1. Introduction & Review of literature

Part I: 2.1 Diabetes- An Overview

Diabetes is a metabolic disorder wherein impairment is seen in conversion of glucose into energy, hence resulting increased glucose in blood [1]. Glucose is supplemented to human system through different sources of food such as carbohydrates, fats, and proteins. Carbohydrates play a major role in the conversion of glucose; liver and muscles also play a vital role in this process [2]. Regulation of blood glucose is essential as it is the only source that provides energy to all cells (brain tissues, retina, and germinal epithelium of the gonads).

Liver acts as a vital organ during the process of glucose conversion and also acts as glucose buffer system. While blood glucose level upsurges, additional glucose is converted into glycogen (glycogenesis) and deposited in the liver; and when the blood glucose is dropped, the stored glycogen is converted into glucose (glycogenolysis) and released into the blood. The storage and release of glucose from liver is mainly regulated by glucagon and insulin [1].

Another organ that plays a significant role in glucose metabolism is the pancreas; Islets of Langerhans in the pancreas are responsible for the endocrine functions. Pancreas contains nearly 1–2 million of islets consisting four different types of cells, namely, A cells or α-cells (secretes glucagon), B cells or β-cells (secretes insulin), D cells or δ-cells (secretes somatostatin) and F cells or PP cells (secretes pancreatic polypeptide) [1]. Insulin is an essential hormone that regulates carbohydrate metabolism and it’s the only hormone that assists in the reduction of blood glucose. There are various parameters that affect regular insulin synthesis by the beta cells. Insulin level is stable during normo-glucose condition however when blood glucose level increases, the production of insulin also increases. Simultaneously, conditions such as excess of protein in the blood stream, β-ketoacids, gastrointestinal hormones (gastrin, secretin, CCK, and GIP), Diabetogenic hormones (glucagon, growth hormone, and cortisol) and
stimulation of parasympathetic nerve tend to stimulate the production of insulin, directly or indirectly [1].

Glucagon is an antagonist of insulin production, which increases the blood glucose level. There are a number of physical, chemical means for stimulation of glucagon such as decrease of blood glucose, excess amino acids, Cholecystokinin and Cortisol (chemical) and stress, exercise (physical), and other factors that inhibit the glucagon production are somatostatin, insulin, free fatty acids, and ketones [3]. When the above process is impaired, the development of diabetes or prediabetes is seen. The term “diabetes mellitus” was coined by Thomas Willis in 1675. He also stated that the cause of diabetes mellitus is due to many factors. Primary diabetes is generally observed with genetic disorders but it might be due to food habits, as it is not related to any disease condition. Secondary diabetes is due to damage of pancreas by other factors or disease [3].

2.1.1 Types of diabetes

Diabetes mellitus is one of the adverse metabolic conditions that develop, irrespective of geographical indifferences. It can be differentiated into three types according to onset of disease conditions in individuals [4, 5].

2.1.1a Type 1 diabetes

Type 1 diabetes or juvenile diabetes is mostly seen in young people, but in special cases it may occur in adults also [4, 5]. The immune system that normally protects from infection malfunctions due to the destruction of β-cells in islets of Langerhans in the pancreas and thus results in lack of insulin production. Type 1 diabetes is insulin dependent individuals and also known as Insulin dependent diabetes mellitus (IDDM).

2.1.1b Type 2 diabetes
Type 2 or adult-onset diabetes is mostly seen with middle or aged groups but sometimes occur within younger age groups also. This type of condition shows defect of the body system in insulin secretion or insulin resistance due to damage of pancreatic beta cells [4, 5]. Type 2 diabetes mellitus are non-insulin dependent individuals as not like type 1 diabetes, otherwise known as non-insulin dependent diabetic mellitus (NIDDM).

2.1.1c Gestational diabetes:

This type of condition is seen in pregnant woman (9.2% prevalence). Women have insulin resistance at the later phase of pregnancy, as the pancreas does not create enough insulin. The body naturally diminishes the ascent of blood glucose after the birth of child. New conceived children will probably be overweight and diabetic. Women influenced with gestational diabetic are more inclined to develop Type 2 diabetes in their later stages. [4, 5].

2.1.1d Aetiological types:

Some Diabetic individuals doesn’t fit easily into any types of diabetes reported before, such kind of diabetes to individual mostly depend on circumstances present during the time of diagnosis. Fig 2.1, demonstrates the order of diabetes mellitus and different categories of glucose control.

2.1.3 Causes of diabetes

2.1.3a Food:

Three sources of food which provides energy to body’s system are carbohydrates, fats, and proteins. Carbohydrate tends to spike the blood glucose more rapidly leading to hyperglycaemia. Fats in the food lead to insulin resistance implying higher amount of insulin is required for the breakdown of fat [28]. Normally liver breaks down glucose to maintain the blood glucose level. On alcohol consumption, liver breaks down alcohol thereby reducing the output of glucose in the blood stream which results in
hypoglycaemia [28]. Protein in the system does not affect the blood glucose level. It has also been studied that increased intake of caffeine shows insulin resistance and also stimulates release of adrenaline.

![Diagram of aetiological types of diabetes]

**Fig. 2.1: Aetiological types of diabetes**

### 2.1.3b Medications:

Drug dosage and timings of diabetic drug affect the regulation of blood glucose level. Other medication tablets, e.g., steroid tablets, along with diabetic medication, may regulate the diabetic condition [29].
2.1.3c Activity:

Physical activities assume an indispensable part in controlling the blood glucose levels. Light movement, for example, energetic strolling, demonstrates the reduced level of glucose, though high intensity action, for example, cardio, running, etc., may bring about the adrenaline reactions and increase the use of glucose in blood bringing about hypoglycaemic shock. In any case, it varies as per the body's condition [29].

2.1.3d Biological:

Anxiety and sickness may influence the immune system and release of epinephrine, glucagon, growth hormone and cortisol. Thus, more glucose is discharged from the liver and the body turns out to be less sensitivity to insulin. In preillness state, some people are much more insulin-sensitive and can tend to run low blood glucose. Similarly, allergies may peak the blood glucose level. Many women are reported of having high blood glucose levels before a few days proceeding to the start of their menstruation cycle; but some women notice a sharp drop in sugar levels. It depends upon the individual's body condition. Smokers are at high risk factors and also show resistance to insulin [30].

