



# Developed methods for the preparation of electrospun nanofibers containing plant-derived oil or essential oil: a systematic review

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## Abstract

Recently, natural health products as alternatives for synthetic/chemical substances have become a growing area of interest. Plant-derived essential oils or oils (E/Os) with a wide range of bioactivities such as anticancer, antibacterial, antifungal, and antioxidant activities are widely used among natural materials. Furthermore, nanofibers (NFs) with distinct properties, including large surface area, many available ingredients for preparation, and various preparation methods have attracted much attention. The present systematic review is an attempt to collect and document the recent studies from 01.01.2013 to 31.12.2018, indicating the loading of E/O in electrospun NFs. First, a summary of the electrospinning process and applications of electrospun NFs in medicine were given. Then, the three manners, which have been introduced for preparing E/O-loaded NFs so far, were described. Moreover, the main techniques for characterization of such NFs, e.g., evaluation of size and morphology, determination of the loaded amount of E/O in NFs, and investigating their release behavior, were explained.

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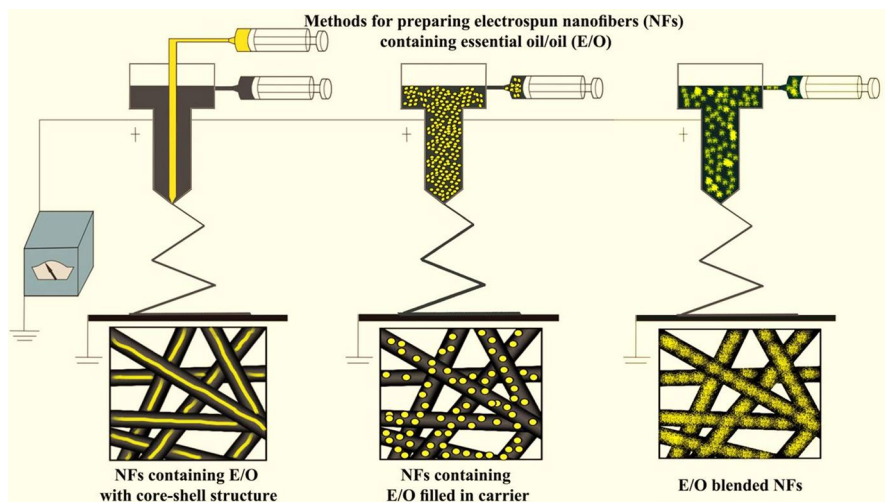
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## Graphic abstract



**Keywords** Essential oil · Electrospun · Nanofibers · Electrospinning · A systematic review

## Nanotechnology

Nanotechnology is defined as targeted manipulations of materials in nanoscale for obtaining size-dependent features or functions [1]. The most common nanomaterials include metallic nanoparticles [2, 3], nanoemulsions [4], polymeric nanoparticle [5, 6], lipidic nanocarrier [7], and nanofibers (NFs) [8]. NFs (diameter ~200 nm) with small pores and large surface areas have enormous applications in medicine, such as tissue engineering, drug delivery, and wound dressing [9]. Various techniques, including template-assisted synthesis, electrospinning, phase separation, self-assembly, solvent casting, and drawing–processing, were developed for the fabrication of NFs based on a literature review [10–12]. Electrospinning is a versatile and easily adaptable technique and the most common method for the preparation of NFs [13–15]. Electrospinning, compared with other approaches such as phase separation and self-assembly, has the advantages of versatility and flexibility in the selection of material and control over fibers' morphology and diameter [16]. The preparation of NFs using drawing–processing also needs viscoelastic material that can undergo substantial deformation during this process [17]. In template-assisted synthesis, the elimination of the template often leads to defects in structures of NFs [18].

