Available online on 15.04.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Review Article

An Updated Perspective of Silk Fibroin-Nanoparticle as a Carrier for Controlled Drug Delivery

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Article Info:

Article History:

Received 09 Jan 2025
Reviewed 03 Feb 2025
Accepted 21 Feb 2025
Published 15 April 2025

Cite this article as:

Roy TC, Uddin ME, Kar A, An Updated Perspective of Silk Fibroin-Nanoparticle as a Carrier for Controlled Drug Delivery, Journal of Drug Delivery and Therapeutics. 2025; 15(4):219-235 *DOI: http://dx.doi.org/10.22270/jddt.v15i4.7116*

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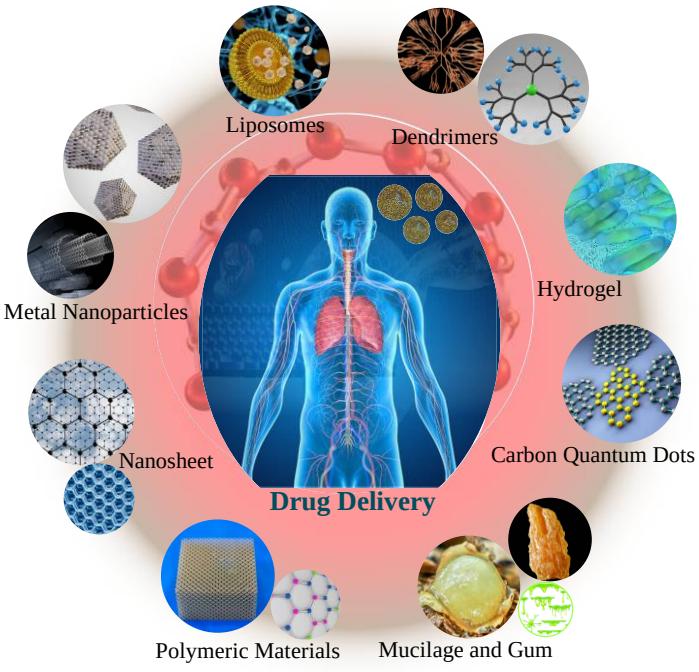
Abstract

This article illustrates a comprehensive review of the use of silk fibroin nanoparticles as a carrier for controlled drug delivery. The article begins by introducing the idea of controlled drug delivery and its importance in modern medicine. The technique, process, and drug-loading capabilities of silk fibroin nanoparticles are then discussed in detail, along with their advantages over other drug delivery systems. The review also examines the potential applications of silk fibroin nanoparticles in various biomedical fields, including cancer therapy, wound healing, and tissue engineering. The paper concludes by highlighting the current challenges and prospects for the development of silk fibroin nanoparticles as an efficient drug delivery system. However, this paper provides valuable insights into the potential of silk fibroin nanoparticles for targeted and controlled drug delivery, making it a useful resource for researchers in the field of drug delivery and biomaterials.

Keywords: Silk fibroin, Biomaterials, Nano-particles, Drug delivery, Controlled release.

Graphical Abstract



1. Introduction:

The drug delivery system (DDS) is designed for delivery of drugs in the target site through a controlled manner that leads to therapeutic efficacy. It consists of a carrier system in which the active drug is dissolved, dispersed, or encapsulated, or onto which the active ingredient is adsorbed or attached[1]. Drug carrier materials play a significant role in the delivery of drugs. These carriers can be processed into different forms such as nanoparticles, microspheres, microcapsules, pills, emulsions, and so on. Among them, nanoparticles have attracted much attention for their ability to be used as an effective carrier in promoting drug efficacy[2]. Nanoparticles as drug carriers were first developed around the 1970s by Birrenbach and Speiser[3]. Controlled drug delivery from implanted polymer-drug depots offers numerous advantages over traditional periodic systemic administration, including enhanced efficacy and cost-efficiency, reduction or elimination of unwanted side effects because of maintaining desirable therapeutic range without peaks and valleys which increases patient convenience and compliance [4]. To meet these needs, many synthetic non-degradable materials or implantable pumps have been proposed, but biodegradable implants alleviate the need for surgical removal of the material after the end of therapy. Naturally derived biodegradable materials can offer superior biocompatibility compared to synthetic degradable materials[5]. Nanostructured materials are synthesized by either "bottom-up" or "top-down" processes. The bottom-up approach starts with atoms, ions, or molecules as “building blocks" and assembles nano-scale clusters or bulk material from them. The "top-down" methods for processing of nanostructured materials involve starting with a bulk solid and obtaining a nanostructure by structural decomposition[6,7]. However, naturally derived materials frequently lack the necessary control of material properties that needed for long-term sustained-release applications. Because of superior encapsulation, controlled drug release, and high biocompatibility, biodegradable polymer nanoparticles have been widely employed as drug delivery systems[8]. A variety of polymeric materials have been employed as a drug delivery matrix. These materials include natural polymers like polysaccharides which include cellulose, chitosan, hyaluronic acid, alginate, dextran, starch, and proteins which include collagen, gelatin, elastin, albumin, and silk fibroin (SF), as well as synthetic biodegradable polymers like poly (lactic acid) (PLA), poly (ε-caprolactone) (PCL), and poly(glycolic acid) (PGA), poly(lactide-co-glycolide) PLGA [9,10]. Silk fibroin may be made into a variety of forms, including fibers, films, 3D scaffolds, and gels, for medication delivery and regenerative engineering research[11]. Generally speaking, amorphous flexible chains strengthened by strong, stiff crystals are what make silkworm cocoons and spider dragline silks semi-crystalline materials. Particle dispersions in a fluid or gel phase are used in many pharmaceutical and medical technologies. Examples include custom coatings, sustained release, and drug delivery systems[12].Silk proteins' capacity to be processed in water, biocompatibility, and biodegradability make them a suitable substrate for drug delivery. A basic aqueous technique is required for the manufacture of silk fibroin particles[13].During processing, the majority of silks take on a variety of secondary structures, such as insoluble spun fibers or soluble protein in the glands. Because of their distinct functions, protein-based nanoparticle drug delivery methods have gained attention. Protein-based carriers have outstanding biocompatibility, non-antigenic, and biodegradability[14]. Furthermore, proteins can a biological reaction in cells and display a variety of functional groups. In particular, covalent drug and ligand attachment can alter the surface of protein nanoparticles to improve therapeutic efficacy[15,16].

