FORMULATION AND EVALUATION BIO- CELLULOSE SHEET MASK OF KOJIC ACID FOR TREATMENT OF HYPERPIGMENTATION OR MELASMA

THESIS SUBMITTED TO THE

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IN

PHARMACEUTICS

SUBMITTED BY

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My Beloved Family

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I wish him all success in life.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"*Formulation and Evaluation of Biocellulose Sheet Mask of Kojic Acid for the Treatment of Hyperpigmentation or Melasma*"** is a bonafide and genuine research work carried out by me under the supervision of **Dr. Bhupen Kalita**, Assistant Professor, Department of Pharmaceutics, ***Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.*** The work embodied in this thesis is original and has not been submitted for the award of degree, diploma, associateship or fellowship of any other university or institution.

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This is to certify that the project proposal no. GIPS/IAEC/M.PH/PRO/09/2021 entitled *The formulation and evaluation of bio-cellulose sheet mask of Kojic acid for hyperpigmentation or melasma* submitted by *Mr. Kamallochan Barman* has been approved/recommended by the IAEC of *Girijananda Chowdhury Institute of Pharmaceutical Science*, Azara, Guwahati-781017 in its meeting held on 25/02/2021 and .....(Number and Species of animals) have been sanctioned under this. 12 New Zealand albino Rabbits & 12 rats & AG HK14 SCF Tg/HRM mice

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Chairman:	Gowranga Das.		02.03.2021
Member Secretary:	Gowranga Das.		02.03.2021
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ABSTRACT

Skin health is an important aspect of glamour and reflects the overall wellbeing of the whole body. Facial and neck hyperpigmentation have been posing a challenge towards a complete cure in skincare and cosmetology. The root cause of hyperpigmentation is excessive production of melanin induced by the enzyme tyrosinase and also excessive production is related to exogenous factor-like cosmetics and perfumes, and endogenous factor-like hormonal imbalance during pregnancy. There are many types of hyperpigmentation including post-inflammatory hyperpigmentation (PIH), periorbital hyperpigmentation, which are a few to mention. The recent addition in skincare is the sheet masks, which are considered to be the most productive and trending technique of drug delivery for skin rejuvenation. Kojic acid is reported to have tyrosinase inhibitor activity, thereby the reason behind selecting them in the development of the desired formulation. In this research the Kojic acid biocellulose sheet mask were subjected to various evaluation parameters such as physical appearance, wet weight, hand-pressed weight, thickness, pH measurement, FTIR study, viscosity study, in-vitro and ex-vivo permeation study, tack properties , in-vivo irritation study. The thickness, weight variation wet weight, hand-pressed were within acceptable limit. However the viscosity was increased in formulations where xanthan gum concentration was increased and on the other pH was increased in formulations where citric acid concentration was increased. The FTIR spectral analysis confirmed the integrity and compatibility of the Kojic acid with the excipients. Also, it is envisaged that this research article may be of interest among dermatologists, other health care researchers, and manufacturing chemists to develop new strategies in skincare.

Keywords: hyperpigmentation, skincare, cosmetic, face mask, sheet mask, dermatology, Kojic acid.

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LIST OF ABBREVIATIONS

Sl No	ABBREVIATIONS	
1	pH	Hydrogen ion concentration
2	℃	Degree Celsius
3	hrs	Hours
4	min	Minute
5	g	Gram
6	kg	Kilogram
7	ml	Milliliter
8	µl	Micro liter
9	mm	Millimeter
10	cm^2	Centimeter square
11	cm^{-1}	Per Centimeter
12	g/kg	Gram per Kilogram
13	g/ml	Gram per milliliter
14	g/cm^3	Gram per cubic centimeter
15	µg/ml	Microgram per milliliter
16	mg/ml	Milligram per milliliter
17	cps	Centipoises
18	DOPA	Dihydroxyphenylalanin
19	CO_2	Carbon dioxide
20	POMC	Proopiomelanocortin melanocytes
21	ACTH	Adrenocorticotropic hormone
22	Alpha MSH	Alpha Melanocyte stimulating hormone
23	UV	Ultraviolet
24	HLH	Hemophagocytic lymphohistocytosis
25	TYR	Tyrosinase
26	PIH	Post inflammatory hyperpigmentation
27	ICHOR	Idiopathic Cutaneous Hyperchromia of the orbital region
28	IPL	Intensed Pulsed Light
29	LPP	Lichen Planus Pigmentosum
30	EDP	Erythema Dichromic Perstans
31	RDA	Recomemded Dietary Allowance
32	DNA	Dioxyribonucleic acid

LIST OF ABBREVIATIONS

33	RNA	Ribonucleic acid
34	NAG	N-acetylglucosamine
35	KA	Kojic acid
35	NCAP	N-Acetyl-4-S-cysteaminylphenol
36	SPF	Sun Protection Factor
37	HQ	Hydroquinone
38	CAGR	Compound Annual Growth Rate
39	FTIR	Fourier-transform infrared spectroscopy
40	QoL	Quality of Life
41	CIR	Cosmetic Ingredient Review
42	DMSO	Dimethylsulfoxide

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1. Skin

The skin is the largest organ in the body, makes up about 15% of the total body weight of an adult, and performs many vital functions, including protecting against external physical, chemical and biological aggressors, as well as preventing excessive water loss through the skin, and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the surface of the body (Kanitakis, 2002)

The skin system consists of the skin and its derived structures. The skin is made up of three layers: the epidermis, the dermis, and the subcutaneous tissue (Kanitakis, 2002). The outermost layer, the epidermis, consists of a certain constellation of cells called keratinocytes, which are used to synthesize keratin, an elongated, thread-like protein with a protective function. The middle layer, the dermis, consists mainly of the fibrillar structural protein collagen. The dermis is located in the subcutaneous tissue, or panniculus, that contains small fat cells known as lipocytes. The thickness of these layers varies greatly, depending on the geographic location in the anatomy of the body. For example, the eyelid has the thinnest layer of the epidermis, less than 0.1 mm, while the palms and soles of the feet have the thickest layer of the epidermis, around 1.5 mm. on the back, where it is 30 to 40 times thicker than the overlying epidermis (James *et al.*, 2019)

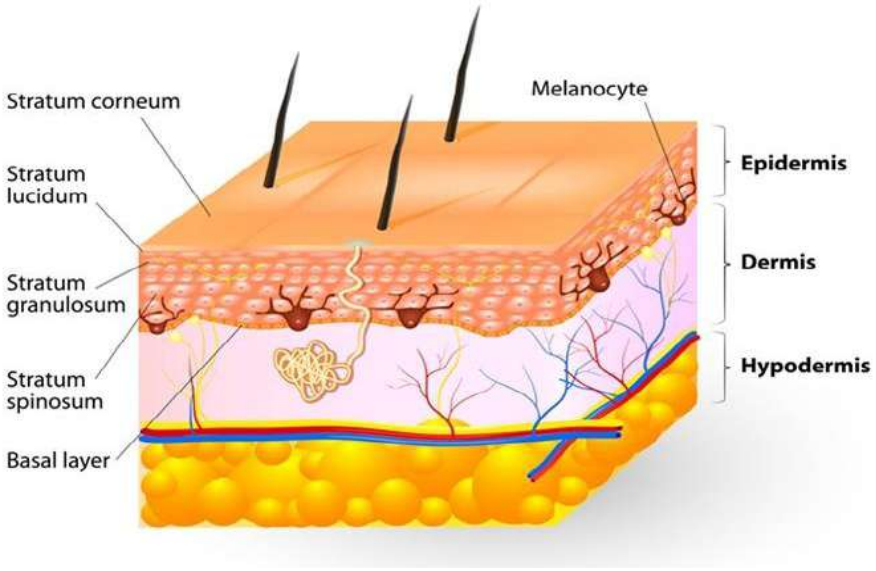


Figure 1.1: Structure of human skin (Image credit: *Megan Knight, BSc*, Melanin Production Pathway, News Medical Life science)

1.2. Melanin

Melanin is the generic name used to refer to perhaps the most ubiquitous, resistant, heterogeneous, and oldest pigments found in nature. Melanin appeared very early in most of the living kingdoms on earth. Therefore, melanin has recently been found in very ancient fossils of dinosaurs, primitive birds, non-aviator antheropods (Zhang *et al.*, 2010) (Wogelius *et al.*, 2011), and primitive cephalopods. These recent findings are likely to make melanin a new biomarker in the evolution of life.

The name "melanin" comes from the ancient Greek melanin, which means "dark" and, according to Borovansky (Borovanskỳ and Riley, 2011), the term was probably first used by the Swedish chemist Berzelius in 1840 to denote a dark pigment that is produced from the membranes of the eyes (Danieles, 1899). The earliest references to the pigmentation of human skin and, to a certain extent, the existence of melanin without using the current name are very old. Pharaonic medicine described some

diseases that affected skin color in the Ebers papyrus (1550 BC) (Dawson and Ebbell, 1938), and one of them was probably vitiligo, although this term appeared much later, derived from the Latin word "vitellus", which means "calf" or pale pink skin (Westerhof, 2006). The first relatively detailed written description of skin pigmentation in mankind comes from Herodotus in Greece, who described the darker skin of the Persians, Ethiopians, and Indians concerning the Greeks.

Structurally, melanins are a group of complex pigments with a relatively diverse and undefined structure. They have been defined in various ways over the past 50 years (Riley, 1980)(D'Ischia *et al.*, 2013), but most of the proposed definitions have some pitfalls in that they are partial or incomplete due to difficulty. to define something with such a wide variety of composition, color, size, occurrence, and function. This is evident as melanin can be found in all living kingdoms. A simple and generalized definition that includes all types of melanin would be "heterogeneous polymer obtained from the oxidation of phenols and subsequent polymerization of intermediate phenols and the resulting quinines.

In humans, melanin exists in three forms: **eumelanin** (which is divided into black and brown forms), **pheomelanin,** and **neuromelanin**. (Maranduca *et al.*, 2019)

Eumelanin and pheomelanin are produced in different amounts in the basal layer of the epidermis of cells called melanocytes. Melanocytes are mature forms of melanocytes that migrate out of the neural crest after the neural tube is closed. Like melanin found in melanocytes, it is wrapped in small circular membrane-bound organelles called melanosomes. Melanocytes are transported from melanocytes to neighboring keratinocytes through dendritic protrusions of the tentacles. The surface of

the melanosome that reaches the keratinocytes is embedded in the nucleus to prevent incident ultraviolet (UV) radiation(D'Alba and Shawkey, 2019).

1.2.1. Biosynthesis pathway of melanin

The first step in the biosynthesis of eumelanin and pheomelanin begins in the same way: tyrosine is converted into dihydroxyphenylalanine (DOPA), which requires tyrosine hydroxylase and tetrahydrobiopterin as a cofactor. The enzyme tyrosinase then converts dihydroxyphenylalanine into dopaquinone, which can take various routes. to form eumelanin or pheomelanin. The primary stimulus for melanogenesis and the subsequent melanosome production is UV radiation, which upregulates the production of proopiomelanocortin melanocytes (POMC) and its secondary products, the alpha-melanocyte-stimulating hormone (alpha-MSH) and the adrenocorticotropic hormone (ACTH). to increase the production of eumelanin. (Interestingly, people with proopiomelanocortin mutations have red hair and Fitzpatrick type 1 skin due to the relatively increased expression of pheomelanin to eumelanin.) Neuromelanin is a dark pigment produced by dopaminergic and noradrenergic cells of the substantia nigra and the locus coeruleus. as a breakdown product of dopamine shown in Figure 1.2(Del Bino, Duval and Bernerd, 2018).

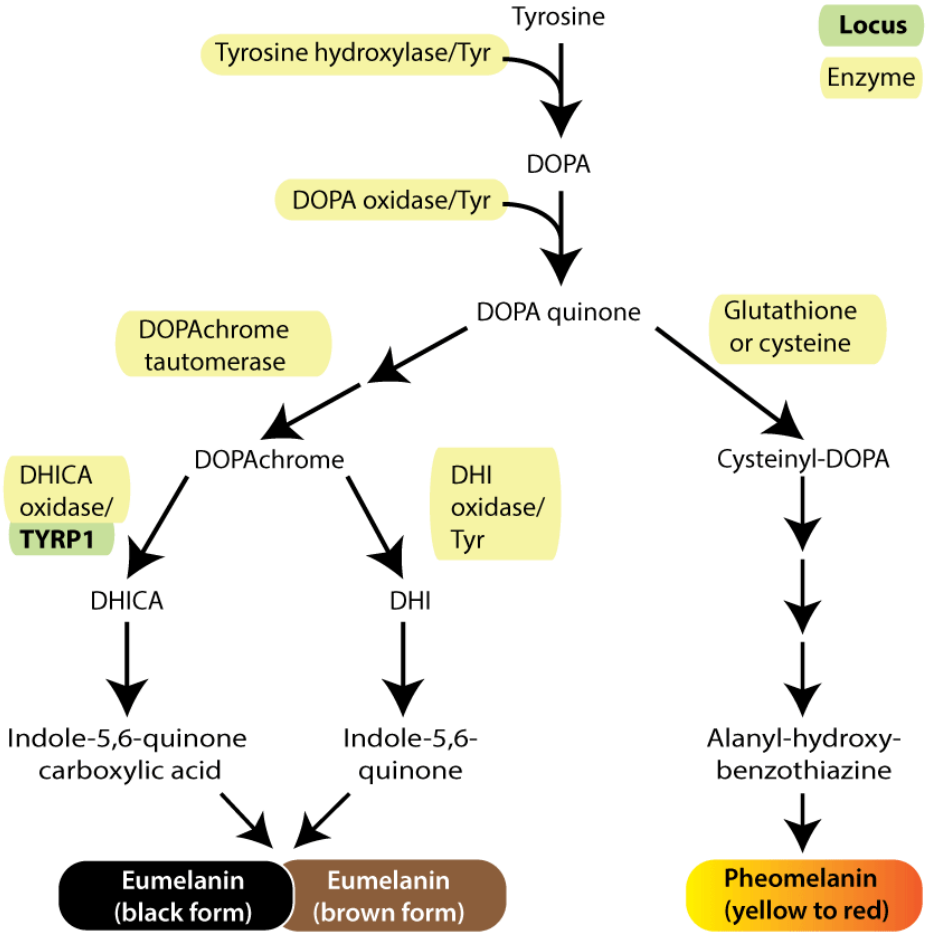


Figure 1.2: Biosynthesis Pathway of melanin synthesis (Image credit: https://i.stack.imgur.com/h5scW.gif)

1.2.2. Function of melanin

In its various forms, melanin fulfills a variety of biological functions, including pigmentation of the skin and hair, and sun protection for the skin and eyes.

Skin pigmentation is the result of the accumulation of melanosomes with melanin in the basal layer of the epidermis. both by the relative ratio of eumelanin (brown-black) to pheomelanin (yellow-red) and by the number of melanosomes within

the melanocytes. Phaeomelanin is responsible for the pink skin that makes up the labia, nipples, vagina, and glans of the penis. In general, lightly pigmented skin contains melanocytes with clusters of two to three melanosomes, while darkly pigmented skin tends to contain individual melanosomes that can melanize neighboring keratinocytes more easily.

The interaction between melanin and ultraviolet radiation is complex. Researchers believe that melanin production in melanocytes increased more than a million years ago as an evolutionary adaptation to the widespread loss of human body hair, antioxidant, and UV absorber. In contrast, populations farther from the equator are relatively richer in pheomelanin, which produces free radicals in response to UV radiation and accelerates cancer development. Since the main stimulus for vitamin D production in the skin is exposed to ultraviolet light, it follows that dark-skinned individuals also tend to have lower vitamin D levels and should be assessed accordingly.

The connection between melanin, the sun, and skin immunology is less clear. Acute and chronic exposure to ultraviolet light induces immune suppression; UVA light is used therapeutically for a variety of skin conditions, including psoriasis. Interestingly, melanin has been attributed immunomodulatory and even antibacterial properties, although the underlying mechanisms are not yet fully understood. Malignant melanin-rich melanocytes are less sensitive to chemotherapy, radiation therapy, or photodynamic therapy, and amelanotic melanomas have a longer overall and disease-free survival than melanotic ones. Therefore, inhibition of melanogenesis has been suggested by some as a therapy for malignant melanoma.

Just as melanin protects the skin from photodamage, it also protects the eye. Melanin is concentrated in the iris and choroid, and those with gray, blue, and green eye colors, as well as albinos, have more sun-related eye problems (Juhasz and Levin, 2018).

1.2.3. Clinical significance of melanin

Every step of melanin formation and melanin transport can be influenced, which leads to a diverse group of diseases (Saleem, 2019):

Melanoblast: Waardenburg syndrome, a group of autosomal recessive (RA) and dominant (EA) characterized a white tuft, skin hypopigmentation, and premature graying of the hair is the result of an altered migration of melanoblasts towards their target tissue (i.e. iris, hair). Various forms also include congenital deafness, heterochromia iridis, sinophrys, and canthorum dystopia. : Vitiligo, a disease characterized by depigmented and photosensitive white spots surrounded by normally pigmented skin and ophthalmic problems, results from the autoimmune destruction of melanocytes (Slominski *et al.*, 2004)

Melanosome: An autosomal recessive inherited disorder caused by partial oculocutaneous Albinism, platelet dysfunction, hemophagohistiocytosis, and hemocytosis) immunodeficiency resulting from mutations in genes likely to regulate lysosomal traffic. Li syndrome, a group of autosomal recessive inherited diseases characterized by hypopigmentation of hair and skin, is the result of mutations in the protein complex responsible for the transfer of mature melanosomes to keratinocytes. Different forms also include neurological impairment, immunodeficiency, and HLH (Tachibana *et al.*, 1996).

Tyrosinase: An autosomal recessive condition characterized by intellectual disability, epilepsy, blonde hair, blonde hair, and blue eyes, and other skin changes, is the result of a deficiency in the enzyme phenylalanine hydroxylase. Pigment changes are due to competitive inhibition of tyrosinase through the accumulation of phenylalanine. Oculocutaneous albinism, a group of autosomal recessive inherited diseases characterized by hypopigmentation and eye problems, is the result of mutations in the tyrosinase (TYR) gene. VogtKoyanagi-Harada syndrome, a disease characterized by progression through phases of meningoencephalitis, uveitis, alopecia with vitiligol-like depigmentation, and recurrent uveitis, is the result of autoimmune destruction of melanosome-bound antigens, possibly including the enzyme tyrosinase itself (Carballo-Carbajal *et al.*, 2019).

Dopaminergic neurons: Parkinson's disease, a neurodegenerative condition characterized by progressive posture and gait difficulties, results from the defect of neuromelanin-producing dopaminergic neurons in the brain. compacta is a pathological characteristic of the disease.

1.3. Hyperpigmentation

Melanin is the most important factor in skin color. The epidermal melanin concentration in melanosomes is twice as high in darker skin types as in lightly pigmented skin types (Iozumi *et al.*, 1993).In addition, the breakdown of the melanosome within the keratinocytes occurs more slowly in darkly pigmented skin than in lighter skin types (Ranu *et al.*, 2011). The melanin content and the melanosomal dispersion It is assumed that the patterns confer protection from UV-induced damage (Taylor, 2002). Kaidbey et al.(Kaidbey *et al.*, 1979) showed that the black epidermis offers an average sun protection factor (SPF) of 13.4. While elevated melanin levels

protect against the harmful effects of UV radiation, including photo damage and skin cancer, it also makes darkly pigmented skin more prone to post-inflammatory pigmentation and structural differences, common diseases require special considerations about ethnic skin color. Additionally, there are many relatively unique skin conditions for people with colored skin and also summarize in table 1.

1.3.1. Causes of Facial Hyperpigmentation

1.3.1.1.Post-inflammatory hyperpigmentation

Post-inflammatory hyperpigmentation (PIH) is the darkening of the skin that occurs after an inflammatory rash or skin injury. Hyperpigmentation is the result of melanocytes' response to skin aggression, which causes an increase in the production and/or redistribution of melanin. The skin is predisposed to this change in pigmentation.

Post-inflammatory changes can occur in both the epidermis and the dermis. The epidermal form of hyperpigmentation leads to increased melanin production, transfer to keratinocytes. In dermal PIH, a damaged basement membrane enables melanin to enter the dermis, where it is phagocytosed by dermal macrophages called melanophages. The epidermis envelops the melanosomes and then returns to the dermis. Melanin in dermal melanophages can persist for years (Masu and Seiji, 1983).

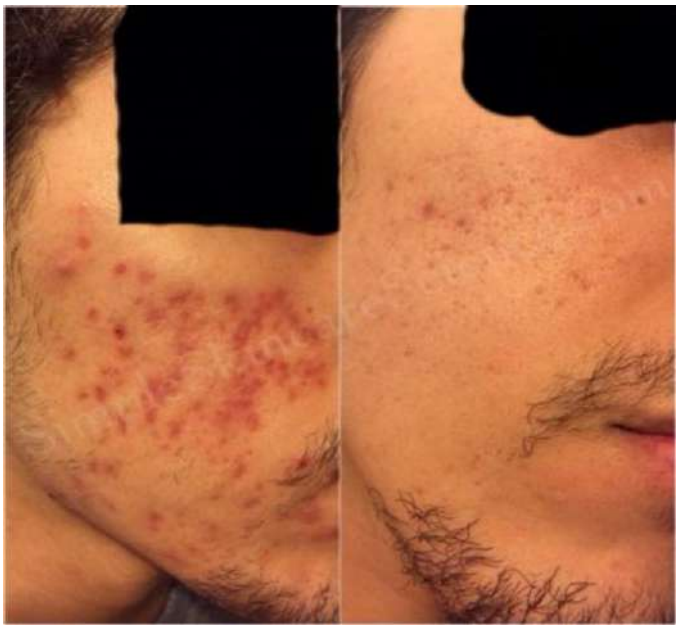


Figure 1.3: Post-inflammatory hypopigmentation (Image source:(Pérez-Bernal *et al.*, 2013))

When the skin recovers from acute inflammatory disease in darker patients, it can become hyperpigmented (PIH). or hypopigmented (known as post-inflammatory hypopigmentation). Lightening or darkening of the skin is associated with many primary conditions including, but not limited to, discoid lupus erythematosus, seborrheic dermatitis, tinea versicolor, atopic dermatitis, and sarcoid (Figure 1.3). Inflammation or injury, e.g. acne, arthropodasault, viral rashes, eczema, psoriasis, trauma. Physical examination findings include small to large hyperpigmentation and spots of varying sizes in any distribution. Although this is usually a clinical diagnosis, in difficult cases a biopsy can be done for histopathological assessment. Disorders such as melasma, morphea, atrophoderma, and other rare etiologies should be considered in patients with no prior evidence of inflammation through history or examination. The time it takes for depigmentation to normalize is highly variable and depends on many factors, including the patient's initial skin tone, the type and intensity of the lesion or

inflammation, and the patient's sun exposure habits. Time can take years and be psychologically stressful. chemical agents, exfoliants, and lasers can be tested; however, they can also cause the original dyspigmentation to deteriorate and should always be used with caution (McDonald and De Silva, 2014).

1.3.1.2.Maturational dyschromia

With mature, dark skin, a darkening of the facial skin tone can also be observed outside of prolonged exposure to the sun. Maturational dyschromia, or a general uneven hue, can be described as diffuse hyperpigmentation that usually occurs in the lateral part of the forehead and cheekbones (Figure 1. 4). A survey found that uneven skin tone was the most common complaint in more than a third of black women. These changes in skin tone are likely due to chronic sun exposure for many years (Baumann *et al.*, 2006). Maturational dyschromia may be misdiagnosed as melasma, acanthosis nigricans, or PIH. This is a diagnosis of exclusion and any type of allergic contact dermatitis or photoallergic dermatitis should be ruled out. Treatment options include sunscreen, skin lightening agents, antioxidants, micro dermabrasion, and/or chemical peels.

