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AQUASOMES: A WATER-BASED NANOPARTICLE SYSTEM FOR ENHANCED BIOAVAILABILITY USED FOR TREATMENT OF OSTEOMYELITIS

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ABSTRACT

Osteomyelitis is an energetic contamination that comes around in provocative pulverization, decay, and bone neoformation, which can development to an inveterate and determined organize. Bacterial infection can happen in the setting of a tremendous bacterial immunization in combination with damage or necrotic tissue and/or the closeness of exterior texture. A cutting edge normally balanced ceramic antigen transport vehicle was illustrated to be reasonable. The aquasomes experienced characterization for assistant examination, atom gauge, and morphology utilizing X-ray powder diffractometry, TEM, and SEM. The hydrophobicity of the gentamicin-loaded Aquasomes was evaluated in vitro and in vivo to choose its ampleness in treating bone maladies caused by both moo and tall levels of minuscule life forms. The bactericidal development of the aquasomes was surveyed against Staphylococcus aureus organisms interior 2 hours and 5 hours, independently. The oxygen- binding properties of the nanocrystalline center, the polyhydroxy oligomeric film coating, the non-covalently bound layer containing the helpful quality parcel, the additional carbohydrate film, and the centering on layer composed of conformationally directed viral layer proteins for quality treatment were examined.

KEYWORD:- Aquasomes, Water-based nanoparticles, Bioavailability, Osteomyelitis.

INTRODUCTION TO OSTEOMYELITIS

Acute bacterial osteomyelitis carried a 50% mortality in the pre-antibiotic period since of overpowering sepsis with metastatic abscesses.[1] Osteomyelitis is a dynamic contamination that comes about in provocative pulverization, corruption, and bone neoformation, which can advance to a constant and diligent stage.[2] Osteomyelitis ordinarily happens in the setting of an expansive bacterial vaccination in combination with injury, corruption or ischemia of tissue and/or the nearness of remote material.[3] It can include distinctive structures such as the bone marrow, cortex, periosteum, and parts of the encompassing delicate tissues, or stay localized. Waldvogel's framework is based on term, instrument of contamination, and nearness of vascular inadequate, giving the taking after classification: a) intense hematogenic osteomyelitis; b) osteomyelitis by contiguity, with or without vascular insufficiency; c) vertebral osteomyelitis; and d) constant osteomyelitis.[4]

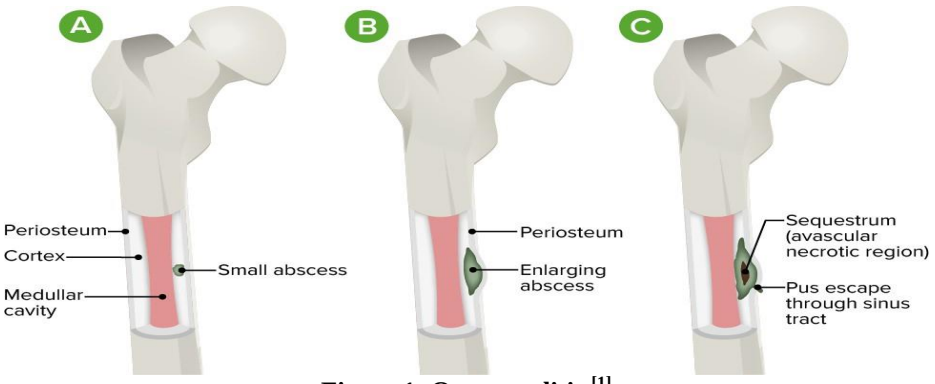

Figure 1: Osteomyelitis.[1]

Etiology of osteomyelitis

Bacteria or organism may cause the contamination. Bloodstream-sourced osteomyelitis is seen most regularly in children, and about 90% of cases are caused by Staphylococcus aureus. In newborn children, S. aureus, Bunch B streptococci (Most common) and Escherichia coli are commonly confined; in children from 1 to 16 a long time of age, S. aureus, Streptococcus pyogenes, and Haemophilus influenzae are common. In a few subpopulations, counting intravenous medicate clients and splenectomised patients, Gram- negative microbes, counting enteric microbes, are critical pathogens.[6]

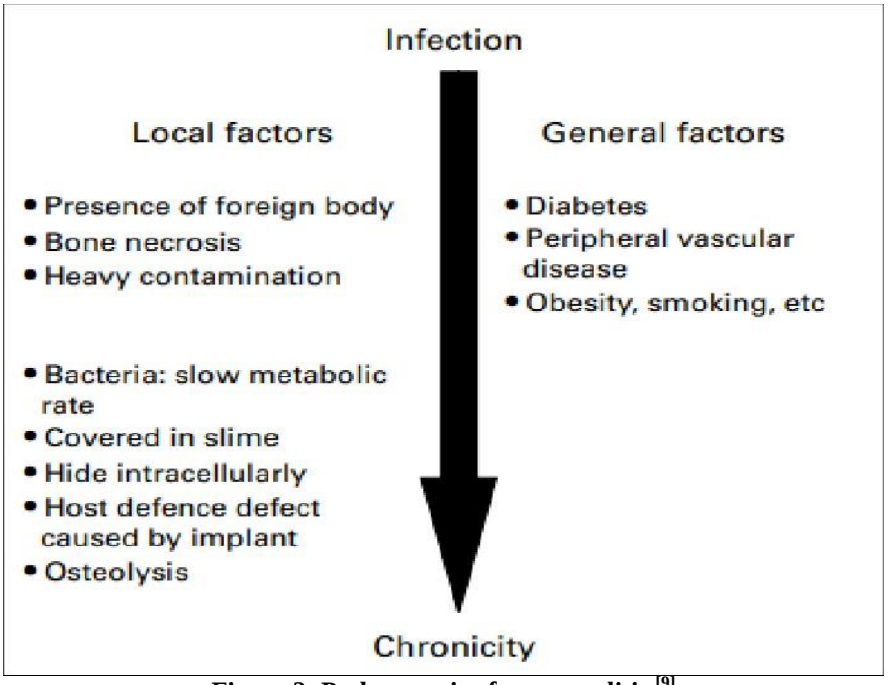

Figure 2: Pathogenesis of osteomyelitis.[9]

Pathogenesis

The disease related with osteomyelitis may be localized or it may spread through the periosteum, cortex, marrow, and cancellous tissue. The bacterial pathogen changes on the premise of the patient's age and the component of disease. The taking after are the two essential categories of intense osteomyelitis hematogenous osteomyelitis and coordinate or touching immunization osteomyelitis. Hematogenous osteomyelitis is a contamination caused by bacterial seeding from the blood. Intense hematogenous osteomyelitis is characterized by an intense contamination of the bone caused by the seeding of the microscopic organisms inside the bone from an inaccessible source. This condition basically happens in children. The most common location is the quickly developing and profoundly vascular metaphysis of developing bones. The clear abating or sludging of blood stream as the vessels make sharp points at the distal metaphysis inclines the vessels to thrombosis and the bone itself to localized corruption and bacterial seeding. Intense hematogenous osteomyelitis, in spite of its title, may have a moderate clinical advancement and treacherous onset. Coordinate or coterminous vaccination osteomyelitis is caused by coordinate contact of the tissue and microbes amid injury or surgery. Coordinate immunization (Contiguous- focus) osteomyelitis is a disease in the bone auxiliary to the vaccination of life forms from coordinate injury, spread from a coterminous center of disease, or sepsis after a surgical procedure.[6]

Treatment

Antibiotic treatment: Acute hematogenous osteomyelitis is best overseen with cautious assessment of microbial etiology and susceptibilities and a four- to six-week course of fitting anti-microbial treatment. After societies have been gotten, an empiric parenteral anti-microbial regimen (Nafcillin furthermore either cefotaxime or ceftriaxone is started to cover clinically suspected life forms. When the culture comes about are known, the anti-microbial regimen is revised. Children with intense osteomyelitis ought to get two weeks of introductory parenteral anti-microbial treatment some time recently they are given a verbal specialist.[7, 8] The choice of anti-microbials, in spite of the fact that constrained by the affectability of etiological specialists, ought to too be based on the choice of fitting through of organization, security of long-term utilize, and cost.[11]

Debridement: Surgical debridement in patients with chronic osteomyelitis can be technically demanding. The quality of the debridement is the most critical factor in successful management. After debridement with excision of bone, it is necessary to obliterate the dead space created by the removal of tissue.[10]

