FORMULATION DEVELOPMENT AND EVALUATION OF UNDEREYE DEPUFFING SERUM

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1. ABSTRACT:

This research aims at the development, formulation and evaluation of a novel under eye depuffing serum for reducing the appearance of puffiness. The main objective of the research study was to formulate a product with ingredients having the ability to reduce puffiness, improve microcirculation, nourishes skin and hydrate the delicate under eye area thereby improve overall skin health. The methodology includes optimizing the serum base formula with DOE, extraction of API, development of quality serum with simultaneous studies. The serum was evaluated for its physical characteristics, stability, skin compatibility, and depuffing activity along with its safety and efficacy studies. Results demonstrated that the formulated serum exhibited desirable physical properties, remains stable overtime, and showed excellent compatibility with the skin. In conclusion, depuffing serum represent a significant contribution

to skincare research. Through meticulous formulation processes and rigorous evaluation methods, the serum has been crafted to address the specific needs for reducing puffiness and revitalizing the delicate under eye area.

Keywords: Serum, Wheatgrass, Under eye puffiness, Depuffing, Optimize etc.

2. INTRODUCTION

2.1. SERUM

The term "cosmeceutical" describes the merging of the pharmaceutical and cosmetics industries. In the field of cosmetics, serum is a concentrated product that is often utilized. Skin type mostly determines skin texture, but regardless of product, texture, or preference, everyone can benefit from the same smart component for healthy skin. To deliver the components straight to the skin, skin serum is a type of skin care product that you can apply to your skin after cleansing but before moisturizing. Because serum is composed of small molecules that can penetrate deep into the skin to provide intensive nutrition to the deeper layer and to bring about a very high concentration of active ingredients with a non-greasy finish, it is especially well suited for this task due to its property of rapid absorption. Thus, address the cosmetic issue as soon as possible and with efficiency. The largest and most protecting organ in the body is the skin. A variety of factors, including UV rays, grime, and makeup left on overnight, can occasionally cause the skin to become dry. **[2]** Serum is appropriate for all ages. Concentrate always yields more than just instant cosmetic results. but also psychological fulfillment following the procedure because results will be noticeable almost right away. **[8]** In addition to providing your skin with a smoother, denser texture, serum also functions as an anti-aging skin treatment, reduces pore formation, intensively hydrates, and prevents wrinkles. Chemical substances known as antioxidants have the ability to attach to free oxygen radicals and stop them from harming healthy cells. **[1]**

2.1.1. Benefits of face serum:

1. Has antiviral and cell-regenerative qualities that soothe inflamed skin. A product with a high concentration of water or oil called cosmetic serum. **[16]**
2. Deep moisturizing: facial serum has the special capacity to both increase and retail skin hydration.

3. Due to its anti-inflammatory qualities, it helps to reduce acne and remove blemishes. It stops the accumulation of germs, which is the primary cause of acne and pimples.
4. Eliminate dark circles and puffiness: Rich in antioxidants and vitamin E, it helps with puffiness and discolouration around the eyes.
5. It lessens the visibility of under-eye circles.
6. By eliminating dead skin cells, it aids in the promotion of collagen formation.
7. Its antioxidant qualities encourage skin that glows. **[5]**
8. Enhances skin elasticity, minimizes pores, hydrates and nourishes the skin, and improves the texture of the skin. **[6]**

2.2. PUFFY EYES

The examination of human skin is a crucial field for research and development in the fields of dermatology, toxicology, pharmacology, and cosmetology. This is because it allows for the evaluation of exogenous drugs' effects, interactions, absorption mechanisms, and/or toxicity towards various cutaneous structures. Humanity has recognized the value of aesthetics since the prehistoric era, and the desire to appear well and attractive has grown across society. contain almost ten times as many physiologically active ingredients as creams, enabling more effective therapy of skin issues. A moisturizer that delivers high-performance ingredients to the skin and fortifies the skin's barrier function is an emulsion-based face serum.

An emulsion is made up of two "immiscible" phases—phases that don't want to mix, such as water and oil. Water and oil are bound together and kept in a stable form by the application of an emulsifier. Using an emulsion gives you the best possibility of introducing potent actives deeper into the skin's tissues. Since the skin acts as a barrier, it is quite difficult for any cosmetic ingredient to get through the dermis; nonetheless, an oil and water combination works well to achieve this amazing result. The moisturizing properties of the emulsion will reinforce the skin's barrier function.

When creating a face serum, stability, and physical attributes are the primary factors that define its quality. Fundamentally, a face serum formulation is an emulsion made of two immiscible liquids. Thickener and emulsifier are added to the system to stop emulsion instabilities.. Emulsifier will balance the system by minimizing the interfacial tension between the two immiscible liquids and at the same time stabilizes the dispersion phase from coalescence. Contributing to the system, thickener also plays an important role as rheology modifier and

provides flexibility to the flow characteristic of the emulsion. pH value is one chemical characteristic that is thought to be significant in formulating. The skin's natural microbiology and function will be disrupted if the pH level is altered. This leads to a host of skin issues, such as severe conditions like acne, excessive sebum secretion, and flaky skin. The skin's natural micro flora remains intact when the pH value falls between 4 and 4.5, but it is eliminated when the pH value falls between 8 and 9. Improving the function of the skin barrier can be achieved by creating a face serum with an appropriate pH range.

