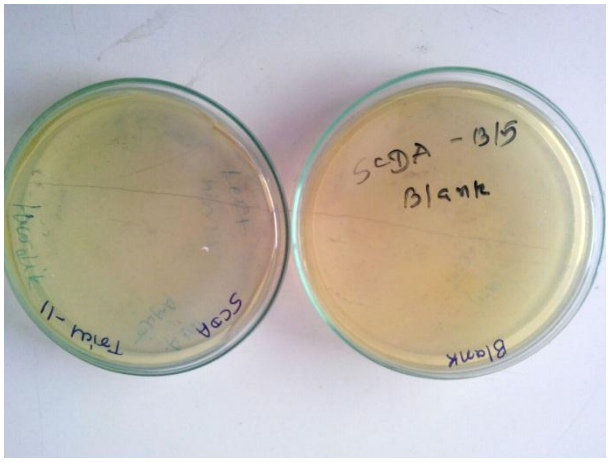

Fig. Trial 23 (Total Bacterial Count + Blank)

Fig. Trial 23 (Total Fungal Count + Blank)

5.1.5.2 Result of Batch 12

TBC	TFC
1000	100
10	10

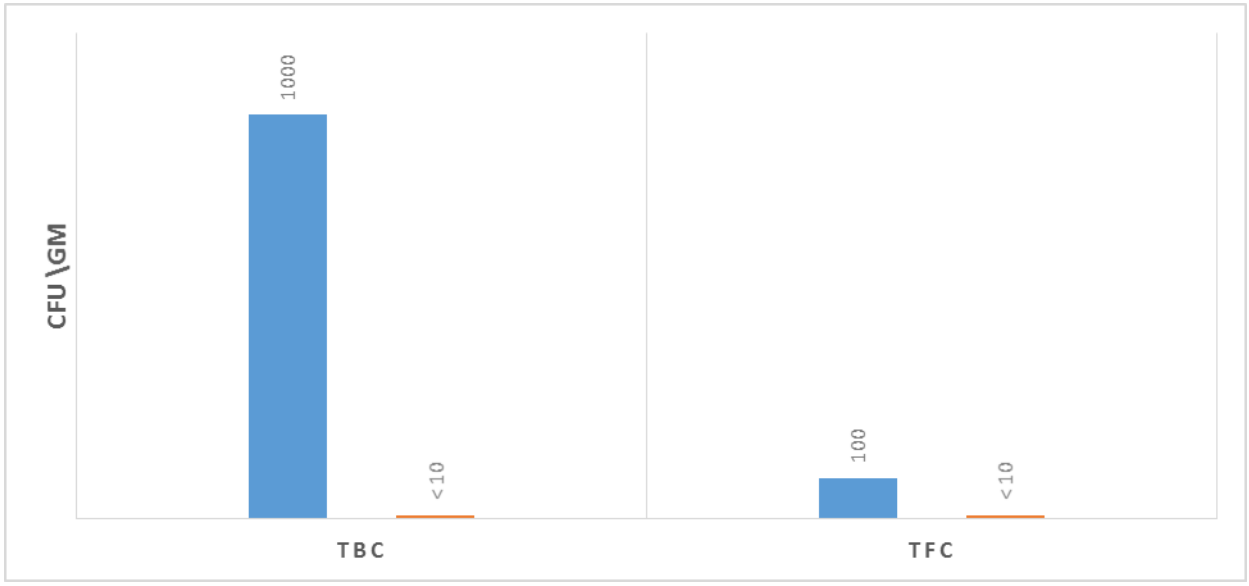

Fig. 24 Graph of Microbial study of Batch 12

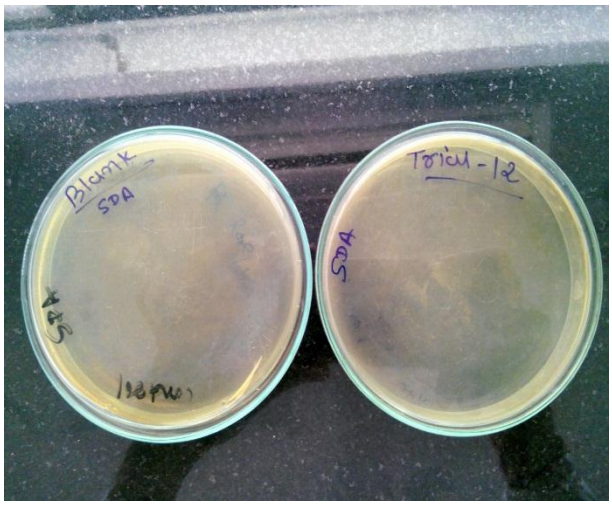

Fig.25 Trial 12 (Total Bacterial Count + Blank)

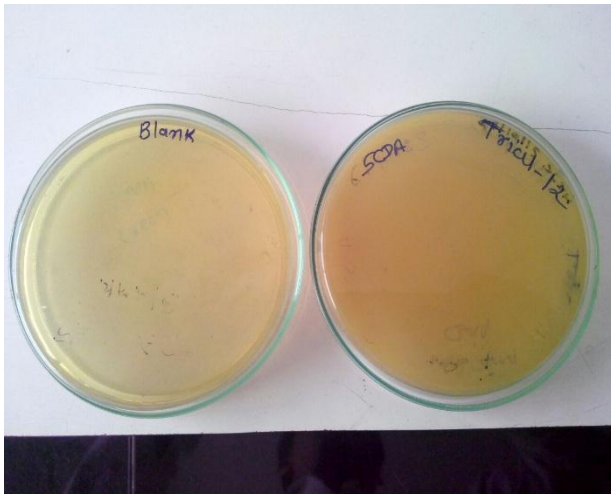

Fig.25 Trial 12 (Total Fungal Count + Blank)

5.1.6 pH testing:

5.1.6.1 Result of all Trials

Batch No	pH
1	4.91
2	4.50
3	4.28
4	4.57
5	4.66
6	4.64
7	4.49
8	3.97
9	4.41
10	4.41
11	4.07
12	4.43

