

# Production of Electrospun Nanofibers from Food Proteins and Polysaccharides and Their Applications in Food and Drug Sciences

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## Abstract

Preparation of nano-microfibers from biopolymers (e.g., proteins and polysaccharides) by using electrospinning technology has been considered by researchers due to the formation of fibers or particles at the nano and micrometer scales, high porosity level, adjustable dewatering behavior, and special mechanical behavior. These products can be used in the microencapsulation of bioactive compounds, stabilization of enzymes and smart packaging. In the electrospinning method, a high voltage is used to create a nanofibers-particles. When the electric field overcomes the surface tension of the droplet, a jet exits the polymeric solution and is formed along the collector surface as it stretches toward the collector panel of the nanofiber. Parameters including molecular weight and polymer microstructure characteristics such as electrical conductivity, viscosity, surface tension, and the electrical potential applied by the device, solution flow rate, distance between the tip of the needle and the collector plate and sometimes the material of the collector plate are effective in the formation of electrospun fibers and particles. In this review, we discussed and evaluated the production stages, the strengths and weaknesses of the fibers produced from proteins and polysaccharides, and their functional properties and potentials, especially in food and drug sciences.

**Keywords:** Electrospinning, Nanofiber, Biopolymers

## Introduction

Electrospinning is an electro hydro dynamic process used to produce nano-microscopic nanoscale fibers, in which the electrical field is applied to a biopolymer solution to produce nano-fiber-particle. Electrospinning consists of several simple parts, including a syringe, syringe pump, high voltage source, and collector plate. In this method, the polymer solution is charged under the electric field, and when the repulsive force of unlike charges overcomes the surface tensile force, a jet of polymer particles is guided from the tip of the needle to the collector plate. The jet moves rotationally and the solvent evaporates during the movement, which leads to the formation of the fibers-particles on the surface of the collector plate (1, 2). Effective parameters in the electrospinning process are divided into two groups: 1- parameters related to polymer solvent properties (molecular weight, conductivity coefficient, surface tension and viscosity), and 2- parameters related to the process (applied voltage, needle gap to collector plate, solution flow rate, and material of the plate collector) (3).

The properties of the polymeric solution have the most effect on the electrospinning and the fiber structure obtained. The first requirement for the electrospinning process is having a solution with electrospinning capabilities. The diameter of the fiber obtained increases by the elevation of the solution's viscosity. In addition, the increase of the electrical conductivity increases the efficiency of electrospinning. The possibility of involving of the chains and their electrospinning increases by an elevation in the molecular weight. Another important factor affecting the electrospinning process is those applied by

the device to the electrospinning current. Considering the applied force resultant, the increase of voltage leads to an increase or decrease of fiber diameter. Increase of the solution current results in increased fiber diameter (4). Owing to their unique features (e.g., high surface-to-volume ratio, high porosity, adjustable dewatering behavior, and mechanical behavior), the fiber obtained by electrospinning can be used in various fields, such as medicine, smart drug delivery, food industry, and tissue engineering (5).

In the electrospinning process, synthetic polymers and biopolymers (proteins and polysaccharides) can be used to produce fibers. The increasing concern of researchers regarding the use of synthetic polymers due to their risks and effects on human health has attracted the attention of scholars to more use of biopolymers. Several biopolymers have been tested for fiber production via electrospinning (6, 7). There are many variations in the use of biopolymers in the electrospinning process, in a way that several biopolymers can be used as a combination, which is carried out by applying a solution containing several compounds (8). In studies conducted on a mixture of polymers for electrospinning, multiple systems, and core-shell structures have been significantly considered for food and pharmaceutical applications (9). In the present research, we evaluated the electrospinning of food biopolymers, including proteins and polysaccharides, important parameters involved in their electrospinning, and the main application of these products.