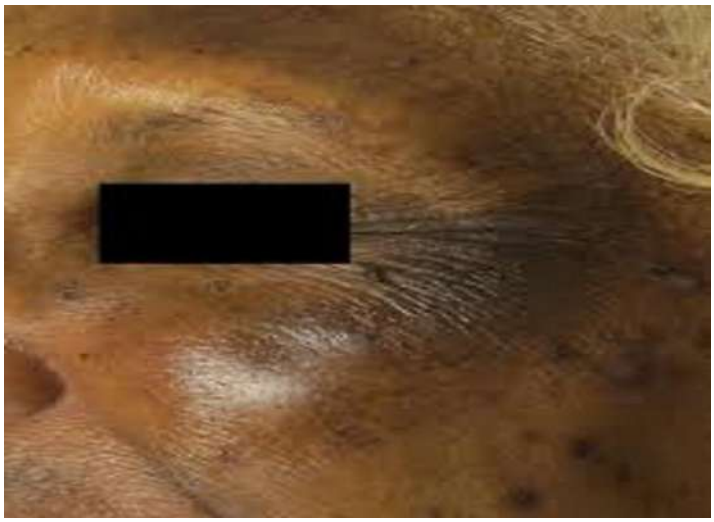


Figure 1.4: Maturational dyschromia (Image source:(Pérez-Bernal *et al.*, 2013)).

1.3.1.3. Periorbital hyperpigmentation

Periorbital hyperpigmentation, also called idiopathic cutaneous hyperchromia of the orbital region (ICHOR), periorbital melanosis, dark circles, or infraorbital pigmentation, is more commonly observed in the skin-colored population and can be a primary or secondary etiology (Sarkar, 2012). The cause of secondary periorbital hyperpigmentation. often has multifactorial pathogenesis, including genetic or constitutional pigmentation, dermal melanocytosis, PIH as a result of atopic and/or allergic contact dermatitis, periorbital edema, excessive subcutaneous vascularization, and shadowing due to sagging skin and cracks associated with aging (Figure 1.5) (Roh and Chung, 2009). It has also been considered that excessive sun exposure, medication, hormonal causes, and the lengthening of pigmented demarcation lines (Malakar *et al.*, 2007), ICHOR is characterized by a bilateral darkening of the orbital skin and eyelid that is not secondary to any systemic or local disease. In a study by Ranu et al. on 200 patients with periorbital hyperpigmentation, possible causes were delineated according to anamnesis, physical examination, and assessment by dermatologists by measuring with a Mexameter (Courage-Khazaka Electronics, Cologne, Germany).noted that the most common forms are vascular (41.8%), characterized by the presence of erythema affecting parts of the lower eyelids with prominent capillaries/telangiectasias, or the presence of bluish discoloration due to visible blue veins; Constitutional form (38.6%), characterized by the presence of brown-black hyperpigmentation of the skin of the lower eyelid along with the shape of the edge of the orbit; PIH (12%); and shadow effects (114%) due to a protruding tarsal muscle or a deep tear duct (Ranu *et al.*, 2011). Other causes included skin laxity, dry skin, hormonal disorders, nutritional deficiencies and other chronic diseases. Verschoore et al. confirmed that not only melanin deposits

but also blood stasis may play a role in the pathogenesis of ICHOR (Ortonne *et al.*, 2012).

Figure 1.5: Periorbital hyperpigmentation (Image source:(Pérez-Bernal *et al.*, 2013))

Regarding the location of the pigmentation, earlier studies by Watanabe et al. and Malakar et al. examined skin biopsies and found the presence of dermal melanocytosis and melanin pigment in higher dermal macrophages. which partly explains the resistance of this disease to various treatments (Watanabe, Nakai and Ohnishi, 2006). Skin lightening creams, chemical peels, intense pulsed light (IPL), Qswitched ruby laser, fat autograft, blepharoplasty, and fat graft combinations, and fillers were used. Tried, but none have provided satisfactory long-term treatment.

1.3.1.4.Rihl melanosis

Rihl melanosis or pigmented contact dermatitis is characterized by a brownish-brown color that can be traced back to dermal melanin deposits; favors the places of use of

contact agents, especially cosmetics. The cases are usually preceded by slight erythema and itching, followed by diffusoreticulated hyperpigmentation (Figure 1.6). It depends on the pathogen. It can be brown or taupe, and it can be toothey have red and blue tones. The diagnosis is supported by closed patch tests for standard series, cosmetic series, fragrance series, and personal products for patients. A photo patch test can also be considered. If the results are unclear or negative, the provocation test or the repeated test for open use can be carried out (WATANABE, NAKAI and OHNISHI, 2006). The treatment includes the complete avoidance of the suspected allergen. Sun protection measures, skin lighteners, and chemical peels can accelerate the resolution of pigment changes.

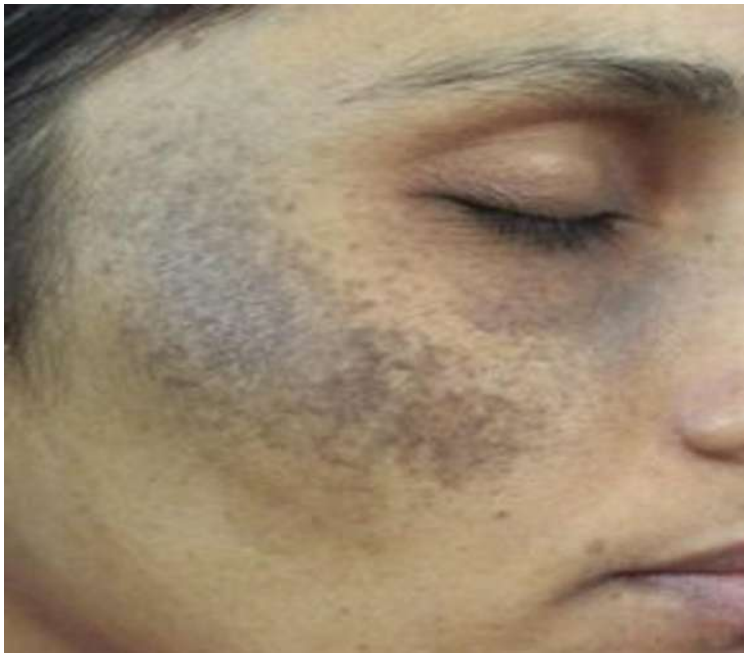


Figure 1.6: Rihl melanosis (Image source:(Pérez-Bernal *et al.*, 2013))

A rarer etiology of facial hyperpigmentation and probably a variant of Riehl's melanosis is called Erythrosa peribucalepigmentaire de Brocq. It is most likely caused by photodynamic substances in cosmetics. Symmetrical reddish-brown pigmentation

around the mouth separated from the vermilion border and may extend to the forehead, temples and corners of the jaw. The pigmentation persists if the cause is not removed (Khanna and Rasool, 2011).

1.3.1.5. Melasma

Melasma is an acquired form of hyperpigmentation that is most common on the face. It is a common hyperpigmentation disorder that affects millions of people around the world. Most affected are Fitzpatrick skin phototypes III and IV (Sheth and Pandya, 2011a), and at least 90% of those affected are women. The exact pathogenesis is unknown. ; However, it is believed that instead of an increase in melanocytes, melasma may be caused by the presence of more biologically active melanocytes in the affected skin (Sheth and Pandya, 2011b). melanocytes produce higher amounts of melanin after exposure to ultraviolet radiation than unaffected skin. Aggravating factors include pregnancy, hormone therapy such as oral contraceptives, and intense sun exposure. Sun exposure worsens melasma, likely due to the ultraviolet-induced upregulation of cytokines that stimulate melanocytes. , there are light to dark brown spots with irregular edges, which are most often symmetrically distributed in the midface, malar and mandible regions and can also be on the forearms (Figure 1.7).Depending on the location of the melanin, the melasma can be divided into different types, with the epidermal type the pigment is brown and the edges are geographically and better defined, while with the dermal type the pigment is rather grayish and the edges are poorly defined. The mixed type occurs when melanin is present in both the epidermis and dermis, and the term indefinite type can be used when it is difficult to classify even with the help of Woods light (Amatya, Jha and Shrestha, 2020). Differential Diagnosis is included PIH, solar lentigos, acanthosis nigricans, and other less common pigment

disorders, including exogenous ochronosis, lichen planus pigmentosum (LPP), and erythema dichromic perstans (EDP). Treatment includes a combined approach of rigorous sunscreen, cosmetic camouflage, topical brighteners, chemical peels. First-line therapy often includes non-destructive modalities that include broad-spectrum light protection and topical compounds that affect the pigment production pathway. Second-line therapy consists of adding chemical peels, but these should be used with caution so as not to induce further post-inflammatory changes. Laser and light therapies show promise; However, like chemical exfoliants, they carry the risk of HPI.

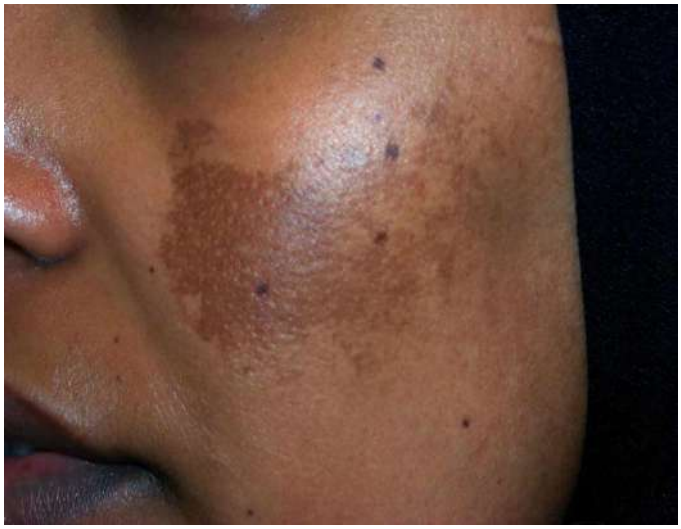


Figure 1.7: Melasma (Image source:(Pérez-Bernal *et al.*, 2013))

1.3.1.5. Exogenous ochronosis

Exogenous ochronosis occurs when foreign matter causes deposition of homogentisic acid dermis, leading to macular and papular hyperpigmentation. It is a rare condition characterized by asymptomatic hyperpigmentation of the face, sides and neck, back, and extensor sides (Figure 1.8) (Bhattar *et al.*, 2015). It is clinically and histologically similar to endogenous ochronosis, also known as alkaptonuria; however, it has no systemic effects and is not a hereditary disease. It has been linked to the use of products

containing hydroquinone, resorcinol, phenol, mercury, and/or pyric acid. It is relatively rare in the United States and most common in Africa. According to a 2007 literature review, 789 cases of exogenous ochronosis have been reported worldwide, only 22 of which have been reported by the etiology of this phenomenon remains elusive but could be due to the use of skincare products that contain resorcinol or the use of resorcinol of hydroquinone in a hydroalcoholic lotion (Levitt, 2007) (Nordlund, Grimes and Ortonne, 2006). In both endogenous and exogenous ochronosis, there is a microscopic deposition of ocher-colored pigment in the dermis. Exogenous ochronosis often requires histological confirmation as it can easily be confused with PIH, pigmented contact dermatitis, and melasma. with dermabrasion, CO_2 laser, glycolic acid peelings, and Qswitched laser (Bhattar *et al.*, 2015) (Tan, 2010)(Charlín *et al.*, 2008)

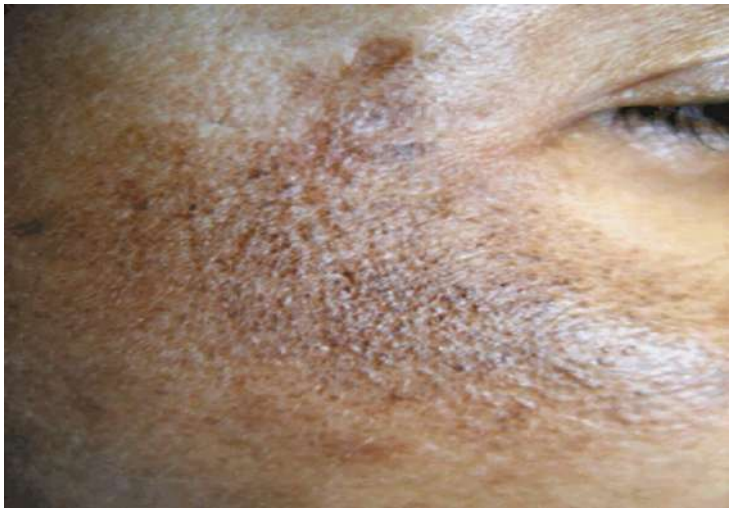


Figure 1.8: Exogenous ochronosis (Image source:(Pérez-Bernal *et al.*, 2013))

1.3.1.6.**Acanthosis nigricans**

Acanthosis nigricans is characterized by hyperpigmented velvety plaques, often in a symmetrical distribution. Although they are most common in the intertriginous areas of

the armpit, groin, and neck, acanthosis nigricans can appear almost anywhere, including the face (Figure 1.9). Acanthosis nigricans is commonly associated with insulin resistance and obesity and is more common in Hispanic and Black people. There is no treatment of choice for acanthosis nigricans. Therapy aims to correct the underlying disease process. Topical medications that have been effective in some cases of acanthosis nigricans include keratolytic (e.g. topical tretinoin 0.05%, ammonium lactate 12% cream) and triple-combination depigmenting cream (tretinoin 0.05%, hydroquinone 4%, fluocinolone acetonides 0.01%) nightly with daily sunscreen (Mishra *et al.*, 2013). Calcipotriol, podophyllin, urea, and salicylic acid have also been reported with variable results (Sinha and Schwartz, 2007).

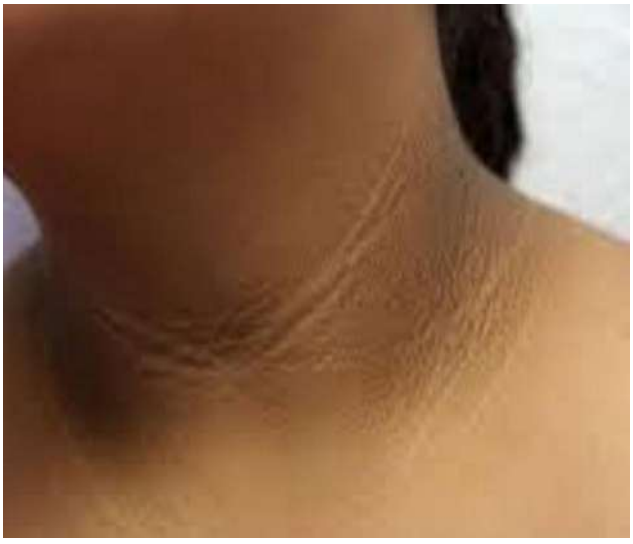


Figure 1.9: Acanthosis nigricans (Image source:(Pérez-Bernal *et al.*, 2013))

1.3.2. Treatment of facial hyperpigmentation

Facial hyperpigmentation can cause significant cosmetic disfigurement with subsequent emotional impact (Khanna and Rasool, 2011) (Pérez-Bernal, Muñoz-Pérez and Camacho, 2000). Treatment continues to be challenging as there is no universally

effective therapy, and existing agents have varying degrees of efficacy. Because the majority of reports regarding treatment consist of small series of patients and anecdotes, it is difficult to evaluate the efficacy of different forms of therapy. In addition, although multiple options do currently exist, some therapies have come under increasing scrutiny, underscoring the need for research into pathogenesis and treatment. Overall, treatment includes removal of provoking factors, photoprotection, and forms of active pigment reduction with either topical formulations or physical modalities (Rigopoulos, Gregoriou and Katsambas, 2007).

1.3.2.1 General instructions

Those with skin types IV–VI need special considerations in preventing and treating various aetiologies of facial hyperpigmentation. Peak hours of sunlight (between 11:00 and 16:00 h) should be avoided in addition to seeking shade and wearing protective clothing (e.g. broad-brimmed hats and long-sleeved shirts). Avoiding provoking triggers is necessary for many types of facial hyperpigmentation including melasma, pigmented contact dermatitis and PIH. If melasma has been induced by the use of oral contraceptives, discontinuation should be considered.

i) Sunscreens

Several studies have shown that light from both the UV and visible spectrum can induce pigmentary changes in the skin (Sheth and Pandya, 2011b) (Pathak, Riley and Fitzpatrick, 1962). Redistribution and oxidation of pre-existing melanin cause immediate pigment darkening and occurs after low-dose UVA exposure and usually fades after 2 h (Mahmoud *et al.*, 2008). After high doses of UVA exposure, persistent pigment darkening may develop, lasting up to 24 h. Both UVA and UVB exposure can

cause melanin synthesis resulting in delayed tanning. Given clinical experience and available data, broad-spectrum UVA and UVB protective sunscreen with an SPF of at least 30 ideally including a physical block (e.g. titanium dioxide or zinc oxide) should be used for prevention of facial hyperpigmentation. Vitamin D is essential for bone health, and as cutaneous synthesis is a well-known source, it is important to counsel patients properly when advocating strict sun protection. Darker-skinned patients have an inherently lower vitamin D level and those that live in environments that do not have regular sun exposure and/or are adhering to strict sun-protective measures may require supplementation and monitoring. The National Academy of Sciences Institute of Medicine recommended dietary allowance (RDA) for vitamin D is 400 international units (IU) for infants/children aged 0–1 years, 600 IU for those aged 1–70 years and 800 IU for those aged ≥ 71 years. Sources include fortified milk, cheese, yogurt and cereal and also oily fish (i.e. salmon and tuna). Fortified foods are rich in both vitamin D and calcium and maintain phosphate levels, which are also needed for bone health (Narla *et al.*, 2020).

ii) Cosmetic camouflage

Physical blocking opaque sunscreens have the dual benefit of camouflaging facial hyperpigmentation and preventing photoinduced darkening. Many of these physical blockers now come in tinted blends to help with camouflaging. In addition, many patients find that the use of make-up helps even out skin tone. Several available brands that provide heavy coverage include Dermablen (Vichy Laboratories, Paris, France), Cover FX (Cover FX Skin Care, Toronto, Ontario, Canada), Covermark/ CM Beauty (CM Beauty, Northvale, NJ, U.S.A.) (Sheth and Pandya, 2011b).

1.3.2.2. Topical treatments

Topical treatments for disorders of facial hyperpigmentation are aimed at disrupting the enzymatic processes of pigment production within the melanocytes. In the process of melanin production, tyrosinase is the rate-limiting enzyme converting L-tyrosinase to L-3,4-dihydroxyphenlalanine (L-DOPA). LDOPA is a required cofactor, and copper is also an important molecule that interacts at tyrosinase's active site. Many compounds target these molecules leading to a decrease in melanization (Grimes *et al.*, 2019).

a. Hydroquinone

Hydroquinone (1,4-dihydroxybenzene) is the gold standard for the treatment of facial hyperpigmentation and has been used for more than 50 years. It is thought to act by inhibition of tyrosinase thus reducing the formation and melanization of melanosomes. This also leads to the destruction of melanosomes and possibly even the inhibition of DNA and RNA synthesis (Briganti, Camera and Picardo, 2003). Being an oxidizing agent, hydroquinone changes from white to brown at which time it needs to be discarded as it is ineffective (Shenoy and Madan, 2020).The efficacy of hydroquinone depends on several factors including location of pigment with epidermal pigmentation responding better than dermal, concentration of hydroquinone and vehicle of administration, hydroalcoholic solution being the most effective. Used in concentrations of 2–5%, the response in melasma becomes evident after 5–7 weeks and therapy may be continued for 3–12 months.25,50 Ennes et al. found that 38% of patients with melasma treated with 4% hydroquinone had a complete response vs. only 8% treated with placebo (Ennes, Paschoalick and Alchorne, 2000). In a nonrandomized trial, 4% hydroquinone and broad-spectrum sunscreen was shown to be efficacious in the treatment of melasma, with 89.5% of subjects showing good to excellent responses

(Amer and Metwalli, 1998). Adverse reactions to hydroquinone are dose and duration dependent. Reactions include asymptomatic transient erythema, irritation, confetti-like depigmentation with higher concentrations and exogenous ochronosis. Uncontrolled access to high concentrations of hydroquinone and/or overuse can increase the risk of adverse events. Over the past few years, concern has been growing over the use of topical hydroquinone. One concern is the potential risks from benzene derivatives after hepatic metabolisms, which are proposed to cause bone marrow toxicity and exert antiapoptotic effects. Topical hydroquinone, however, bypasses the liver initially, and the major route of metabolism is via water soluble, renally excreted molecules (Westerhof and Kooyers, 2005). In a review of hydroquinone safety issues, Nordlund et al. maintained that there does not appear to be more than a theoretical risk of malignancy and very low risk of other side-effects, including exogenous ochronosis, when using prescription topical preparations of hydroquinone under physician supervision.61 In addition, there have been no reports to date of skin or internal organ malignancies occurring in humans as a result of topical hydroquinone application(Nordlund, Grimes and Ortonne, 2006)

b. Azelaic acid

Azelaic acid is a 9-carbon dicarboxylic acid derived from Pityrosporum ovale, which is antiproliferative and cytotoxic to melanocytes. It acts as a weak, reversible, competitive inhibitor of tyrosinase. Another possible mechanism of action includes decreased free radical formation. It has been used in the treatment of both melasma and PIH. In patients with melasma, 20% azelaic acid was found to be as effective as 4% and superior to 2% hydroquinone, but without side-effects (Baliña and Graupe, 1991).