Introduction to delivery system

The Aquasomes are a sort of nanoparticulate carrier framework. In any case, they are not fair straightforward nanoparticles; instep, they comprise of three layers and are self- assembled structures. These structures are made up of a strong stage nanocrystalline center that is coated with an oligomeric film. This film is where biochemically dynamic atoms are adsorbed, with or without modification. Aquasomes take after "bodies of water" and their water-like characteristics defend fragile organic atoms, protecting their basic astuteness and permitting for a tall degree of surface presentation. This highlight is utilized in focusing on bioactive particles such as peptide and protein hormones, proteins, antigens, and qualities to particular areas. The three-layered structures are shaped through non-covalent and ionic bonds, and these carbohydrate-stabilized ceramic nanoparticles are alluded to as "aquasomes." The consolidation of pharmacologically dynamic particles is accomplished through copolymerization, dissemination, or adsorption onto the carbohydrate surface of pre-formed nanoparticles.[12]

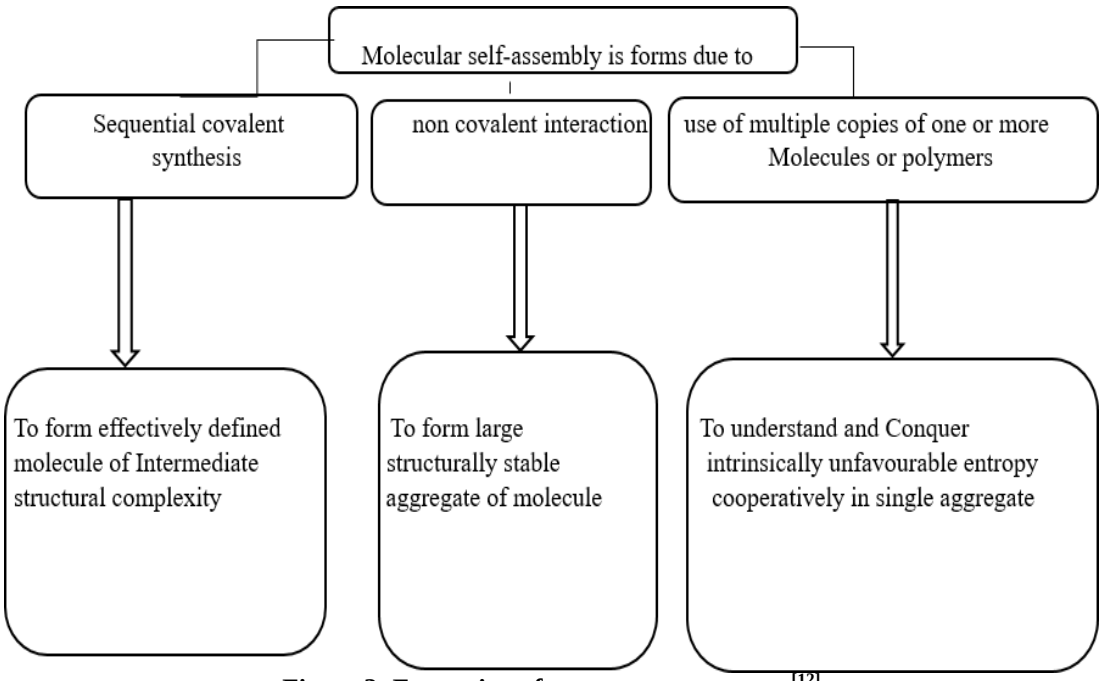

Figure 3: Formation of aquasomes structure.[12]

Principle of self-assembly: The concept of self-assembly implies that the person components of a last item actually expect particular basic courses of action in two or three measurements. When macromolecules self-assemble in a watery environment, whether to deliver shrewdly nanostructured materials or as portion of organic forms, three physicochemical forms basically administer the handle: the intuitive of charged bunches, parchedness impacts, and auxiliary stability.[13]

I. **Interaction between charged groups:** The nearness of charged bunches, such as amino, carboxyl, sulfate, and phosphate bunches, encourages the long-range approach of self-assembly subunits. These charged bunches too play a part in stabilizing the tertiary structures of collapsed proteins.[13]

II. **Hydrogen Holding and Lack of hydration effect:** Hydrogen bonds help in base combine coordinating and the stabilization of auxiliary protein structures, such as alpha helices and beta sheets. Particles that shape hydrogen bonds are hydrophilic, driving to a noteworthy degree of organization in the encompassing water particles. On the other hand, hydrophobic atoms, which cannot shape hydrogen bonds, repulse water, subsequently organizing the encompassing environment. This organized water diminishes the generally disorder/entropy of the encompassing medium. As organized water is thermodynamically troublesome, the atoms lose water and get dried out, driving to self-assembly.[13]

III. **Stacking of the medicate of choice to this assembly:** The last step includes stacking the medicate onto the coated particles through adsorption. To do this, a arrangement of the sedate at a known concentration is arranged in a reasonable pH buffer, and the coated particles are scattered into it. The scattering is at that point cleared out overnight at a moo temperature for sedate stacking or lyophilized after a few time to get the drug-loaded definition, known as aquasomes. The coming about planning is at that point characterized utilizing different techniques.[14]

Objectives

Initially, aquasomes give security for bio-actives. Different other conveyance frameworks, such as

prodrugs and liposomes, have been examined for the same reason, but they are helpless to hurtful intelligent between the medicate and carriers.[53, 72, 73]

In such occurrences, aquasomes demonstrate to be a noteworthy carrier due to the carbohydrate coating in aquasomes anticipating inconvenient denaturation intelligent between the medicate and strong carriers.[71]

Additionally, aquasomes keep up atomic adaptation and ideal pharmacological movement. Dynamic particles regularly have characteristics such as a interesting three-dimensional compliance, the capacity for inner atomic improvement impacted by atomic intuitive, and the capacity for bulk development. Dynamic particle denaturation can happen due to components such as pH, solvents, salts, and temperature when in the fluid state. Hence, the bio-active experiences various biophysical confinements beneath these conditions.[36]

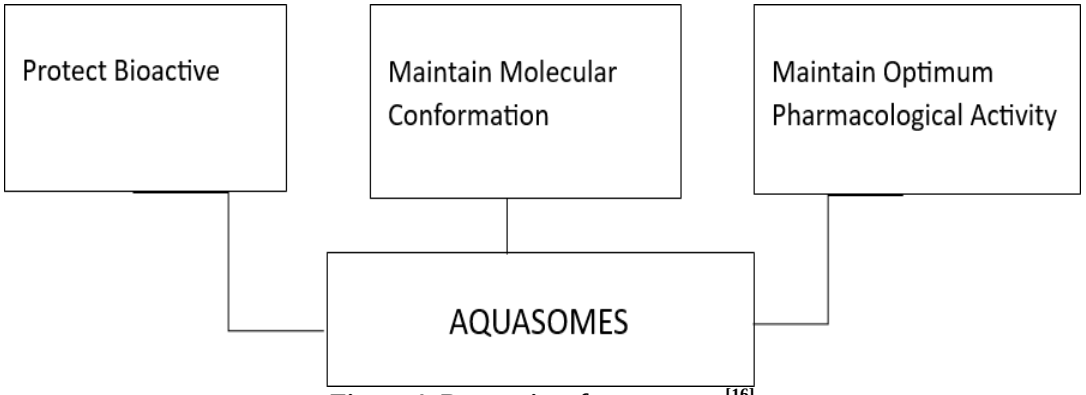

Figure 4: Properties of aquasomes.[16]

Properties of aquasomes[16-18]

- Aquasomes have expansive measure and dynamic surface thus can be proficiently stacked with significant sums of operators through ionic, non-co-valent bonds, vanderwaals strengths and entropic strengths.
- As strong particles scattered in fluid environment, show physical properties of colloids.
- Aquasomes instrument of activity is controlled by their surface chemistry.
- Aquasomes provide substance through combination of particular focusing on, atomic protecting, and moderate and supported discharge prepare.
- Aquasomes water like properties gives a stage for protecting the conformational judgment and bio chemical solidness of bio-actives.
- Aquasomes due to their measure and structure solidness, dodge clearance by reticuloendothelial framework or corruption by other natural challenges.

