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A Review of Diabetic Foot Ulcer Infections and Lyophilized Wafer Formulation

Rupalben Kaushalkumar Jani[1*], Goswami Kaushal Puri[1] and Shrestha Ubana[1]

[1]Department of Pharmaceutics, Parul Institute of Pharmacy and Research, Parul University, Waghodia Road, Vadodara-391760, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author RKJ designed the study and wrote the protocol. Author GKP wrote the first draft of the manuscript. Author SU managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Diabetic foot difficulties are the most usually occurring problems globally, resulting in economic disasters for the patients, families, and society. In patients with diabetes, the risk of emerging foot ulcers is 25% high. It has also been in the record that one lower limb amputation occurs every 30 seconds in patients with diabetes worldwide. Novel methods of drug delivery and wound dressing have to develop to solve the lower limb amputation crisis. One such novel method is "Lyophilized wafer formulation." It is an upcoming medicated dressing material that can enhance wound healing and the potential to ingest vast quantities of exudates from Chronic wounds. That can have been formulating by lyophilizing hydrogel of absorbent polymers such as Calcium Alginate, Carrageenan, Thiolated Chitosan, and plasticizer to enhance flexibility withstand the day to day mechanical stress, covered with some adhesive and protective backing layer. Unless it passes evaluation tests such as Fourier-transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM), Exudate handling property, Folding endurance, *In-vitro*, *In-vivo* drug release profile, and Gamma-irradiation sterilizes wafer formulation, and it should not administrate directly. Lyophilized wafer formulation will be the most acceptable medicated dressing material in the future that will be useful to treat the normal wound. The wound formation because of

**Corresponding author: E-mail: rupal.jani@paruluniversity.ac.in;*

diabetic foot ulcer (DFU) infections as there will be site-specific delivery of the drug, packed with an advantage to self administer and easy termination of the drug that can achieve just by removing the wafer case of drug toxicity.

Keywords: Calcium alginate; wafer formulation; lyophilization method; exudate handling property; In-vitro; In-vivo drug release studies; diabetic foot ulcer; lyophilizer.

1. INTRODUCTION

1.1 Diabetic Foot Ulcers

Diabetic foot ulcers describe foot abrasions as (ulcers). It may affect the skin, soft tissue, and bone in the lower limbs, contributing to more contagious diabetic patients, leading to outcomes such as lower-limb amputations [1]. A foot ulcer can occur in anyone, and it looks like a cover of broken-down skin, usually on the lower leg or feet. A foot ulcer can affect both Type one(1) and Type two(2) diabetes [2]. Colonization by *Staphylococcus aureus*, *Streptococcu*s group B, *Enterococcus, Clostridium perfringens*, *Enterobacteriaceae* and other pathogenic species in DFUs*, S. aureus,* including methicillin-resistant *S. aureus* (MRSA) is the most common bacteria found in DFU infections [3]. Another significant complication of DFUs is osteomyelitis, and each person with infected DFUs needs to ovoid [1]. Typically, when blood glucose levels are elevated or fluctuated, the skin wounds may not heal or repair themselves properly due to insufficient oxygen supply and clotting factors towards the wound site [4]. The foot ulcer can start even in a mild injury, leading to a change in the wound's pH, ranging from 6.0 to 8.0 [5]. Persons suffering from chronic leg ulcers release 5 grams of exudate per 10 cm^2/24h as per records [3].

It takes more than 12-14 weeks for a wound to heal in a Diabetic Foot ulcer/chronic wound as healing is slow due to inadequate delivery of Nutrients, blood cells and, oxygen in the blood at the wound site. Some of the common sites are shown in Fig. 1 [4].