Overall, azelaic acid is well tolerated with the most commonly reported side-effects including pruritus, mild erythema, scaling and burning.

c. **Retinoids**

Retinoids reduce hyperpigmentation through multiple mechanisms including promoting loss of melanin through the stimulation of keratinocyte turnover, reducing melanosome transfer and allowing for greater penetration of other active ingredients (Ortonne, 2006).Retinoids are thought to inhibit tyrosinase transcription, interrupt melanin synthesis and inhibit tyrosinaserelated proteins 1 and 2. Available retinoids (retinoic acid, tretinoin, adapalene, tazarotene) have been used to treat melasma and PIH, either as monotherapy or combined with other agents including hydroquinone and topical steroids. As monotherapy, higher percentages of retinoic acid (0.1%) have been found to be more effective for melasma than lower percentages (0.05%, 0.025%), however, also more irritating (Kimbrough Green *et al.,* 1994). In a randomized controlled trial, 0.1% tretinoin was found to be more effective than the vehicle for treating patients with melasma (Gasparian, Geng and Hawy, 2021). Importantly, it took longer, 24 weeks, in these studies to see significant improvement. Adapalene (0.1%) has been found to be as efficacious as tretinoin (0.05%) with significantly less irritation in the treatment of melasma (Dogra, Kanwar and Parsad, 2002). Once-daily application of 0.1% tazarotene cream was shown to be effective against PIH, achieving significantly greater reductions compared with vehicle in overall disease severity and in the intensity and area of hyperpigmentation within 18 weeks (Grimes and Callender, 2006). In a study comparing 0.1% tazarotene cream and 0.3% adapalene gel, both were found to be effective and well tolerated in the treatment of PIH; however, tazarotene was more effective (Tanghetti *et al.,* 2010).Topical 0.05% isotretinoin gel applied daily with SPF

28 sunscreen has been evaluated in the treatment of melasma in Thai patients; however, this application did not show increased efficacy vs. vehicle and sunscreen alone. Retinoids have been found to be effective when combined with other agents including but not limited to hydroquinone, lactic acid and ascorbic acid. Irritation is the most common side-effect and may even cause hyperpigmentation so should be used cautiously (Leenutaphong, Nettakul and Rattanasuwon, 1999).

d. Kojic acid

Kojic acid is produced by Aspergillus oryzae and Penicillium spp (Zolghadri *et al.*, 2019). It inhibits the production of free tyrosinase by inhibiting tyrosinase through chelation of copper at the enzyme's active site. The agent is usually available over the counter in a 2% concentration. When used alone, 1–4% formulations are only modestly efficacious; however, it can also be used in combination formulations. Studies have found mixed results. For melasma, in one split-face trial, a gel containing glycolic acid, hydroquinone and kojic acid showed more improvement (60%) in patients than a gel that contained only glycolic acid and hydroquinone (47.5%) (Grimes *et al.*, 2019). In another split-face trial comparing a combination glycolic acid and kojic acid preparation with a glycolic acid and hydroquinone preparation, authors found no statistically significant difference in clinical efficacy (Garcia and Fulton, 1996). Important to note is that kojic acid is a known sensitizer and may cause contact dermatitis and erythema (Draelos, 2007).

e. Ascorbic acid

Ascorbic acid, also known as vitamin C, has antioxidant properties and reduces melanogenesis by interacting with copper at the active site of tyrosinase and by

reducing dopaquinone by blocking dihydrochinindol-2-carboxyl acid oxidation (Oe, Iana and Raelos, 2007). In a randomized trial, patients with melasma found greater subjective improvement to one side of the face treated with 4% hydroquinone cream (93%) than the other side of the face treated with 5% ascorbic acid cream (62.5%).78 However, ascorbic acid had a better safety profile causing significantly less irritation than hydroquinone; therefore, it may be a useful adjunctive treatment in those unable to tolerate hydroquinone. In another study, a 25% L-ascorbic acid formulated with a penetration enhancer was found to improve melasma significantly (Hwang *et al.*, 2009). Because of its instability in aqueous solution, esters like magnesium ascorbyl-2-phosphate (MAP) with similar properties have been used (Matsui *et al.*, 2018). MAP was found to reduce pigmentation significantly in 19 of 34 patients with melasma and senile freckles but only in three of 25 patients with normal skin.81 Ascorbic acid can be used alone or in combination therapy. Overall, this molecule is rapidly oxidized, highly unstable and does not work well alone; it is, therefore, usually combined with other agents such as liquorice extracts and soy to increase efficacy.

f. Liquorice derivatives

Liquorice is the root of the perennial herb, Glycyrrhiza glabra. Liquorice extract has anti-inflammatory properties and contains glabridin, which inhibits tyrosinase in vitro (Yokota *et al.*, 1998). Other active ingredients are liquiritin and isoliquiritin, which, respectively, disperse melanin and contain flavonoids. In a split-face trial, a 20% liquiritin cream was found to be effective at 4 weeks in the treatment of epidermal melasma (Amer and Metwalli, 2000). The use of 1 g per day for 4 weeks is often used before any benefit is seen.

g. Soy

Soybean trypsin inhibitor reversibly inhibits the protease-activated receptor-2 pathway that is needed for melanosome transfer (Berardesca *et al.*, 2020). Inhibition of this pathway caused a dose-dependent loss of pigmentation by as early as 4 weeks at the highest tested dose (Seiberg *et al.*, 2000). Additional work on this pathway has shown that soymilk and the soybean-derived serine protease inhibitors can inhibit both baseline and UVB-induced pigmentation in vitro (Paine *et al.*, 2001). In a multiagent comparative trial, soy extract was shown to have a modest effect in lightening solar lentigines (Hermanns *et al.*, 2002).

h. Arbutin/deoxyArbutin

Arbutin is the naturally occurring b-D-glucopyranoside derivative of hydroquinone that is derived from the bearberry plant, and deoxyArbutin is a dehydroxylated derivative of arbutin. Arbutin is hydrolyzed in the skin to hydroquinone and produces skin lightening by direct, dose-dependent inhibition of tyrosinase (H. Kim *et al.*, 2012). The synthetic deoxyArbutin is a more potent tyrosinase inhibitor, and in guinea pig and human tests has been shown to be more rapidly effective (Boissy, Visscher and deLong, 2005). Although good controlled clinical trials are lacking, initial in vitro and in vivo experiments have demonstrated its safety and efficacy in hypermelanotic disorders. It has been used in Japan at concentrations of 3%; of note, at higher concentrations it may cause paradoxical hyperpigmentation (Hamed *et al.*, 2006) .

i. Niacinamide

Niacinamide reduces pigmentation by reversibly preventing the transfer of melanosomes from melanocytes to the keratino-cytes (Greatens *et al.*, 2005). It is an

important component of many over-the-counter lightening creams. Hakozaki et al. showed that niacinamide decreases hyperpigmentation compared with vehicle alone after 4 weeks of use (Hakozaki *et al.*, 2002). By enhancing percutaneous absorption, its efficacy was doubled by low-frequency sonophoresis (Hakozaki *et al.*, 2006).

j. N-Acetylglucosamine

N-Acetylglucosamine (NAG) is a carbohydrate and represents the monomeric unit of chitin, the main component of the cell walls of fungi and the exoskeletons of arthropods such as crustaceans and insects. It is proposed to work by inhibiting the conversion of protyrosinase to tyrosinase. NAG has been shown to decrease melanin synthesis and downregulate the expression of various pigmentation-related genes (Bissett *et al.*, 2007). Clinical studies have used either NAG alone or in combination with niacinamide. In an 8-week, double-blind, placebo-controlled, split-face clinical trial, 2% NAG reduced the appearance of facial hyperpigmentation. In a 10-week, double-blind, vehiclecontrolled, parallel-group study, the combination 2% NAG and 4% niacinamide was significantly better than vehicle in reducing the detectable area of facial spots and the appearance of hyperpigmentation (Kimball *et al.*, 2010). Overall, NAG has high tolerance, ease of formulation and stability in solution making it a suitable depigmenting agent.

k. Glycolic acid

Glycolic acid directly inhibits tyrosinase and also reduces hyperpigmentation due to its effects on epidermal remodelling and accelerated desquamation (Usuki *et al.*, 2003). A combination of 10% glycolic acid and 4% hydroquinone was shown to be effective in

treating melasma. Side-effects include irritation and erythema, which resolves upon withdrawal and moisturization (Sun *et al.*, 2020).

l. Lignin peroxidase

Lignin peroxidase is a novel method of skin lightening and acts by targeting, enzymatically oxidizing and breaking down melanin in the skin. In a randomized, double-blind, placebocontrolled, split-face, single-centre study of 51 patients, patients were randomized to receive day and night lignin peroxidase cream on one side of the face and either 2% hydroquinone or placebo on the other (Mauricio, Karmon and Khaiat, 2011). The application of lignin peroxidase cream provided a significantly more rapid and observable skin-lightening effect than 2% hydroquinone or placebo. Overall, lignin peroxidase is well tolerated with minimal to no side-effects.

1.3.3. Miscellaneous agents

There are multiple other agents either under investigation or combined with other products to enhance skin-lightening effects. N-Acetyl-4-S-cysteaminylphenol (NCAP) acts as an alternative substrate for tyrosinase, inhibiting its activity. In a retrospective analysis of 12 patients with melasma who used 4% NCAP, 66% showed marked improvement as early as after 2–4 weeks (Jimbow, 1991). Other agents known to affect melanin pigmentation are thiotic acid (a-lipoic acid), gentisic acid and mulberry extract. In addition, a-tocopheryl ferulate absorbs UV radiation and significantly slows melanogenesis, possibly by inhibiting tyrosine hydroxylase. Flavonoids including catechin, ellagic acid and aloesin are naturally occurring polyphenols with antioxidant properties.

1.3.4. Combination products

Topical agents when combined have the ability to give better therapeutic results as they act on different stages of melanogenesis. Combining agents can also reduce side-effects. For example, topical steroids reduce hydroquinone- and retinoidinduced irritation, while retinoids can counter steroid-induced atrophy. Other agents can be used as stabilizers, and sunscreens are also often added to combination agents.

As the gold standard in the treatment of hyperpigmentation disorders, hydroquinone is often combined with different agents including retinoids, corticosteroids, glycolic acid, kojic acid and ascorbic acid. The most extensively studied and widely used is the combination therapy of hydroquinone, retinoic acid and corticosteroid. First proposed by Kligman and Willis, the original combination contained 5% hydroquinone, 0.1% tretinoin and 0.1% dexamethasone (Kligman and Willis, 1975). This combination treatment was found to be effective in melasma, ephelides and PIH, and time to see benefit with twice-daily usage was approximately 3 weeks. Researchers also found that fluorinated steroids were more effective than nonfluorinated steroids. The theory behind the effectiveness of this combination is that tretinoin prevents the oxidation of hydroquinone and improves epidermal penetration while the topical steroid reduces irritation from the other two ingredients and also decreases cellular metabolism, which inhibits melanin synthesis (Lynde, Kraft and Lynde, 2006).

Since this discovery, multiple dual and triple combination therapies have been studied. Due to the irritation, the combination has been modified to reduce hydroquinone to 2–4% and tretinoin to 0.025–0.05%, and the concomitant use of regular photoprotection of at least SPF 15 has been shown to increase efficacy of topical therapy significantly (Trivedi, Yang and Cho, 2017). One of the most successful combination formulations

has been 4% hydroquinone, 0.05% tretinoin and 0.01% fluocinolone acetonide. In a multicentre, investigator-blinded, randomized, prospective trial in patients with melasma, this triple combination cream was found to be more efficacious than dual-combination creams containing either hydroquinone plus tretinoin, hydroquinone plus fluocinolone or tretinoin plus fluocinolone with nightly use (Torok *et al.*, 2005) Seventy-seven per cent of patients on the triple-combination treatment achieved complete or near complete clearance compared with a maximum of 468% of patients on the dual-combination treatment (Nasrollahi *et al.*, 2019). A more recent trial comparing the triple-combination regimen with 4% hydroquinone alone in patients with melasma also confirmed the superiority of the combination regimen, 35% achieving clearance vs. 5.1% (Ferreira Cestari *et al.*, 2007) . Another combination using 6% hydroquinone, 0.05% tretinoin, 0.05% triamcinolone acetonide and 01% ascorbic acid nightly with daily photo protection was found to be efficacious, with five of six patients with epidermal or mixed melasma showing moderate to significant improvement over an 8-week period (Guevara and Pandya, 2001).

Side-effects of combination treatment include erythema, irritation, pruritus and desquamation. In treating disorders of hyperpigmentation, especially melasma, triple-combination regimens appear to be the most clinically effective treatment. While efficacious, the cost can be prohibitive. However, although associated with increased upfront costs, a cost/benefit analysis of combination therapy vs. hydroquinone alone for melasma found that the combination regimen led to a 30% greater rate of clearance with lower overall cost (Cestari *et al.*, 2007).

Hyperpigmentation Disease	Causes	Features	Treatment	Reference
Postinflammatory hyperpigmentation (PIH)	Inflammation (acne, eczema, allergic reaction), trauma to the skin cause melanocytes to produce melanin.	Presence of erythema in inflammation	Skin lightening agents, tyrosine inhibitor, laser	(Rigopoulos, Gregoriou and Katsambas, 2007)
Periorbital hyperpigmentation	Caused by rubbing and scratching of skin around the eye.	Dark circle in eye	Skin lightening agents, tyrosine inhibitor, combination product	(Ranu *et al.*, 2011)
Riehl melanosis	Due to allergic reaction of sensitizing chemicals in cosmetics.	Mild erythema followed by hyperpigmentation of brown grey color.	Allergen free cosmetics and soap, Sun Protective creams, Skin lightening agents	(Pérez-Bernal, Muñoz-Pérez and Camacho, 2000)
Melasma	Due to birth-control pills, pregnancy and hormone therapy cause melasma.	History of pregnancy, symmetric distribution of dark brown patches with irregular border in face and umbilicus	Combination product, kojic acid, skin lightening agents	(Sheth and Pandya, 2011a)
Exogenous ochronosis	Occurs due to topical contact with phenol or resorcinol.	Banana shaped yellow-brown deposit on dermis	Laser, glycolic acid, kojic acid	(Zawar and Mhaskar, 2004)
Acanthosis nigricans	Caused by type 2 diabetes, affects of hormonal level, and certain medicine including steroids.	Pigments in neck and axillae	Skin lightening acid, kojic acid, salicylic acid.	(Sinha and Schwartz, 2007)
Maturational dyschromia	Sun exposure.	Darkening of malar cheeks and forhead.	Skin lightening agen, sun protective agents like sunscreen, antioxidant	(Woolery-Lloyd and Kammer, 2011)

Table.1.1: Different types of hyperpigmentation, causes, features and treatment

1.4.Sheet mask

Sheet Mask is a type of beauty essential these days, that is basically shaped in the form of a face, imbued with essential nutrition-packed serums. These masks that come with an opening at eyes and nostrils nicely shape up with your facial structure so that every inch of your face gets covered and gets enriched by the concentrated serum.

These beauty innovations brought by the cosmetic and beauty care industry of South Korea and Japan are widely gaining popularity as they are used for treating various skin related problems as shown in Figure 1.10. The masks are generally made up of different types of materials like cotton wool, coconut pulp, gel, microfibres, paper, cellulose, etc and they are drenched in a variety of serums depending upon the type of skincare issue being treated.

Figure 1.10: Sheet mask

These sheet masks are very different from usual masks and peel-off masks as in here, you don’t have to apply the cream or gel and then wash it off. One usually has to

cleanse the face using a face wash or a facial cleanser, apply some soothing toner and then just put on the sheet mask and relax for about 20 to 30 minutes. The extra serum inside the packet can be applied to other parts like the neck, hands and chest area. After that, just remove the sheet mask and you are ready to go. It can be used every alternate day when you don't have much time to spend on your skincare routine and is much beneficial if applied before going to sleep so that overnight the enriched serum seeps into the skin and gives the desired effect.

The concentrated serum used in the sheet masks is diluted in little water and consists of essential vitamins and hyaluronic acid. The sheet prevents evaporation of the serum and helps it to completely seep inside the skin cells. When used regularly, it gives the same effect as a spa and bestows a radiant glow to the skin.

Different sheet mask material:

a) **Cotton**

Fits well on the face and has a white fabric. It does not contain harsh chemicals that may irritate the skin. It is soft, absorbent, and allows air and moisture move around freely, allowing your skin to stay comfortable and moisturized.

Fiber masks feature a non-woven fabric; they have a coarse texture that feels and behaves like paper. Fiber masks are very inexpensive and have some capacity to replenish moisture to your skin. They evaporate very quickly, however, and their paper-like texture lacks the "contouring" to remain in place for very long. If you use a low-end fiber mask, you will need to lie down to keep it from falling off.

b) Microfiber

Feels like soft silk and is white in color. It is usually a little thicker and softer than regular cotton sheets. It also conforms onto the face better. This material is eco-friendly because is biodegradable and does not contain harmful chemical ingredients.

c) Cupra

Made from “cotton linters”. These are short fibers that are left behind after processing raw cotton. This sheet mask material is white but has a transparent appearance when applied on the skin. It allows for moisture control and ensures that your skin is getting essence that is evenly distributed.

d) Tencel

This fiber is naturally derived from trees, it is a newer fiber that is considered a type of high-quality lyocell fiber. It is biodegradable and eco-friendly. It has a white/light cream color and feels like silk on the skin.

e) **Rayon**

Rayon masks are the classic sort of sheet mask material. It is has a thicker feel to it compared to Tencel. This material does tend to slip because of the thickness, but it's still comfortably soft and grabs onto the essence well.

f) Hydrogel

This sheet mask material has a transparent look and requires careful handling! Don’t pull or tug on a hydrogel mask as it may rip and you’ll be left with sticking puzzle

pieces to your face. They are 100% soluble and feels like a cool, thin layer of film on your face.

g) Bio-cellulose

This is an all natural fiber and really sticks to our skin. It's very similar to hydrogels but bio-cellulose sheet masks are usually thinner. The strong adhesion allows active ingredients to really sink in.

h) Foil

Foil sheet mask materials really help prevent the liquid, nutrients, and active ingredients from evaporating. It has a cotton layer on the side where it touches your skin making it super comfortable to wear.

i) **Charcoal**

This sheet mask material is a black material and feels super smooth on the skin. Charcoal powder is compressed and infused into the sheet to detox the skin and help balance out excess oils.

j) PULP

Pulp masks have a finer texture than fiber masks, but the limitations of the two mask types are similar. The fit of a pulp mask will be a bit uneven, with gaps noticeable between the mask and your skin (adhesion loss). As with a fiber mask, the serum in a pulp mask will evaporate fairly quickly. Rayon cellulose is an example of a mask created from pulp.

1.4.1 Different skin care regimen with sheet mask:

i) Brightening Sheet Mask:

With time, our skin loses the natural glow and radiance and needs proper care to regain it back. Most of the sheet masks under this category are enriched with vitamin C, collagen and powerful antioxidants that prevent and treat harmful free radical damage. It also reduces dark spots and restores the healthy glow to provide younger-looking skin

ii) Hydrating Sheet Mask:

Infused with hydrating ingredients like seed oil, glycerine, fruit extracts, these sheet masks effectively soothe and hydrate the skin. It gets rid of dry patches and crepe skin and provides a firm, plump and rejuvenated appearance.

iii) Acne-Control Sheet Mask:

Environmental pollutants, unhealthy food habits, and hormonal changes lead annoying zits and pimples. Most of the women are plagued due to this and hence the anti-acne sheet masks are everyone's hot favourite. Imbued with soothing and calming ingredients like algae, tea tree oil, aloe vera and salicylic acid these sheet masks help in controlling oil secretion, clears pores, removes dirt and prevents breakouts. It also reduces irritation and inflammation in acne-prone skin.

iv) Anti-aging or Cellular Repair Sheet Mask:

We all love to showcase a youthful skin minus the annoying signs of aging like wrinkles, fine lines, spots, etc. Although reversing the age clock, might seem like a pretty tough job, these sheet masks saturated with ingredients like aloe vera, witch

hazel, hyaluronic acid, and essential oils not only reduces the various signs of aging but also bestows a radiant, glowing rejuvenated skin.

v) Detoxifying Sheet Mask:

Most of the working women out their will agree that their daily routine consists of facing a lot of harmful pollutants, debris, dirt, and allergens onto their skin which ultimately leads several underlying skin conditions. The detoxifying sheet masks mostly containing ingredients like charcoal, aloe vera, essential oils and caffeine hold high significance in removing the harmful toxins and allergens from the skin. It nourishes the skin from within and helps to get smooth radiant skin.

1.4.2 Biocellulose Sheet mask

The sheet mask is a trending product connected with smart consumers for safeness and effectiveness. According to Mintel's data, the Asia-Pacific area is identified at present as among the world's largest facial care markets. Sheet masks are produced from various types of fabric. Sheet mask of coarse fiber is made up of a very less expensive mask. While advanced sheet mask is manufactured by natural polymer, also some gentle sheets are made up of cotton pulp, hydrogel, and bio-cellulose. These all are soaked in the solution made up of active ingredients used as pampering of the skin, brightening the skin tone, providing relaxation, reduce fine lines and wrinkles(P *et al.*, 2019).

Biocellulose is used in this formulation because biocellulose is a more environmentally friendly alternative to the fibers used in other traditionally manufactured sheet masks because it's 100% compostable.

- Biocellulose fabrics are tightly woven and very durable. Masks made from Biocellulose will not snatch or tear.
- Biocellulose is toxin-free, while paper masks are often treated with chemicals.
- Biocellulose has the capability of donating liquid as well as absorbing quality which makes it the best carrier for delivering nourishing ingredients to our skin.
- Biocellulose holds on to our skin, and they are best for deeper ingredient penetration.

1.5. Kojic Acid

1.5.1 Chemical and physical data

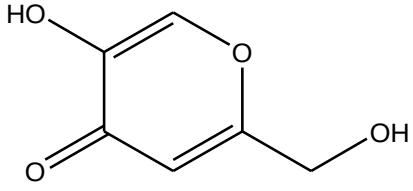
1. **Nomenclature**

Chemical Name: 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one

IUPAC Systematic Name: Kojic acid

Synonym: 5-Hydroxy-2-(hydroxymethyl)-4-pyrone; 2-hydroxymethyl-5-hydroxy-γ-pyrone

2. **Structural and molecular formulae and relative molecular mass**



5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one

Molecular Formula: $C_6H_6O_4$

Relative molecular mass: 142.11

3. Chemical and physical properties of the pure substance

(a) Description: Prismatic needles from acetone (Burnett *et al.*, 2010)

(b) Melting-point: 153.5 °C .

(c) Solubility: Soluble in water (43.85 g/L; Dialog Corp., 2000), acetone, chloro- form, diethyl ether, ethanol, ethyl acetate and pyridine; slightly soluble in benzene (das Neves *et al.*, 2020)

(d) Dissociation constants: pKa, 7.90, 8.03.

4. Technical products and impurities

Kojic acid is commercially available at a purity greater than 98.0% and as a solution for spraying (TCI America, 2000; Tokyo Kasei Kogyo Co., 2000). Impurities may include heavy metals (10 mg/kg max.) and arsenic (4 mg/kg max.) (Jarchem Industries, 2000).

5. Analysis

Methods for the analysis of kojic acid in commercial foods, flavouring compounds, cosmetic products and microorganisms have been reported. These methods include voltammetry, spectrophotometry, column chromatography with ultraviolet detection, thin-layer chromatography, gas chromatography with or without flame ionization, electron capture or mass spectrometry detection, and high-performance liquid chromato- graphy with photodiode-array or ultraviolet detection (Owens, Welty and

Lucas, 1970) (Scott, Lawrence and van Walbeek, 1970) (Mehlig and Shepherd, 1949)(Qureshi, Prentice and Burger, 1979)(Tanigaki, Obata and Tokuyama, 1980).

1.5.2 Mechanism of Kojic acid

Kojic acid works by blocking tyrosine from forming, which then prevents melanin production. Decreased melanin production may have a lightening effect on the skin.

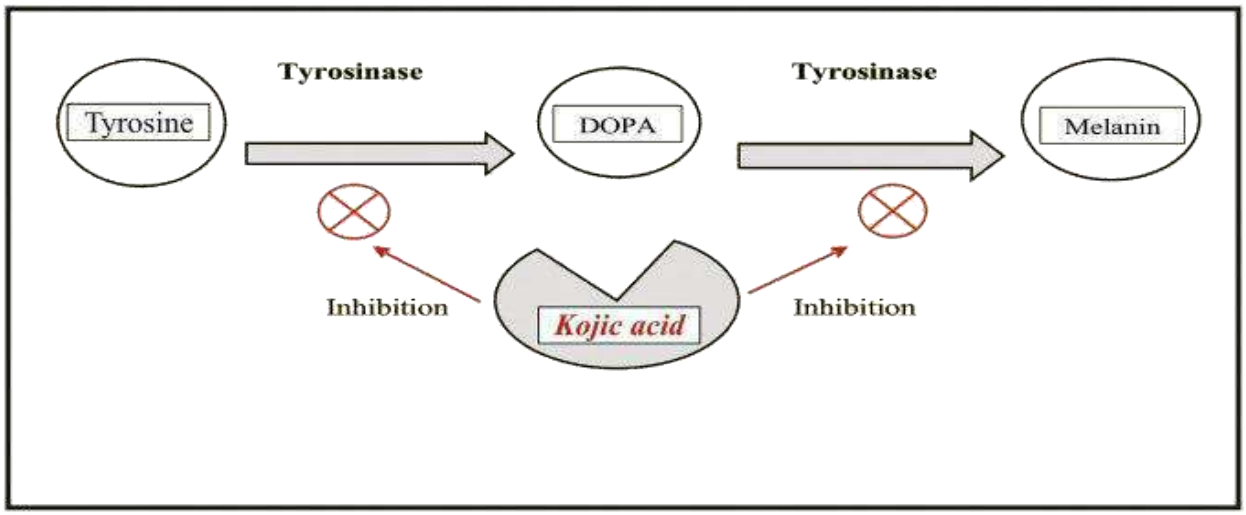


Figure 1.11: Mechanism of Kojic acid

1.5.2 Production

Kojic acid is a natural antibiotic agent obtained from koji malt (Aspergillus oryzae). Koji malt has been used for the production of miso, soya sauce and sake in Japan for a long time ((Feng *et al.*, 2019); Jarchem Industries, 2000).