Structure of aquasomes

The three-layered structure of aquasomes consists of a core coated with a polyhydroxy oligomer onto which the drug is loaded. Biochemically active molecules can interact with the coated core through various forces such as Van der Waals forces, entropic forces, and ionic and non-covalent bonds. The unique structure of aquasomes allows them to transport a wide range of substrates (Chemicals), enabling applications such as the delivery and protection of proteins and peptides, as well as the delivery of nucleic acids for gene therapy applications. The solid core of Aquasomes, which can be made of ceramic or polymeric material, provides structural stability to the nanoparticle itself and can lead to improved solubility and biocompatibility of the drug. Various core designs have demonstrated an impact on the controlled release properties of the drug molecule. Ceramic calcium phosphate, naturally present in the body, is a commonly used core material. Another frequently utilized core material is hydroxyapatite, which is found in bone. Studies have indicated that hydroxyapatite cores contribute to the targeted delivery of encapsulated hepatitis B antigens intracellularly. The drug is adsorbed onto the carbohydrate coat, which forms the second layer of aquasomes. Carbohydrate's role as a dehydroprotectant has been demonstrated to act as a natural stabilizer for maintaining the shape of soft drugs. Additionally, the carbohydrate coat's dehydroprotectant property helps protect the biochemically active molecule from dehydration and protein degradation.[15]

Aquasomes vary in size from 60 to 300 nm, which is why they are considered a type of nanoparticle drug carrier.[19] Their nanoscale dimensions result in a high surface area to volume ratio. A smaller core leads to a higher surface area to volume ratio, thus enhancing the drug loading capacity of the aquasomes. The tri-layer structure of aquasomes is formed through non-covalent and ionic bonds, as well as the physicochemical properties of their components. Initially, calcium phosphate nanoparticles are created, followed by the adsorption of a carbohydrate coat onto the core surface through electrostatic interactions. Additional layers are then incorporated to attain the desired size, with crosslinked polymers contributing to further stabilization.[16]

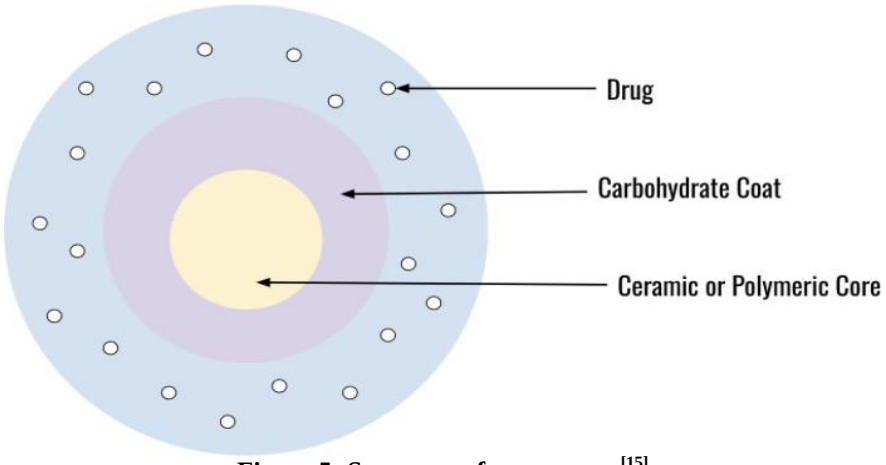

Figure 5: Structure of aquasomes.[15]

Composition of aquasomes

Core material: Ceramic and polymeric are the most commonly utilized materials for core preparation. The polymers utilized comprise acrylates, albumin, and gelatine, while the ceramic materials include nano-crystalline carbon ceramic (diamond), calcium phosphate (brushite), and tin oxide.[34]

As ceramics are inherently crystalline, these materials offer a high level of order and structural regularity. This high level of order results in increased surface energy, leading to effective binding of carbohydrates. The use of calcium phosphate as a core material is beneficial because it naturally occurs in the body and is extensively used in the form of bio composites.[31-33]

Ceramic is simple to produce, environmentally degradable, affordable, and compatible with living organisms. Therefore, it is well-suited for creating aquasomes and for use in drug delivery.[34, 35]

Coating material: Mostly preferred coating materials include citrate, chitosan, cellobiose, sucrose, trehalose, and pyridoxal-5-phosphate.[36] Using carbohydrate films as coating material prevents soft drugs from changing shape. Carbohydrates act as dehydroprotectant and natural stabilizers by maintaining the molecular conformation of biochemically active molecules, ensuring structural integrity, creating a water-like environment for the biochemically active molecules, and safeguarding the three-dimensional conformations of drug molecules.[19, 23]

Bioactive material: The coating material's surface contains bioactive molecules that can interact with the film through non-covalent and ionic interactions. Aquasomes with these properties have crucial medical uses in delivering antigens and xenobiotics.[36, 37]

Advantages of aquasomes

- **Interaction between charged group:** The presence of charged groups aids in the long- range attraction of self-assembling subunits and also contributes to the stabilization of folded protein structures. Both intrinsic chemical groups and adsorbed ions from the biological environment impart a charge polarity to most biological and synthetic surfaces. It is noteworthy that the majority of biochemically relevant molecules are amphoteric. Amino, carboxyl, sulphate, and phosphate groups are examples of charged groups that promote the long-range approach of self-assembling subunits. The initial phase of self-assembly involves the long-range interaction of constituent subunits, which begins at an intermolecular distance of approximately 15 nm. In the case of hydrophobic structures, long-range forces may extend up to 25 nm. Additionally, charged groups play a crucial role in stabilizing the tertiary structures of folded proteins.[18,55]

- **Hydrogen Bonding and Dehydration effect:** The hydrogen bond plays a crucial role in matching base pairs and stabilizing secondary protein structures like alpha helices and beta sheets. Molecules that participate in hydrogen bonding are hydrophilic, leading to a high degree of organization in the surrounding water molecules. In the case of hydrophobic molecules, which cannot form hydrogen bonds, their aversion to water aids in organizing the surrounding environment. This organized water reduces entropy levels and is thermodynamically unfavourable, causing the molecules to dehydrate and self- assemble.[18,55]

- **Structural stability of protein in biological environment:** The molecule's hardness and softness are mainly determined by the interaction between charged groups and hydrogen bonds external to the molecule, as well as by the van der Waals forces experienced by hydrophobic molecules internally. These factors also play a role in maintaining the internal secondary structures and providing sufficient softness to allow for the maintenance of conformation during self-assembly. Self-assembly results in altered biological activity, and it is

important to buffer the van der Waals forces. In aquasomes, sugars assist in molecular plasticization.[18,55]

Disadvantages of aquasomes[56,57]

- Its preparation method is time-consuming.
- Maintaining the drug concentration is essential for accurate drug loading in aquasomes.
- Burst release caused by poorly soluble drugs can lead to toxicity.
- Long-term storage can result in leaching and aggregation.
- Despite their low transfer efficiency, they are also expensive.

Applications of aquasomes

- Aquasomes are used in imaging or diagnosis and act as biological labels because they can conjugate with antibodies, proteins, and nucleic acid. Aquasomes helps to prevent the denaturation of molecules by providing a favourable environment for proteins and peptides. Aquasomes works as a reservoir, which releases the drug molecule in a continuous and controlled manner.[56,57]

Methods for the preparation of aquasomes

Core preparation: The preparation method for the core varies based on the type of core being utilized. Typically, core materials such as nanocrystalline tin oxide, carbon ceramic (diamond), calcium phosphate, and hydroxyapatite are employed. Nanocrystalline calcium phosphate and hydroxyapatite are commonly chosen as core materials for aquasomes among these options:

i. Co-precipitation

The addition of a 0.19 N solution of diammonium hydrogen phosphate drop by drop to a 0.32 M solution of calcium nitrate is carried out while maintaining a pH of 8 to 10 by adding concentrated aqueous ammonia solution. This process takes place at 75°C in a three-necked flask equipped with a reflux condenser, a thermometer containing a CO_2 trap, and a charge funnel. Following 4 to 6 days of continuous stirring at 75°C and pH 8–10, the resulting precipitates are filtered, washed, and then dried overnight at 100 °C before being sintered at 800 to 900 °C. An aliquot of 3 ml from a 0.1 g/L solution of methylcellulose (used as a dispersant) was combined with 1440 ml of deionized water containing 0.152 M of calcium nitrate tetrahydrate and 0.090 M of diammonium hydrogen phosphate. Following this, 115 ml of 24% NH_4OH was added to the solution, and the mixture was heated at 60 to 70°C on a hot plate with vigorous mechanical stirring for 3 hours. The precipitate that formed was separated from the supernatant via filtration and then washed five times with deionized water. After being dried overnight at 100°C, the precipitate was ultimately calcined in an air atmosphere at 1000°C for 6 hours, followed by light grinding.[20,21,22,23]

ii. Self-precipitation

The pH 7.2 of the simulated body fluid includes sodium chloride (134.8 mM), potassium chloride (5.0 mM), sodium hydrogen carbonate (4.2 mM), calcium chloride (2.5 mM), disodium hydrogen phosphate (1.0 mM), magnesium chloride (1.5 mM), and disodium sulphate (0.5 mM). Hydrochloric acid is used to adjust the pH to 7.26 daily. The solution is then transferred to a series of 100 ml polystyrene bottles, which are tightly sealed and stored at 37 ± 1°C for one week. Afterward, the precipitate that forms on the inner surface is filtered, thoroughly washed with double distilled water, and dried at 100°C.[21]

iii. Sonication

Disodium hydrogen phosphate solution is added gradually to a calcium chloride solution while being sonicated at 4°C for 2 hours. The resulting precipitate is separated through centrifugation, and the liquid above the precipitate is poured off. The separated cores of the precipitate are washed, then suspended again in distilled water, and finally filtered using a membrane filter to obtain cores with the desired particle size.[24]

