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DRUG DELIVERY BY USING SILK NANOSPHERE: A REVIEW

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ABSTRACT

Silk fibroin (SF) is a protein-based biomacromolecule that has excellent biocompatibility, biodegradability, and immunogenicity. The development of SF-based nanoparticles for drug administration stands out because of its high restriction limit in terms of various drugs, regulated drug discharge qualities, and other factors. Silk fibroin (SF) is a protein-based biomacromolecule that has excellent biocompatibility, biodegradability, and immunogenicity. Because of the high restriction limit in terms of different drugs, regulated drug discharge qualities, and mild planning circumstances, the advancement of SF-based nanoparticles for drug administration stands out sufficiently to be noted. The modified or recombinant SF-based nanoparticles can be designed to work on the restorative proficiency of drugs incorporated in these nanoparticles by adjusting the molecule size, the substance composition, and the method. They can be used to deliver small particle pharmaceuticals, protein and growth factor drugs, quality treatments, and so on in this manner. This study summarises recent progress on SF-based nanoparticles, including synthetic design, characteristics, and planning methods. The use of SF-based nanoparticles as transporters for therapeutic medicines is also being investigated.

Keywords *– Silk fibroin, Nanoparticles, Drug administration, Planning methods.*

1.INTRODUCTION

A drug delivery system consists of a drug carrier in which the active component is adsorbed or attached, or a drug carrier in which the active ingredient is dissolved, disseminated, or encapsulated. Drug carrier materials serve an important role in drug delivery. These carriers can be made into nanoparticles, microspheres, microcapsules, tablets, emulsions, and other drug-controlled release systems.

Nanoparticles have gotten a lot of interest because of their ability to operate as an effective carrier for improving therapeutic efficacy. Birrenbach and Speiser [1] were the first to use nanoparticles as drug carriers in the 1970s. They were originally colloidal particle systems with sizes ranging from 1 to 1000 nm, and their "size effect" gave them distinct properties. Nanopaticles have been shown to preserve drugs from degradation, improve biological stability, drug absorption into a specific tissue, bioavailability,

retention time, intracellular penetration, and lower patient costs and toxicity concerns [2,4]. Modulating the surface characteristics, composition, and milieu can also be used to generate the desired drug release pattern biodistribution [4].

Because of their superior biocompatibility, improved encapsulation, and controlled drug release capabilities, biodegradable polymer nanoparticles have been widely exploited as drug delivery methods. Synthetic biodegradable polymers like poly (lactic acid) (PLA), poly (-caprolactone) (PCL), and poly (glycolic acid) (PGA), as well as natural polymers like cellulose, chitosan, hyaluronic acid, alginate, dextran, and starch, as well as proteins like collagen, gelatin, elastin, albumin, and silk fibroin, have all been used as drug delivery matrix [5].

Because of their particular functions, protein-based nanoparticle drug delivery methods are gaining popularity. Protein-based carriers are biodegradable, antigenic-free, and have high biocompatibility. Proteins also have different functional groups and can cause a biological reaction in cells. Covalent attachment of medicines and ligands to the surface of protein nanoparticles can be used to improve therapeutic efficiency [6].

Silk Fibrin (SF) is a biomacromolecule made up of proteins. Films, three-dimensional scaffolds, hydrogels, electrospun fibres, and spheres have all been employed as biomaterials in biomedical applications. SF-based nanoparticles are a promising drug delivery strategy because of their biodegradability, high biocompatibility, improved cell adhesion and proliferation, chemical alteration potential, and cross-linking capability. This review will focus on the utilisation of SF-based nanoparticles as drug delivery carriers. The chemical structure and characteristics of SF are described, as well as the manufacturing processes for SF-based nanoparticles and their use as therapeutic drug carriers.

2. SILK FIBROIN CHEMICAL STRUCTURE AND PROPERTIES

Silk fibroin (SF) is a natural polymer produced by silkworms and spiders. Dragline silk from the spider Nephila clavipes and tamed silkworm Bombyx mori are the most well-known silks. SF is a natural protein polymer that the US Food and Drug Administration has approved as a biomaterial (FDA). Because of the more aggressive nature of spiders and the more complicated and lower volumes of silk combinations created in orb webs, commercial production of spider silks has been limited compared to the established supply chain available for silkworm silk [7,8].

2.1Silk Fibroin's Basic Structure

SF is a protein-based biomacromolecule with cumbersome tedious particular hydrophobic spaces, which are intruded on by little hydrophilic gatherings. The essential design of Bombyx mori SF is for the most part made out of glycine (Gly) (43%), alanine (Ala) (30%) and serine (Ser) (12%) [9]. SF is a heterodimeric protein with a weighty (H) chain (~325 kDa) and a light (L) chain (~25 kDa) associated by a solitary disulfide bond at cys-172 of the L-chain and cys c-20 (20th buildup from C end) of the H chain [10,11]. Additionally, a 25 kDa silk glycoprotein, P25 related with disulfide-connected weighty and light chains by noncovalent communication [12]. The chains of SF additionally contain amino acids with massive and polar side chains, specifically tyrosine, valine, and acidic amino acids [13]. The H-chain of SF contains exchanging hydrophobic and hydrophilic squares like those seen in amphiphilic block co-polymers. It is hydrophobic and gives translucent like highlights to the silk string [14]. The hydrophobic spaces of H chains contain Gly-X rehashes, with X being Alanine (Ala), Serine (Ser), Threonine (Thr) and Valine (Val) and can shape hostile to resemble β-sheets and result in the strength and mechanical properties of the fiber. The hydrophilic connections