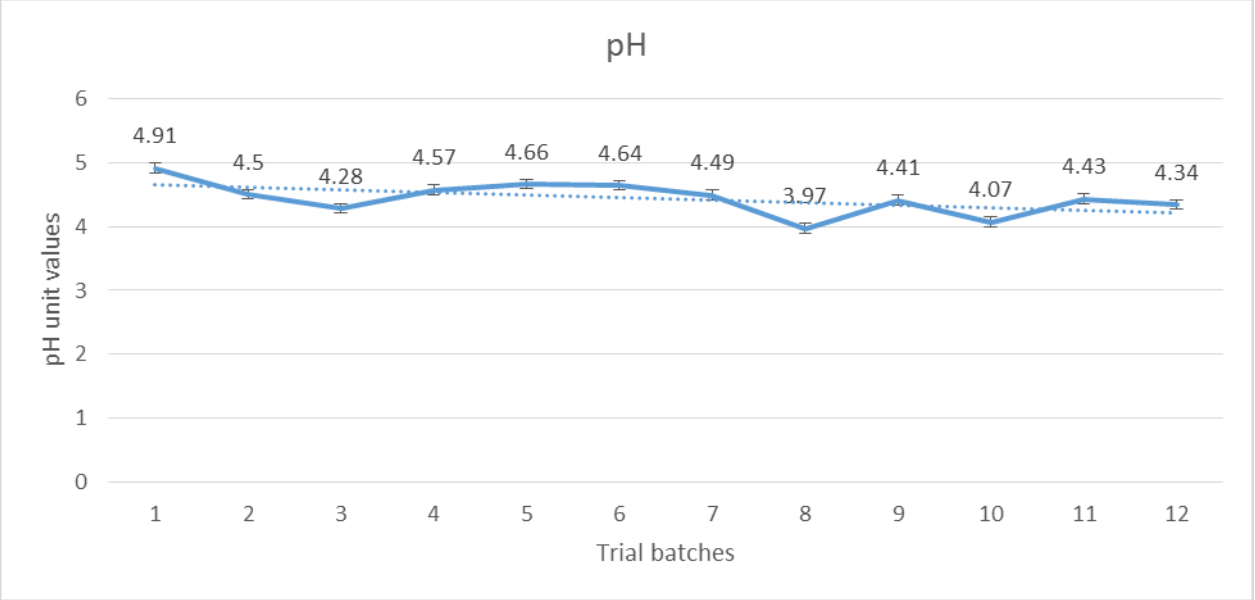

Fig. 26 Graph of pH results of all Trial Batches

5.1.6.2 Comparison of pH drift of all Trial Batch

Batch No	pH	pH drift
1	4.91	0.29
2	4.5	0.34
3	4.28	-
4	4.57	-
5	4.66	-
6	4.64	-
7	4.49	-
8	3.97	2.3
9	4.41	1.6
10	4.41	1.3
11	4.07	-0.4
12	4.43	-0.1

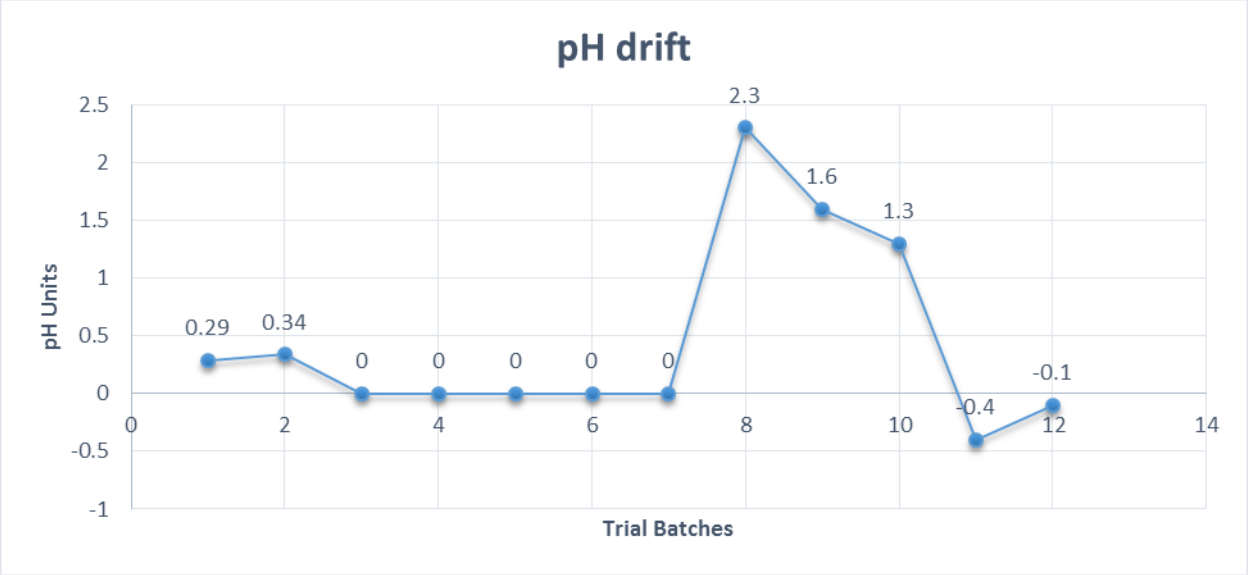

Fig. 28 Graph of pH drift of all stable emulsion at definite storage periods (25°c)

5.1.6.3 Table -29 Correlation with thermal stability and pH drift; Trial 11

Condition	Time	Phase Separation	pH	pH Drift
40°C/75%RH	Initial	Stable	<4.5	0
	1 month	Stable	4.43	4.25
25°C RT	Initial	Stable	<4.5	0
	1month	Stable	4.46	4.16
	2 month	Ongoing	Ongoing	-
55°C	Initial	Stable	<4.5	0
	1month	Stable	4.45	4.22
Sunlight	Initial	Stable	<4.5	0
	1month	Stable	4.42	4.28