The size, composition, and %yield of particles are influenced by sintering, pH, and time. Uncontrolled pH leads to the formation of large, elongated particles. The particles have a range of micrometer sizes. Maintaining the pH between 8 and 10 and avoiding sintering leads to the production of particles that are extended to round and are ≤1.0 μm. However, sintering leads to the creation of round particles in the nanoscale range.[25,26] The percentage yield from the uncontrolled and controlled processes without sintering was similar, at 37% and 36% respectively. However, the process with controlled pH and sintering resulted in a higher percentage yield of 60%. Stirring the slurry for one day while maintaining a pH between 8 and 10 led to the formation of large, elongated particles with a lower percentage yield of 33%. Sintering resulted in the formation of similar particles with a size of 500–1000 nm and the same percentage yield. Stirring the slurry for 4–6 days improved the particle size (250–1000 nm) and type (from elongated to spherical), resulting in a percentage yield of 61%. Sintering in this case caused the formation of spherical particles (100 to 200 nm) with a percentage yield of 60%. Therefore, maintaining the pH between 8 and 10 during the sintering process led to the formation of spherical nanoparticles in the nano meter size range with an increased percentage yield. Additionally, increasing the duration of stirring followed by sintering also led to the formation of spherical nanoparticles with an increased percentage yield.[25] After stirring the slurry for 4 to 6 days, the particle size (250 to 1000 nm), shape (from elongated to spherical), and yield percentage (61%) improved. Sintering resulted in the formation of spherical particles (100–200 nm) with a yield percentage of 60%. Therefore, maintaining the pH between 8 and 10 during the sintering process led to the formation of spherical nanoparticles in the nanometer size range,

along with an increased yield percentage. Furthermore, increasing the stirring duration and subsequent sintering also led to the formation of spherical nanoparticles with an increased yield percentage. The self-precipitation method resulted in the formation of spherical particles (1 to 5 μm) but with a low yield because a monolayer precipitate formed on the container surface. The introduction of seeding led to an acceleration in the crystallization rate, resulting in the production of particles with irregular sizes and shapes. This suggests that the particle size, shape, and percentage yield of the ceramic core are influenced by the pH, duration of stirring, and sintering process.[26]

Coating of the core with polyhydroxy oligomer

Carbohydrate is introduced into a core dispersion and then subjected to sonication followed by lyophilization. Coating can also be achieved through adsorption via direct incubation and addition of nonsolvent.[27] The addition of carbohydrate to a ceramic core dispersion, followed by sonication and lyophilization, resulted in the coating of the cores. A core to coat ratio of 1:4 or 1:5 led to the formation of spherical coated particles. Increasing the Sonicator power up to 15W or 20W resulted in the formation of small spherical discrete particles (< 200 nm). Similarly, extending the sonication time up to 60 minutes led to the formation of small, spherical particles (< 200 nm), but at 90 minutes, small aggregates began to appear. The optimization of carbohydrate and antigen adsorption was conducted using the Langmuir adsorption isotherm. Upon analysis, it was determined that the binding constant of cellobiose-coated aquasomes exceeded that of trehalose-coated aquasomes, and trehalose- coated aquasomes exhibited a higher amount of sugar adsorbed per milligram compared to cellobiose-coated aquasomes. Therefore, trehalose demonstrated lower adsorption efficiency than cellobiose, but formed a stronger bond. In terms of packing, trehalose exhibited less packing than cellobiose, but its arrangement led to the achievement of the lowest adsorption energy. The drug loading of ceramic cores was prepared without carbohydrate coating to observe its effect on drug loading. It was noted that the drug loading was lower in comparison to the carbohydrate-coated core. Therefore, the carbohydrate film on the ceramic core enhances the rate of drug adsorption.[20]

Nanocrystalline carbon ceramic, diamond particle

Diamond particles, nanocrystalline carbon ceramic can also be utilized for core synthesis following thorough cleansing and sonication. The typical characteristic of diverse cores is their crystalline nature, with a size ranging between 50 and 150 nm when incorporated into the manufacturing processes, displaying a clean appearance and acting as reactive species. The most commonly employed material for core production is a highly uniform ceramic structure, which collectively offers a high level of surface energy, facilitating the adhesion of a polyhydroxy oligomeric surface film. The centrifuged precipitated cores have the sodium chloride removed by washing with an ample amount of distilled water, which is produced during the reaction. To aggregate the particles to the required size, the precipitate is re-suspended in distilled water and passed through a fine membrane filter.[29]

Loading of drug

The coated particle is loaded with the preferred drug through adsorption. A drug solution with a known concentration is made in an appropriate pH buffer, and the coated particles are introduced into it. The mixture is then freeze-dried to produce the aquasomes.[28]

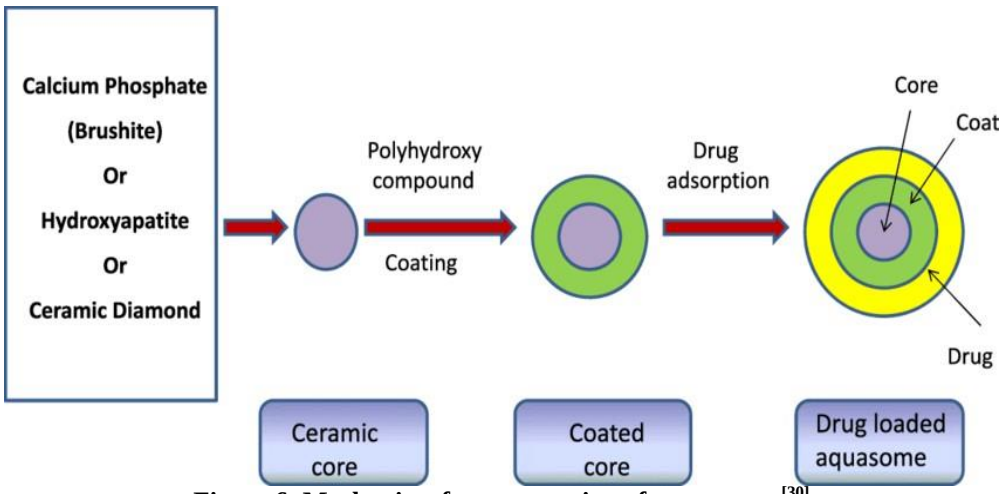

Figure 6: Mechanism for preparation of aquasomes.[30]

Characterization of aquasomes

Characterization of core

i. Size distribution

The particle size of aquasomes should fall within the 60 to 300 nm range. Aquasomes are primarily used for drug release and targeting drug molecules. Therefore, the particle size and distribution play a significant role as they affect important colloidal properties like surface area, rheology, film gloss, and packing density.[36] Understanding the physical and chemical properties of aquasomes is also facilitated by particle size and distribution analysis. Size distribution analysis and

morphological characterization are typically conducted using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).[36, 37] In the case of TEM, particle size is determined after negative staining with phosphotungstic acid, while for SEM, samples are coated with gold on a specimen stub using double-sided adhesive tape to determine the particle size. They found the particle size around 90 nm which was well within a range of 60–300 nm and was significantly less as compared to pure pimozide drug (210 nm). This concludes that the reduction of particle size to nano meter range has been occurred in aquasomes formulations.

ii. Structural analysis

The analysis of structure is extremely important as it helps in identifying and determining the structure of a molecule. Structural analysis can assess whether the specific structural design will be able to withstand internal and external stresses, and it also describes the forces expected for the design. FTIR spectroscopy can be utilized for structural analysis. By using the potassium bromide (KBr) sample disk method, both the core and the coated core can be examined by recording their IR spectra in the wave number range of 400 to 4000 cm^{-1}. The characteristic peaks are observed and compared with reference peaks. This method is used to analyze the drug, ceramic core, core coated with carbohydrate, and drug- loaded formulation. The stability of the drug in the formulation can be estimated through FTIR analysis.[21,39–42]

iii. Crystallinity

The thermal and mechanical properties of aquasomes can be influenced by the level of crystallinity. Therefore, X-ray diffraction study is conducted to comprehend the kinetics and process of crystallization. The ceramic core undergoes preparation and analysis to determine its crystalline or amorphous characteristics using X-ray diffraction.[42,43]

