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Recent Advances in the Fabrication of Gelatin based Nanoparticles Using Desolvation Techniques and Their Pharmaceutical Applications

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Abstract

Gelatin based nanoparticles (GNPs) produced using desolvents of gelatine have become a high interest as a biocompatible and versatile nanoparticle system for pharmaceutical and biological field. In this manuscript, an in depth analysis of the basic principles of desolvation is presented which includes solvent induced aggregation, polymer solvent thermodynamics, and gelatin type, molecular weight distribution, and critical process parameters. Classical two step desolvation and optimized one step processes are analyzed and compared to new invention scenarios of green, microfluidics assisted, ultrasonics, supercritical fluid and AI guided fabrication processing strategies, focusing on scalability, reproducibility and environmentally inspired strategies. Advances in cross linking methodologies that employ chemical, natural and physical stabilization approaches are reviewed in the light of stability, biodegradation, and drug release behaviour of nanoparticles. Characterization techniques are highlighted and the relevance of *invitro* and in vivo correlations for clinical translation is made. Finally, the wide ranging pharmaceutical potential of GNPs in drug, gene, and vaccine delivery, diagnostics, and regenerative medicine is outlined, making desolvation derived gelatin nanoparticles a promising platform for next generation precision nanomedicine.

Keywords: Gelatin Nanoparticle; Desolvation; Nanocarriers; Biomedical Applications

Introduction

In the last twenty years, nanoparticles made of biopolymers have been gaining a popularity in pharmaceutical and biomedical studies because of the high level of biocompatibility, finely adjustable physicochemical characteristics, and flexibility structure. Gelatin is one of these systems and has become a promising polymeric matrix by which nanoparticles can be produced especially a partially hydrolyzed derivation of collagen. The amphoteric nature of gelatin, which is combined with the ability to degrade, lack immunogenicity, to form stable colloidal suspensions, makes this polysaccharide applicable for drug delivery, gene delivery, and regenerative medicine practices [1]. In addition to this, gelatin nanoparticles (GNPs) require alterable surface functionalities in which reactive amino, carboxyl, and hydroxyl residues can be conjugated via surface decoration with ease, making them useful in conjugation with targeting ligands, imaging probes, and therapeutic molecules.

The desolvation technique is now the most effective and reproducible process of forming gelatin-based nanoparticles. It entails controlled solubility addition of a non solvent, usually acetone, ethanol or isopropanol, in an aqueous gelatin solution, and as a result, phase separation and nanoparticle aggregates are produced. The method provides fine control of the size of particles, surface charge, and encapsulation efficiency by controlling the following parameters: pH, solvent polarity, gelatin concentration, and the speed of solvent injection [2]. In comparison to other fabrication methods of nanoparticles, e.g. coacervation or emulsification, desolvation avoids the toxic surfactants or oil phases, and high purity and biocompatible nanocarriers are produced.

An improved way of controlling the mono dispersity of the nanoparticles was realized through the classical two-step desolvation process that was initially proposed by Coester and Langer, the low molecular weight gelatin fractions were eliminated before the formation of nanoparticles. The process was, however, long and consumed a lot of material. To overcome these issues, a number of various adjustments to the one step desolvation protocol have been devised, combining pH adjustment, solvent turnover control and cross linking in one step, leading to significant increases in scalability and reproducibility [3]. Innovations in green and cross linker free desolvation methods are also described in the modern methods, using biological based reagents like genipin or polydopamine instead of glutaraldehyde to reduce cytotoxicity, though without achieving any mechanical stability.

In the recent years, microfluidic process assisted, ultrasonic deserted, supercritical fluid based desolvation have been developed as next generation methods that can settle to very narrow particle size distribution and giant manufacturing of GNPs on an industrial scale [4]. Also, computational modeling and artificial intelligence have made it possible to exert predictive control of nanoparticle formation kinetics, with size, porosity, and drug release profile fine tuning becoming possible.

In addition to fabrication, gelatin nanoparticles have proven to have a wide pharmaceutical application. They can be degraded to amino acids in a natural manner, and this makes them an ideal carrier of chemotherapeutics, peptides, and nucleic acids [5]. Gene delivery vectors, controlled release formulations and vaccine delivery and regenerative scaffolding applications have increased the biomedical potential of magnetic or metallic cores through the incorporation of genetic acids into nanocomposites. Due to the field converging on the concept of precision nanomedicine, the desolvation derived GNPs are at the juncture of material science, green chemistry, and pharmacological discovery, and they will form the basis of the next generation of effective, sustainable, and multifunctional nanotherapeutics.