2. Silk-fibroin Features:

Silk fibroin, often known as Bombyx mori, is a naturally occurring protein derived from silkworm cocoons. Its predominant amino acid composition consists of glycine, alanine, and serine. It stands out due to its fibrous structure and elevated levels of hydrophobic amino acids. Bombyx mori silk fibroin is a stable protein possessing good mechanical properties, such as high elasticity and tensile strength[17]. It is resilient to a broad variety of environmental factors. Silk fibroin from Bombyx mori can be made by dissolving the cocoons in aqueous alkali solutions and spinning the resulting solution to form fibers. After that, several products, including hydrogels, films, and fibers, can be made from the silk fibroin fibers by treating them[18,19]. Silk fibroin from Bombyx mori has been extensively studied for its possible biological applications, particularly in the field of drug administration. Because of its ability to prevent encapsulated medications from degrading and its biocompatibility and biodegradability, it is a desirable material for drug delivery. Silk fibroin from Bombyx mori can be used to make silk fibroin nanoparticles (SFNs), which can encapsulate a variety of medications, proteins, and other biomolecules and can be tailored in terms of size, shape, and release kinetics[20]. Additionally, research has been done on the potential applications of Bombyx mori silk fibroin, which include tissue engineering, wound healing, and cell growth scaffolding[21]. Because it is biocompatible, biodegradable, and non-toxic, silkworm (Bombyx mori) silk fibroins, a naturally occurring protein derived from the cocoons of silkworms, have drawn a lot of attention as possible drug delivery vehicles. Its exceptional mechanical properties, which make it suitable for a range of biomedical applications, and its ability to stop encapsulated drugs from deteriorating, make it an ideal material for drug delivery[22]. Because it can be functionalized with other biomolecules to enhance its targeting and release kinetics, it is adaptable[32]. A protein-based biomolecule with good biocompatibility, biodegradability, and minimal immunogenicity is called silk fibroin (SF). The development of SF-based nanoparticles for drug administration has drawn a lot of interest because of their versatile drug-binding capability, controlled release characteristics, and easy production. The therapeutic efficacy of pharmaceuticals contained in qualified or recombinant SF-based

nanoparticles can be enhanced by programming changes to the particle size, chemical structure **(Figure 1)**, and characteristics. As a result, they can be utilized to deliver gene medications, protein and growth factor drugs, and small molecule drugs (such as anti-cancer drugs)[24,25,26].

The main components of the naturally occurring protein known as silk fibroin are serine, glycine, and alanine. The two primary domain types that comprise this fibrous protein are the crystalline domains, which are composed of densely packed beta-sheet structures, and the amorphous domains[20], which are composed of random coil patterns. The chemical composition of silk fibroin has a large number of hydrophobic amino acids, such as alanine and glycine, and a small number of charged and polar amino acids, such as tyrosine and histidine. Silk fibroin have self-assemble structures in water because of its hydrophobic qualities[21]. Silk fibroin is a highly stable protein that can withstand a wide range of environmental conditions, such as high temperatures, pH changes, and contact with organic solvents[28]. The presence of intramolecular and intermolecular hydrogen bonds, which support the structural maintenance of the protein, is responsible for its stability[29]. Silk fibroin's exceptional tensile strength makes it an ideal material for biomedical applications, such as wound dressings and surgical sutures. Its exceptional mechanical properties enable it to be utilized in the production of numerous materials, such as films, fibers, and hydrogels [30].

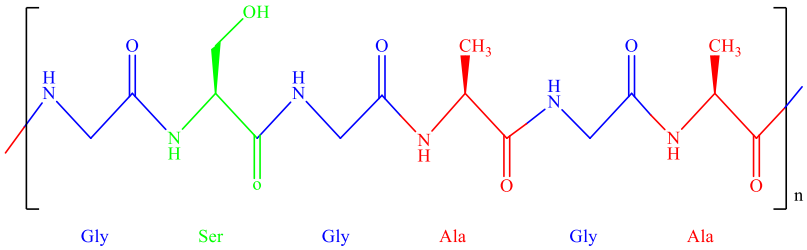


Figure 1: Primary structure of silk fibroin.

Overall, due to its chemical makeup and physical characteristics, silk fibroin is a desirable material for the utilization of various biomedical applications. It is especially useful as a drug delivery vehicle due to its biocompatibility, biodegradability, and ability to stop the encapsulation of pharmaceuticals from degrading[31].

Fibroin, the main component of silkworm silk fiber, and spider silk fiber's equivalent, saproin, are two significant families of silk proteins. The two protein chains that make up the B. mori silk fibroin are the heavy chain (H-fibroin), which has a molecular weight of around 350 kDa, and the light chain (L-fibroin, Mw ~ 26 kDa), which is covalently bonded at the carboxy-terminus of the two subunits by a disulfide bond[33,34,35]**(Figure 2a& 2b)**.

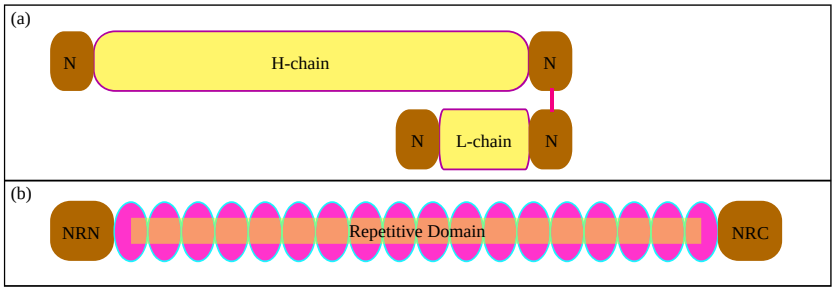


Figure 2: (a) A highly repetitive heavy chain and a non-repetitive light chain joined by covalent bonds make up silkworm fibrin. (b) Non-repetitive amino-(NRN) and carboxy-terminal (NRC) domains encircle the primary repetitive core domain of spider silk spidroins[36].

Like silk fibroin from Bombyx mori, the main constituents of these proteins are serine, glycine, and alanine[37]. However, spider silk fibroins are not the same as silkworm silk fibroins in terms of composition and structure. Spider silk fibroins differ from silkworm silk fibroins in that they have a reduced amount of hydrophobic amino acids and a higher degree of secondary structure, such as beta-sheet and alpha-helix. As a result, spider silk fibroins have exceptional mechanical properties, such as high tensile strength, high extensibility, and high toughness[38]. Spider silk fibroins have been thoroughly studied for their possible applications in biomedicine, particularly as a drug delivery system. Spider silk fibroins have strong biocompatibility and biodegradability, making them an effective material for biomedical applications[39]. Additionally, many biomolecules can be functionalized onto spider silk fibroins to enhance their targeting and release kinetics. Spider silk fibroins are highly versatile and can be employed in many different activities, including wound healing, tissue engineering, and cell growth scaffolding. Because it is non-toxic,

biocompatible, and biodegradable, spider silk fibroins—a naturally occurring protein derived from spider silk—have been thoroughly studied as possible drug delivery vehicles[40]. Its remarkable mechanical properties make it suitable for various biomedical applications; additionally, its versatility stems from its capacity to be functionalized with various biomolecules to enhance its release kinetics and targeting abilities[41]. Furthermore, the presence of various chemical groups in the structure of SF, such as amines, phenol, alcohol, thiol, and carboxyl, promotes the interaction of SF with particular cell types' antibodies and biomolecules. It is feasible to include different medications inside or on the surface of SF fibers by turning on the various SF functional groups. SF is thus shown as a potent drug carrier with a variety of biomedical applications. **(Figure 3)**[37].