Information available in 2000 indicated that kojic acid was manufactured by two companies in China and one company each in Japan, Switzerland and the USA (CIS Information Services, 2000).

1.5.3 Use

The most important applications of KA are as follows:

- Bleaching properties and skin protection in contrast to ultravioletlight in cosmetic products
- Dental care products

In some studies, melanogenic inhibitory properties of KA has been proven in vitro. Due to the carcinogenicity of HQ and its prohibition in Asia, the FDA has introduced KA as an alternative for HQ. Recently, chelates of KA and manganese and zinc metals have been introduced asprotective agents against gamma and radio rays (Lee *et al.*, 2006).In many studies, various derivatives of KA such as KA ester, KA laureate, KA dipalmitate and, KA ethyl phosphonate with aldehyde have been reported to be more effective than KA (Ashari *et al.*, 2009). A schematic of cosmetic applications of KA is shown in Figure.1.12. Moreover, due to the presence of a pyron ring in the structure of KA, it is used to assess iron in mineral stones. KA metal chelates are used in controlled release in drug delivery and catalysts.

Several studies have reported that KA acts as an antibiotic against human tubercle bacilli, gram-negative and gram-positive microorganisms in in-vitro. In addition, the derivatives of KA called azido metal kojates are reported to act as antifungal and antibacterial agents on several specieso f Bacillus, Staphylococcus, Saccharomyces, Aspergillus, Rhizopus, and Fusarium. Also, zinc derivatives of azidometalkojates have cytotoxic activity on the hella tumor cells. In addition, other derivatives of KA act as antifungal agent on several species of Phythium graminicola, Fusarium oxysporum, and Rhizoctonia solani. In other studies, insecticidal properties of KA has been shown

on Heliothis zea, Spodoptera frugiperda, Musca domestica, and Drosophila melanogaster insects. It also causessterility in male and female species (Synytsya *et al.*, 2008).

KA and its derivatives have become increasingly important due to various biological activities, including antimicrobial and antiviral(Singh *et al.*, 2016), antitumor (Nawarak *et al.*, 2008), antidiabetic (Xiong and Pirrung, 2008), anticancer (Cho *et al.*, 2010), anti-speck (Uchino *et al.*, 1988), anti-parasitic (Rodrigues *et al.*, 2014), and pesticidal and insecticidal activities. In addition, KA and its derivatives are used as antioxidant, anti-proliferative, anti-inflammatory, radio protective and skin-lightening agent in drug and cosmetic products, due to their tyrosinase inhibitory activity(Novotný *et al.*, 1999). Furthermore, KA could be developed as a chemo sensitizer to enhance efficacy of commercial antifungal drugs or fungicides (J. H. Kim *et al.*, 2012).

Potential application of KA and its derivatives has been studied in veterinary medicine, cosmetic and chemical industry (Garcia and Fulton, 1996)(Ravi Kumar, 2000)

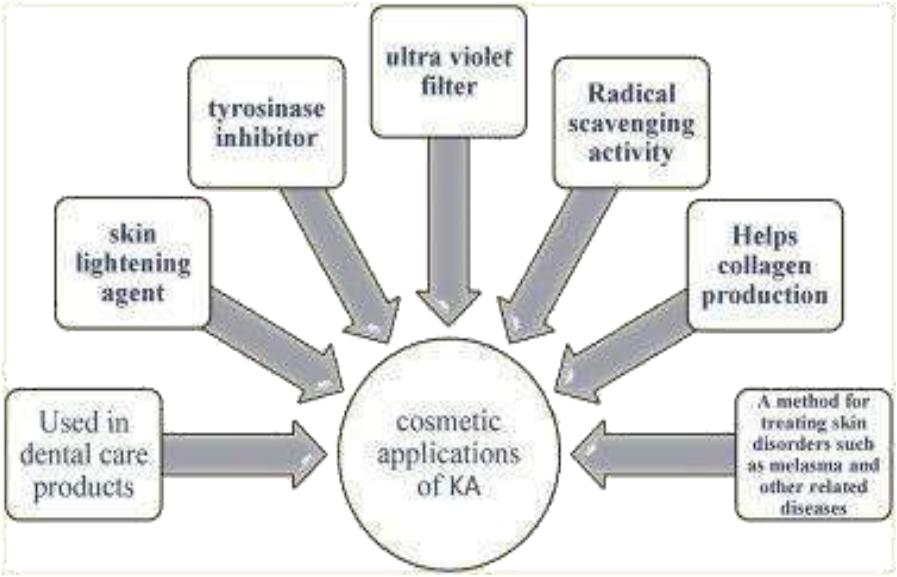


Figure 1.12: Application of Kojic acid.

1.6. Marketed review of sheet mask

According to a new report published by Allied Market Research titled, " **Sheet Face Mask Market by Product Type, Price Point and Distribution Channel: Global Opportunity Analysis and Industry Forecast, 2019–2026**," The global sheet face mask market size was valued at $206.0 million in 2018 and is anticipated to reach $392.1 million by 2026, with a CAGR of 8.76% during the forecast period. The cotton/microfiber sheet segment was the highest contributor to the market, with $62.4 million in 2018, and is estimated to reach $107.5 million by 2026, at a CAGR of 7.4% during the forecast period.

Premiumization trend in cosmetic products has played a crucial role in the adoption of facial mask products. The increased awareness toward sustainability and environment footprint largely influences purchase decision of consumers. Nowadays, consumers are more concerned about materials and active ingredients used in the manufacturing and packaging of skincare products. They also check the reusability of raw materials used during manufacturing. This has resulted into adoption of innovative ingredients and technologies for manufacturing environmentally-friendly sheet masks. Such adoption of novel technologies and materials is expected to propel the revenue growth of the overall sheet face mask market during the forecast period.

Increased number of geriatric population and rise in popularity of beauty enhancer products boosts the sheet face mask market demand. Moreover, growing awareness among generation X of Asia, especially female population, drives the revenue growth of the sheet face mask market. Sheet face mask market trends of multi-functional cosmetic products is also followed among consumers, where consumers are showing

interest in single products with all skin benefits. Generation X is adopting sheet face masks not only for appearance but also to supplement their routine skin care regime.

The sheet face mask market report is segmented on the basis of product type, price point, distribution channel, and region. By product type, it is categorized into cotton/microfiber sheet, hydrogel sheet, knit sheet, bio-cellulose sheet and others. The global Cotton/Microfiber Sheet segment was valued at $62.4 million in 2018 and is anticipated to reach $107.5 million by 2026, with a CAGR of 7.4% during the forecast period. By price point, it is divided into mass and premium. By distribution channel, Sheet Face Mask Market is categorized into retail pharmacies, convenience stores, E-commerce, and supermarkets/hypermarkets. Region-wise, Sheet Face Mask Market segment is analyzed across North America (the U.S., Canada, and Mexico), Europe (Germany, France, Spain, Italy, and Rest of Europe), Asia-Pacific (China, India, Japan, Australia, South East Asia, and Rest of Asia-Pacific), and LAMEA (the Middle East, Latin America, and Africa).

Key Findings of the Study:

- The Hydrogel Sheet segment is expected to grow at a CAGR of 8.8% during the forecast period.
- Asia-Pacific region is anticipated to dominate the sheet face mask market share, registering a CAGR of 8.5% during the forecast period.
- North America is projected to exhibit exponential growth throughout 2026, registering the highest of CAGR 11.0% during the forecast period.

- The Supermarkets/Hypermarkets distribution segment is anticipated to dominate the global sheet face mask market analysis, with a CAGR of 8.3% during the forecast period.

- The E-commerce segment is estimated to grow at a CAGR of 11.7% during the sheet face mask market forecast period.

The key players operating in the global sheet face mask industry include BioRepublic SkinCare, ES Cosmetics, Estee Lauder Companies Inc., Innisfree Corporation, Kracie Holdings, Ltd., L'Oréal, Lancome Paris, Sephora Inc., The Face Shop and Tonymoly Co Ltd. Other key players operating in Sheet Face Mask Market value chain are Amorepacific Corporation, Bio Natural Inc., Boss Biological Technique Ltd., Decleor Paris, Erno Laszlo, It's Skin, Luxaderme, Orgaid, Star Skin Beauty Group AG, and Starskin, Yunos Co. Ltd.

Figure 1.13: Marketed formulation of sheet mask.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Review of articles:

- Pérez-Bernal et al., 2013; found that facial and neck pigmentations are significant cosmetic problems. They are common in middle-aged women, related to endogenous (hormones) and exogenous factors (cosmetics, perfumes, sun exposure), and often represent paramount causes of emotional distress. Although melasma is the most common cause of facial pigmentation, there are many other forms including drug-induced and post inflammatory hyperpigmentationoften proves challenging. Several methods of treatment are available depending on the condition, and a thorough understanding of the underlying aetiologies of facial hyperpigmentation is essential for selecting the best treatment options (Pérez-Bernal *et al.*, 2013) .

- Jiang et al., 2018 interviewed that QoL (quality of life) and self-esteem appear to be significantly impacted by hyperpigmention/melasma. Patients refused to leave their house, felt inferior to others, and incessantly thought about their melasma. Physicians who treat patients with melasma should aim to not only improve pigmentary changes but also improve QoL and self-esteem. Further research into this subject area should be performed, perhaps by conducting prospective studies using a validated tool that is specific for self-esteem such as the Rosenberg Scale for self-esteem of patients with melasma from different backgrounds and skin types before and after treatment (Jiang *et al.*, 2018).

- Camacho et al., 2000; says that treatment of melasma and other facial pigmentations has always been challenging and discouraging. It is important to

avoid exposure to the sun or to ultraviolet lamps, and to use broad-spectrum sunscreens. Several hypopigmenting agents have been used with differing results. Topical hydroquinone 2 to 4% alone or in combination with tretinoin 0.05 to 0.1% is an established treatment. Topical azelaic acid 15 to 20% can be as efficacious as hydroquinone, but is less of an irritant. Tretinoin is especially useful in treating hyperpigmentation of photo aged skin. Kojic acid, alone or in combination with glycolic acid or hydroquinone, has shown good results, due to its inhibitory action on tyrosinase. Chemical peels are useful to treat melasma: trichloroacetic acid, Jessner's solution, Unna's paste, α-hydroxy acid preparations, kojic acid, and salicylic acid, alone or in various combinations have shown good results. In contrast, laser therapies have not produced completely satisfactory results, because they can induce hyperpigmentation and recurrences can occur. New laser approaches could be successful at clearing facial hyperpigmentation in the future (Pérez-Bernal, Muñoz-Pérez and Camacho, 2000).

- Piantavini et al., 2013 developed UV spectrophotometric method for the quantification of KA in raw material and in final pharmaceutical formulations. The method is selective to quantify KA in creams. According to the validation results, the method is linear (5-50 μg/mL found 215 to 240nm), precise, accurate and robust. Additionally the method takes advantage of simple and accessible reagents and equipment, while having a low operating cost.(Piantavini *et al.*, 2013)
- Devi et al., 2015 reported that the FTIR spectrum of the sample kojic acid shows the peak wave number values for the functional groups similar to that of standard kojic acid. The bands appear for functional groups at 3270.8 cm-1 ,

3179.43 cm-1 (- OH), 2925.17 cm-1 , 2854.05 cm-1 (aliphatic- CH), 1660.59 cm-1 (cyclic-C=O), 1611.11 cm-1 (C=C), 1472.61 cm-1 (deformation of-CH2), 1074.04 cm-1 (cyclic C-O-C), 943.58 cm-1 , 863.66 cm-1 and 775.65 cm-1 (1, 4 α –di substituted ring) (Devi *et al.*, 2015)

- Burnett et al., 2010 analyzed the properties of kojic acid are found such as solubility, pka(7.90 to 8.03), melting point (152-154℃), log P(-0.64 aprrox) . Also Kojic acid was not a toxicant in acute, chronic, reproductive, and genotoxicity studies. While some animal data suggested tumor promotion and weak carcinogenicity, kojic acid is slowly absorbed into the circulation from human skin and likely would not reach the threshold at which these effects were seen. The available human sensitization data supported the safety of kojic acid at a use concentration of 2% in leave-on cosmetics. Kojic acid depigmented black guinea pig skin at a concentration of 4%, but this effect was not seen at 1%. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the 2 end points of concern, dermal sensitization and skin lightening, would not be seen at use concentrations below 4%; therefore, this ingredient is safe for use in cosmetic products up to that level (Burnett *et al.*, 2010).

- Saeedi et al., 2019; concluded that Kojic acid (KA) is a natural metabolite produced by fungi that has the ability to inhibit tyrosinase activity in synthesis of melanin. The major applications of KA and its derivatives in medicine are based on their biocompatibility, antimicrobial and antiviral, antitumor, anti-diabetic, anticancer, anti-speck, anti-parasitic, and pesticidal and insecticidal properties. In addition, KA and its derivatives are used as anti-oxidant, anti-proliferative, anti-inflammatory, radio protective and skin-lightening agent in skin creams, lotions, soaps, and dental care products. KA has the ability to act

as a UV protector, suppressor of hyperpigmentation in human and restrainer of melanin formation, due to its tyrosinase inhibitory activity. Also, KA could be developed as a chemo sensitizer to enhance efficacy of commercial antifungal drugs or fungicides. In general, KA and its derivatives have wide applications in cosmetics and pharmaceutical industries. The most significant advantage of KA is its wide applications in cosmetics and medical industry. It acts as a brightening ingredient in whitening creams, skin lotions, and bleaching soaps, and also in dental and medical care product (Saeedi, Eslamifar and Khezri, 2019).

- Gonçalez et al., 2013; observed the results suggest that the kojic acid (KA) is iron chelator employed in treatment of skin aging, and inhibits tyrosinase, promotes depigmentation. Nanotechnology-based drug delivery systems, such as liquid crystalline systems (LCSs), can modulate drug permeation through the skin and improve the drug activity. This study is aimed at structurally developing and characterizing a kojic acid-loaded LCS, consists ofwater (W), cetostearyl isononanoate (oil—O) and PPG-5-CETETH-20 (surfactant-S) and evaluating its in vitro skin permeation and retention. Three regions of the diagram were selected for characterization : A(35% O, 50%S,15% W),B(30% O, 50%S,20% W) andC(20% O, 50% S, 30% W), to which 2% KA was added. The formulations were subjected to polarized light microscopy, which indicated the presence of a hexagonal mesophase. Texture and bioadhesion assay showed that formulation B is suitable for topical application. According to the results from the in vitro permeation and retention of KA, the formulations developed can modulate the permeation of KA in the skin. The in vitro cytotoxic assays showed that KA-unloaded LCS and KA-loaded LCS didn’t present cytotoxicity.

PPG- 5-CETETH-20-based systems may be a promising platform for KA skin delivery. Hence, the proposed system is feasible for the incorporation of KA and its controlled release also may be a promising platform for KA skin delivery and to treat topical hyperpigmentation and skin aging (Gonçalez, Corrêa and Chorilli, 2013).

- Khezri et al., 2020; concluded Kojic acid (KA) as tyrosinase inhibitor shows insufficient skin penetration and several adverse events due topical administration. KA solid lipid nanoparticles (KA-SLNs) were prepared using high speed homogenisation followed by ultra-probe sonication method for improve its effectiveness. KA-SLNs was optimised by Glyceryl mono-stearate (GMS) and Cholesterol (Chol) as lipid excipients and span 60 (SP 60) and Tween 20 (Tw 20) as co-emulsifiers (particle size 156.97±7.15nm, encapsulation efficiency 59.02±0.74%, drug loading 14.755±1.63%, polydispersity index (PDI) of 0.388±0.004 and zeta potential (ZP) of -27.67± 1.89mV). Optimum formulation (KA-SLN3 dispersion) was stable at 4 and 25 ℃ for 3months. Also, TEM image confirmed these results. The results of XRD, DSC and ATR-FTIR analysis indicated that KA was well encapsulated within the SLNs either in molecularly dispersed state and stabilised in amorphous form and there was no chemical interaction between drug and other ingredients. Controlled release was achieved with this formulation. KA-SLN3 dispersion have more tyrosinase inhibition potency in comparison with pure KA. Also, the results of the ex vivo and in vitro percutaneous absorption show that KA-SLN3 dispersion improved percutaneous delivery of KA as a promising and potential novel topical preparation and might open new avenues for treatment of hyperpigmentation disorders. Hence, the presence of lipid ingredient in the KA-

SLN3 dispersion carriers seems to have a capability in the development and efficiency of cosmetic and pharmaceutical preparations due to their easier cellular uptake and can be used as a promising and potential novel cosmetic preparation and might open new avenues for treatment of hyperpigmentation disorders (Khezri *et al.*, 2020).

- Kusumawati et al., 2020; results that Sheet Mask has been widely used by East Asians, sheet masks are generally made of non-woven fabric, paper fibers, bio-cellulose, and so on. Red rice with a slightly purplish red color is very suitable for normal to dry skin. As in the analysis of red rice content, there are protein levels, fat content, water content, vitamin C in brown rice which is very good for moisturizing the facial skin. One of the natural ingredients studied is Virgin Coconut Oil (VCO). In this study, a physical evaluation test was carried out on red rice and VCO samples. The purpose of this research is to find out what formulation produces good physical properties. Red rice was extracted coldly using 96% ethanol solvent and VCO using the fermentation method. The results of the phytochemical test of red rice extract contained flavonoids, saponins, terpenoids, and quinones. Based on research results, brown rice extract and VCO have stronger antioxidant activity compared to VCO. Based on the results that have been done on the preparation of sheet mask of red rice extract and VCO, it can be concluded that the most optimal formula in F1 with an active substance concentration of 0.5% more preferred by respondents in hedonic testing with the resulting odor, color and texture parameters (Kusumawati, Wulan and Ridwanuloh, 2020).

- Reveny et al., 2017; found results indicate that facial mask is popular facial-care product amongst consumers, especially the one that contains vitamin E as anti-aging. Face mask is easy to use, and it has a better active- ingredient penetration effect. Biocellulose is a natural cotton-mask replacement which is more eco-friendly with higher occlusive effect. The aim of this research is to formulate a biocellulose mask with vitamin E as anti-aging and to evaluate its effectiveness against volunteer's facial skin.The evaluation conducted to the biocellulose mask include the mask's weight and thickness. Essence evaluation includes homogeneity test, viscocity test, pH test, stability test, irritation test and anti-aging effectivity test using skin analyzer. Parameters measured include moisture, evenness, pore, spots, and wrinkles. The results showed that biocellulose could be formulated as a facial mask preparation and higher vitamin E concentration in the essence result in a better anti-aging effectiveness (Reveny, Tanuwijaya and Stanley, 2017).

- Leny et al., 2020; prepared s the purpose of this sudy was to find out the mask sheet by using black soybean extract as a moisturizer and to know its ability to increase water content or moisture on the skin. Sheet masks were formulated with the addition of black soybean seed extract with a concentration of 1%, 3%, and 5%, then an evaluation of the formula was carried out in the form of homogeneity, pH, skin irritation test, stability, and the activity test to moisturize whites. The results of the evaluation of the formula showed that the sheet mask was homogeneous, pH value 6.1-6.8, did not irritate the skin, and was stable in storage for 4 weeks. The highest percentage increase in humidity occurred in the group using positive control followed by the Black Soybean Sheet Mask (BSSM) 5%. There is a significant difference between the positive control

group, BSSM 3 and 5% against negative control, except BSSM 1%. In the second to fourth week, the 5% BSSM group showed an increase in numbers so that it did not differ significantly from positive control, so this was marked as the optimum concentration. The conclusion of this research is that black soybean seed extract can be formulated in sheet mask form and can increase skin moisture (Leny *et al.*, 2020).

- Sumbul Fatma Hasan Khan *et al* prepared sheet mask for wrinkles. A lot of claims are made about how to make wrinkles go away. Some methods can be painful or even dangerous. To overcome these problems we can use anti-aging sheet mask which is the latest and easiest method to get rid from wrinkles. Sheet masks are face-shaped sheet fabrics soaked in nutrition-packed solution called serum. The sheet is made up of variety of materials including papers, fibers or gel types. It is observed that sheet masks are super simple and hassle-free. There are several disadvantages of conventional sheet mask like there shelf life is less and some of them may prone to oxidative decay. To overcome this problem powdered form serum is suggested. By using ingredients in powdered serum, the shelf life of the product can be increased.(Sumbul Fatma Hasan Khan *et al.*, 2021)

- Russ et al., 2017 reviewed that thickeners are used to enhance the stability and performance of a cosmetic product, as well as to improve consistency, volume and viscosity. In terms of their chemistry, thickeners can be divided into natural thickeners obtained from sources like xanthan gum; chemically modified natural thickeners known as semi-synthetics; and purely chemically synthesized

polymers. Also they reviewed Xanthan Gum provides exceptional rheology control in face masks (Russ and Kasper, 2017).

- Das A et al., 2017 describe the methods for performing skin irritation study healthy rabbits (average weight 1.2-1.5 kg). The samples were applied on the dorsal surface of the rabbit skin, hair of which was removed by shaving and cleaned with rectified spirit. Adhesive tape USP was used as control patch. The formulations (transdermal patch) were removed after 24 hrs and the skin was examined for erythema/edema(Das and Ahmed, 2017).

- Berben et al., 2018 developed for the prediction of the passive permeability of various biological barriers. In addition to cutting costs of cell-and tissue-based permeation tools, cell-free permeation models also proved to be more robust against exogenous substances like pharmaceutical excipients and/or food (digestion) components. As a result, these systems allow formulation scientists to simultaneously investigate dissolution of the formulation of interest and drug permeation without compromising barrier integrity. While cell-free permeation systems were initially intended to predict intestinal drug permeation, their utility towards non-oral drug delivery including blood-brain barrier, transdermal and buccal delivery was also demonstrated in recent years by adjusting the composition of the permeation barrier (Berben *et al.*, 2018).

- Zsiko et al., 2019 present various experimental models used in dermal/transdermal research and summarize the novel knowledge about the main in vitro methods available to study skin penetration. These techniques are: Diffusion cell with rat skin and dialysis membrane, skin-PAMPA, tape

stripping, two-photon microscopy, confocal laser scanning microscopy, and confocal Raman microscopic method (Zsikó *et al.*, 2019).

- Ahmed et al., 2020 develop the methods for permeation n with ear pinna Skin from Goat and cow were taken and also swiss albino rat skin. And after study they found permeation and their localization of cow skin were quantitatively same as goat skin but it differ from rat skin (Ahmad *et al.*, 2020).

- Ghafari et al., 2019; evaluated tyrosinase enzyme plays a crucial role in melanin biosynthesis and enzymatic browning process of vegetables and fruits. Hence, tyrosinase inhibitors are important in the fields of medicine, cosmetics and agriculture. In this study, novel N-(2-morpholinoethyl)cinnamamide derivatives bearing different substituent on phenyl ring were designed, synthesized and evaluated for their tyrosinase diphenolase inhibitory activity. The compounds were found to be better tyrosinase inhibitors (IC50s were in micro molar range) than cinnamic acid. (E)-3-(3- chlorophenyl)-N-(2-morpholinoethyl)acrylamide (B6) exhibited the highest inhibition with IC50 value of 15.2 ±0.6 μM which was comparable to that of kojic acid. The inhibition kinetic analysis of B6 indicated that the compound was a mixed-type tyrosinase inhibitor. In silico ADME prediction indicated that B6 might show more skin penetration than kojic acid. Molecular docking analysis confirmed that the active inhibitors well accommodated in the mushroom tyrosinase active site and it was also revealed that B6 formed the most stable drug-receptor complex with the target protein. Therefore, cinnamamide B6 could be introduced as a potent tyrosinase inhibitor that might be a promising lead in cosmetics, medicine and food industry. Hence, for their inhibitory effect on mushroom tyrosinase, some of the compounds

exhibited better tyrosinase inhibitory activity (in low micromolar range) than cinnamic acid. Compound B6, containing 3-Cl on phenyl ring, was the most potent derivative against tyrosinase with IC50 value of 15.2 ± 0.6 μM which was comparable to that of kojic acid (Ghafari *et al.*, 2019).

