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CUBOSOMAL HYDROGEL-BASED DRUG DELIVERY SYSTEMS FOR DIABETIC WOUND HEALING: A COMPREHENSIVE REVIEW

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Abstract:
Wound healing in diabetes is a serious problem due to slow wound closure, inflammation, and vulnerability to infections. Standard methods of topical treatment suffer from inadequate penetration and inability to provide continuous release of drugs. Mupirocin is a commonly used topical antibiotic that shows efficacy against Gram-positive bacteria but has limited performance due to low adherence and brief time of stay on the wound. The recent advancements in the field of nanotechnology have provided Cubosomes as novel carriers of drugs because of the unique characteristics such as bicontinuous cubic structure, high load, and controlled release.
This paper provides information about the benefits of using mupirocin-loaded cubosomal hydrogels (cubogels) for diabetic wound healing. Information concerning the mechanism of diabetic wounds and limitations of standard therapies is provided. Also, the advantages of cubosomes and hydrogel carriers are presented. Furthermore, recent developments in the field of nanocarrier-based treatments of wounds are discussed in order to identify the gaps in the current state of knowledge. Despite the fact that many efforts were put into creating innovative formulations, there are not enough studies devoted to the use of cubosomal hydrogels for mupirocin treatment of diabetic wounds.
Keywords: *Cubosomes, Cubosomal Hydrogel, Diabetic Wound Healing, Topical Drug Delivery, Advanced Drug Delivery Systems*

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I. INTRODUCTION:

1.1 Skin Wound Healing

The skin is the largest organ of the human body, which works as a protecting shield against external physical, chemical, and microbial insults. Injury to the skin causes wound healing, which involves different cell-based and molecular processes to repair the damaged tissues. The wound healing process is regulated by keratinocytes, fibroblasts, endothelial cells, immune cells, cytokines, growth factors, and extracellular matrix (ECM). Under physiological conditions, wound healing takes place in an organized manner, but some factors such as infection, diabetes, and insufficient vascularization can affect the wound healing process leading to chronic wounds [1].

1.2 Physiological Skin Wound Healing

Skin wound healing is a natural process, which consists of three overlapping stages: inflammation, proliferation, and remodeling. Inflammation stage clears out the debris and activates the tissue repair process using the inflammatory cells and growth factors. Proliferation stage includes the proliferation of fibroblasts, collagen deposition, angiogenesis, and formation of granulation tissues. In the remodeling stage, collagen becomes mature and strengthens the healed tissue; however, the skin regains just 70% of its original strength even after healing. For that promote healing include oxygen, nutrition, and cell coordination while absence of these factors hinders the healing process thus making the wound more vulnerable to infections particularly when dealing with patients who have diabetes [2]

1.3 Chronic Wounds and their Connection with Diabetes

The process of wound healing is complex involving several biological interactions. If the process of wound healing is impeded by diseases, there results in delayed healing which ends up resulting in chronic wounds. Such wounds fail to progress through the healing stages and tend to be stuck in the inflammatory stage[3].The common causes of chronic wounds include inadequate blood flow, immune system issues, diabetes, medications, previous damage to tissues, and external factors like pressure and excessive moisture. Treatment of such wounds requires adequate wound care among other treatments including maintaining good glucose level[4].

1.4 Wound Infection

Infection is one of the main reasons for impaired wound healing. While microorganisms usually inhabit wounds, infection sets in when bacteria proliferate beyond the capabilities of the host to protect itself.[5]. Redness, swelling, warmth, pain, and purulent drainage are the most common clinical signs of wound infections [6]

1.5 Wound Management and Dressings

Wound management involves wound cleansing, debridement, prevention of infections, and appropriate dressing of wounds. Debridement ensures removal of necrotic tissues, reduction of bacteria, and provision of favorable conditions for formation of healthy granulation tissue[7] The present-day wound dressings ensure creation of conditions that promote epithelialization, debridement, and wound healing. Dressings are selected depending on the nature of the wound, presence of exudates, and presence of infections while moist dressings are more common than traditional gauze dressings [8]

Table1: Types of wound dressings and commercially available products

Type of dressings	Commercially available products
Gauze	Curity, Vaseline Gauze, Xeroform
Films	Bioclusive, Blisterfilm, Cutifilm, Flexigrid, OpSite, Tegaderm
Hydrocolloids	Aquacel, Comfeel, DuoDERM, Granuflex, Tegasorb
Hydrocolloid	Carrasyn, Curagel, Nu-Gel, Purilon, Restore, SAF-gel, XCell
Foams	3M Adhesive Foam, Allevyn, Lyofoam