2.1.4 Symptoms of diabetes

Various symptoms occur depending on types of diabetes [31]

- Blurred vision
- Unusual thirst
- Frequent urination
- Slow healing wound
- Unexplained tiredness
- Rapid weight loss
• Numbness and tingling in hands or feet

2.1.5 Detailed report on type 2 diabetes mellitus

Type 2 diabetes increases the risk of many diseases such as vascular disease, blindness, diabetic foot ulcer, and end stage of foot ulcer. It is almost seen in 90-95% of the population, and there is rising prevalence of diabetes worldwide [32]. This metabolic disorder is responsible for 3.2 million deaths every year (WHO 2008) [35]. Detection of T2DM is unpredictable, thus causing a lot of damage to the body’s system [33, 34]. Type 2 diabetes mellitus is described by high blood glucose level with typical indicators of thirst, polyuria, polydipsia, and weight reduction. Resistance in insulin intervened signal pathways results in decreased glucose transportation from blood to muscle and fat cells. The significant risk is vascular injury stimulating coronary illness, which is accelerated by the increased level of lipids and hypertension. Administration of diabetes incorporates: control of blood glucose level and lipids and diminish hypertension. Dietary admission of beta-glucans has appeared to decrease all these risk elements to improve the treatment of diabetes and related difficulties. Furthermore, beta-glucans likewise promote injury recuperating and alleviate ischemic heart damage [33, 34]. Be that as it may, the components behind the impact of beta-glucans on diabetes and related difficulties should be further concentrated on utilizing pure beta-glucans.

2.1.5a Epidemiological data

Diabetes mellitus is one of the degenerative ailments around the world. Individuals with diabetes mellitus are at high risk in having coronary illness while contrasted to non-diabetics. More than 60 % of individuals with end-stage renal disease are diabetic. Diabetes is the main source of retinopathy (28.5%) in the United States. Diabetes mellitus is among the main 10 reasons for death either straightforwardly or by implication; yet our comprehension of its pathophysiology and administration is
WHO appraises that by 2025 upwards of 200-300 million individuals worldwide will create type 2 diabetes [36].

Facts

- Currently 382 million people have been diagnosed with diabetes; by 2035 the number will rise to 592 million. 175 million people with diabetes are still undiagnosed.
- Every 6 second a person dies from diabetes; Diabetes has caused 5.1 million deaths in 2013.
- In Western Pacific, 138 million adults have diabetes.
- In Africa, 76% death under the age of 60.
- In India, 65.1 million developed diabetes in 2013; it is the largest contributor to regional mortality with 1.1 million deaths [37].

Part II

2.2 Wound healing

2.2.1 Type 2 diabetes Wound Healing:

Diabetic mellitus is usually characterized into micro-vascular, macro-vascular, and neuropathic ailments due to their secondary complications. Micro-vascular vessels are connected with faster rate of atherosclerosis and amplified inclination of periphery vascular affliction, myocardial infraction, and cerebrovascular damage [38].

Wound healing is impaired in diabetic condition and both are inter-related. Healing of wound in diabetes mellitus is characterized by reduced tensile strength of wound [39]. In mammals, diminished perfusion results in peripheral arterial disease as well as peripheral neuropathy contribute to weaken
healing process which ensued as an indication of diabetic complications. Cross linking of matrix protein and advanced glycation may also involve in an unusual growth expression or factors secondary to diabetes [40]. Involvement of growth factor is not only limited to wound healing but also retinopathy or nephropathy [41]. In normal wound healing process, the apoptosis takes place resulting in the removal of granulation tissue, including fibroblast and small vessels, occurring late as scars. But while in diabetes, the apoptosis occurs throughout the healing process resulting in aberrant control of cell death. In a non-diabetic individual, process happens at the end stage of healing during scar formation.

Hyperglycaemia leads to increased release of reactive oxygen species that mediates mitochondria release of cytochrome C [42]. The activation of caspase 3 induced myocardial cell apoptosis and inhibition of glucose by insulin prevent myocardium cell death. Dysregulation of apoptosis in body system leads to the weakened wound healing. Diabetic wound is caused by peripheral vascular disease and neuropathy; it is also associated with micro-vascular complication of diabetes mellitus which provides evidence of increased apoptosis all over body [43–45].

Risk factors in diabetes:

- Peripheral vascular disease
- Peripheral neuropathy
- Callus formation
- Limited joint mobility

2.2.2 Normal wound healing:

Wound healing is a complex process. Apoptosis and necrosis are the part of the normal development of healing. Apoptosis is complex network of biochemical and molecular pathway that regulates cell death. It is one of the main mechanisms in the process of wound healing. This helps in the removal of
inflammatory cells and evolution of granulation tissue in the scarred area [46–54]. In healthy individuals, the repair, wound mechanism follows as:

- Neutrophils are first cells that arise during wound, as they prevent invasion from bacteria. Their activity is implicated in local and distinct tissue damage through free oxygen radicals and protease.
- Neutrophils that enter the wound would have eliminated micro-organism, under apoptosis and are later consumed by macrophages.
- Thus, further leading to the cycle of inflammation process.
- Migration of inflammatory cells to colonise the provisional matrix.
- Proliferation of fibroblast and vascular cell apoptosis.
- Synthesis of extracellular matrix to reconstruct the dermal architecture.

2.2.1a Common types

The common types of wound are shown in Fig. 2.2 [55, 56].

2.2.2b Phases of wound healing

There are four phases of healing; it is a continuous and steady process merging with the next phase (Fig 2.3) [56, 57].

- Inflammatory
- Migratory
- Proliferation
- Maturation
Haemostasis and inflammation: Bleeding usually occurs during skin injury and serves to flush out bacteria or antigen from injured site. Bleeding activates haemostasis which initiates an exudate component such as clotting factor. Fibrinogen elicits the clotting mechanism resulting in coagulation and together the formation of fibrin network to stop bleeding. Clot further dries up and forms scab which gives protection to the wounded site. This is simultaneously followed by inflammation, lasting for a few minutes to 24hrs or 3 days. Necrotic tissue release yellowish colour mass, and platelet liberated from
blood vessels becomes activated as they contact with mature collagen and forms aggregates as part of clotting mechanism.

**Migration:** It involves the movement of epithelial cells and fibroblast to the wounded site. These cells regenerate from margin towards the centre site of the wound, to form a scab accompanied by epithelial thickening.

**Proliferation:** It is the next step after the migratory phase; observe skin tightening and epithelial thickening take place until collagen bridges the wound. The proliferation of fibrin and collagen synthesis lasts for 2 weeks that result in a decrease in blood vessels and oedema.

**Maturation:** it is the final stage of the healing phase. During this phase, the tensile strength of skin gets increased and re-epithelization of skin takes place up to 12 months, according to the condition of the wound.

![Fig. 2.3 Phases of wound healing](image-url)
2.2.2c Controls affecting wound healing

**Intrinsic factors:**

Health status: Blood flow and better circulation to wounded sites helps in faster healing of wounds, e.g., Angiogenesis is an important parameter in wound healing, anaemic condition decreases the haemoglobin content that leads to slow blood circulation in the wounded site [58, 59].

Immune function: If immune system is weak, then the body’s loses its ability in pervading the pathogens entering through the wounded site [58, 59].

![Flow Chart of Factors Affecting Wound Healing](image_url)
**Diabetes:** It is one of the cause of chronic wound, delay in capillary response to the injury site. Hyperglycaemia is a prevailing condition in diabetes that results in the delay of wound healing, as it lacks the synthesis of insulin and more prone to infection [58,59].