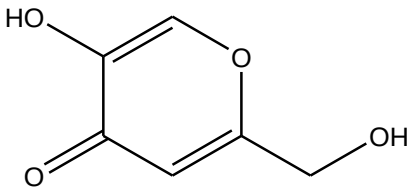
- Dehghani et al, 2019; developed a series of novel tyrosinase inhibitors. Compounds D5 and D12 were found to be promising tyrosinase inhibitors with the IC50 values of 19.72 ± 1.84 and 20.63 ± 0.79μM, respectively. The kinetic analysis disclosed a competitive mode of inhibition for D5. Docking studies were performed to delineate the binding affinity of the active derivatives in the binding site of tyrosinase. Docking studies showed good correlation with experimental results. Some derivatives also indicated good free radical scavenging activity. It can be concluded that the compounds can serve as structural outlines and promising leads in order to design and develop potential tyrosinase inhibitors which is used hyperpigmentaion study (Dehghani *et al.*, 2019).
- Nakano et al, 2021 studied that post-inflammatory hyperpigmentation (PIH) is a common cutaneous condition that can cause a disfigured appearance. However, the pathophysiology of PIH remains poorly understood, at least in part, because an appropriate animal model for research has not been established. In order to analyze the pathomechanism of PIH, we successfully induced PIH in a hairless version of transgenic mice (hk14-SCF Tg/HRM) that have a human-type epidermis containing melanin by repeated hapten application of 2,4-dinitrofluorobenzene. Histopathologic observation showed epidermal hyperplasia, predominant infiltrations of inflammatory cells, and melanin-

containing cells in the dermis just after elicitation of the atopic dermatitis-like condition. At week 2, the findings were similar to the characteristics of PIH, that is, an increase of melanin without spongiosis or liquid degeneration in the epidermis and an increase in dermal melanophages. Dynamic analysis of melanin showed that the melanin in the dermis remained for a longer duration than in the epidermis. Furthermore, immune histochemical staining revealed that the majority of cells containing melanin were positive for the anti-CD68 antibody, but negative for the anti-F4/80 antibody. These data suggest that novel treatments of PIH should be targeted against macrophages and should eventually lead to the development of new treatment modalities (Nakano *et al.*, 2021) .

2.2 API Review

2.2.1 Kojic acid

- Source: Kojic acid is a chelation agent produced by several species of fungi, especially *Aspergillus oryzae*, which has the Japanese common name *koji*. Kojic acid is a by-product in the fermentation process of malting rice, for use in the manufacturing of sake, the Japanese rice wine.
- Synonym: kojic acid, 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one, 5-Hydroxy-2-(hydroxymethyl)-4-pyrone, 5-Hydroxy-2-hydroxymethyl-4-pyrone
- Chemical name: 5-Hydroxy-2-(hydroxymethyl)-4-pyrone
- Molecular formula: $C_6H_6O_4$
- Molecular weight: 142.11
- Chemical structure: The structure of Kojic acid is given below:



5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one

Figure 2.1: Chemical Structure of Kojic acid

- Physico-chemical Properties
  - Physical State and appearance: Solid, Prismatic needles.
  - Colour: white colour.
  - Density (bulk): 1.5±0.1 g/cm^3
  - Boiling point: 401.7±45.0 °C

- Melting point: 151.5- 154°C
- Solubility: Soluble in water (92.3mg/ml), ethanol, ethyl ether, acetone, DMSO, slightly soluble in benzene, Sparingly soluble in pyridine.
- LogP: -0.64 (approx)
- Dissociation constant: 7.66(approx)

- Stability: The principle problem with using Kojic acid is stability. The molecule is highly reactive and degrades within weeks in solution. The process is accelerated by heat, UV, or low pH. Degraded Kojic acid has little or no function for skin lightening and is a distinctive yellow colour. Prior solutions to the stability issue have included airless packaging to remove most of the oxygen, coloured packaging to prevent UV exposure, and unfortunately, cream formats to disguise the yellow discolouration. These options are far from ideal.

- Incompatibility: Kojic acid (5-hydroxy-2-hydroxymethyl-gamma-pyrone) is a bacterial metabolic product used intensively in the food industry. In the presence of visible light and molecular oxygen it was found to cause breakage of calf thymus DNA. Such degradation was considerably enhanced in the presence of the transition metal ions Fe(III), Fe(II) and Cu(II). The cleavage of DNA in the presence of Fe(III) did not appear to have any preferred site(s) or sequence(s) for strand scission. Kojic acid catalysed the reduction of transition metal which in the case of Cu(II) was found to play an essential role in the degradation of DNA. Kojic acid also reduced oxygen to superoxide and hydroxyl radicals were formed in the presence of metal ions. The involvement of these active oxygen species in the reaction was established by the inhibition of DNA breakage by superoxide dismutase,

catalase, iodide, mannitol, formate and sodium azide.

- Storage: Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

- Pharmacology: Kojic acid (KA) is a natural metabolite produced by fungi that has the ability to inhibit tyrosinase activity in synthesis of melanin. The major applications of KA and its derivatives in medicine are based on their biocompatibility, antimicrobial and antiviral, antitumor, antidiabetic, anticancer, anti-speck, anti-parasitic, and pesticidal and insecticidal properties. In addition, KA and its derivatives are used as anti-oxidant, anti-proliferative, anti-inflammatory, radio protective and skin-lightening agent in skin creams, lotions, soaps, and dental care products. KA has the ability to act as a UV protector, suppressor of hyperpigmentation in human and restrainer of melanin formation, due to its tyrosinase inhibitory activity. Also, KA could be developed as a chemo sensitizer to enhance efficacy of commercial antifungal drugs or fungicides. In general, KA and its derivatives have wide applications in cosmetics and pharmaceutical industries.
- Uses: Kojic acid has been used as monotherapy to treat melasma. Kojic acid is widely used as a food additive for preventing enzymatic browning, and in cosmetic preparations as a skin-lightening or bleaching agent. aboratory reagent used as standard chemical for tyrosinase inhibitor assays. The major applications of KA and its derivatives in medicine are based on their biocompatibility, antimicrobial and antiviral, antitumor, antidiabetic, anticancer, anti-speck, anti-parasitic, and pesticidal and insecticidal properties. In addition, KA and its derivatives are used as anti-oxidant, anti-proliferative, anti-inflammatory, radio protective and skin-lightening agent in skin creams, lotions,

soaps, and dental care products. KA has the ability to act as a UV protector, suppressor of hyperpigmentation in human and restrainer of melanin formation, due to its tyrosinase inhibitory activity. Also, KA could be developed as a chemo sensitizer to enhance efficacy of commercial antifungal drugs or fungicides. In general, KA and its derivatives have wide applications in cosmetics and pharmaceutical industries.

(Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Kojic-acid accessed on 30th July, 2021)

2.3. EXCIPIENTS REVIEW

2.3.1. Sorbitol

- Non proprietary Name: Sorbitol
- Synonym: D-glucitol; D-Sorbitol; Sorbogem; Sorbo
- Chemical name: (2S,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol
- Molecular formula: $C_6H_{14}O_6$
- Chemical structure: The structure of sorbitol is given below:

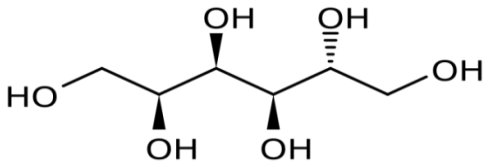


Figure 2.2: Chemical Structure of sorbitol.

- Physico-chemical Properties
  - Physical State and appearance: Crystalline powder, Liquid, white hygroscopic powder.
  - Colour: White
  - Density (bulk):1.489 g/cm^3
  - Boiling Point: 295 °C
  - Melting point: 88 to 102 °C
  - pH: 7.0
  - pKa:13.6
  - Moisture content
  - Specific gravity
  - Solubility: 2750000 mg/L (at 30 °C), Very soluble in water, slightly soluble in ethanol

- Stability: Sorbitol will form water-soluble chelates with many divalent and trivalent metal ions in strongly acidic and alkaline conditions. Addition of liquid polyethylene glycols to sorbitol solution, with vigorous agitation, produces a waxy, water-soluble gel with a melting point of 35-40 °C. Sorbitol solutions also react with iron oxide to become discolored. Sorbitol increases the degradation rate of penicillins neutral and aqueous solution.
- Storage: The bulk material is hygroscopic and should be stored in an airtight container in a cool, dry place.

- Pharmacology: Sorbitol is a sugar alcohol found in fruits and plants with diuretic, laxative and cathartic property. Unabsorbed sorbitol retains water in the large intestine through osmotic pressure thereby stimulating peristalsis of the intestine and exerting its diuretic, laxative and cathartic effect. In addition, sorbitol has one-third fewer calories and 60 % the sweetening activity of sucrose and is used as a sugar replacement in diabetes.
- Uses: Sorbitol occurs naturally and is also produced synthetically from glucose. It was formerly used as a diuretic and may still be used as a laxative and in irrigating solutions for some surgical procedures. It is also used in many manufacturing processes, as a pharmaceutical aid, and in several research applications. Such as bulking_agent, humectants, sequestrant, stabilizer, sweetener, texturizer and thickener.

  (Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Sorbitol accessed on 30th July, 2021)

2.3.2. Xanthan Gum

- Non proprietary Name: Xanthan gum
- Synonym: Xanthan, Xanth, Glucomannan, Glucomannan Mayo, Galactomannane, Rhodopol 23, Xanthan, Xantempo
- Molecular formula: ($C_{35}H_{49}O_{29}$)$_n$
- Chemical structure: The structure of xanthan gum is given below:

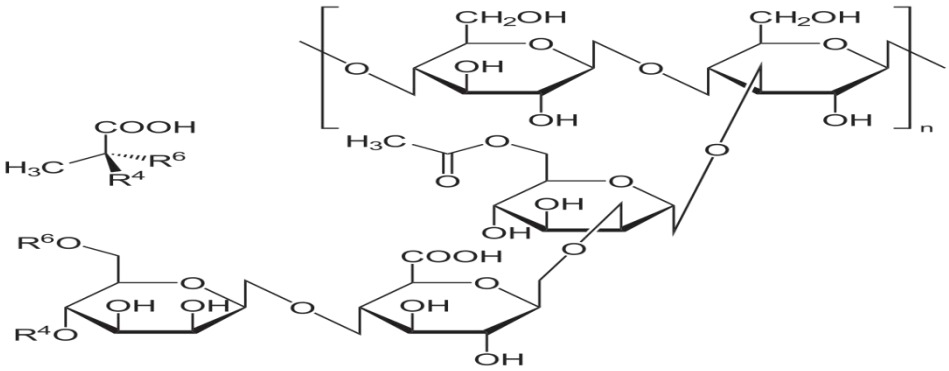


Figure 2.3: Chemical structure of xanthan gum.

- Physico-chemical Properties
  - Physical State and appearance: white to tan powder
  - Colour: Yellowish white
  - Density (bulk):1.5gm/cm^3
  - Melting point: 64.43 °C
  - Solubility: Soluble in water giving a highly viscous solution, practically insoluble in organic solvents
  - Stability: Stable. Combustible. Incompatible with strong oxidizing agents.
- Storage: Xanthan gum is a stable material. Aqueous solutions are stable over a wide pH range (pH 3–12), although they demonstrate maximum stability at pH 4–10 and temperatures of 10–60°C. Xanthan gum solutions of less than 1% w/v concentration may be adversely affected by higher than ambient temperatures:

for example, viscosity is reduced. Xanthan gum provides the same thickening, stabilizing, and suspending properties during long-term storage at elevated temperatures as it does at ambient conditions. In addition, it ensures excellent freeze–thaw stability. Solutions are also stable in the presence of enzymes, salts, acids, and bases. Vanzan NF-ST is especially designed for use in systems containing high salt concentrations as it dissolves directly in salt solutions, and its viscosity is relatively unaffected by high salt levels as compared with general purpose grades.

The bulk material should be stored in a well-closed container in a cool, dry place.

- Uses: In foods, pharmaceuticals, and cosmetics as stabilizer and thickening agent. For rheology control in water-based systems. In oil and gas drilling and completion fluids. xanthan gum (corn starch gum) serves as a texturizer, carrier agent, and gelling agent in cosmetic preparations. It also stabilizes and thickens formulations.

(Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/XC-Polymer accessed on 30th July, 2021)

2.3.3. Propylene Glycol

- Non proprietary Name:
- Synonym: propylene glycol, 1,2-propanediol, propane-1,2-diol,1,2-Propylene glycol, Glycol, Propylene Monohydrate
- Chemical name: propane-1,2-diol
- Molecular formula: $C_3H_8O_2$ or $CH_3CHOHCH_2OH$
- Chemical structure: The structure of propylene glycol is given below:

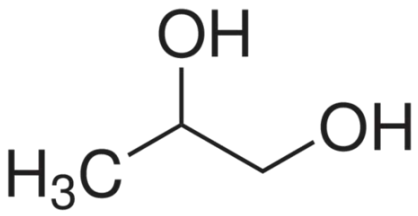


Figure 2.4: Chemical structure of propylene glycol.

- Physico-chemical Properties
  - Physical State and appearance: Thick odorless colorless liquid.
  - Colour: Colorless viscous liquid
  - Density (bulk):1.04 gm/cm^3
  - Boiling point: 187.6 °C
  - Melting point: -60 °C
  - Solubility: Solubility in water: miscible, Soluble in benzene
  - Stability: At cool temperatures, propylene glycol is stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid. Propylene glycol is chemically stable when mixed with ethanol (95%), glycerin, or water; aqueous solutions may be sterilized by autoclaving.

- Storage: Propylene glycol is hygroscopic and should be stored in a well-closed container, protected from light, in a cool, dry place.

- Pharmacology: Propylene Glycol is a propanediol that exists as a clear, colorless and hygroscopic liquid and consists of propane where the hydrogens at positions 1 and 2 are substituted by hydroxyl groups. Propylene glycol is used as an organic solvent and diluent in pharmaceuticals and many other industrial applications.
- Uses: Propylene glycol is used as a vehicle for IV administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, c hlordiazepoxide, multivitamins, nitroglycerin, pentobarbital sodium, phenobarbital sodium, and trimethoprim-sulfamethoxazole.

  Also used as Humectant, Skin conditioning , Solvent, Viscosity controlling, exfoliant (remove dead cell from the skin)

  (Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Propylene-glycol accessed on 30th July, 2021)

2.3.4. Glycerin

- Non proprietary Name:

Synonym: 1,2,3-Propanetriol, 1,2,3-Trihydroxypropane, Glycerin, Glycerine, Glycerol

- Chemical name: propane-1,2,3-triol
- Molecular formula: $C_3H_8O_3$
- Chemical structure: The structure of glycerin is given below:

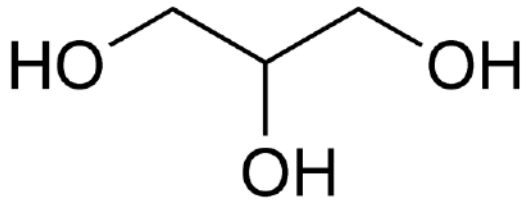


Figure 2.5: Chemical Structure of glycerin.

- Physico-chemical Properties
  - Physical State and appearance: Glycerine appears as a colorless to brown colored liquid.
  - Colour: Clear, colorless syrupy liquid
  - Density (bulk):1.261 gm/cm^3
  - Boiling point : 290℃
  - Melting point: 18.2℃
  - Solubility: Miscible with ethanol, slightly soluble in ethyl ether, insoluble in benzene, carbon tetrachloride, chloroform, carbon disulfide, petroleum ether; Solubility in water: miscible.
  - Stability: Mixtures of glycerin with water, ethanol (95%), and propylene glycol are chemically stable. Glycerin may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20 °C.

- Storage: Glycerin oral solution should be stored in tight containers at a temp less than 40 °C, perferably between 15-30 °C; freezing should be avoided. Glycerin ophthalmic soln should be protected from light and stored in tight glass or plastic containers.

- Pharmacology: Glycerin is commonly classified as an osmotic laxative but may act additionally or alternatively through its local irritant effects; it may also have lubricating and fecal softening actions. Glycerin suppositories usually work within 15 to 30 minutes.
- Uses: Cosmetics: Denaturant, Humectant, Solvent; Food additives; Glycerol has a ubiquitous use pattern and can be found in industrial, professional and consumer products. Glycerol is used as a constituent in numerous products and as an intermediate in industrial applications for the manufacture of products such as soaps/detergents and glycerol esters. It is found in consumer products such as pharmaceuticals, cosmetics, tobacco, food and drinks and is present in numerous other products such as paints, resins and paper. For example, it is used as a down hole lubricant in oil and gas fields and as a wetting agent in pesticide formulations. There is no single use which dominates the use pattern.

  (Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Glycerol accessed on 30th July, 2021)

2.3.5. Citric acid

- Synonym: citric acid, 2-hydroxypropane-1,2,3-tricarboxylic acid, Anhydrous citric acid
- Chemical name: 2-hydroxypropane-1,2,3-tricarboxylic acid
- Molecular formula: $C_6H_8O_7$ or $CH_2COOH\text{-}C(OH)COOH\text{-}CH_2COOH$
- Chemical structure: The structure of citric acid is given below:

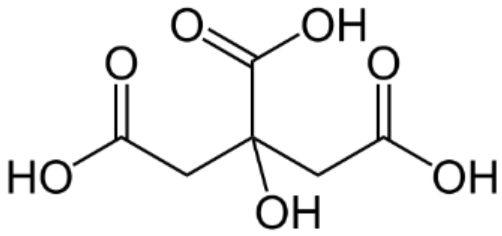


Figure 2.6: Chemical structure of citric acid.

- Physicochemical Properties
  - Physical State and appearance: Citric acid appears as colorless, odorless crystals with an acid taste.
  - Colour: Colourless.
  - Boiling point: Decomposes
  - Melting point: 153°C
  - Density: 1.665 gm/cm^3
  - Solubility: Very soluble in water, freely soluble in ethanol, soluble in ether
  - Storage: Crystalline citric acid, anhydrous, can be stored in dry form without difficulty, although conditions of high humidity and elevated temperatures should be avoided to prevent caking. Storage should be in tight containers to

prevent exposure to moist air. Several granulations are commercially available with the larger particle sizes having less tendency toward caking.

- Pharmacology: Anhydrous Citric Acid is a tricarboxylic acid found in citrus fruits. Citric acid is used as an excipient in pharmaceutical preparations due to its antioxidant properties. It maintains stability of active ingredients and is used as a preservative. It is also used as an acidulant to control pH and acts as an anticoagulant by chelating calcium in blood.
- Uses: Cosmetics: Buffering, Chelating, Gel Base; Flavoring Agent.

(Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Citric-acid accessed on 30th July, 2021)

2.3.6. Sorbic acid

- Synonym: sorbic acid; (2E,4E)-hexa-2,4-dienoic acid; Potassium Sorbate ; 2,4-Hexadienoic acid
- Chemical name: 2,4-Hexadienoic acid
- Molecular formula: $C_6H_8O_2$ or $CH_3CH=CHCH=CHCOOH$
- Chemical structure: The structure of sorbic acid is given below:

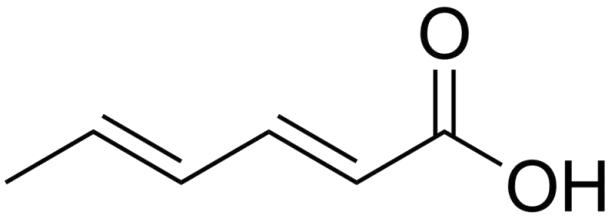


Figure 2.7: Chemical structure of sorbic acid.

- Physicochemical Properties
  - Physical State and appearance: Sorbic acid appears as white powder or crystals
  - Colour: Colorless needles or white powder
  - Density (bulk): 1.204gm/cm^3
  - Boiling point: 228 °C with decomposition.
  - Melting point: 134.5°C.
  - Solubility: Slightly soluble in water, soluble in ethanol.
  - Stability: Sorbic acid is stable and no degradation occurs even if stored at room temperature for a long time. However, in solutions and in foods, it undergoes autoxidation during storage, forming carbonyls and other compounds.
  - Storage: Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature 2 - 8 °C. Storage class (TRGS 510): Non Combustible Solids.

- Uses: Sorbic Acid E200 can be used in Food, Beverage, Pharmaceutical, Health & Personal care products, Agriculture/Animal Feed/Poultry. Sorbic acid E200 is used a food preservative in dairy foods like cheese and yogurt, dried fruit, fish, meat, pickles, olives, soups, prepared salads, jelly, syrups, wine, beer, soft drinks and baked goods such as breads, bagels and pastries against fungi and yeasts. Sorbic Acid E200 is not effective against bacteria. Sorbic acid E200 is used in its salt forms (sodium sorbate, potassium sorbate, and calcium sorbate) as a preservative in the production of drinks and food to prevent the growth of any mold, yeast, and fungi. In Cosmetics: Preservative and Antioxidants.

(Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Sorbic-acid accessed on 30th July, 2021)

CHAPTER 3
AIM & OBJECTIVES

3. AIM & OBJECTIVES

3.1. Aim of the study:

The aim of the study is to treat hyperpigmentation and melasma by formulating Kojic acid Biocellulose sheet mask and evaluate the formulation for effective results.

Hyperpigmentation and melasma are the common, usually harmless condition in which patches of skin become darker in color than the normal surrounding skin. This darkening occurs when an excess of melanin, the brown pigment that produces normal skin color, forms deposits in the skin. Hyperpigmentation and Melasma have been shown to have a significant impact on the quality of life and self-esteem of those affected. Many patients reported a significant negative effect on their quality of life and self-esteem.

Sheet masks are great because it allows the skin to 'rest' while it is saturated in hydrating, brightening and giving an extra boost to our current skin routine when we need it the most. In 21st century, peoples living busier lifestyle with having their proper physical training (exercise) and skin care routine.

So overcome this hyperpigmentation and melasma, formulation of Kojic acid sheet mask is the main aim of the study. Along with the treatment of hyperpigmentation and melasma, it will provide relaxation to the skin and gain confidence of the patient.

3.2. Objectives:

- To reduce melanin pigments.
- To give relaxation and massage to the skin.
- Pre-formulation analysis of Kojic acid and excipients.
    - Solubility study
    - Partition coefficient of Kojic acid
    - Dissociation constant of Kojic acid
    - Melting point of the Kojic acid
    - FTIR analysis of Kojic acid and excipients.
- To formulate different formulations of Kojic acid biocellulose sheet mask by varying the concentration of citric acid and thickening agent i.e. Xantham gum. Formulate the bio-cellulose sheet mask of Kojic acid.
- To characterize all the formulations based on their physico-chemical parameters:
    - Physical appearance
    - Wet weight
    - Hand-pressed weight
    - Water hold capacity (WHC)

- Thickness of the sheet mask
- Homogeneity of the sheet mask
- Measurement of p^H
- Viscosity of the sheet mask

- To characterize the formulations for analytical parameters:
  - Drug content of the formulations.
  - Compare the tack properties with marketed formulation.
  - Permeation study of the optimized formulation.
  - *in-vitro* hyperpigmentation study
  - *in-vivo* irritation study by using rabbit.
  - *in-vivo* hyperpigmentation study by using HRM mice.