Characterization of coated material

i. Carbohydrate coating

The presence of carbohydrate on the core is assessed using the anthrone reaction, Concanavalin A-induced aggregation method,[44, 23] and the phenol sulphuric acid method.[45,46] The anthrone reaction, a calorimetric method, is utilized to quantify the total sugar content. The application of sugar onto the ceramic core can be confirmed through the anthrone method, which identifies the unbound residual sugar or sugar remaining after coating. The anthrone reaction is a rapid and suitable technique for distinguishing between free and polysaccharide-bound carbohydrates. Under acidic conditions, carbohydrates undergo hydrolysis to produce hydroxymethyl furfural. This furfural then reacts with an anthrone reagent, resulting in the formation of a blue-green coloured complex, and its absorbance can be measured at 620 nm using a UV-visible spectrophotometer with glucose as a standard. The phenol-sulfuric acid method is a colorimetric technique utilized for the estimation of total carbohydrates, including mono-, di-, oligo-, and polysaccharides present in the sample. This method is known for its speed and simplicity. In the phenol-sulfuric acid method, carbohydrates are dehydrated to form a furfural derivative in the presence of concentrated sulphuric acid, which then undergoes a reaction with phenol to produce a yellow-gold colour.[45, 46]

ii. Zeta potential measurement

The zeta potential indicates the electrostatic attraction or repulsion between particles. The surface charge intensity and nature of aquasomes are crucial for its electrostatic interaction with bioactive compounds and its interaction with the biological environment. Measuring the zeta potential allows for predicting storage stability. To prevent particle aggregation, it is important to achieve a high zeta potential value, whether negative or positive.[23,28]

Characterization of drug-loaded aquasomes

i. Drug loading

Aquasomes that are successful should possess a high capacity for loading drugs, which in turn reduces the amount of matrix material required for administration. A smaller hydroxyapatite core size results in a larger surface area, thereby increasing the drug loading capacity in the formulation. In aquasomes, drug loading is achieved by incubating the hydroxyapatite-coated core in a concentrated solution of drug/protein. The diffusion and subsequent drug release from the aquasomes are determined by the coating of carbohydrate on the core. The drug payload is determined by accurately weighing and dispersing the aquasomes formulation in distilled water.[47,48,49]

ii. In-vitro drug release studies

The primary objective of studying aquasomes is to effectively administer drugs and comprehend how the drug molecule is released as needed. It is crucial to investigate the drug release mechanism and the factors influencing it. The release of drugs from aquasomes is regulated by the surface chemistry. The rate of drug release depends on the drug's solubility, desorption of adsorbed drug, and drug diffusion through the matrix. When the drug is included during the formulation process, the system will initially display a small burst effect followed by sustained release. If the particle is coated with a polymer, the drug release occurs through diffusion or desorption from the polymeric membrane. Additionally, the release of the drug is influenced by the particle size, with smaller particles having a higher surface area-to-volume ratio, leading to faster drug release from aquasomes.[47,50,51,52]

iii. In-process stability studies

The aquasomes are stabilized by polyhydroxy oligomer using covalent, ionic, and exotropic forces, serving as a non-denaturing solid carrier for delivering drug

molecules. To assess stability, the formulation was stored in sealed vials under nitrogen in a refrigerator for one month. The formulation underwent 5 freeze-thaw cycles between 4 and 25 °C for 24 hours. It was observed that there was no desorption of haemoglobin from the formulation because trehalose created a pseudo-hydrated layer around the protein, stabilizing the haemoprotein molecules.[53,54]

Gentamicin-loaded aquasomes as potential antibiotic delivery systems for the treatment of bone infections

One of the major and prevailing complications in orthopaedic surgical procedure and fracture treatments are pathogenic infections which may lead to rejection from host, disunions, or ultimate bone destruction. The infection may be restricted to one portion of the bone or can involve several regions including the marrow, cortex, periosteum, and the surrounding soft tissue. Among pathogenic microorganisms, *S. aureus* is by far the most commonly involved in osteomyelitis in humans, followed by Enterobacteriaceae and Pseudomonas species. The early colonization of host tissues or implanted biomaterials is believed to depend on The ability to adhere S. aureus has various adhesins on its surface, each of which interacts specifically with a different host protein, such as fibrinogen, fibronectin, collagen, vitronectin, laminin, thrombospondin, bone sialoprotein, elastin, or von Willebrand factor. Both S. aureus and S. epidermidis are capable of creating biofilms, which are microbial communities characterized by cells attaching to a substratum or interfacing with each other. These biofilms are challenging to treat with antimicrobial agents.[58,59]

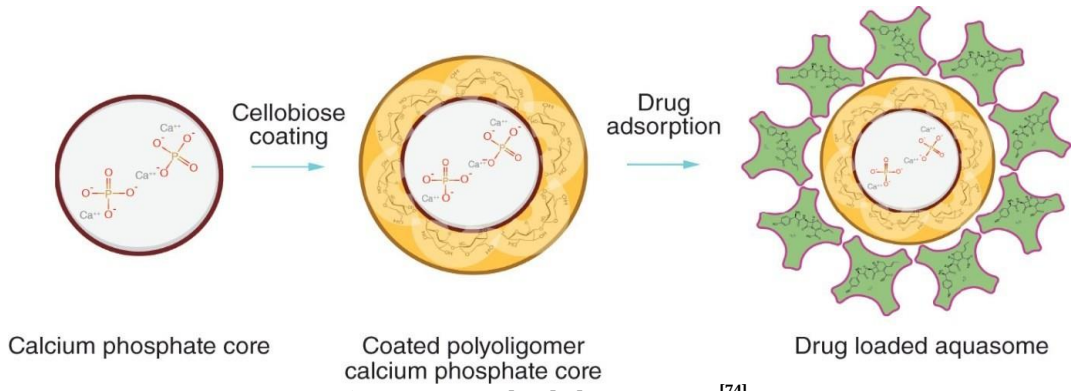

Figure 7: Drug loaded aquasomes.[74]

Hydroxyapatite as a biomaterial for antibiotic local delivery

Drug delivery systems that demonstrate the characteristics of biocompatible bio ceramics are in high demand and are therefore manufactured in forms such as powders, blocks, cements, scaffolds, porous devices, and coatings for this specific application. Synthetic hydroxyapatite (HA) is a prominent example often cited for this characteristic. HA constitutes the inorganic part of bone composition. Chemically and structurally, synthetic HA closely resembles the mineral phase of bone, displaying impressive osteogenic and osteoconductive attributes. HA demonstrates outstanding biocompatibility due to its chemical and structural resemblances to the inorganic phase of human bone. Research studies have extensively examined the utilization of ceramic materials HA as carriers for antibiotics to treat bone infections, due to their close resemblance in chemical composition to the mineral phase of bones.[60,61,62,63]

HA cement loaded with gentamicin has been studied in vitro and in vivo to determine its efficacy in treating chronic osteomyelitis following trauma. After 7 days of culture, no growth was observed in animals treated with HAC/gentamicin. The HAC/gentamicin-treated group showed no histopathological signs of infection, whereas other groups exhibited various stages of chronic osteomyelitis. The antibacterial activation of micro-porous HA was assessed by testing three different antibiotics, including gentamicin, in comparison to dense HA. Antibacterial inhibition tests were conducted on various pathogenic bacteria to evaluate the adsorption of antibiotics and their microbiological effectiveness after being loaded onto the HA. The findings indicated a significantly higher adsorbed amount on the micro-porous HA compared to the dense HA, along with an observed prolongation in the release of antibiotics.[64,65]

Preparation of Gentamicin-loaded aquasomes

(a) Coating phase

Weighing 100 mg of nano-sized hydroxyapatite (HA) powder, it was then placed in freeze dryer vials with a capacity of 10 ml. A 0.1 M trehalose solution was prepared using distilled water, and 10 ml of the trehalose solution was added to each vial containing the HA powder. The resulting suspension was mixed for 2.5 hours.[66]

(b) Freeze Drying and Secondary drying phases

The HA-trehalose suspension underwent further centrifugation at 1000 rpm for 5 minutes. The supernatant was then discarded, leaving behind the coated HA nanocores. These coated HA nanocores were rinsed with 2.5 ml of phosphate buffer saline solution and centrifuged again at 100 rpm for 5 minutes.