between these hydrophobic spaces is non-monotonous and extremely short contrasted with the size of the hydrophobic rehashes [15]. It comprises of cumbersome and polar side chains and structures the formless piece of the optional construction. The chain compliance in nebulous squares is arbitrary curl, which gives versatility to silk [7,16]. The L-chain is hydrophilic in nature and generally versatile. P25 protein could assume a huge part in keeping up with the uprightness of the complex. The molar proportions of H-chain:L-chain:P25 are 6:6:1 [17,18].

2.2. Silk Fibroin Secondary Structure

Silk fibroin crystal formations are divided into two categories: Silk I and Silk II. Silk I is a transition state that contains amorphous structures, random coils, and -helical structures. Silk II is made up of antiparallel-sheet crystal formations that render silk fibroin insoluble in water. Silk I is found in the silk gland, whereas Silk II is found in the form of spun silk fibre [19]. Thus, studying the silk spinning mechanism can reveal the components that influence the structure transformation process of silk fibroin. This knowledge can subsequently be applied to the study of SFNP production mechanisms. The change of Silk I (random coil and -helical structure) to Silk II (highly organised -sheet) is the basis for the production of SFNPs [20]. Due to their intrinsic electrostatic repulsion, silk fibroin molecules form loose amorphous forms in water solution. Water, on the other hand, tends to bond with these silk fibroin molecules and produce a hydration film. External treatment causes the creation of -sheet structures, which leads to the self-assembly of molecular chains and the synthesis of SFNPs [21].

SFNPs are frequently made using the self-assembly approach. It is well understood that molecular aggregation, a thermodynamic process, determines self-assembly, which can be influenced by external environmental influences [22]. The soluble and irregular Silk I can be changed into non-soluble Silk II with external stimulation such as metal ions, low temperature, organic solvent, and ultrasound [23]. The -sheet structures of Silk II, on the other hand, undergo conformational reversion to amorphous structures of Silk I at high concentrations of neutral salt and other specific circumstances (e.g., acid, ROS, enzyme, and hyperthermia) [24,25]. As a result, the transition of silk fibroin crystal structures is a complicated and multi-factorial regulated process, which is critical to the multi-responsive property of SFNPs [26].

2.3 SFNPs that respond to multiple stimuli

Drug release in sick tissues can be controlled spatially and temporally using multi-responsive nanotherapeutics [26]. The Kaplan lab was the first to discover that SFNPs had a pH-dependent drug release feature. In compared to the buffers with pH values of 7.4 and 6.0, the release rate of doxorubicin (DOX) from SFNPs was dramatically increased in the buffer (pH 4.5). They theorised that the loss of negative net charges in the buffer (pH 4.5) weakened the electrostatic interaction between silk fibroin molecules and DOX, causing DOX to be released from NPs more quickly Also looked at how DOX released from PEGylated SFNPs in acidic buffers with and without lysosomal enzymes [27]. They discovered that the rate of DOX release was greatly increased in the mimicked lysosomal fluid (lysosomal enzyme and acidic environment), indicating that DOX is released more quickly in tumour cells' lysosomes. Our research recently revealed that SFNPs are not only pH responsive, but also have apparent ROS/GSH/hyperthermia-responsive characteristics. We also uncovered a possible mechanism for their hyperthermia-responsive pH/ROS/GSH characteristics [28]. Protons, H_2O_2 molecules, and hyperthermia can break down hydrogen bonds in -sheet structures, while GSH can break down internal disulfide bonds into sulfhydryl groups. Protons, H_2O_2 molecules, heat, and GSH treatments relax the tight structures of SFNPs, resulting in faster drug release from these NPs [29]. These findings show that SFNPs have

pH/ROS/GSH/hyperthermia/lysosomal enzyme-responsive characteristics, implying that they can enhance selective drug release in targeted cells via microenvironmental stimuli.

3.Silk Fibroin-Based Nanoparticle Preparation Methods

Desolvation, salting out, mechanical comminution, electrospraying, supercritical fluid technology, and other technologies are also accessible for the creation of SF-based nanoparticles. Each method has advantages and disadvantages, therefore choosing the best method for forming SF-based nanoaprticles for drug delivery applications is critical.Fabrication of SF nanoparticles is a difficult task that requires further investigation. Because of the high molecular weight and protein composition of SF, nanoparticle production is challenging to manage. Furthermore, when exposed to heat, salt, pH change, or severe shear, SF tends to self-assemble into fibres or gels. Silk Fibrin (SF) is a biomacromolecule made up of proteins. Films, three-dimensional scaffolds, hydrogels, electrospun fibres, and spheres have all been employed as biomaterials in biomedical applications. SF-based nanoparticles are a promising drug delivery strategy because of their biodegradability, high biocompatibility, improved cell adhesion and proliferation, chemical alteration potential, and cross-linking capability. This review will focus on the utilisation of SF-based nanoparticles as drug delivery carriers. The chemical structure and characteristics of SF are described, as well as the manufacturing processes for SF-based nanoparticles and their use as therapeutic drug carriers [30].