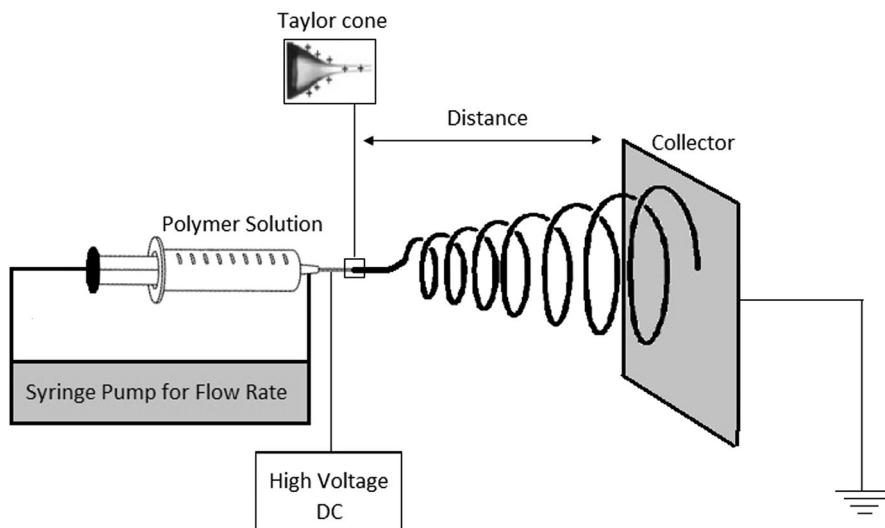
## Electrospinning

An electrospinning setup typically consists of a high-voltage source, a polymer solution or melt, an electrically conductive blunted needle, a syringe pump, and a collector [19, 20]. The electrospinning process consists of three continuous stages, including jet initiation, elongation, and solidification into NFs. During jet initiation, the polymer solution is subjected to an electric field, which was filled into a syringe connected to the spinneret. When the high voltage is applied to the spinneret, typically in the range of 10–30 kV, the surface of the fluid droplet gets electrostatically charged at the tip. As a result, two forces affect droplets, i.e., electrostatic repulsion between the surface charges and the columbic force applied by the external electric field. Due to these electrostatic interactions, the liquid drop elongates into a conical object known as the Taylor cone [16, 21]. When critical electric field intensity is applied between spinneret (positive) and collector (negative), the electrostatic forces on the surface are sufficient to overcome the surface tension holding the droplet together. That leads to a forcible ejection of the liquid jet from the tip; the fluid jet continues to be ejected steadily and elongated. The solvent evaporates before reaching the collector, leading to the formation of a randomly oriented of thin polymeric fibers on the collector [16]. By using types of a collector, such as metallic plate, drum, parallel electrode, and an array of counter electrodes, the morphology and structures are customizable [22].

In fixed environmental conditions, (temperature and relative humidity) optimizations of two types of parameters during the electrospinning are crucial. Instrumental factors include the applied voltage, the injection rate of polymer, and the distance between the needle and collector. Polymer solution parameters are concentration or viscosity, conductivity, and solvent volatility [23, 24]. A schematic of electrospinning and main parameters is shown in Fig. 1.

*Applied voltage* Applying high voltage into polymeric solution via a metallic needle will cause a spherical droplet to deform into a Taylor cone. At a critical voltage, depending on the used polymer, ultrafine NFs could be harvested. The formation of smaller-diameter NFs by applying higher voltage is attributed to the stretching of the polymer solution in correlation to the charge repulsion within the polymer jet. Interestingly, an increase in the diameter of NFs, as well as the formation of beads or beaded NFs, could occur when a higher voltage than the critical value is applied. That is attributed to the decrease in the size of the Taylor cone and the increase in the jet velocity for the same flow rate [25, 26].

*Flow rate* Uniform and beaded electrospun NFs could be prepared via a critical flow rate for a polymeric solution, which varies from polymer to polymer. Increasing the flow rate (higher than a critical value) leads to an increase in the pore size and fiber diameter. Also, due to incomplete drying of the NFs jet during the flight between the tip and collector, beads formation is inevitable [21, 27].



**Fig. 1** Electrospinning and main parameters

*Distance between tip and collector* The morphology of obtained electrospun NFs is critically dependent on the distance between the needle tip and collector and varies with the polymers system. When this distance is kept small and defective, large-diameter NFs are formed. However, by increasing this amount, the diameter of the NFs is decreased [28, 29].

*Polymer concentration* The stretching of the charged jet is significantly related to the concentration of the polymeric solution. In low concentration, the polymer chains break into fragments before reaching the collector, due to the applied electric field and surface tension. These fragments cause the formation of beads or beaded NFs. Increasing concentration (viscosity) of the polymeric solution leads to better chain entanglement, which can overcome the surface tension and ultimately result in uniform and beadless electrospun NFs [30, 31].

*Solution conductivity* This factor affects the formation of the Taylor cone as well as controlling the diameter of the NFs. At very low solution conductivity, the surface of the droplet will have no charge to form a Taylor cone; i.e., no electrospinning will start. Increasing the conductivity of the solution to a critical value leads to an increase in charge on the surface of the droplet to form a Taylor cone, which also causes a decrease in the fiber diameter. Increasing the conductivity beyond a critical value results in hindering the Taylor cone formation and electrospinning [17, 32].