Following this, the coated HA nanocores were manually freeze-dried by freezing the HA slurries at –20°C to expedite the freezing process while lowering the shelf temperature of the freeze dryer to –32°C, a temperature slightly higher than the Tg of trehalose, which is –34°C. The coated HA nanocore slurries were then transferred to the cooled freeze dryer shelves, and the condenser and vacuum pump (500µBar) were activated to initiate the freeze-drying process. This freeze-drying step lasted for 12 to 16 hours. Subsequently, the shelf temperatures were increased to 30°C to remove any remaining non-sublimed moisture content. This drying step lasted for 4 hours.[66]

Drug loading phase: Distilled water was used to prepare solutions of gentamicin sulphate at a concentration of 2 mg/ml. Then, 10 ml of the 2 mg/ml gentamicin solution was introduced into each vial that held freeze-dried coated HA nanocores. The resulting suspension underwent coating and subsequent freeze-drying, following the same process detailed in phases (a) and (b) for coating and freeze-drying, and secondary drying.[66]

Characterization of gentamicin-loaded aquasomes

i. **In-vitro Release Study of Gentamicin Loaded Aquasomes:** Using the partial replacement method, 1 ml samples were taken and replaced with fresh pre-warmed release medium of the same volume at hourly time intervals for the duration of 8 hours. Samples were analyzed using UV spectrophotometry at the maximum detection wavelength (λ max) of gentamicin, 257 nm. Gentamicin standards were prepared with medium as diluent and a calibration curve was used to determine corresponding gentamicin concentrations in supernatant solutions according to Beer-Lambert's law.

ii. **Bacterial zone inhibition assay:** Any colonies present were counted and the colony forming units (CFU) were calculated using the below formula:

CFU/ml = Average colony count x 50 x Dilution used

iii. **In-vitro cell toxicology assay:** A thiazolyl blue tetrazolium bromide assay was performed to measure cell death after exposure of cells to different concentrations of gentamicin for 24 hours, representing short term exposure to aquasomes (acute toxicity). The cell viability was calculated using the below formula:

$$\text{Cell Viability (\%)} = \frac{[\text{Diameter of Treated Cell} - \text{Diameter of Blank}] \times 100\%}{[\text{Diameter of Untreated Cell} - \text{Diameter of Blank}]}$$

Gentamicin, an aminoglycoside antibiotic, is commonly utilized to prevent bacterial infections near bone implants due to its broad spectrum of activity against bacteria, particularly gram-negative bacteria. Its mechanism of action involves binding to the 30S subunit of the bacterial ribosome, thereby disrupting protein synthesis. Orally administered gentamicin is ineffective, a common trait among aminoglycosides, as it is absorbed from the small intestine, passes through the portal vein to the liver, and becomes inactivated. As a result, it can only be administered intravenously, intramuscularly, or topically. However, delivery via these routes is often ineffective because the drug struggles to reach the infection site in bone tissue, especially in necrotic or avascular tissue post-surgery. Increasing systemic doses to overcome this limitation is not feasible due to organ toxicity associated with higher antibiotic concentrations. The effectiveness of gentamicin-loaded aquasomes in killing S. aureus bacteria was assessed for potential use in treating localized bone infections. Bone infections are mainly caused by S. aureus, so the goal of this study was to simulate a bone disease situation and demonstrate how effective gentamicin-loaded aquasomes are as a method for delivering antibiotics.[66]

The findings indicate that aquasomes loaded with gentamicin have demonstrated effectiveness as a delivery system for antibiotics at the nano level, showing great potential for treating bone infections caused by both low and high levels of bacteria. For low and high levels of S. aureus bacteria (O.D=0.5; O.D=1), gentamicin released from the loaded aquasomes exhibited bactericidal activity against S. aureus within 2 hours and 5 hours, respectively. Statistical analysis revealed that the disparities between the samples of colony- forming units (CFUs) from the test groups (gentamicin loaded-aquasomes) and the control groups (S. aureus) were statistically significant ($p<0.0001$). S. aureus is commonly associated with osteomyelitis, and thus, demonstrating the bactericidal effect of gentamicin-loaded aquasomes holds promise for the in vivo treatment of bone infections. These results demonstrate that gentamicin-loaded aquasomes can offer a protective bactericidal barrier for 24 hours at the site of local prophylactic application.[66]

Benefits of Gentamicin-loaded Aquasomes in Osteomyelitis[67-70]

i. **Precise delivery:** Gentamicin can be encapsulated within aquasomes, enabling targeted delivery to infected bone tissue, thereby increasing local drug concentration while minimizing systemic exposure and side effects.

ii. **Controlled release:** The structure of aquasomes allows for the controlled and sustained release of gentamicin, ensuring prolonged therapeutic levels at the infection site. Enhanced Solubility: Aquasomes can improve the solubility of gentamicin, thereby enhancing its bioavailability and effectiveness in combatting bacterial infections in the bone.

iii. **Biocompatible materials:** The materials used to create aquasomes are often biocompatible, reducing the risk of adverse reactions and enhancing patient safety.

iv. **Stability:** Gentamicin-loaded aquasomes can provide stability to the drug, protecting it from degradation before it reaches the target site.
v. **Biofilm disruption:** The localized high concentration of gentamicin can help disrupt bacterial biofilms, which are often resistant to standard antibiotic treatments in osteomyelitis.
vi. **Reduced dosing frequency:** With sustained release mechanisms, gentamicin-loaded aquasomes may reduce the need for frequent dosing, improving patient compliance and convenience.
vii. **Versatile administration:** Aquasomes can be designed for various routes of administration, including local injection, which is particularly advantageous in targeting osteomyelitis.

Other applications of aquasomes

i. **Insulin delivery:** Aquasomes were created by using a calcium phosphate ceramic core for delivering insulin through injections. The core was coated with various disaccharides like cellobiose, trehalose, and pyridoxal-5-phosphate. Afterward, the drug was attached to these particles using an adsorption method. The extended effectiveness was due to the slow release of the drug from the carrier and the structural integrity of the peptide. Additionally, this study also achieved the optimal controlled release of insulin.[43,67]

ii. **Oral delivery of acid-labile enzyme:** The ceramic core containing the acid-labile enzyme Serrato peptidase was delivered orally in nanosized form. Chitosan was used to prepare the nanocore through colloidal precipitation under sonication at room temperature. The enzyme was protected by encapsulating the enzyme-laden core in alginate gel. TEM images revealed that the particles were spherical and had an average diameter of 925 nm. The particles exhibited an enzyme-loading efficiency of around 46%. These aquasomes were shown to preserve the structural integrity of the enzymes, leading to enhanced pharmacological response.[68]

iii. **As oxygen transporter:** Hydroxyapatite cores were prepared using carboxylic acid- terminated half-generation poly(Amidoamine) dendrimers as templates or crystal modifiers. These cores were then coated with trehalose, followed by haemoglobin adsorption. The particle size was determined to be in the nano meter range, with a loading capacity of approximately 13.7 mg of haemoglobin per gram of the core. The oxygen-binding properties of the aquasomes were examined and compared to those of fresh blood and haemoglobin solution. Hill coefficient values were calculated for fresh blood, haemoglobin solution, and the aquasomes formulation, revealing that the properties of haemoglobin, including its oxygen-carrying capacity, were retained by the aquasomes. Studies conducted in rats demonstrated that aquasomes show promise as an oxygen carrier. Additionally, it was observed that the formulation maintained its oxygen-binding characteristics for over 30 days.[54]

iv. **Antigen delivery:** The excipients commonly used to enhance antigen resistance tend to alter the antigen's conformation through surface adsorption or by shielding the functional groups. A new organically modified ceramic antigen delivery vehicle was proven to be effective. Due to its high surface energy, diamond was the primary option for adsorbing and bonding with cellobiose, providing a colloidal surface capable of forming hydrogen bonds with the proteinaceous antigen.[69]

v. **Delivery of drug:** Aquasomes containing indomethacin were created by developing an inorganic core of calcium phosphate enclosed with a lactose film and then adsorbing indomethacin as a low-solubility drug. The aquasomes underwent characterization for structural analysis, particle size, and morphology using X-ray powder diffractometry, TEM, and SEM. The particle size of the drug-loaded aquasomes was determined to be within the range of 60–120 nm. SEM and TEM techniques verified the spherical shape of the aquasomes.[43]

vi. **For delivery of gene:** Aquasomes are being researched for their ability to deliver genes, demonstrating an effective delivery system for genetic material. Research indicates that aquasomes can safeguard and preserve the structural integrity of the gene segment. A proposed five-layered structure consists of a ceramic nanocrystalline core, a polyhydroxy oligomeric film coating, a non-covalently bound layer containing the therapeutic gene segment, an additional carbohydrate film, and a targeting layer composed of conformationally conserved viral membrane proteins for gene therapy. The aquasomes vehicle is anticipated to offer the potential benefits of viral vectors while simultaneously reducing the risk of irrelevant gene integration.[69]

vii. **For delivery of enzymes:** Aquasomes are also utilized for transporting enzymes like DNAase and pigment/dyes because the activity of enzymes varies with molecular conformation, and the cosmetic properties of pigments are sensitive to molecular chains. DNAase, an enzyme used to treat cystic fibrosis, was effectively attached to aquasomes, targeted to a specific site, and produced a significant therapeutic effect. A noticeable preservation of biological activity was seen with DNase immobilized on the solid phase of a colloidal calcium phosphate nanoparticle coated with polyhydroxy oligomeric films.[70]

viii. **For vaccine delivery:** The use of aquasomes for delivering vaccines offers several advantages. These include the ability to stimulate both cellular and humoral immune responses to antigens attached to the aquasomal surface. In vaccine delivery, the outer surface of aquasomes, to which antigens are covalently linked, is made up of polyhydroxy oligomers or sugar molecules like cellobiose, trehalose, maltose, sorbitol, and lactose. Additionally, it contains substances like pyridoxal-5-phosphate and sodium citrate, which have allosteric effects and protect the protein from denaturation and degradation. The presence of a carbohydrate sheath on ceramic particles confirms the surface characteristics of aquasomes, such as their three-dimensional conformations, the ability for internal molecular rearrangement initiated by intermolecular interactions, and autonomous bulk movement.[60]