2. Diabetes and Wound Healing

2.1 Impact of Diabetes on Wound Healing

The process of normal wound healing involves the process made up of different processes, which intersect each other, and they are divided into three phases: inflammation, proliferation, and remodeling. Inflammation is subdivided into hemostasis and leukocyte infiltration. Proliferation and remodeling phases lead to the development of angiogenesis, re-epithelialization, and scarring. They all are affected by diabetes. [9]

2.2 Inflammation in Diabetic Wounds

In case of wounds occurring in people suffering from diabetes, there is an extended inflammatory phase causing delays in wound healing and formation of chronic wounds. The normal process of changing pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages in order to induce tissue healing is not possible in diabetic wounds, thereby causing persistent inflammation and increasing the production of pro-inflammatory cytokines. There is also excessive activity of neutrophils, generation of ROS, and NETs that worsen the problem of inflammation. [10]

2.3 Angiogenesis in Diabetic Wounds

Angiogenesis is of paramount importance in the proliferative phase in supplying blood. The process of angiogenesis is controlled by proangiogenic and antiangiogenic factors. Diabetic wounds fail to go through the stage of angiogenesis due to low secretion of proangiogenic factors, high amount of antiangiogenic factors, imbalance in miRNAs, excessive secretion of matrix metalloproteinases, and lack of factors which regulate vascular maturation. Lack of blood vessels results in deficient supply of oxygen and migration of leukocytes.[11]

2.4 Scar Formation in Diabetic Wounds

The process of remodeling involves collagen remodeling and scar formation. [12] Differences between scar tissue and normal skin are associated with the lack of appendages and collagen fibers which are very dense. Scar formation in diabetic people differs from scar formation in healthy people owing to low production of collagen, wrong collagen fiber construction, and tensile strength of collagen fibers. Senescence of fibroblasts leads to inability of wound contraction and therefore the process of healing does not take place via wound contraction but rather via granulation and re-epithelization, thus scar formation occurs[13]

2.5 Post-Surgical Problems in Wound Healing

There is a greater possibility of having surgical infection, wound dehiscence, wound healing issues and scar formation in surgical procedures among patients with diabetes. Besides hyperglycemia, there are several factors that can be attributed to such complications. The inflammation caused by obesity can lead to increased infection during superficial and deep surgeries.The results of surgeries done on diabetic patients are worse than non-diabetic patients during surgeries like abdominal panniculectomy, abdominal wall reconstruction, breast reconstruction, cosmetic surgery, hand surgery, and facial fracture fixation due to infection, flap necrosis, re-surgeries, and prolonged hospital stay. [14]

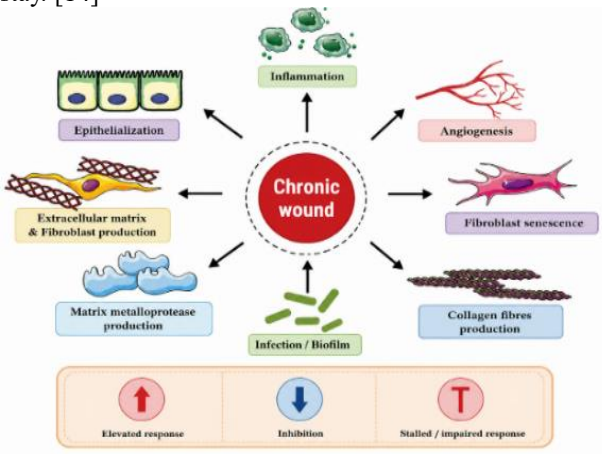

Figure1: Pathophysiology of Chronic Wound Healing

3. Current Therapies for Wound Healing

Wound management involves the prevention of infections, promotion of tissue regeneration, and the maintenance of good health of tissues. Conventional methods of wound treatment consist of wound cleansing, debridement, infection prevention, and using the proper dressings to ensure a wet environment[15]. Popular agents used topically in treating infections include Mupirocin, Silver Sulfadiazine, Povidone-Iodine, and also gauze, hydrogel, hydrocolloid, alginate, and foam dressings [16]. In case of severe infection, systemic antibiotics, analgesics, and anti-inflammatory drugs are required along with glycemic control [17]. Modern therapies for wound healing include PRP, NPWT, growth factors, stem cell therapy, and the use of nanotechnology in drug delivery systems. Yet infections, poor vascularization, and inflammation remain some of the prevalent challenges of chronic and diabetic wounds [18].