Principles of Desolvation

Principle of Solvent - Induced Aggregation

The principle of controlled solvent induced aggregation can be regarded as the basic process in the desolvation technique. Introducing a non solvent, e.g. ethanol, acetone or isopropanol, lowers the gelatin solubility in the aqueous layer. Through this decrease, gelatin chains also become dehydrated and convert into coacervation to form nanoparticles. This process can be said to be a fine balance between the solute-solvent interaction, the polarity of the solvent, and the intermolecular forces of the gelatin macromolecules. In the case of the addition of the desolvating agent, the hydrogen bonding of water molecules to the hydrophilic groups of gelatin is weakened, and the hydrophobic regions of gelatin converse. The interactions end up in the separation of phases and the nanoparticles

are formed [6]. The stability of these nanoparticles is normally induced by the rate of diffusion through the solvent followed by cross linking which freezes the structure so that no additional aggregation or coalescence is possible.

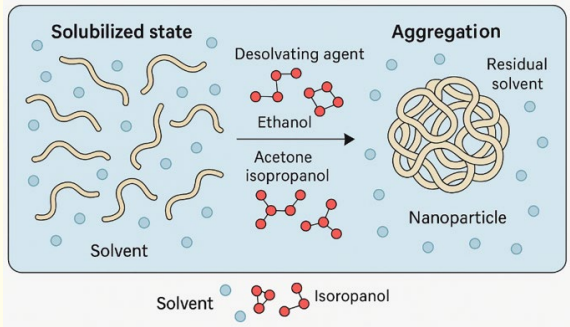

Figure 1: Principle of Solvent-Induced Aggregation.

Polymer Solvent Interactions Thermodynamics

Thermodynamically, the process of desolvation may be considered as a process in which the liquid-liquid phase separation occurs because of the variation of the Gibbs free energy. The balance between the enthalpic and entropic contributions to the total free energy of the system ensures the derivation of the total free energy of the system. Introduction of a non solvent elevates the system enthalpy because the affinity between the polymer and solvent is reduced and the number of interchain interactions also rises [7]. Expelling of the water molecules of the hydration shell of gelatin chains has been found to be entropically favoured to increase movement of the chains and allow aggregation. Critical desolvation point is a point at which the Gibbs free energy of the aggregated state is lower in comparison with that of the dissolved state thus supporting nucleation of nanoparticles. The polarity of the solvent, the dielectric constant of the solvent and its miscibility with water have a direct effect on the free energy landscape [8]. Besides, solvent addition rate will influence the outcome of nucleation as compared to growth to determine whether the end product is monodispersed and of the same size. Slow addition gives rise to homogenous nucleation, but high addition rate may give rise to polydispersed aggregates as nucleation and growth may occur simultaneously.

Serum Type (A and B) and Bloom Strength

There are two main forms of gelatin Type A, which is a product of acid treated collagen, and Type B, which is a product of alkali treated collagen. Type A gelatin has a net positive charge at a physiological pH with Type B having a net negative charge. This difference has a strong influence on the process of desolvation changing the electrostatic interactions and colloidal stability [9]. Typically Type A gelatin will just be in small and stable Nanoparticles when in slightly acidic conditions, but Type B gelatin prefers neutral and slightly basic conditions. The strength of the bloodstream that measures the rigidity of the gel and is proportional to its molecular weight is the key factor influencing the size and mechanical stability of nanoparticles. The greater the intensity of network, the more compact nanoparticles with decreased porosity and slower drug release are obtained with high bloom gelatin, whereas low bloom gelatin results in softer, more permeable networks to be used in fast release.

Effects of Molecular Weight Distribution

The viscosity of gelatin, its diffusivity, and the kinetics of its interaction of desolvation depends on the molecular weight distribution of gelatin. Extensive dispersion causes a heterogeneity in chain entanglement and aggregation behaviour leading to polydisperse nanoparticles. Improved homogeneity and better control over nucleation is obtained by removing low molecular weight fractions,

normally in the first method of the two step desolvation procedure [10]. On the other hand, the small chains cannot be retained to enhance nanoparticle elasticity and drug encapsulation. Hence, mechanical and release properties in nanoparticles can be customized by enzymatic degradation or selective filtration of molecular weights.