CONCLUSION

Aquasomes as a drug delivery system for bone infections. Aquasomes, a drug-loaded nanoparticles, have been evaluated for their efficacy in treating bone infections caused by bacteria. Osteomyelitis, a progressive infection, can progress to a chronic stage due to bacterial inoculation, trauma, necrosis, or foreign material. The quality of debridement is crucial for successful management. Aquasomes have a three-layered structure, with a solid core and a carbohydrate coat. The method for preparation of core varies based on the type of core used. The presence of carbohydrate on the core is assessed using various methods. The drug loading process involves incubating the hydroxyapatite-coated core in a concentrated solution of drug/protein. The surface chemistry of aquasomes regulates drug release and helps in the long-range attraction of self-assembling subunits and stabilization of folded protein structures. Aquasomes are often biocompatible, reducing the risk of adverse reactions and enhancing patient safety. Diamond was the primary option for adsorbing and bonding with cellobiose due to its high surface energy. Aquasomes can safeguard and preserve the structural integrity of the gene segment, making them a promising option for vaccine delivery.

REFERENCES

1. Joyner AL, Smith DT. Acute staphylococcus osteomyelitis. Surg Gynecol Obstet, 1936; 63: 1-6.
2. Smith IM, Austin OM, Batchelor AG. The treatment of chronic osteomyelitis: a 10 year audit. Journal of Plastic, Reconstructive & Aesthetic Surgery, 2006; 1, 59(1): 11-5.
3. Gristina AG. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science, 1987; 25, 237(4822): 1588-95.
4. Waldvogel FA, Medoff G, Swartz MN. Osteomyelitis: a review of clinical features, therapeutic considerations, and unusual aspects. New England Journal of Medicine, 1970; 22, 282(4): 198-206.
5. A b Carek, P.J.; L.M. Dickerson; J.L. Sack (2001-06-15). "Diagnosis and management of osteomyelitis.". Am Fam Physician, 63(12): 2413–20.
6. Bhowmik D, Bhanot R, Gautam D, Rai P, Kumar KP. Osteomyelitis-symptoms, causes and treatment. Research Journal of Science and Technology, 2018; 10(2): 165-77.
7. Mader JT, Mohan D, Calhoun J. A practical guide to the diagnosis and management of bone and joint infections. Drugs, 1997; 54: 253-64.
8. TR T. Oral antibiotic therapy for skeletal infection of children. II. Therapy of osteomyelitis and suppurative arthritis. J. Pediatr, 1978; 92: 485-90.
9. Walikhan O, Bismellah N. Epidemiologic study of chronic osteomyelitis in Paktya and Kabul, Afghanistan.
10. Tetsworth K, Cierny III G. Osteomyelitis debridement techniques. Clinical Orthopaedics and Related Research®, 1999; 1, 360: 87-96.
11. Lazzarini L, Lipsky BA, Mader JT. Antibiotic treatment of osteomyelitis: what have we learned from 30 years of clinical trials?. International journal of infectious diseases, 2005; 1, 9(3): 127-38.
12. Jain NK, editor. Advances in controlled and novel drug delivery. CBS publishers & distributors, 2008.
13. Kossovsky N, Gelman A, Sponsler E. Cross linking encapsulated hemoglobin with solid phase supports: lipid enveloped hemoglobin adsorbed to surface modified ceramic particles exhibit physiological oxygen lability. Artificial Cells, Blood Substitutes, and Biotechnology, 1994; 1, 22(3): 479-85.
14. Jain NK, Umamaheshwari RB. Control and novel drug delivery systems. Pharmaceutical product development, 2006; 21: 419-55.
15. Jagdale S, Karekar S. Bird's eye view on aquasome: Formulation and application. Journal of Drug Delivery Science and Technology, 2020; 1, 58: 101776.
16. Shahabade Gururaj S, Bhosale Ashok V, Mutha Swati S, Bhosale Nilesh R, Khade Prashant H. An overview on nanocarrier technology-Aquasomes. Journal of Pharmacy Research, 2009; 2(7).
17. Wani SU, Yerawar AN. Aquasomes: A novel nanocarrier for drug delivery. ChemInform, 2011; 15: 42(46).
18. Gholap AD, Borude SS, Mahajan AM, Gholap MA. Aquasomes: A potential drug delivery carrier. Pharmacol Online, 2011; 3: 230-7.
19. Kossovsky N, Gelman A, Rajguru S, Nguyen R, Sponsler E, Hnatyszyn HJ, Chow K, Chung A, Torres M, Zemanovich J, Crowder J. Control of molecular polymorphisms by a structured carbohydrate/ceramic delivery vehicle—aquasomes. Journal of controlled release, 1996; 1, 39(2-3): 383-8.
20. Vengala P, Aslam S, Subrahmanyam CV. Development and in vitro evaluation of ceramic nanoparticles of piroxicam. Latin American Journal of Pharmacy, 2013; 1, 32(8): 1124-30.