*Solvent volatility* Formation of smooth and beadless electrospun NFs are dependent on the selection of appropriate solvent. The polymer should be completely soluble in the solvent, and it has a low boiling point. The use of highly volatile solvents causes an increase in the rate of evaporation and drying of the jet at the needle tip.

This drying will block the needle tip and will hinder the electrospinning process. In contrast, the use of high-boiling solvents prevents them from drying during the flight of the NFs jet. The deposition of NFs containing solvent on the collector will cause the formation of beaded NFs [25, 33]. A summary of the mentioned factors is given in Table 1.

## Application of NFs in medicine

*Drug delivery* Drug delivery using NFs is based on increasing drug dissolution in their high surface area as a carrier [34]. Interestingly, cargoes are not limited to drugs; numerous kinds of DNA and RNA and proteins were also incorporated with electrospun NFs [35]. This combination can either be loaded into the electrospun NFs or coated on their surface. Both types of mentioned methods can provide a controlled and sustained release of a drug at the target site [25]. The possibility for loading of several medications on/into NFs and proper release profiles leads to enhanced therapeutic efficacy and reduced toxicity [36].

*Tissue Engineering* Unique properties of electrospun NFs such as the higher surface area-to-volume ratio, porosity, and manipulatable mechanical properties have led to their extensive use in tissue engineering [37]. Also, almost every extracellular matrix (ECM) of connective tissue, such as cartilage, bone, and skin, is based on NF structures [38, 39]. NF-based scaffolds not only have shown an impact on the cell-to-cell interaction but have also increased the interaction between the cells and matrix. Therefore, the growth of many cells on these scaffolds has been reported to be excellent [40]. Besides that, other desirable properties for tissue-engineered scaffolds can be achieved by electrospun NFs mesh, for example, regulating porosity, adjusting surface morphology, and capability for surface functionalization [41–43].

*Wound dressing* Wound healing is a dynamic and intricate process, including homeostasis, inflammation, proliferation, and remodeling. An ideal wound dressing should provide an excellent moist environment for the wound site to enhance wound healing, as well as should have the ability to handle pathogens, especially antibiotic-resistant bacteria [44, 45].

**Table 1** Parameters affecting the electrospun NFs

Parameters	Effects
Higher voltage	Decrease in fiber diameter
Higher flow rate	Generation of beads in fiber
Higher distance between tip and collector	Decrease in fiber diameter
Higher polymer viscosity (concentration)	Increase in fiber diameter
Higher solution conductivity	Decrease in fiber diameter
Higher solvent volatility	Decrease in fiber diameter

NF-based scaffolds attract cells to the dermal layer, which can excrete vital extracellular materials (e.g., cytokines, collagen, and growth factors) that assist in the repair of damaged tissues [46]. Numerous such scaffolds were prepared via raw materials such as chitosan, gelatin, and collagen; their role in wound healing has been proven [44, 47]. Furthermore, electrospun NFs can be used in the fabrication of cosmetic masks, skin cleaner, and bandage [48]. Based on the mentioned properties of electrospun NFs, they provided numerous advantages over wound dressing agents prepared using conventional methods [49]. In Fig. 2, schematics of three mentioned applications of E/O-loaded NFs are illustrated.

## Plant-derived oil and essential oil

The use of renewable raw materials has a vital role in sustainable development in countries. Some of the most widely applied renewable raw materials are included, such as plant-derived essential oils or oils (E/Os). Essential oils (EOs) are natural liquid oils containing a mixture of many chemical components [50]. Hydro-distillation via the Clevenger-type apparatus is the most common method for extraction of EOs from different parts of plants, such as floret, roots, leaves, peel, bark, stem, and fruits [51]. Furthermore, plant oils are one of the most important renewable raw materials as a primary substance for surfactants, cosmetic products, lubricants, flooring materials, coating, and resin applications [52]. Nowadays, E/Os are