Important Process Conditions: pH, Temperature, Ionic Strength

Of all the process variables, pH has the most significant effects on desolvation behaviour. Close to the isoelectric point of gelatin (PI 1 = 4.7-5.2 of Type A and 5.0 -5.5 of Type B), the repulsive force caused by the charges is minimal as well, and the aggregation process occurs quite easily [11]. Nevertheless, when it is too acidic or too basic, it may trigger the chain denaturation or precipitation. Temperature affects viscosity and chain mobility and medium heating (354ºC) which can improve the rates of desolvation through a reduction in the viscosity of solution, with no serious damage to structure. The strength of the ions screening effects are regulated by ionic strength especially low ionic strength stabilizes the colloidal and it enhances the repulsive interactions between particles and high ionic environments are favorable to coacervation and tightening the electrical double layer. The parameters are important to control the rate of nanoparticle nucleation, stability, and yield, and their effective control is required to produce high quality and reproducible gelatin nanoparticles.

Classical and Modern Methods of Desolvation

Mechanism and Limitations of Traditional Two step Desolvation

Two step desolvation technique forms the basis approach used to obtain gelatin nanoparticles with high mono dispersity. The method that is used in the first phase involves the stepwise addition of a non solvent (either ethanol or acetone) to a dilute aqueous gelatin solution to isolate low molecular weight fractions selectively. A low molecular weight fraction is discarded to produce a high molecular weight fraction that is redissolved in water to repeat the desolvation step. When a solvent is added in a controlled way the gelatin molecules are aggregated into colloidal sized structures which are stabilized by cross linking reagent like glutaraldehyde or genipin [12]. This two step purification is carried out to guarantee structural homogeneity and better particles stability However, the approach is labor intense, solvent intense, and needs numerous separation processes and fine control of temperature and pH. It is also not scalable because of the variability of batches making it less applicable in continuous production or industrial production.

One-step Desolvation: Optimization and Scalability

To overcome these shortcomings, a more efficient, reproducible and scalable approach, the one-step desolvation method, has been brought up. The same reaction vessel proceeds to desolvation, followed by the creation of nanoparticles. The gelatin solution is first brought to a pH which is close to its isoelectric point so as to induce minimum repulsion, and a desolvating agent is added slowly with continuous stirring. This order uniform nucleation and growth of nanoparticles eliminates the necessity of clearing low molecular weight fractions [13]. This is quicker and more control over the characteristics of the particles by setting the values of solvent ratio, temperature and mixing rate. Its simplicity makes it compatible with aseptic pharmaceutical production, continuous flow production, and high throughput production, making it more reproducible, and having a higher yield and uses of less solvent.

Relative Holistically of Desolvating Agent

The desolvating agent used must have a significant effect on the dynamics of nanoparticle formation. Ethanol is also a preferred choice because of moderate polarity and low toxicity which enable it to form small homogeneous nanoparticles, but needs more volumetric solvents. Acetone is a more polar and volatile matter which dehydrates and phases rapidly, producing more generous yields of nanoparticles with wide distribution of their size. Isopropanol which has intermediate polarity provides a trade off between the size of particles and the efficiency of the process [14]. Polarity and dielectric constant of every solvent, as well as the miscibility, determines the degree of gelatin dehydration and coacervation rate which ultimately determines nanoparticle uniformity and encapsulation efficacy.

Process Kinetics and Phase Separations Dynamics

The rate of stirring and the addition rate of solvent are critical factors that impact on the supersaturation and aggregation processes. Gradual, slow solvent addition leads to gradual nucleation and uniform particle growth, and a rapid, uncontrolled solvent addition leads to polydispersive nanoparticles [15]. The ideal stirring effect is achieved, resulting in a fully diffused solvent be it in either the excess or the solvent, to prevent the effects caused by high supersaturation of a solvent and subsequent large aggregates. Moreover, the phase separation is determined by the solvent polarity by changing the dielectric constant of the aqueous system so that the gelatin water forces are weakened and allow intermolecular hydrogen bonding of gelatin chains. It is a process that undergoes two important steps first being in the initial nucleation of nanoclusters and subsequent controlled growth and stabilization [16]. By regulating the solvent polarity and diffusion rate properly, uniform and stable gelatin nanoparticles with customized surface and mechanical characteristics applicable to advanced pharmaceutical and biomedical advances will be obtained.