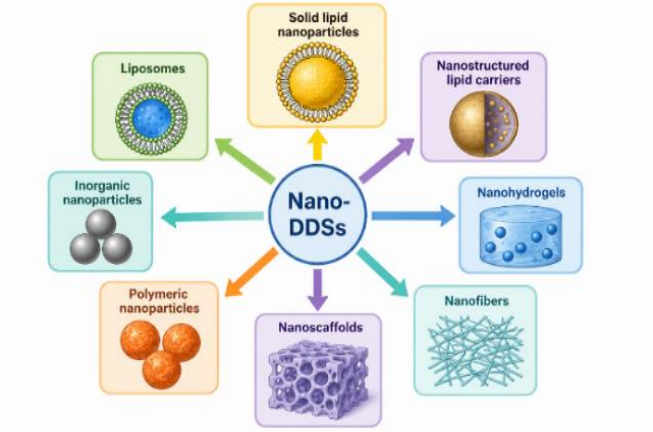

Figure2: Nanoparticle Drug Delivery Systems Used in Wound Management

3.1 Challenges with Traditional Wound Healing

There are a lot of challenges when it comes to traditional wound healing techniques like dressing with gauze, antiseptic application, and systemic antibiotics. Lack of good diffusion, poor availability and uncontrolled delivery of the drug are major challenges associated with this type of wound healing. Biofilms and drug resistance make it hard to manage infections and inflammations. In addition, traditional dressings cannot provide sufficient moisture required to facilitate efficient wound healing process. They should be changed very often which may end up damaging the newly formed tissues. Also, this method does not tackle any molecular imbalances in chronic wounds hence making it difficult to treat the patients [19]

4. Nano Drug Delivery System for Wound Healing and Skin Regeneration

Nanodrug delivery systems increase the efficacy of treatment through the sustained delivery of the drugs without breakdown. These systems assist in wound healing and skin regeneration since they help in providing moisture, cell proliferation and rapid healing of tissues. [20]. There are various delivery

systems that include liposomes, polymeric nanoparticles, inorganic nanoparticles, lipid nanoparticles, nanofibers, nanohydrogels, among others.[21]

4.1 Liposomes

Liposomes are phospholipids vesicles that can load hydrophilic and lipophilic drugs. Liposomes improve drug stability, increase skin permeability, produce wet wound environment, and controlled release of the drug, thereby helping in wound healing. Deformable liposomes have been seen to be more efficient in chronic wounds and diabetic individuals[22]

4.2 Polymeric Nanoparticles

Polymeric nanoparticles made of biodegradable polymers, such as PLGA and chitosan, protect against degradation and ensure sustained release of the drug. They facilitate collagen deposition, neovascularization, and epithelialization, which in turn help in tissue regeneration [23]

4.3 Inorganic Nanoparticles

The inorganic nanoparticles, especially silver and zinc oxide nanoparticles, have good antimicrobial, anti-inflammatory, and wound healing properties. They reduce the bacterial load and promote tissue regeneration by growth factors responsible for wound repair [24]

4.4 Lipid Nanoparticles

Lipid nanoparticles are solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). They increase drug stability, skin penetration, and sustained release of the drug. The biocompatibility and wound healing potential make them suitable for drug delivery [25]

4.5 Nanofibrous Structures

Nanofibers resemble the extracellular matrix and act as cell attachment sites for producing collagen, development of blood vessels, and tissue regeneration. Nanofibrous structures are also employed for delivering therapeutics as part of the wound healing process [26]

5. Cubosomes

The third type of liposomal carriers are cubosomes that are novel nano-carrier systems based on bicontinuous cubic liquid crystalline phases dispersed in aqueous solution and stabilized with surfactants such as Poloxamer 188, Poloxamer 407, Tween 80, and polyvinyl alcohol (PVA). Cubosomes are generally obtained from amphiphilic lipids, such as glyceryl monooleate (GMO) or phytantriol, that form self-assembled threedimensional cubic structures in aqueous solution. It is characterized by presence of aqueous channels separated by continuous lipid bilayers that allows for simultaneous encapsulation of hydrophilic, lipophilic and amphiphilic drugs[27]