**Age factors:** As we age, the oil content of the body diminishes thus resulting in tearing of skin, furthermore loses sensory cells. It is more prone to physical and chemical damage [58, 59].

**Body build:** Obese individuals may have issue with wound healing because of the inability of the body to deliver oxygen and nutrients to wounded site. Even underweight individuals also face same problems with the healing process [58, 59].

**Nutritional status:** Proteins, carbohydrates, fats, vitamins, and trace elements have played a vital role in wound repair. Amino acid (arginine) as a supplement can improve the rate of wound healing [58, 59].

**Extrinsic factors:**

**Mechanical stress:** Immobile and sudden pressure, results in tissue damage.

**Debris:** The debris released on wound such as scab, eschar, wound dressing debris will disturb the healing process [58, 59].

**Temperature:** Temperature plays an essential role in wound healing for eg

a) Moist conditions cause difficulty in wound healing, hence leading to various infections.

b) Increased temperatures may lead to changes at the wound site with risk of cellular breakdown, thereby limiting healing process.

**Desiccation:** Exposed wound may significantly cause more to pain, itchiness and have scab material during healing [58, 59].
Maceration: It will cause destruction of tissue and slows down healing.

Infection: Bacterial colonization of wound site may cause difficulty in healing process.

Other factors can be alcohol, smoking, and synthetic medicines.

2.2.2e Overview of types of wound

Complex and chronic wound [60, 61]

- Leg ulcer
- Pressure wounds
- Postoperative wound
- Neoplastic
- Diabetic wound

Acute wound [60, 62]:

- Antiseptic
- Inflammation occurs

2.2.3 Epidemiology

Wound healing is a complex process and has been subjected to intense research work for a long time. Diabetic individuals have 15% of lifetime risk in developing a foot ulcer and in 50–70% of population, lower limb amputation was also observed. Worldwide statistical data indicate that every 30 seconds one leg is amputated in diabetic foot ulcer patients [63–66].
2.3A Nanotechnology

2.3A.1 Nanotechnology:

Nanotechnology can be characterized as designing of particles with a remarkable property at a nanoscale. In 1959, Richard Feynman proposed a thought regarding nanotechnology in the discussion "A lot of room at the base" and Norio Taniguchi (1974) instituted the term nanotechnology. A nanometre is one billionth size of the meter. Atomic force microscope and scanning tunnelling microscope are the two filtering tests that lead the way into the field of Nanotech [67]. Emergences of Nanoparticles have prompted its applications in different parts, for example, electronics, biomedical, cosmetics, catalyst, and other materials. Nanotechnology is not a new technology. In Ayurveda or sages era, some medicines are in the form of churunam (fine powder) and size of powder was in nanosize. Still today it is available in the worldwide market.

Bottom up approach

The materials and devices are fabricated from atomic segments. Bottom up, or self-get together, ways to deal with nanofabrication use substance or physical powers working at the nanoscale to mass essential units into bigger structures [68]. As segment size abatements in nanofabrication, bottom up methodologies give an undeniably vital supplement to best down systems. Motivation for bottom up methodologies originates from organic frameworks, where nature has saddled substance strengths to make basically every one of the structures required by life. Specialists want to reproduce nature’s capacity to deliver little groups of particular molecules, which can then self-assembly into more-expand structures [68].

Top down approach
Nanoparticles are built without nuclear level. The most widely recognized top-down way to deal with creation includes lithographic designing strategies utilizing short-wavelength optical sources [69]. A key favourable position of the top-down methodology-as created in the manufacture of incorporating circuits that the parts are both designed and constructed set up, so that now get together step is required [69].

2.3A.2 Micro-Nano

Day by day the progress of nanotechnology has been noticed in biomedical field such as drug delivery system, medical imaging, and lot more military purposes [70–75]. There are different kinds of metallic nanoparticles such as gold, silver, platinum, iron, copper, etc., have shown their unique application in the biomedical sector, e.g., gold and ceramic oxide nanoparticles are used in tumour and anti-inflammatory, silver nanoparticles are proved as an antimicrobial agent, anti-inflammatory, disinfectant and less toxicity to human system. Hence, silver is further taken for the studies of wound healing [76-83].

2.3A.3 History of nanotechnology

The history of Nano science’s and nanotechnologies is summarized in the flow chart (Fig. 2.5) [84–86].

2.3B Nanoparticles (silver)

2.3B.1 Introduction about silver nanoparticles

Novel strategies are being developed to probe and manipulate single atoms and molecules. Among all nanoparticles, the metallic nanoparticle, such as silver has application in different ranges, e.g., gadgets, cosmetics and biotechnology. The benefits of nanoparticles are to penetrate through the skin and target the specific site. Earlier nanoparticles were synthesized by using different physical and chemical methods. Biosynthesis/Biological method was later reported. The present study emphasises on
synthesis of silver nanoparticles by using biological sources. Silver nanoparticles have differing properties like catalysis, attractive and optical polarizability, electrical conductivity, antimicrobial action and surface plasmon reverberation [81-83].
2.3B.2 History of Nanosilver

Nanosilver is a type of nanomaterial which has been subjected into lot of investigations. Discussions were carried with base of silver nanoparticles as new and availability in this modern era. But the history of nanosilver goes back more than 120 years and the impact of it with the medical field and lots of other applications are very strong [87]. Metallic silver is third metal known to ancient Egypt and to Caldean as early as 4000 B.C.E. Over a millennium, silver has been used in various medical conditions. Hippocrates used in various treatment such as ulcer and wound healing. Mostly silver nitrate was used medically and as in published pharmacopeia in Rome 69 B.C.E. Silver nitrate used as a medical agent by Gabor 702-705, In 980 AD, Avicenna used silver fillings for blood purifier. By 1800, a wide range of silver used in the transportation of water, wine, milk, etc., have shown longer period of storage [88]. The 120 years of nanosilver has been given in Table 2.1. Among history silver has gone, but has not reported in the name “Nano”.

Table 2.1: 120 years of Nanosilver

<table>
<thead>
<tr>
<th>Year</th>
<th>Inventor and Invention</th>
<th>Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1889</td>
<td>Lea M. C.-Citrate stabilized colloidal silver</td>
<td>7–9 nm</td>
<td>[89–91]</td>
</tr>
<tr>
<td>1902</td>
<td>Further by protein stabilization</td>
<td>—</td>
<td>[92]</td>
</tr>
<tr>
<td>1897</td>
<td>Collargol</td>
<td>10 nm$^{23}$</td>
<td>[93]</td>
</tr>
<tr>
<td>1907</td>
<td>Termed as nano range</td>
<td>10 nm$^{23}$</td>
<td>[94]</td>
</tr>
<tr>
<td>1953</td>
<td>Moudry—Gelatin stabilizing silver nanoparticles</td>
<td>2–20 nm</td>
<td>[95]</td>
</tr>
<tr>
<td>1970’s</td>
<td>Silver impregnated water filters</td>
<td>&gt;100 nm</td>
<td>[96,97]</td>
</tr>
<tr>
<td>Last 20 decades in</td>
<td>Collargol Argyol</td>
<td>&gt;100 nm</td>
<td>[98]</td>
</tr>
</tbody>
</table>
2.3 B.3 Properties and application of silver nanoparticles

Silver nanoparticles have diverse properties such as [99,100]

- Catalyst
- Magnetic and optical polarizability
- Electrical conductivity
- Microbial activity
- Raman scattering

The properties of it varies according to different parameters

**Diameter, surface area and volume:**

- It has unique properties depending upon the size.
- Solubility and stability also vary according to the nature of nanoparticles
- High surface area, compared to volume ratio, is important for catalyst properties

**Shape and crystallinity:**

- It can be produced with various shapes and size
- Anisotropic shape formed with stabilizing polymer, binds with one crystal face and move in one direction—faster complex SNPs from highly faceted.