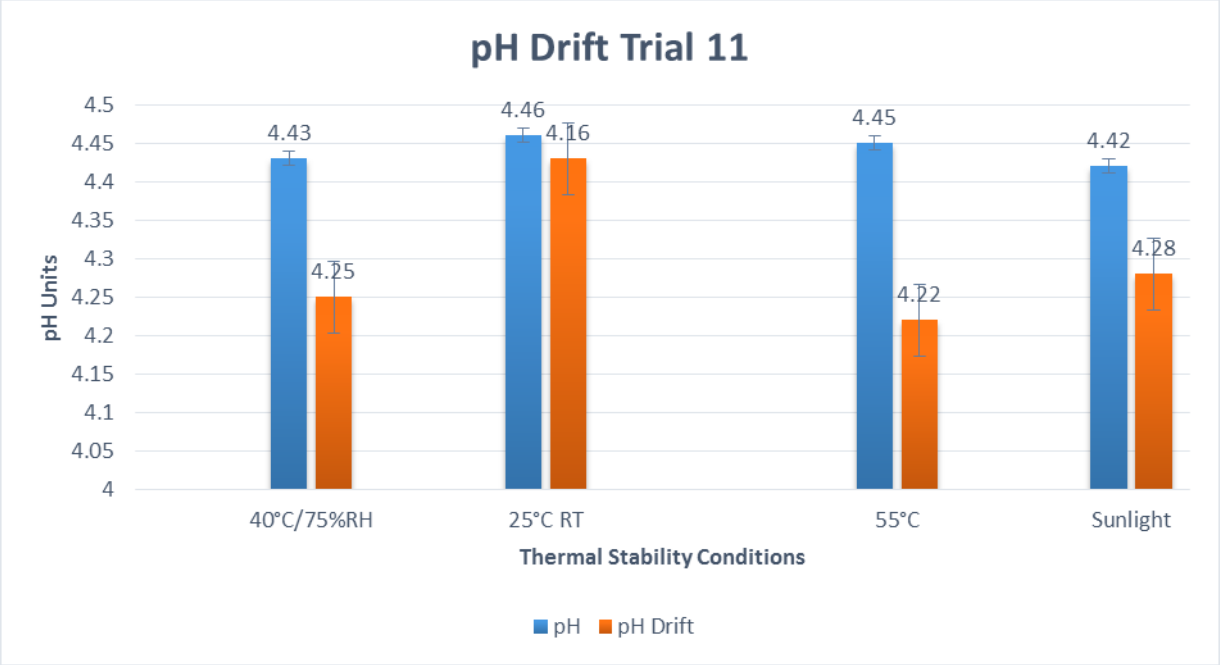

Fig. 30 Graph of pH Drift of Trial 11 at different Thermal stability conditions

5.1.6.4 Table 31- Correlation with thermal stability and pH drift; Trial 12

Condition	Time	Phase Separation	pH	pH Drift
40°C/75%RH	Initial	Stable	<4.5	0
	1 month	Stable	4.35	4.21
	2 month	Ongoing	Ongoing	-
25°C RT	Initial	Stable	<4.5	0
	1month	Stable	4.43	4.34
	2 month	Ongoing	Ongoing	-
55°C	Initial	Stable	<4.5	0
	1month	Stable	4.36	4.22
Sunlight	Initial	Stable	<4.5	-
	1month	Stable	4.36	4.28

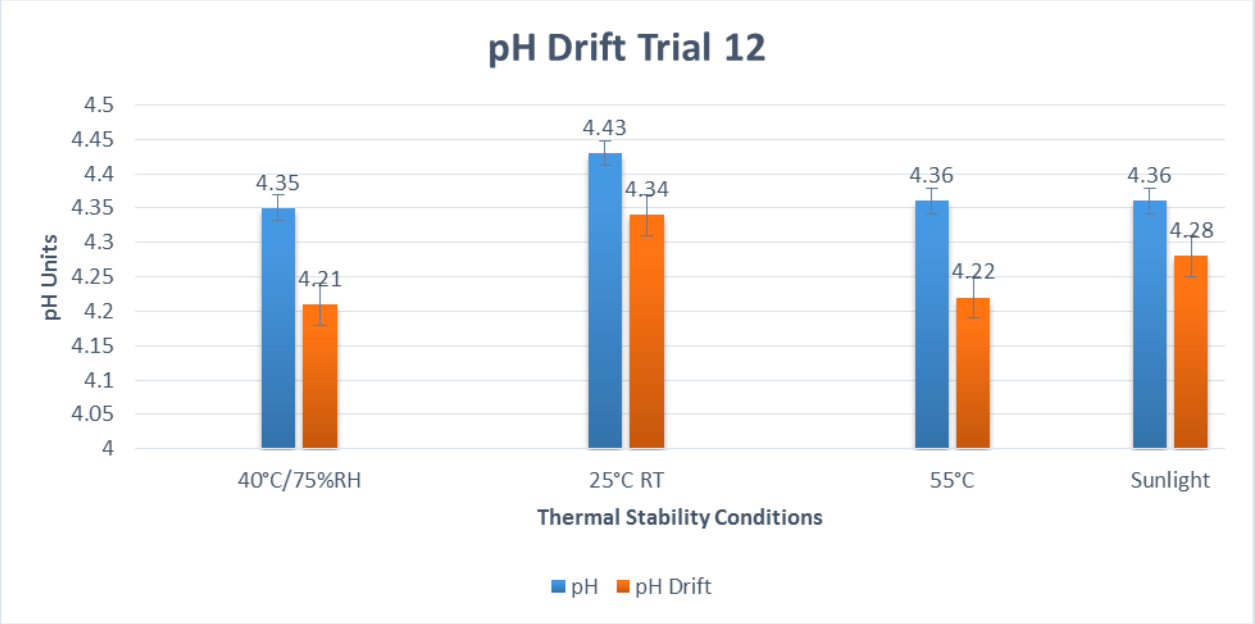

Fig. 32 Graph of pH Drift of Trial 12 at different Thermal stability conditions

5.1.7 Skin Test Meter (Corneometry)

Protocol adherence

With regards to appointment days, few subjects changed one of their appointments during the study, as described below:

For the appointment at T+4 days

- Variation of appointment hour by +5 hours: subject n° 02 (07381) and 04 (14274)
- Variation of appointment day by ±1 day: subject n° 03 (14752).

No other deviation from the protocol has been observed during the study time course.

Study population

Five (05) subjects were enrolled in the study and none of them was absent during the whole study.

Therefore, 05 subjects have completed the study, and the objective to keep at least 5 subjects at the end of the test has been achieved.

Table 33: Description of the main characteristics of the panel enrolled at T0

Number of subjects	05
Mean age in years	57.4
Median age (years)	57

Min age (years)	55
Max age (years)	61
Skin type Dry skin:	23%
Normal skin:	27%
Combination skin:	25%
Greasy skin:	5%