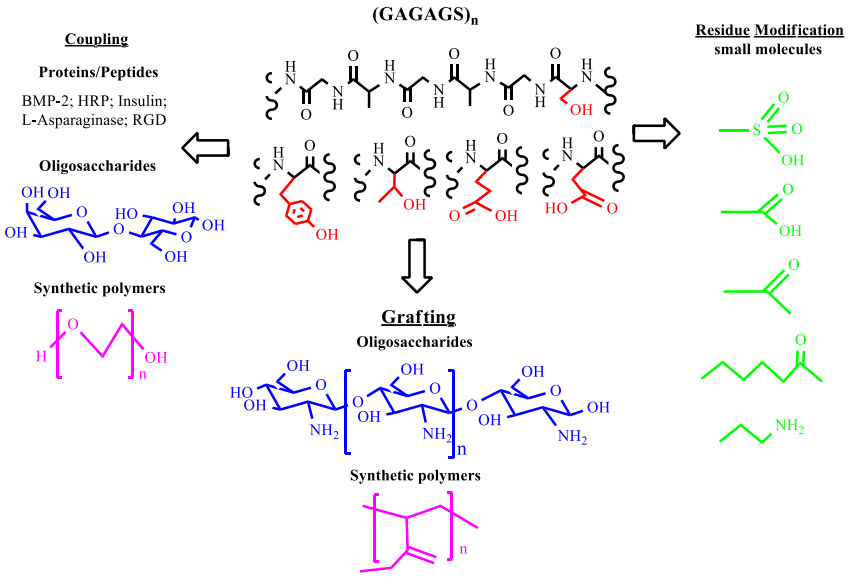


Figure 3: Different types of modification strategies for changing the surface properties of SF[43].

Silk fibroin (SF) is an attractive material for use in many applications, including the delivery of medications, due to its many physical properties. The following are some of the fundamental physical traits of SF: High elasticity, high toughness, and high tensile strength are some of SF's excellent mechanical qualities. Owing to these properties, SF finds utility in numerous fields such as tissue engineering and wound healing[38].

- Biocompatibility: Being biocompatible means that SF does not trigger an immune response when it comes into contact with living tissue. For these reasons, SF is a sought-after material for biomedical applications[45].
- Biodegradability: SF can be broken down by the body's enzymes and other systems because it is biodegradable. SF is a useful option in applications where the material must be removed after a specific period[46]. SF is attracted to water due to a characteristic known as hydrophobicity. This property makes SF a promising material for use in hydrogels and other water-based systems[47].
- Hydrophobicity: Numerous hydrophobic amino acids are present in SF, and they can be changed to suit the requirements of the application. Two properties of SF that make it suitable for usage in circumstances where the material will be exposed to extremes of heat or cold are thermal stability and a wide temperature range[48].
- Transparency: SF films and fibers can be employed in optical applications since they are transparent[49]. Because SF is porous, the rate at which drugs and other substances are released from SF-based drug delivery systems can be controlled by altering its structure. These qualities make SF an attractive material for many biomedical applications, such as wound healing, tissue engineering, and medication administration[50,51].

3. Preparation Methods of Silk Fibroin-Based Nanoparticles:

Many methods, such as salting out, mechanical comminution, desolvation, electrospraying, and supercritical fluid technologies, can be used to create SF-based nanoparticles.

3.1. Salting out:

Salting out is a widely used technique in chemistry and molecular biology for the purification and precipitation of proteins and nucleic acids. This process involves the addition of a high salt concentration to a solution containing a biomolecule of interest. He increased ionic strength disrupts the solvation shell around the biomolecule, thereby promoting aggregation and precipitation. Typically, ammonium sulfate is the salt of choice because of its high solubility and its ability to effectively precipitate a wide range of proteins [46]. This process is usually carried out in a stepwise manner, gradually increasing the salt concentration to selectively precipitate different proteins based on their solubility properties. This method not only aids in the concentration of proteins but can also be used to enhance their purity by removing contaminants that remain soluble under similar salt conditions [47]. For optimal results, various parameters such as pH,

temperature, and specific salt concentration must be carefully controlled. The resulting precipitate was collected via centrifugation, washed, and suspended in a suitable buffer for further analysis or purification [48]. These are a few benefits and drawbacks of the salting-out technique used to create silk fibroin nanoparticles. It should be mentioned, nevertheless, that the salting-out technique is still a practical and popular way to create silk fibroin nanoparticles.

Advantages	Disadvantage	Particle size
Ease of Preparation: The salting out method is a simple and easy procedure to create silk fibroin nanoparticles. It doesn't require intricate procedures or specialized tools[52]. Scalability: It is easy to scale up or down the salting-out process to meet the needs of different applications[53]. High Yield: High yields of silk fibroin nanoparticles can be produced by the salting out procedure, which is advantageous for large-scale manufacturing[54]. Cost-Effective: The salting out method is less expensive than other methods for producing silk fibroin nanoparticles since it doesn't require costly materials or specialized equipment[55].	Limited Control Over Particle Size: The silk fibroin nanoparticles' size cannot be precisely controlled using the salting out approach, which may be problematic for applications that demand particular particle sizes[53]. Poor Particle Stability: The salting out approach can produce silk fibroin nanoparticles that are unstable and may congregate over time, hence decreasing their efficacy in drug delivery applications[57]. Limited Modification Capabilities: Since the salting-out process makes it difficult to easily functionalize or modify the nanoparticles, it might not offer as much adaptability as other methods for creating silk fibroin nanoparticles[54].	A few factors that could influence the size of the silk fibroin nanoparticles produced by the salting out procedure include the temperature, pH of the solution, and the amount of salt employed. Reducing the pH of the solution and increasing the quantity of salt usually results in smaller particle sizes[55,56]. However, because the particle size distribution can be vast and unpredictable, it might be difficult to get consistent results. The particle size of the silk fibroin nanoparticles produced by the salting out process can potentially be affected by contaminants such as proteins or salts in the starting material[57].Overall, the salting out method for managing silk fibroin nanoparticle size may not be as suitable for applications that require precise particle sizes as alternative methods like electro-spinning or mechanical comminution. Nonetheless, the salting out process remains a useful and well-liked method for producing silk fibroin nanoparticles because of its simplicity and scalability[62].

One technique for separating and purifying proteins from natural sources, such as silk fibroin (SF), is the salting-out process **(Figure 4)**. It is predicated on the theory that the increased electrostatic attraction between the protein molecules causes the proteins in a solution of proteins to "precipitate," or separate from the solution when salt is added. The target protein is dissolved in water together with any other possible contaminants or impurities. The mixture is salted, frequently using a salt with a high molecular weight, such as ammonium sulfate. As the concentration of salt increases, the proteins begin to precipitate out of the solution. Before the precipitate is recovered by centrifugation or filtering, the protein is rinsed and dried to remove any remaining salt.

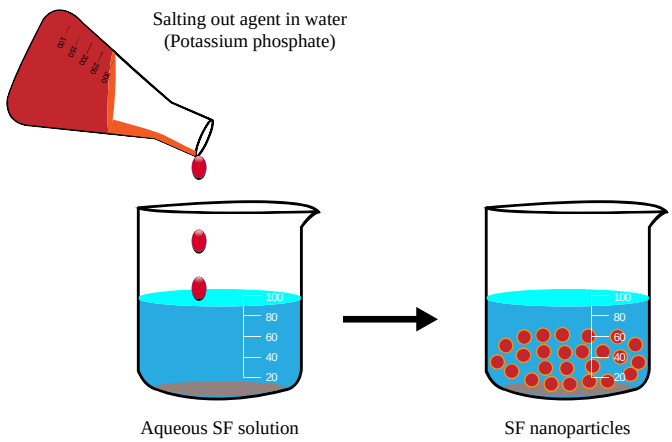


Figure 4. Diagrammatic representation of the salting out process used to create SF nanoparticles salting-out [63].