**Nanoparticle surface:**
• Tannic acid, citrate, PVP gave very good capping agent for SNPs
• Especially PVP binds strongly to SNPs surface and provides greater stability than citrate and tannic acid

**Particle stability:**

• Zeta potential helps to measure particle stability > or < 20 mv have sufficient electrostatic repulsion for stability nature.

**Surface chemistry and functionalization:**

• PVP or tannic acid is used as capping agent. In the biological application, SNPSs were coated with BSA and PEG for stability and has property to flip the surface with positive and negative charge.

**Measuring SNPs concentration:**

• Depending on concentrations, the properties of silver nanoparticles also varies. ICP-MS is used in measuring the concentration of SNPs.

### 2.38.4 Types of silver nanoparticles [100]

**Silver colloids:** Silver colloids are available to the market in five types. In that mesosilver is the purest colloidal silver and others are not true or pure.

**Nano xact:** They are the aggregated spherical nanoparticles that are suspended into water molecules.

**Biopure silver:** It is highly concentrated and available in pure form.
**OECD-silver:** This silver was given an Organization for Economic Cooperation and Development direction. Its definition has been chosen as Nano toxicology principles with PVP and citrate surfaces.

**Custom silver:** It has been customized according to the custom organization, bio functionalized shells, and suspension media

**Silver nanoparticles:** They have high optical efficiencies and tend to cooperate between the wavelength 550-950 nm.

### 2.3B.5 Methods of synthesizing nanoparticles

**Chemical reduction:** It is one of the processes used in the reduction of silver nanoparticles with the help of ionic salts in the appropriate medium in the vicinity of surfactants utilizing diminishing specialist, e.g., sodium citrate, sodium borohydrate [101–104].

**Solvo thermal synthesis:** This is an adaptable low temperature mechanism; polar solvents under pressure and at temperature over their building focuses are utilized. Under solvo thermal conditions, the dissolvability of reactants increments fundamentally, empowering response to happen at lower temperature [101–104].

**Sol gel technique:** It is one of the wet chemical strategy/method utilized for the manufacturing of metal oxides from the availability chemicals which represents an integrated system of discrete patches or polymers. This precursor sol can either be deposited on the substrate to form a film or caste into a suitable compartment with fancied shape or used to synthesize particles or powders [101-104].

**Laser ablation:** This is the procedure of irradiating so as to expel materials from a strong surface with a laser shaft. Subsequently, they create nanoparticles [101-104].
**Biological method**: Biological method follows bottom-up approach with reduction or oxidation process. As the name subjects, the biological sources such as microorganism and plants are used for the reduction, capping and stabilizing nanoparticles. The advantages are eco-friendly, cost effective, faster production, and non-toxic production with any chemicals [101–104]. The systematic biological pathway has been indicated in Fig. 2.7

![Biological pathway of silver nanoparticles](image)

**Fig. 2.6 Biological pathway of silver nanoparticles**

### 2.3B.6 Characterization

There are various techniques followed to validate and confirm the synthesis of silver nanoparticles [105–107].

- **Scanning electron microscope, field emission electron microscope and transmission electron microscope**: Basically used to determine the size and morphology of particles.
- **X-ray diffraction**: To study the crystal structure and size.
- **Fourier Transform infrared spectroscopy**: To identify the functional groups of biological sample.
• **Energy dispersion X-ray spectroscopy:** To determine the composition information.

• **Dynamic light scattering:** To determine the particle size, and is commonly used in chemicals and pharmaceuticals industries.

• **Atomic force microscopy:** To identify the mechanical properties of sample.

### 2.3B.7 Applications

Silver nanoparticles have a strong role in the field of anti-microbial and anti-inflammatory applications till date. There are other various medical applications such as wound dressing material, silver impregnated catheters, vascular prosthesis, ventricle drainage catheters, orthopaedics, and surgical mesh available in the market. Currently, a lot of research is carried out on Nano drug delivery system; liposomes are used to extend the life of drug, biochips as nanodevice used in synthetic neuron, and usage of Nano robots for the microsurgery [108–120].

### Part IV

#### 2.4 Ganoderma lucidum

*Ganoderma lucidum* is also known as Lingzhi, Reishi or red mushroom. In Greek, *Ganoderma lucidum* was named according “Ganos” brightening, “derma” skin, and “lucidum” shiny. It is a much demanded mushroom, particularly in the Far East countries for over 4000 years and it’s termed as “King of herbs” due to its medical and spiritual properties. The main constituents are protein, fat, carbohydrate, and fibre [121–126].

#### 2.4.1 Background
Lingzhi was recognized as medicinal mushroom over 2000 years in ancient script for its medicinal properties. 1400 A.D. art associated with Taisom shows the existence of *G. lucidum*. The first book that is fully dedicated for medicinal herbs and their value is by Shen Nong Ben Cao Jing written during eastern dynasty of China (25–220 AD). It is known as the Classic Book of Material Medica or Shen Nong Herbal Classics [127].

The benefits of *G. lucidum* extracts are recognized from one generation to other, including its cancer cure, good fortune, good health, longevity, and immortality. In 1280–1368 A.D., Yuvan Dynasty represented hidden art of mushroom, scarving of jade or deer antlers, furniture, and carpet design. In 221–207 B.C., Ling Zhi was the first emperor to report the identification of mushroom [128–129].

### 2.4.2 Taxonomical and Scientific Classification

*Ganodermataceae* family depicts polypore basidiomycetes having double walled basidospore identification of 219 species inside the family of *Ganoderma*. *G. lucidum* (P. Karst) is the species type. Basidiocarp of this variety have glossy surface or outer covering that connected with thickened wall pilocystidia implanted with extra melanin [130] . It is one of the species that is grown irrespective of geographical barrier and temperature, but the bioactive and medicinal components vary according to locations, temperature, attitude, etc. A few taxonomists, likewise, consider macromorphological elements to be of constrained worth in the recognizable proof of *Ganoderma* species because of its high phenotypic plasticity [131]. More dependable morphological attributes for *Ganoderma* species are thought to incorporate spore shape and estimate, connection shading and consistency and the microanatomy of the pilear crust. Chlamydospore creation and shape, enzymatic studies and, to a lesser degree, the reach and optima of development temperatures have additionally been utilized for separating morphologically comparative species. Biochemical, hereditary, and atomic methodologies have additionally been utilized as a part of *Ganoderma* species scientific classification [130,131].
Scientific Classification

Kingdom : Fungi
Phylum : Basidiomycota
Class : Agaricomycetes
Order : Polyporales
Family : Ganodermataceae
Genus : Ganoderma
Species : lucidum

2.4.3 Components and their therapeutic applications:
Fig 2.7 List of *G. lucidum* properties

**Triterpenoids [132,133]:**

- The major components are ganoderic acid A, B, C, D lucidnic acid and ganodermanontriol.