Acute and Chronic are two types of wounds. Healing of both wounds consists of four cycles, but healing time may differ according to wound type. Below, the healing period mention for the chronic injury [4,6].

i. Hemostasis: In this cycle, coagulation factors occur when fibrinogen forms a fibrin network to stop bleeding. This activity may take quite a few minutes to 3 days [6,7].
ii. Inflammation: This cycle occurs continuously along with Hemostasis, and it can take 24 hrs to 20 days for the process [7,8].
iii. Cell proliferation/granulation: This cycle also occurs always along with Hemostasis, in which the formation of granules occurs by ingrowth of capillaries and lymphatic vessels. This process can take nearly 7-40 days [7,9].
iv. Remodeling or Maturation: In this phase, complete regeneration of skin takes place at the site of the wound just left with a scar and this process takes about 40 days to 2 years [6,7].

1.2 Pathophysiology

DFUs have complex pathogenesis, such as Diabetic Neuropathy and Peripheral Arterial Disease (PAD), which are the key variables affecting their development, with trauma being a triggering factor. Above all, these variables together take part in various stages of ulcer growth, as before and after a pause in wound healing, its occurrence [1,10].

1.2.1 Diabetic neuropathy

- Hyperglycemic-induced oxidative stress (OS) on nerve cells contributes to neuropathy, affecting sensitive, motor and autonomous nerves.
- Confines the alteration to NADPH of Nicotinamide Adenine Dinucleotide (NAD) by obstructing glucose-6-phosphate dehydrogenase activity [2].
- Increased nicotinamide adenine dinucleotide phosphate (NADPH) from certain enzymes via the polyol metabolic pathway absorbs. Such as Aldose reductase and Sorbitol dehydrogenase. Which further decreases by pathway activation of the hexosamine.
- The sweat gland is damage by autonomic nerve damage, and the foot may progress in a state of reduced capacity to moisturize the skin, resulting in dermal cracks and skin membrane breakdown [11].

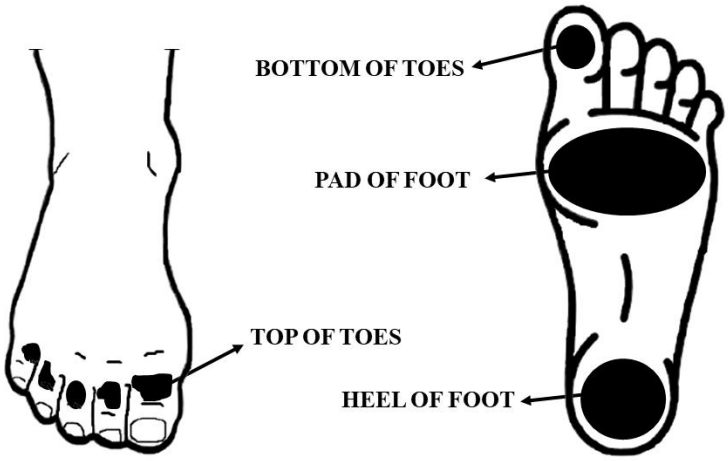

Fig. 1. Some of the most common sites for Diabetic ulcers on foot

1.2.2 Role of immunology in the pathogenesis of DFU'S

- Distinct resistant features occur in persons who have Diabetes that involve an abridged healing response in DFUs. Cellular resistant response alterations with enhanced T-lymphocyte apoptosis are some of these responses. Boost of pro-inflammatory cytokines. The deficiency of chemotaxis, adherence, phagocytosis, and intracellular killing functions of polymorphonuclear cells. The prohibition of fibroblast proliferation. Impairment of the basal keratinocyte layer with condensed epidermal cell movement prevents wound healing [10,12,13].
- High sugar levels in the blood are also an effective mediator for bacteria's progress, such as *Staphylococcus aureus (S. aureus)* and *β-hemolytic streptococci,* primarily aerobic *Gram-positive cocci* [14].

1.2.3 Peripheral Arterial Disease (Pad)

- It is in the records that 78% of DFU patients still have PAD. In the peripheral arteries of the foot, hyperglycemia induces changes and begins at the cellular level. A crucial feature of microcirculation dysfunction in endothelial cell dysfunction is a decrease in vasodilators, especially in nitric oxide synthesis.
- With consequent persistent vasoconstriction, Plasma Thromboxane A2 levels turn out to be elevated. Plasma hypercoagulation, which leads to an elevated risk of ulceration and ischemia [10,11].