3.1 Desolvation

The desolvation/coacervation process is the most generally utilized strategy to plan protein-based nanoparticles because of relatively gentle circumstances. The desolvation (straightforward coacervation) process diminishes the solvency of the protein prompting stage detachment. The expansion of desolvating specialist prompts adaptation changes in protein structure bringing about coacervation or precipitation of the protein [6,31,32]. Figure 1 shows the schematic chart of the desolvation strategy for planning SF nanoparticles. In short, the protein is at first broken down in a dissolvable and afterward step by step separated into a non-dissolvable stage. By stage division, a stage with a colloidal part/coacervate and a second stage with a dissolvable/non-dissolvable blend are shaped. In this cycle, the dissolvable should be miscible with the non-dissolvable. A steady molecule size is reached after an underlying interaction period so that further desolvation (expansion of non-dissolvable) exclusively prompts an expanded molecule yield. As the coacervation cycle is quicker and more effective at states of zero net charge (isoelectric point of the protein), the pH of the protein arrangement is of significant significance and can be changed towards the ideal circumstances with respect to molecule size and interaction yield.

SF particles with an average diameter of 980 nm using polyvinyl alcohol (PVA) as an emulsifier in the particle creation process to prevent the agglomeration of silk particles. SF solution was thoroughly combined with ethanol and vortex for 10 seconds. After that, the PVA solution was added to the silk/ethanol combination and vortexed for 10 seconds more. Finally, the ternary solution was frozen for 24 hours to create SF particles.

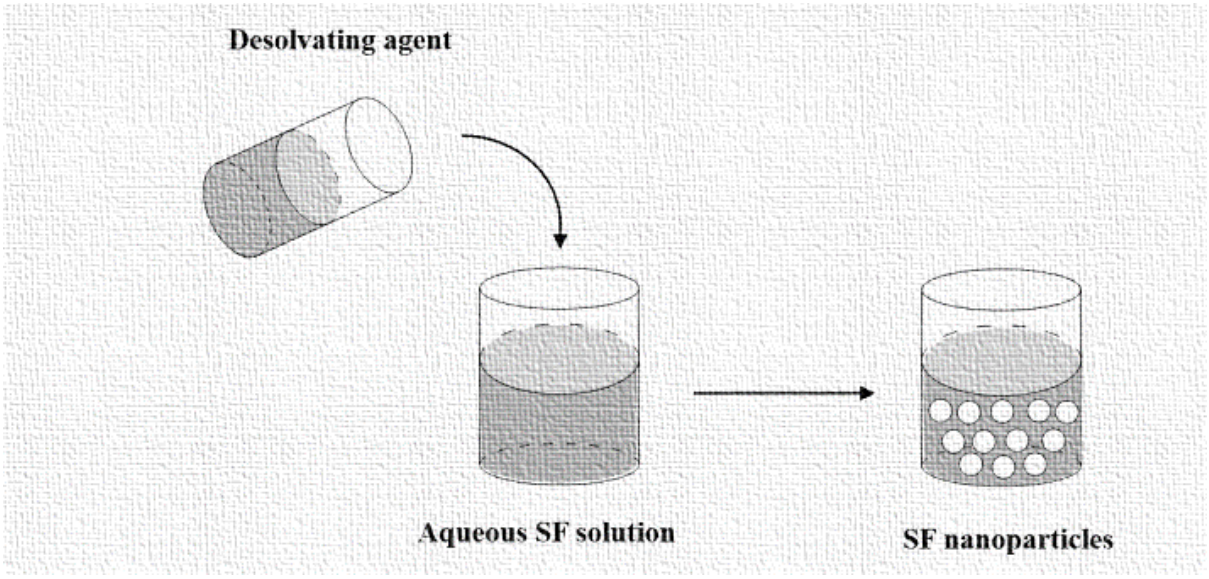

Fig. 1. The desolvation procedure for making silk fibroin (SF) nanoparticles is depicted in this diagram.

3.2. Extraction of salt

The salting out of a protein solution to generate protein coacervates is a straightforward method for making protein-based nanoparticles. Hydrophilic and hydrophobic regions of proteins exist. Hydrophobic portions interact with water molecules, allowing proteins to form hydrogen bonds with the water molecules in the environment. The salt ions attract some water molecules as the salt concentration rises, removing the water barrier between protein molecules and increasing protein-protein interactions. As a result, the protein molecules develop hydrophobic contacts with one another and precipitate out of the solution.