21. Vengala P, Dintakurthi S, Subrahmanyam CV. Lactose coated ceramic nanoparticles for oral drug delivery. Journal of Pharmacy Research, 2013; 1, 7(6): 540-5.
22. Tas AC. X-ray diffraction data for flux-grown calcium hydroxyapatite whiskers. Powder Diffraction, 2001; 16(2): 102-6.
23. Goyal AK, Khatri K, Mishra N, Mehta A, Vaidya B, Tiwari S, Vyas SP. Aquasomes—a nanoparticulate approach for the delivery of antigen. Drug development and industrial pharmacy, 2008; 1, 34(12): 1297-305.
24. Weber M, Möller K, Welzeck M, Schorr J. Artificial Self-Assembling Systems for Gene Delivery. Felgner, P, 1996; 28-9.
25. Vengala P, Aslam S, Subrahmanyam CV. Development and in vitro evaluation of ceramic nanoparticles of piroxicam. Latin American Journal of Pharmacy, 2013; 1, 32(8): 1124-30.
26. Patil S, Pancholi SS, Agrawal S, Agrawal GP. Surface-modified mesoporous ceramics as delivery vehicle for haemoglobin. Drug delivery, 2004; 1, 11(3): 193-9.
27. Kommineni S, Ahmad S, Vengala P, Subramanyam CV. Sugar coated ceramic nanocarriers for the oral delivery of hydrophobic drugs: Formulation, optimization and evaluation. Drug Development and Industrial Pharmacy, 2012; 1, 38(5): 577-86.
28. Umashankar MS, Sachdeva RK, Gulati M. Aquasomes: a promising carrier for peptides and protein delivery. Nanomedicine: Nanotechnology, Biology and Medicine, 2010; 1, 6(3): 419-26.
29. Jagdale S, Karekar S. Bird's eye view on aquasome: Formulation and application. Journal of Drug Delivery Science and Technology, 2020; 1, 58: 101776.
30. Asfour MH. Advanced trends in protein and peptide drug delivery: a special emphasis on aquasomes and microneedles techniques. Drug delivery and translational research, 2021; 11: 1-23.
31. Beherei HH, El-Bassyouni GT, Mohamed KR. Modulation, characterization and bioactivity of new biocomposites based on apatite. Ceramics international, 2008; 1, 34(8): 2091-7.
32. Mohamed KR, Mostafa AA. Preparation and bioactivity evaluation of hydroxyapatite-titania/chitosan-gelatin polymeric biocomposites. Materials Science and Engineering: C, 2008; 1, 28(7): 1087-99.
33. El Kady AM, Mohamed KR, El-Bassyouni GT. Fabrication, characterization and bioactivity evaluation of calcium pyrophosphate/polymeric biocomposites. Ceramics International, 2009; 1, 35(7): 2933-42.
34. Paul W, Sharma CP. Bioceramics, towards nano-enabled drug delivery: a mini review. Trends Biomater Artif Organs, 2005; 1: 7-11.
35. Hnatyszyn HJ, Kossovsky N, Gelman A, Sponsler E. Drug delivery systems for the future. PDA Journal of Pharmaceutical Science and Technology, 1994; 1, 48(5): 247- 54.
36. Pandey S, Badola A, Bhatt GK, Kothiyal P. An overview on aquasomes. Int J Pharm Chem Sci, 2013; 2(3): 1282-7.
37. Crowe JH, Crowe LM, Chapman D. Infrared spectroscopic studies on interactions of water and carbohydrates with a biological membrane. Archives of biochemistry and biophysics, 1984; 1, 232(1): 400-7.
38. Jain SS, Jagtap PS, Dand NM, Jadhav KR, Kadam VJ. Aquasomes: A novel drug carrier. Journal of Applied Pharmaceutical Science, 2012; 30: 184-92.
39. Manikiran SS, Krishna CS, Rao R. Aquasomes: role to deliver bioactive substances. Research Journal of Pharmaceutical Dosage Forms and Technology, 2010; 2(6): 356-60.
40. Narang N. Aquasomes: Self-assembled systems for the delivery of bioactive molecules. Asian Journal of Pharmaceutics (AJP), 2012; 6(2).
41. Pavani V, Dintakurthi S, Aslam S, Gollagudem R, Pabbapi K. Aquasomes: a novel drug carrier system. ChemInform, 2013; 28: 44(22).
42. Nanjwade BK, Hiremath GM, Manvi FV, Srichana T. Formulation and evaluation of etoposide loaded aquasomes. Journal of Nanopharmaceutics and Drug Delivery, 2013; 1, 1(1): 92-101.
43. Rojas-Oviedo I, Salazar-Lopez RA, Reyes-Gasga J, Quirino-Barreda CT. Elaboration and structural analysis of aquasomes loaded with indomethacin. European journal of pharmaceutical sciences, 2007; 1, 32(3): 223-30.
44. Kommineni S, Ahmad S, Vengala P, Subramanyam CV. Sugar coated ceramic nanocarriers for the oral delivery of hydrophobic drugs: Formulation, optimization and evaluation. Drug Development and Industrial Pharmacy, 2012; 1, 38(5): 577-86.
45. Vengala P. Carbohydrate stabilized ceramic nanoparticles for the delivery of a poorly soluble drug, lornoxicam. Asian Journal of Pharmaceutics (AJP), 2017; 11: 11(03).
46. Nielsen SS. Phenol-sulfuric acid method for total carbohydrates. Food analysis laboratory manual, 2010; 47-53.
47. Singh R, Lillard Jr JW. Nanoparticle-based targeted drug delivery. Experimental and molecular pathology, 2009; 1, 86(3): 215-23.
48. Dasgupta S, Bandyopadhyay A, Bose S. Reverse micelle-mediated synthesis of calcium phosphate nanocarriers for controlled release of bovine serum albumin. Acta biomaterialia, 2009; 1, 5(8): 3112-21.
49. Lowry D, Abdulrazzaq F. Effect of Temperature and Time on the Coating and Loading Processes of Aquasome Manufacture. CRS Newsletter: A publication of the Controlled Release Society, 2012; 29(4).
50. Kumar JM, Kumar VV, Mounica R, Bolla SP, Pavani M. Aquasomes-the best carriers for protein and peptide delivery. Asian Journal of Pharmaceutical Research and Development, 2013; 1.
51. Izumimoto M, Zhu YongNing ZY. Influence of

saccharides on the stabilization of frozen hemeprotein.
52. Sharma S, Gupta A, Niranjan H, Goyal M. Aquasome: A Novel Drug Delivery Approach Using Nanocrystaline Biomaterial. Current Research in Pharmaceutical Sciences. 2012 Apr 7; 16-21.
53. Cherian AK, Rana AC, Jain SK. Self-assembled carbohydrate-stabilized ceramic nanoparticles for the parenteral delivery of insulin. Drug development and industrial pharmacy, 2000; 1, 26(4): 459-63.
54. Khopade AJ, Khopade S, Jain NK. Development of hemoglobin aquasomes from spherical hydroxyapatite cores precipitated in the presence of half-generation poly (amidoamine) dendrimer. International journal of pharmaceutics, 2002; 8, 241(1): 145- 54.
55. Chaudhari MJ, Pandya DP, Thakkar PP, Soni AM, Modi DA. Aquasomes: a novel drug delivery system. ChemInform, 2013; 17: 44(38).
56. Gholap AD, Borude SS, Mahajan AM, Gholap MA. Aquasomes: A potential drug delivery carrier. Pharmacol Online, 2011; 3: 230-7.
57. Kallingal A, Suresh AM. Aquasomes nanocarriers for delivery of small molecules. Int J Med Phar Sci, 2021; 11(07): 1.
58. Gupta AK, Gupta D, Gupta V. Aquasomes: a self-assembled nano-particulate carrier system. Int J Cur Res Rev, 2021; 13(4): 44-52.
59. El-Husseiny M, Patel S, MacFarlane RJ, Haddad FS. Biodegradable antibiotic delivery systems. The Journal of Bone & Joint Surgery British Volume, 2011; 1, 93(2): 151-7.
60. Soriano I, Evora C. Formulation of calcium phosphates/poly (d, l-lactide) blends containing gentamicin for bone implantation. Journal of controlled release, 2000; 31, 68(1): 121-34.
61. Mizushima Y, Ikoma T, Tanaka J, Hoshi K, Ishihara T, Ogawa Y, Ueno A. Injectable porous hydroxyapatite microparticles as a new carrier for protein and lipophilic drugs. Journal of controlled release, 2006; 10, 110(2): 260-5.
62. Itokazu M, Yang W, Aoki T, Ohara A, Kato N. Synthesis of antibiotic-loaded interporous hydroxyapatite blocks by vacuum method and in vitro drug release testing. Biomaterials, 1998; 1, 19(7-9): 817-9.
63. Arcos D, Vallet-Regi M. Bioceramics for drug delivery. Acta Materialia, 2013; 1, 61(3): 890-911.
64. Baro M, Sanchez E, Delgado A, Perera A, Evora C. In vitro–in vivo characterization of gentamicin bone implants. Journal of Controlled Release, 2002; 30, 83(3): 353-64.
65. Joosten U, Joist A, Frebel T, Brandt B, Diederichs S, Von Eiff C. Evaluation of an in situ setting injectable calcium phosphate as a new carrier material for gentamicin in the treatment of chronic osteomyelitis: studies in vitro and in vivo. Biomaterials, 2004; 1, 25(18): 4287-95.
66. Chai F, Hornez JC, Blanchemain N, Neut C, Descamps M, Hildebrand HF. Antibacterial activation of hydroxyapatite (HA) with controlled porosity by different antibiotics. Biomolecular engineering, 2007; 1, 24(5): 510-4.
67. Udo-Chijioke O. Aquasomes: multilayered nanoparticular drug delivery systems, 2016.
68. Paul W, Sharma CP. Porous hydroxyapatite nanoparticles for intestinal delivery of insulin. Trends Biomater Artif Organs, 2001; 14: 37-8.
69. Rawat M, Singh D, Saraf S, Saraf S. Development and in vitro evaluation of alginate gel–encapsulated, chitosan-coated ceramic nanocores for oral delivery of enzyme. Drug development and industrial pharmacy, 2008; 1, 34(2): 181-8.
70. Kossovsky N, Gelman A, Sponsler EE, Hnatyszyn HJ, Rajguru S, Torres M, Pham M, Crowder J, Zemanovich J, Chung A, Shah R. Surface-modified nanocrystalline ceramics for drug delivery applications. Biomaterials, 1994; 1, 15(15): 1201-7.
71. Chaudhary JS. Aquasomes; A new approach for delivering therapeutics: an overview. Asian Journal of Pharmaceutics (AJP), 2018; 18: 12(02).
72. Haberland ME, Fless GM, Scanu AM, Fogelman AM. Malondialdehyde modification of lipoprotein (a) produces avid uptake by human monocyte-macrophages. Journal of Biological Chemistry, 1992; 25, 267(6): 4143-51.
73. Patel S, Aundhia C, Seth A, Shah N, Pandya K, Patel D. Aquasomes: a novel approach in drug carrier system.
74. Çağdaş M, Sezer AD, Bucak S. Liposomes as potential drug carrier systems for drug delivery. Application of nanotechnology in drug delivery, 2014; 25, 1: 1-50.
75. Shanmugam B, Srinivasan UM. Aquasomes nano-formulation for controlled release of drug and improved effectiveness against bacterial infections. Therapeutic Delivery, 2024; 1, 15(2): 95-107.