After being purified, the protein might be used for several things. The salting out method is widely used to extract proteins, such as silk fibroin, from natural sources since it is inexpensive and simple to use. Some of its disadvantages include the potential for denaturing the protein and the need to eliminate salt before utilizing the protein in specific applications. It is significant to highlight that the salting out methodology has recently advanced to overcome some of the limitations of previous methods, such as the requirement of low-molecular-weight salts and the requirement to purify SF using aqueous two-phase systems (ATPS). Some experts claim that the salting out method is a simple and affordable way to purify proteins. Additionally, they emphasize that it is a widely used approach in both industry and research and has been well-established for several years[64]. Some, however, highlight the disadvantages of the salting out technique. For example, the protein may be denatured by the high salt concentrations used in the procedure, which could affect the protein's structure and functional properties. Some experts assert that the salting-out method can be improved by using salts with low molecular weight or by combining it with other methods of purification such as size-exclusion chromatography[65]. While the salting out approach is widely used to purify proteins, scientists have differing opinions about its effectiveness and limitations. It is a simple, low-cost process, but its effectiveness can be enhanced by using low-molecular-weight salts or by combining them with other purification methods.

3.2. Desolvation:

Desolvation is a crucial method used to purify and concentrate biomolecules, especially proteins and nucleic acids. This technique involves stripping water molecules from the solvation layer surrounding a biomolecule, which leads to precipitation or aggregation. By diminishing the hydration shell, desolvation strengthens the interactions between biomolecules, aiding in their separation from impurities. The procedure typically starts by altering solution conditions, such as ionic strength, pH, or temperature, to induce desolvation. Commonly used agents in this process include organic solvents (like acetone or ethanol) and salts that interfere with solvation dynamics [62]. In the case of proteins, desolvation can trigger conformational changes, often resulting in precipitation, which is then harvested through centrifugation or filtration. One of the key advantages of desolvation is its ability to selectively isolate specific proteins based on their distinct solubility characteristics. It can also enhance the yield and purity of the target biomolecule by effectively eliminating soluble contaminants [63,64]. This approach is extensively utilized in protein crystallization, where precise management of desolvation is essential for producing high-quality crystals. These are a few benefits and drawbacks of the desolvation technique used to create silk fibroin nanoparticles (**Figure 5**).

Advantages	Disadvantages	Particle size
Desolvation can yield uniformly sized and shaped silk fibroin nanoparticles, which makes it appropriate for applications involving controlled drug delivery[66]. Simplicity: Desolvation is an easy-to-scale process that can be used to produce silk fibroin nanoparticles on a big scale[67]. High loading efficiency: One essential element of regulated drug administration is the production of nanoparticles with high drug loading efficiency, which can be achieved through desolvation[68].	Low yield: Low yields from the desolvation of silk fibroin nanoparticles may make large-scale synthesis impractical[69]. Certain conditions must be met for desolvation, which might be challenging to control and uphold. These requirements include using specific solvents and pH levels[70]. Impurities: Due to the use of solvents during desolvation, the final silk fibroin nanoparticles may contain impurities, which could negatively impact their stability and performance[71].	The silk fibroin nanoparticles generated during desolvation can have different sizes depending on several factors, including the concentration of the silk fibroin solution, the type of solvent used, the length of time and temperature of the desolvation process, and others. It's important to remember that while smaller particle sizes can increase the effectiveness of medicine loading and release, they can also make handling and stability more difficult. Therefore, it is necessary to carefully monitor and optimize the particle size of silk fibroin nanoparticles produced during desolvationto meet the specific goals and requirements of a given drug delivery application[72]. The normal range of sizes for the silk fibroin nanoparticles produced during desolvation is 50 to 500 nm, though this can vary based on the specific setup and conditions[73].

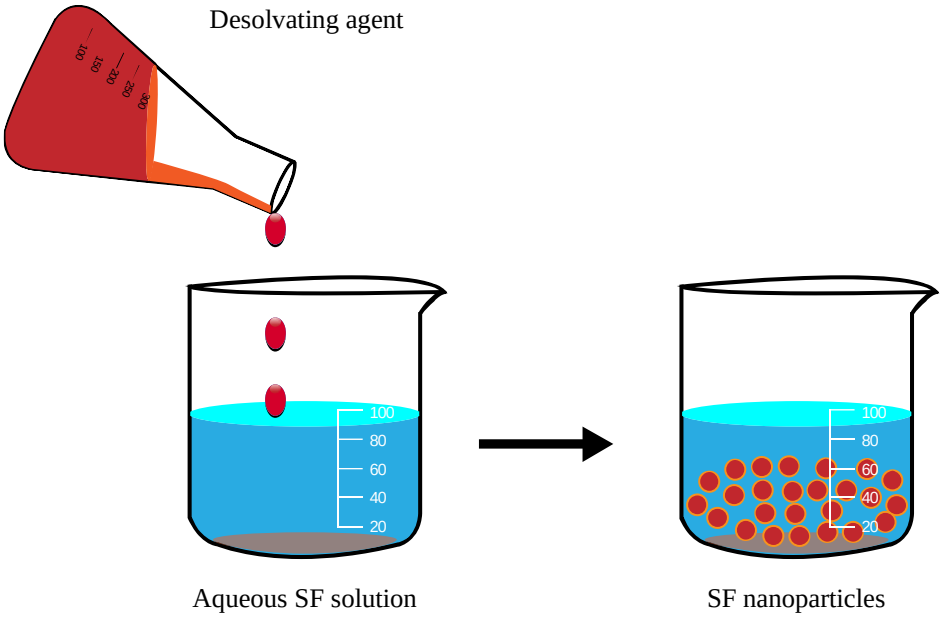


Figure 5: Schematic diagram of the desolvation method for preparing silk fibroin (SF) nanoparticles[74].

While desolvation offers certain advantages in the production of silk fibroin nanoparticles for controlled drug delivery, some disadvantages need to be considered. The process of desolvation involves removing solvent molecules from a substance, typically a protein or other biomolecule. It is a crucial phase in the purification and separation of the molecule. Industry experts generally view the desolvation method favorably. They stress how effective it is at eliminating solvent molecules and how it may be used in conjunction with other purification methods, such as chromatography, to create extremely pure protein or biomolecule samples. Because desolvation procedures maintain the chemical and physical properties of the protein or biomolecule, they are also often preferred over alternatives like precipitation and lyophilization[75]. Experts also note that desolvation can be a time-consuming procedure and that the necessary equipment can be expensive. Additionally, certain proteins or biomolecules may be more difficult to dissolve than others based on their solubility qualities[76]. Recent research has shown that the desolvation process can be improved by utilizing state-of-the-art technology, such as supercritical fluid technology, which provides a gentle, efficient, and effective way of desolvation and drying process **(Figure 6)**. To summarize, experts think that desolvation is a good method for getting rid of solvent molecules from proteins or biomolecules, and it can be used with other methods of purification to get extremely pure samples[77].