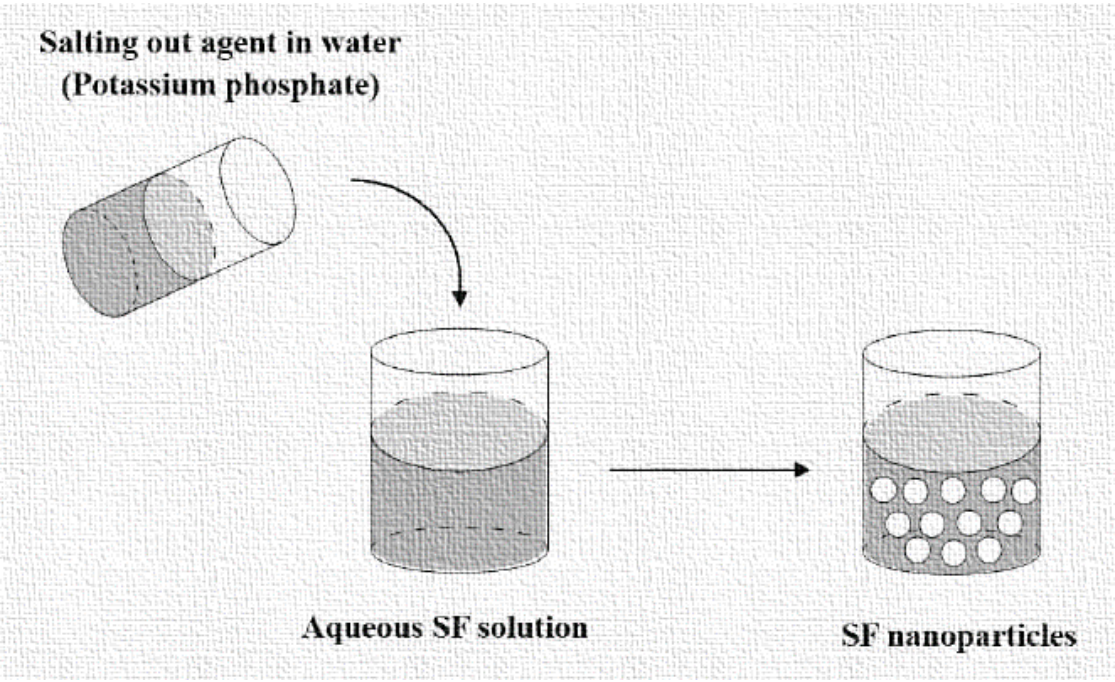

Fig. 2. The salting out procedure for making SF nanoparticles is depicted in this diagram.

3.3. Technologies for Supercritical Fluids

New supercritical fluid (SCF) technologies have recently been discovered as viable alternatives to traditional particle preparation methods, overcoming the drawbacks of traditional methods [33,34]. Supercritical fluids (SCFs) are substances that exist at temperatures and pressures that are higher than their critical values (Pc; Tc). SCFs have remarkable thermophysical capabilities, allowing them to permeate and dissolve material like a gas. Supercritical CO2 (scCO2) is the most widely used SCF and has been shown to have great potential in the field of micronization of materials due to its favourable critical conditions (Tc = 31.1 °C, Pc = 7.38 MPa), non-toxicity, non-flammability, and low costs in pharmaceutical, nutraceutical, and food applications [35,36].

Up to this point, the most well-known strategies for molecule development utilizing scCO2 incorporate the quick extension of supercritical arrangements (RESS), particles from gas-soaked arrangements or suspensions (PGSS), and gas or supercritical liquid antisolvent (GAS or SAS) [36,37,38]. Specifically, arrangement upgraded scattering by supercritical liquids (SEDS), a changed SAS process, has been broadly used to plan miniature or nanoparticles. Figure 3 shows the schematic outline of the SEDS interaction for planning SF nanoparticles. In this interaction, the arrangement containing solute and supercritical CO2 (scCO2) are atomized through an uncommonly planned coaxial spout to get beads with little size and upgrade blending to increment mass exchange rates. In this interaction, a spout with two coaxial entries permits the presentation of scCO2 and an answer into the high-pressure vessel where strain and temperature are controlled [40]. At the point when the arrangement contacts the scCO2, the high speed of the scCO2 separates the arrangement into tiny drops and improves mass exchange and common dispersion among SCFs and the drops momentarily, bringing about stage partition and supersaturation of the polymer arrangement, along these lines prompting nucleation and precipitation of the polymer molecule [41].

In the SEDS interaction, the scCO2 goes about as an enemy of dissolvable. Moreover, scCO2 is utilized as a "scattering specialist" to work on mass exchange among SCFs and the drops. Along these lines, tiny particles can be created. Furthermore, the molecule size dispersion and morphology of the polymer can be constrained by changing the boundaries of the SEDS cycle, including the convergence of solute, stream pace of arrangement, temperature, and tension of scCO2.