Formulating a product like face serum or any other formulation might be challenging. This is because the traditional One Factor at a Time (OFAT) method was highly time consuming and may result in unnecessary experiment that consume costs. OFAT was done by changing one factor at a time while keeping the other factor as constant. It requires large number of tests. On the contrary, optimization using Design Expert software could save time, costs and labor. **[6]**

The skin under the eyes is some of the thinnest layer in the body. Puffy eyes can be caused by several factors such as fluid retention due to high alcohol or salt intake, emotions especially crying, allergies, hormone changes, insufficient sleep and other factors as well. **[3]**

2.2.1.Etiology/ epidemiology:

Causes of puffy eyes can be divided depending on the underlying mechanism.

- Increased Capillary Hydrostatic Pressure.
- Regional Venous Hypertension (often unilateral).
- Increased Plasma Volume.
- Drugs and chemicals.
- Protein Loss.
- Malnutrition/mal absorption.
- Increased Capillary Permeability.
- Aging**. [9]**

2.3. ACTIVE HERBAL INGREDIENT: WHEATGRASS

- Wheatgrass is the young shoot of *Triticum Aestivum* and belongs to the family Poaceae.
- It shows various activities like anti-inflammatory, anti-obesity, anti-cancer, antiflatulence, detoxification, antioxidant, antibiotic, skin rejuvenation and protective properties for heart.
- Superoxide dismutase (SOD) and cytochrome oxidase, two antioxidant enzymes that have the ability to convert reactive oxygen species (ROS) into hydrogen peroxide and oxygen molecule, are known to be present in wheatgrass. Although wheatgrass has been studied for potential health benefits, its activity in serum formulation may be related to its antioxidant properties, potential anti-inflammatory effects, and potential ability to support detoxification processes. **[26]** A significant crop farmed in temperate climates is T. aestivum. From 47°N to 57°S, it can grow in most types of climate. T. aestivum grass, which is obtained after 6–10 days of germination, was likewise observed to preserve the restoration of plasma glucose and insulin levels together with hepatic glycogen. T. aestivum has the capacity to flush the body of heavy metals and carcinogens, as well as toxins from the blood and hepatic region. **[25]**

2.3.1. The key ingredients of wheatgrass and their uses:

2.3.1. Vitamin A: It enhances the skin luster and provides glow to the outer skin. It helps to cure the black spots and blemishes below the eyes and improves the eyesight.

2.3.2. Vitamin B: It is helpful in preventing premature aging.

2.3.3. Vitamin C: Wheatgrass contains more Vitamin C than an orange. A powerful antioxidant, it is essential for health and vitality and healing of sores and wounds. It is also a natural source for antibiotic elements

2.3.4. Vitamin E: It dilates the capillaries and enables free flow of the blood. Without enough of this fat-soluble vitamin, we would face muscle degeneration, sterility, and slower healing of wounds and infections.

2.3.5. Vitamin K and B-complex vitamins: Wheatgrass is also a source of vitamin B17, also known as amygdaline, which some studies suggest can help ward off cancer. In addition to these vitamins, wheatgrass contains 17 amino acids and

92 different minerals the human body uses and needs. The nutrients in wheatgrass are also said to assist in fighting cancer.

2.3.6. MSM - MSM is a Sulphur bearing molecule found in all living organisms destroyed in processed food. It helps our body use vitamins, helps to reduce allergies, helps detoxify the body and increase oxygen, and takes out inflammation.

2.3.7. Proteins and Amino acids

2.3.8. Minerals

- Iron: Iron deficiency creates shortage of haemoglobin in blood, iron salts in wheatgrass have no side effects.
- Calcium: Acting as a buffer to restore balance to blood pH.
- Potassium: Helpful for the radiance and luster of youth, hypertension, helps maintain a smooth mineral balance, firms the skin, and promotes overall beauty.
- Zinc
- Sodium: Regulates the extra cellular fluid volume. It also regulates the acid-base equilibrium and maintains proper water balance.
- Magnesium: As it aids eliminative functions and is also responsible for drawing fat out of the liver.

2.4. Nutrients and other substances:

Chlorophyll is a source of alkaline, preventive, and curative elements. Wheat grass juice contains these elements as well as other elements. Human blood has a pH value of 7.4. The wheat grass juice's rapid absorption in the blood can be attributed to its pH of 7.4. **[10]** One of the main ingredients in wheatgrass extract, chlorophyll, has been shown to improve blood coagulation and fortify the immune system by preventing the metabolic activation of carcinogens. **[27-28]** Wheatgrass has glabrous, simple, hollow or pithy culms. Its leaves are flat, narrow, 20–38 cm long and 1.3 cm broad, with a height of around 1.2 meters. **[36**] Research has demonstrated the possible anti-inflammatory and anti-aging effects of wheatgrass. **[38]** Additionally, 33.26 g of potassium per 100g of wheatgrass is added. Potassium is essential for controlling the balance of minerals and fluids in body cells. **[39]** Wheatgrass's anti-inflammatory qualities have a positive impact on reducing pain and swelling.**[41]**