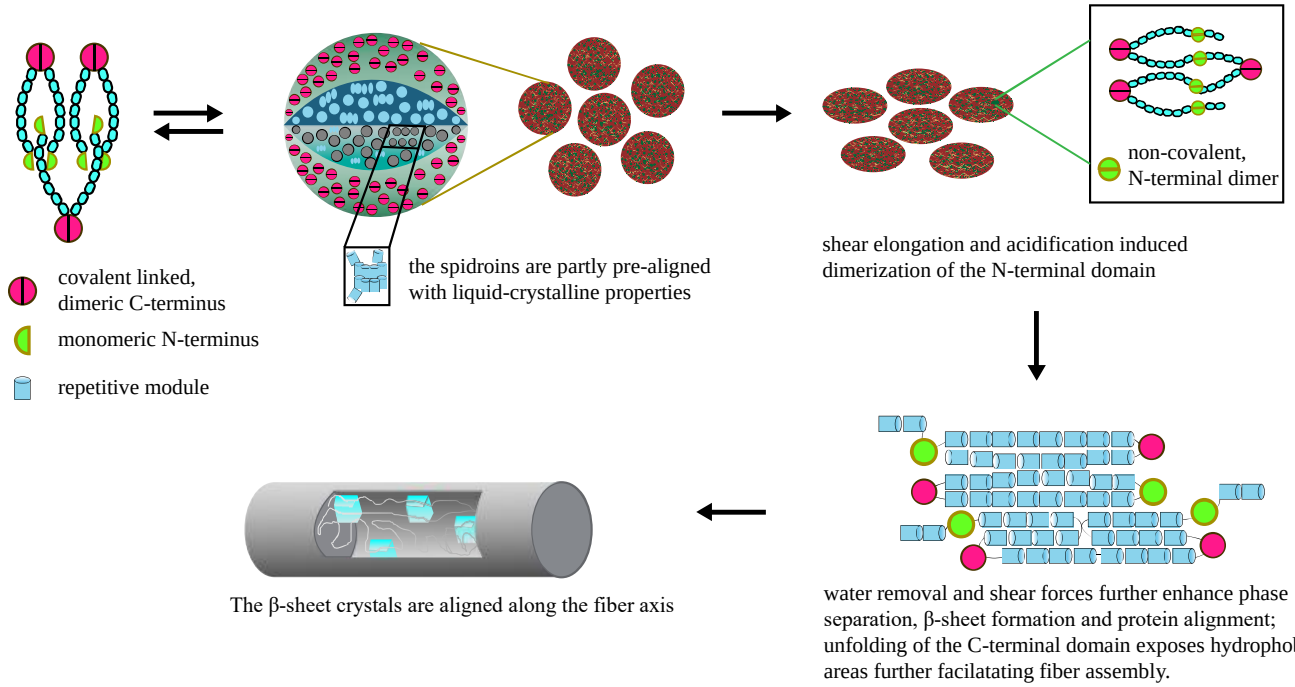


Figure 6: Schematic formation mechanism of the hierarchical assembly from molecular silk fibroin to microfibers [78].

They also mention that some proteins or biomolecules may be more difficult to dissolve than others and that desolvation can take a lengthy period. Recent research has shown that the procedure can be enhanced by applying state-of-the-art technologies, like supercritical fluid technology.

3.3. Electrospraying:

Electrospraying is an innovative technique utilized for the production of fine aerosol droplets from liquid solutions and is frequently employed in the fields of nanotechnology, material science, and pharmaceuticals. This process utilizes an electric field to induce the formation of a spray from a charged liquid, which is subsequently directed towards the target substrate or collector. The electrospraying process commences with the application of a high voltage to a liquid feed, which typically contains polymers, biomolecules, or nanoparticles [74]. The electric field causes the liquid to form a Taylor cone at the tip of the nozzle, from which a fine jet emerges. As the jet traverses through air, it undergoes solvent evaporation and fragmentation, resulting in the generation of ultrafine particles or fibers. One of the significant advantages of electrospraying is its capacity to produce uniform and controllable particle sizes, which is critical for applications such as drug delivery, where precise dosage and release profiles are desired [75]. The parameters that influence the electrospraying process include the solution viscosity, concentration, applied voltage, and distance between the nozzle and collector [52]. Electrospraying is particularly valuable for encapsulating active compounds and for enhancing their stability and bioavailability. This methodology has gained prominence for the production of nanofibers, nanoparticles, and microcapsules for various biomedical and industrial applications. The electro-spraying approach (**Figure 7**) for the manufacture of silk fibroin nanoparticles has the following benefits and drawbacks.

Advantages	Disadvantages	Particle size
High-monodispersity: Nanoparticles with an incredibly uniform size, shape, and chemical composition can be produced via electrospraying. This could lead to better-controlled drug release and more effective medication loading[79]. Scalability: Because electro-spraying is an easy technique to scale up or down to meet the needs of different drug delivery applications, it is a versatile and reasonably priced method for producing silk fibroin nanoparticles[80]. Controlled particle size: The applied voltage, flow velocity, and distance between the electrode and the collecting surface are some of the variables that can be changed to precisely regulate the particle size of silk fibroin nanoparticles created by electrospraying[81].	Sophisticated setup: The precise apparatus required for electro-spraying can be costly to set up and maintain[82]. This arrangement consists of a collection surface, a microfluidic delivery system, and a high-voltage power source. Low-yield: When dealing with thick or extremely viscous fluids, electrospraying has the potential to yield nanoparticles with poor yields [83]. Degradation: The stability and efficacy of the silk fibroin material as a drug delivery vehicle may be jeopardized by electrospraying-induced degradation[84].	A few factors that could influence the size of the particles produced by electrospraying are the properties of the solution being sprayed, the voltage applied, the separation between the nozzle and the collector, and the solution's flow velocity[85]. Nanoparticles that range in size from a few micrometers to tens of nanometers may generally be produced via electrospinning. Some advantages of electro-spinning for nanoparticle manufacturing include the ability to produce very monodisperse particles, the ability to produce particles from a variety of materials, and the process's scalability for large-scale manufacturing. Some disadvantages include the need for specialized equipment and the possibility of aggregate growth during the spray process[86].

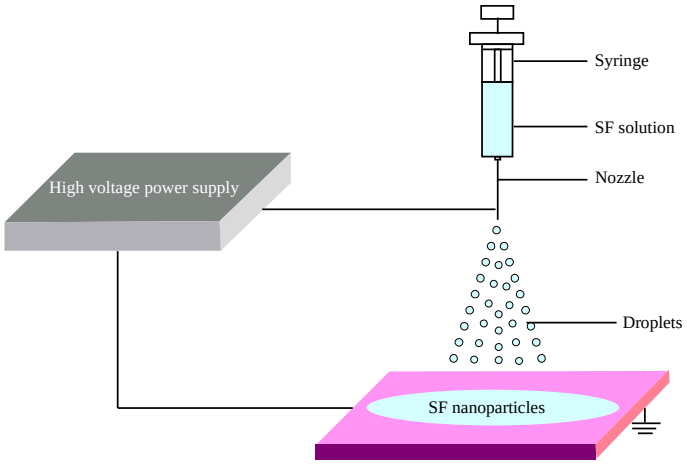


Figure 7: Schematic illustration of the SF nanoparticle preparation process using electrospraying[87].