## 2. Electrospinning of Protein Fibers

The presence of the third and fourth structures that prevent their chains from being pulled in

the form of a chain complicates the electrospinning of proteins. In addition, compared to stranded proteins, spherical proteins have a low ability to be pulled during the electrospinning process due to their significantly low interaction with each other. To increase the electrospinning potential of proteins, they must be completely dissolved in the solvent and their secondary structures must be denatured. While the electrospinning of casein seems easy due to its spiral structure, there is no electrospinning ability in aqueous solutions due to the formation of casein clusters after dissolving in water (10).

As mentioned, proteins must be completely solved in the solvent and denatured in order to have pullability during the electrospinning process (11). With high solubility, solvents can cause the denaturation and electrospinning of proteins by destructing the bonds of the third and fourth structures. The electrospinning of gelatin and collagen can be carried out using solvents such as Hexafluoro-2-propanol (HFP) or acidic solutions (12, 13). Application of carrier polymer is another method used for electrospinning of proteins. Generally, carrier polymer is a polymer that has electrospinning ability and can give the electrospinning ability to protein by being added to it without the protein having this ability itself. An example of a polymer with a high electrospinning ability is polyvinyl alcohol, which gives the electrospinning ability in combination with soy protein isolate (14, 15).

For proteins used in medical applications, polyvinyl alcohol and polyethylene oxide are often selected as carrier polymers (15, 16). Another method for electrospinning of proteins is the application of cross linkers, which cause the formation of different properties in the fiber and include natural and

synthesis linkers, such as Genipin, glutaraldehyde, N-3-dimethylaminopropyl-n-ethylcarbodiimide hydrochloride (EDC), and EDC with N-hydroxy sulfo succinimide. Use of crosslinkers in gelatin electrospinning increases the diameter and leads to smearing, ultimately increasing the stability of the produced fibers. Another advantage of using polymer carrier and crosslinkers is the improvement of mechanical stretching and fiber stability (17, 18).

There are several crosslinkers that create different features in fibers. The effect of natural and synthetic crosslinkers, such as genipin, glutaraldehyde, N3 dimethylaminopropyl, N-ethylcarbodiimide hydrochloride (EDC), and EDC with N-hydroxysulfosuccinimide (NHS) on collagen electrospinning has been evaluated, demonstrating that EDC and EDC-NHS are the most appropriate choices and establish fiber stability for up to three months (19). However, one of the disadvantages of crosslinkers (e.g., glutaraldehyde) is probably their toxicity. The toxicity of the fibers is due to the inactive compounds remaining in the crosslinkers, which remain in the fibers due to inadequate washing (20). Oleuropein is olive leaf extract that can be used as a natural crosslinker for protein fibers (21).

Protein fibers that are stable in an aqueous environment and without cross linkers, such as laminin fibers produced by Neal et al., are much more than fibers produced by food industry connectors (22). In Table 1, a number of electrospun proteins and solvents used for electrospinning are presented. Subsequently, some of the most important electrospun proteins have been assessed.

**Table 1. Electrospun protein fibers**

| Protein                         | Solvent/carrier                              | References   |
|---------------------------------|--|--------------|
| Gelatin                         | poly(caprolactone)/hexafluoroisopropanol     | (23)         |
|                                 | Acetic acid                                  | (24)         |
|                                 | Water  | (25)         |
|                                 | 2,2,2-Trifluoroethanol                       | (26)         |
|                                 | Hexafluoro-2-propanol                        | (27)         |
| Collagen                        | Acetic acid/Dimethyl sulfoxide               | (28)         |
|                                 | Hexafluoro-2-propanol                        | (29, 30)     |
|                                 | Trifluoroacetic acid                         | (18)         |
|                                 | PHBV/ Hexafluoro-2-propanol                  | (31)         |
| Soy                             | Polyvinyl alcohol/water                      | (32)         |
|                                 | polyethylene oxide/water                     | (33)         |
| Zein                            | Ethanol                                      | (21, 34, 35) |
|                                 | Isopropanol                                  | (36, 37)     |
|                                 | Dimethylformamide                            | (38)         |
|                                 | 2,2,2-Trifluoroethanol                       | (39)         |
|                                 | Acetic acid                                  | (40)         |
| Amaranth                        | Pullulan/formic acid                         | (41, 42)     |
|                                 | Hexafluoro-2-propanol                        | (43)         |
| Casein                          | polyethylene oxide/water; triethanolamine    | (44)         |
|                                 | Pullulan/water                               | (45)         |
| Whey                            | Guar gum/water                               | (8)          |
|                                 | polyethylene oxide/water                     | (14, 46)     |
| Algae                           | polyethylene oxide/water                     | (47)         |
|                                 | poly methylene oxide/aqueous sodiumhydroxide | (47)         |
| Fish sarcoplasmic proteins (SP) | Hexafluoro-2-propanol                        | (48-50)      |