Wheatgrass is said to be able to treat a wide range of illnesses, such as the common cold, coughs, bronchitis, fevers, infections, and sore mouths and throats. Wheatgrass was used in traditional medicine to cure a variety of conditions, including gout, cystitis, rheumatic pain, chronic skin conditions, and constipation. Proponents claim that wheatgrass increases oxygen levels in the body and can prolong the lives of cancer patients by equating haemoglobin, which carries oxygen in the blood, with chlorophyll, the substance that gives wheatgrass and other plants their green color. They contend that a diet high in wheatgrass boosts immunity, eradicates dangerous germs from the digestive tract, and cleanses the body of waste products and toxins. Because it enriches the blood, wheatgrass also increases metabolism and the body's enzyme systems. By widening the blood vessels all over the body, it also helps lower blood pressure. **[12]**

2.5. Mechanism of action:

The structural building block of chlorophyll is a tetra pyrrole ring with a magnesium atom in the core. This ring has a striking resemblance to the iron-centred heme group found in haemoglobin. By inhibiting P450 cytochrome enzymes and causing the creation of Phase II enzymes, chlorophyll possesses significant biological activity. This indicates that the plant is healthy for people and has anti-inflammatory and anti-proliferative properties. **[13]** Furthermore, it has the capacity to prevent oxidative DNA damage**. [29]** Clinical research regarding the efficacy of herbal medicines was first funded by the National Institutes of Health's National Centre for Complementary and Alternative Medicine in the United States in 2002.

A survey of one thousand plants was finished in 2010, and 356 of those plants had clinical trials published assessing their pharmacological activities & medicinal uses. One of these plants, wheatgrass, has been used for thousands of years in Indian culture and is well renowned for its extraordinary medicinal qualities**. [35]** Vegetable wheatgrass is collected before the plant produces a blossom head. Per 3.5 grams, wheatgrass has an impressive amount of nutrients, including 18.5 mg of chlorophyll, 15 mg of calcium, 38 mg of lysine, 7.5 mg of vitamin C, and an abundance of micronutrients, including amino acids and B complex vitamins.**[37]** Wheatgrass is a physiochemical that contains alkaloids, carbohydrates, saponins, gum, and mucilage. Because of its high water-soluble chlorophyll content—roughly 70% of it is soluble in water—it has a higher water-soluble extractive value than alcohol-soluble extractive value.**[36]**

A study on Effect of various treatments on formalin induced paw edema in rats done by Sai Krishna Priya Dasari, et al. has found that Wheatgrass tablet showed 73.379 % Inhibition as compared with standard and with respect to control. This confirms wheat grass possess a significant anti-inflammatory activity against chronic paw edema induced by formalin in experimental model**.[42]**

3. MATERIALS AND METHOD

3.1. MATERIALS USED:

3.1.1.ACTIVE PHARMACEUTICAL INGREDIENT:WHEATGRASS

Fig. 1. Wheatgrass and powder.

Synonym: Gehu, Kanak in ayurveda calls it as godhuma.

Biological source: *Triticum aestivum* Linn.

Family: Poaceae

Geographical source: Found throughout the north temperate zone, India, United states. **Morphological characteristics:** Wheatgrass (*Triticum aestivum*) typically has narrow, bladelike leaves that are bright green in color. The leaves are flat and grow from the base of the plant in a tufted manner. The stems are hollow and jointed, and they can grow up to around 2 feet in height.

Colour: Green.

Odour: The odor of wheatgrass is often described as fresh, earthy, slightly sweet and pleasant.

Taste: The taste of wheatgrass is typically described as slightly bitter, grassy, and earthy.

Chemical constituents: Wheatgrass is packed with nutrients, including vitamins, minerals, amino acids, and enzymes. Some of its key chemical constituents include chlorophyll, vitamins A, C, and E, iron, calcium, magnesium, and amino acids like lysine and tryptophan.

Solubility: Powder is soluble in water and alcohol.

Uses: It is used to treat cold, coughs, fevers, gougout, infections, joint pain, reduces inflammations, etc.

3.1.2.CULTIVATION AND COLLECTION OF WHEATGRASS:

Wheat seeds were purchased from the local market in Nagpur.Seeds of Triticum aestivum were soaked in tap water for 24 hours before being covered with a damp cotton cloth and left to grow for 48 hours or until it gives sprouts. Next day, the seeds were spread and germinated in trays containing soil. On perforated soil bed trays within, the sprouted wheat seed were next set. The trays were monitored daily and watered as per the need. Wheatgrass plant of 6-8 inches was used**.[1]** The trays were positioned so that the young plants could get regular airflow and sunlight, and the sprouts were watered twice daily. Wheatgrass is the result of the sprouted seeds transforming into young leaf blades. The wheat grasses were harvested on the 7-10th day after the sprouting period. **[11]** It must be cut at the edges so that it can give the second crop rinse the grass lightly as it doesn't need heavy washing as it is grown from organic seeds from organic soil**.[42]**