5.1.7.1 Results of the corneometry measurements

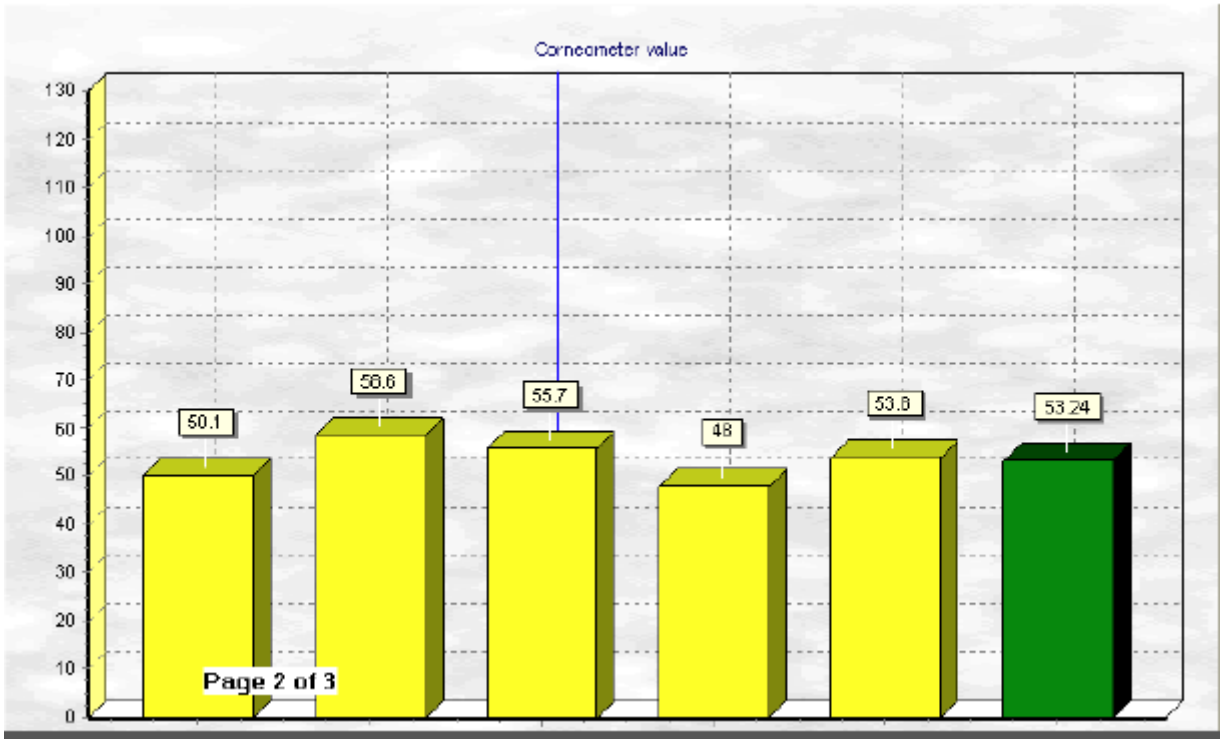

Fig. 34 Image of a Corneometer reading of a sample (Trial Batch 12) on computer monitor at T0

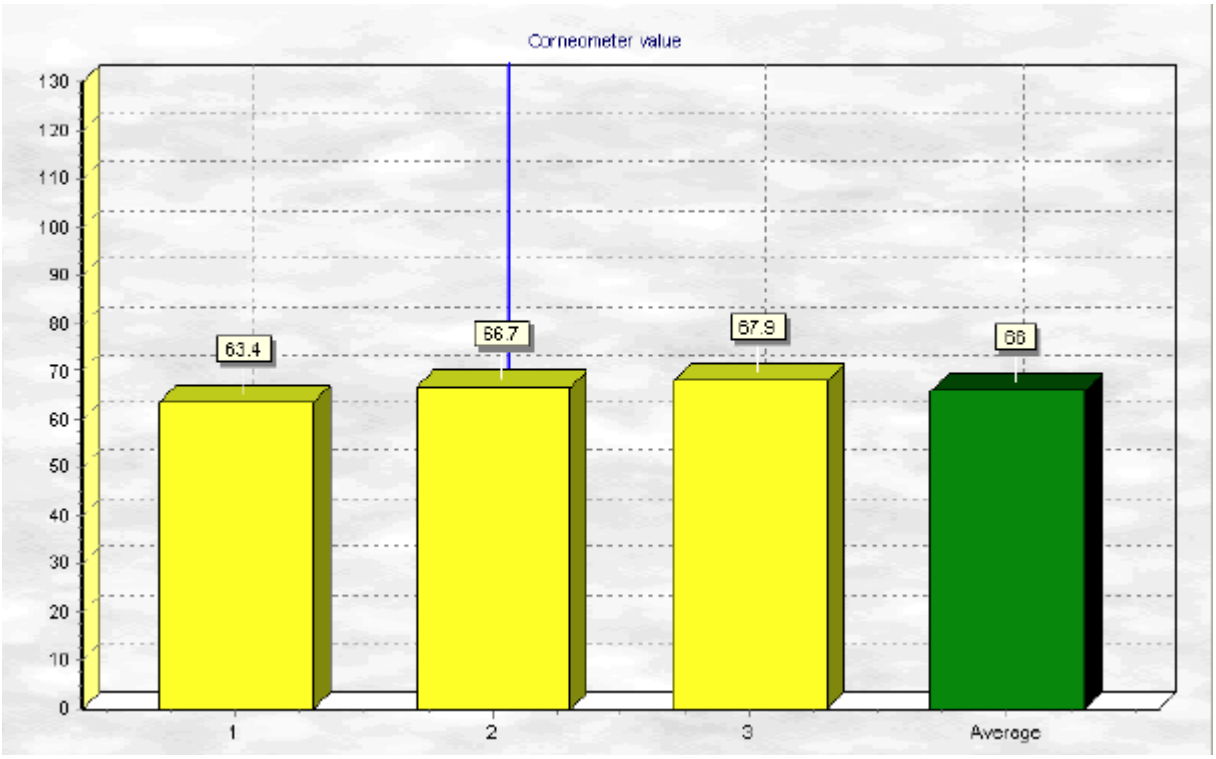

Fig. 35 Image of a Corneometer reading of a sample (Trial Batch 12) on computer monitor at T2