5.1 Cubosome Structure

The key structural elements of the cubosomes are associated with the high organization of the bicontinuous cubic liquid crystal phase that is defined by the structure of the lipid bilayers divided into two aqueous channels. This unique structural design ensures the incorporation of hydrophilic drugs into the aqueous channels and lipophilic drugs into the lipid bilayers.The components of cubosomes include a lipid phase and a stabilizer. The lipid molecules form a cubic phase due to the presence of water, while the role of the stabilizer is to prevent particle aggregation and to ensure the stability of the nanoparticle suspension [28]

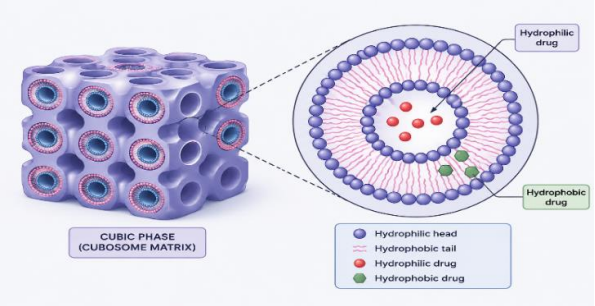

Figure 3: Illustration of Drug Encapsulation within Cubosomes

5.1.1 Cubic Phases Types

Based on the structure of cubic phases, they are categorized into three types:

• **Primitive Cubic Phase (Im3m):** The structure has large aqueous channels that make it suitable for use in the encapsulation of hydrophilic drugs.

• **Double Diamond Cubic Phase (Pn3m):** It is the most commonly used phase in cubic phases utilized in the cubosomal system. This is because it has a high drug loading and release control ability.

• **Gyroid Cubic Phase (Ia3d):** It is a complex 3-dimensional phase that has large aqueous channels which create a high surface area for drug delivery purpose [29]

Cubosomes can be found in open or close form based on whether aqueous channels are in contact with the surroundings or not.

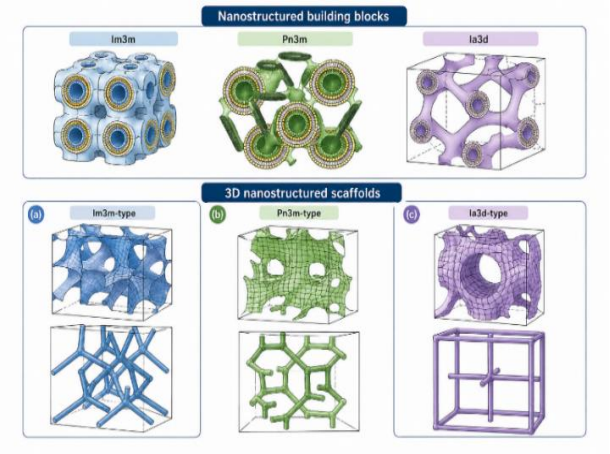

Figure 4: Underlying minimal surfaces and skeletal graphs (centre of water channels) for the inverse bicontinuous cubic phases. (a) Pn 3 m (Diamond), (b) Ia 3 d (Gyroid) and (c) Im 3 m (Primitive)

5.2 Components of Cubosomes

The three major components of cubosomes are; lipids, stabilizers and aqueous phase. They play a significant role in formation, stability, loading capacity and performance of the cubosomes. [30]

5.2.1 Lipids

Lipids constitute the major component that forms the cubic liquid crystalline phase. They are amphiphilic in nature, that is, they possess both lipophilic and hydrophilic regions thus enabling self-assembly in the presence of water. The common lipids used in formulation of cubosomes are Glyceryl Monooleate (GMO) and Phytantriol.

5.2.1.1 Glyceryl monooleate

It is the common biocompatible and biodegradable lipid that constitutes the cubosomal formulation. It is a glycerol molecule that is esterified with Oleic acid and has the ability to spontaneously form cubic phases in the presence of water. Glyceryl Monooleate possesses high drug-loading capacity and can incorporate both lipophilic and hydrophilic drugs. Due to its bioadhesive nature, it extends the residence time of the formulation in the area of administration.

5.2.1.2 Phytantriol

Another type of lipid that forms stable cubic LC phases is phytantriol. The lipid is more chemically stable compared to GMO because of its resistance to hydrolysis. Cubosomes produced from phytantriol typically demonstrate increased structural stability and improved shelf life. This lipid is extensively utilized in the development of topical and transdermal drug delivery systems.Selection of the lipid influences particle size, drug encapsulation efficiency, rate of release, and stability of the cubosome formulation

5.2.2 Stabilizers

Stabilizers are surface-active agents employed to prevent cubosomal nanoparticles' aggregation and coalescence during the production process and storage period. They provide colloidal stability of the dispersion by adsorption on the particle surface.