**Polysaccharide [134,135]:**

- 200 types of polysaccharides were isolated from this family, but polysaccharides isolated from *Ganoderma* are different.
- As the polysaccharide derived from *Ganoderma* have beta glucans.
- It is used as potent anti-tumour drug.

**Protein [132]:**

- Few proteins such as lectin and ribonuclease are present.

**Germanium [132]:**

- It is vital compound of Ganoderma and has very good medicinal properties.
- It has been associated with antimutagenic, immune-modulatory, anti-oxidant, and anti-tumour effects

**Adenosine [132]:**

- It suppresses the platelet aggregation and acts as a cardiac depressant used in pharmaceutical industry as an anti-arrhythmic.

**Therapeutical Applications:**
Fig. 2.8 List of applications reported in *G. lucidum* [137–142]

Part V

2.5 Transdermal drug delivery system

The skin is an external barrier that provides us with a complete protection against bacterial infections, thereby maintaining the homeostasis of the body. Skin loss occurs frequently as a consequence of blazes, injury, and clutters as the skin is the main organ in contact with the external factors. Generally, wounds are categorized into three classes, for example, superficial wound, partial thickness wound, and full thickness wound [143]. The essential guideline of ideal wound healing is to minimize tissue damage, give satisfactory tissue perfusion, oxygenation, appropriate sustenance, and
moist injury recuperating environment to re-establish its natural capacity [144,145]. Traditionally, there are a lot of therapeutic options are available to treat wounds, but it takes prolonged time to heal. Due to recent advancements in the field of drug delivery, a rapid recovery can be observed against infections. The transdermal medication conveyance is a novel medication conveyance framework which has been as of late created. The bio adhesive patches containing distinctive constituents of medications are administrated into the skin. It is non-obtrusive, advantageous, and effortless technique for medication conveyance; additionally it is significantly toxic properties, for example, gastrointestinal poisonous quality [146,147]

2.5.1 Administration of drugs:

Drugs that are dermally administered through the skin categorized as (1) those applied for local action and (2) for systemic effects. Local action impact incorporates those connected on or at the surface of the skin, which applies the activity on the stratum corneum or regulating the function on epidermis or dermis. The product that come in these criteria are creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions. Transdermal drug delivery system (TDS) or transdermal patches go under the criteria of systemic impact [148]. Transdermal drug delivery system represents discrete dose type of medication to the general flow through the skin. It overcomes drawbacks of oral drug delivery system by providing an end to gastro-intestinal lethality and hepatotoxicity [149]. Transdermal patch gives uniform scattering of medication into target side unlike the oral dosage forms which forms blood hikes and troughs [150]. Therapeutical applications to the skin to ease the ailments have been followed since the time of ages and have shown beneficial outcomes.

2.5.2 Advantages of transdermal drug delivery [151-153]:

- It is a convenient method.
- It requires only weekly applications.
• It also acts as an alternative route to administrate drug by avoiding oral dosage and reducing the toxicity.

• The relatively consistent plasma levels are a good indicator for transdermal drug delivery.

2.5.3 Limitation of transdermal drug delivery [153,154]:

• Local irritation at the site of application may be due to adhesive or other excipients used in the formulation of film/patch.

• Its molecular weight should be less than 500 Da.

2.5.4 Involvement of transdermal delivery in the society

2.5.4a Historical perspective:

From earlier era, the use of ointments and plaster has been followed without the advancement and interaction of drugs. Mustard plaster was applied for severe chest congestion. Paste of mustard was applied on chest with strip of flannel and wrapped with cotton cloth to hold the drug on the place [155,156]. The moisture and heat liberated from the body activates myrosin enzyme by hydrolysing a sinergen glycoside causing the release of active ingredients allyl isothiocyanite; it is one of transdermal absorption component because it has low molecular weight 90 Da. Now mustard plaster has been commercialized and is available in pharmacy. Laterally, lot of other plaster has been released at 20th century and recognized by United States pharmacopeia and national formulary [157,158].

Belladonna plaster containing belladonna root alkaloids was used to act as analgesic. Most remarkable in transdermal medication were super mercurial ointment for treatment of syphilis along with other salvaresan and arsenicals were in market before the discovery of penicillin. But there are a lot of controversies of absorption mercury vapours by humans and it requires specific chemicals to clean the surface. Hence, number of studies was carried out for bioavailability [155–163].
2.5.4b Future perspective:

In the last 10 years, Nicotene patch had modernized smoking cessation. Patients were being administrated with patches for eg; nitro-glycerine for angina, clonidium for hypertension, scopolamine for motion sickness and estradiol for estragon deficiency [155-158]. Advancement in biotechnology has led in designing new products, whereas transdermal drug delivery technology is appealing and patience acceptance as it minimizes and avoids the limitation allied by previous technology or development. Other factors for limitation are fitting molecular weight, lipophilicity and potency requirement for transdermal absorption [155–158]. Molecular absorption enhancement and absorption enhancement by energy output are the new absorption enhancement technology that has opened its path in future transdermal drug delivery system [163].

2.5.5 Characteristics of several drugs permeation through transdermal:

Transdermal permeation is a slow process of diffusion driven by concentration gradient between high in delivering system and zero in prevailing system of skin. For continuous delivery of drug towards the site, continuous contact of film with considerable amount of time is required [156].

Transdermal patches are currently marketed in transdermal delivery system. Functional parts of film are

- An impermeable backing
- A reservoir holding active ingredients
- An adhesive to hold the patch
- A protective cover that is peeled away before applying it

Patches falls into two categories; reservoir and matrix system. Formulation of patches is a complex process. The rate and amount of absorption depends on many factors such as nature of drugs, concentration in reservoir or matrix and space covered by patch. The release of drug also depends on
the site covered by patch. To keep the favourable absorption concentration large amount of drug are placed in the film [156,162,164–169]. There are various methods to enhance the penetration methods and are given in Fig. 2.8 [170,171]

2.5.6 Transdermal film:

A transdermal patch is defined as an adhesive patch kept over the dermal layer to release a particular dose of medication released through the skin with fore-ordained rate of discharge to venture into the circulation system. Today the most widely recognized transdermal film present in the market is based on semipermeable membranes that are known as patches [172–179].