1.3 Classification of Diabetic Foot Ulcer and Severity

A foot ulcer due to diabetes is classified either as Neuropathic or Ischaemic. Roughly 45% to 60% of all population are purely neuropathic diabetic ulcerations, despite the fact up to 45% are due to neuropathic and ischemic constituents [2,9,10].

1.3.1 Neuropathic

Neuropathic diabetic foot ulceration occurs due to the Motor, Sensory, and Autonomic Nervous system's failure [2,12].

i. Motor: It is a type of condition in which ankle motion decreases due to which plantar burdens on the barefoot increase. This type of disorder occurs due to the failure of motor nerves near the foot.
ii. Sensory: This condition occurs due to the loss of protective sensation in the foot area due to the sensory nerve's improper functioning.
iii. Autonomic: In this condition, the foot's skin becomes dry by forming cracks and fissures, making way to enter bacteria and fungi. These may occur due to the dysfunction of microvascular thermoregulators, arteriovenous blockage, and sympathetic failure.

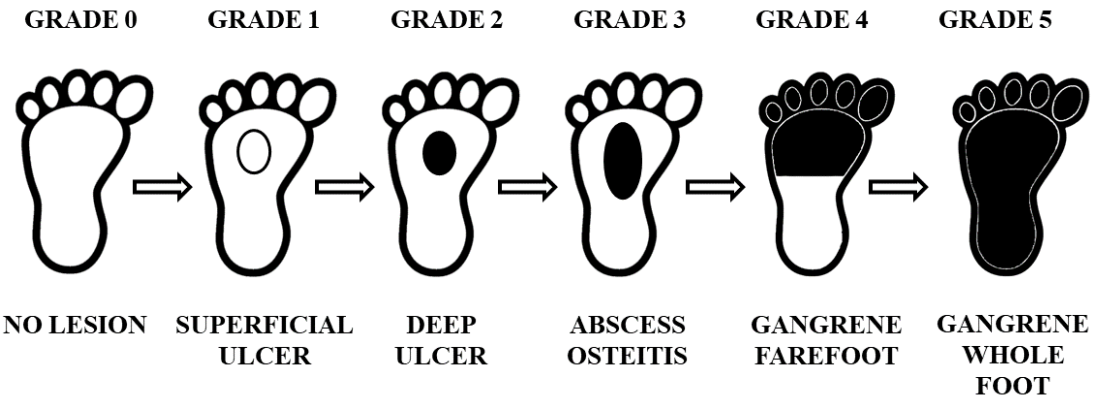

Fig. 2. Pictorial representation of Wagner's grade of foot ulcer

1.3.2 Ischaemic

Ischaemic diabetic foot ulceration occurs due to the narrowing of blood vessels. It contributes to less oxygen and nutrients provide to the tissues, and in this condition, oral or intravenous administration of antibiotics is less effective lead to the amputation of the limb.

Doctors use Wagner Grade to describe the seriousness of a foot ulcer. By using numbers from 0 to 5, six ranking systems classify diabetic foot ulcers [2,12,15,16].

Grades 0 to 5 by Wagner are as follows and presented in (Fig. 2).

0. There is no diabetic foot ulcer, but a significant risk of spreading one is present.

1. Full skin thickness includes a surface ulcer that does not affect the underlying tissues yet.

2. A deep ulcer permeates past the surface, down to the muscle and ligaments. There are also no abscesses or bones involved.

3. A profound ulcer arises with the tenderness of the subcutaneous connective tissue or a cyst. It may involve thew (muscle), ligament(tendon), near joint bones, and bone infections.

4. The tissue (limited to the foot and forefoot) around the ulcer region has started to decay. Gangrene is called this disease.

5. To become extensive, Gangrene spread from the localized region of the ulcer. The whole foot involves.

1.4 Causes for Diabetic Foot Ulcer

There are many reasons, which cause Diabetic Foot Ulcer. Some of them are as given below [1,2,10,11,17].