5.2.2.1 Poloxamer 407

The most frequently used stabilizer for preparation of cubosomes is poloxamer 407. It is a non-ionic triblock copolymer that acts as a stabilizing agent due to creation of the protective layer on the surface of cubosomesInstead of poloxamer 407, the following stabilizers can be used: Poloxamer 188, Tween 80, and PEG derivatives.

5.2.2.2 Poloxamer 188 (Pluronic® F68)

Poloxamer 188 is a non-ionic triblock polymer consisting of segments of polyethylene oxide (PEO) and polypropylene oxide (PPO). Poloxamer 188 is widely used as a stabilizer in cubosome delivery systems because it serves as a steric stabilizer that adsorbs to the surface of the cubosomes and thereby avoids agglomeration and ensures colloidal stability. Furthermore, poloxamer 188 helps in decreasing the particle size, increasing particle uniformity, increasing the drug entrapment efficiency, and also helps in the sustained release of drugs. [31]

5.2.2.3 Tween 80 (Polysorbate 80)

The Tween 80 surfactant is a non-ionic surfactant and functions as an excellent stabilizer in the cubosome formulation, which decreases the interfacial tension between the lipid and aqueous phases. It forms a protective barrier over the surface of the cubosomes and thus prevents aggregation, resulting in the formation of stable dispersions of narrow particle size distribution.Tween 80 has been found to be a very good enhancer for the permeation of drugs. Due to its good biocompatibility and emulsifying properties, Tween 80 can be used as a good stabilizer for the cubosomes made of phytantriol and glyceryl monooleate. [32]

5.2.2.4 Polyvinyl Alcohol (PVA)

The polyvinyl alcohol (PVA) is an artificial and water soluble polymer that acts as a steric stabilizer in the cubosome formulation. It adsorbs to the surface of the cubosomes to prevent the aggregation. The presence of the polymer ensures stability of the formulation because of the even distribution of particle sizes and prolonged shelf-life of the dispersion. Because of its nontoxic, biocompatible and biodegradable nature, PVA has been widely used in the lipid based nanoparticle formulations. [33]

5.3 Aqueous Phase

Water forms the continuous outer phase in case of the cubosome formulation and plays an important role in the formation of the bicontinuous cubic structure. Hydrolysis of the lipid with water helps the formation of the channels through which the lipids assemble, forming the aqueous connections, which help the entrapment of hydrophilic drug. The concentration of water determines the viscosity, particle size and stability of the formulation, thus becoming important for the formation of the cubosome. [34]

6 Hydrogel Cubosome System:

Hydrogel cubosomes are the next generation of topical drug delivery systems which have the properties of cubosomes as well as hydrogels. Cubosomes are the latest drug delivery system comprising of amphiphilic lipids such as Glyceryl Monooleate (GMO), stabilized by Poloxamer 407 that can trap both hydrophilic as well as lipophilic drugs and release the entrapped drug sustainably. Cubosomes within hydrogel system increase the viscosity, spreadability, and residence time of the delivery system in the applied area. Hydrogels provide moisture to the surroundings of the wound while cubosomes ensure the entry of drug into the skin. This results in the wound healing process as well as decreases the dose frequency [35]

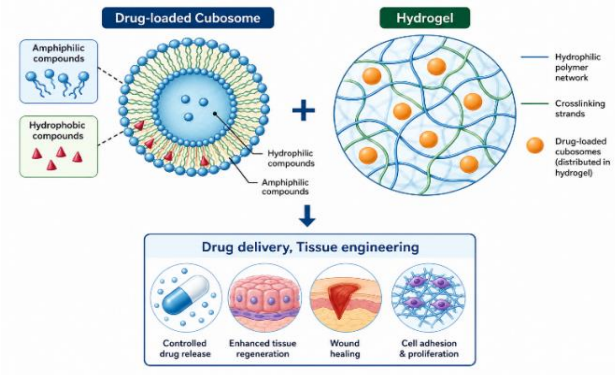

Figure 5: Drug-Loaded Cubosomes Embedded in Hydrogel Matrix for Tissue Regeneration