Crosslinking Strategies of Gelatin Nano particles in the Desolvated State

Traditional Cross-linkers (Glutaraldehyde, Formaldehyde)

Crosslinking is the next important step to stabilize gelatin nanoparticles after desolvation. By covalently linking polymer chains, it is possible to prevent the dissolution of polymer chains, increase the mechanical robustness, and make the particles less susceptible to enzymatic degradation. Conventionally, glutaraldehyde and formaldehyde have been most commonly used as chemical cross linkers owing to the high reactivity of the cross linkers to amino groups in gelatin [17]. They contain these aldehydes, which form covalent bonds among the lysine residues forming a dense 3D network which stabilizes the nanoparticle matrix. In particular, glutaraldehyde is more manipulable in terms of the density of crosslinking and rigidity of particles, and allows tuning drug release profiles with a high degree of accuracy. Nevertheless, the possible cytotoxicity of the remaining residues (action of aldehyde groups) and the purification required to eliminate any unreacted crosslinker limit its biomedical application [18]. Formaldehyde offers a different stabilization, being volatile and less selective, and poses other handling and safety issues. In the light of these disadvantages, aldehyde based crosslinking is used as a standard to compare newer methods of stabilization.

Genipin (Natural outstanding), Tannic Acid (Genipin, Polydopamine), Polydopamine (Natural outstanding)

Natural and biomimetic cross linkers have become popular in order to overcome the constraints of the traditional agents, as they are environmentally friendly. Genipin, which is formed by the interaction of gardenia jasminoides, reacts with the amino groups of gelatin in a heterocyclic ring-opening reaction to produce blue colored, strongly stable cross-linked products. Genipin is not very toxic to the cells and offers considerable biodegradability, that is why it is a good choice in drug delivery and in tissue repair [19]. Tannic acid is a polyphenolic acid that reacts to gelatin with hydrogen bonding and hydrophobic reactions, creating flexible networks that are securely stable without adding reactive aldehyde residues. Polydopamine is inspired by mussel adhesive proteins and self polymerizes under mild conditions, binding to gelatin surfaces to act as a cross linker and provide a functional coating. These green strategies not only guarantee biocompatibility, but also add further functionalities to it namely, antioxidant activity, surface reactivity and stimuli responsiveness behaviour.

Physical Stabilization Through Heat & pH Modulation

Beyond the traditional approaches to chemical crosslinking, physical methods of stabilization can be used to improve gelatin nanoparticle integrity and avoid introducing a foreign agent. In particular, controlled thermal treatment leads to a partial denaturation of the gelatin chains, thus favoring the formation of hydrogen bonds and hydrophobic associations which contribute overall to strengthening the matrix. In the instance where the temperature is maintained above the gelation threshold the resultant internal cohesion is significantly enhanced, all the while retaining the structural integrity of the encapsulated drug molecules. An analogous approach is achieved by adjusting the pH near the isoelectric point of the gelatine; by reducing the electrostatic repulsion forces between gelatine molecules, the polymer chains are supposed to aggregate spontaneously, achieving self stabilization [20]. Such forms of physical modalities are particularly beneficial for delicate biomacromolecules that may experience a loss of function when they are

chemically modified.

Effect on Biodegradation, Mechanical Integrity, and Release of Drugs

The extent and nature of crosslinking have a tremendous effect on the kinetics of biodegradation, mechanical resiliency, and drug release profile of gelatin nanoparticles. High crosslinking density reduces the rate of enzymatic degradation with concomitant increase in drug retention whereas loosely crosslinked constructs exhibit accelerated erosion rate and show pronounced burst release effect [21]. Thus, chemically crosslinked nanoparticles typically exhibit higher rigidity and slower release, whereas systems stabilized by natural or physical processes provide a varying rate of degradation that is compatible with the natural biological processes. Striking an optimal equilibrium between structural stability and biodegradability is therefore of prime importance when adapting gelatin nanoparticles to specific therapeutic indications, to ensure efficacious delivery, intrinsic biocompatibility, and predictable in vivo performance.

Advanced Process Changes and Innovations

Microfluidic Assisted Desolvation and Continuous Manufacturing

The recent development in process engineering has prepared the way for the integration of microfluidic technology with desolvation based nanoparticle synthesis. Microfluidic enhanced desolvation has the benefit of providing a mode of action that allows one to have fine control over mixing rates, solvent diffusion, and temperature gradients, thus enabling the formation of uniform particles under conditions that are both highly reproducible and scalable [22]. In this configuration the gelatin and the desolvating agent feed through micro channels in which rapid mixing by laminar flow precipitates instantaneous phase separation. The precise control of the flow rate obviates the existence of macroscopic gradients of solvent concentration, hence reducing polydispersity of nanoparticles and enabling the continuous generation of monodispersed nanoparticles [23]. This continuous manufacturing approach not only improves the scalability and reproducibility, but the amount of solvents is also reduced and the processing time. The applicability of this is especially interesting for the production of clinical grade nanoparticles in the context of the confines of Good Manufacturing Practice (GMP) guidelines.