- Poor blood circulation
- Wearing poor fitting shoes
- Walking barefoot
- Insufficiently well-controlled diabetics
- Cigarette Smoking, become overweight, not having exercise, and improper cleaning of foot
- Uneven growth of foot nail
- History of prior ulcer/amputation

1.5 Risk Factors due to Diabetic Foot Ulcer

In Diabetic Foot Ulcer, the major risk factors in patients are as below [2,9,18,19].

Results

- ✓ Amputation of Limb
- ✓ Improper wound healing
- ✓ Longer wound healing time

Reasons

- ✓ Improper glycemic control
- ✓ Trauma

2. DIABETIC FOOT ULCER MANAGEMENT REMEDIES

2.1 Debridement

It should perform every 7-14 days. In this process, dead skin or debridement is removed by

Table 1. Available marketed products for topical therapies for wound healing and antimicrobial property

Antibiotics	Marketed product	Company
Neomycin	Ointment	Neopharm Co., Ltd.
Silver Sulfadiazine	Cream	Pfizer Laboratories.
Bacitracin	Powder	Auro Pharma Inc
Mafenide	Powder	Mylan Bertek Pharmaceuticals Inc.
Povidone-Iodine	Liquid	Baxter Laboratories
Chlorhexidine	Solution	Cardinal Health
Benzethonium Chloride	Liquid	Rite Aid Corporation

cutting it with a scalpel and tweezers or using a strong water jet. It prevents microbial growth and promotes wound healing. Cleaning of the lesion after debridement removal is carried by (0.9% NaCl) isotonic saline solutions [1].

2.2 Topical Therapies

It can also control by giving topical Antibiotics and Antiseptics such as Bacitracin, Neomycin, Mupirocin, Polymyxin B, Silver Sulfadiazine, Mafenide, Povidone-iodine, Chlorhexidine and Benzethonium Chloride which are existing in the form of Creams, Powders and Ointments [10] given in (Table 1).

2.3 Dressings

It could also control by different dressing materials such as Hydrocolloids, Hydrogels, Foam, Calcium Alginate Dressing, and Wafers [1,10]. Among the above wound dressings, wafer formulation is the furthermost effective dressing in the sense of moisture handling capacity, Exudate absorbing capacity, wound healing, and antibiotic delivery in a single sauce in 24 hrs [3,20].

3. WAFER FORMULATION

As per et al. Anthony David Auffret, the lyophilized composite of the polymer substrate and plasticizer is known as a wafer. Along with stable crystalline particles of a pharmaceutically active compound, mainly used as a wound-healing agent. Which can be placed on an open wound, whether it is an Acute or Chronic wound [21].

3.1 Lyophilizer

- Freeze-drying or Lyophilization, also known as Cryodesiccation, is a very widely used method to formulate pharmacological and biological products to prolong shelf life. For example, vaccines and injectables are freeze-dried for ease of storage and shipping and later reformed to their original liquid form before use.
- Here, the hydrogel is freeze-dried to produce a crystalline, porous material known as 'Sponges' or 'Wafers.' The method of extracting water from the substance by first freezing it into ice and then sublimating it in the water vapor beneath reduced pressure is Freeze-drying or Lyophilization [22].
- The principle of freeze-drying can describe using a water phase diagram in (Fig. 3). The Freeze-drying process does not damage the integrity of the material's crystalline structure because the water gets removes in the form of vapor. The freeze-drying process involves 3 (three) phases Freezing, Primary drying, and Secondary drying.
- The solution is freeze to well below the triple-point temperature for pure water so that the evaporation can take place via sublimation, thus avoiding the liquid phase. By using a vacuum pump, the pressure is managed effectively underneath the triple point. The vapor formed is drawn out frequently to prevent a pressure upsurge that would stop sublimation, which accomplishes by chilling the condenser below the model temperature (making a temperature slope).
- The vapor is dragged to the chiller surface and imprisoned in the form of ice. Primary drying (sublimation) eradicates the bulk of the icy free water by heating in a series of thermal ramps to room temperature beneath reduced pressure.
- Additionally, elevating the temperature at the termination of the primary drying process to above room temperature eradicates the residual humidity, which adsorbs onto the model (secondary drying) [23]— a representation of a distinctive freeze-dryer as given away in (Fig. 4).