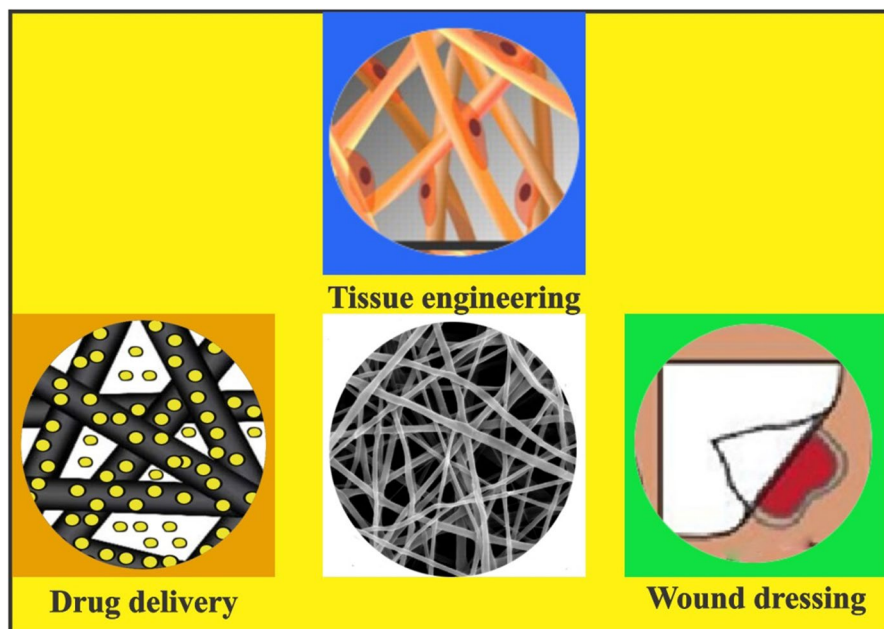


Fig. 2 Three primary applications of E/O-loaded NFs in medicine

commonly used in medicine as an antibacterial or antifungal agent [53], antioxidant-based materials [54], larvicidal bioassays [55], and natural anticancer drugs [56].

Recently, researchers give special attention to improving the mentioned properties of E/Os [57] as well as developing the sustained release formulations using NFs [58]. Excellent review articles have already been published on the applications of NFs in the medical and health sciences. For instance, in one study, reports were categorized by used herbal substance and described the advantages of using NFs and plant-derived elements simultaneously [59]. In another review paper, researches have been conducted in which plant-derived materials (extracts and EOs) were loaded into NFs. The documents have been categorized in the areas of antibacterial, tissue engineering, and food industry by application of the prepared products [16]. Finally, another review article has been found, addressing the biological applications of NFs in drug delivery, wound dressing, and antibacterial activity. Unlike the previous articles, the focus of this article was on collecting information about embedding of metallic nanoparticles (Ag-, Zn-, and Cu-derivative nanoparticles) and common drugs (Ampicillin, Rifampin, and Vancomycin) into NFs [21].

For the first time, in this research, literature was systematically reviewed from 2013.01.01 to 2018.31.12 for collecting data about the methods for the preparation of electrospun NFs containing E/Os and their detailed characterization.

### Method for data collection and inclusion criteria

PubMed Web site (<https://www.ncbi.nlm.nih.gov/pubmed/advanced>) was searched from 2013.01.01 to 2018.31.12 using six special terms, independently (Table 2). Five terms were used for searching for NFs, and one word was assigned to find E/Os (oil\*[Title]). Then, every five words were searched binary with “oil\*[Title]”

**Table 2** Details of systematically search (2013.01.01–2018.31.12) and selected papers

Step no.	Search keyword	Number of papers
1	Nano fiber*[Title]	40
2	Nanofiber*[Title]	3963
3	Nanofibrous*[Title]	935
4	Electrospun*[Title]	2557
5	Electrospin*[Title]	732
6	Oil*[Title]	20,420
7	Step 6+5	7
8	Step 6+4	33
9	Step 6+3	17
10	Step 6+2	36
11	Step 6+1	0
12	Total results of steps 7–11	93
13	Ignoring duplicated papers	76
14	Reviewing abstracts and including final papers	25

(steps 7–11): Totally, 93 papers were collected (step 12). After ignoring duplicated documents, 76 articles were selected for review (step 13). In the last stage, the papers about E/O-loaded NFs were included, in which finally 25 were selected and reviewed.

## Methods for preparing electrospun NFs containing E/O

Three different approaches have been developed for preparing electrospun NFs containing E/O. First, E/Os were mixed with a polymer solution; then, the mixture was subjected to electrospinning for making E/O-blended NFs. Second, E/Os were first loaded into a carrier and then was electrospun for the harvesting of NFs containing E/O filled in a carrier. Third, NFs with core–shell structure (E/O and polymer) were prepared. The examples for these three modes are given in Tables 3, 4, and 5.

### E/O-blended NFs

There are 17 reports listed in Table 3. Preparation of E/O-blended NFs has been performed in two steps. First, a homogenous solution of polymer and E/O was prepared using an appropriate solvent. Then, the mixture was subjected to electrospinning.