Microwave and Ultrasonic Assisted Nanoparticle Formation

Microwave and ultrasonic assisted desolvation routes are fast catching up as energy efficient means to make nanoparticles. Microwave irradiation can increase the evaporation of the solvent and also uniform heat is achieved on the gelatin solution, so rapid nucleation and uniform nanoparticle formation is obtained [24]. This modality reduces the response time, enhances yield and overcomes aggregation of particles by preventing local overheating. In contrast, ultrasonic assisted desolvation utilizes acoustic cavitation to create localized high energy microenvironments, collapse of microbubbles increases mixing efficiency and transient shear forces are imparted creating uniformity in desolvation [25]. Ultrasonication also increases the dispersion of the solvent and uniformity of the particles, whilst at the same time decreasing the need for chemical stabilizers. The use of both approaches makes for sustainable processing by reducing solvent waste and energy input.

Supercritical CO2 and Solvent Free Desolvation Techniques

There is a need for environmentally benign, solvent free routes to the synthesis of nanoparticles and supercritical carbon dioxide (scCO2) is one of them. Working as the desolvent as well as antisolvent medium, scCO2 precipitates gelatin nanoparticles by decreasing solubility and not introducing solvent residues that would be toxic [26]. The process uses mild temperature and pressure conditions and thus, it maintains structural integrity of the encapsulated bioactive. Supercritical desolvation removes the need for post processing purification steps, making it of interest for large scale production on a pharmaceutical grade. The tunable density and diffusivity of CO2 offer an option for an accurate control of the size and porosity of nanoparticles, as well as their surface morphology.

Artificial Intelligence and Process Modeling in Nanoparticle Design

The use of artificial intelligence (AI) and computational modelling is transforming the fabrication of nanoparticles using a technique called "predictive process control". Machine learning algorithms can crunch through large volumes of data of formulation parameters, like solvent ratios, flow rates and temperature, to predict the optimum conditions for desired particle characteristics. Computational fluid dynamics (CFD) models are used to visualize the mixing of solvents and dynamics of phase separation between the solvents in microreactors [27]. These digital tools help optimize processes in real time, minimize experiment trial and error, and increase reproducibility in industrial scale manufacturing.

S. No.	*Technique / Platform*	*Principle / Mechanism*	*Key Advantage / Applications*
1	Electrohydrodynamic (EHD) Desolventisation	High voltage electrostatic atomization of droplets of gelatin produce nanoparticles by solvothermal evaporation.	Solvent free, uniform particle size, suitable for heat-sensitive drugs, scalable process.
2	Photon-Induced Nanoassembly (PIN)	Controlled laser/UV exposure triggers localized desolvation and crosslinking.	Spatially selective synthesis, on-demand fabrication, optical and surface modification potential.
3	Cryo-Desolvation	Low-temperature solvent removal prevents biomolecule denaturation.	Preserves protein activity, smooth morphology, ideal for vaccines and peptide carriers.
4	Bio-Catalyzed Crosslinking Reactors	Enzymatic crosslinking during desolvation using transglutaminase or laccase.	Eco-friendly stabilization, enhanced biocompatibility, enzyme-assisted functionalization.
5	AI-Guided Digital Twin Systems	Virtual models simulate desolvation to optimize parameters in real time.	Predictive control, high reproducibility, enables smart automated nanomanufacturing.
6	Bioprinted Nanocomposite Scaffolding	Incorporates GNPs into 3D/4D printed matrices for local delivery.	Customizable scaffolds, controlled drug release, regenerative medicine applications.
7	Plasma-Assisted Desolvation	Cold plasma activates gelatin for solvent-free nanoparticle formation.	Green synthesis, sterilization effect, improved surface reactivity.
8	Quantum Dot–Enhanced Nanostructuring	Quantum dots guide gelatin aggregation and crosslinking energetics.	Optical tunability, theranostic imaging, nanoscale structural precision.

Table 1: Advancements in Nanoparticles Designing.