## 2.1. Soy

Recognized as one of the most cost-effective protein products, electrospinning isolated soy protein is considered due to its biocompatibility and high maintenance capability (51). In addition, it has features of wound healing and reconstruction of damaged tissues (52). However, this protein cannot perform electrospinning alone and requires a carrier polymer for fiber formation (53, 54). Therefore, polyethylene oxide and polyurethane are used as a carrier polymer for electrospinning of isolated soy protein. Isolated soy protein fibers have been applied for microencapsulation of zinc oxide and ciprofloxacin hydrochloride, resulting in positive results such as antimicrobial activity in the laboratory environment (55-57). Moreover, isolated soy protein is employed to package food and produce food foils (53).

## 2.2. Gelatin

Owing to its compatibility with the environment, degradability, and low cost, gelatin, which is obtained from the thermal or chemical degradation of collagen, has been greatly emphasized as a natural polymer. While gelatin is a water-soluble polymer, the electrospinning of its aqueous solution alone is not possible since gel quickly forms and coalesces in the capillary tube during the electrospinning process. The formation of a triple helix depends on the gelatinization of gelatin, which inhibits its electrospinning. Therefore, attention has been paid to the

combination of solvent and environmental parameters that prevent the formation of this triple helix (58). Researchers have proposed the use of solvents such as formic acid to performing gelatin electrospinning (59). The gelatin/poly caprolactone fibers were produced, and the considerably high level of cell proliferation on the poly caprolactone gelatin scaffolds was shown (60).

### 2.3. Zein

Zein is the major storage protein from corn and a prolamin. In addition, it is a secondary product of bioethanol production in the industry, widely applied to create nano/microfiber since 1960 (61-63). Electrospinning of zein is carried out with food and food industry purposes (64). The loading of curcumin in zein fibers showed that curcumin was released slowly and constantly and revealed anti-oxidant and antibacterial properties. Zein fibers are exploited to improve the oxidative stability of fish oil. When placed in zein fibers, omega 3 fatty acids (Omega 3) are more protected against oxidation, compared to omega-3 fatty acids that have a free state (65-67).

### 2.4. Amaranth

The amaranth protein isolates have an herbal origin. Along with pullulan, this protein is electrospun for microencapsulation of folic acid (41), curcumin (42), folic acid and quercetin (68). For example, quercetin and

folic acid are released in electrolytic fibers continuously and slowly, and their antioxidant properties are more likely to be preserved, compared to the free mode (68).

### 2.5. Marine Proteins

The health providing and bio-activity benefits of fish proteins have made increased their use in various areas, including pharmaceutical and food industry (69-73). Electrospinning of the proteins obtained from *Botryococcus braunii* algae was carried out applying polyethylene oxide as a carrier (69, 70). In addition, researchers have studied the electrospinning of sarcoplasmic protein (soluble in water), which includes peptides and proteins with high molecular weights. Fish sarcoplasmic protein is economically justifiable due to its abundance in industrial Wastewater. In addition, nano/microfibers produced from fish sarcoplasmic protein cannot be dissolved in an aqueous environment and are decomposed by proteolytic enzymes (74).