The use of cubosomal hydrogels for the delivery of drugs and maintaining therapeutic concentrations for long periods makes them very suitable for treating chronic wounds including diabetic wounds. Besides, they have a high level of biocompatibility, stability, and drug loading capacity; therefore, they are promising carriers for antimicrobial and wound healing agents. Consequently, cubosomal hydrogels can be considered as an innovative and efficient method for the promotion of diabetic wound healing [36]

6.1 Hydrogel and Role of Carbopol in Topical Drug Delivery

A hydrogel refers to a three-dimensional polymer network which is able to absorb and retain a large amount of water and hence create a moist condition favorable for wound healing. These materials are classified into three categories namely natural (gelatin, chitosan, alginate, collagen, hyaluronic acid); synthetic (Carbopol, PVA, PEG, PVP); semi-synthetic (HPMC, Na-CMC, methyl cellulose). Among these Carbopol is one of the most common synthetic polymers used in topical formulations due to its good gelling ability, viscosity, and stability Carbopol hydrogels show good spreadability, residence time, biocompatibility and moisture wound condition. They not only increase the physical stability of cubosomal formulation but also release the drug in a controlled manner. [37]

7. Mechanism of Drug Release from Cubosomes

Drug release from cubosomes primarily occurs through diffusion. Diffusion is driven by the concentration difference between the cubosome and the surrounding environment. Usually, the process obeys Fick's law of diffusion and the Higuchi diffusion equation. Drug release depends upon many factors such as drug solubility, diffusion constant, partition constant, pore size, cubic liquid crystalline geometry, lipid composition, pH, temperature, and ionic strength of the release medium. The unique bicontinuous cubic nanostructure of cubosomes facilitates a controlled and sustained drug release mechanism[38]

Table 2. Advantages and Disadvantages of Cubosomes as Drug Delivery Systems

Advantages	Disadvantages
Their high capacity to load hydrophilic as well as lipophilic drugs because of their bicontinuous cubic structure.	Formulation process is difficult and involves optimization of lipids and stabilizers.
They have excellent biocompatibility and biodegradability because of the use of physiological lipids.	Viscous cubic structures can cause difficulty during manufacture and handling.
They increase the solubility and bioavailability of poorly soluble drugs.	Physical instability could arise when stored for a long period because of particle aggregation.
They protect the drugs against chemical and enzymatic degradation.	It is difficult to prepare and involves specialized machinery.
Large scale production needs special high-pressure homogenization machines and process control.	Scaling up and largescale production pose problems.

8. Methods of Preparation of Cubosomes

8.1 Top-Down Approach

The top-down approach is the most widely used method for cubosome preparation. In this method, a bulk cubic phase is first prepared using the mixture of lipids such as glyceryl monooleate (GMO), phytantriol and water and stabilizer Poloxamer 407. Then, cubosomes are obtained from bulk cubic phase through fragmentation using high pressure homogenization and probe sonication. Cubosomes prepared by the top-down approach exhibit precise internal cubic structure, uniform particle size distribution and excellent stability. However, this approach requires higher energy and time, and therefore affects the stability of temperature sensitive [39]

8.2 Bottom-Up Approach

Bottom-up approach, also called solvent dilution method, entails dissolving lipids in hydrotropic solvent followed by diluting in aqueous solution with a stabilizer. In bottom-up approach, cubosomes are obtained as a result of spontaneous assembly of lipids during dilution process. This is a low-energy approach and therefore best suited for temperature sensitive drugs. Normally, this approach results in efficient drug entrapment with minimal energy consumption. However, residual solvent may affect formulation stability[40]

8.3 High Pressure Homogenization

High-pressure homogenization converts the bulk cubic phase to nanosize cubosomes through passing through a narrow homogenization valve at high pressure. High shear forces and cavitations produced in the process give rise to cubosomes with good physical stability and narrow particle size distribution. The technique is highly reproducible and is ideal for industrial-scale production but it needs special equipment and high energy usage [41]

8.4 Sonication

Sonication uses ultrasonic energy to convert the bulk cubic phase to cubosomal nanoparticles through cavitation mechanism. It is an easy and rapid technique that does not require expensive equipment and is extensively used in laboratory scale production and particle size reduction. But long sonication time may lead to overheating and destruction of thermally sensitive drugs and contamination with the probe tip [42]

8.5 Solvent Evaporation Technique

Solvent evaporation technique implies dissolution of lipids and drugs in a volatile organic solvent followed by emulsification in an aqueous stabilizer. The solvent is evaporated leaving the lipids assembly in cubosomal nanoparticles. This technique allows high drug loading and good particle size control, but complete solvent evaporation is essential to eliminate toxicity [43]