3.1.3.EXTRACTION OF WHEATGRASS POWDER

The extraction of wheatgrass by lyophilization, also known as freeze-drying, involves several steps:

1. Harvesting: Wheatgrass is typically harvested when it reaches the desired height, usually around 6-8 inches on 7^{th} day of its growth as it has maximum therapeutic and medicinal content and activity on 7^{th} day.
2. Preparation: The harvested wheatgrass is thoroughly cleaned to remove any dirt or debris and grind in the mixer blender with water to obtain juice. Strain it to remove the solids . this juice is filtered in a Vacuum Filtration to remove the particle.
3. Freezing: The wheatgrass juice is then quickly frozen to preserve its freshness and nutrients. This is usually done using specialized freezing equipment.
4. Lyophilization: The frozen wheatgrass is placed in a vacuum chamber, where it undergoes a process called sublimation. In this process, the frozen water in the wheatgrass directly transitions from a solid to a gas, bypassing the liquid phase. This removes moisture from the wheatgrass while preserving its nutritional content and flavor. Lyophilized wheatgrass powder retains most of its nutrients and can be reconstituted by adding water before consumptions. This process is also called as freeze drying.
5. Packaging: Once the lyophilization process is completed, the dried wheatgrass powder is packaged into containers or sealed bags as to protect it from moisture and oxidation**.[42]**

3.2. PHYTOCHEMICAL SCREENING

Several chemical assays were performed on the wheatgrass aqueous extract to ascertain whether secondary plant components were present.

1. Sugar-Reduction Test: Two milliliters of the extract were combined with five milliliters of Fehling's solution A and Fehling's solution B (1:1), and the mixture was heated in a water bath for five minutes. The presence of free reducing sugars was indicated by a brick-red precipitate.
2. Test for the presence of Anthraquinones: 0.5 g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken; the presence of a pink, red or violet color in the ammoniacal (lower) phase indicated the presence of anthraquinones.
3. Saponin Test: Boiling on a water bath produced an aqueous extract. After the extract was put into a test tube, it was given a good shake, allowed to stand for ten minutes, and the outcome was recorded. Saponins can be identified by a thick, sticky foam.
4. Test for Flavonoids: Extract of the sample was reduced to dryness on the boiling water bath. The residue was treated with dil. NaOH, followed by addition of dilute HCl,

solubility and color was noted. A yellow solution with NaOH, which turns Colorless with dil. HCl, confirms flavonoids.

5 Tannin test: 5 ml of water was mixed with 0.5 g of the extract, and then a few drops of 10% ferric chloride were added. Tannins would be present if a precipitate turned blue-black, green, or blue-green.

6 Alkaloids test: On a water bath, 0.5 g of extract and 5 ml of 1% aqueous hydrochloric acid were mixed. The filtrate was then treated with Dragendorff's reagent. The presence of alkaloids in the extracts would be indicated by turbidity or precipitation with any of these reagents.

7 Terpenoids test (Salkowski test): 5 ml of extract was combined with 2 ml of chloroform, and 3 ml of precisely mixed sulfuric acid was added to create a layer. The interface developed a reddish-brown coloring that indicated the presence of terpenoids.

8 Phenolic Content Total Calculation Using the Folin-Ciocalteu reagent, the phenolic compounds present in each of the extracts were identified, following the method suggested by Singleton and Rossi (15). This method was based on the observation that phenolics reduce a mixture of phosphate tungsten and molybdenum oxides complex to produce a blue-colored result. 250 µl of FC (2 N Folin-Ciocalteu) reagent was added to 50 µl of a sample or standard, along with 3.5 ml of water. After mixing, the liquid was allowed to sit at room temperature for eight minutes. After adding 750 µl of a 20% sodium carbonate solution to the combination above, it was allowed to sit at room temperature for two hours. At 765 nm, absorbance was measured following incubation. A gram of dry extract's total phenolic content (TPC) was quantified as milligrams of gallic acid equivalents. **[1]**

3.3. **EXCIPIENTS:**

3.3.1. Carbopol: USP: Carbopol Chemical name and CAS registry number: Carboxy methylene (54182 – 57 – 9) (2007 – 3) for Carbopol 973 (76050 – 42 – 5) for Carbopol 940 Functional category : Suspending agent, Emulsifying agent, binder, viscosity increasing agent. Application: Carbopol are mainly used as in liquid or

semi solid cosmeceuticals formulations which includes cream, gels and ointments. Carbopol are also employed as a emulsifying agent in the preparation of oil in water emulsion

3.3.2. Disodium EDTA: Synonym: Disodium dihydrogen ethylenediamine tetra acetate Disodium edentate, disodium salt, dehydrate Chemical name: C10H14O8N2Na2.2H2O CAS no.: 139 – 3 Molecular weight: 372.24 Functional category: Stabilizer, chelating agent. Description: White crystals, it is odorless Melting point: 252°C Solubility: 10gm in 100ml of water Storage: Stored in well closed container.