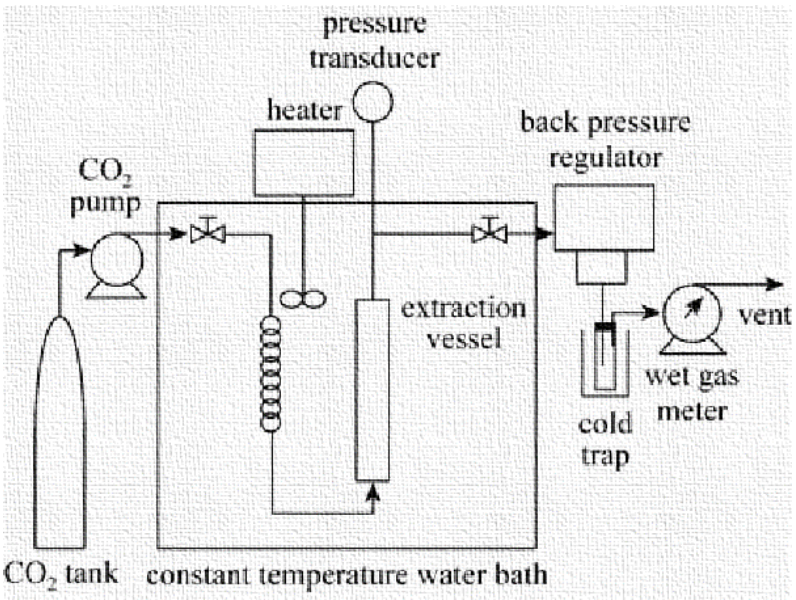

Fig.3. The SEDS process for making SF nanoparticles is depicted in this diagram.

3.4 Electrospraying

Electrospraying (electrohydrodynamic spraying) is an emerging approach for the quick and high throughput generation of nanoparticles that uses electrical forces to atomize liquids. The electrospraying process for preparing SF nanoparticles is shown schematically in Figure 4. The liquid pouring out of a capillary nozzle with a high electric potential is driven to scatter into minute droplets by the electric field in electrospraying [42,43].

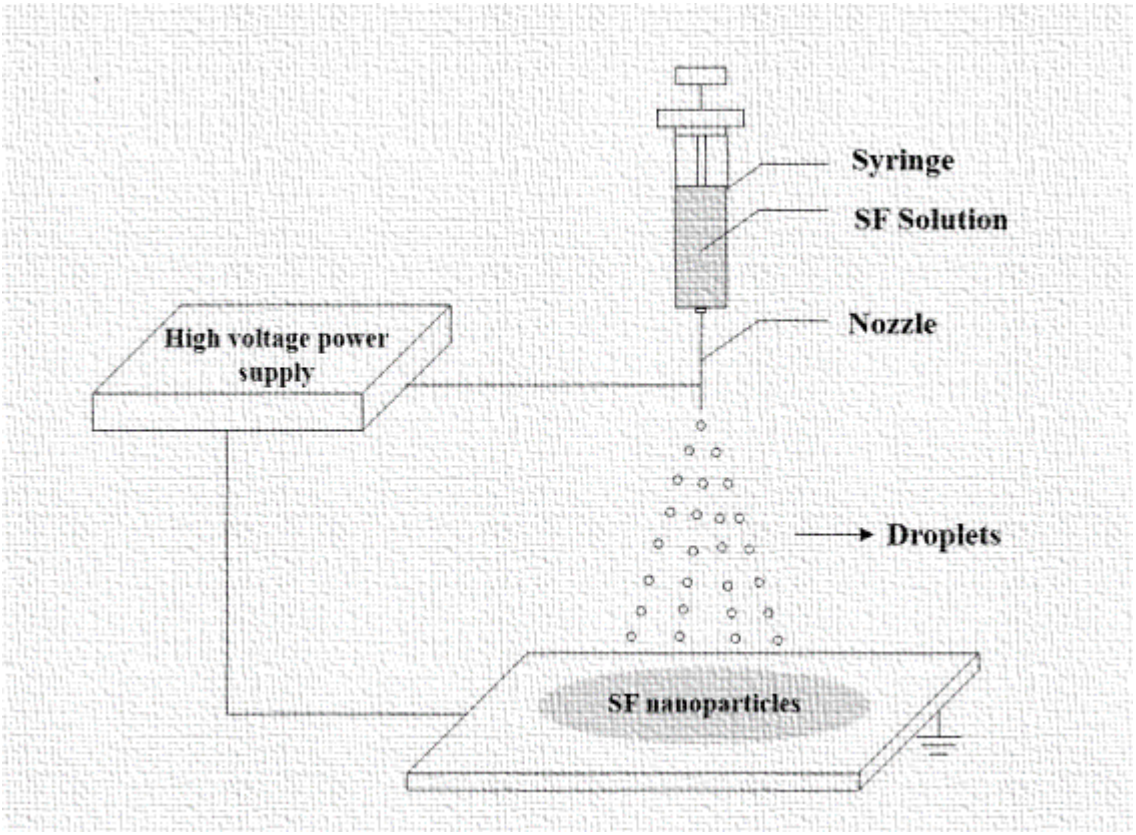

Fig.4. The electrospraying process for making SF nanoparticles is depicted in this diagram.

3.5. Comminution Mechanical

Crushing, grinding, milling, and other methods are used to reduce solid materials from one average particle size to a lower average particle size. The approach typically comprises high-energy dry/wet milling with milling additives, with milling periods ranging from a few hours to several days [44,45,46]. The schematic diagram of the mechanical comminution process for particle preparation is shown in Figure 5. The method is simple to use and expand. The process, however, still has problems guaranteeing that all of the particles are milled appropriately. More milling contaminants will arise from a longer milling duration. Furthermore, the particle size distribution is very broad. Impurities and any grinding aids employed during the preparation must also be eliminated [46].