8.6 Spray Drying Technique

In the spray drying process, cubosome dispersions are dried in the form of dry powder through atomization in the presence of hot air, with the quick evaporation of solvent. The powder is characterized by increased stability, increased shelf life, and easy reconstitution prior to administration. However, exposure to high temperatures may cause changes in thermolabile drugs and proteins [44]

8.7 Heat Treatment Technique

Heat treatment is one of the techniques that can be used after cubosomes production to enhance the efficiency of their formation and stabilization. In this way, the structure becomes more stable due to lipid reorganization from vesicles into stable cubic phases, which causes reduced particle size heterogeneity. However, it cannot be applied to heat labile drugs [45]

8.8 Melt Dispersion Technique

The melt dispersion process involves melting and dispersing of lipid phase into the aqueous phase containing stabilizer under stirring, followed by cooling of lipids molecules leading to cubosomal nanoparticles formation. It is a solvent-free method that is easy and cost-effective; however, the heat can affect thermolabile substances [46]

8.9 Microfluidization Technique

Microfluidization is a high pressure process in which lipids and water phases collide inside microchannels to form cubosomes by virtue of shear force. It produces nano particles having a uniform size distribution, reproducibility, and good physical stability, hence can be used on a large scale. Its use is hindered by the expensive cost of the specialized equipment used for the process [47]

9. Characterization of Cubosomes

It is necessary to characterize the cubosomes in order to determine the physicochemical properties of cubosomes, their structure, loading capability of drugs, and their stability. Different analytical methods have been applied to determine the presence of cubosomes and to check whether they can act as drug delivery systems or not[48]

9.1 Particle Size Analysis and Polydispersity Index (PDI)

The particle size is one of the parameters that affects drug release, skin penetration, cellular uptake, and stability of formulations. The measurement of this parameter is done through Dynamic Light Scattering (DLS). Particle sizes for cubosomes are normally between 100–500 nm, whereas Polydispersity index (PDI) shows the homogeneity of particles' sizes. If the value of PDI is less than 0.3, it means that there is good stability of the formulation. Small particle sizes increase the surface area, thus favoring drug release and bioavailability [49]

9.2 Zeta Potential

Zeta potential determines the amount of surface charge on nanoparticles of cubosomes, as well as their stability. It is measured through electrophoretic

light scattering, and values above ±30 mV signify good physical stability by reducing aggregation of particles. Surface charge affects drug release and interaction of nanoparticles with the biological membrane [50]

9.3 Morphological Analysis

The morphological characterization is carried out using TEM, SEM, and Cryo-TEM methods. This allows one to obtain information about the particle shape, morphology of the surface, size, and stability of the structure, proving the formation of a characteristic cubic nanoscale structure and presence of agglomerates.

9.4 Entrapment Efficiency

Entrapment efficiency characterizes the percentage of the drug entrapped in the cubosomes, which shows the capacity of drug loading in the system. It is usually estimated using dialysis or ultrafiltration and quantification of the free drug with UV-Visible spectrophotometry or HPLC. High entrapment efficiency corresponds to a better retention of the drug and its sustained release [51]

9.5 In Vitro Drug Release

The main mechanism of drug release from cubosomes is diffusion from the channels of interconnecting cubic liquid crystalline structure. The rate of the process is affected by various parameters such as drug solubility, pore size, composition of lipids, pH, temperature, and release medium. The in vitro drug release is usually studied using dialysis bag or pressure ultrafiltration method [52]

9.6 Cubosome Stability Studies

Stability studies analyze the physical and chemical stability of the cubosomes in storage conditions. Various parameters including particle size, PDI, zeta potential, drug content, and entrapment efficiency are assessed at different temperatures and time points. DSC and viscosity are also considered as techniques for analyzing phase transition and stability of the formulation [53]

9.7 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) analysis is performed to assess the compatibility of the drug with the excipients and the chemical interaction between them. The lack of major shifts or absence of characteristic peaks implies the good compatibility of the drug in the cubosomes and assures its chemical stability in the system [54]

10. Characterization of Cubosomal Hydrogel

10.1 pH Determination

The pH of cubosomal hydrogel becomes a very important factor in terms of its skin compatibility. The product with optimal pH ensures the absence of irritation and improves the convenience of its application. Keeping the pH close to that of the skin ensures the enhancement of wound healing and formulation stability. Thus, the pH determination is recognized as an important parameter for the quality control of topical hydrogels. [55]