- A distinctive freeze-dryer comprises temperature-controlled ramps, a condenser to trap water detached from the substantial, a freezing system to supply the shelves and condenser with coolant, and a lower vacuum device for the chamber pressure. The freeze-drying stages are generally programmed via computer through suitable freeze-dryer software.
- Freeze-drying of polymer gels to acquire wafers can generally undertake with the help of a laboratory-scale freeze-dryer [23,24].

3.2 Lyophilization (Freeze Drying)

The primary Lyophilization method consists of the following steps: Graphically explained in (Fig. 5).

- It is a Freeze drying process in which polymer substrate and a plasticizer, preservative and antibacterial or antifungal medicament homogenously mixed with the high-speed stirrer such as a Mechanical stirrer or Magnetic stirrer [24].
- Later this homogenous mixture or gel is poured into a mold or petri-dish previously greased with the greasing agents. Then it is frozen at approximately -0°C to -80°C (Primary drying) for overnight or a few hours as per formulation requirement [23,25].
- Then, the frozen formulation dried in a series of thermal ramps (secondary drying) in the presence of Vaccum to remove ice formed between the matrix of polymer and drug. This cycle is carried out even for few hours to days [23].

3.2.1 Advantages of wafer formulation

Wafer formulation has the same advantages as Transdermal Patches. It consists of the following benefit [26].

- Avoidance of first-pass drug metabolism.
- Decreased plasma concentration levels of drugs, with reduced side effects.
- Reduction of variations in drug plasma levels, Utilizing drug candidates with a reduced half-life and short therapeutic index.
- Simple termination in the presence of toxicity during the delivery of drugs can achieve by removing the patch.
- Dosage frequency reduction achieves, and an improvement in patient compliance may be observed.
- Transdermal wafer formulation can deliver a steady infusion of a drug over a prolonged period.
- It is also possible to prevent side effects or clinical deficiencies often associated with alternating dosing
- By preventing particular issues associated with the medication, transdermal delivery will improve many drugs' healing value. E.g., GI irritation, lesser absorption, disintegration due to the hepatic first-pass effect.
- The basic medicine regimen can be improved by patient compliance and reduced inter and intra-patient fat variability.

3.3 Role of Polymers in Wafer Formulation

3.3.1 Calcium alginate

When calcium alginate (CA) consumed by living tissue, these compounds' evident lack of toxicity significantly attracted alginates. Calcium alginate (CA), and alginic acid calcium salt, are derived from brown seaweed and consist of alternating β-(1–4) D-mannuronic acid (M-blocks) and alpha-(1–4) L-guluronic acid sequences (G-blocks). The proportionate dispersal of the blocks is contingent upon the source. The seaweed uses different conformational structures with mannuronic and guluronic acid residues [27]. The conformational study showed that the M-block residues of the di-equatorial mannuronic acid. Flexible, flat ribbon-like chain conformation show. On the other hand, in G-block, the diaxially related guluronic acid residues show rigid structures [28]. Due to its hemostatic properties, calcium alginate has applications in heavily exuding lesions like DFUs and pressure-induced leg ulcers. It interchanges calcium ions with blood-present sodium particles (wound exudate) that activate growth factors, including growth factors derived from platelets and cytokines, which play a crucial part in cell enrollment and extracellular deposition matrix [29].