In one of the reports, a composite of PVA-CHI-GT (polyvinyl alcohol–chitosan–gelatin) was used for blending with *Zataria multiflora* EO. Briefly, the PVA solution (6% w/v) was prepared by dissolving in distilled water (stirring for 4 h at 80 °C). Then, the CHI-GT solution (3:1% w/v) was prepared by dissolving in an aqueous solution of acetic acid. Next, the CHI-GT solution was mixed with prepared PVA solution to a final concentration of 30% v/v acetic acid (stirred for 24 h). Then, various amounts of the EO (0, 2, 5, and 10% v/v) were added to the polymeric solution and stirred for an additional 24 h [60]. In step 2, each prepared polymer solution (containing different amounts of EO) was loaded into a 5-mL syringe with a blunted stainless-steel needle. A DC voltage of 21 kV was applied. The polymer feed rate and distance between needle and collector were fixed at 0.2 mL/h and 15 cm, respectively [60, 61]. The obtained *Z. multiflora* EO-blended NFs were reported as beadles and uniform fibers with a diameter of  $95 \pm 14$ ,  $154 \pm 27$ ,  $187 \pm 40$ , and  $218 \pm 58$  nm with various amounts of EO (0, 2, 5, and 10%, respectively) [60].

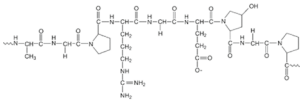
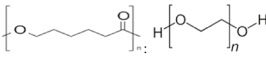
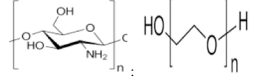
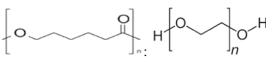
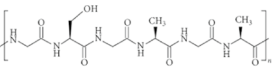
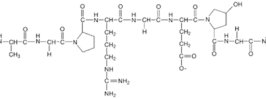
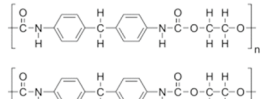
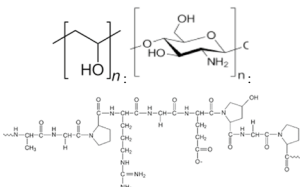
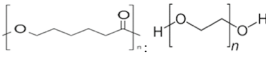
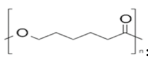
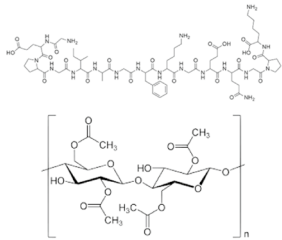
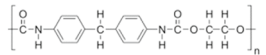
### NFs containing E/O filled in the carrier

Some examples for the preparation of NFs containing E/O filled in the carrier and their applications are listed in Table 4. In two of the researches, polymeric carriers were used, while in others, beta-cyclodextrin or its derivate was used.

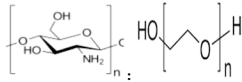

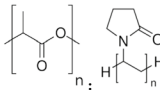
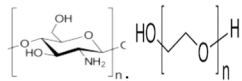
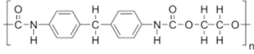
In such formulations, E/O was first loaded into the carrier; then, the carrier containing E/O was electrospun for preparing the NFs containing E/O filled in a carrier. However, in one report, clove oil was filled in PVA microcapsules and then grafted onto the previously developed nylon NFs via chemical cross-linking [78].



**Table 3** E/O-blended NFs and their applications

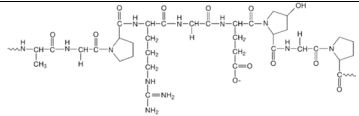
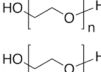
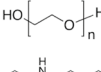
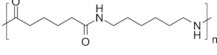
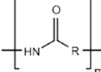


Polymer solution	Chemical structure	E/O	Application	References
GT		Corn oil	Encapsulate of hydrophobic compounds	[64]
PCL <sup>a</sup> :PEG <sup>b</sup>		Emu oil	Control of inflammation	[65]
CHI <sup>c</sup> :PEO <sup>d</sup>		chrysanthemum EO	Antibacterial as beef packaging	[66]
PCL:PEG		Emu Oil	Support for survival, proliferation, and maintaining the stemness of ADSCs <sup>em</sup>	[67]
SF <sup>e</sup> :GT <sup>f</sup>		Thyme EO	Drug delivery	[68]
PU <sup>g</sup>		Grape seed oil, honey, and propolis	Bone tissue regeneration	[69]
PU		Indhulekha oil	Tissue engineering purpose	[70]
PVA <sup>h</sup> :CHI:GT		Zataria multiflora EO	Wound dressing	[62]
PCL:PEG		Emu oil	Cytoprotection, proliferation and epidermal differentiation of ADSCs	[71]
PCL:COL <sup>i</sup>		Emu oil	Reinforce the cell adhesion and enhance ADSCs proliferation	[72]
CA <sup>j</sup>		Oregano EO	Antimicrobial Activity	[73]
PU		Bio oil <sup>TM</sup>	Wound healing application	[74]