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS AND METHODS:

4.1. MATERIALS

4.1.1. Chemicals :

All the materials used in the formulations, evaluations are Kojic acid (Batch no:RHA/18/1, Mfg By Morril Fox, India), sorbitol, xanthan gum, propylene glycol, glycerin, citric acid, sorbic acid and reagents. The Korean biocellulose sheet mask from Evana Skin, Mumbai are used.The chemicals and solvents used were of analytical reagent grade and were used as such without any further purification. The water used was of high grade after double distillation and deionization.

4.1.2 Equipments:

Sr. No.	Equipment	Company
1	Electronic balance	Model BS 124S, Labtronic
2	Magnetic stirrer	Rolex
3	p^H meter	Systronics
4	FTIR spectrophotometer	Model ALPHA-E, Bruker, Germany
5	Viscometer	Brookfield LDV-E

6	UV Visible spectrophotometer	Shimadzu
7	Texture analyzer TA.XT Express	Stable Micro system
8	Homogenizer	Rolex

Table 4.1: List of equipments and apparatus.

4.1.3. Animal Used

New Zealand albino Rabbit are used. Animals are approved by the IAEC of Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati-17 with project proposal no GIPS/IAEC/M.Ph/PRO/09/2021.

4.2. METHODS

4.2.1. Determination of wavelength (λ_{max}) of Kojic acid

The UV Visible spectroscopy of Kojic acid at maximum absorption was done by phosphate buffer with Kojic acid of 100 g /mL. Then 400 μL of the 100 μg/mL KA solution and 2 ml of 0.2M, pH 7.4 phosphate buffer solution was loaded in a quartz cell and lambda max was measured.(Gonçalez, Corrêa and Chorilli, 2013).

4.2.2. Analytical Curve of Kojic Acid in Phosphate Buffer

The stock solution 1mg/ml was prepared then 1ml from the stock solution was taken in 100ml of phosphate buffer 7.4. Then different concentration was made by serial dilution and UV absorption was taken in 226nm.(Gonçalez, Corrêa and Chorilli, 2013)

4.2.3. Solubility of Kojic acid by shake flash method

The supersaturated sample was prepared in three different solutions with a volume of 10ml, the solutions are phosphate buffer (pH 4), phosphate buffer (pH 8), and distilled water. After vigorously shaking, solutions are kept overnight. The sample was collected from the top and dilutions were made and UV absorbance was taken. ((TONY)TONG, 2007)(Glomme, März and Dressman, 2005) .

4.2.4. The partition coefficient of Kojic acid by shake flash method

10mg of Kojic acid was added to 10 ml water and mix properly then the same amount of n-octanol was added in a tube. The solution was shaken vigorously and kept

overnight. The sample was collected from the top and dilutions were made and UV absorbance was taken (Schönsee and Bucheli, 2020).

4.2.5. Dissociation constant of Kojic acid

10 ml of 0.5% w/v solution of Kojic acid in water is pipetted out in a conical flask. By using methyl red as an indicator, the solution was titrated with 0.5 N NaOH. Similarly, 10 ml of 0.5%w/v Kojic acid was taken and titrated up to 50% neutralization point. Then the pH of the neutralized solution was checked in the pH meter. Because at 50% neutralization point that is when [ionized drug]=[unionized drug] i.e. pH=pKa.

4.2.6. Melting point

A little amount of the drug sample was taken in a dry capillary tube of 1 mm internal diameter forming a column about 3mm high. Heat the melting point apparatus to a temperature 5-10 ℃ below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 10℃ per minute.The melting point was measured by using melting point apparatus.

4.2.7. Fourier Transform Infrared spectroscopy (FT-IR) Study

FT-IR studies were performed pure KojicAcid (KA), sorbitol, glycerin, xanthan gum, citric acid, prpelene glycol, physical mixture of KA and sorbitol (1:1), KA and propelene glycol (1:1), KA and citric acid (1:1), KA and Xanthan Gum(1:1), KA and Glycerine(1:1)and KA and excipents mixture were performed in an FT-IR spectrophotometer (Bruker, Germany). A small quantity of sample was placed just below the probe on to which the probe was tightly fixed and scanned in the wave number region 4000-500 cm-1 . The obtained IR spectra were interpreted for functional groups at their respective wave number (cm-1).

4.2.8.Preparation of biocellulose sheet mask

In the formulation Kojic acid is used as API. In the excipients sorbitol and glycerine is used as humectants that will reatian or preserve the moisture. Xanthan gum is used as thickening agent.propylene glycol is used as exfoliant for removing of dead cells. Citric acid used as gell base and it also maintain the pH. Sorbitol is used as preservative.

The components of the biocellulose sheet mask preparation were shown in Table 4.2

Materials	**F1 (%)**	**F2 (%)**	**F3(%)**	**F4(%)**	**F5(%)**
Kojic acid	0.6	0.6	0.6	0.6	0.6
Sorbitol	0.6	0.6	0.6	0.6	0.6
Xabthan Gum	0.6	0.15	0.3	0.45	0.075
Propylene Glycol	0.6	0.6	0.6	0.6	0.6
Glycerin	1.5	1.5	1.5	1.5	1.5
Citric acid	1.2	1.65	1.5	1.35	1.42
Sorbic acid	0.3	0.3	0.3	0.3	0.3
Distilled water	qs	qs	qs	qs	qs

Table 4.2: Formulation of Biocellulose sheet mask.

The weighted amount of sorbitol, xanthan gum and propylene glycol were mixed with little amount of distilled water in homogenizer. The weighted amount of Kojic acid, citric acid,sorbic acid and glycerin was mixed with previous solution in homogenizer. Then make up the volume of solutuion. Deep the sheet mask in the solution and hand-pressed to remove the extra solution.

4.2.9. Biocellulose Physical Evaluation

The thickness, weight and after hand-pressed weight of produced biocellulose are going to be evaluated. The thickness of biocellulose mask is measured using a digital screw gauge. The weight of biocellulose mask measured with wetand hand pressed weight biocellulose mask. The measurements of thickness and weight are done on three different masks, the results are averaged.

4.2.10. Homogeneity test

A certain amount of preparations were applied on a piece of glass or other suitable transparent material, preparations should show a homogeneous composition and no visible coarse grains.

4.2.11. pH Analysis

The pH of the formulations were determined by using digital pH meter. The glass electrode was calibrated with the solutions determined for the equipment (pH of 4.00 and 7.00),. The analysis of pH of formulation were done in triplicate and average values were calculated (Dantas *et al.*, 2016).

4.2.12. Viscosity Study

Determination of viscosity is made by using a Brookfield viscometer with 62 as the number of spindle and 12 as the speeds.. At each speed, the corresponding dial reading was noted (Dantas *et al.*, 2016).

4.2.13. Accelerated Stability and Physicochemical Analysis of Kojic Acid sheet mask

The F5 was submitted to accelerated stability tests. Stability tests will performed at different conditions for sheet mask to note the effect of these conditions on the storage of creams. These tests on samples will kept at 8°C ± 0.1°C (in refrigerator), 25°C ± 0.1°C (R.T), 30°C(in oven), 40°C ± 0.1°C (in oven) and 40°C ± 0.1°C (in oven) with 75% relative humidity (RH). Samples are observe with respect to change in color, liquefaction and phase separation. (Dantas *et al.*, 2016)

4.2.14. Drug Content

A specified area of all the formulation of sheet mask (1 cm∗1 cm) was dissolved in 50 mL phosphate buffer and shaken continuously for 24 h. Then the whole solution was ultrasonicated for 15 min. After filtration, the drug was estimated spectrophotometrically at wavelength of 226 nm and determined the drug content.(Prajapati, Patel and Patel, 2011)

4.2.15. *in-vitro* Permeation Study

An in vitro permeation study was carried out by using modified Franz diffusion cell with a diffusional area of 1.766 cm^2 . Franz diffusion cell with a receptor compartment capacity of 30 mL. The dialysis membrane was used for the

determination of drug from the prepared sheet mask. The dialysis membrane having a pore size 0.45 µ was mounted between the donor and receptor compartment of the diffusion cell. The prepared sheet mask (F5) was placed on the dialysis membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads, and the temperature was maintained at 32 ± 0.5◦C, because the normal skin temperature of human is 32◦C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically at 226nm. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal (Prajapati, Patel and Patel, 2011).

4.2.16. Skin irritation test

White New Zealand rabbits weighing 2–5 kg were acclimatized before the beginning of the study, and were divided into two groups (n = 3)—group 1: control; group 2: test group with Kojic acid sheet mask. The rabbit backs clipped to remove all hair (6 cm^2) 24 h prior to the application of the formulations. The formulation F5 was applied to the hair-free skin (4 cm^2). Next, the sheet mask will apply for 30 min. Animals are then examined for signs of skin irritation, such as erythema, at 24 h after each application of the formulations. The mean scores will recorded (ranging from 0–4) according to the degree of erythema: 0 = no erythema; 1 = slight erythema (barely perceptible or light pink); 2 = moderate erythema (dark pink); 3 = moderate to severe erythema (light red); 4 = severe erythema (extreme redness). The edema will measure and scored similarly. (Castro *et al.*, 2011) (Das and Ahmed, 2017).

4.2.17. Tack Properties

A texture analyzer was used to determine the adhesion efficiency of the TAF sheet mask. Prior to running the test samples, the instrument was calibrated and parameters, such as target force, approach speed, return speed, as well as distance and hold time, were optimized. A sheet mask (width and length of about 1.0 inch × 1.0 inch, respectively) was cut and adhered on to the sample holder after removal of release liner. As the test run was initiated, the probe was allowed to touch the adhesive film with a target force and hold time of 5 g and 1 s, respectively. This resulted in the creation of a bond between the probe surface and sheet mask. Further, as the probe was pulled off, it resulted in debonding between the two surfaces. The absolute force that is required to peel off the adhesive film was measured and recorded and compare with the marketed formulation. (Banga et al., 2019).

4.2.18. *ex-vivo* permeation study

Ear pinna Skin from Goat was used for the permeation studies. The skins was cleaned with cold water followed by the removal of non-dermatome skin by the scalpel. Optimized formulations i.e. F5 was performed on a modified Franz diffusion cells (1.766 cm^2 diffusional area) and receiver chamber (30.0 ml capacity) for the ex vivo permeation on goat skin. Phosphate buffer saline (pH 7.4) filled in the receptor compartment followed by the stirred with a magnetic bar. The receptor compartment was maintained a constant temperature (32.0 ± 1°C). After stabilization, 1cm^2 of F5 Sheet mask was kept into every donor compartment with goat skin. Samples were withdrawn at diferrent points (5,10,15,20,25 and 30 minute) and analysis is done by UV spectrophotometer at 226 nm (Ahmad *et al.*, 2020).

4.2.19. *in-vitro* hyperpigmentation study with mushroom tyrosinase

Mushroom tyrosinase was assayed using L-DOPA as the substrate. The enzyme diphenolase activity will monitor spectro- photometrically by observing dopachrome formation at 475nm. All the test samples will first dissolve in DMSO at 40mM and dilute to the require concentrations. Initially, 10ml of tyrosinase ($0.5mgml^{-1}$) will mixe with 160ml of 50mM phosphatebuffer (pH=7.4) and then 10ml of the test sample in 96-well microplates will add. After the mixture will pre-incubate at 28°C for 20min, 20ml of L-DOPA solu tion (0.5mM) will add to the phosphate buffer. DMSO without test compounds will use as the control, and ascorbic acid will use as a positive control. Each assay will conduct as three separate replicates. The inhibitory activity of the tested compounds will express as the concentration that inhibited 50% of the enzyme activity (IC50) (Dehghani *et al.*, 2019) . The percentage inhibition ratio was calculated according to the following equation.

$$\text{Inhibition (\%)} = 100(\text{Abs}_{\text{Control}}-\text{Abs}_{\text{Compound}})/\text{Abs}_{\text{Control}}.$$

4.2.20. *in-vivo* hyperpigmentation Study

Hk14-SCF Tg/HRM mice were previously developed and have melanocytes in their epidermis, which mimics human skin . In this study, male 16- to 20-week-old mice will used unless. 2,4-dinitrofluorobenzene (DNFB; Wako) will use as a hapten to induce chronic contact dermatitis. The melanin content (melanin index) of the dorsal skin of 5 mice will measured and after formation of pigments the sheet as apply for every consecutive 3 days. And finally The melanin content (melanin index) of the dorsal skin of 5 mice will measure (Nakano *et al.*, 2021).

CHAPTER 5
RESULTS AND DISCUSSIONS

5. RESULTS AND DISCUSSIONS:

5.1. RESULTS

5.1.1. The wavelength (λ_{max}) of Kojic acid:

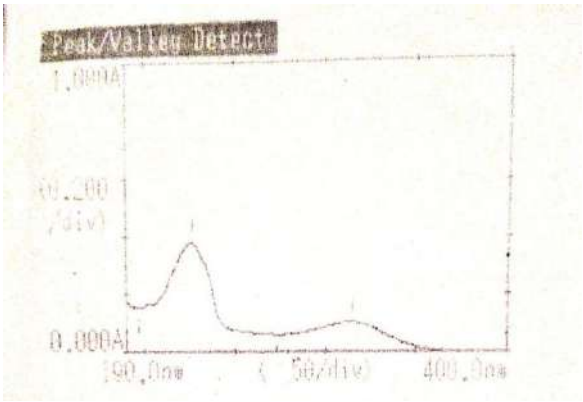


Figure 5.1: λ_{max} of Kojic acid in UV Visible spectroscopy.

5.1.2. Analytical Curve of Kojic Acid in Phosphate Buffer 7.4

Sl No	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.1767
3	4	0.2875
4	6	0.4066
5	8	0.5401
6	10	0.6189

Table 5.1: Different absorbance reading for standard curve.

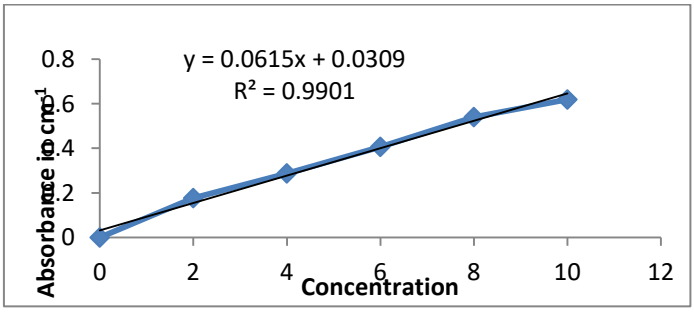


Figure 5.2: Standard curve of Kojic acid.

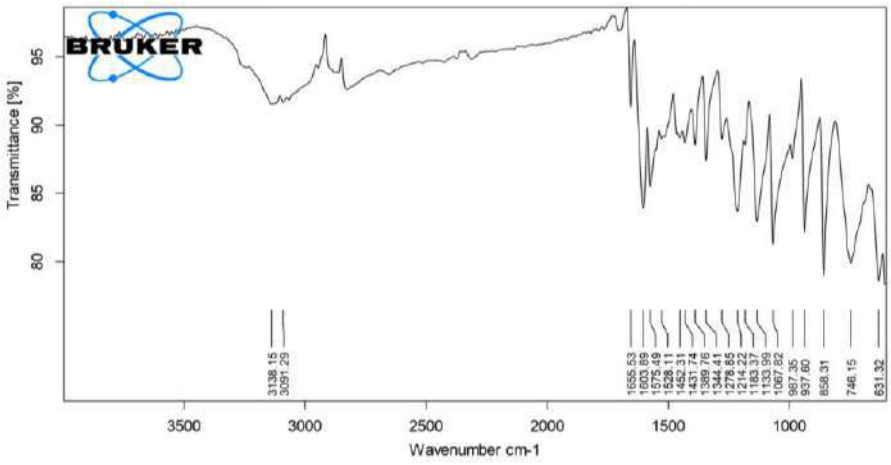
5.1.3. Solubility of Kojic acid:

Sl No	Sample solution	Solubility (mg/ml)
1	Water	3.179783± 0.002059
2	Basic Phosphate buffer (pH 8)	3.001463± 0.035529
3	Acidic Phosphate buffer(pH 4)	3.07832± 0.00291

Table 5.2 : Solubility of the Kojic acid

*All values are expressed as Mean ± SD (n=3)

5.1.4. FTIR analysis:



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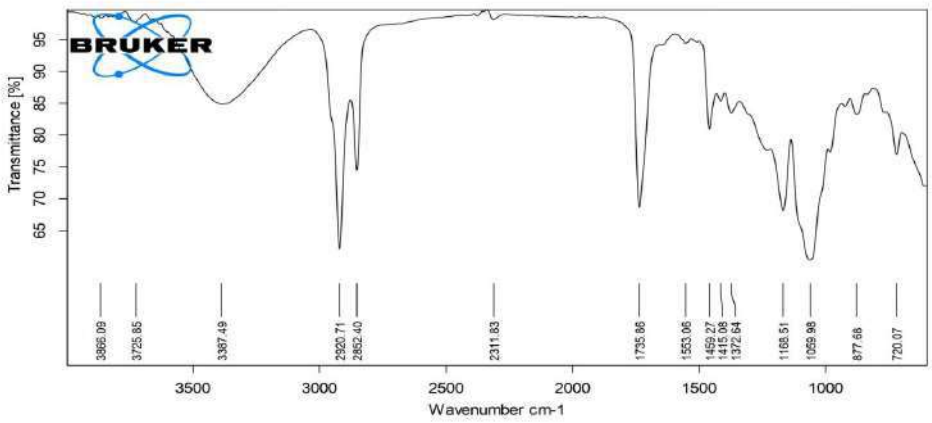
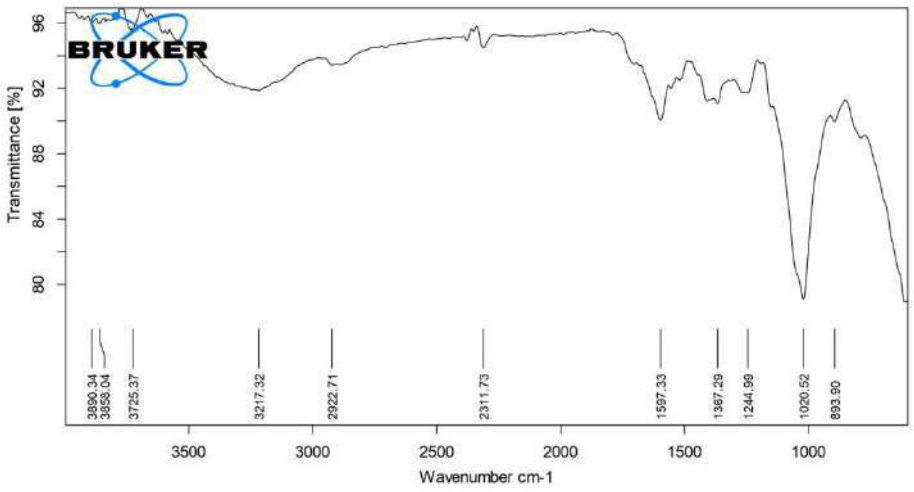


(b)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88
84
80
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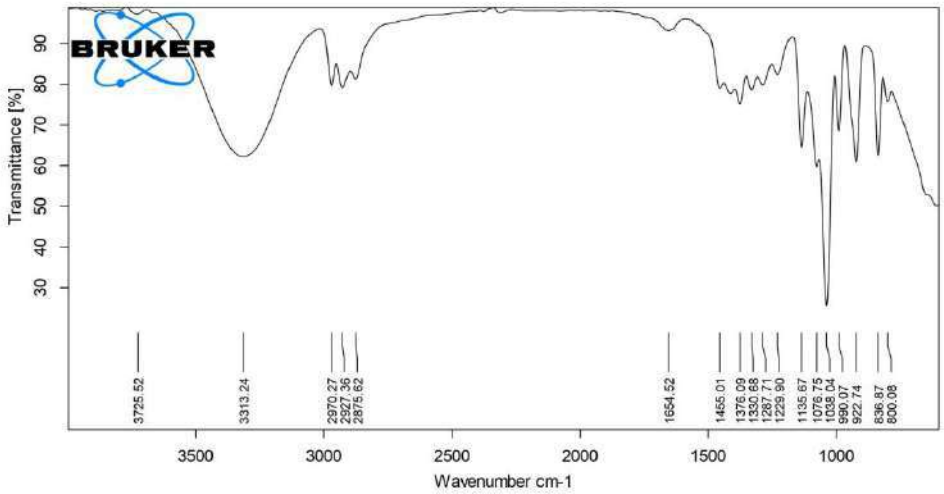
3858.04
3725.37
3217.32
2922.71
2311.73
1597.33
1367.29
1244.99
1020.52
893.90
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

(c)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Transmittance [%]
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1455.01
1376.09
1330.68
1287.71
1229.90
1135.67
1076.75
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3500
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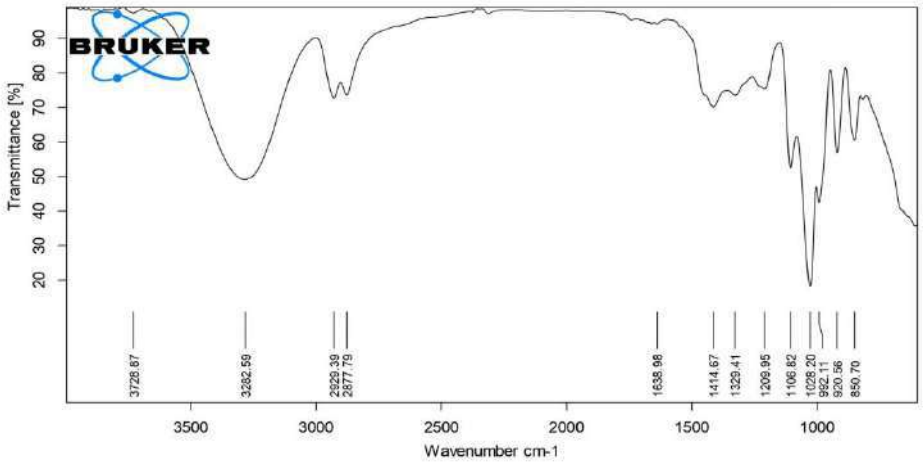
2000
1500
1000
Wavenumber cm-1

(d)


BRUKER
Transmittance [%]
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1414.67
1329.41
1209.95
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992.11
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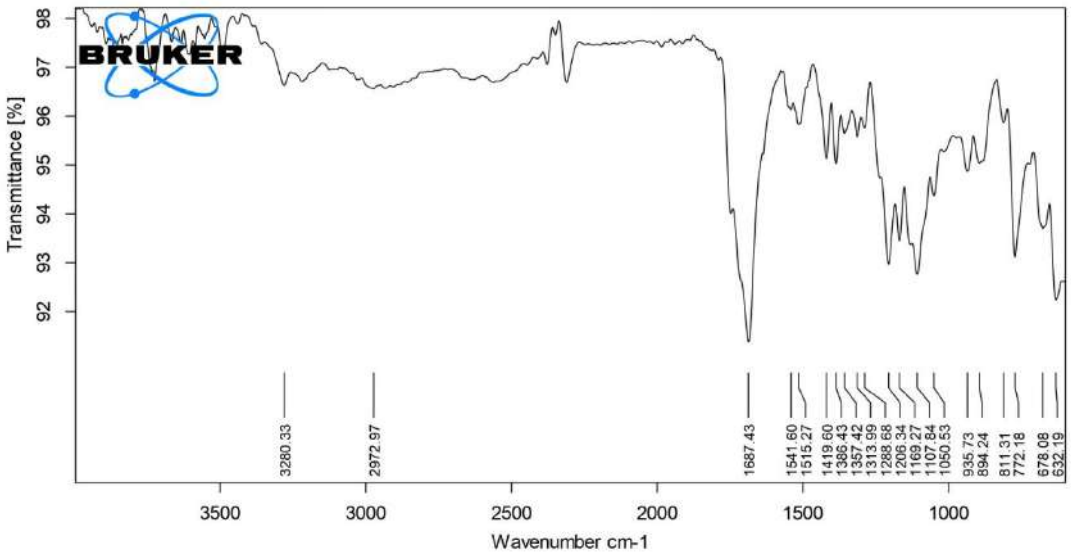
2000
1500
1000
Wavenumber cm-1

(e)



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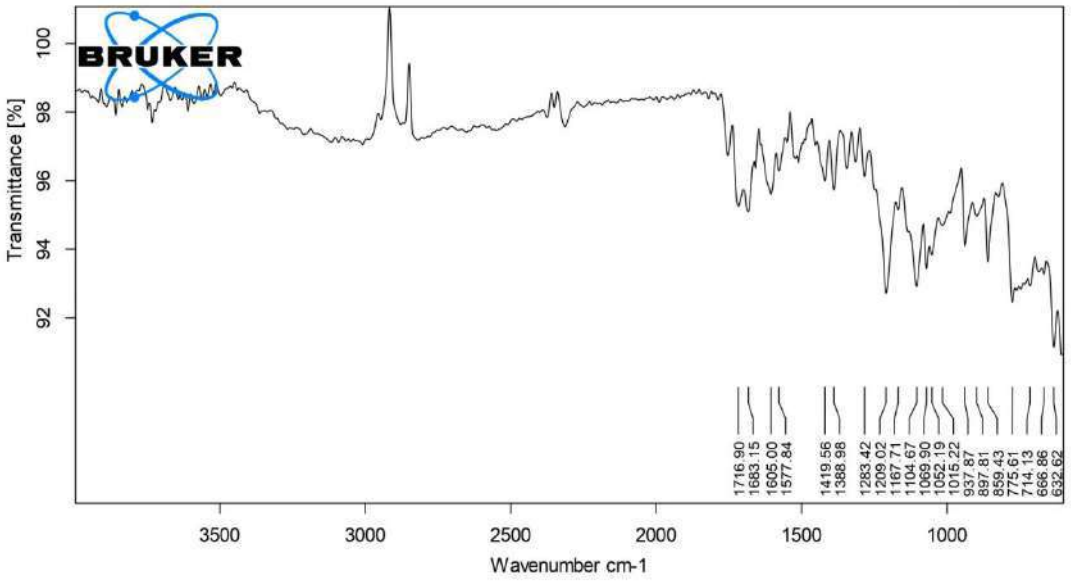


(g)

Figure 5.3: FTIR spectra of (a) Kojic acid, (b) Sorbitol, (c) Xanthan gum, (d) Propylene glycol, (e) Glycerin, (f) Citric acid (g) Mixture of Kojic acid and excipients.