Fig 2.9: Various methods used to enhance the Skin penetration

2.5.7 Technologies for development:
A number of technologies have been developed to provide rate control release and skin permeation of drugs. It was classified into four basic approaches [158-162,180].

2.5.7a Polymer membrane permeation-controlled TDDS

In this system, drug reservoir has been sandwiched between the drug impermeable support overlay and rate controlling polymeric film. The drugs are allowed to discharge just through the rate-controlling polymeric film. In the drug supply compartment, the drugs solids are scattered homogeneously in a strong polymer network, suspended in an unleached viscous fluid medium (e.g., silicone liquid) to frame a paste like suspension, or broke down in a releasable dissolvable (e.g., alkyl liquor) compound to form clear drug solution. The rate controlling layer can be either a microporous or a nonporous polymeric film with particular drug permeability. The rate of medication discharge from this TDD framework can be custom-made by fluctuating the organization of the drug repository plan and the permeability coefficient and/or thickness of the rate-controlling layer [158-162,180].

2.5.7b Polymer matrix diffusion-controlled TDDS

In this approach, the process of medication supply is framed by homogeneously scattering the medication solids in a hydrophilic or lipophilic polymer lattice, and the sedated polymer shaped is then formed into medicated disks with a characterized surface zone and controlled thickness. This drug reservoir containing polymer disk is then mounted onto an occlusive base plate in a compartment manufactured from a drug impermeable plastic backing. Rather than covering the adhesive polymer straightforwardly on the surface of the mediated disks, as demonstrated prior in the main kind of TDD systems, in this system the adhesive polymer is connected along the perimeter of the patch to shape a segment of adhesive edge encompassing the sedated plate [158-162,180].

2.5.7c Drug reservoir gradient-controlled TDDS
Drug reservoir slope controlled TDDS to beat the non-zero-order \((Q \text{ versus } t\%)\) drug discharge profiles, polymer matrix drug scattering sort TDD system can be altered to have the medication stacking level differed in an incremental way, shaping a gradient of drug reservoir repository along the diffusional way over the multilamine adhesive layers [180]. The rate of drug discharge from this sort of drug reservoir gradient can be expressed by

\[ \frac{dQ}{dt} = \frac{K_a}{rD_a} h_a(t) \times L_d(h_a). \]

**2.5.7d Microreservoir dissolution-controlled TDDS**

The drug delivery system can be recognized as a hybrid of the reservoir and matrix dispersion drug delivery system. In this approach the drug reservoir is framed by first suspending the drug solids in an aqueous arrangement of water-miscible medication solubilizer, e.g., polyethylene glycol [80].

**2.5.8 Types of transdermal films**

**2.5.8a Single layer drug adhesive:**

Adhesive layer of system contains the drugs that not only adhere to other layers but also are responsible for releasing the drugs [181,182]. The rate of release follows the diffusion phenomenon. The rate of release of drug is expressed as

\[ \frac{dQ}{dT} = C_r/1/P_m + 1/P_s \]

Where \(C_r\) is the drug concentration in the reservoir compartment; \(P_s\) is the permeability coefficient of the adhesive layer; \(P_m\) is the permeability coefficient of the rate controlling membrane
2.5.8b Multiple layer drug adhesive

It is almost similar to a single layer adhesive, but in here there are two layers of adhesive are sandwiched with a single layer of membrane in between them. Release of drug follows the diffusion phenomenon and results in immediate release of drug along with adhesive layer [181,182]. The rate of release of drug is expressed as

\[
\frac{dQ}{dT} = \left( \frac{K_a}{r} \cdot D_a/h_a \right) C_r
\]

Where \( K_a/r \) is the partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer.
2.5.8c Drug reservoir in adhesive

Drug reservoir is immersed in the backing and membrane layer followed by adhesive layer and release liner. This drug can be available in many forms such as gels, solutions, suspension, or dispersed in solid matrix. Release of the drug is controlled by controlling the membrane (microporous or nonporous) [181, 182]. The rate of drug release from this drug reservoir system is given by

\[
dQ/dT = \frac{K_d}{r} \cdot \frac{D_d}{h_d(t)} A(h_a)
\]

where \( h_a \) is the thickness of adhesive layer; \( A \) is the thickness of the diffusional path.
2.5.8d Drug matrix in adhesive

Drug availability nature in this particular system is dispersed homogenously in a hydrophilic or lipophilic polymer matrix and designed by semisolid matrix having drug solution or suspension form which is in direct contact with the release liner [181,182]. The rate of release of drug is calculated by the following equation: 

\[ \frac{dQ}{dT} = AC_pD_p^{1/2}/2t \]

where \( A \) is the initial drug loading dose dispersed in the polymer matrix; \( C_p \) is the solubility of the drug; \( D \) is the diffusivity of the drug in the polymer.

2.5.9 Skin permeation

2.5.9a Anatomy and physiology of skin

Skin is the largest organ and receives about one-third of blood. It is multilayered and is divided into three categories; epidermis, dermis and hypodermis [183,184].

**Epidermis**: It is the outer layer of skin which gives protection from immediate attacks and also tones the skin.
1. Stratum corneum is otherwise known as horny layer of skin. Though the layer is flexible, it is impermeable. Hence prove the principle barrier for penetration. The layer constitutes 75 to 80% proteins, 5–15% lipids, and 5–10% ondansetron material.

2. Viable epidermis is found just beneath stratum corneum. It consists of various layers such as stratum lucidum, stratum granulosum, stratum spinosum, and the stratum basale beneath stratum corneum.

**Dermis:** It is the layer found next to epidermis and contains tough connective tissue, hair follicles, and sweat glands. The continuous function of blood supply is important in regulating the body temperature. It provides nutrients and oxygen to the skin removing toxins and waste products. Dermal concentration of permeate very low and concentration difference across the epidermis provide essential driving for transdermal permeation.

**Hypodermis:** It is a subcutaneous tissue made up of fat and connective tissues. This layer helps to regulate temperature, provide nutrition, and mechanical protection. During transdermal drug delivery system, drug need to pass through all these three layers to reach the circulation, while topical drug
delivery penetration through stratum corneum is only necessary, thus retention of drug in skin layer is desired.

Fig. 2.14 Anatomy of skin [185]

2.5.9b Drug delivery routes

Drug has been delivered through different routes or pathways according to the drugs [186]. Drug is administrated and passed through the sweat glands, then followed by sebaceous gland or has been penetrated through the layers of hair follicles. Mostly drug that has passed through it are from ointments such as topical delivery system. But in case of transdermal drug delivery, the drug has been passed through stratum corneum.

* Sweat gland
* Sebaceous gland
* Hair follicles
* Stratum corneum: intercellular route is the major pathway for permeation of most drugs across the stratum corneum

![Fig 2.15: Routes of drug delivery](image)

2.5.9c Methods of skin penetration

**Transcorneal penetration**

**Intracellular penetration**

Drugs go through the cells of the stratum corneum. It is mostly used in the hydrophilic medications. As stratum corneum hydrates, water accumulates the external surface of the protein fibres. Polar atoms seem to go through this immobilized water [187–190].