3.3.2 Carrageenan (CAR)

Carrageenan is a sulfated natural polymer formed from red seaweed generally used in food

manufacturing as a thickening medium [30]. Various grades are assorted according to the number of sulfate groups present Kappa (k) CAR (one sulfate group) gives rise to a thermoreversible sol-gel in an aqueous medium, which undertakes dispersal subsequent random-coil development in the early phase. At low temperatures, galactose sequences within the CAR chains turn in a double-helix manner. The sugary taste of galactose might help cover some drug's unpleasant taste, thus evading flavoring and sweetener agents [31]. Numerous spots for hydrogen bonding impart bioadhesive features. However, this could improve by ionic bond development between the negatively charged sulfate group, and the mucin existing in the oral mucosa is a positive charge [32]. Poloxamer 407 is a block copolymer encompassing Polyethylene glycol (PEG) and propylene oxide [33]. It is a non-ionic surfactant with the capability to escalate drugs' solubility (e.g.ibuprofen) with high log P [34].

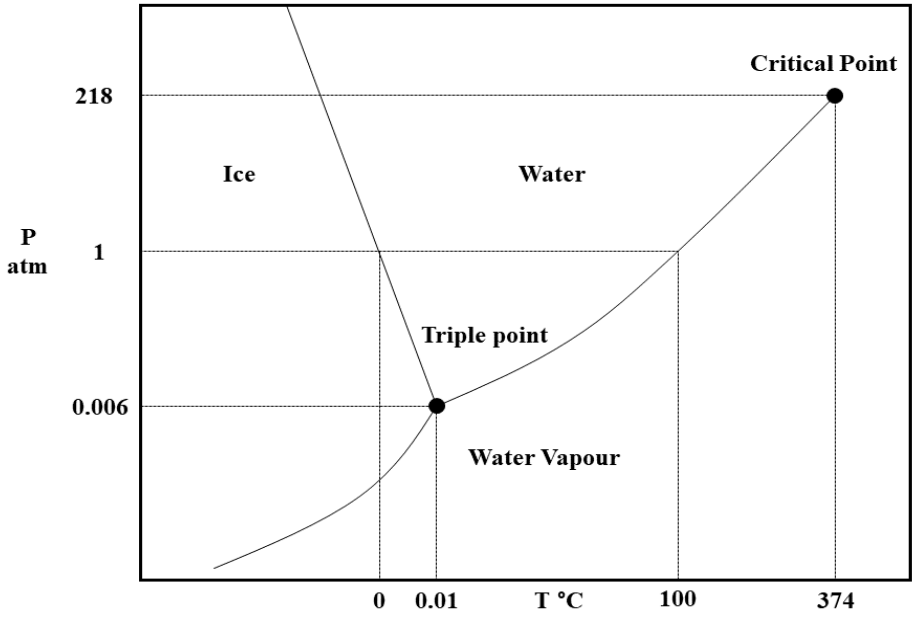

Fig. 3. Represents phase diagram of water

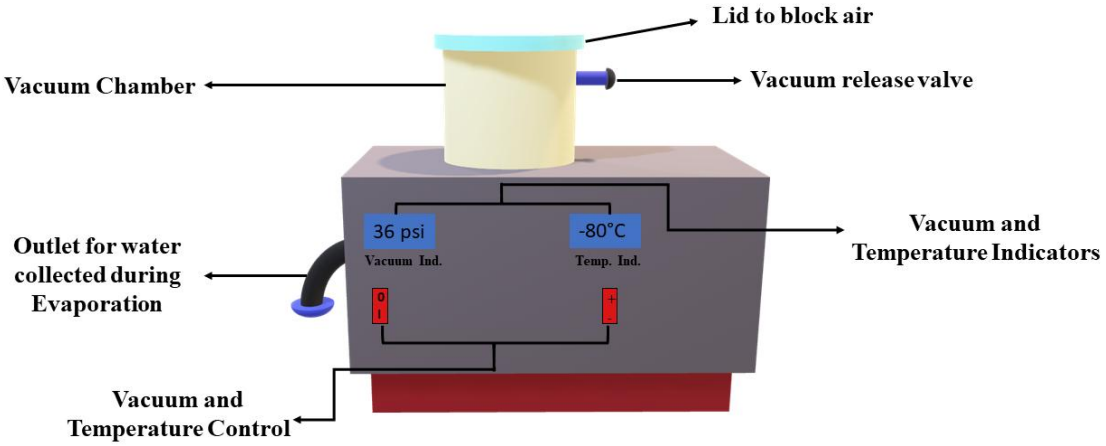

Fig. 4. Graphical representation of freeze drier components

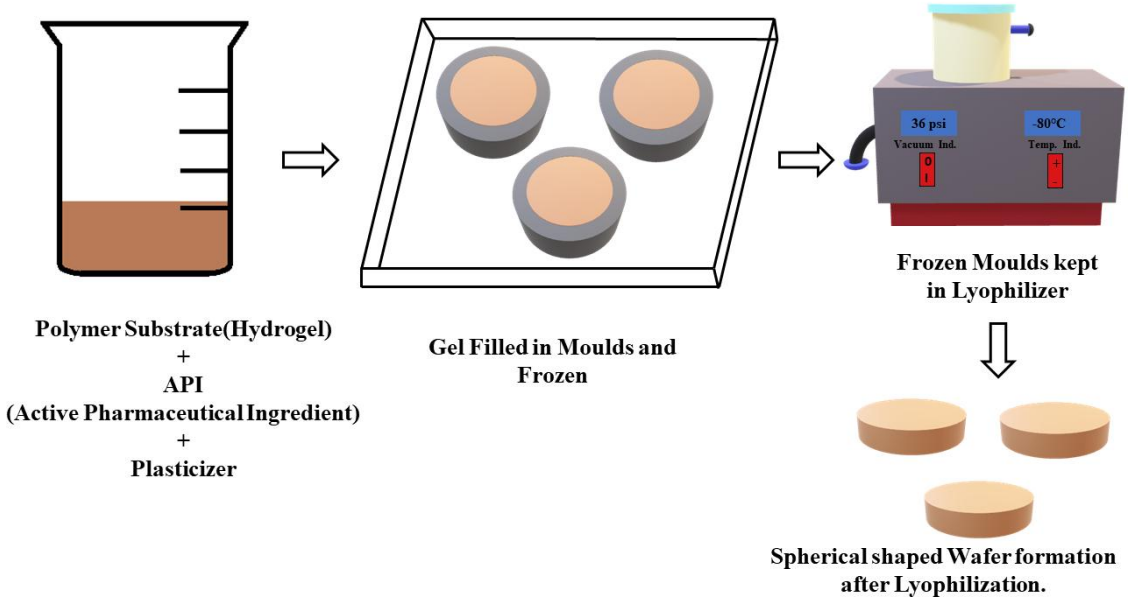

Fig. 5. Graphical presentation of preparation of wafer formulation

3.3.3 Thiolated chitosan

Synthetic polymers, such as thiolated Chitosan that is stated to be up to 10 times additionally bioadhesive than Chitosan but still biodegradable, can be formulated with wafers [35].