Overall, electro-spaying is a promising method for producing silk fibroin nanoparticles for medication delivery; however, careful consideration of the advantages and disadvantages is necessary to ensure optimal performance and results.

Electrospraying is a technique used to create microscopic droplets or particles of a liquid by subjecting it to a high electric voltage, which causes the liquid to break down into tiny droplets or particles. It is used in several fields, such as material science, biotechnology, and medicine delivery. The majority of expert opinions regarding electrospraying are positive. They emphasize that it is a versatile procedure that can be used to produce particles with a variety of sizes and shapes as well as ones composed of materials that are difficult to produce using other methods. Furthermore, monodispersity and considerable surface functionality can be produced via electrospraying[88]. Experts also note

that the process of electrospraying may be difficult and that the necessary equipment can be expensive. Additionally, certain materials may be more difficult to electrospray than others based on their unique properties. Recent research indicates that electrospraying has been applied to produce drug delivery systems, protein particles, and other biomolecules. This method can also be used to generate particles made of metal, ceramic, or polymer. According to experts, electrospraying is a versatile technology that can be used to produce particles with a wide range of sizes and forms as well as ones composed of materials that are difficult to produce using other methods. They do, however, also note that the electrospraying method can be challenging and that the necessary supplies could be expensive. The method's versatility in manufacturing particles for many applications has been exhibited by recent investigations.

3.4. Mechanical Comminution:

Mechanical comminution is a widely utilized technique for size reduction of solid materials and is primarily employed in the fields of materials science, mineral processing, and pharmaceuticals. This process involves the application of mechanical forces to break down larger particles into finer ones, thereby increasing the surface area and enhancing the reactivity or bioavailability of materials [82]. The mechanical comminution process typically encompasses several methods such as crushing, grinding, milling, and pulverization. Various types of equipment can be employed depending on the desired particle size and material characteristics, including ball mills, hammer mills, jet mills, and attrition mills [21]. Each method operates on distinct principles, with some relying on impact forces and others utilizing shear or compression forces. Key parameters influencing the efficacy of mechanical comminution include the type of material being processed, the size and morphology of the feed particles, and operating conditions such as speed, duration, and moisture content. The optimization of these parameters is essential for achieving the desired particle size distribution and minimizing energy consumption. Mechanical comminution is particularly advantageous in industries where precise particle size is crucial, such as the formulation of pharmaceuticals, where it can enhance the dissolution rates of active ingredients. Additionally, it plays a vital role in the recycling of materials, facilitating the recovery of valuable components from waste products [83]. These are a few benefits and drawbacks of the mechanical comminution technique used to create silk fibroin nanoparticles.

Advantages	Disadvantages	Particle size
Particle size control: These are some advantages and disadvantages of the method of mechanical comminution that is employed to produce nanoparticles of silk fibroin[89].	Required time: Grinding by hand requires time, especially when handling big quantities of material[92].	The particle size of the silk fibroin nanoparticles produced by mechanical comminution depends on several factors, including the size of the grinding apparatus, the amount of time spent grinding, the type of grinding medium employed, and the properties of the silk fibroin [95].
Scalability: The mechanical comminution technique is readily scaleable up or down to accommodate roughly silk fibroin[90].	Requires specialized equipment: Specialized tools, such as a homogenizer or ball mill, are required for the mechanical comminution method[84]; these tools might be costly and aren't always available in lab settings[93].	The particle size of the silk fibroin nanoparticles created by mechanical comminution usually varies from a few hundred nanometers to several micrometers. Both the targeted particle size for the application and the specifics of the mechanical comminution process used will determine the exact size[96]. It's critical to keep in mind that the size of the silk fibroin nanoparticles may have a substantial impact on their effectiveness as drug delivery vehicles. While smaller particle sizes are often preferable for greater medication delivery efficacy, larger particle sizes can be beneficial for improved stability and handling.
High yield: When using mechanical comminution, one can frequently obtain nanoparticle yields that are suitable for applications requiring large amounts of material [91].	Degradation might occur: The mechanical stress from grinding could cause the silk fibroin to break down, which would reduce the quality of the finished nanoparticles[94].	

Overall, there are certain advantages to mechanical comminution, such as scalability and control over particle size, but there are also disadvantages, such as the requirement for specialized equipment and the potential for deterioration of silk fibroin. As with any approach, the selection of mechanical comminution will depend on the particular needs of the application. Mechanical comminution is one method of reducing materials to very small particles[97]. It is a widely used technology in a variety of industries, such as mining, food processing, and pharmaceuticals. Reviews of the mechanical comminution method by industry specialists have generally been positive. They stress how simple it is to scale up for large-scale operations and how

efficient and fast it can be used to reduce particle size **(Figure 8)**. Furthermore, mechanical comminution processes are usually preferred over alternatives like chemical or heat treatments since they do not alter the chemical or physical characteristics of the material being comminuted[98]. Experts also note that mechanical comminution procedures can require expensive and energy-intensive equipment. Additionally, some materials may be more difficult to comminute than others based on their mechanical properties[99]. The mechanical comminution process can be enhanced with the use of high-pressure homogenization (HPH) and ultrasonic technologies, which will make the process more precise, effective, and gentle[100].