### 2.6. Whey Protein Isolates

The whey protein isolate is water soluble and forms more particles when electrospun separately (75). The electrospinning of the protein is carried out with the concomitant use of a carrier, including pullulan (76) and polyethylene oxide (46). Fibers produced by the electrospinning of whey protein isolates are applied for microencapsulation of

probiotic bacteria to increase their survival (77). In addition, whey protein isolates are exploited for encapsulation of rosemary extract. Evaluation of its release demonstrated that release depends on pH, which argues the possibility of its use in smart drug delivery systems (46). Furthermore, whey protein isolates are electrospun along with dextran and the resulting fibers have acceptable morphology (78). Electrospinning of whey protein isolates along with Guar gum in aqueous solution was carried out, where the major part of the resulting fibers was whey protein isolates (8).

### 2.7. Casein

Casein is an insoluble milk protein, accounting for 70% of milk proteins (79). However, casein alone cannot be electrospun and must undergo this process along with a carrier such as polyvinyl alcohol, polyethylene oxide, and pullulan. Casein fibers with polyethylene oxide were prepared to stabilize the lipase enzyme, and the activity of the lipase enzyme in polyvinyl alcohol fibers was six times higher than that of casein and polyethylene oxide fibers, which can be attributed to the high level of polyvinyl alcohol fibers, which increases the level of access to the enzyme (44).

### 3. Polysaccharide Electrospinning

Polysaccharides are the most abundant

renewable resources in the environment. In Table 2, a number of electrospun polysaccharides and solvents used for their electrospinning are presented. The electrospinning capacity of the polysaccharides depends on the length of the molecular chains and the interconnection of the molecular chains to each other during stretching (6, 80). Therefore, molecular weight, the concentration of biopolymer solution, their conformation structure and dilution in shear thinning affect their electrospinning properties. The concentration required for the electrospinning of polysaccharides is a concentration in which the polysaccharide molecules become closer to each other so that the molecular bonding of the chain to the adjacent chain could be possible.

This situation is associated with stretching and electrospinning. Polysaccharides with a random spiral structure and a low-shear thinning behavior (e.g., amylose and starch amylopectin) are suitable for electrospinning. In addition, spherical polysaccharides have a lower tendency for electrospinning (6, 81). In Table 2, a number of electrospun polysaccharides and solvents used for their electrospinning are presented. In the next part, some of the most important electrospun polysaccharides will be evaluated.

**Table 2. Electrospun polysaccharide**

| Polysaccharide | Solvent   | Reference            |
|----------------|---|----------------------|
| Chitosan       | Trifluoroacetic acid/Dichloromethane<br>Acetic acid   | (82-85)              |
| Starch         | Formic acid<br>Dimethyl sulfoxide<br>95%  | (81, 86-88)          |
| Alginate       | Pullulan/water  | (89)                 |
| Pullulan       | Redistilled water<br>Dimethyl sulfoxide<br>and Dimethyl<br>sulfoxide/water<br>95% Formic acid | (81, 87, 88, 90, 91) |

### 3.1. Chitosan

Chitosan is a polysaccharide derived from chitin, which is produced by thermochemical reactions. Chitosan can be found in weak acids such as acetic acid. One of the unique features of chitosan is its antimicrobial property, which is due to the presence of positive charge amine groups in its structure that react with bacterial cell membranes and cause bacterial death through their destruction (92, 93). Several attempts have been made to make electrospun chitosan nanofibers by changing the electrospinning conditions

(needle distance to the collector plate, voltage, solvent flow rate), as well as the physical and chemical properties of chitosan, such as the molecular weight (Mw) of chitosan or the change of solvent type (94-96). In a study, chitosan fibers were used for microencapsulation of the essential oil. Moreover, the researchers assessed the effect of rheological properties on electrospinning of chitosan (97). Moreover, the electrospun chitosan nanofibers are applied for microencapsulation of glucose oxidase and hydrogen peroxide, which have revealed their antimicrobial properties (98). Essential oils of cinnamon (99), silver particles (100, 101), curcumin (102) and lysosome (103) were electrospun in chitosan fibers.