3.3.3. Tri ethanol amine: Synonym: TEA, Triethyl olamine, trihydroxy triethyl amine Chemical name: 2,2,2notrilotriethanol CAS No.: (102 – 71 – 6) Empherical formula: C6H15NO3 Molecular weight: 149.19 Structural formula: N(CH2CH2OH) Functional category: Alkalizing agent, Emulsifying agent Description: Clear colorless to pale yellow color Viscous liquid acing a slight ammonical Odour.

3.3.4. Glycerin: Synonym: glycerin, glycol G-100; 1,2,3 – propanetriol Trihydroxy propane glycerol Chemical name: Propane-1,2,3-tiol Empherical formula: C3H8O3 Molecular weight: 92.09. Functional category: Humectant, emollient, solvent, plasticizer Specific gravity: 1.249 Description: Glycerin is clear, colorless, odorless, viscous, hygroscopic liquid. Melting point: 17.8°C Boiling point: 290°C Solubility: It is slightly soluble in acetone, practically Insoluble in benzene, chloroform and oil Miscible with ethanol. Storage: Store in well closed container.

3.3.5.Water: Synonym: Aqua; hydrogen oxide Chemical name: Water CAS no: 773218-5 Empherical formula: H2O Molecular formula: 18.0 Description: Water is clear, colorless and tasteless Liquid. Melting point: 0°C Boiling point: 100°C Solubility: Miscible with most polar solvent. Storage: Water for specific purposes should be Stored in appropriate container.

3.4. CHEMICALS:

All the chemicals used during this project work are of the standard quality and of laboratory grade from the quality vendors.

Sr. no.	Chemicals	Grade	Supplier

1.	Demineralised water	EP Grade	ASHI Enterprises
2.	Glycerine	EP Grade	ASHI Enterprises
3.	Disodium EDTA	EP Grade	ASHI Enterprises
4.	Allantoin	AR Grade	DKSH India Pvt ltd
5.	Propylene glycol	EP Grade	ASHI Enterprises
6.	Xanthan gum	AR Grade	ASHI Enterprises
7.	Carbopol 940	AR Grade	ASHI Enterprises
8.	Triethanol amine	AR Grade	ASHI Enterprises
9.	Euxyl PE	AR Grade	Yasham speciality ingredients

3.5. **EQUIPMENTS USED:**

Sr. No.	**Apparatus**	**Supplier**
1.	Electric stirrer	Remi motors ltd., Mumbai
2.	Lyophilizer	LABCONCO
3.	Viscometer	Brookfield
4.	pH meter	Microprocessor
5.	Spreadability test apparatus	Fabricated at Dadasaheb Balpande college of Pharmacy
6.	Glasswares	Gupta Agency, Ambala cantt,. Haryana
7.	Mortar and pestle	Gupta Agency, Ambala cantt,. Haryana
8.	Weighing machine	Shimadzu
9.	DOE software	System solutions, Mumbai

3.6. **BASE FORMULA OPTIMIZATION**

The preparation of serum base is very important before incorporating active ingredient.

Sr. No.	**Ingredients**	**BF1**	**BF2**	**BF3**
1.	DM water	q.s to 100	q.s to 100	q.s to 100
2.	Disodium EDTA	.1	0.1	0.1
3.	Allantoin	0.1	0.1	0.1
4.	Propylene glycol	2	3	3
5.	Glycerin	3	5	5
6.	Xanthan gum	0.2	0.2	0.25

7.	Carbopol 940	0.1	0.2	0.2
8.	TEA (10% soln)	1.5	1.3	1.6
9.	Euxyl PE 9010	0.5	0.5	0.5

3.7. **FORMULATION DEVELOPMENT :**

Design of experiments (DOE) is a systematic, efficient method that enables scientists and engineering to study the relationship between multiple input variables (aka factors) and key output variables. It is a structured approach for collecting data and making discoveries.

DOE used to optimize the process of manufacturing a part, identify the cause of a quality problem, or reduce the variability of a process, which is a measure of quality, also used for exploratory work, optimization, study response factor relationship and comparison.

Std	**Run**	**Factor 1 A:Xanthan gum (gm)**	**Factor 2 B:Triethanol amine (ml)**	**Response 1 Viscosity (cps)**	**Response 2 pH**
7	(F1)	0.2	0.6	1542	7.67
4	(F2)	0.2	0.35	669	6.72
8	(F3)	0.4	0.6	13800	7.53
2	(F4)	0.4	0.1	852	5.31
1	(F5)	0.2	0.1	4700	5.59
5	(F6)	0.4	0.35	4656	6.94
9	(F7)	0.6	0.6	7600	7.32
6	(F8)	0.6	0.35	19700	8.69
3	(F9)	0.6	0.1	25600	7.37

- Design type: 3 level factorial (3^2) where, variable: 2 and level factors: 3 ✦Design model: Quadratic.
- Runs: 9.00
- Subtype: Randomized.

- Study type: Response surface.
- Independent factors: Xanthan gum and triethanolamine.
- Dependent factors: pH and viscosity.