Alkaline hydrolysis was used to degum the silk fibres first. Then, using attritor milling, SF nanoparticles with a volume median particle size d(0.5) of 7 m in diameter were created, which were then decreased to 200 nm with a narrow particle size distribution by bead milling. The pH and milling time were adjusted to control particle size. High pH, on the other hand, may induce chemical

damage to silk. To solve the problem of alkali degradation.[47] used a bead milling process with the biocompatible surfactant Tween 80 to make SF nanoparticles. Instead of using repelling charges at high pH in the milling of submicron particles, the surfactant can be utilised to aid milling and avoid aggregation. By attritor milling silk snippets, silk particles with a volume median particle size (d(0.5)) of 7 m were created as a predecessor. The precursor particles were subsequently bead milled with 0.5-mm beads and Tween 80 to produce SF nanoparticles with a d(0.5) of 200 nm and a narrow particle size distribution by using 30% Tween 80 on the weight of powder.

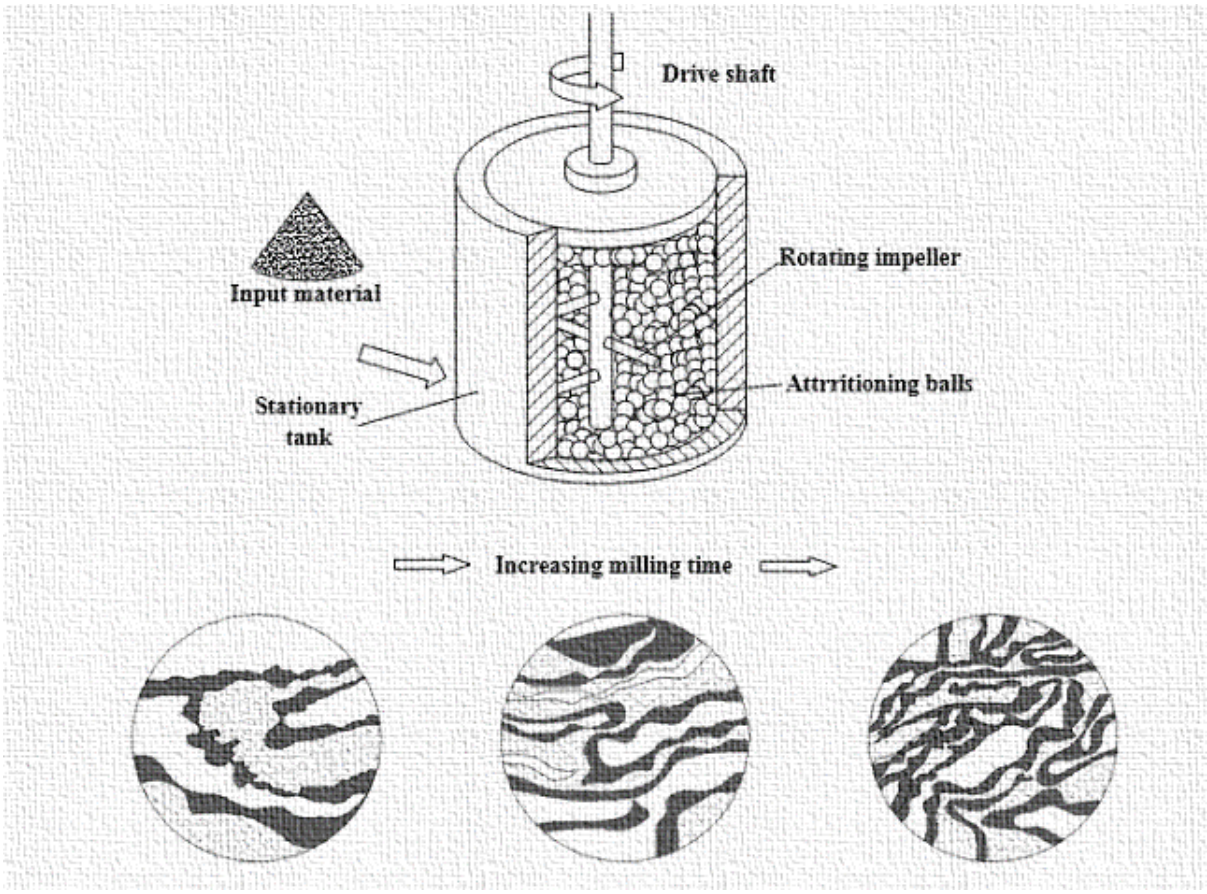

Fig.5. The mechanical comminution method for particle preparation is depicted in this diagram.

3.6 Method of Microemulsion

A microemulsion is a surfactant-assisted thermodynamically stable dispersion of two immiscible liquids (water and oil). Water-in-oil (w/o), oil-in-water (o/w), and water-in-sc-CO2 (w/sc-CO2) are the three forms of microemulsions. The aqueous phase in w/o microemulsions generates nanometer-size droplets in a continuous hydrocarbon-based continuous phase, and it's usually towards the oil apex of a water/oil/surfactant triangle phase diagram. Surfactant self-assembly is thermodynamically driven in this region, resulting in reverse or inverted micelles. Surface area can be reduced by spherical reverse micelles [48,49]. By adding a solvent to the microemulsion, such as ethanol, the precipitate can be extracted by filtering or centrifuging the mixture. The fundamental benefit of this approach is that it allows for improved particle size control by altering the nature and amount of surfactant and cosurfactant, as well as the oil phase and reacting conditions. Triton X-100 is used as a surfactant in this technique. To begin, the SF aqueous solution was stirred into the Triton X-100 cyclohexane mixture. The surfactant was subsequently removed, the

microemulsion was broken, and the particles were recovered using a mixture of methanol and ethanol. Fluorescent dye-encapsulated SF nanoparticles were also generated by combining a colour dye (rhodamine B) solution with the aqueous SF solution. The average size of the SF nanoparticles with and without fluorescent dye was around 167169 nm. The fluorescent molecules in the SF nanoparticles were found to be stable, indicating that these nanoparticles could be useful in molecular imaging and bioassays.