Among the numerous bioadhesive polymers employed, Chitosan (CS) is one of the utmost generally reported. Because of its well-established bioadhesive properties [36]. Due to its biocompatibility and biodegradation properties. CS has been used principally to enhanced mucoadhesion, infiltration enhancement, and drug delivery via mucosal routes such as GI, buccal, and ocular mucosa [37]. The bioadhesive property of CS is reporting to be enhanced by the thiolation of the parental CS mediety ensuing in the formation of numerous thiolated CS derivatives [38]. The thioalkylamidine substitute incorporates without the establishment of N-substituted non-thiol substrates to obtain thiolated Chitosan. By means of the immobilization of thiol groups, mucoadhesion was enormously improving due to disulfide bonds with mucus glycoproteins [39].

3.4 Role of Backing Layer and Plasticizer in Wafer Formulation

The key role of a backing layer in Topical Drug delivery is to protect the patch from environmental stress, keep the patch adhere to the required site, and protect the photosensitive drugs by reflecting the light. It also needs to be flexible, comfortable, and empathize with the adhesive and excellent printability. Some of the backing ingredients are Vinyl, Polyethylene, Polyester films, Aluminum and Polyolefin films. The most common release liners are Polyacrylate, Polyethylene, Polyvinylchloride, Silicon, Teflon, and Paper fabric will be using as a backing layer [40,41].