Formulation table of the optimized batch:

Sr.no.	Ingredients	Function	Quantity
1.	Demineralised water	Solvent base	94 ml
2.	Glycerine	Humectant	3 ml
3.	Disodium EDTA	Chelating agent and stabilizer	0.1 gm
4.	Allantoin	Emollient	0.1 gm
5.	Propylene glycol	Humectant	2 ml
6.	Xanthan gum	Viscosity modulator	0.4 gm
7.	Carbopol 940	Lubrication of eyes, stabilizer, suspending agent, thickener	0.1 gm
8.	Triethanol amine	Balancing pH	1.5 ml
9.	Euxyl PE 9010	Preservative	0.35 ml
10.	Wheatgrass powder	Active pharmaceutical ingredient	1 gm
11.	Rose essence	Fragnance/ perfume	0.1

3.8. **FORMULATION PROCESS**

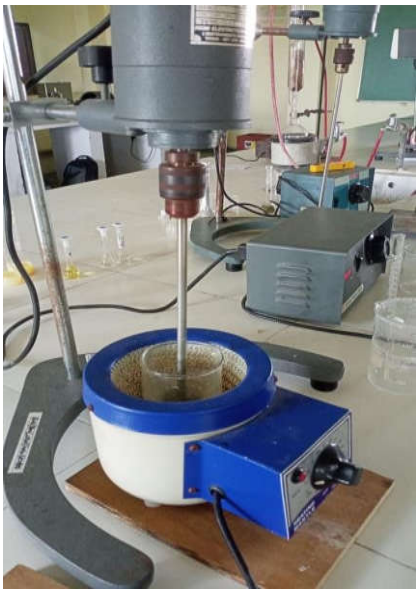
1. Juice of wheatgrass was prepared by grinding with water.
2. Once the juice was lyophilized, it was transformed into a powder form.
3. Wheatgrass powder was obtained after freeze drying.
4. Serum was prepared using magnetic stirrer as it ensures uniform distribution of components and helps to achieve a constituent texture and composition.

5. Wheatgrass Juice Extraction and Lyophilization: On 7th day wheatgrass was cut with sterilized knife and after washing immediately took for extracting juice. Extracted juice was preserved into deep freezer and then lyophilized by using Martin ChristLyophilization unit. The process carried out in three phases i.e., freezing, primary drying and secondary drying. The lyophilized Wheatgrass Juice Powder was

6. further used for experimental studies**. [43]**

Fig. Wheatgrass juice **Fig. Lyophilizer**

Fig.



Wheatgrass powder **Fig. In-process serum formulation**

3.9. **DEVELOPMENT OF COSMETIC SERUM:**

1. All the ingredients were weighed according to the given quantity.

2. Allantoin and EDTA were added slowly to water with continuous stirring on electric stirrer.
3. Humectants were added once allantoin was dissolved.
4. Polymer was added slowly with stirring on.
5. Gum was triturated with mortar pestle, passed through sieve to obtain very fine particles and then sonicated for 30 mins with water which was added slowly with stirring on and making sure that there were no lumps.
6. Once all ingredients were dissolved Triethanolamine was added.
7. Lastly preservative and perfume were added.

3.10. EVALUATION PARAMETERS

3.10.1. Physical Evaluation: Visual observations were made on the formulation's color and look. The consistent dispersion of extracts during the formulation process. Both touch and visual appearance were used to confirm this test.

3.10.2. pH Value: The calibration of a pH meter was done with a typical buffer solution. After accurately measuring and dissolving nearly 1 milliliter of the face serum in 50 milliliters of pure water, the pH of the mixture was determined. Since the skin has an acidic spectrum, the skin serum's pH should be between 4.1 and 6.7. **[2]**

3.10.3. Spreadability Test: The serum base should not increase friction during the rubbing process and should distribute readily without excessive drag. The Spreadability device, consisting of a wooden board with a scale and two glass slides with two pans hung on each side on a pulley, was used to calculate spreadability. To compress the excess material to a consistent thickness, it was sandwiched between the two glass slides and 100 g of weight was applied to one of the slides for five minutes. To the pan was added weight (250 g). Spread ability was measured as the number of seconds needed to complete the task of separating the two slides.

S = m * l/t m – weight tied on

upper slide l – length of glass slide

t – time in s **[14]**

3.10.4. Rheological Study: Using a spindle type model S64, the Brookfield® Viscometer measures the formulation's viscosity at different rpm of 5 ml of the serum. Before the measurement, the spindle will be immersed in the serum for roughly five minutes in a large mouth container. **[4]**

3.10.5. Stability Studies: A pharmaceutical product cannot be fully developed or formulated without a thorough stability analysis performed on it to ascertain its chemical and physical stability and, consequently, its safety. The stability studies are conducted in accordance with ICH recommendations. For a few months, a short-term accelerated stability study was conducted for the developed formulation. The samples were held at various temperatures, including 3-5°C, 250°C with a relative humidity of 60%, and 40°C with a relative humidity of 75%. **[6]**

4. RESULT AND DISCUSSION

4.1. AUTHENTICATION OF PLANT:

Authentication is the process of identifying a plant species. It is taken up for research use is a necessity to achieve satisfactory results and also to maintain efficacy and therapeutic property of the preparations in which these plants are used. Plant was grown at college and authenticated at Rashtrasant Tukadoji Maharaj Nagpur University by Head of department, Botany, RTMNU, Nagpur.