Sr. No.	IR spectrum	Groups	Peak (cm^{-1})	Stretching/Deformation
1	Kojic acid	-OH(Free)	3608.81	Stretching
		-OH (Alcohol)	1067.82	Bending
			1278.85	Stretching
		-OH (Phenol)	1214.22	Bending
			1344.41	Stretching
		C–H	2861.15, 2824.42	Aliphatic stretching
		C=O	1655.53	Aliphatic stretching
		C=C	1603.89	Aromatic stretching
		C-O-C	987.37	Stretching
		1, 4 α –di substituted ring	746.15	
2	Sorbitol	-OH (Alcohol)	1059.98	Bending
			1168.51	Stretching
		C-H (Alkane)	1372.64	Bending
			2920.71	Stretching
3		-OH (Alcohol)	1067.82	Bending
	Xanthan Gum		1244.99	Stretching
		-OH (Phenol)	1367.29	Stretching
		C-H	2922.71	Stretching
			1597.52	Bending
		C-O-C	1020.52	Stretching
4	Glycerin	C-H	2929.39	Stretching
			1414.67	Bending
		-OH (Alcohol)	1106.82	Bending
			1329.41	Stretching
5	Propylene glycol	C-H	2970.27	Stretching
			1455.03	Bending
		-OH (Alcohol)	1076.75	Bending
			11287.71	Stretching
6	Citric acid	C-H	2972.97	Stretching
			1419.60	Bending
		-OH (Alcohol)	1050.53	Bending
			1313.99	Stretching
		C=O	1687.43	Stretching

Table 5.3: FTIR interpretation data.

5.1.5. Physical evaluation of KA Biocellulose sheet mask:

Biocellulose mask	Wet weight (g)	Hand pressed weight (g)	Water hold capacity (%)	Thickness (mm)
F1	134.21	24.6	82.00	1.25
F2	139.73	23.8	79.33	1.22
F3	128.89	24.2	80.66	1.30
F4	132.32	26.4	88	1.25
F5	132.04	26.6	88.66	1.30
Average	133.43	25.12	83.73	1.26

Table 5.4: Parameters obtain from physicochemical evaluation.

5.1.6. Homogeneity test, pH analysis and viscosity of KA Biocellulose sheet mask:

Parameter	F1	F2	F3	F4	F5
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.3	6.8	6.4	6.4	6.7
Viscosity (cps)	14260	4666.66	8970.55	10220	2466.66

Table 5.5: Parameters of homogeneity test, pH analysis and viscosity.

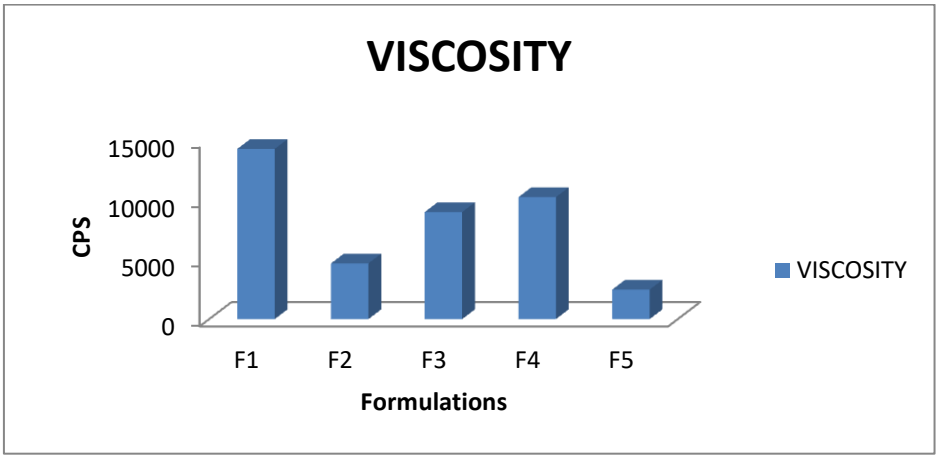


Figure 5.4: Viscosity analysis.

5.1.7. Drug Content

Sl No	Formulation	% Drug Content
1	F1	94.20054±0.544034
2	F2	92.32611±0.146149
3	F3	95.49684±0.509228
4	F4	93.98374±0.461302
5	F5	95.60976±0.566357

Table 5.6: Drug content study of KA biocellulose sheet mask.

*All values are expressed as Mean ± SD (n=3)

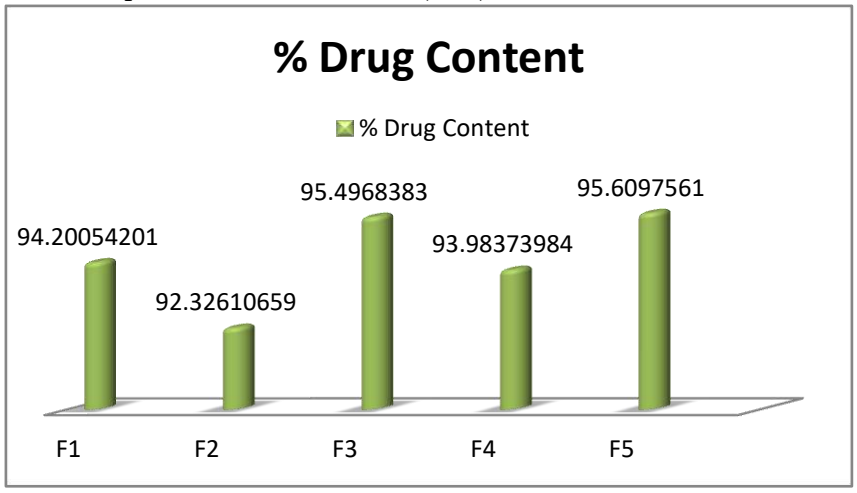
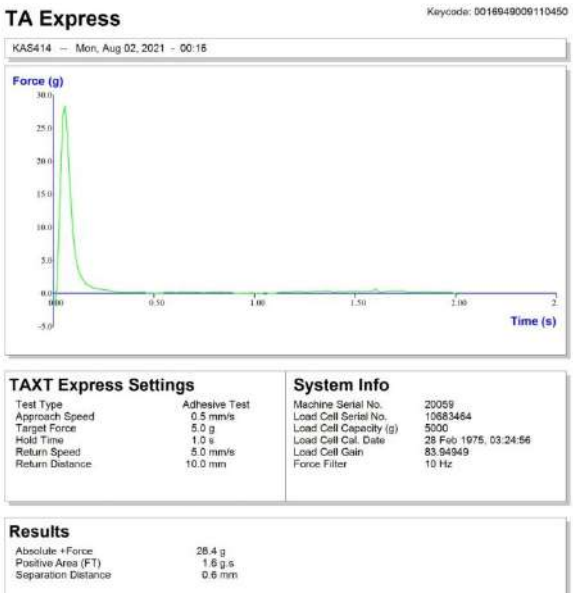
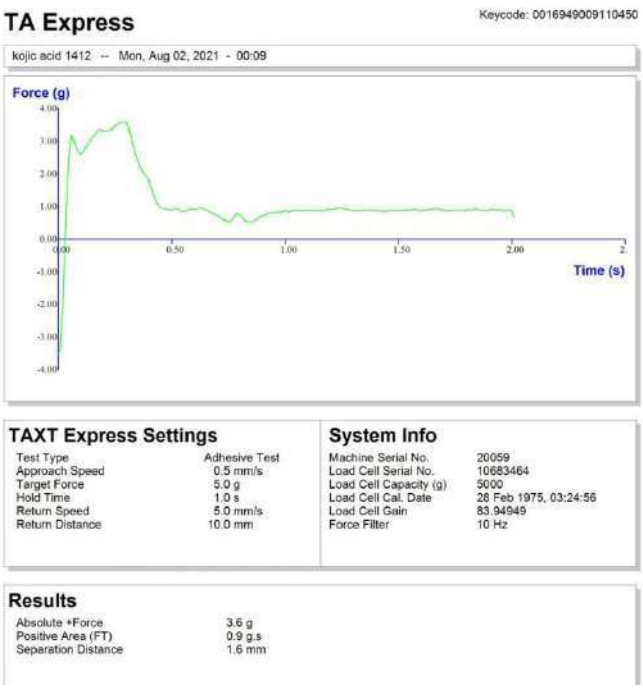


Figure 5.5: % drug content.

5.1.8. Tack properties with marketed formulation:



(a)



(b)

Figure 5.6 : Adhesive test (a) KA biocellulose sheet mask, (b) Marketed formulation of Garnier sheet mask.

5.1.9 Permeation study

Sl No	Permeation study	Formulation code	Flux(µg/cm2/min)	Kp (cm/min)
1	*In-vitro* permeation study	F5	0.0191	0.031833
2	*Ex-vivo* permeation study	F5	0.0266	0.04433

Table 5.7: *in-vitro* permeation and *ex-vivo* permeation study.

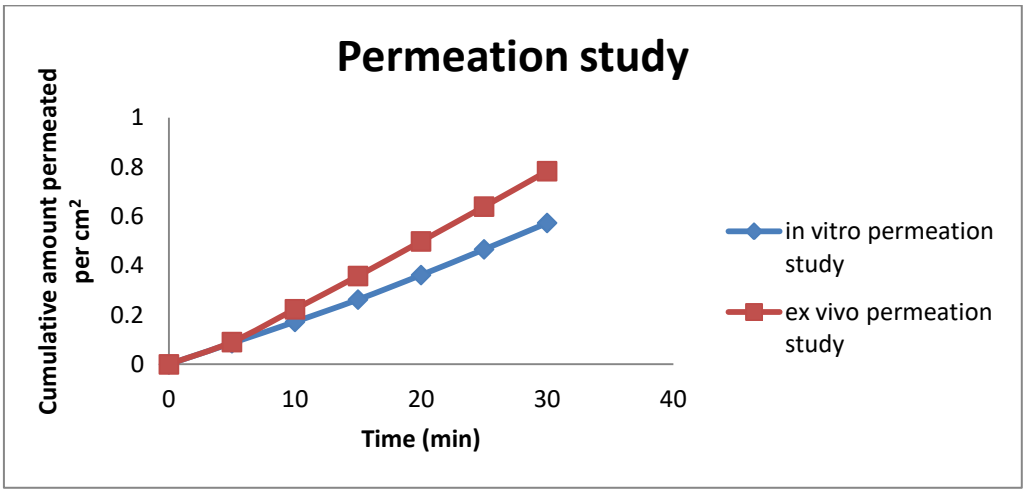


Figure 5.7: Permeation study.

5.1.10. *in-vivo* irritation study:

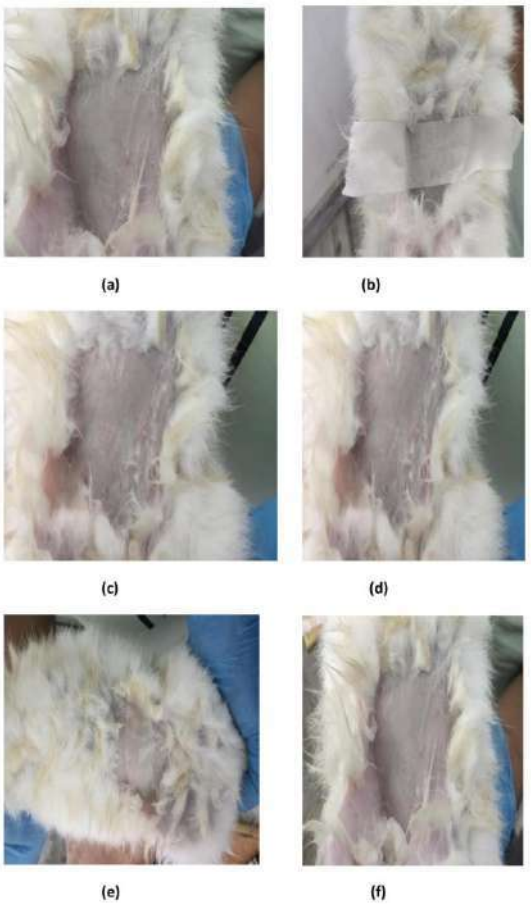


Figure 5.8: Photographs of skin irritation study, (a) Before application of adhesive tape USP, (b) application of adhesive tape USP, (c) After application of adhesive tape USP, (d) before application of sheet mask, (e) application of sheet mask, (f) after application of sheet mask.

5.2. DISCUSSIONS:

5.2.1. The wavelength (λ_{max}) of Kojic acid:

The figure 5.1 illustrates the spectrum scan of Kojic acid, which may observe that the Kojic acid has a higher absorption at a wavelength equal to 226 nm.

5.2.2. Analytical curve of Kojic acid:

The analytical curve of KA in a phosphate buffer (7.4) solution was performed to analyze the results solubility; partition coefficient; drug content and permeation study because the phosphate buffer solution was used for dilution. The analytical curve in phosphate buffer is illustrated in table 5.1 and figure 5.2.

5.2.3. Solubility, Partition coefficient, Dissociation Constant and melting point of Kojic acid:

Kojic acid is reported in many literatures (Burnett *et al.*, 2010) (Saeedi, Eslamifar and Khezri, 2019) found to be highly hydrophilic in nature with water solubility. The Kojic acid in water solution found 3.179783± 0.002059 that is better soluble than Phosphate buffer of different pH described in Table 5.2. It was also found that there are colour changes in phosphate buffer (pH 8.0) when it was kept for overnight as shown in figure 5.9. There may be any degradation of Kojic acid as Kojic acid changes colour to orange- red during degradation reported in literature(Shah, Khan and Ali, 2017).

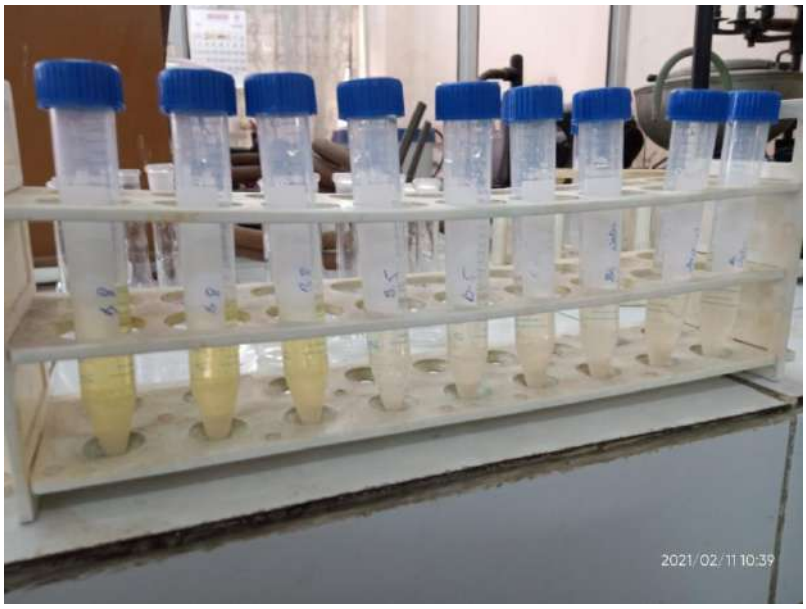


Figure: 5.9: Photograph shows that changes of colour with Phosphate buffer (8.0) during solubility study.

Partition coefficient can be considered as an important factor for predicting skin permeability from an aqueous environment to the lipophilic stratum corneum. For intra-dermal absorption the permeation should possess (octanolwater) partition coefficient in the range -1.0 to 4.0 (Higaki *et al.*, 2002) (Chandrashekar and Shobha Rani, 2008). The Partition coefficient values of Kojic acid is found -0.05989. The dissociation constant (pKa) value was found 8.0 and melting point was found 153 ℃ which is under range as reported in literature (Burnett *et al.*, 2010). These are the important factors in designing formulations and evaluation of the sheet mask.

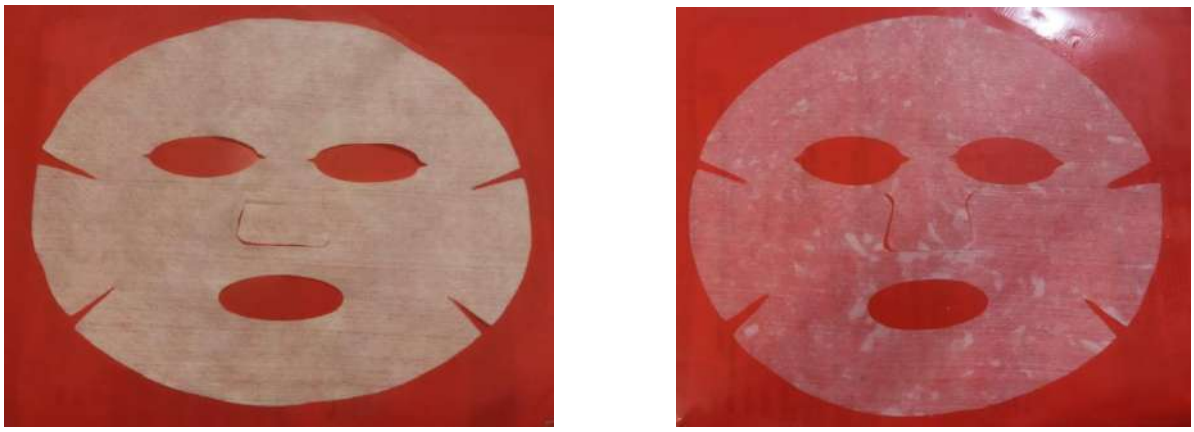
5.2.4. FTIR analysis: Figure5.3 and table 5.3

The Kojic acid bands appear for functional groups at 3608.81 cm^{-1} (- OH free), 1214.22 cm^{-1} (-OH phenol bending), 1344.41 cm^{-1} (-OH phenol bending), 2861.15 cm^{-1} (aliphatic- CH), 1655.53 cm^{-1} (cyclic-C=O), 1603.89 cm^{-1} (C=C), 987.37 cm^{-1} (cyclic C-O-C), 746.15 cm^{-1} (1, 4 α –di substituted ring). Also, the bands of excipients and

mixture of excipients and drug are summarizes in Figure 5.3 and table 5.3. it confirms that drug is compatible with excipients.

5.2.5. Physical evaluation KA biocellulose sheet mask:

The biocellulose sheet mask thickness and weight data in the Table 5.4 shown that the average biocellulose mask's thickness was 1.26 mm, and the biocellulose wet and hand-pressed weight was 133.43 g and 25.12 g. The water hold capacity was 83.73%. These show that biocellulose sheet masks have high hydrophilic properties. If the biocellulose water content is below 5% or less than one time of dry biocellulose, it will decrease the hydrophilic properties(Clasen *et al.*, 2006). Because the hydrogen bond in biocellulose forms a covalent bond between solution (formulation) and dry biocellulose sheet, due to the lack of water content in the dry biocellulose sheet mask, its bond with the formulation(Dean, 2011). Hence the strength of water holding capacity of the biocellulose sheet mask is good. That is why biocellulose sheet mask is the best to absorb the solution.



(a) (b)

Figure 5.10: Biocellulose sheet mask (a) before Deeping to the solution, (b) after Deeping to the solution.

5.2.6. Homogeneity test:

The data in the table 5.5 above shows that each formula was homogeneous.

5.2.7. pH Analysis:

The pH value of the prepared biocellulose sheet mask was 6.3-6.7 shown in table 5.5 which is within the range of permitted pH requirements for cosmetics. Also, this pH fulfils the requirement for the water holding capacity of biocellulose sheet mask (Dean, 2011).

5.2.8. Viscosity Study:

The viscosity of the formulations is found in different ranges because of the concentration of thickening agents i.e. Xanthan gum. The viscosity of formulation F1, F2, F3, F4, and F5 were found to be 14260, 4666.66, 8970.55, 10220 and 2466.66 respectively. Out of these values, F5 has the best viscosity range for biocellulose sheet masks. So F5 will be the best formulation for further evaluation (Table 5.5 and Figure 5.4). Xanthan gum is an exceptional rheology control agent that is very effective even at low concentrations. It provides different flow properties depending on concentration, allowing the texture and rheology of any product to be tuned and controlled to meet specific needs. Xanthan gum is a visco-elastic material that can behave more like an elastic solid or more like a viscous fluid, depending on the concentration. It possesses pseudoplasticity, meaning viscosity decreases with increasing external stress, and provides yield stress controlling stability and resistance to flow.

Water retention is an important feature in sheet masks to prevent drying and to ensure a refreshing sensation during use. With its high water-retaining capacity, xanthan gum ensures optimum moisture retention for a long-lasting fresh skin sensation.

Apart from its rheological and water-binding benefits, xanthan gum has the advantage of being of natural origin, tasteless and odorless, allergen free. Thus, xanthan gum has all the qualities needed to make it the perfect thickening agent for face masks (Russ and Kasper, 2017).

5.2.9. Drug Content:

All the formulation contains 92-96% of drug as shown in Table 5.6 and Figure 5.5. The result means uniform distribution of the drug in the sheet mask. The optimize Formulation (F5) of KA biocellulose sheet mask .contain 95.60976±0.566357% of drug.