Characterization of Gelatin Nanoparticle

Morphological and Size Analysis (DLS, SEM, TEM, AFM)

Comprehensive characterization of gelatin nanoparticles (GNPs) is crucial in the determination of their physicochemical and functional characteristics. Dynamic Light Scattering (DLS) is commonly used to obtain the hydrodynamic diameter, polydispersity index (PDI) and size distribution of nanoparticles suspended in a solution. This technique provides both rapid and precise information about the uniformity of the colloidal and about the stability of dispersion of the colloids [28]. Scanning Electron Microscopy (SEM) & Transmission Electron Microscopy (TEM) High resolution images of surface morphology and internal architecture & high resolution visualization of surface texture and shape for SEM and direct observation of particle crystallinity and internal matrix density for TEM [29]. Atomic Force Microscopy (AFM) complements such techniques as it provides 3D surface topology at the nanometer scale and is very useful in the assessment of surface roughness and coating uniformity. Collectively, these analytical tools define the morphology

and function relationship that is extremely important in predicting nanoparticles performance in biological system [30].

Structural Integrity (FTIR, Nuclear Magnetic Resonance (NMR), X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC))

Evaluation of structural integrity of gelatin nanoparticles after desolvation and crosslinking is imperative to ensure that chemical and secondary structures are preserved. Fourier Transform Infrared Spectroscopy (FTIR) is used to identify functional groups, and help to detect possible chemical modifications from crosslinking or drug incorporation [31]. Nuclear Magnetic Resonance (NMR) spectroscopy provides detailed information about the interaction between polymers and drugs and conformational changes on the molecular level within the gelatin backbone. X-Ray Diffraction indicates the difference between crystalline and amorphous regions, and tells how the desolvation process affects the ordering of molecules [32, 33]. Differential Scanning Calorimetry (DSC) is used to measure thermal transitions (glass transition, melting points, etc.), which provides information on the stability of the nanoparticle and crystallinity; encapsulation of drugs and data on how dispersed the encapsulated drug is. Together, these techniques validate that the structural properties are maintained or suitably modified so to support the function to be performed.

Surface Charge, Colloidal Stability (Electrophoretic Mobility)

Zeta potential measurement is a key parameter in assessing nanoparticle surface charge and colloidal stability where different values of more than ± 30 mV are commonly regarded as an indication of high resistance to aggregation. Electrophoretic mobility studies are a complement to zeta potential analysis as they measure the migration of nanoparticles in an electric field which helps to understand the distribution of charge or the effect of pH or ionic strength on the colloidal behaviour [34-36]. Surface charge information is also used to make predictions for cellular uptake and biodistribution since the interactions between the electrostatic field and biological membranes are important in determining the internalisation efficiency.

Degradation and Release Kinetics

Assessment of degradation in vitro and drug release kinetics of GNPs is crucial for predicting the therapeutic performance of GNPs. Controlled degradation tests commonly performed in simulated physiologic media assess the rate of decomposition of fibers and the time dependent disintegration of the remainders of the fibers. Drug release profiles are conventionally obtained by using dialysis or dissolution studies, thus providing a quantitative determination both of diffusion controlled, and erosion controlled mechanisms [37, 38]. Mathematical models like Higuchi, Korsmeyer-Peppas and first order kinetics are used to characterize release behaviour and optimal formulation.

In Vitro - In Vivo Correlation (IVIVC) and Pharmacokinetics Modeling

The establishment of an IVIVC is of paramount importance to the translation of performance in the laboratory to efficacy in the clinic. By correlating invitro release information with pharmacokinetic parameters, like absorption, bioavailability, and half life, IVIVC models allow prediction of invivo results with great precision [39, 40]. Advanced pharmacokinetic modelling taking into consideration nanoparticle breakdown, release kinetics, and systemic distribution therefore, assists in dosage optimization and optimizes the regulatory approval process. Such comprehensive characterization ensures that gelatin nanoparticles are up to the stringent standards that are required for pharmaceutical and biomedical applications [41].

Pharmaceutical and Biomedical Applications

Drug Delivery Systems of Hydrophilic and Hydrophobic Drugs

Gelatin nanoparticles (GNPs) have proven to be interesting drug delivery tools because of their intrinsic biocompatibility, surface tunability and the ability to encapsulate a vast range of therapeutic agents [42]. Their amphiphilic character makes them efficient in loading hydrophilic drugs in electrostatic & hydrogen-bonding interactions, hydrophobic drugs entrapped in gelatin matrix or adsorbed in hydrophobic domains of surface modifications. Precise control over desolvation conditions allows the particle size and

porosity to be fine tuned and guaranteed control of release kinetics [43]. The inherent biodegradability of gelatin ensures controlled degradation of the matrix and thus extended localized delivery and minimal systemic toxicity of drug delivery.