**Table 3** (continued)

Polymer solution	Chemical structure	E/O	Application	References
CHI:PEO		Cinnamaldehyde oil	Loading of hydrophobic molecules for drug delivery	[75]
PLA <sup>k</sup>		<i>Matricaria chamomilla</i> EO	Differentiation of MSCs <sup>a</sup> into IPCs <sup>b</sup>	[76]
PLA:PVP <sup>l</sup>		Copaiba	Antimicrobial activity	[77]
CHI:PEO		Cinnamaldehyde oil	Antimicrobial activity	[78]
PU		Virgin olive oil	Suitable for biomedical applications	[79]

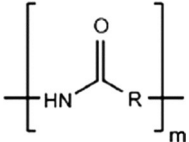
<sup>a</sup>Polycaprolactone, <sup>b</sup>Polyethylene glycol, <sup>c</sup>Chitosan, <sup>d</sup>Polyethene oxide, <sup>e</sup>Silk fibroin, <sup>f</sup>Gelatin, <sup>g</sup>Polyurethane, <sup>h</sup>Polyvinyl alcohol, <sup>i</sup>Collagen, <sup>j</sup>Cellulose acetate, <sup>k</sup>Poly lactide acid, <sup>l</sup>Polyvinyl pyrrolidone, <sup>m</sup>Adipose tissue-derived stem cells, <sup>n</sup>mesenchymal stem cells, and <sup>o</sup>insulin-producing cells

**Table 4** NFs containing E/O filled in the carrier and their applications

Polymer solution	Chemical structure	Carrier	E/O	Application	References
GT		CHI NPs	Clove oil	Antibacterial activity	[83]
PEO		beta-Cyclodextrin	Tea tree oil	Antibacterial packaging	[84]
PEO		beta-Cyclodextrin proteoliposomes	Cinnamon EO	Antibacterial activity	[82]
Nylon 66		PVA microcapsules	Clove oil	Acaricidal Effects	[80]
Zein <sup>a</sup>		beta-Cyclodextrin	Eucalyptus EO	Antimicrobial activity	[85]
PLA		beta-Cyclodextrin	Cinnamon EO	Antimicrobial packaging	[86]
PVP		2-Hydroxypropyl-beta-Cyclodextrin	Plai oil	Topical application	[87]

<sup>a</sup>Type of protein

**Table 5** NFs with core–shell structure (E/O and polymer)

Shell polymer	Chemical structure	EO as core	Application	Reference
Zein		Orange EO	Antimicrobial activity	[88]

The steps for preparing polyethylene oxide (PEO) NFs containing cinnamon EO filled in beta-cyclodextrin proteoliposomes are as follows. First, the EO was loaded into beta-cyclodextrin proteoliposomes as a carrier. Then, a certain amount of PEO powder was dissolved in distilled water (25% w/v) and stirred for 3 h at room temperature. Next, the PEO solution was mixed with EO-beta-cyclodextrin proteoliposomes in the ratio of 2:8 v/v (stirring 12 h at room temperature). Finally, for fabricating NFs containing EO filled in the carrier, the prepared solution was electrospun. The parameters were adjusted as DC voltage of 25 kV, the distance between the syringe needle tip and collector of 12 cm, and the polymer solution flow rate of 0.6 mL/h [79, 80]. Morphology analysis of the obtained NFs showed a rough surface with the diameters mostly ranged within 500–650 nm [80].

### NFs with core–shell structure

Among the references, just in one research, orange EO was loaded into the electrospun NFs as core material (Table 5). For preparing NFs with a core–shell structure, two separate syringe pumps are required. Furthermore, the special blunted needle consists of an inner needle (for feeding core) aligned with a surrounded needle (for feeding shell, see Fig. 3) which is also needed [86, 87]. In the mentioned report for preparing core–shell NFs, first, zein powder as shell polymer was dissolved in an aqueous solution of ethanol (80% v/v) with stirring at 300 rpm for 1 h [88]. Then, the solution was loaded into a 5-mL syringe connected to the outer inlet of the needle. The orange EO was loaded into another syringe and perfused into the inner inlet needle. The zein solution flow rate ranged from 1.5 to 3.0 mL/h, and for EO was 1.5 mL/h. The other operational parameters were as follows: DC voltage, 19 kV, and the distance between the needle tip and collector, 15 cm [88]. The minimum diameter of the obtained NFs was around 750 nm [88].