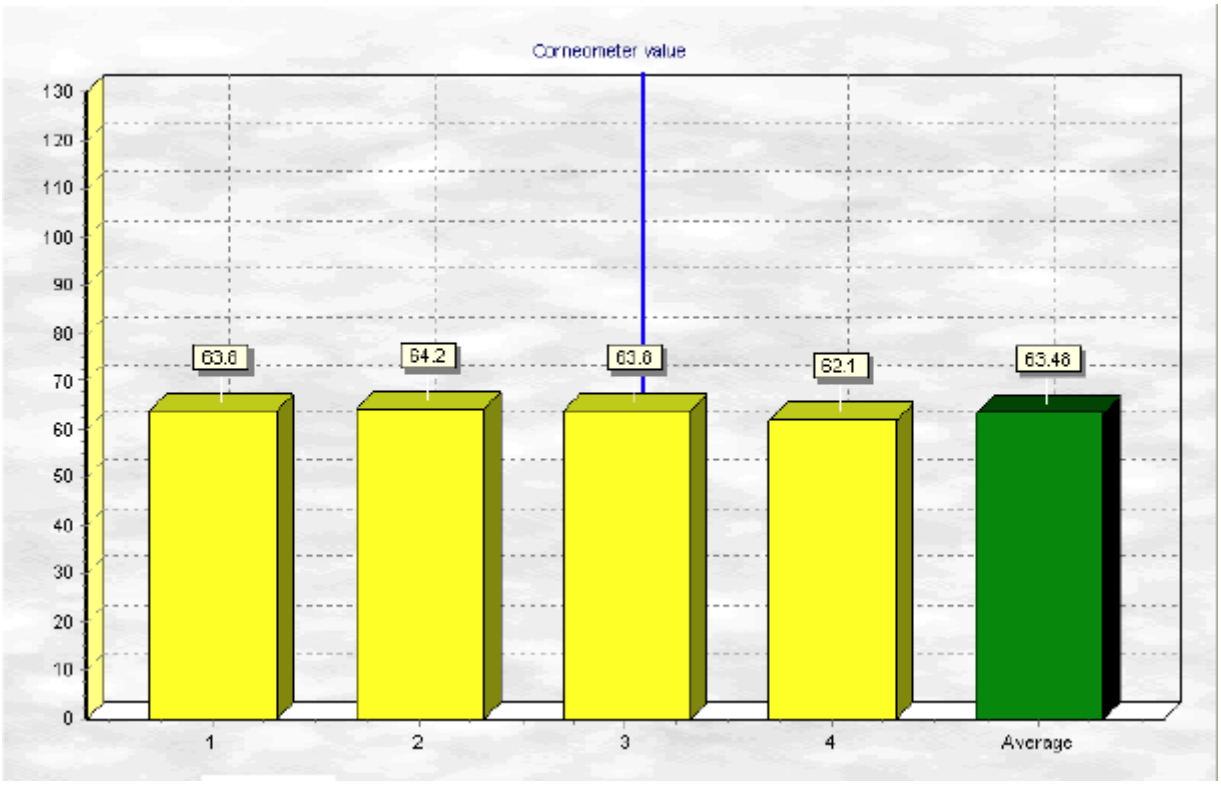

Fig. 36 Image of a Corneometer reading of a sample (Trial Batch 12) on computer monitor at T4

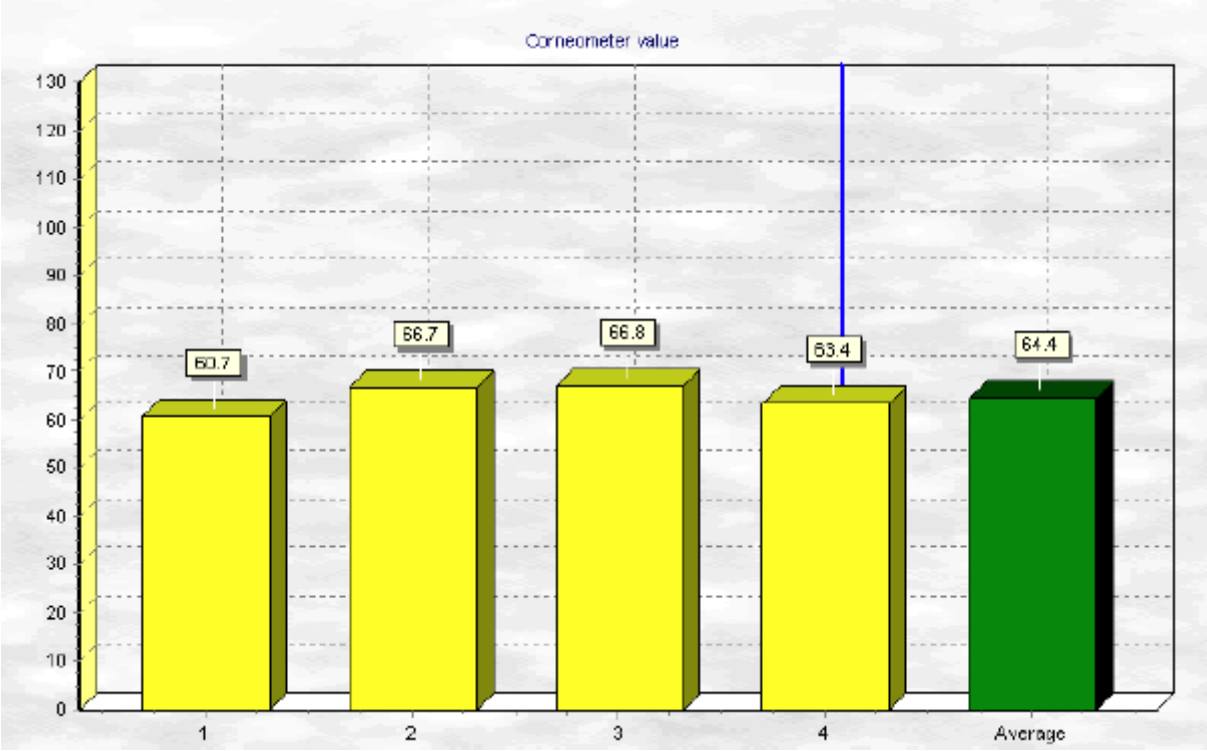

Fig. 37 Image of a Corneometer reading of a sample (Trial Batch 12) on computer monitor at T6