**Intercellular penetration**

A non-polar substance passes through the skin by intercellular penetration route. These particles break down and diffuse through the non-watery lipid matrix between the protein fibres [187-190].
Transappendegeal penetration

It is also called as the shunt pathway. In this course, the drug might transverse through the hair follicles; the sebaceous pathway of the pilosebaceous contraption or the aqueous pathway of the salty sweat organs. The transappendegeal pathway is thought to be of minor significance due to its moderately smaller range (less than 0.1% of aggregate surface). However, this course might be of some significance for vast polar compounds [187–190].

The transdermal permeation can be visualized by number of series:

* Adsorption of a penetrant molecule onto the surface layers of stratum corneum.
* Diffusion through stratum corneum and through viable epidermis.
* Finally through the papillary dermis into the microcirculation.

There are various methods to enhance the penetration methods and are shown in Fig. 2.14

2.5.10 Properties

2.5.10a Physiochemical properties [191]

* Molecular weight should be below or approximately 1000 Daltons
* Drug should have affinity on both lipophilic and hydrophilic phases
* Low melting point
* pH range should be between 4 and 6. Thus, results in significant and uniform distribution off drug

2.5.10b Biological properties [191]

* Slow release rate of drug with few mg per day
* Drugs should have half-life $t_{1/2}$

* Drugs need to be non-irritating and non-allergic

* Drugs that degrade in gastrointestinal track are important parameter noted on transdermal drug delivery system

**2.5.11 Evaluations of transdermal film**

There are various parameters to evaluate the transdermal films such as [192,193]

* Thickness of the patch

* Weight uniformity

* Folding endurance

* Percentage moisture content

* Content uniformity test

* Moisture uptake

* Drug content

* Shear adhesion test

* Peel adhesion test

* Water vapor transmission studies

* Quick stick (peel-tack) test

* Probe tack test

* *In vitro* drug release studies

* *In vitro* skin permeation studies

* Skin irritation study

* Stability studies
In vivo toxicity or irritation study

2.5.12 Regulatory and market of transdermal film

Prior to marketing, the product needs approval from U.S. Food and Drug Administration (FDA) for demonstrating safety and efficacy [163].

Table 2.2 Transdermal film in market [194–196]

<table>
<thead>
<tr>
<th>Product</th>
<th>Active drug</th>
<th>Type of transdermal patch</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estraderm</td>
<td>Estradiol</td>
<td>Membrane</td>
<td>Postmenstrual syndrome</td>
</tr>
<tr>
<td>Duragesic</td>
<td>Fentanyl</td>
<td>Reservoir</td>
<td>Pain relief patch</td>
</tr>
<tr>
<td>Transderm-Scop</td>
<td>(Scopolamine)</td>
<td></td>
<td>Motion sickness</td>
</tr>
<tr>
<td>Alora</td>
<td>Estradiol</td>
<td>Matrix</td>
<td>Postmenstrual syndrome</td>
</tr>
<tr>
<td>Climara</td>
<td>Estradiol</td>
<td>Matrix</td>
<td>Postmenstrual syndrome</td>
</tr>
<tr>
<td>Androderm</td>
<td>Testosterone</td>
<td>Membrane</td>
<td>Hypogonadism in males</td>
</tr>
<tr>
<td>Captopress</td>
<td>TTS Clonidine</td>
<td>Membrane</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Combidpatch</td>
<td>Estradiol</td>
<td>Matrix syndrome</td>
<td>Postmenstrual</td>
</tr>
<tr>
<td>Esclim</td>
<td>Estradiol</td>
<td>Matrix</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>Deponit</td>
<td>Nitroglycerine</td>
<td>Drug in adhesive</td>
<td>Angina Pectoris</td>
</tr>
<tr>
<td>FemPatch</td>
<td>Estradio</td>
<td>Matrix</td>
<td>Postmenstrual syndrome</td>
</tr>
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<td>Lidocaine</td>
<td>Drug in adhesive</td>
<td>Anesthetic</td>
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<td>Drug in adhesive</td>
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<td>Testoderm</td>
<td>TTS Testosterone</td>
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<tr>
<td>Product</td>
<td>Drug</td>
<td>Formulation</td>
<td>Use</td>
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<tr>
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</tr>
<tr>
<td>Habitrol</td>
<td>Nicotine</td>
<td>Drug in adhesive</td>
<td>Smoking Cessation</td>
</tr>
<tr>
<td>Prostep</td>
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<td>Smoking Cessation</td>
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<td>Diclofenac diethylamine</td>
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<td>Anti-inflammatory</td>
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<td>Nitroglycerine</td>
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<td>Matrix</td>
<td>Angina Pectoris</td>
</tr>
<tr>
<td>Transderm Nitro</td>
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<td>Reservoir</td>
<td>Angina Pectoris</td>
</tr>
<tr>
<td>OxytrolR</td>
<td>Oxybutynin</td>
<td>Matrix</td>
<td>Overactive bladder</td>
</tr>
<tr>
<td>Nuvelle TS</td>
<td>Estradiol</td>
<td>Drug in adhesive</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>Fematrix</td>
<td>Estrogen</td>
<td>Matrix</td>
<td>Postmenstrual syndrome</td>
</tr>
<tr>
<td>Climaderm</td>
<td>Estradiol</td>
<td>Matrix</td>
<td>Postmenstrual syndrome</td>
</tr>
</tbody>
</table>

**2.5.13 Basic Components Required For Transdermal Film**

**2.5.13a Polymers:**

It is one of the basic components of transdermal system. Selection of polymers and designing are vital. There are two categories of polymers natural polymer, for e.g., zein, gelatin, cellulose,
chitosan, etc., and synthetic polymers, for e.g., hydrid rubber, polyisobutylene, silicon rubber, nitrile, neoprene, etc. [175,177,180].

**Characteristics of polymers selection**

* Should be stable and non-responsive with the drug moiety.
* Easily accessible, created and fabricated into desired formulation.
* The properties of polymer ought to be such way the drug should be easily penetrated through skin.
* Mechanical properties should be stable, when the high concentration drug is substituted.

**2.5.13b Release liners:**

The film is covered by a protective liner until the storage. Outer covering (release liner) is removed and discarded before the application of films. It is composed of non-occlusive (paper fabric) or occlusive (polyethylene, polyvinylchloride) and release coating layer made up of silicon or teflon, other materials used as release liner are polyester foil and metalized laminate [175,177,180,182,186,197–201].

**2.5.13c Backing Membrane:**

Backing membrane was designed considering various parameters, such as [175,177,180,182]

* It should be flexible.
* Low water vapour transmission so as to promote skin hydration and thus greater skin permeability.
* Should be chemical resistance and good tensile strength.
* Non-irritant.
2.5.13d Drugs:

Drugs selected should be considered with various physiochemical, pharmacokinetic, and pharmacological properties for transdermal system development [175,177,180,182,186,197–201].