- Biological source: Triticum aestivum Linn, belonging to the family Poaceae.
- Grown and cultivated at botanical garden, DBCOP.

Fig. 2. Wheatgrass shoot authentication

4.2. **QUALITATIVE PHYTOCHEMICAL EVALUATION:**

Compounds	**Observations**	**Results**
Reducing sugar	Blue color of fehling turns to brick red	+ve
Saponins	Appearance of frothing	+ve
Flavanoids	Appearance of yellow color	+ve
Alkaloids	Appearance of turbidity	+ve
Terpenoids	Reddish green color	+ve
Anthraquinones	Pink color	+ve
Tannins	Bluish green	+ve

4.3. **DESIGN OF EXPERIMENTS:**

4.3.1. Viscosity:

If the concentration of xanthan gum increases then the viscosity of serum gets increased.

When the triethanolamine concentration increases, it doesn't show any effect on serum viscosity.

4.3.2. pH:

When the concentration of triethanolamine increases then it leads to increase in pH of the serum.

If the concentration of xanthan gum increases then the pH of the serum also increases.

Both xanthan gum and triethanolamine are the independent variable and shows effect on serum pH.

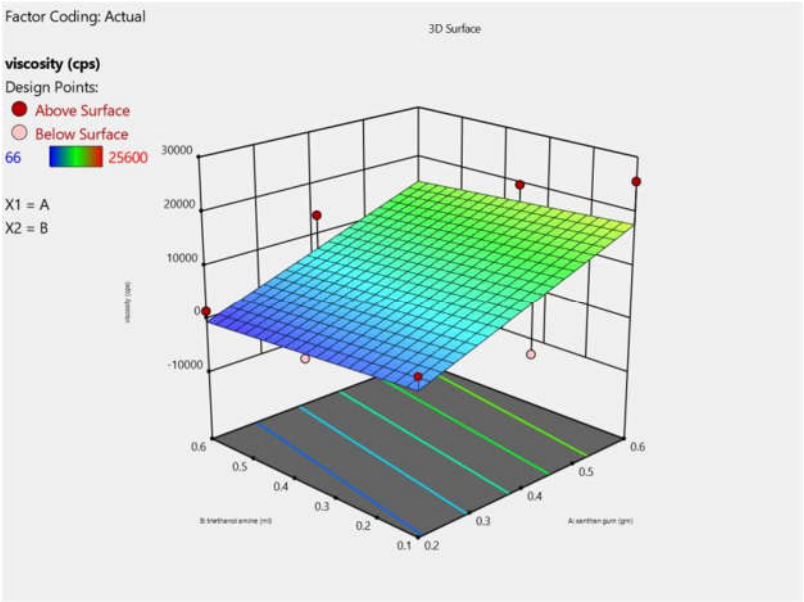


Fig.2: 3D surface plot of viscosity (cps): Effect of xanthan gum (X1) a concentration of triethanolamine (X2) on viscosity (Y2).

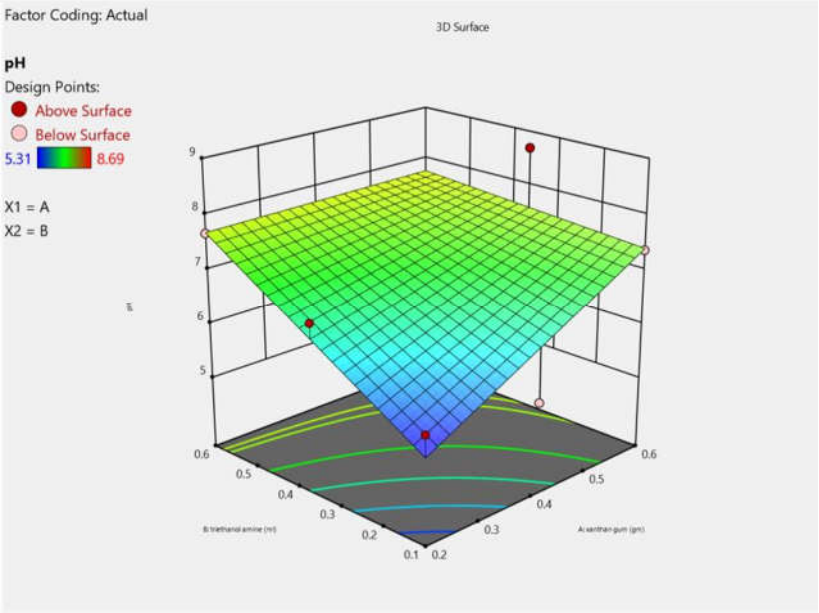
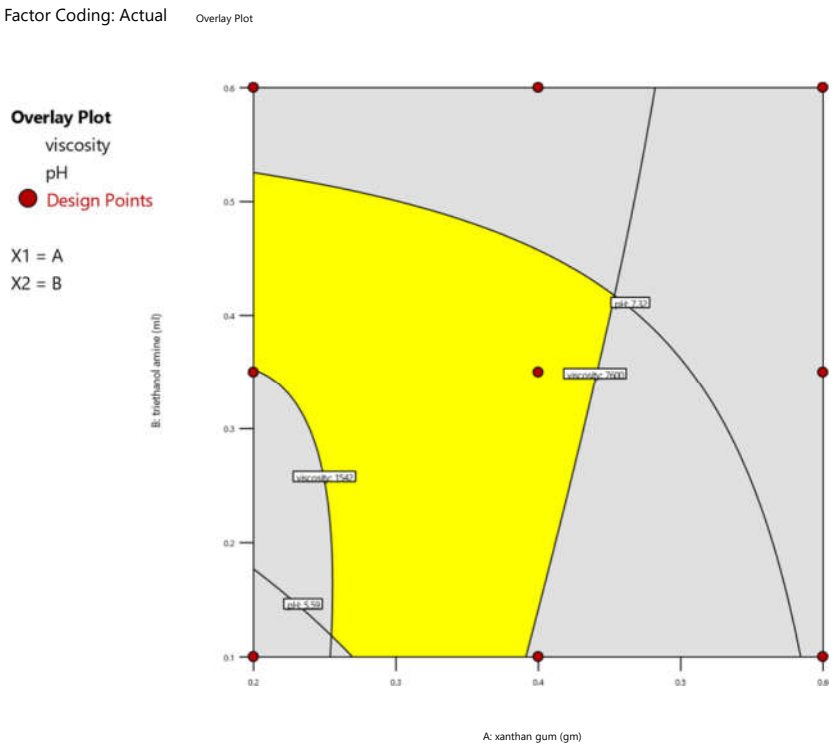