3.7 Fields of Electricity

Prepared an SF gel system (e-gel) comprising SF microspheres with diameters ranging from 200 nm to 3 m under modest electric fields. The electric fields approach for producing SF nanoparticles is depicted schematically in Figure 6. Briefly, five sets of SF solution were incubated at 70 °C for 6, 12, 24, 48, and 72 hours. The electrodes were then immersed in an aqueous solution of SF (0.8 wt%), and 25 V d.c. was delivered to a pair of conductive electrodes for 3 minutes. A visible gel formed at the positive electrode within seconds of the voltage being applied. SF gels were placed in liquid nitrogen after being washed with ddH2O. Finally, freeze drying was used to create the SF microspheres.

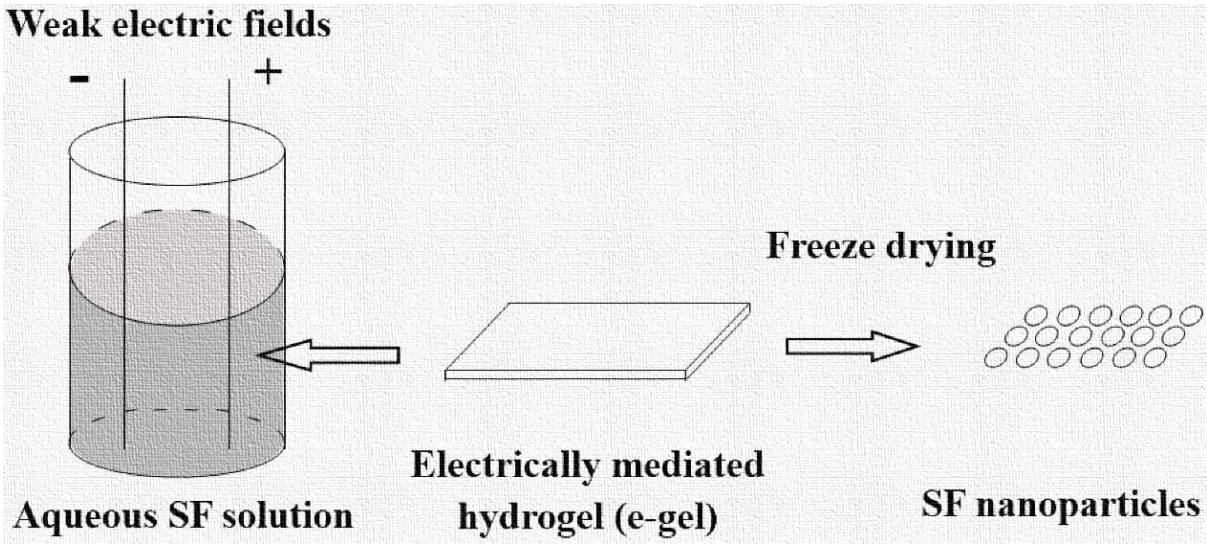

Fig. 6. Electric fields method for preparing SF nanoparticles.

3.8. Technique of the Capillary-Microdot

The developed capillary-microdot approach was used to create SF-encapsulated curcumin nanoparticles fewer than 100 nm in size. The capillary-microdot approach for manufacturing SF nanoparticles is shown schematically in Figure 7. Curcumin was mixed with SF solution to make a pharmacological suspension. A microcapillary was used to pour the suspension onto glass slides. The slides were then lyophilized after being frozen overnight. The resulting dried dots, which included SF-encapsulated curcumin nanoparticles, were scraped off the slides and crystallised using methanol. Phosphate-buffered saline (PBS) was used to rinse the nanoparticles recovered by centrifugation, and they were suspended in PBS for further examination.