Characteristics of drugs

* Low molecular weight ≤1000 Da
* Solubility nature
* Range of melting point (200 °C)
* Lipophilicity, undergo extensive presystemic metabolism, non-ionic and non-irritant are considered to suitable for delivery system

2.5.13e Penetration enhancers:

Incorporation of protein enhancers results in the formulation and to improve the diffusivity and solubility of drugs through the skin [175,177,180,182,186,197–201].

Properties need to be considered

* Should be non-irritant, non-sensitizing, non-phototoxic, and non-comedogenic.
* Should not have pharmacological activity, as it should not bind to receptors
* Should be readily formulated into dermatological preparations
* Should be adheres and equally spread to the skin

2.5.13f Plasticizers:

Plasticizers used can be synthetic and natural. Along with brittleness, ductility, adhesiveness, and strength of the film also improved. Plasticizers have been used in many formulations ranging from 5
to 20% (w/w, dry basis). For example, glycerol or sorbitol, phosphate, phthalate esters, fatty acid esters and glycol derivatives such as PEG 200, and PEG 400 [175,177,180,182,186,197–201].

2.5.13g Solvents:

Various solvents used to prepare drug reservoir are water, methanol, chloroform, acetone, isopropanol, dichloromethane, etc. [197–201].

2.5.14 Components used in current preparation of transdermal film

2.5.14a Chitosan

Chitosan is substance derived from chitin, a polysaccharide which exists in cell wall of fungi and also in the exoskeletons of crustaceans. They are being processed with the removal of the shells from shellfish such as shrimp, lobster, crabs, etc. [202]. It is obtained by the partial deacetylation of chitin; natural polymer composed of β-(1-4)-linked D-Glucosamine, randomly distributed. It consists of two types of monomers; chitin-monomers and chitosan-monomers [203]. Commercially, chitosan is produced between 3800 and 20,000 Daltons, which are the structural element in the exoskeleton of crustaceans and cell wall of fungi [204]. The degree of deacetylation (%DD) can be determined by ultra violet-visible spectrophotometer, Fourier transform infrared (FTIR) and nuclear magnetic resonance spectroscopy. A common method for the synthesis of chitosan is from the N-deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent, this yield up to 98% product.

Chitosan is biocompatible, non-antigenic, non-toxic, bio-functional, bio-degradable and enhances the transport of polar drugs across epithelial surfaces [205]. Chitosan has been used over a wide range of applications, such as wound healing agent, drug carrier, chelating agent, membrane filter for water treatment and bio-degradable coating or film for food packaging. It is also used as a latent biomaterial that can be used for nerve repair. Chitosan is used in water purification by spreading the
powder over the surface where any toxic substances such as grease, oils, or dangerous heavy metals are immediately absorbed, and the scum is then easily removed [206]. Chitosan is an antibacterial agent and also used in food preservative agent to prolong the shelf life. Purified chitosan are used for medicinal purposes where they have contact with skin to undergo a strict inspection process, this promotes in healing wounds. Chitosan and its derivatives, such as trimethylchitosan (where the amino group has been trimethylated), have been used in non-viral gene delivery [206].

The novel characteristic features of chitosan not only relive its property in the pharmaceutical industry but also in drug targeting and delivery. In our study chitosan was extracted from chitin taken from Indian prawn (Fenneropenaeus indicus), marine decapod with estuarine juvenile is one of commercialized prawn species in the world and has been known in different names Indian white prawn, Tugela prawn, white prawn, banana prawn, Indian banana prawn and red leg banana prawn. It is found in the Indo-west Pacific from eastern, India, Malaysia and Indonesia [205,206].

**2.5.14b Sacchachitosan**

In order to provide an alternative source of chitin or chitosan from crustacean shells, fungi was used. It follows the same procedure with slight modification such as deacetylation, alkali treatment; production cost should be estimated due to reduction of sodium hydroxide and process time.

The term “sacchachitin” was initially utilized by [207] in 1997 for portrayal of uncommon glucosamine subsidiary isolated from G. tsugae. This current Su’s sacchachitin is a copolymer using β-1, 3-glucan (around 60%) and N-acetyl glucosamine (roughly 40%), having a filament structure. This pulp was applied as a skin substitute. This showed quicker injury mediating in contrast with the popularized skin substitute, i.e.; the chitin sheet made of crab shell (Beschitin). Moreover, numerous specialists showed that sacchachitin displays intense antibacterial [208,209] and antiviral exercises [210,211]. It is weavable skin substitute made from residual fruiting body of G. tsugae and has demonstrated to
promote wound healing by inducing cell proliferation, increase the secretion of cytokines and growth factor and decrease the matrix proteinase during the healing of wound.

Sacchachitosan is a deacetylated form of sacchachitin. It is also showed as a good promoter of cell proliferation and may be developed as an ocular drug [212]. There are only handpicked papers portraying about sacchachitin and synthesized from G. tsugae. In our study, extraction and isolation of sacchachitosan was carried out from G. lucidum which is reported for the first time.

2.5.14c Gelatin

Gelatin is Latin word known as “gelatus” which means stiff and frozen. It is translucent, brittle on drying, and possesses many applications. It is derived from collagen obtained by animal by-products and it’s a mixture of proteins and peptides, soluble in most polar solvents and it extracted from beef bones [214]. Porcine and bovine wastes are the good quality of gelatin obtained. The other sources are fish bones and connective tissues. The mechanical property of gelatin affects denaturation, acid used to adjust pH, hydrophilic nature of films. Thermal properties enhances hydrophilic of the films were examined by differential scanning spectroscopy and thermo gravimetric analysis [214].

In food technology, commonly used as gelling agent in granny candies, gelatin desserts, candy corn, ice cream, etc., and used as stabilizer, thickener and texturizer in yoghurt and cream cheese. In pharmacy and biomedical applications, gelatin-biopolymer in tissue engineering such as wound dressing and bone scaffolding [214]. Casting method was carried out in the formation of film [213].

2.5.14d Glycerol

Glycerol is naturally derived compound from plants or animals. It is widely used in pharmaceutical field due to its vibrant properties. It is colourless, odourless, and viscous in nature. Some
of them used for substitute for weight loss, helps body maintain water during dehydration and reduce the pressure in the eye during glaucoma [215].

Glycerol is used in biopolymer field and incorporated into hydrocolloid films, as indeed hygroscopic molecules were added in the formation of film to avoid the brittleness. It is one of the eco-friendly and biodegradable plasticizer used [216, 217].

2.5.14e Silver nanoparticles

Silver nanoparticles are used as a drug in the transdermal film matrix. It also acts as a binding agent between chitosan / sacchachitosan and gelatin.

2.5.14f Water

Usually, biopolymers and plasticizer are hygroscopic. This property of biopolymers and plasticizer may affect the moisture content of the film. Water is a natural solvent having use in biopolymer technology and also acts as plasticizer in hydrocolloid films. Other examples of plasticizer are polypols, mono-, di- and oligosaccharides. [218-220].