Fig.2.2: 3D surface plot of pH: Effect of xanthan gum(X1) an concentration of triethanolamine(X2) on pH(Y1).



The results of DOE shows that optimized batch of serum formulation was determined to be Run No. 6

5. RESULTS OF EVALUATION PARAMETERS

The Faces serum was formulated as a transparent, white, viscous liquid that had a glossy, smooth, uniform texture. It took only a second to redisperse the formulation. It felt emollient and slipperier after usage, and it was easy to wash off with no residue left behind. **[7]**

pH Value: It was discovered that the formulation's pH was 7.6. Given that skin has an acidic pH of 4.1–6.7, this formulation range is appropriate for skin.

Determination of Spread ability: Spread ability of liquid formulation, or the face serum's capacity to cover the skin, is a crucial factor in how well a prescribed dose is applied to the skin. It was discovered that face serum spreads 5 to 6 cm.

Determination of Viscosity: One important factor in the formulation of a topical is its viscosity. Low-viscosity topical treatments clean the skin more quickly than viscous ones. Moreover, extremely viscous solutions may cause unwanted skin effects. The Face Serum's viscosity was measured at 2580 cps.

Stability Studies: Stability experiments were conducted on the formulation to account for both chemical and physical changes. There were no discernible differences in the formulation's characteristics**.[6]**

During the irritancy examination, it also shown no redness, irritation, or inflammation. You can use the serum on any kind of skin. Regular use of serum increases the formation of collagen, greatly improves texture, and leaves skin feeling firm, smooth, and youthful. Particularly high vitamin C concentration is essential for skin renewal. **[6]**

Stability study table

Sr no.	Days	Parameters				
		pH	Homogeneity	Appearance	Spreadability	Viscosity
1	0th	7.83	H	NCC	ES	2446
2	5th	7.83	H	NCC	ES	2445

3	10th	7.82	H	NCC	ES	2546
4	15th	7.83	H	NCC	ES	2548
5	20th	7.83	H	NCC	ES	2648
6	25th	7.82	H	NCC	ES	2649
7	30th	7.83	H	NCC	ES	2749

H: Homogenous; NCC: No change in color; ES: Easy to spread;

The serum was found to be homogenous, no change in color, easy to spread, and easy removal after application till 30th day.

Evaluation results table

Sr. no.	**Parameter**	**Result**
1.	Colour	Pale yellow
2.	Odour	Pleasant
3.	pH	7.6
4.	Viscosity	2580 cps
5.	Spreadability	5-6 cm
6.	Texture	Smooth
7.	Skin irritancy	Non irritant
8.	Stability studies	Stable at room temp
9.	Washability	Washable

6 CONCLUSION:

The aim of this research was to study about that exactly are depuffing serums and their history along with their overall importance. The study includes its proper selection and correct sequence of Application. the research study could lead to safe and cost effective undereye depuffing serum to reduce puffiness. The formulated

serum incorporated wheatgrass juice may be really effective for people to get rid of undereye puffiness.

The pH was found to be between 6.0 to 8.0, texture of serum was found to be uniform and smooth, viscosity was found to between 2580 cps and spreadability was found to be 5-6 g cm/sec. So, from the above results it was found that optimized formulation shows good results.

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Wheatgrass also contributes 33.26 g potassium/100g and this mineral plays an important role in regulating fluids and minerals in body cells [30]

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