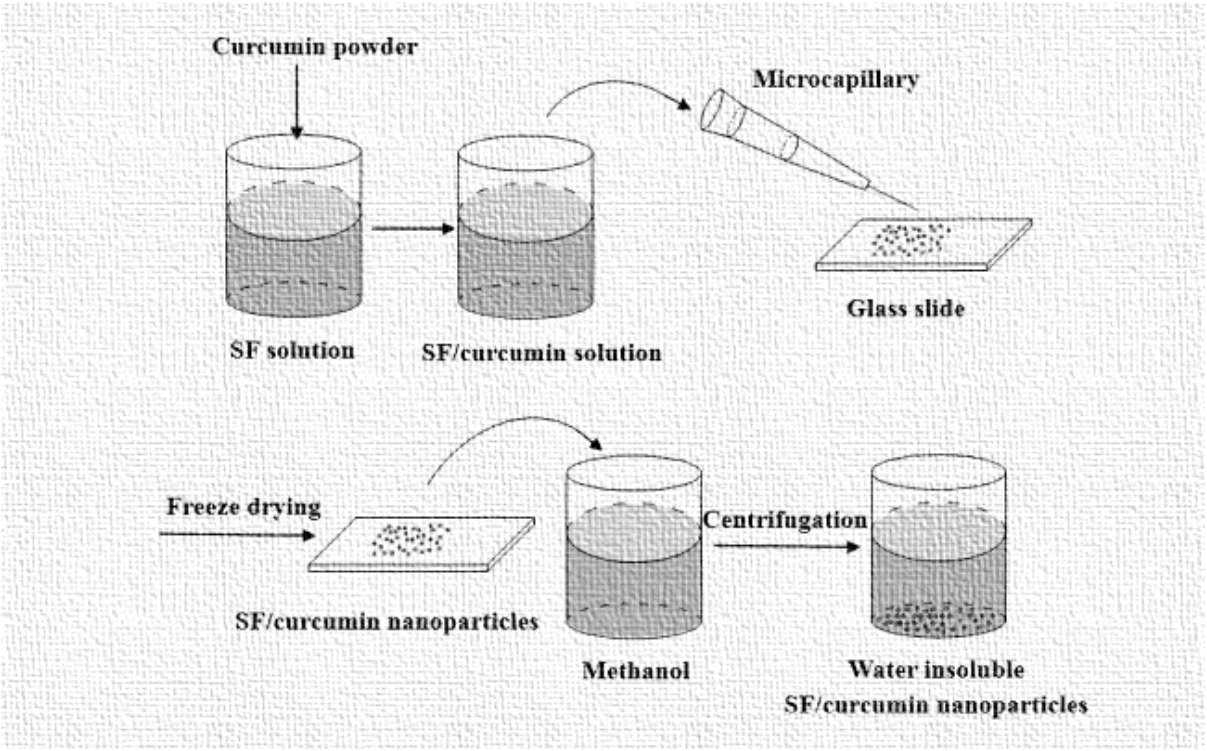

Fig.7. The capillary-microdot approach for making SF nanoparticles is depicted in this diagram.

3.9 Method of PVA Blend Film

Separation of SF solution into micro- and nanoparticles in SF/PVA blend films at a weight ratio of 1/1 to 1/4 employing PVA as a continuous phase to separate SF solution into micro- and nanoparticles [50]. The method was based on the separation of SF and polyvinyl alcohol phases (PVA). The PVA blend film method for manufacturing SF nanoparticles is shown schematically in Figure 8. In a nutshell, the SF/PVA blend solution was first dried into a film. Then, by dissolving the film in water and centrifuging the PVA out, water-insoluble SF particles could be created. Only water and PVA, an FDA-approved chemical, were used in the procedure, making it environmentally benign.

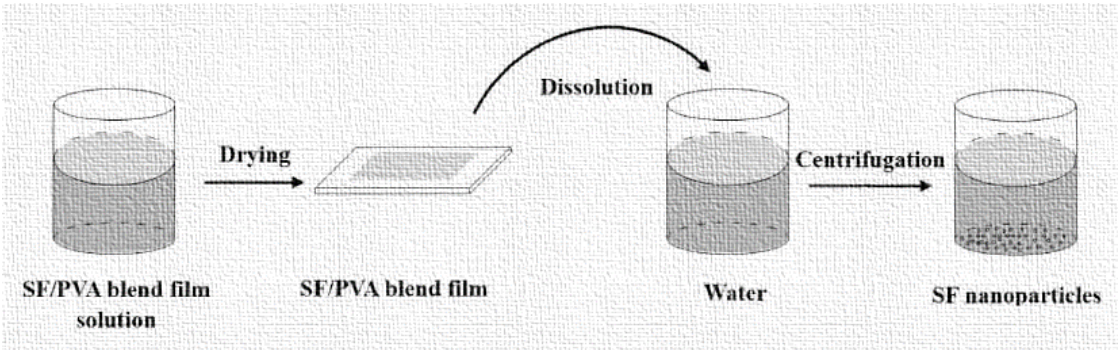

Fig. 8.PVA blend film technique for SF nanoparticle preparation schematic diagram

5.CONCLUSIONS AND FUTURE PERSPECTIVES

Fibroin has a strong mechanical resistance, the nanoparticle production process is complicated by its high stability, which is caused by the high number of hydrogen bonds in its secondary structure, which is typically in the form of antiparallel -sheets. Despite the remarkable features of SF nanoparticles that make them a prospective therapeutic carrier, certain significant obstacles remain. Each method of nanoparticle preparation has advantages and disadvantages, and it is critical to continue developing new nanoparticle fabrication techniques to meet varying demands. Furthermore, because SF varies between species and individuals within the same species, predicting SF degradation and drug release kinetics of SF-based nanoparticle drug delivery systems is difficult. To improve therapeutic efficiency, surface engineering strategies such as genetic engineering or surface chemical modification can be developed. Silk fibroin-based nanoparticles could offer a larger range of applications as technology advances.

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