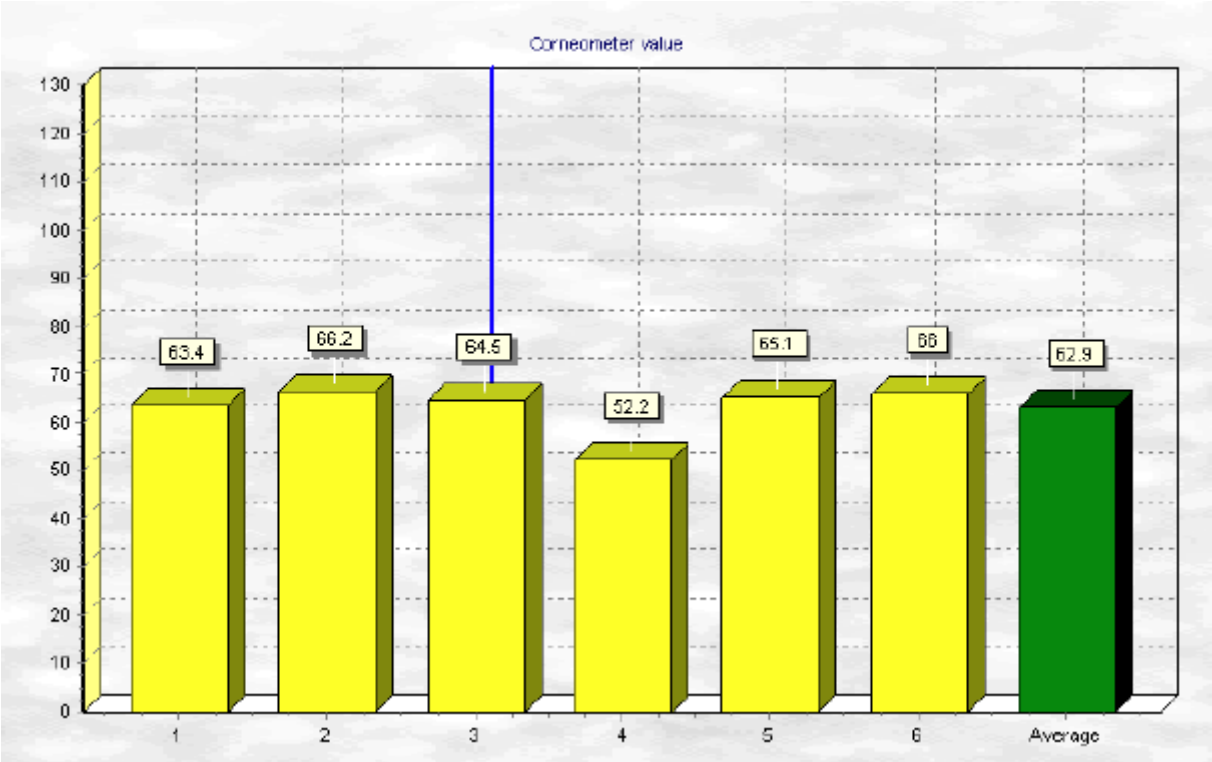

Fig. 38 Image of a Corneometer reading of a sample (Trial Batch 12) on computer monitor at T8

5.1.7.2 Table 39 - Summary of the Corneometer moisturizing measurements performed on the skin (Facial Site) treated with Sample (Trial 12)

TIME	READING	Difference w.r.t baseline	Percentage of Hydration improvement
T0(untreated)	52.2 ± 0.3	-	-
T2	70.8 ± 0.3	18.6	20.6
T4	69.3 ± 0.3	17.1	13.2
T6	68.1 ± 9.0	15.9	13.04
T8	68.9	16.7	13.19

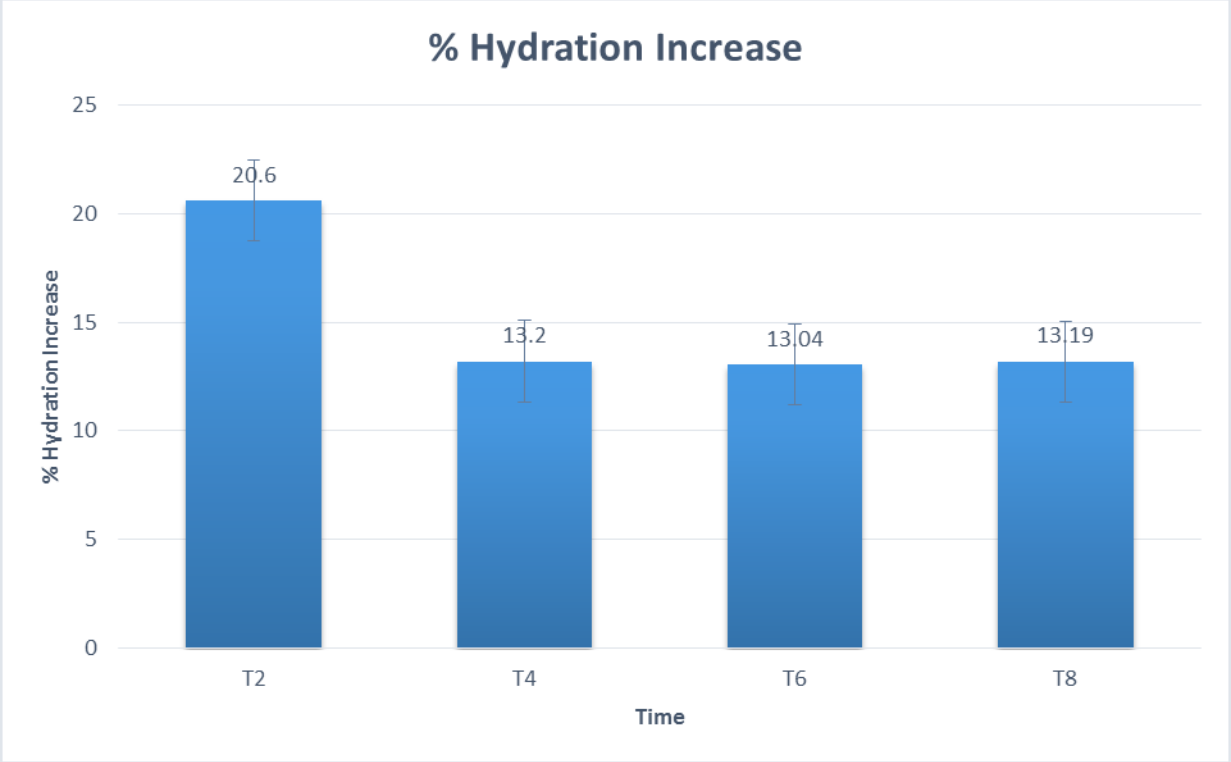

Fig. 40 Graph of Percentage hydration improvement to baseline during 8 Hours after Application