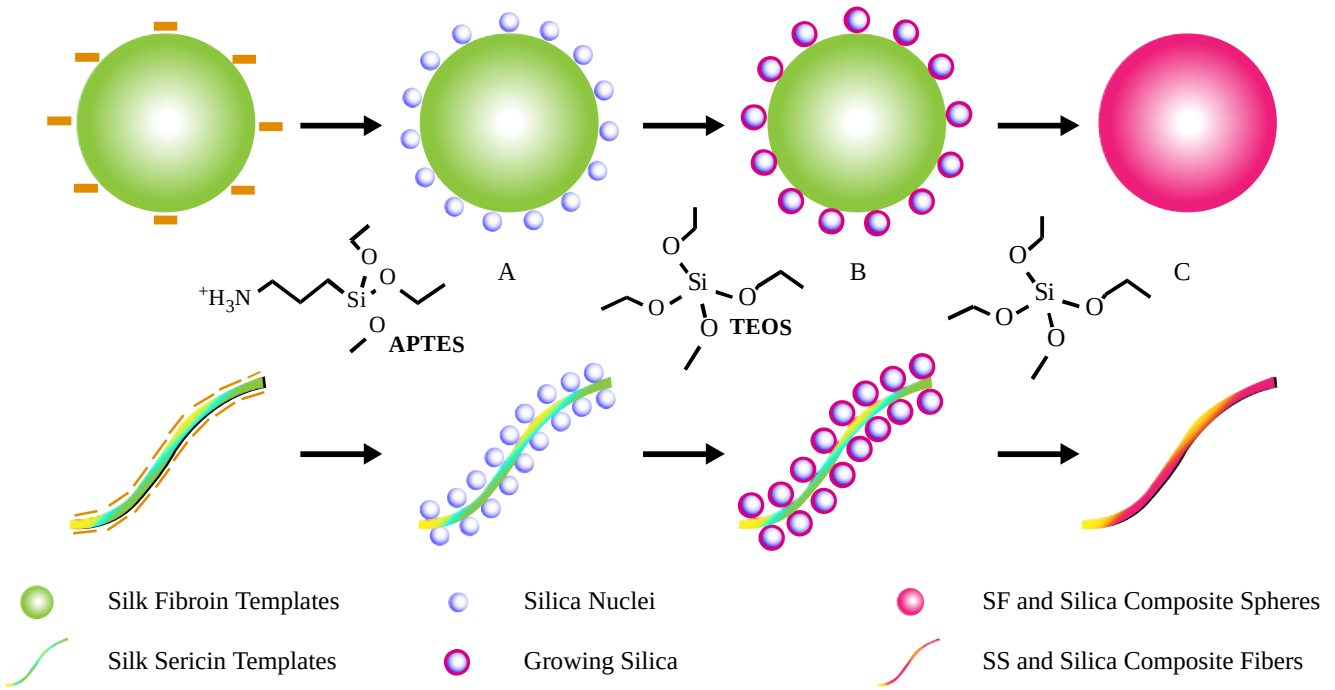


Figure 8: Diagram illustrating the nucleation and self-assembly of silica into nanocomposites[101].

However, mechanical comminution is a simple and efficient method of reducing particle size, but they also point out that it can be energy-intensive and that comminution may be more difficult for some materials than for others. Recent research has shown that the process can be improved by utilizing state-of-the-art tools like ultrasonic and high-pressure homogenization (HPH).

4. Silk fibroin biomaterials for controlled-release medication delivery:

A natural protein-based biomaterial called silk fibroin has been extensively explored for its possible application in the distribution of controlled-release medications. The use of silk fibroin biomaterials for controlled-release medication administration is generally viewed favorably by experts, and several researchers have highlighted its benefits. Silk fibroin is easily processed into a range of forms, including hydrogels, microparticles, and nanoparticles, which can be used to encapsulate pharmaceuticals. It has been demonstrated to be both biocompatible and biodegradable. Furthermore, research indicates that a broad spectrum of medications, including important macromolecules like proteins and nucleic acids as well as tiny compounds, may be present in silk fibroin[102]. Furthermore, silk fibroin has been shown to have low toxicity and immune-stimulating properties, which makes it a great choice for controlled-release drug administration[103]. There are still issues to be resolved before silk fibroin is widely used as a drug delivery system animal. One of the main issues is enhancing the stability of silk fibroin-based delivery methods to prevent drug leakage. Controlling the rate at which medications are released from silk fibroin-based delivery systems is another problem that has to be resolved[104]. All things considered, the fabrication of silk fibroin biomaterials **(Figure 9)** could be used to administer controlled-release medications, however further research is needed to address the problems that need to be fixed.

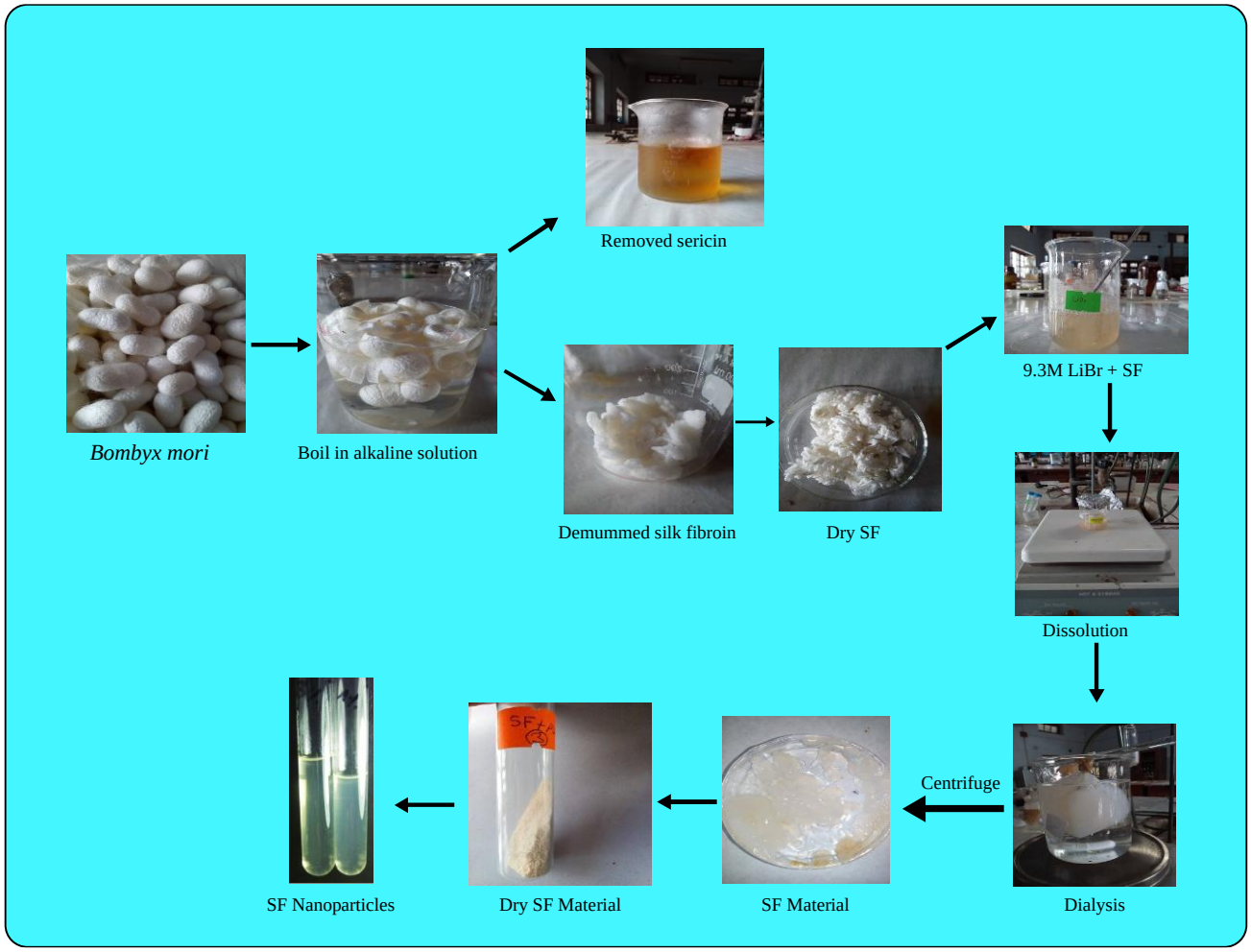


Figure 9: Production of Particles of Silk [105].

5. The Applications of Nanoparticles Based on Silk Fibroin for Drug Delivery:

Silk fibroin-based nanoparticles have been studied as a possible drug delivery strategy because of their biocompatibility, biodegradability, and low immunogenicity. Silk fibroin is a naturally occurring protein that can be easily extracted from silkworm cocoons and processed into nanoparticles using methods like solvent evaporation, electrospraying, and electrospinning. There are several scenarios in which drug delivery via silk fibroin-based nanoparticles could be employed.