Besides, plasticizers such as Dibutyl phthalate, triethyl citrate, Polyethylene glycol, propylene glycol, and glycerin add plasticity to the wafer formulation [40].

4. WAFER EVALUATION PARAMETERS

4.1 Characterization Studies

a. **X-Ray Diffraction (XRD):** The need for this study is to evaluate the physical nature (Crystalline or Amorphous) of pure drug, polymer, blank wafer formulation, and drug-loaded wafer formulation [3].
b. **HPLC analysis:** This study analyzes the data and separates the analyte to know the components' purity and mobile phase selected based on the drug's solubility [42].
c. **FTIR studies:** In this study, the compatibility, i.e., any chemical interaction between the polymer and drug, can be

detected by Fourier Transform Infrared Spectroscopy (FTIR) instrument [3,43,44].

4.2 Post Formulation Studies

a. **SEM (Scanning Electron Microscopy):** In this evaluation parameter, the Surface morphology of wafer formulation will study by having a magnification look at 200x at a low fast-tracking electrical energy of 1.0kV [3,45].
b. **Texture analysis:** In this study, the mechanical hardness and in-vitro adhesion of wafer formulation will determine using a texture analyzer operated by Texture Exponent 32 software [5,46].
c. **Fluid(exudate) handling properties:** The Fluid exudate handling properties of the wafer formulation can be determined by pouring 5 g of SWF in a petri dish placed on the weighing machine and then immersing the wafer formulation in 5 g SWF for 3 hours to know the exudate handling property. At the same time, it will also be used to measured to know the water-absorbing capacity, water holding capacity, and rate of water uptake by the wafer formulation just by pouring a more weighed quantity of SWF until it forms into liquid [3,47].
d. **Folding Endurance:** The wafer formulation should be folded repeatedly in one place until breaks show the elasticity of the wafer formulation. It should withstand a minimum of 300 folds manually [48].
e. **DSC studies:** Differential Scanning Calorimetry (DSC) is an instrument used to measure enthalpy changes due to change in the material's physical and chemical nature due to exposure to a different temperature [3,4,48].
f. ***In-vitro* studies:** In this study, the drug release profile of drug-loaded wafer formulation will be studying using culture plates containing human primary epidermal keratinocytes, Franz diffusion cell, and culture plates containing Gram-Positive and Gram-Negative bacterial growth [46,47,49].
g. ***In-vivo* studies:** This study will be performing on the animal by following the guidelines as per the Ethics committee with the permission of IAEC (Institutional Animal Ethical Committee). In a study conducted by et al. Mahalaxami Rathnanand, Diabetes should be induced in the rats of either sex with Streptozotocin, and excision of 1×1 cm^2 should be made on the rats after anesthetized with the help of Ketamine HCL + Xylazine. Afterward, wafer formulation is placed with adhesive paper tape, and shrinking of the wound to be measured with a scale and observed for 14 days [29,46,47].
h. **Gamma-irradiation:** It is a Gamma radiation emitting device that will help sterilize the wafer formulation and know any rheological changes in wafer formulation due to gamma rays [50,51].
i. **Stability studies:** As per ICH guidelines, a short-term stability study will be carried at 40°C ± 2°C at 75±5 RH for six months [52–54].

5. CONCLUSION

This review explains the development and stages of diabetic foot ulcer infections, some major bacterial species involved in them, and risks of diabetic foot ulcer infections, along with an example of some marketed medications used to control such conditions. Along with diabetic foot ulcer infections, this review hypothesizes the wafer formulation's safe outcomes, suggests a common method to prepare it and describes the different stages of the process carried in a lyophilizer. Furthermore, this review suggests the importance of wafer formulation and selection of polymers, plasticizers, backing layers. It also throws some lights on various evaluation parameters before developing the wafer formulation and tests after preparing the formulation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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