Targeted and Controlled Release Formulations

Targeting can be accomplished by functionalizing the surface of GNP with ligands, antibodies and peptides that bind to specific tissues or cell receptors. The rate of degradation of gelatin can be manipulated through the density and molecular architecture of crosslinking, so that controlled release sound as physiological stimuli such as pH, temperature, or enzymatic activity can be developed [44]. Such responsive nanoparticles selectively deliver therapeutics to pathological sites (e.g. tumor microenvironments or inflamed tissues) and thus improve the treatment's effectiveness and mitigate negative effects. Multi-layered GNPs and composite systems further allow for a dual or a sequential drug delivery, providing a synergistic pharmacological benefit [45].

Applications of Gene and Peptide Delivery

GNPs are especially suitable for the delivery of genes and peptides due to their cationic surface potential and mild conditions of fabrication which preserve macromolecular integrity [46]. Positively charged GNPs may be able to complex with nucleic acids (DNA, siRNA or mRNA) that are negatively charged, which will improve cellular uptake through endocytosis, and from endosomal endocytosis. The matrix of gelatin protects these molecules against enzymatic breakdown and increases the frequency of transfection. For peptide therapeutics GNPs offer sustained release and enhanced stability and address the problems of short half life and low bioavailability often seen with free peptides.

Diagnostic and Imaging Probes (MRI, Fluorescent GNPs)

The introduction of diagnostic agents inside of GNPs has led to the creation of theranostic platforms that are capable of simultaneous therapy and imaging. Incorporation of superparamagnetic iron oxide or gadolinium complexes produces magnetic resonance imaging (MRI) contrast which allows real time tracking of nanoparticles in vivo [47]. Fluorescent tagging by dye or quantum dot helps in optical imaging which can be used in biodistribution and cellular localization studies. These hybrid nanoparticles bring the opportunity to non invasively monitor the therapeutic delivery and the efficacy, and thereby advance precision medicine approaches.

Regenerative Medicine and Tissue Engineering Scaffolding

In regenerative medicine, GNPs play a pivotal role in the promotion of cell adhesion, proliferation and differentiation due to their bioactive surface and to their structural similarity with the components of the extracellular matrix [48]. When embedded in scaffolds or hydrogels GNPs give the controlled release of growth factors and signaling molecules which are used to direct the regeneration of tissues. Their tunable degradation synchronizes the resorption of the scaffolds to the remodeling of the surrounding tissue making them ideal for use in wound healing, bone regeneration and cartilage repair.

Vaccines Delivery and Immunomodulation Platforms

GNPs are becoming efficient vaccine platforms with capacities to encapsulate antigens as well as to deliver in a sustained manner to the immune system. Their biodegradable nature induces long duration antigen exposure while surface modifications can increase their uptake into antigen-presenting cells. GNPs can also co-encapsulate adjuvants to modulate immune responses to culminate in stronger and durable immunity [49, 50]. These attributes position gelatin based nanoparticles as next generation sub unit and mucosal vaccine formulation platforms that harmonizes their safety, efficacy and controlled immunomodulation.

Biocompatibility, Toxicity and Regulatory Considerations

Cytocompatibility Studies and Hemocompatibility Studies

Biocompatibility is one of the basic requirements for clinical translation of GNPs. Extensive cytocompatibility studies have shown that gelatin being a natural source derived biomolecule has minimal cytotoxicity if purified and crosslinked under controlled condi-

tions. The low energy process aspects of the desolvation process preserves native peptide sequences to stimulate favorable interactions with cellular membranes [51]. GNPs promote cell adhesion and proliferation as they are recognized by the integrins like Arg-Gly-Asp (RGD), resulting in a higher compatibility with many cell populations including fibroblasts and keratinocyte cells and endothelial cells. Hemocompatibility studies have shown additional findings of a low hemolytic behavior and negligible interference with coagulation parameters as long as surface charge and crosslinking density are optimized. Surface modifications that use hydrophilic polymers such as polyethylene glycol (PEG) or polysaccharides can also be used to further reduce the rate of nonspecific protein adsorption, reducing the potential for hemolysis and thrombogenicity when intravenously administered.