In this manner, to get NFs with proper core–shell structure control of the injection speed of the inner and outer fluid is crucial; the very high or meager speed of the internal fluid is not suitable [89]. At a very lower flow rate, an insufficient amount of core solution is delivered; thus, incorporation of the core into the shell does not continuously occur, while higher flow rates cause an increase in the size of the core until the shell solution is insufficient to surround the core solution and subsequently results in a mixing of core and shell solutions [90]. In fact, the shell cannot uniformly encapsulate the fast-moving of the core, and the overall process becomes unstable [91]. Therefore, at a lower ratio of feed rates of the core and the

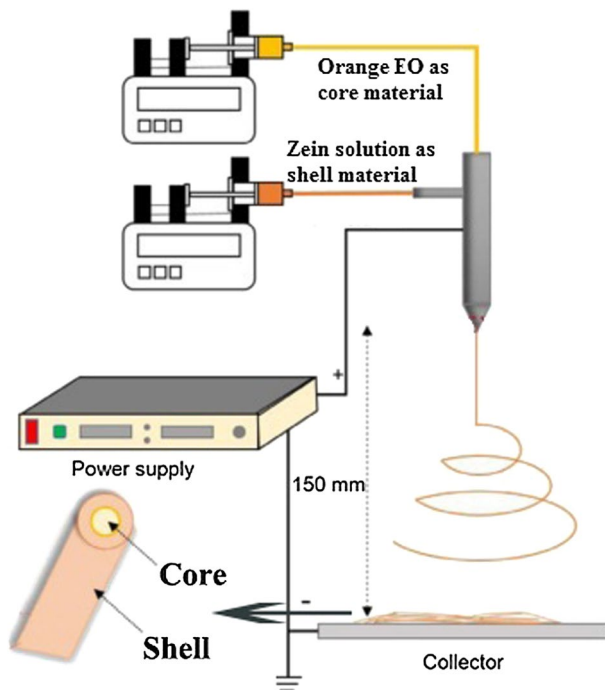


Fig. 3 Schematic for preparing NFs with core–shell structure (EO–polymer) [82]

sheath, better stable core/sheath structures of nanofibers with higher porous density are achievable [92, 93].

For a better comparison of three mentioned methods for loading of E/Os in NFs, drawbacks and advantages of each approach are listed in Table 6.

## Characterizations of electrospun NFs containing E/O

### Morphology and structure

The first properties to be investigated in NFs are diameter and morphology. It is now widely accepted that at the nanoscale, the surface-to-volume ratio is increasing, leading to new features in fibers. Furthermore, as discussed in the previous sections, one of the advantages of the electrospinning method is the regulation of the morphology of nanofibers for different purposes. For instance, random NFs are used for preparing of mats as wound dressing or aligned NFs in tissue engineering [94, 95]. Scanning electronic microscopy (SEM) is commonly used for determining the size and surface morphology of NFs [84, 85]. Besides, transmission electron microscopy (TEM) is applied to confirm the size, morphology, and core–shell structure of NFs [62, 96].

**Table 6** Comparison of developed methods for loading of E/O into NFs

	E/O-blended NFs	NFs containing E/O filled in the carrier	NFs with core-shell structure
Advantages	No need for extra process	Protection of EO for evaporate Requires low amounts of E/Os Possibility of choosing the right carrier according to the NFs polymer	Forming regular fibers Loading high amounts of E/Os into NFs
Drawbacks	The possibility of damage to the volatile components of EO Lack of a homogeneous mixture of polymer and E/Os Requires high amounts of E/Os	Requires initial loading of E/Os into the carrier Carrier size limitation Probability of changing the morphology of NFs	Requires accessories such as special needles and extra pumps Challenging in the optimization of process Requires high amounts of E/O

## Evaluation of E/O loaded into NFs

In the previous section, developed methods for loading of E/Os into electrospun NFs have been described. The purpose of loading E/Os is to utilize its properties as well as to take advantage of NFs simultaneously. Therefore, confirming and quantifying the amount of E/Os loaded into NFs is critical. In the reviewed articles for both parameters, few methods were presented as follows.

*Qualitative confirmation* The FT-IR analysis is frequently used to confirming the successful loading of E/O into the NFs. The FT-IR spectra of the E/O-loaded NFs with no-E/O-loaded NFs and free E/O are compared. By observing new peaks related to major constituents of E/O in the spectrum of NFs-E/O in comparison with the spectrum of NFs, the loading of E/O can be confirmed [70, 97]. Furthermore, by following SEM images of E/O-NFs and observing the NFs with a larger diameter in comparison with no-E/O-loaded NFs, it was concluded that E/O was loaded into the prepared NFs [64, 81].