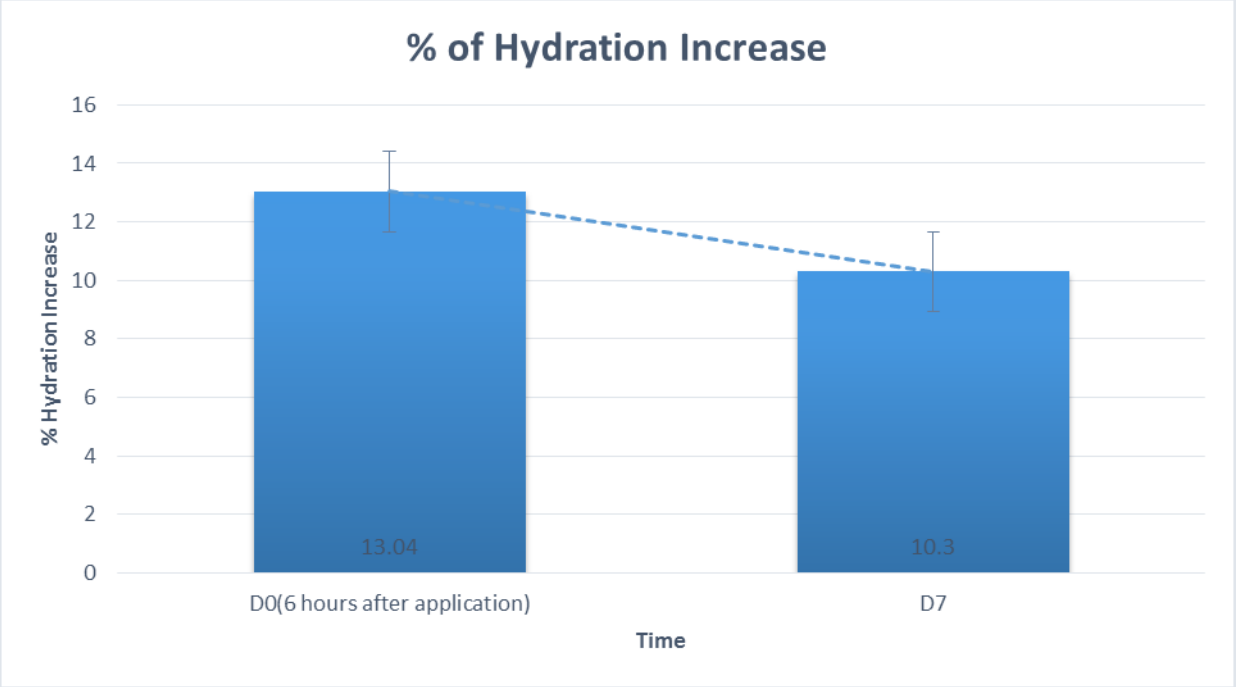

Fig 41. Graph of Percent hydration improvement to baseline at Day 7

5.1.8 Sensory Analysis

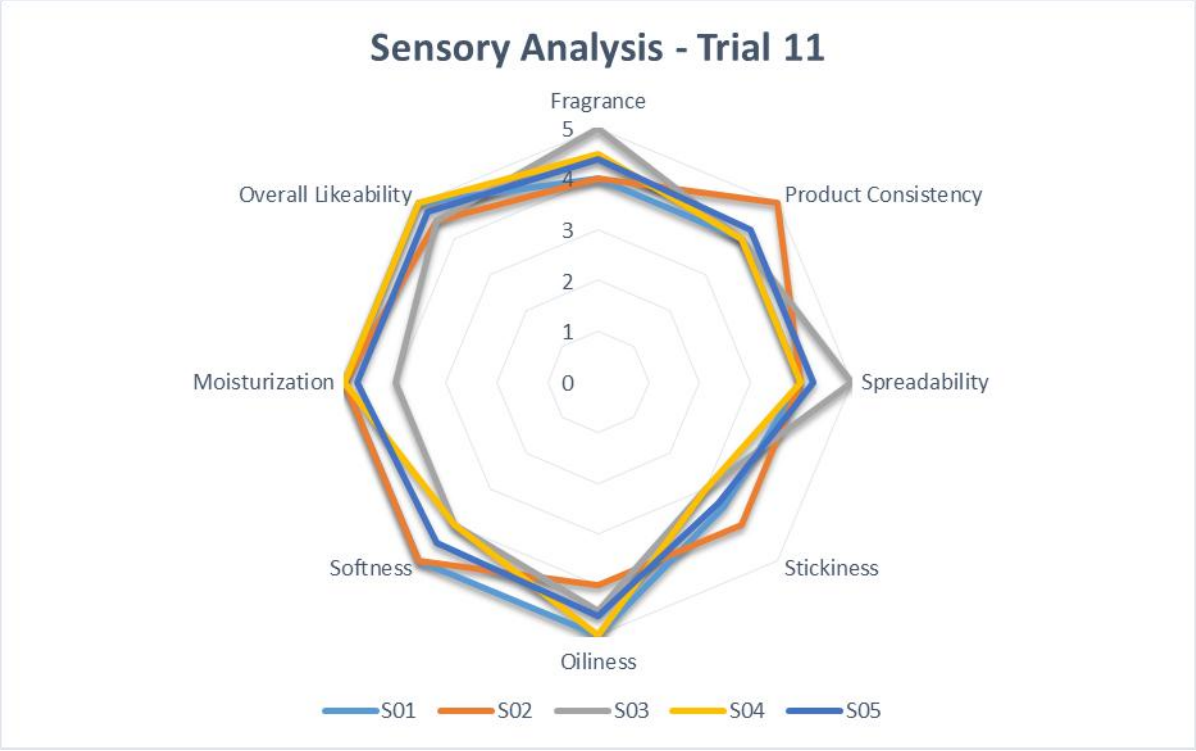

Fig. 42 Graph of Sensory Analysis of Trial 11

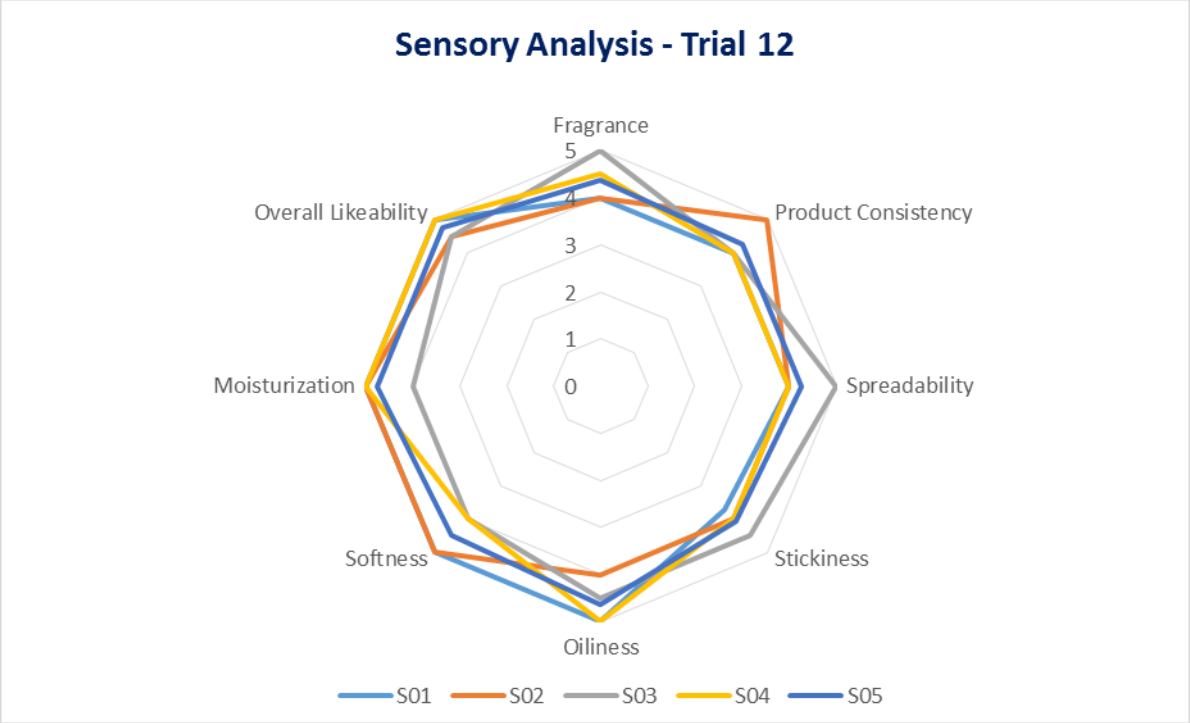

Fig. 43 Graph of Sensory Analysis of Trial 12

5.1.9 Table 44 - Centrifuge Test:

Batch No	Emulsion Separation Test
1	Failed
2	Failed
3	Failed
4	Failed
5	Failed
6	Failed
7	Failed
8	No Separation
9	Failed
10	Failed
11	No Separation
12	No Separation