- Cancer treatment: Due to their capacity to selectively target tumor cells and release the medicine under controlled circumstances, silk fibroin-based nanoparticles have been studied as a possible anticancer drug delivery system.
- Gene therapy: Silk fibroin-based nanoparticles have been shown to carry plasmid DNA, which can be used to introduce genetic material into cells.
- Vaccines: Silk fibroin-based nanoparticles have been studied as possible vaccine carriers because they can strengthen the immune response to the vaccination.
- Ocular drug delivery: Due to its ability to both reduce the number of doses required and enhance drug retention in the eye, silk fibroin-based nanoparticles have been studied as a potential drug delivery technology.
- Dermal drug delivery: Since silk fibroin-based nanoparticles may improve the drug's skin penetration and reduce the number of doses required, they have been studied as a possible carrier for dermal pharmaceuticals.
- Biomedical imaging: Silk fibroin-based nanoparticles are used as a contrast agent in computed tomography (CT) and magnetic resonance imaging (MRI) procedures.

While silk fibroin-based nanoparticles have great potential as drug-delivery vehicles **(Table 1)**, further research is needed to fully understand their properties and optimize their use in a range of applications.

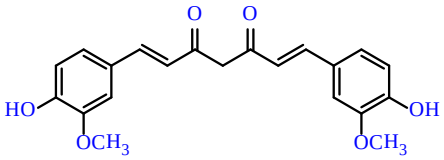
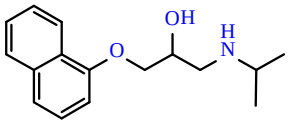
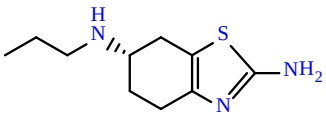
Drug	Formula	Molecular Weight
curcumin		368.39
propranolol hydrochloride		259.34
pramipexole		211.32

Table 1: Model medications contained in nanoparticles of silk fibroin [106].

6. Expert Opinions:

Various reviews and research papers provide expert opinions regarding the use of silk fibroin-based nanoparticles for drug delivery. In a paper, the authors assessed the potential of silk fibroin-based nanoparticles as a drug delivery vehicle and highlighted the advantages of using them, such as their controlled drug release, biocompatibility, and biodegradability. Additionally, they discussed the challenges that must be overcome for silk fibroin-based nanoparticles to realize their full potential[107,108].The authors of the review study discussed the application of silk fibroin-based nanoparticles for cancer therapy. They discussed the advantages of using silk fibroin-based nanoparticles for targeted drug administration and assessed the current status of research in this area[109]. An article discussed the usage of silk fibroin-based nanoparticles for the delivery of ophthalmic medications. They highlighted the advantages of using silk fibroin nanoparticles for the delivery of ocular medications, such as their regulated release capabilities and biocompatibility. They also highlighted the challenges that must be solved for silk fibroin-based nanoparticles to fulfill their full potential in the delivery of ocular medications[110,111].An article mentioned using silk fibroin-based nanoparticles for gene therapy. The advantages of using silk fibroin-based nanoparticles for gene transfer were highlighted, including their controlled gene release properties, biocompatibility, and biodegradability. They also discussed the challenges that must be solved for silk fibroin-based gene therapy nanoparticles to realize their full potential[112].

An article described the use of silk fibroin-based nanoparticles for protein delivery. They highlighted the advantages of using silk fibroin-based nanoparticles for protein delivery, including their regulated protein release capabilities, biocompatibility, and biodegradability. They also discussed the challenges that must be overcome for silk fibroin-based nanoparticles to fulfill their full promise in protein delivery[113,114].An article described the usage of silk fibroin-based nanoparticles for the transport of small compounds. They highlighted the advantages of using silk fibroin-based nanoparticles for the transport of small compounds, such as their controlled release properties, biocompatibility, and biodegradability. They also discussed the challenges that must be solved for silk fibroin-based nanoparticles to fulfill their full potential in the delivery of tiny molecules of therapy[115].An article mentioned using silk fibroin-based nanoparticles as a cancer treatment. The advantages of silk fibroin-based nanoparticles for cancer therapy were highlighted, including their regulated drug release, biocompatibility, and biodegradability [116,117]. They also discussed the challenges that must be solved for silk fibroin-based nanoparticles to be fully utilized in cancer treatment[118].A paper described the use of silk fibroin-based nanoparticles for gene editing. The advantages of using silk fibroin nanoparticles for gene editing were highlighted, including their capacity for regulated drug release, biocompatibility, and biodegradability. They also discussed the challenges that must be solved for silk fibroin-based gene editing nanoparticles to realize their full potential[119].An article described the use of silk fibroin-based nanoparticles for targeted medication delivery. The advantages of using silk fibroin nanoparticles for targeted drug delivery were highlighted, including their ability to encapsulate a range of medications and their biocompatibility and biodegradability. They also discussed the challenges that must be solved for silk fibroin-based nanoparticles to fulfill their full potential in the targeted drug delivery industry[120].An article mentioned using silk fibroin-based nanoparticles to heal wounds. They highlighted the advantages of using silk fibroin-based nanoparticles for wound healing, such as their ability to deliver drugs with controlled release and biocompatibility[121,122]. They also discussed the challenges that must be solved for silk fibroin-based nanoparticles to heal wounds to the fullest extent possible[123,124]. An article examined the application of silk fibroin-based nanoparticles for skin delivery. They highlighted the advantages of using silk fibroin-based nanoparticles, such as their ability to stop drug degradation and their biocompatibility and biodegradability[125,98], for the transportation of

medications through the skin. Additionally, they discussed the challenges that must be solved for silk fibroin-based nanoparticles intended for skin distribution to realize their full potential[127].These are just a few instances of current expert opinions; there are many more publications on the use of silk fibroin-based nanoparticles for medication administration and other therapeutic applications. Like with any new technology, more research is necessary to fully understand its features and make the most of it in a variety of applications.

7. Conclusions:

The emerging ideas may be included in this investigation that explore the use of silk fibroin-based nanoparticles for medication delivery: due to their biocompatibility, biodegradability, and capacity to encapsulate a variety of medicines, silk fibroin-based nanoparticles offer promise as a vehicle for regulated drug delivery. Research on targeted medication delivery via silk fibroin-based nanoparticles is ongoing and has the potential to increase treatment efficacy and lessen negative effects. The current difficulties in creating silk fibroin-based nanoparticles for drug delivery include heightening their stability, managing drug release, and enhancing targeting. To fully grasp the promise of silk fibroin-based nanoparticles for medication administration and to address the issues that must be resolved, more study is required. Future work on this project will focus on realizing the full potential of silk fibroin-based nanoparticles, and the knowledge gained could be useful to the development of cutting-edge controlled drug delivery systems.

Conflict of Interest: The authors declare no potential conflict of interest with respect to the contents, authorship, and/or publication of this article.

Author Contributions: All authors have equal contribution in the preparation of manuscript and compilation.

Source of Support: Nil

Funding: The authors declared that this study has received no financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting in this paper are available in the cited references.

Ethical approval: This study does not involve experiments on animals or human subjects.

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