10.2 Rheological Studies

Rheological studies give information about the flow and deformability of cubosomal hydrogels in various conditions of applied stresses. Majority of topical hydrogels show non-Newtonian pseudoplastic behavior, which means that their viscosity depends on the shear rate and decreases with its increase. This property helps to spread the product during its application easily. [56]

10.3 Spreadability

Spreadability is a significant parameter indicating how easily the hydrogel can be spread across the skin or the wound surface. Spreadability ensures uniform distribution of drugs, which increases therapeutic efficacy. Additionally, good spreadability makes drug application convenient since the force required for application is minimized. Factors influencing spreadability of the cubosomal hydrogels include viscosity, concentration of polymers, and formulation of the hydrogel[57]

10.4 Swelling Index

Swelling index determines the water absorbing properties of the hydrogel and its expansion during hydration. The significance of swelling index is attributed to the fact that this property is necessary for wound healing applications because it creates moist environment in the wounds, facilitates oxygen diffusion, and absorbs wound exudates. Swelling is influenced by the type of polymer, its cross-linking and environmental factors[58]

10.5 Bioadhesion Strength

Bioadhesion is a term describing the ability of the hydrogel to adhere to biological tissue for a long time. Bioadhesive properties make the formulations stay at the site of action for a longer time, increasing the duration of drug delivery. Additionally, it leads to decreased losses of the formulation and less frequent drug administration[59]

11 Applications of Cubosomes

There has been much interest recently in cubosomes due to their special structure, namely the bicontinuous cubic phase, their capacity to deliver large quantities of drug, biocompatibility and ability to encapsulate both hydrophilic and lipophilic drugs. This leads to controlled delivery of the drug with improved efficiency.

11.1. Topical and Transdermal Drug Delivery

Due to such features as good bioadhesion and penetration enhancement, cubosomes find wide applications in topical and transdermal drug delivery. They provide good retention of the drug at the application site, allow to achieve sustained drug delivery and promote the passage of the drug through the skin. These properties make them good carriers for treatment of wounds, burns, psoriasis, acne, etc.

11.2. Diabetic Wound Healing

Cubosomal hydrogels can be effectively used for the treatment of diabetic wounds because they ensure the sustained delivery of the drug, maintain the moist wound environment and have increased antimicrobial action.

11.3 Oral and Ocular Drug Delivery

Cubosomes improve the solubility and bioavailability of low aqueous solubility drugs upon oral delivery. In ocular delivery, the bioadhesive properties of Cubosomes prolong precorneal residence time and improve drug absorption, resulting in better therapeutic effect on ocular diseases.

11.4 Cancer Therapy

Cubosomes are promising delivery systems for targeted cancer therapy because of the capability of these nanoparticles to encapsulate the anticancer drugs and enhance their accumulation at tumor site. These nanoparticles provide controlled release of drugs, decrease systemic toxicity, and improve the effectiveness of chemotherapeutic drugs.

11.5 Gene, Protein, and Vaccine Delivery

The interconnected aqueous channels of cubosomes allow for efficient encapsulation of proteins, peptides, nucleic acid, and vaccine antigens. These particles offer protection against degradation and sustained release of these biomolecules

12.Future Directions

Cubosomal hydrogels have proved promising for wound healing in diabetes, but further work is required before their use in clinics. Future research needs to involve clinical trials, development of methods for mass production at reasonable costs, and preparation of smart cubosomes responsive to stimuli for targeted delivery of drugs. The inclusion of antimicrobial substances, growth factors, or antioxidants in combination therapies can improve wound healing efficiency. Moreover, there is a need for regulations that would guarantee success of commercializing cubosomal hydrogels. Development in personalized medicine might also allow the use of customized cubosomal formulations.

LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviation	Full Form
GMO	Glyceryl Monooleate
P407	Poloxamer 407
P188	Poloxamer 188
PVA	Polyvinyl Alcohol
PEG	Polyethylene Glycol
PLGA	Poly(lactic-co-glycolic acid)
PRP	Platelet-Rich Plasma
NPWT	Negative Pressure Wound Therapy
DLS	Dynamic Light Scattering
PDI	Polydispersity Index
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
HPMC	Hydroxypropyl Methylcellulose
Na-CMC	Sodium Carboxymethylcellulose
PVP	Polyvinylpyrrolidone
Pn3m	Double Diamond Cubic Phase
Ia3d	Gyroid Cubic Phase
Im3m	Primitive Cubic Phase

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