*Quantitative calculations* two methods were used for determining the loaded amount of E/O into NFs: One method is based on weighting the ingredients of E/O-loaded NFs, and the other method was dependent on using analytical instruments (UV–Vis or GC–MS).

*A: Weighting method* E/O-loaded NFs were milled and dissolved in the mixture of water and ethanol (50:50) without exposure to sonicator [81, 97], and the mixture was centrifuged. After discarding the supernatant, the obtained plaque was dried. Using Eq. 1, E/O content in the NFs was calculated [97]:

$$\text{E/O content (\%)} = \frac{\text{Weight of E/O-loaded NFs} - \text{Weight of dried powder}}{\text{Weight of dried powder}} \times 100. \quad (1)$$

*B: Apparatus-dependent methods* E/O-loaded NFs were milled and dissolved in the mixture of water and ethanol (50:50) and centrifuged. In the next step, the amount of E/O in the supernatant was determined using UV–Vis or GC–MS analysis. For GC–MS analysis, the sample should be extracted by *n*-hexane. Using Eqs. (2) and (3), the E/O content and entrapment efficiency were calculated, respectively [5, 85]:

$$\text{E/O content (\%)} = \frac{\text{Weight of E/O in supernatant}}{\text{Weight of E/O-loaded NFs}} \times 100 \quad (2)$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{E/O content (mg)}}{\text{Theoretical amount of primary E/O (mg)}} \times 100. \quad (3)$$

The theoretical amount of primary E/O was calculated by the feeding rate in the optimized electrospinning condition.

## Release behavior

In most researches, one method with a slight difference was developed to determining released E/O from NFs. First, E/O content of NFs was determined (as described above). Then, EO-loaded NFs were added to a PBS solution. The amount of released E/O into PBS during different periods (1–15 days) was determined using UV–Vis or GC–MS analyses (described in the previous section). The release rate was calculated by Eq. (4) [64, 82]:

$$\text{Release rate (\%)} = \frac{\text{Released E/O (mg)}}{\text{E/O content (mg)}} \times 100. \quad (4)$$

Additionally, another method was reported for determining the release rate. A defined number of identical pieces of E/O-loaded NFs were placed in a petri dish and stored under constant conditions (room temperature and natural relative humidity). For the determination of the released E/O, the samples at different times were analyzed by UV–Vis or GC–MS technique, as described above. The obtained data were analyzed to determine the remaining amount of E/O in the specimens at each time point. By subtracting the remaining amount from the theoretical amount of primary E/O, the amount of released E/O is measurable [85].

## Future perspectives

Most of the process of synthesis of NFs is done by the device (except the preparation of polymeric solution); thus, human error is significantly reduced. Accordingly, it is possible to repeatability and mass production of NF-based products. On the other hand, more side effects of synthetic products in the food, pharmaceutical, and health industries are revealed every day. Therefore, the use of natural products has again attracted the attention of people all over the world.

Plants and their derivatives include a variety of extracts, oils, and EOs which are excellent sources of healthy food, as well as the production of health and even pharmaceutical products. Furthermore, NFs can be made from natural polymers such as chitosan, gelatin, silk, and cellulose. As described in detail in the preceding sections, the loading of E/Os into NFs is expanding, recently. The mentioned applications for NFs containing E/Os in the articles reviewed in this study are mainly focused on antibacterial activity, wound dressing, and tissue engineering. Nevertheless, some other applications are also conceivable such as in food packaging, bed net containing green insecticides, and mosquito-repellent-impregnated clothing in areas with a high prevalence of vector-borne diseases.

The number of articles published in this field is minimal among NF-related papers. However, it is anticipated that in the coming years, products based on E/Os and NFs will be highly developed.

## Conclusions

Reviewing the literature, three manners for loading of E/Os in electrospun NFs were developed, until now. Most simply, E/O was mixed with a polymer solution and then directly subjected to electrospinning for preparing E/O-blended NFs. In another manner, E/O was first loaded into the carrier and then was electrospun for the fabrication of NFs containing E/O filled in a carrier. The development of electrospun NFs containing E/O with a core–shell structure is the latest developed manner. In that manner, core and shell substrates were injected using two separate syringes connected to a blunted needle (with two distinct inner and outlet existence). Furthermore, methods for characterization of E/O-loaded NFs, including determining size and morphology, calculating loading, and release, were summarized. Due to the increasing use of E/Os in medicine as well as various products made from NFs, it seems shortly many products will be made using E/O-loaded NFs.

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## Compliance with ethical standards

**Conflict of interest** There is no conflict of interest to the authors.

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