5.1.10 Table 45 - Product evaluation data for final batch (Trial 12)

Sr No.	Evaluation parameters	Observation
1	Specific Gravity	0.986
2	Loss on drying (LOD)	74.674
3	Viscosity (mPas.s)	180k
4	Thermal Stability	Passed 1 month at 55°C, 45°C 75% RH and at RT
5	Microbial testing	Pass (<10 Cfu)
6	Centrifuge	No separation
7	Corneometry	10% increase (6hours)
8	pH	4.34
9	% Hydration Increase (Corneometry)	~13.1 % Across 8 Hours

5.2 Discussion

Effective moisturizing of the skin is one of the potential alternative route that can improve undesirable characteristics of skin aging and its effects. Aquaxyl being a derivative of two plant sugars, xylitol and glucose reinforces the antidehydration shield together with the activation of water reserves like hyaluronic acid booster and the movement of Aquaporins is a tailor made active for elderly care therapy with extremely beneficial effects. Therefore Aquaxyl was chosen as a moisturizing active and attempt was made to deliver Aquaxyl in Topical dosage form. With a primary aim to design an emulsified, low pH (around 4) cosmetic composition to normalize and improve aged skin functions having improved storage stability and improved pH stability, different types of emulsifiers and thickeners were used. Inititally, Batch-1 was made with carbopol (Acrypol TR-2) thickening system which proved ineffective as the emulsion was not stable. Therefore in Batch- 2 it was changed to Polyethylene Glycol 150 Distearate system which caused the emulsion to have a higher pH and emulsion would break apart. Batch-3 was made with a natural thickening system using Xanthan Gum which caused the emulsion to have a sticky after feel, thermal instability, and contained the characteristic stringyness caused due to higher concentration of xanthan gum. A similar attempt was made to change the concentrations of emulsifiers and thickeners to formulate an aesthetically pleasing and stable emulsion in batch no.4 but it failed to eliminate the characteristic stringiness of

xanthan gum. Batch no 5 was then formulated using a carbopol system (Acrypol ST-100) which has dramatically low pH stable properties but the trial failed thermal stability test along with centrifuge test. Therefore to overcome the above problem of visually unappealing, thermal instability, stringiness in emulsions, and higher pH values, a different type of xanthan gum (Dehydroxy) was used in successive trials along with changing the preservative system from phenoxyethanol to a natural preservatives sodium benzoate and potassium sorbate which are uniquely active at pH range of 4 serving the primary objective of the product and introducing a low pH stable starch based structuring agent. Attempts were made to improve product characteristics in batch no 6, 7, 8 and 9 all of which failed in various product stability testing. Batch no-10 having a lower concentration of natural thickener Xanthan gum had effectively passed the low pH criteria of the formulation and was found stable in thermal stability test. However, its pH 4.07 was very low, and as is with such low pH emulsion systems which experience a downward pH drift during storage, the formula started to drift lower in pH range which caused irritation on skin during application. Batch 10 also failed in centrifuge test due to lower thickening causing an emulsion instability. Lastly, Batch no 11 and 12 were selected for final analysis having passed various stability testing and evaluation parameters. Batch no 11 caused a primary feeling of stickiness and perfume instability during storage period in the sensory analysis by the panel members and hence the xanthan gum was switched to a very versatile carbopol polymer system Ultrez 30 (Lubrizol) in Batch no.12. The resulting final formulation of Batch no. 12 is a successful emulsion with very low pH stable (4.43) profile having a pH drift of 0.15, an improved phase stability, improved storage profile, improved thermal stability (1 month @55^{o}c), improved hydration property of ~13% lasting almost 8 hours in dry and aged skin.

6. Conclusion

There is an increasing demand for face- and body-care formulations tailor-made for the cosmetic treatment of elderly skin. Modern topical formulations not only deliver excellent moisturizing and superfatting capabilities, but also many products, especially face care products, contain one or more actives counteracting the signs of intrinsic and/or photoaging.

Effective moisturizing of the skin is one of the potential alternative route that can improve undesirable characteristics of skin aging and its effects. Aquaxyl is a derivative of two plant sugars, xylitol and glucose. Its mode of action, reinforces the antidehydration shield (Loricrin, essential lipids) together with the activation of water reserves (hyaluronic acid booster) and their movement (Aquaporins, Tight Junctions)

It is a tailor made active for elderly care therapy with extremely beneficial effects. Topical delivery system provides a means to fast absorption into the skin as well as increase the intensity of action and provide ease of applicability directly on site. Therefore Aquaxyl was chosen as a moisturizing active and attempt was made to deliver Aquaxyl in Topical dosage form. Face moisturizing cream of Aquaxyl was prepared by Hot-emulsion method using Xanthan Gum and Ultrez-30 as polymer. In present study various formulations were evaluated using physicochemical evaluation parameters such as specific gravity, Loss on drying (LOD), Viscosity, Thermal Stability and Centrifuge test. In-vitro skin testing study was performed using Courage Khazana skin testing meter with Corneometer CM825 probe.

Result revealed that the product showed good physical characteristics. Microbial load determination was confirmed by using IS 14648 studies. Structural XL was selected as structuring agent and 0.1-5% of Cetyl alcohol was suitable coemulsifier for preparation of face moisturizer containing Ultrez-30 polymer.

In vitro study was also performed. Results indicate in-vitro diffusion profile is similar to ex-vivo diffusion profile. Average moisture content of three transdermal patch of batch H5 was determined spectroscopically and % drug content was found to be 95.05 %

Different concentration of cetylalcohol and silicones were used for preparation of suitable base cream. Batch 11 and 12 (0.1-5 % of Cetylalcohol and 0.1-5 silicone) showed optimum viscosity and pH range as compared to all other batches. It showed acceptable physicochemical and sensory properties.

Thus, a emulsified, low pH cosmetic compositions having improved storage stability and improved pH stability, containing active Aquaxyl was successfully designed and evaluated.

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Design and Evaluation of Face Moisturizer with Specific Needs of Senior Citizen

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