### 3.2. Starch

Starch is a plant protein storage that consists of two parts, namely amylose and amylopectin. Early studies have focused on the electrospinning potential of amyloid due to the linearity and ability to establish intramolecular bonding (81, 87, 88). Amylopectin content of starch plays an important role in the electrospinning of starch and should be below 65% since helix structure of starch increases the electrospinning ability and leads to the formation of starch nanofibers (104). Starch derivatives such as hydroxypropyl are electrospun in combination with polyethylene oxide, and the resulting fibers are used in tissue engineering (105).

### 3.3. Alginate

In a research, the electrospun nanofiber of alginate was obtained using a polar solid solvent such as glycerol (106). In addition to reporting a diameter of 120-300 nm for the fiber produced, researchers demonstrated that the fiber diameter had a direct relationship with alginate concentration and the ratio of water and glycerol to the solvent. The presence of glycerol in alginate solutions improves the alginate electrospinning, which is the result of the destruction of intracellular and exogenous hydrogen bonds of alginate chains. To facilitate alginate electrospinning, the combination with other polymers, such as polyethylene oxide, was evaluated (107). Generally, the electrospun alginate/chitosan mixture can be used for the treatment of wounds and drug delivery applications (108). Furthermore, the fibers obtained from alginate-pectin-polyethylene oxide mixture can be exploited for microencapsulation of folic acid to increase its sustainability against the light (109).

### 3.4. Cellulose

Cellulose is insoluble in a wide range of solvents due to its chemical structure (110). The electrospinning process of this polysaccharide is limited, and the electrospinning ability of cellulose significantly depends on molecular weight

and solvent selection (111). The cellulose-based electrospun nanofibers have been assessed for non-immobilizing of enzymes and microencapsulation of drug/vitamin (112). The cellulose acetate nanofibers with a 230 nm diameter are used for non-immobilizing of Lipase *Candida rugose*, which showed the significant increase in thermal stability and relatively high durability relative to the equivalent free enzyme (113). In addition, the electrospun cellulose acetate fibers are applied as biosensors to detect the considerably low concentrations of viologen and cytochrome C in aqueous solutions (114).

### 3.5. Dextran

A bacterial polysaccharide composed of D-glucopyranose units, dextran has several applications in areas of drug delivery, medicine and food industry owing to its biocompatibility and biodegradability features (115). The crosslinking of dextran by glutaraldehyde occurs with regard to the water solubility of dextran, and the resulting fiber with a diameter of 200 nm can be used in biosensors (116, 117).

### 3.6. Pullulan

Pullulan is also water soluble and fibers with a diameter of 100-700 nm are obtained in their electrospinning from aqueous solution. In addition, this compound is used as a carrier



polymer (90, 118), and its fibers with a diameter of several hundred nm to several  $\mu\text{m}$  were obtained by electrospinning of the solution containing a mixture of DMSO/water as a solvent. The electrospun fibers can be used for microencapsulation of bioactive compounds and active packaging (119, 120). Furthermore, pullulan fibers have been exploited along with amaranth protein for microencapsulation of curcumin and quercetin (42, 68).

#### 4. Conclusion

Electrospinning is a cost-effective and simple technique for nanofiber production with the use of polymers. Given the high potential of nanofibers in various fields (e.g., medicine, smart drug delivery, active food packaging and filtration) and with regard to the increasing concerns of researchers about the adverse effects of synthetic compounds, the electrospun polymer nanofibers are expected to be produced at industrial scales for application in the mentioned fields, especially as a foundation for antibacterial compounds.

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#### Declarations

##### *Conflict of interest*

We declare that we have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the article.

##### *Authors' contributions*

All authors contributed equally to this work.

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