5.2.10. Tack Properties:

Tack Properties test was used to evaluate the peel adhesion force. An ideal transdermal patch/ sheet mask should peel off after application on skin, without causing delamination. There is no significant difference in the Kojic acid biocellulose sheet mask (F5) and marketed formulation of Garnier Sheet Mask as shown in Figure 5.6 and it is under the range of acceptable value (Banga *et al.*, 2019). The absolute force that is required to peel off biocellulose sheet mask (F5) is 28.4 g and force required to peel of Garnier sheet mask is 3.6 g. So it can be concluded that F5 can peel off without any damage to the skin.

5.2.11. Permeation Study:

Figure 5.7 table 5.7 A study of percutaneous absorption of kojic acid in human volunteers found the potential for dermal transfer into the blood to be very low (Burnett *et al.*, 2010). The *in-vitro* permeation study with dialysis membrane shows flux 0.0191 µg/cm_2/min and permeability coefficient (Kp) 0.031833 cm/min. The *ex- vivo* study shows flux 0.0266 µg/cm_2/min and permeability coefficient (Kp) 0.04433 cm/min. The table 5.7 and Figure 5.7 confirm that a very less amount of drug reaches the blood circulation and it can assume that there is deposition of Kojic acid in the epidermis of skin as literature confirms that kojic acid (KA) shows insufficient skin penetration (Khezri *et al.*, 2020).

5.2.12. Irritation study:

On performing skin irritation study test for formulation F5 on healthy male rabbits for hrs and comparing it to the control (adhesive tape USP); no edema or erythema was observed on the site of application of the sheet mask indicating that they are non-irritable to the skin hence can be considered safe for Kojic acid biocellulose sheet mask. The observed results are depicted in Figure 5.8.

CHAPTER 6
CONCLUSION

6. CONCLUSION

This work demonstrates the successful development of biocellulose sheet mask through successful incorporation of mixture of Kojic acid, sorbitol, xanthan gum, glycerin, propylene glycol, citric acid and sorbitol. The development of the Kojic acid biocellulose sheet mask was designed by varying the ratios of xanthan gum as a thickening agent and citric acid to maintain the pH.

Kojic acid is reported to have tyrosinase inhibitor activity, thereby the reason behind selecting them in the development of the desired formulation. The Kojic acid biocellulose sheet mask were subjected to various evaluation parameters such as physical appearance, wet weight, hand-pressed weight, thickness, pH measurement, FTIR study, viscosity study, in-vitro and ex-vivo permeation study, tack properties , in-vivo irritation study. The thickness, weight variation wet weight, hand-pressed were within acceptable limit. However the viscosity was increased in formulations where xanthan gum concentration was increased and on the other hand pH was increased in formulations where citric acid concentration was increased. The FTIR spectral analysis confirmed the integrity and compatibility of the Kojic acid with the excipients. The drug content test shows 92-96%. The permeation study shows less flux which is acceptable because Kojic acid should deposited in the epidermis.

It is hoped that the preparation could provide treatment against hyperpigmentation with the formulation of Kojic acid with the help of biocellulose sheet mask.

CHAPTER 7
FUTURE WORK

7. FUTURE WORK

7.1. *in-vitro* hyperpigmentation study with mushroom tyrosinase

Mushroom tyrosinase was assayed using L-DOPA as the substrate. The enzyme diphenolase activity will monitor spectro- photometrically by observing dopachrome formation at 475nm. All the test samples will first dissolve in DMSO at 40mM and dilute to the require concentrations. Initially, 10ml of tyrosinase ($0.5mgml^{-1}$) will mixe with 160ml of 50mM phosphatebuffer (pH=7.4) and then 10ml of the test sample in 96-well microplates will add. After the mixture will pre-incubate at 28°C for 20min, 20ml of L-DOPA solu tion (0.5mM) will add to the phosphate buffer. DMSO without test compounds will use as the control, and ascorbic acid will use as a positive control. Each assay will conduct as three separate replicates. The inhibitory activity of the tested compounds will express as the concentration that inhibited 50% of the enzyme activity (IC50) (Dehghani *et al.*, 2019) . The percentage inhibition ratio was calculated according to the following equation.

$$\text{Inhibition (\%)} = 100(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Compound}})/\text{Abs}_{\text{Control}}.$$

7.2. *in-vivo* hyperpigmentation Study

Hk14-SCF Tg/HRM mice were previously developed and have melanocytes in their epidermis, which mimics human skin . In this study, male 16- to 20-week-old mice will used unless. 2,4-dinitrofluorobenzene (DNFB; Wako) will use as a hapten to induce chronic contact dermatitis. The melanin content (melanin index) of the dorsal skin of 5 mice will measured and after formation of pigments the sheet as apply for every consecutive 3 days. And finally The melanin content (melanin index) of the dorsal skin of 5 mice will measure (Nakano *et al.*, 2021).

7.3. Expected result from *in-vitro* and *in-vivo* hyperpigmentation study:

As many literatures confirm that Kojic acid used for inhibit tyrosinase activity in synthesis of melanin (Saeedi, Eslamifar and Khezri, 2019), (Gonçalez, Corrêa and Chorilli, 2013)(Khezri *et al.*, 2020). It can expect that biocellulose sheet mask will give effective result in the *in-vitro* and *in-vivo* hyperpigmentation study.

CHAPTER 8
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8. REFERENCES

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1. RESEARCH PAPER

The article **Design and Molecular docking of Sulfonamide Derivatives** has been published in **International Journal of Current Pharmaceutical Research** in July, 2021.

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Original Article

DESIGN AND MOLECULAR DOCKING OF SULFONAMIDE DERIVATIVES

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ABSTRACT

Objective: Sulfonamides are a sulfa-related group of antibiotics, which are used to treat bacterial infections and some fungal infections. Some sulfonamides are also devoid of antibacterial activity, such as thiazide diuretics, etc. In this study, an effort was made to find out some novel and potent Sulfonamide derivatives as diuretic agents.

Methods: Here, 30 three-dimensional sulphonamides are designed and docking simulation with PDB ID 1AZM which was downloaded from www.rcsb.org. All the molecules were also screened through a preliminary property filter (Molinspiration Property Calculator).

Results: Among the 30 different molecules designed, 5 molecules were found to have a very good affinity towards the target protein.

Conclusion: These molecular properties define if a molecule can be orally active in our body.

Keywords: Docking, Sulphonamide, SAR

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INTRODUCTION

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target-specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy, and stability of complexes. At present, a docking technique is utilized to predict the tentative binding parameters of the ligand-receptor complex. The main objective of molecular docking is to attain a ligand-receptor complex with optimized conformation and to possess less binding free energy [1, 2].

A sulfonamide is a functional group (a part of a molecule) that is the basis of several groups of drugs, which are called sulphonamides, sulfa drugs, or sulpha drugs. The original antibacterial sulfonamides are synthetic (non-antibiotic) antimicrobial agents that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, e. g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based upon the antibacterial sulphonamides [3].

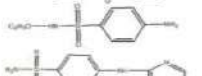
The discovery of sulphonamide diuretic i. e, thiazide diuretics in 1957-58 was the beginning of a new era in the treatment of edema and hypertension. In general, diuretics such as carbonic anhydrase inhibitors, thiazides, and loop diuretics are sulfonamide compounds. Loop diuretics are considered safer and high ceiling diuretics. Their efficacy has a linear relationship with their doses, to the contrary of thiazides which are low-ceiling diuretics. These properties can be attributed to the reason that the sulphonamide derivative shows diuretic activity [4, 5].

Thiazides are sulfonamide-related organic acids that are secreted into the proximal tubule by an organic secretory mechanism.

MATERIALS AND METHODS

The various kind of software which are used. Marvin sketch is used to design 2D and 3D structures of molecules as described in table 1. Molinspiration property calculatorb is used to determine the physicochemical property predictions. Arguslab It is used for docking study of the designed molecule.

Table 1: Molecule and the structure

Molecule	Structures
S1A	
S1B	
S1C	
S1D	
S1E	

Research paper published on in International Journal of Current Pharmaceutical Research

2. REVIEW ARTICLE

i. The article **Enhancing of Oral Bioavailability of Poorly Water Soluble Antihypertensive Drugs** has been published in **International Journal of Current Pharmaceutical Research** in July, 2021

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Review Article

ENHANCING OF ORAL BIOAVAILABILITY OF POORLY WATER-SOLUBLE ANTIHYPERTENSIVE DRUGS

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ABSTRACT

Among various routes of drug delivery, Oral administration is the most convenient route because of its high patient compliance. Although oral drug delivery is effective for drugs with high aqueous solubility and epithelial permeability; however for poorly aqueous soluble drug the membrane permeability, chemical, and enzymatic stability of drugs are the major limitations in successful oral drug delivery. Almost 70% of the new drug candidates which shows poor bioavailability, the antihypertensive drugs are among those. Novel drug delivery systems are available in many areas to overcome the problems associated with hydrophobic drugs and the nanotechnology-based drug delivery system is the most potential to beat the challenges related to the oral route of administration with some important advantages such as the colloidal size, biocompatibility, lowered dose size, reduced toxicity, patient compliance and drug targeting. The foremost common nanotechnology-based strategies utilized in the development of delivery systems are nano-emulsions, nano-suspensions, dendrimers, micelles, liposomes, solid lipid nanoparticles, polymeric nanoparticles, carbon nanotubes, Self-Nano-emulsifying Drug Delivery System, proliposomes, nano-crystals, and so forth, which give controlled, sustained, and targeted drug delivery. The appliance of those systems within the treatment of hypertension continues to broaden. This review focuses on various nano-carriers available in oral drug administration for improving solubility profile, dissolution, and consequently bioavailability of hydrophobic antihypertensive drugs.

Keywords: Nanoparticles, Anti-hypertension, Hydrophobic drugs, Oral bioavailability, Nanocarriers

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INTRODUCTION

Oral delivery of a drug is the most convenient, common, and widely used route of administration for compliance like effortless administration, no assistance, patient conformity as compared to the other routes; for example intravenous, pulmonary, and intramuscular drug delivery. Because of the low bioavailability and low absorption upon oral administration several compounds failed and did not succeed in the research and development [1]. The drugs which have poor oral bioavailability are not capable to reach the minimum effective concentration to exhibit the therapeutic action [2]. The reasons for the poor bioavailability of the drugs depend on some parameters like dissolution rate, permeability, aqueous solubility, susceptibility to efflux mechanisms, first-pass metabolism. In recent years, almost 70% of new drug candidates are showing poor bioavailability [3]. Among these drugs, some antihypertensive drugs show poor bioavailability.

Presently available anti-hypertensive drugs can be classified into the following categories-(a) ACE inhibitors, (b) Angiotensin antagonist, (c) Calcium channel blocker, (d) Diuretics, (e) Central sympatholytics, (f) α-Adrenergic blocker, (g) Vasodilator, (h) β-Adrenergic blocker [4]. The majority of these drugs bear a few significant drawbacks like low permeability, low bioavailability, comparatively short half-life, and undesirable side effects. To overcome such types of challenges related to antihypertensive drug therapy, novel drug delivery systems present an opportunity for formulation scientists which can provide the following characteristics: (1) Enhanced bioavailability, (2) Low dosing frequency, (3) Reduced side effects, and (4) Increased selectivity [5].

Oral drug delivery confrontation

Although oral drug delivery is effective for drugs with high aqueous solubility and epithelial permeability, efficient oral administration of the poorly water-soluble drug may be a challenge. Presently, most of the new chemical entities are lipophilic and consequently have poor aqueous solubility [6]. On the idea of the biopharmaceutical classification system (BCS), a variety of latest therapeutic entities are characterized under BCS class II (low solubility and high permeability) or BCS class IV (low solubility and low permeability). Besides, the oral bioavailability of certain drugs is also suffering from their poor gastrointestinal permeability. To achieve effective therapeutic action, these drugs need to be given at a high dose for example antiviral drugs. Moreover, chemical and enzymatic barriers presented by the gastrointestinal tract (GIT) also affect the oral administration of medicine. The change in GIT pH and the presence of sort of enzymes significantly affect the oral bioavailability of medicine like antihypertensive, antibiotics, antihyperlipidemic agents, and so forth. Furthermore, drugs with high first-pass metabolism such as repaglinide, Β-blockers, calcium channel blockers, and ACE inhibitors even have low oral bioavailability. These drugs also present a challenge in formulation development for oral administration [7].

Fig. 1: Reasons for poor oral bioavailability of poorly water-soluble drugs

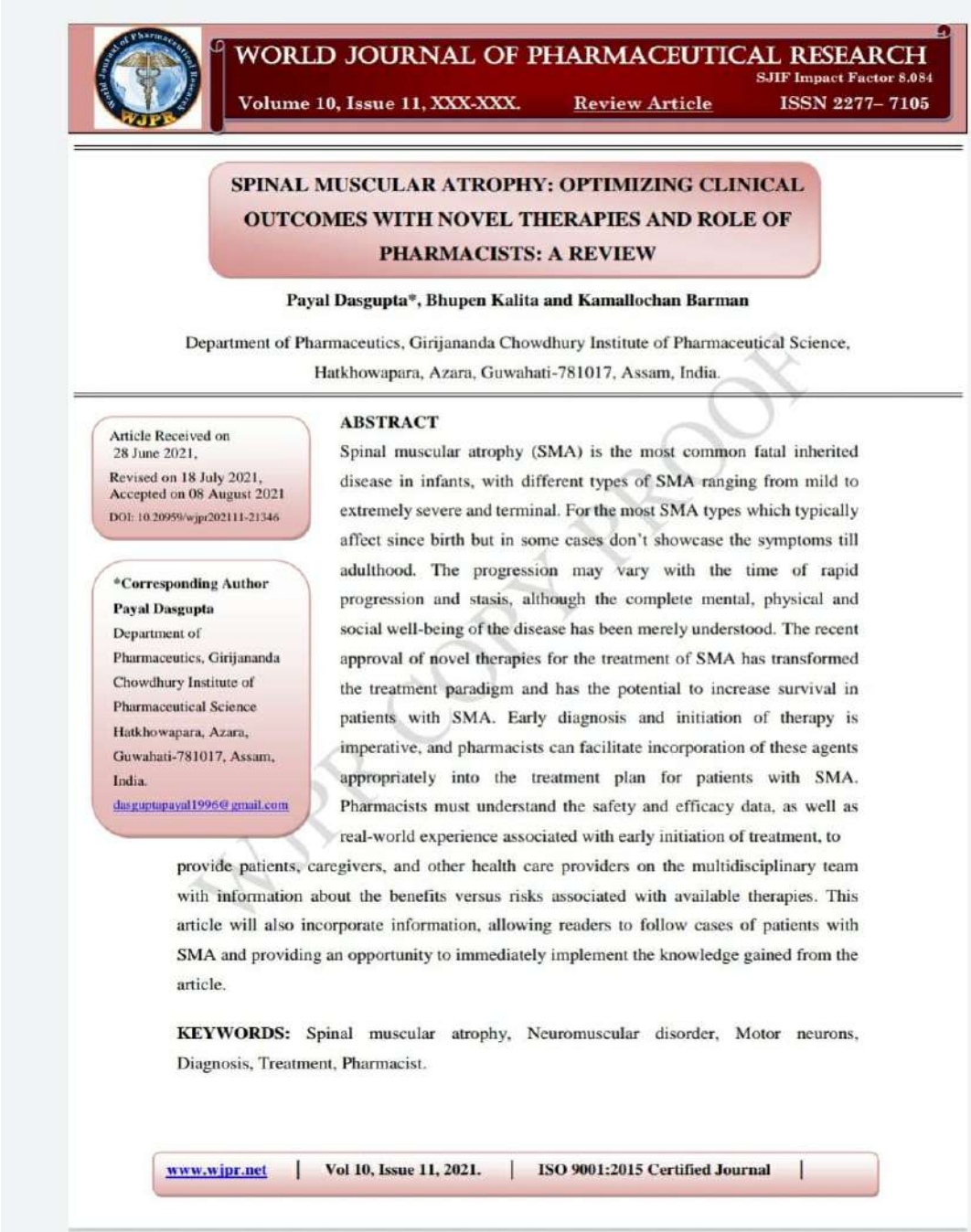
Nanotechnology in delivery of poorly soluble drugs

Drugs with poor solubility possess difficulty in formulation by applying conventional approaches as they show problems like slow

Review article published on in International Journal of Current Pharmaceutical Research

ii. The review article **Spinal Muscular atropy: Optimizing clinical outcomes with noval therapies and role of Pharmacists: A review** has been published in **World Journal of Current Pharmaceutical Research** in August, 2021

- Dasgupta P, Kalita B**, Barman K**; Spinal Muscular atropy: Optimizing clinical outcomes with noval therapies and role of Pharmacists: A review; World Journal of Current Pharmaceutical Research, Vol.10, Issue 11, 2021.



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SPINAL MUSCULAR ATROPHY: OPTIMIZING CLINICAL OUTCOMES WITH NOVEL THERAPIES AND ROLE OF PHARMACISTS: A REVIEW

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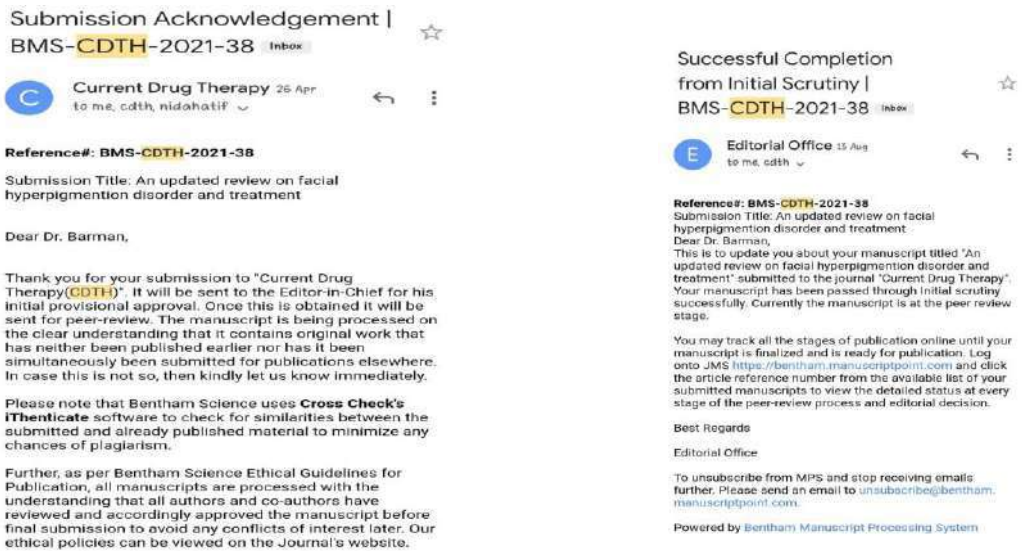
ABSTRACT

Spinal muscular atrophy (SMA) is the most common fatal inherited disease in infants, with different types of SMA ranging from mild to extremely severe and terminal. For the most SMA types which typically affect since birth but in some cases don't showcase the symptoms till adulthood. The progression may vary with the time of rapid progression and stasis, although the complete mental, physical and social well-being of the disease has been merely understood. The recent approval of novel therapies for the treatment of SMA has transformed the treatment paradigm and has the potential to increase survival in patients with SMA. Early diagnosis and initiation of therapy is imperative, and pharmacists can facilitate incorporation of these agents appropriately into the treatment plan for patients with SMA. Pharmacists must understand the safety and efficacy data, as well as real-world experience associated with early initiation of treatment, to provide patients, caregivers, and other health care providers on the multidisciplinary team with information about the benefits versus risks associated with available therapies. This article will also incorporate information, allowing readers to follow cases of patients with SMA and providing an opportunity to immediately implement the knowledge gained from the article.

KEYWORDS: Spinal muscular atrophy, Neuromuscular disorder, Motor neurons, Diagnosis, Treatment, Pharmacist.

Review article published on in World Journal of Current Pharmaceutical Research

3. **Review article under process:**
    a. The article **An updated review on facial hyperpigmentation disorder and treatment** is under review process in **Current Drug Therapy** with corresponding author **Kamallochan Barman** and co-author: Bhupen Kalita, Mayuri Phukan, Robina Jidung and Payal dasgupta.



Submission Acknowledgement | BMS-CDTH-2021-38 Inbox

Current Drug Therapy 26 Apr
to me, cdth, nidahatif

Reference#: BMS-CDTH-2021-38

Submission Title: An updated review on facial hyperpigmention disorder and treatment

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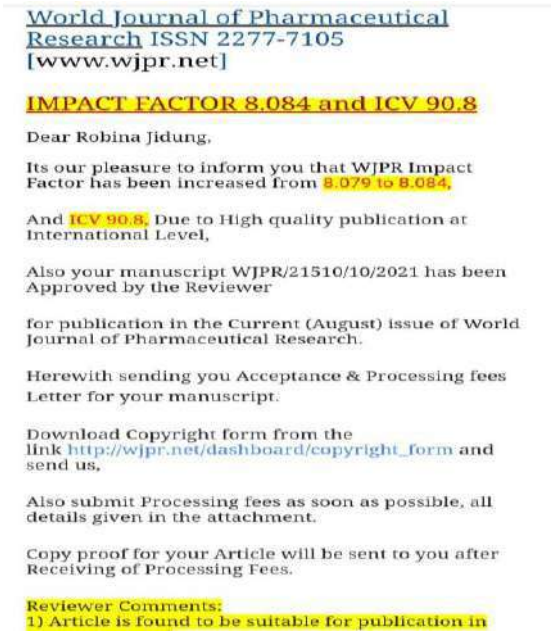

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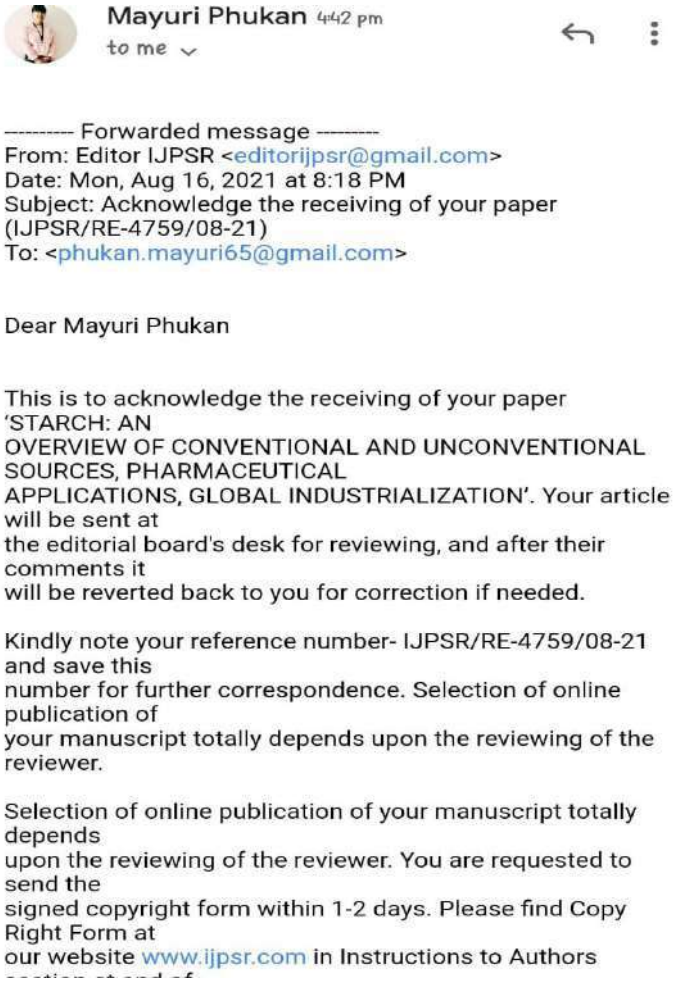

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