Immunogenicity and Pathways of Biodegradation

The immunogenic profile of GNPs is dependent mainly on the source of GNPs, the crosslinking chemistry and the residual impurities. Gelatin from porcine or bovine collagen can cause immune system reactions if the gelatin is not adequately purified, but recombinant gelatin or gelatin from fish eliminate such risks [52]. Crosslinkers like glutaraldehyde have the risk of introducing antigenic residues, therefore biocompatible crosslinks genipin and polydopamine are used for medical grade preparations. Enzymatic hydrolysis is mediated by proteolytic enzymes (collagenase, matrix metalloproteinases) and controls the biodegradation of gelatin. The crosslinking density, the molecular weight of the polymers and environmental conditions collectively govern the degree of its degradation, the amino acids and the peptides which are formed are easily metabolized, guaranteeing adequate clearance and avoiding accumulation and toxicity. Controlled degradation ensures adjustable drug release timing of nanoparticle destruction and drug release.

Pharmacokinetic and Biodistribution Challenges

The translational potential of gelatin nanoparticles (GNPs) is subjected to many limitations that are often suppressed by the complex interplay between physicochemical properties of the nanoparticles and the host biological environment. Size, surface charge, and hydrophilicity are of paramount importance to the determination of systemic persistence and tissue specific accumulation. Nanoparticles that are smaller than the 200 nm threshold generally do not get cleared quickly by the reticuloendothelial system (RES); on the other hand, larger and cationic entities display a hepatic and splenic predilection. A possible solution is to use polyethylene glycol (PEG) or similar stealth coatings to reduce the amount of opsonization and phagocytic uptake to increase the circulatory residence [53]. However, it remains necessary to exert a rigorous optimization of these parameters and a thorough in vivo validation, often using sophisticated imaging modalities and pharmacokinetic modeling, in order to reach reproducible biodistribution profile under variable physiological conditions.

FDA and EMA Regulatory of Gelatin Nanocarriers

From the perspective of regulation, the two largest authorities in the world, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have approved gelatin as a Generally Recognized as Safe (GRAS) excipient that supports its use as a part of the pharmaceutical formulations. However, once engineered with the nanoscale, GNPs undergo strong safety, stability and reproducibility assessment protocol [54]. The regulatory guidance highlights the importance of thorough characterization which includes particle size distribution, particles surface chemistry characterization, residue solvent screening, and endotoxins. Adhering to Good Manufacturing Practice (GMP) standards and International Organization for Standardization (ISO) norms is mandatory in the clinical grade manufacturing. Moreover, nanomedicines require extensive preclinical and toxicological studies to prove long term safety and bioequivalence and consistency of batches. Conformity with such changing requirements is necessary for the successful translation of gelatin based nanocarriers from bench to bedside.

Emerging Trends and Future Perspective

The future paths of gelatin based nanotechnology are being set by innovations focusing on sustainability, precision and multifunctionality. Recombinant and plant derived gelatin are two examples of ethical and environment sustainable alternatives to animal derived derivatives [55]. Produced by microbial or yeast expression systems, recombinant gelatin allows for specific manipulation of

molecular weight and amino acid composition which eliminates the risk of zoonotic contamination as well as religious restrictions. These engineered variants provide a way to have customizable degradation kinetics and mechanical attributes, consistent with the regulatory and ethical requirements for biomedical applications. Emerging stimuli responsive and functionalized GNPs are the next generation of nanocarriers that are able to respond to discrete physiological cues like pH, temperature, or the presence of an enzyme. These "smart" nanoparticles are used to enable site and controlled release of drugs because they should increase therapeutic precision and reduce any off-target drug activities [56, 57]. Their versatility makes them especially promising in the intervention of oncologists, inflammatory and chronic patients.

Conclusion

Gelatin architecture based nanoparticles, prepared by desolvation procedures, have become a very versatile tool for pharmaceutical and biomedical purposes. Their innate biocompatibility, their tunable physicochemical properties, as well as their biodegradability, make it possible to encapsulate and control the delivery of a variety of therapeutic agents, from small molecules to genetic agents. Continuous optimization of desolvation processes as varied as microfluidic helper techniques, green solvent free processes, and AI optimization have had a significant impact on enhancing scalability, reproducibility and environmental sustainability. The incorporation of functional polymers, bio crosslinkers and hybrid nanostructures has extended further to enhance their therapeutic possibilities, including targetable, stimuli responsive and multimodal delivery systems. Emerging innovations like recombinant sources of gelatins, gene editing payloads and theranostic prowess are stimulating the journey towards precision medicine. Moving going forward, the continued convergence of material science, bioengineering, and computational modeling will continue to shape gelatin nanoparticle technology such that it can be brought to fruition from the laboratory research setting to clinically viable, safe, and effective nanotherapeutic solutions.

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